University of Southampton

Faculty of Medicine

Clinical and Experimental Sciences

Exploring the Psychoneurobiology of the Placebo Response in Gambling, Depressive, and Anxiety Disorders

by

Dr Nathan Thomas Matthew Huneke (MBChB MRes MRCPsych)

ORCID ID 0000-0001-5981-6707

Thesis for the degree of Doctor of Philosophy in Clinical Neurosciences

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Abstract

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The discovery of new therapeutic agents for psychiatric disorders is potentially hindered by the large placebo responses seen in psychotropic drug trials. Through improved understanding of the placebo response, we might understand how to better design clinical trials and identify novel therapeutic targets. However, the placebo response in psychiatric disorders has been scarcely explored. Trial-level variables, such as duration of the trial or number of centres, appear to influence placebo response rate. Within-patient factors, such as previous experience of treatment, expectations, and neurobiology, are also likely to influence placebo response. Which factors matter, and how they influence placebo response in psychiatric disorders, remains unknown.

There are large placebo response rates in clinical trials of gambling disorder (sometimes exceeding 70%). I aimed to identify predictors of placebo and medication response in gambling disorder through a pooled analysis of individual patient data from six treatment studies. Multiple linear regression models demonstrated that baseline severity and number of weeks completed in the trial were predictors of medication response (p's < 0.01). By contrast, predictors of placebo response included increased baseline depressive symptoms, reduced baseline anxiety symptoms, and non-Caucasian ethnicity (p's < 0.05). These results were robust to choices made in the analysis.

It was noteworthy that symptoms of anxiety and depression were predictors of placebo response in gambling disorder. It is possible that biomarkers associated with placebo effects on anxiety or depressive symptoms might be relevant in other psychiatric disorders. I carried out a systematic review of the functional neuroimaging literature to identify neural correlates of placebo response in patients with anxiety or depression. Due to a small number of included studies and significant heterogeneity in study design, I was not able to carry out a formal meta-analysis. The rostral anterior cingulate cortex and default mode network, the ventral striatum, orbitofrontal cortex, and dorsolateral frontal cortex appeared to be key anatomical nodes in placebo antidepressant or anxiolytic effects. Important neurotransmitters might include endogenous opioids, dopamine, and serotonin.

The review highlighted a relative lack of research into placebo anxiolysis. This could be due to a lack of convenient experimental paradigms free of confounders. I developed and tested two experimental paradigms designed to induce placebo anxiolysis in healthy volunteers. The first combined verbal suggestions of improvement and a conditioning procedure with administration of a placebo nasal spray in the 7.5% CO_2 inhalational model of anxiety. The conditioning procedure induced significant expectations of improvement ($p < 0.001$) but this did not translate into reduced anxiety compared with a control group who did not undergo conditioning ($p's > 0.350$). In the second, volunteers with symptoms of social anxiety disorder were shown a placebo abstract video accompanied by suggestions that it would reduce their anxiety before completing a novel online social interaction task (InterneT-based Stress test for Social Anxiety Disorder, ITSSAD). The placebo again did not reduce anxiety compared with a control group who did not receive verbal suggestions of improvement ($p's > 0.236$). An exploratory analysis suggested that expectations did not influence outcomes. Acute anxiety is associated with nocebo effects, and it is possible that such experimental models are not conducive to inducing placebo effects.

My findings highlight several gaps in the literature for future study, as well as a few challenges. Further research is needed to explore whether application of knowledge regarding predictors of placebo response improves detection of medication efficacy in clinical trials, to understand how to optimise prospective neuroimaging study design into placebo effects in mental disorders, and to explore the interaction between autonomic arousal and placebo conditioning.

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Research Thesis: Declaration of Authorship

Print name:	Nathan Huneke
Title of thesis:	Exploring the Psychoneurobiology of the Placebo Response in Gambling, Depressive, and Anxiety Disorders

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

This work was done wholly or mainly while in candidature for a research degree at this University;
Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
Where I have consulted the published work of others, this is always clearly attributed;
Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
I have acknowledged all main sources of help;
Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
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Chapter 1 Introduction

Psychiatric illness is a leading cause of mortality and disability. Psychiatric disorders are very common, affecting more than one billion people globally[1]. Anxiety disorders, major depressive disorder, alcohol use disorder, and dementia are all estimated to affect at least 5% of the adult population[2,3]. Psychiatric disorders result in impairments in social and occupational functioning, causing possibly more lost working years than musculoskeletal disorders[4-6]. Indeed, affective disorders, anxiety disorders, substance misuse disorders, schizophrenia, and dementia are among the largest causes of disability worldwide[2,7,8]. Psychiatric illness is also associated with excess mortality. Mental disorders are thought to reduce life expectancy by 10 years, on average[9]. The result is significant costs in terms of lost productivity as well as social and healthcare costs. It is estimated that the global cost of psychiatric disorders will be $6 trillion by 2030[10]. In sum, psychiatric disorders are associated with significant disability and are socioeconomically costly.

Considering this large unmet need, there is a requirement to develop improved treatments for psychiatric disorders. Broadly, treatment options for psychiatric disorders fall under three categories: biological, psychological, and social. A crucial function of social interventions is education of patients and their families regarding psychiatric illness[11,12], but some treatments target specific symptoms: for example, physical activity programmes designed to increase activity levels in depression[13] or cognitive remediation to improve cognitive functioning in schizophrenia[11]. Several disorder-specific psychological interventions have empirical evidence for their use, including exposure therapy for specific phobia, interpersonal and social rhythm therapy for bipolar affective disorder, and cognitive-behavioural therapy for depression[14]. Biological treatments aim to reduce symptoms of psychiatric disorders through surgical (e.g. anterior cingulotomy), neurostimulatory (e.g. transcranial direct current stimulation), or pharmacological (e.g. selective serotonin reuptake inhibitors) therapy[15-17]. None of these treatments are 'ideal' at present. Access to psycho-social interventions is limited by scarce availability[18,19]. In addition, many patients do not improve with initial treatments. In patients with anxiety disorders, the probability of response to first line psychological or pharmacotherapy is approximately 50-70%, while the probability of remission is approximately 40-55%[20,21]. Similarly, less than half of patients with depression achieve remission following first-line psychological or pharmacotherapy[22,23]. Current treatments also cause unwanted adverse effects, such as deterioration in symptoms during psychotherapy, or metabolic side-effects with antipsychotic medication[24,25]. We need novel treatments with improved efficacy and side effect profiles.

Despite this clear need, and substantial investment into research and development for mental health, treatment efficacy has not much improved in the past 60-70 years[16,19]. Below, I discuss the challenges with novel treatment discovery for psychiatric disorders. A full discussion considering all possible treatment modalities is outside the scope of this thesis. I will instead focus on novel psychopharmacological treatment discovery; however, it should be noted similar discussions regarding novel psychosocial treatment discovery are also ongoing (for example, as described in this *Lancet Psychiatry* Commission report[19]).

1.1 Challenges with psychopharmacological treatment discovery

In recent times, many pharmaceutical companies have 'pulled out' of neuroscience research, including into treatment development for psychiatric disorders. The challenge of developing novel medications for psychiatric indications is highlighted by the average 13 year development time, the longest of all disease areas[26,27]. In addition, potential neuropsychiatric drugs show a large amount of attrition from phase I trials to approval, with failure to progress often occurring at a late stage[26-28]. Of the 60% of compounds that progress to phase II trials, only a third will progress to phase III trials, less than half of these will be reviewed by regulatory bodies, and only 8.2% will be approved[29]. There are likely several reasons for these challenges, and I discuss these further below.

1.1.1 Poor understanding of the neurobiology of psychiatric disorders

Most medications with a novel mechanism of action used in the treatment of psychiatric disorders have been discovered by serendipity[27]. Subsequent medications have mostly iterated on these mechanisms of action, for example D2 receptor blockade in schizophrenia or altering monoaminergic system function in depression[16,26]. However, not all patients respond to these treatments, suggesting that other systems might be involved in the aetiology of these disorders. For example, the discovery that ketamine acts as a rapid-acting antidepressant has implicated the N-methyl-D-aspartate (NMDA) receptor in the pathophysiology of depression[30]. Subsequently, dextromethorphan-bupropion has been developed, an antidepressant that also targets the NMDA receptor[31]. In addition, due to a lack of knowledge of the pathophysiology of psychiatric disorders, we might not be appropriately stratifying patients[32]. For instance, intravenous immunoglobulins can be effective for those with psychosis of autoimmune origin[33], but are unlikely to outperform D2 antagonists in those with non-autoimmune psychosis. Improved understanding of the neurobiology of psychiatric disorders might reveal novel tractable treatment targets or allow more personalised medicine in future.

1.1.2 Poor validity of preclinical studies

Before a novel compound is tested in clinical studies in humans, it is assessed in pre-clinical *in-vitro* and *in-vivo* animal models[34]. However, as stated above, the promise shown in pre-clinical studies often does not translate in the first clinical studies in humans[27,34,35]. There are probably two reasons for this, both of which relate to the same underlying issue, namely that efficacy in humans is measured through reports of symptoms. It is challenging to validly model human psychiatric symptoms in animals[26,36]. In addition, there are very few predictive and prognostic biomarkers for psychiatric disorders[16,26,27,37]. Such models and biomarkers are vital for pre-clinical studies. As a result, it is unclear how valid the readouts from pre-clinical studies are. Work is ongoing to improve the 'disconnect' between pre-clinical and clinical studies[16,37]. For example, there is some evidence that electroencephalography (EEG) markers translate from rodents to humans[38], suggesting EEG could be used as a translatable readout. Another approach to bridge this gap has been to develop experimental models in humans that mimic important clinical features of a disorder[39-41].

1.1.3 Placebo response

Another factor that needs to be considered is the substantial placebo response seen in psychotropic drug trials. The placebo response is an important and clinically relevant effect. Approximately 30% of patients in the placebo arm of randomised antidepressant drug trials show a clinically significant improvement[42-46]. Two meta-analyses in patients with anxiety disorders have shown that within-group pre-to-post effect size for placebo treatment ranges from 0.65 to 1.29[47,48]. In antipsychotic trials, the magnitude of the placebo response has increased over the past 40 years, while effect sizes for medication have remained stable[49,50]. There is additionally evidence that medication and placebo response are correlated, but that placebo response rate varies more than medication response rate[45,51-53]. Consequently, large placebo effects can reduce the 'assay sensitivity' of a clinical trial, resulting in failure to demonstrate efficacy of the investigational treatment[52-55]. For this reason, improved understanding of placebo effects, their biomarkers, and interplay with drug targets, is required[34,56].

1.1.4 Summary

There is a need for improved psychopharmacological treatments for psychiatric disorders. However, there are several challenges that inhibit the translation of promising novel compounds into effective treatments. These include a poor understanding of the neurobiology of these

disorders, limited validity of pre-clinical models, and the substantial placebo response that occurs in clinical trials.

Although much work is being done to improve our neurobiological understanding, to identify biomarkers, and improve translation across pre-clinical and clinical studies[16,27,37], the placebo response in psychiatric disorders has received comparatively little attention[56]. By improving our understanding of the placebo response in psychiatry, I believe we can improve clinical trial design, enhance the effectiveness of our current treatments, and possibly identify novel therapeutic targets[56]. This is my focus in the rest of this thesis. In sections 1.2 to 1.4, I summarise current knowledge regarding the placebo response. Next, I explore what is known about the psychoneurobiology of placebo effects on emotions in section 1.5. Finally, I discuss gaps in the literature and future directions in section 1.6.

1.2 Placebo response and psychotropic clinical trial design

In conditions predisposed to large placebo response rates, the clinical trial as an assay may exhibit reduced sensitivity to detect separation between active medication and placebo. It is important to understand which factors increase the placebo response rate in a trial, so that we can design trials to make this rate predictable, allowing accurate power calculations for subsequent studies[56]. Antidepressant trials are a good example of this principle in action. A 2016 meta-analysis showed that the placebo response rate in antidepressant trials increased from 1978 to 1991, but from 1991 it remained constant at 35–40%[42]. A meta-regression was also performed and showed that trials lasting longer than 4 weeks, multi-centre trials, and trials with flexible dosing regimes were all associated with increased placebo response rates[42]. The important finding is that once certain methods became standard practice (between 1990 and 2000), e.g. duration of 8 weeks, multi-centre trials being the norm, and fixed dosing schedules, the placebo response rate also became more constant. It is likely impossible and might even be unhelpful to completely eliminate the placebo response[57]. Nonetheless, this result suggests we can standardise certain factors in trial design and reduce variability of placebo response rate trial to trial. This would allow easier estimation of expected effect sizes for medication across trials, and allow assurance that clinical trials are properly powered to detect medication effects, reducing the likelihood of ‘failed trials’.

It is probable that the methodological factors that need to be standardised will differ between diverse conditions. For example, in antipsychotic trials increased sample size, shorter trial duration, shorter pre-trial washout, the rating scale used, studies outside the United States and shorter duration of illness are associated with an increased placebo response rate[58]. In attention-deficit hyperactivity disorder (ADHD) trials, year of publication, selective reporting of risk of bias,

risk of bias of the blinding assessor, incomplete outcome data, baseline severity, current depression, and region the trial took place in are significantly associated with placebo effect size[51]. The factors that are important need to be identified for all psychiatric conditions.

Consideration also needs to be given to within-subject factors that increase placebo response rate. Possibly the most consistent predictor of increased placebo response is reduced baseline severity of illness, which has been found in unipolar and bipolar depression, acute mania, and ADHD[45,46,51,59-61]. It should be noted, however, that baseline severity and change from baseline artifactually correlate[62]. Still, there is indirect evidence that psychological mechanisms are involved in driving placebo response in clinical trials in patients with psychiatric disorders. For instance, increased probability of being randomised to active treatment (e.g. due to multiple active treatment arms) is associated with higher placebo response rate[60,63]. This likely reflects increased beliefs in the patient that they have been randomised to active medication. This is corroborated by the observation that a medication's effects are greater in open-label trials than in double-blind trials, i.e. in situations when the patient is certain to be receiving active treatment[64,65].

Whether placebo responses result from statistical artifact or represent psychoneurobiological phenomena has been the subject of much debate over the past few decades. In the next section, I summarise this debate and the current consensus.

1.3 Placebo effect versus placebo response

A placebo (from the Latin for “I shall please”) is an inert substance or sham procedure that is given to a patient either as a control when testing the efficacy of a treatment, or as a form of psychological reassurance. The first recorded uses of this term appear in the late 18th Century[66,67]. Records indicate that physicians at this time routinely administered placebos to patients as a form of reassurance or to satisfy the patients’ demands for a treatment[66,67]. Following the second world war, placebo-controlled trials became a common method to measure the efficacy of a treatment[67]. The term “placebo effect” entered medical discourse following HK Beecher’s influential 1955 paper, in which he described how administration of a placebo in the context of a randomised-controlled trial can exert powerful therapeutic effects[67,68].

In the past three decades, this assertion has been questioned, with the observation that some patients improve despite receiving no treatment at all[69-74]. Indeed, a number of meta-analyses have shown that natural history or “no treatment” arms of clinical trials often show a statistically significant spontaneous improvement in symptoms, and that this improvement can be similar in size to the improvement seen in the placebo arm[70-72]. However, this is not uniform across

conditions. For example, specific phobia and insomnia show a relatively smaller effect of spontaneous improvement compared with placebo treatment[72]. Further, placebo pills show a much larger effect on symptoms than waiting list in anxiety disorders[48]. In addition, the context of a clinical trial, in which there is uncertainty regarding the treatment being received and its efficacy, is likely to reduce the effect of placebo. This has been demonstrated by a meta-analysis comparing clinical analgesic trials with studies in which the primary aim was to induce and investigate placebo analgesia (mean effect size of placebo was 0.15 and 0.95, respectively)[75]. In the latter, the placebo effect is maximised through the use of verbal suggestions or conditioning procedures. Further supporting the idea that uncertainty in clinical trials reduces the effect size of placebo are meta-analyses showing that the more active treatment arms a psychotropic trial has the greater the placebo effect[60,63]. Overall, it appears that receiving a placebo treatment under certain conditions can cause effects on symptoms over and above spontaneous improvement.

Achievement of a placebo response appears to involve many factors. As described above, one non-specific factor includes spontaneous improvement. Non-specific factors can be thought of as causing "apparent placebo effects", as they lead to clinical improvement in the placebo arm of a trial, but are not directly related to the placebo itself[69,74]. Other such non-specific factors include natural course of the illness, features of a clinical trial such as repeated ratings of symptoms, unidentified parallel interventions such as changes in a patient's diet over the course of a trial, sampling bias due to dropouts of the least improved patients in the placebo arm, and natural fluctuation in symptom severity (regression to the mean)[69,76]. [77-80] (see Figure 1.1). These mechanisms are described further in section 1.4 below.

To aid placebo research in the future, definitions that capture apparent and true placebo effects were recently reached by expert consensus. "Placebo response" was defined as "all health changes that result after administration of an inactive treatment (i.e., differences in symptoms before and after treatment"), including apparent and true effects[81]. The "placebo effect" was defined as "changes specifically attributable to placebo and nocebo mechanisms", which includes true placebo effects only[81]. The terms "placebo response" and "placebo effect" are used in the rest of this thesis in line with these definitions.

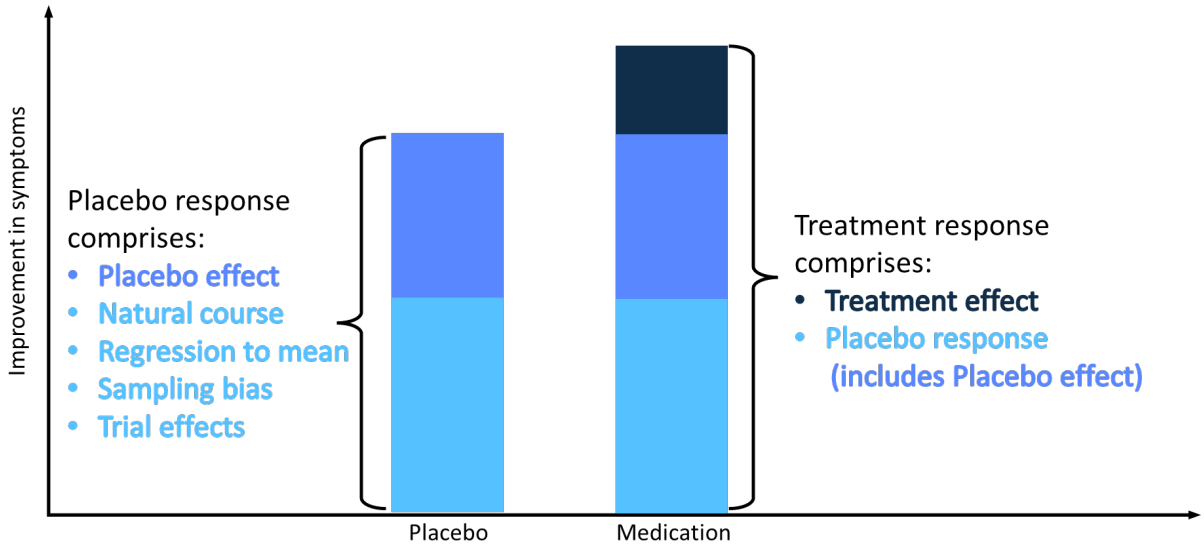


Figure 1.1 Cartoon showing the relationship between placebo response and placebo effect. Placebo effect refers to changes in symptoms resulting directly from specific placebo mechanisms, while placebo response includes the placebo effect and additionally consists of non-specific effects, such as natural course of the illness and regression to the mean. Similarly, treatment response comprises the specific effect of treatment *and* placebo response which *includes* the placebo effect.

1.4 Mechanisms of placebo effects

Placebo treatment affects a diverse set of biological systems leading to improvements in symptoms. Placebo effects have been demonstrated experimentally in many physiological systems and conditions including: acute pain[79,82-85] and chronic pain[86], immune function[77,78], Parkinson's disease[87,88], ADHD[89], nausea[90,91], itch[92], irritable bowel syndrome[93], short-term memory performance[94], mood[95], major depression[65,96,97], and social anxiety disorder[98]. Although a placebo contains no active ingredients, it is not inert, as it is administered within a specific context. This involves both external context, such as clinical equipment, verbal suggestions and social cues by the health practitioner, and an internal context, including previous experience of treatment, emotional state, and expectations[79,99-101]. These contexts exist prior to the treatment being given, but internal associations are also updated after receiving the treatment based on its results[76]. It is thought that this interplay between prior expectations and learning leads to the recruitment of 'top-down' brain control systems that regulate incoming sensory information[79,99,101]. Below, I outline in more detail our current understanding regarding the psychoneurobiology of placebo effects.

1.4.1 Psychological mechanisms: expectation and learning

Placebo effects are known to involve both expectation and learning. The role of expectation can be demonstrated by use of the 'open-hidden paradigm'. In these experiments, active medication is either administered in full view of the participant or is administered in a hidden fashion, either by a machine or with instructions that no medication is being administered. Several studies have shown that open administration of treatment in acute pain, Parkinson's disease, depression and social anxiety disorder is superior to hidden administration[65,98,102-105]. Since the treatment is the same, the difference in efficacy can be attributed to a placebo effect mediated by expectations[54,100,101,105]. Expectations of therapeutic benefit can also be induced or manipulated through verbal suggestion before the placebo is given. Such verbal suggestion has been shown to produce placebo analgesia[106], and placebo effects on mood[95].

Placebo effects can also be induced in the absence of conscious expectation through learning, either via classical conditioning or observational learning. An example classical conditioning paradigm involves pairing an active medication (unconditioned stimulus) with a neutral stimulus (for example, a green drink) for a number of doses. Eventually, the neutral stimulus becomes a conditioned stimulus that can evoke a conditioned response similar to that created by the active medication[54]. Such paradigms have effectively induced placebo immunosuppression[78], placebo

effects in ADHD[89], and placebo analgesia[107]. An alternative classical conditioning paradigm involves pairing a placebo with a reduction in aversive stimulus intensity to experimentally create a 'successful treatment'. For example, pairing the application of a moisturising cream with a reduction in pain stimulus intensity leads to reductions in subjective pain when stimulus intensity is returned to moderate levels[84,85,108]. However, classical conditioning is not the only form of learning that is important, as learned placebo effects can also occur through observation. For example, after observing a demonstrator experience pain relief, the degree of placebo analgesia may be similar to the analgesia experienced by those undergoing conditioning directly[82].

The terms 'expectation' and 'expectancy' are frequently used interchangeably in the literature. However, *explicit* expectancies produced via verbal suggestion or other means differ from *implicit* expectancies that can be produced via classical conditioning. To capture this difference, it has been suggested that the term 'expectation' should be used to described explicit, verbalised constructs; while 'expectancies' should refer to psychophysiological 'predictions' made without full conscious awareness[109,110]. The terms 'expectation' and 'expectancy' are used in line with this suggestion in the rest of this thesis.

It is likely that expectations, expectancies, and learning do not operate independently, but rather are inter-related and reinforce one another. Although conditioning or verbal suggestions alone can induce placebo effects, placebo effects are stronger when conditioning and suggestions are paired[76,92,111,112]. Moreover, previous experience of treatments can affect subsequent efficacy of placebo treatments. Placebo analgesia is reduced in participants who have experienced an ineffective analgesic treatment previously, even if route of administration is changed[113-115]. Likewise, placebo analgesia is greater in those who have experienced a successful treatment previously[114]. It is likely that learning takes place during these experiences, and this creates an expectation about future treatments. This becomes self-reinforcing, with a positive expectation causing a positive experience, which in turn creates greater expectations for the future[76]. However, if symptom relief is paired with a different explanation, for example that an experimenter has reduced the aversive stimulus intensity, then a placebo effect can be prevented[84,108]. It appears that to reliably induce a placebo effect, one requires both positive treatment experiences (learning) and a belief that the treatment is the cause of symptom relief (expectations)[76].It appears then that the generation of expectations or expectancies results from *suggestions*, either verbal or behavioural in origin. One could argue, therefore, that an individual's *suggestibility* could be an important determinant of individual placebo responsiveness[116]. However, placebo and suggestion are typically viewed as distinct concepts, possibly due to suggestion being poorly defined[117]. There is growing interest in incorporating measures of suggestibility in future placebo research as this might inform understanding about how

expectations and expectancies are generated[118,119]. A full discussion about the definition and measurement of suggestibility is outside the scope of this thesis, but I have summarised potential measures that might be relevant in Table 1.1.

Table 1.1 A non-exhaustive list of possible approaches that could be used to quantify placebo-relevant individual differences in suggestibility and related factors

Concept	**Example measures**	**Potential addition to knowledge**
Direct verbal suggestibility	Harvard Group Scale of Hypnotic Susceptibility (HGSHS:A)[120] Stanford Hypnotic Susceptibility Scale: Form C (SHSS:C)[121]	Individuals higher in direct verbal suggestibility might develop more definite expectations as a result of verbal suggestions
Interrogative suggestibility	Gudjonsson Suggestibility Scale[122]	The tendency for someone's behaviour to be affected by messages within formal questions. This might influence response biases in placebo research
Conformity	Asch's test of conformity[123]: participant and confederates asked to announce which of three lines are identical to a standard presented line. On some trials confederates unanimously answer incorrectly. Conformity is measured as the number of times the participant also answers incorrectly	Might be relevant for production of expectancies resulting from social observation
Post-event recall	Could be assessed by measuring stability of memories in response to differing language in questions about those events. For example, participants who were asked how fast two cars were going when they 'smashed into each other' after watching a car accident on video were more likely to report seeing broken glass one week later compared with those questioned with less extreme language[124]	Might influence response biases in placebo research
Interoceptive awareness and sensitivity	Scales such as the multidimensional assessment of interoceptive awareness (MAIA)[125] Tasks such as heartbeat detection task[126] for interoceptive sensitivity	Heightened awareness of internal bodily states might interfere with placebo-related expectations/expectancies

The above information was summarised from the following citations[116-119,127]

These psychological placebo mechanisms can also be important in determining outcomes in clinical trials. For example, medications in open-label trials produce larger effects than the same drug in a double-blind trial[64,65]. A patient's expectations will subsequently be updated through experience and learning[76]. Such learning effects might confound crossover designs[54], and previous treatment experiences might influence a patient's expectations on entry to a clinical trial[128]. These possibilities have yet to be empirically tested, and the potential size of the effects is unknown.

1.4.2 Neurobiological 'top-down' control mechanisms

The interaction between expectations and learning described above is thought to recruit neurobiological systems involved in top-down control of incoming bottom-up sensory information. Placebo analgesia has been explored considerably and so our understanding of the 'top down' mechanisms involved in this placebo effect are well understood.

Placebo analgesia consistently causes reduced activity within the pain processing network[129], but it is perhaps more informative to discuss the regions that show increased activation under a placebo manipulation. A meta-analysis of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies conducted in 2014 showed that placebo analgesia consistently increases activity in prefrontal regions, such as rostral anterior cingulate cortex, ventromedial prefrontal cortex, orbitofrontal cortex, and dorsolateral prefrontal cortex, and in the ventral striatum and periaqueductal grey matter[129].

The anterior cingulate cortex and periaqueductal grey matter are known to be key regions in the endogenous opioid system, a system that is important in top-down pain control[130]. Consistent with this, placebo analgesia is reliably inhibited by opioid antagonists such as naloxone[83,102,131]. Functional neuroimaging studies suggest that placebo analgesia increases endogenous opioid activity within anterior cingulate cortex, dorsolateral prefrontal cortex and periaqueductal grey matter[132-135]. Endogenous opioids might also be important in placebo effects in other domains. Recent evidence suggests a role for endogenous opioid release in placebo antidepressant effects[96,136]. Therefore, the importance of the endogenous opioid system might not be limited to pain.

The endogenous opioid system is not the sole mediator of placebo effects. For example, when the non-opioid analgesic ketorolac is administered for 2 days and then switched to a placebo on day 3, placebo analgesia is not reversed by naloxone but is instead reversed by rimonabant, a cannabinoid type 1 (CB1) receptor antagonist. Interestingly, rimonabant does not block placebo

analgesia that has been conditioned with morphine[137]. This suggests that the endocannabinoid system might be important in non-opioid placebo analgesic effects.

The ventral striatum and ventromedial prefrontal and orbitofrontal cortices are also reliably activated by placebo analgesia. The ventral striatum is an important centre of dopaminergic neurotransmission and has connections with ventromedial prefrontal and orbitofrontal cortices[138]. This suggests that the dopamine system might also be important in mediating placebo effects. Indeed, in one study, degree of dopamine release in the nucleus accumbens explained 25% of the variance in placebo analgesia in healthy volunteers[139]. Further, placebo effects in Parkinson's disease have been demonstrated to have a dopaminergic basis[88,140]. It is unclear whether dopamine is important in placebo effects in other domains.

Although placebo analgesia has been investigated in detail and understanding of the mechanisms is accelerating, the neurobiology of placebo effects in psychiatric disorders has been relatively poorly investigated. Accumulating evidence suggests a link between the neurobiology of placebo effects and the neurobiology of the experience of different affective states. There could therefore be an intriguing interaction between placebo mechanisms and psychiatric symptoms. I discuss the potential neurobiological overlaps further below.

1.5 The psychoneurobiology of placebo effects on emotions

For decades, emotions have been thought of as discrete, innate, and universal entities (e.g. the so-called 'basic emotions': 'anger', 'fear', 'happiness', 'sadness', 'disgust', and 'surprise')[141,142]. More recently, however, this assertion has been questioned. If there were 'basic emotions', one would expect to be able to delineate each emotion through some distinct, observable phenomena[143]. Instead, evidence suggests that subjective experience of emotion, objective measures of behaviour such as facial expression, and their associated functional neuroanatomy correlate poorly[143]. A possible explanation is that emotions are not discrete entities, but are instead 'constructed' through evaluation of internal physiological changes and external cues through the lens of previous experience[141,143-145]. Indeed, it is possible that these constructions are integral to perception of the world[146]. For example, when perceiving a strawberry, both the visual information of a small, soft, red object and physiological information such as salivation and mild heightened level of arousal would be used by the brain to inform the prediction that this is a strawberry. Importantly, the pleasurable, mild heightened level of arousal might also become conscious leading to the object being perceived as both a strawberry and pleasant[146]. It is thought that affective information is required for accurate perception: a red object causing pleasurable, mild arousal is more likely to be a strawberry than a traffic light[146]. In other words, an emotion is a

type of constructed perception resulting from an interaction between prior predictions and incoming, noisy, sensory information.

The idea that emotions and perceptions are constructed in concert is potentially relevant for understanding placebo effects. It has been theorised that placebo analgesia, and perhaps placebo effects more generally, are mediated by a reduction in negative emotions in anticipation that a distressing symptom might soon improve (Figure 1.2)[79,147]. This is supported by evidence that increased anxiety is known to reduce placebo, and increase nocebo (the opposite of placebo), effects on pain[109,148-150]. Increased anxiety has also been correlated with nocebo effects on itch[151]. Perhaps the altered affective information results in changes to predictions regarding symptom intensity. This raises the question of whether the 'top-down' neurobiological regulatory systems that mediate placebo effects also mediate regulation of emotions. If so, this would suggest that key neurobiological systems in placebo effects are clinically relevant in psychiatry. Such systems might be important therapeutic targets.

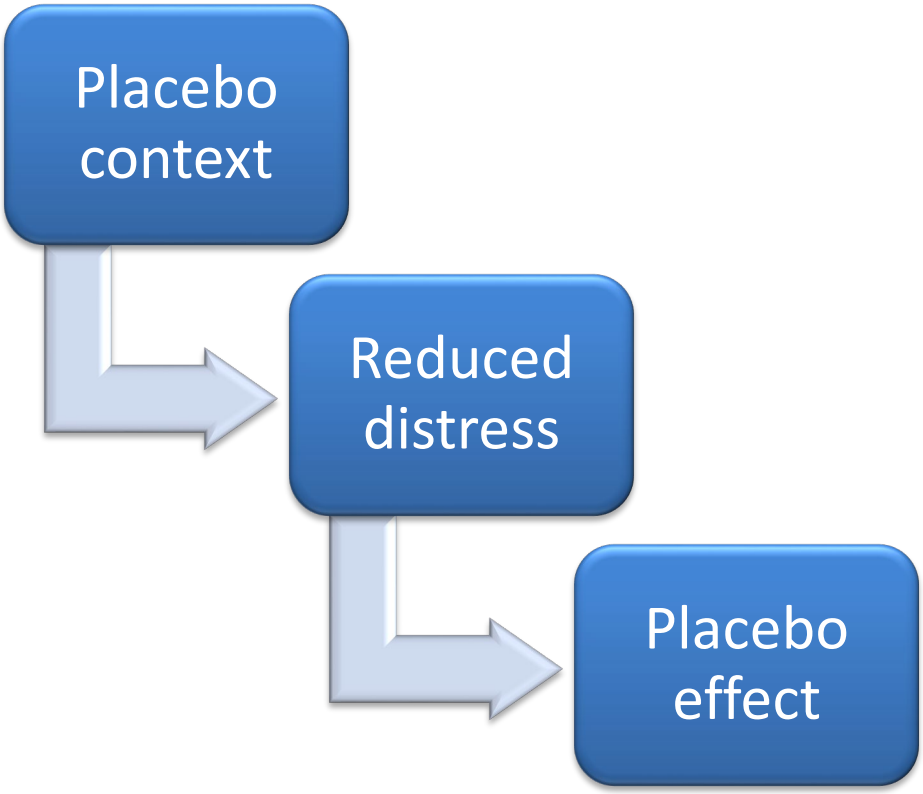


Figure 1.2 One theory of placebo effect is that reduction in distress regarding symptoms is the mediating step between placebo context and placebo effect.

Another consideration is that placebo mechanisms might alter the predictive model about the emotional meaning of physiological changes, potentially reducing the subjective (or perceived) intensity of distressing emotions. Conditioning procedures and/or verbal suggestions appear to be capable of causing placebo effects on feelings of sadness[95,152,153], disgust[154], and unpleasantness[155,156], and preventing rumination[157] in healthy volunteers. Experiments have also attempted to induce placebo effects on anxiety in healthy volunteers[158,159]. These studies can offer clues regarding psychoneurobiological systems that are important in both placebo effects and construction/regulation of emotions. Below, I describe behavioural and neuroimaging experiments in which placebo effects on emotion have been studied.

1.5.1 Sadness

Placebo effects on sadness in healthy volunteers have been explored in three studies, all by the same research group[95,152,153]. First, the effects of a placebo nasal spray for sadness were investigated in 128 healthy female participants[95]. The placebo nasal spray contained sesame oil and a low dose of capsaicin (0.0007%) to produce a mild stinging sensation, as placebos that cause mild side-effects are more effective than 'inactive' placebos[160]. Participants were randomised to one of four conditions: 'citalopram' (suggestions the nasal spray would reduce feelings of sadness), 'ondansetron' (suggestions the nasal spray would increase feelings of sadness), 'placebo' (suggestions the spray would have no effects on sadness), and 'no treatment' (no nasal spray). The experimenters were blinded to treatment condition, other than for the 'no treatment' group who did not receive a nasal spray. Following this, participants watched a sequence from *The Champ* to temporarily induce sadness. Subjective mood was measured before and after the sadness induction using a modified version of the sadness sub-scale from the Positive and Negative Affect Schedule-Expanded Form (PANAS-X), where each item was scored from 0 (*"not at all"*) to 10 (*"very much"*). Repeated measures multivariate analysis of variance (ANOVA) showed significant effects of time and group*time interactions, driven by increased sadness in all groups, except the 'citalopram' group in which sadness remained unchanged[95]. *Post-hoc* tests revealed the effect size of the placebo was moderate to large (Hedges' g ranged from 0.59 to 0.87)[95]. Interestingly, subjective sadness in the 'ondansetron' group did not differ from the 'placebo' and 'no treatment' groups, implying the suggestions of increased sadness did not result in a nocebo effect. A follow up study with the same placebo, sadness induction, and outcome measures investigated whether deception was necessary to cause placebo effects on sadness in 147 healthy volunteers (68% female)[153]. Participants were randomised to differing experimental conditions relating to whether the placebo was open-label or deceptive and the style of information presented (rational facts versus a personal story). The personal story recounted the

experience of a fictitious participant deriving benefit from the placebo, while the rational facts were presented with scientific terminology and stated the 'drug' would protect from sadness through re-uptake inhibition of serotonin or the placebo would protect from sadness through conditioning mechanisms. The result was five experimental groups: scientific rationale (deceptive or open-label), personal rationale (deceptive or open-label), and a 'no treatment' control group. Interestingly, there were no differences in sadness depending on type of rationale, but the open-label placebo groups exhibited significant increases in sadness following the film while the deceptive placebo groups exhibited no change in sadness[153]. Indeed, post-film sadness was similar in the control and open-label groups, and significantly lower in the deceptive groups[153].

Finally, the effect of a placebo on autonomic measures following sadness induction has been assessed in 120 healthy female volunteers[152]. The study design was similar to those described above, with an identical placebo nasal spray and identical subjective measures of sadness. However, sadness was instead induced through the presentation of negative self-evaluation statements combined with mood congruent music. Skin conductance level and heart rate were measured during and after the sadness induction. Participants were randomised to a 'citalopram' group, a 'placebo' group (suggested not to affect sadness), and a 'no treatment' group that did not receive the placebo. As with the above studies, the experimenter was blinded to 'citalopram' and 'placebo' condition, but was not blind to the 'no treatment' condition. In this study, sadness significantly increased following the induction procedure in the 'placebo' and 'no treatment' groups, but not the 'citalopram' group[152]. Further, skin conductance level was significantly reduced in the 'no treatment' group compared with the 'citalopram' group with a moderate effect size ($d = 0.60$), but the difference between 'citalopram' and 'placebo' groups was not significant[152]. There were no significant differences between groups in heart interbeat interval.

The results from the studies above suggest that expectations can 'protect' healthy volunteers from experiencing sadness during an acute experiment. However, there are some potential confounds. When the placebo was given openly, the effect on sadness did not differ from no treatment. By contrast, open-label placebos have shown effects on symptoms in a variety of conditions including back pain, cancer-related fatigue, allergic rhinitis, irritable bowel syndrome, and major depression (for a review see[161]). Indeed, an open-label placebo also reduces ratings of unpleasantness in healthy volunteers (see section 1.5.3)[156]. Interestingly, in these studies that showed an effect of open-label placebo, there was no 'active' treatment arm. Perhaps participants' knowledge that there was another 'drug' group in the above studies affected expectations regarding potential benefits of the 'placebo'. Moreover, the effects of the placebo were larger on subjective outcomes compared with autonomic outcomes. This might reflect responder bias: the participants might have given responses that they believed were the expected

responses, or that they believed would please the researcher[162,163]. The risk of responder bias should have been reduced through blinding the experimenter, although the experimenter was not *completely* blind in these studies. Thus, sadness reporting might have been increased in the 'no treatment' groups, for example. Finally, most participants in these studies were female, and it is unknown whether the results would generalise to a male-predominant or more balanced sample.

1.5.2 Rumination

The effect of a placebo nasal spray on acute induction of rumination in healthy volunteers has been investigated in a single study[157]. Ninety-one participants (47 females, 52%) underwent a negative mood induction that involved a combination of negative autobiographic recall and listening to sad music. Following this, they were randomised to either a control group that received no treatment or a deceptive placebo group, in which participants were told they would receive an antidepressant nasal spray called 'Ramiprazol-direct'. In reality, as with other studies by the same group above, the nasal spray contained only a low dose of capsaicin (0.0007%). Following this, participants completed a rumination task, in which they were asked to read statements designed to direct their attention inwards (e.g. "*Think about how tired you feel"*) and consider associated thoughts and feelings for 8 minutes. State rumination was subsequently assessed via a validated questionnaire called 'Questionnaire to assess state rumination'. An ANOVA with repeated measures showed a significant interaction of time*group, driven by a significantly lower rumination score following rumination induction in the placebo group (d = 0.57)[157]. However, there was no placebo-expectation control group. The experimenter and participant were additionally unblind to experimental condition. It is therefore unclear whether the lower rumination score was the result of placebo mechanisms (such as expectation) or another phenomenon such as responder bias or disappointment at not receiving a treatment for those in the 'no treatment' group.

1.5.3 Unpleasantness and distress

Three studies have been published in which placebo effects on 'unpleasantness' or 'distress' were investigated[155,156]. The first involved a conditioning paradigm followed by an fMRI experiment in fifteen healthy female volunteers[155]. On day 1, participants were shown unpleasant and neutral pictures from the International Affective Picture system (IAPS) and asked to rate feelings of unpleasantness. Following this, they were given an infusion of midazolam 0.015mg/kg, an anxiolytic drug, and repeated the picture viewing. This reduced subjective ratings of unpleasantness. They were then given an infusion of flumazenil 0.25mg, which reverses the effects of midazolam. They then viewed the pictures again and unpleasantness ratings increased.

On day 2, participants were given two infusions of 5ml saline in the fMRI scanner. During one infusion participants were told they were receiving midazolam, while during the other infusion they were told they were receiving flumazenil. This manipulation produced significantly reduced unpleasantness ratings and bilateral amygdala activity during the placebo condition ("midazolam") compared with the control condition ("flumazenil")[155]. Placebo also activated lateral orbitofrontal cortex (OFC) and rostral anterior cingulate cortex (rACC), regions typically associated with placebo analgesia, and rACC activity correlated positively with degree of placebo response[155]. It should be noted that although the conditioning procedure involved an anxiolytic drug, participants were asked to rate the pictures' 'unpleasantness'. Unpleasantness is a complex emotional concept, and so it is unclear which underlying emotion was manipulated. Potential emotions could include anxiety, disgust, or sadness.

In two related studies, investigators explored whether an open-label placebo treatment could reduce 'emotional distress' when viewing negative images[156]. Sixty-two healthy volunteers (23 females) took part in experiment 1, while 198 volunteers (all female) participated in experiment 2. In both experiments, participants were randomised to open-label placebo or control. In the former, participants read an article about placebo effects, including information that placebo effects can occur without deception. In the latter, participants read an unrelated article about pain. Following this, the experimenter gave the participants a placebo saline nasal spray with differing instructions. In the placebo group, the experimenter reminded participants that the placebo would work if the person believed it would; while for the control group the experimenter stated the nasal spray was important for obtaining good quality physiological readings. The experimenter was not blind to experimental condition. Following administration of the nasal spray, participants completed an image viewing task. In this task, participants were shown neutral and negatively valenced images and then asked to rate how 'distressed' the images made them feel. Experiment 1 focussed on self-report behavioural results, and showed that participants in the open-label placebo reported significantly less distress following negative pictures than the control group[156]. Experiment 2 focussed on the sustained late positive potential (LPP), an EEG marker of emotional distress. Participants in the open-label placebo group exhibited a gradual reduction in LPP throughout the picture presentation while those in the control group showed no change in magnitude in the LPP[156]. Although the experimenter was not blinded, the authors argued that since the open-label placebo affected an EEG biomarker, the placebo effect on distress was likely over and above responder bias[156]. Nevertheless, it remains unclear which emotion was manipulated, as 'emotional distress' is a vague construct. Moreover, experiment 2 included only female participants and it is unknown if similar effects on LPP would be seen in male participants.

1.5.4 Disgust

Investigators have attempted to image placebo effects on disgust through a crossover study in 34 healthy female volunteers[154]. During two fMRI sessions, participants were shown images of either a disgust, fear or neutral valence. Each fMRI session was separated by 1 week. During one of these sessions, participants received a placebo pill with verbal suggestions that the pill contained pulverised bark of the angostura tree, a powerful anti-disgust herbal medicine. In the other session, participants received no tablet. After each scan, participants were shown the images that they had seen during the scan again and were asked to rate the intensity of fear and disgust elicited for each category. Paired t-tests showed that both fear and disgust ratings were reduced in the placebo condition. Compared with the no-placebo condition, in the disgust vs neutral contrast, the placebo was associated with reduced activation of the left insula. No regions showed increased activity. Exploratory analyses of the fear vs neutral contrast showed no significant activation changes. An exploratory psychophysiological interaction (PPI) analysis in the placebo condition showed reduced functional connectivity between bilateral amygdala and the insula and orbitofrontal cortex, and increased connectivity between dorsomedial prefrontal cortex and amygdala in the disgust vs neutral contrast[154]. A potential confounder in this study is that the pictures were repeated multiple times, which might have altered emotional responses to them. The authors checked for order effects to attempt to rule this out and there was no difference between participants who received placebo first or second. However, participants were also shown the images after the scan in the presence of the experimenters to rate their emotional response, which could introduce recall bias or responder bias. It is therefore unclear whether the imaging results truly represent the reported emotional responses.

1.5.5 Anxiety

Placebo anxiolysis has also been explored in three related studies, all through an unpredictable threat of shock paradigm[158,159]. In these studies, healthy volunteers underwent an experimental task comprising 12 runs. In 6 of these runs, the participants were given a placebo with verbal suggestions that it was an anxiolytic drug (either intranasal lorazepam or laughing gas), while in the other 6 runs they were given a placebo and told it was inert. Each run involved 7-12 trials of either threat or no threat valence, indicated by differing geometrical cues. In threat trials, the probability of receiving a painful electric stimulus was 25-33%, and participants were aware of this. In all three experiments, placebo reduced subjective fear and skin conductance responses globally, regardless of whether the trial was threat or non-threat[158,159]. Furthermore, event-related potentials measured through electronencephalography were also attenuated in all placebo runs regardless of trial type[158]. This was interpreted as reduced sustained arousal and

vigilance resulting from placebo treatment. Supporting this, the authors also found increased alpha activity and increased frontal theta power on EEG in the inter-trial intervals, suggesting reduced arousal and possibly recruitment of top-down control mechanisms[158]. The third study also demonstrated evidence for placebo-attenuated responsiveness to salient cues during fMRI scanning. Regardless of trial type, placebo reduced activity within the salience network, and increased activity within the rostral anterior cingulate cortex[159]. Further, placebo administration increased functional connectivity between the rostral anterior cingulate cortex and the salience network, and this was generalisable to the entire default mode network[159].

There are, however, some problems with this placebo paradigm. It is unclear whether the reduced arousal that was measured truly represents placebo anxiolysis, or instead represents another phenomenon. Often, anxiety in an unpredictable shock paradigm is measured through startle responses rather than subjective fear ratings or skin conductance as in the above studies[164]. Indeed, in healthy volunteers unpredictable threat of shock has been associated with *reduced* skin conductance responses compared with predictable shocks[165]. As such, it is unclear whether what has been interpreted as reduced arousal seen in this placebo manipulation is indeed a measure of anxiety reduction. Moreover, this placebo procedure was carried out under single-blind conditions, and so the possibility of responder bias cannot be discounted.

1.5.6 Summary

In sum, placebo effects on sadness, rumination, and unpleasantness have been investigated behaviourally, while neuroimaging correlates of placebo effects have been assessed in disgust, unpleasantness, and anxiety. There was some evidence in these studies that treatment expectations could influence the experience of emotion. However, there were a number of confounds that mean the results should be interpreted with caution, particularly issues around blinding, possible gender bias, and potential for responder bias.

The above issues notwithstanding, the neuroimaging correlates of placebo effects on emotion suggest there is at least one neurobiological system that might be important in both placebo effects and other forms of emotion regulation. Reduced feelings of ‘unpleasantness’ were related to reductions in amygdala activity, and associated with increased rACC and lateral OFC activity[155]. Consistent with this, placebo effects on disgust were associated with reduced connectivity between the OFC, insula and bilateral amygdala[154]. Increased rACC activity was also associated with reduced arousal following placebo administration, as well as increased functional connectivity between the default mode and salience networks[159]. In line with these findings, the default mode network, and the ventromedial prefrontal cortex which includes the rACC in

particular, has recently been hypothesised to be central in generating placebo effects[76]. Studies of placebo analgesia have shown expectancy- and placebo-induced reductions in bilateral amygdala activity as well as increases in rACC, OFC, dorsolateral prefrontal cortex, and periaqueductal grey matter[129]. These findings suggest that the top-down control of emotion and pain might share common mechanisms. Indeed, automatic emotion regulation processes are known to recruit regions including ACC, OFC and medial prefrontal cortex, while voluntary processes additionally recruit lateral prefrontal cortex, to downregulate amygdala activity[166,167]. A potential common neurotransmitter system might be the endogenous opioid system, as the ACC, OFC, and the amygdala are known to be important nodes in this system (see Figure 1.3)[130,168]. The endogenous opioid system is important in regulation of pain transmission, and in placebo analgesia[79,130]. Accumulating evidence suggests this system also regulates a range of other physiological processes including gastrointestinal function, immunity, and affective states[168-170]. If activation of the endogenous opioid system through expectancies leads to psychiatric symptom relief, then by extension it is possible that this system might be a relevant treatment target for psychiatric disorders. However, it is unclear whether the experimental emotion inductions used in placebo research to date are ecologically valid for psychiatric disorder. For example, some evidence suggests that dysfunctional cognitions are only observed during experimentally induced sad mood in those predisposed to clinical depression[171]. Similarly, threat of shock induces a functional anxiety response to a genuine stressor as opposed to the dysfunctional anxiety that is characteristic of anxiety disorders[172]. We need experimental paradigms that are more ecologically valid to fully understand the psychoneurobiological mechanisms of placebo effects in patients with psychiatric disorders.

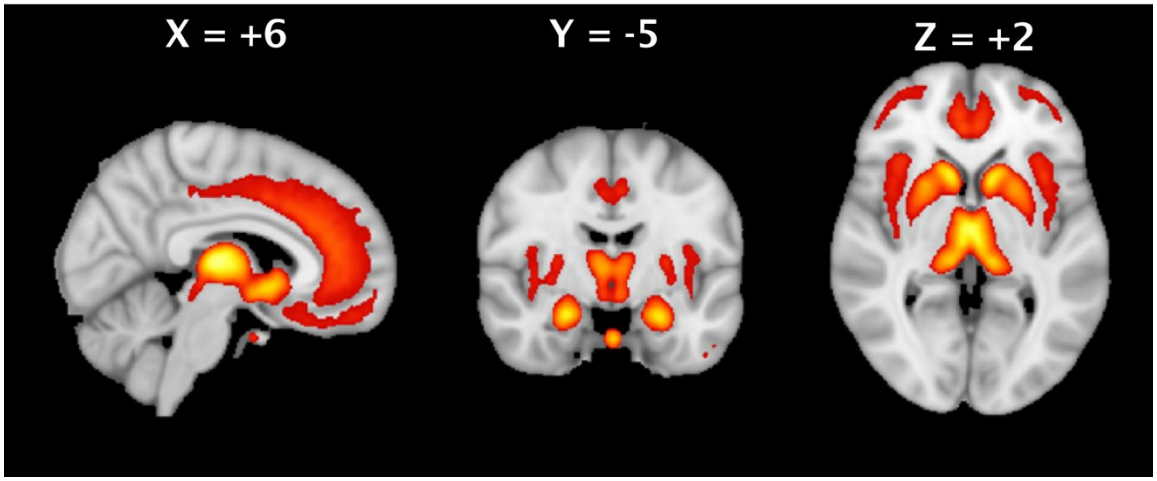


Figure 1.3 Distribution of μ-opioid receptors in the brain, demonstrated by mean [^{11}C]carfentanil non-displaceable binding potential from PET imaging of 89 healthy volunteers, visualised on the standard Montreal Neurological Institute (MNI) template.

Image adapted with permission from[168], statistical parametric map available from https://neurovault.org/media/images/4841/mean_carfentenil_BPND_88_1mm.nii.gz

1.6 Overall summary and gaps in the literature

Improved treatments are needed for psychiatric disorders. However, the discovery of new therapeutic agents is potentially hindered by the large placebo responses seen in psychotropic drug trials. By understanding the placebo response, we might understand how to better design clinical trials and identify potential novel therapeutic targets for psychiatric disorders.

In this chapter, I have summarised our current understanding concerning how placebo mechanisms operate in, and influence the outcome of, clinical trials. Variables at both the trial level, for example duration of the trial or number of centres, and at the within-subject level, such as expectations and previous experiences, are likely to influence placebo response rate. However, the relative importance of these variables likely differs between conditions. Analyses have been carried out exploring these in depression[42], bipolar affective disorder[46], schizophrenia[58] and ADHD[51], but predictors of placebo response rates have been scarcely explored in other conditions. Whether predictors of placebo response rate differ from predictors from medication response rate is also unknown for the majority of psychiatric disorders.

I have also discussed our current understanding of the mechanisms that drive placebo effects. Psychological mechanisms include expectations and learning, which likely interact to recruit top-down neurobiological control systems. The best understood of these neurobiological systems is the endogenous opioid system, mainly through research into placebo analgesia. There is evidence to suggest these psychological mechanisms and the endogenous opioid system might also be important in placebo effects on emotion, which might be clinically relevant for psychiatry. This comes from studies in which attempts were made to induce placebo effects on sadness, rumination, disgust, feelings of unpleasantness, and anxiety. However, these experimental paradigms had confounders that mean it is unclear whether they truly measured placebo effects on emotion. It is also unclear whether these experimental paradigms are clinically relevant. There are two approaches that could address this. First, we can investigate the neuroimaging correlates of placebo effects in patients with psychiatric disorders to determine whether similar brain regions and neurotransmitters are associated. Second, we can explore placebo mechanisms in an experimental paradigm that has little risk of bias and high construct and ecological validity for clinical symptoms. Such an experimental paradigm does not yet exist.

In the next chapter, I summarise my aims for this thesis, my research questions, and work packages intended to address these.

Chapter 2 Aims and Work Packages

2.1 Aims

The overall aim of this thesis is to improve our understanding of the psychoneurobiology of placebo effects in psychiatric disorders. I believe that progressing knowledge in this area will lead to improvements in the design of psychotropic clinical trials and potentially lead to identification of novel treatment targets. More specifically, my aims for the studies described in this thesis are to:

1. Advance knowledge regarding predictors of medication and placebo response in patients with psychiatric disorders.
2. Explore whether neuroimaging correlates of placebo effects in patients with psychiatric disorders correspond with those seen in experimental studies in healthy volunteers. This would help to identify whether findings from these studies are likely to be relevant in clinical populations.
3. Develop a novel experimental placebo paradigm that has little risk of bias and high construct and ecological validity for clinical symptoms.

Below I describe the work packages intended to meet these aims.

2.2 Work packages

2.2.1 Work package 1: Predictors of treatment response in gambling disorder

As I discuss in Chapter 1, interest in predictors of medication and placebo response in psychotropic trials is growing. A disorder that shows large placebo response rates (sometimes exceeding 70%) is gambling disorder[173]. The pathophysiology of gambling disorder is thought to involve the opioidergic and dopaminergic systems, which are also implicated in placebo effects[56,79,174]. Yet, only one study has explored predictors of placebo response in this patient group[175], and one study has investigated predictors of treatment response in trials of opioid antagonists for gambling disorder[176]. I sought to ascertain predictors of both medication and placebo response in patients with gambling disorder, to advance knowledge in this area.

2.2.1.1 Research question

Which factors predict treatment response in clinical trials of gambling disorder, and do the predictors differ between active medication and placebo?

2.2.1.2 Approach

Predictors of placebo response in clinical trials of psychiatric conditions have been investigated through meta-analysis and meta-regression[42,46,51,58] and through analysis of individual patient-level data[43,175]. Meta-regression allows one to explore how differences in study characteristics might influence the effect size of placebo treatment. If there are differences on average in the patient populations of included studies, then these can also be included in a meta-regression model. However, important information can be lost through meta-regression compared with analyses of individual patient data, as by definition the outcome modelled is an average effect. Analyses of individual data allow one to assess the effect of within-subject variables on the interindividual degree of response with greater statistical power and less risk of bias than can be achieved with meta-regression[177-179]. Therefore, pooling data from multiple trials was the best approach to answer my research question. This approach has been used in the past to explore predictors of placebo response in patients with anxiety and depressive disorders. Study-level predictors, such as studies conducted in Europe, and within-subject predictors, such as baseline severity, were found to be significant[43].

I was fortunate to be able to collaborate with Professor Jon Grant (University of Chicago) and Professor Sam Chamberlain (University of Southampton) who had access to a suitable dataset. This dataset included individual patient data from multiple clinical trials in gambling disorder all carried out by the same research group. I report the results of an investigation into the predictors of treatment response in this dataset in Chapter 3.

2.2.2 Work package 2: Ascertain neuroimaging markers of placebo response in patients with depressive or anxiety disorders

Attempts have been made to generate placebo responses in experimentally-induced emotional states in healthy volunteers including sadness[95,152,153], disgust[154], rumination[157], feelings of unpleasantness[155,156], and anxiety[158,159]. However, these experiments were affected by confounds, and the relationship between these experimentally-induced emotional states and clinical psychiatric symptoms is unclear (see section 1.5). Some of these studies involved neuroimaging. The findings of these studies suggest that brain regions associated with the endogenous opioid system might be important in placebo effects on emotion. If activity in similar brain regions is

associated with placebo responses in patients, then this would increase confidence that the data from these healthy volunteer studies are clinically relevant. The most relevant clinical conditions to the emotional domains studied thus far are depressive and anxiety disorders. Both depressive and anxiety disorders exhibit altered processing of negatively valenced emotional information, and this is detectable through neuroimaging[180,181]. I sought to investigate the functional neuroanatomical correlates of placebo responses in patients with these disorders.

2.2.2.1 Research question

What are the functional neuroanatomical correlates of placebo response in patients with depressive or anxiety disorders?

2.2.2.2 Approach

This question could have been answered by experimentally inducing placebo responses in patients with depressive or anxiety disorders as they underwent neuroimaging. However, even if there were an experimental paradigm with low risk of bias, it would remain unclear whether any observed neural activity would also mediate 'naturally-occurring' placebo effects such as those seen in clinical trials. Another approach could have been to carry out a placebo-controlled trial and measure neuroimaging correlates of improvement in the placebo group. But, although placebo response rate in psychotropic trials averages 30%[42,44,50], there is no guarantee that any patients would respond. Further, I would also likely need to scan a large number of patients to achieve adequate statistical power. I decided that a more feasible approach was to systematically search the literature of clinical trials in patients with depressive or anxiety disorders that have involved neuroimaging for data concerning neural correlates of placebo response.

A systematic review is an in-depth, comprehensive summary of the literature regarding a specific topic. Using explicit and reproducible methods, the literature is systematically searched, critically appraised and synthesised, which reduces the chance of errors or bias[182]. Where identified research is similar, the results can be statistically synthesised in a meta-analysis[182]. This approach is ideal for the current research question. Such approaches have previously been used to explore neuroimaging correlates of placebo analgesia[129,183]. I report the results of a systematic review of functional neuroimaging correlates of placebo response in patients with depressive or anxiety disorders in Chapter 4.

2.2.3 Work package 3: Develop a novel experimental placebo paradigm with high validity for clinical psychiatric symptoms

To test hypotheses regarding the psychoneurobiology of placebo effects on psychiatric symptoms, an experimental paradigm is needed that reliably and reproducibly generates a placebo response. Even though there are large placebo response rates in clinical trials for psychiatric disorders, it is not practical to conduct all placebo research in patients. Patient research is complex, expensive, and challenging in terms of recruitment and confounders, such as comorbidity and other treatments[41]. In addition, there are potential ethical questions to consider. If an active treatment is known to be beneficial, then withholding this to conduct placebo research would arguably not be in patients' best interests. Using a placebo is easier to justify ethically when the efficacy of an active treatment is unclear; but this uncertainty might reduce the effect size of placebo[75,184]. Indeed, experimental manipulation in healthy volunteers maximises placebo response[75,184], and recruitment of these participants is relatively simple, potentially speeding up research in this field. Experiments in healthy volunteers will be important in the future, but the experimental paradigm must be reliable, easy to reproduce, and have clinical validity.

2.2.3.1 Research question

Is it possible to induce a placebo response in an experimental model of psychiatric symptoms in healthy volunteers?

2.2.3.2 Approach

Human experimental models have been developed for several psychiatric disorders. Models of psychosis include administration of apomorphine (non-selective dopamine agonist)[185], NMDA antagonists such as ketamine[186], or serotonin receptor 2A agonists such as psilocybin[186]. Human experimental models of depression include depletion of dietary tryptophan[186] or administration of human endotoxin[187]. These models induce symptoms or experiences in healthy volunteers similar to those seen in their respective psychiatric disorders. However, these models are not ideal for research into placebo mechanisms as they involve administration of a drug or special diet designed to induce symptoms, which could be considered a nocebo. Expectancies regarding the additional pill, diet, or injection might confound expectancies regarding the placebo itself. Another experimental model of depression involves measurement of emotion processing biases[188]. As an example, treatment with a selective serotonin reuptake inhibitor (a first-line treatment for depression) relatively improves recall of positive emotional events compared with negative ones in healthy volunteers[189]. This shift in emotional processing biases is thought to lead to improvements in depressive symptoms. This does not involve administration of a 'drug' or

'nocebo'. However, I have carried out research previously that suggests placebo responses in this model are minimal[190]. Therefore, the models described thus far are not ideal for the development of a novel experimental placebo paradigm.

In contrast with many psychiatric symptoms, fear and anxiety are easy to trigger and quantify in the laboratory[41]. Several experimental models of clinical anxiety in healthy volunteers have been developed (see Table 2.1). These include pharmacological challenges, such as administration of the benzodiazepine antagonist flumazenil[39], behavioural challenges, such as the Trier Social Stress Test (TSST)[40], and physiological challenges, such as inhalation of carbon dioxide (CO_2)[40].

Table 2.1 A non-exhaustive list of experimental models of clinical anxiety used in healthy volunteers

Pharmacological challenges	**Physiological challenges**	**Behavioural challenges**
• Caffeine • Cholecystokinin • Flumazenil (benzodiazepine antagonist) • Yohimbine (alpha-2 adrenergic antagonist)	• Inhalation of CO_2 • Hyperventilation • Sodium lactate infusion	• Unpredictable threat of electric shock • Darkness • Trier Social Stress Test

List of models gathered from the following citations[39-41,172]

As discussed above, pharmacological models of anxiety are not ideal for development of an experimental placebo paradigm. However, physiological challenges that allow manipulation of the intensity of an aversive stimulus for the participant could be an ideal basis for a clinically-relevant placebo paradigm. Inhalation of air enriched with 7.5% CO_2 (or 7.5% 'CO_2 challenge') mimics the subjective, autonomic and neurocognitive features of generalised anxiety disorder[191-193]. In addition, anxiety in this model is ameliorated by standard pharmacological and psychological treatments for generalised anxiety disorder[194,195]. Since this model is treatment-responsive, has construct validity for clinical anxiety, and allows for control of the anxiogenic stimulus intensity, it is ideal for use in an experimental placebo procedure. I report an attempt to create a novel placebo procedure using this anxiety model in Chapter 5.

Similarly, a behavioural challenge that causes dysfunctional anxiety (as seen in an anxiety disorder) could be used to generate clinically-relevant experimental placebo responses. The TSST is a reliable and ecologically valid method for inducing acute stress under controlled conditions[196], and has been used as an experimental medicine model of social anxiety disorder since it induces social-evaluative threat[40]. Measures of stress and anxiety in the TSST are also ameliorated by standard anxiolytic treatments, e.g. alprazolam[197], and experimental treatments, e.g. buprenorphine[198]. This suggests that the TSST has good construct validity for social anxiety symptoms. In Chapter 6, I report the results of a study in which I attempted to generate a placebo response in a modified version of the TSST for use with videoconferencing software.

Chapter 3 Predictors of Placebo Response Differ from Predictors of Medication Response in Gambling Disorder

3.1 Background

Gambling is a recreational activity that is found throughout the world. However, in some individuals, gambling behaviour can become persistent, recurrent and maladaptive, leading to problems with relationships, employment and financial difficulties[199]. The prevalence of gambling disorder is approximately 1%, with a further 3-4% exhibiting subclinical problem gambling behaviours[200,201]. Furthermore, patients with gambling disorder often experience comorbid psychiatric disorders, such as depression or anxiety disorders[200,202]. Therefore, gambling disorder yields a significant public health burden. At present, there are no licensed treatments for gambling disorder. One contributing factor to this is the large placebo response rate seen in clinical trials for gambling disorder treatments. Placebo response rates can surpass 70%[173]. As a result, it can be challenging to demonstrate medication benefits over placebo.

Improved understanding of which factors predict placebo response could inform better designs of clinical trials. For example, standardising certain predictors (e.g. trial duration), or controlling for them in the analysis (e.g. including previous experience of treatment as a covariate), might improve assay sensitivity of the placebo-controlled trial[56]. Predictors of placebo response in clinical trials of psychiatric conditions have been explored through meta-analysis and meta-regression[42,46,51,58]. Meta-regression enables an assessment of how variations in study characteristics might influence a treatment's (or placebo's) effect size. However, important information can be lost through meta-regression compared with analyses of individual patient data, as by definition the outcome in the model is an average effect. By contrast, analyses of individual patient-level data allow estimation of the effect of within-subject variables on the interindividual degree of response. For example, state anxiety and noradrenaline levels have been associated with interindividual variability in placebo-induced interleukin-2 suppression[203].

Two previous studies in gambling disorder have involved pooled analyses of patient-level data to explore predictors of treatment response[175,176]. The first pooled data across two placebo-controlled trials of opioid antagonists (naltrexone and nalmefene)[176]. A family history of alcoholism and the strength of gambling cravings were significantly related to medication response, while placebo response was associated with younger age[176]. The second pooled data

from 152 patients randomised to placebo from several clinical trials in gambling disorder[175]. Predictors including number of weeks completed in the trial, ethnicity, and reporting enjoyment as a trigger to gambling behaviour, were significantly associated with placebo response[175].

In both studies described above, patients were dichotomised into responders and non-responders[175,176]. However, it is unlikely that there is an underlying dichotomy in placebo responsiveness. For example, if the threshold for response is determined to be 35% improvement, then a patient who improves by 33% is classed as a non-responder, despite improving a great deal. Dichotomising the outcome into "responder" and "non-responder" likely causes loss of information regarding its relationship with predictor variables, reducing statistical power[204,205]. Further, the threshold for labelling treatment response in gambling disorder remains unclear[206]. A further limitation of these studies is that the comparison with medication response was limited. Either a single medication class[176] or patients treated with placebo only[175] were included. However, it is important to know which variables predict medication response *and* placebo response in terms of clinical trial design. To maximise the assay sensitivity of the clinical trial, placebo response rate needs to be predictable and potential medication response rate needs to be maximal[54]. I therefore chose to include data from medication *and* placebo arms of clinical trials in gambling disorder, with the aim of identifying predictors that transcended any particular class of medication.

In this study, I aimed to identify predictors of placebo response in gambling disorder, and whether these differed from predictors of medication response. Individual patient-level data were pooled from six treatment studies all carried out by the same group of researchers in a quasi-meta-analysis (see section 2.2.1.2). These treatment studies were:

1. A 16-week randomised-controlled trial of the SSRI paroxetine compared with placebo in outpatients with gambling disorder in five centres across Spain and the USA. Paroxetine was initiated at 10 mg/day, titrated to 20 mg/day during week 2, and was prescribed with flexible dosing up to 60 mg/day thereafter. There was no significant difference in response rate between groups (59% in the paroxetine group versus 49% in the placebo group)[207].
2. A 12-week open-label trial of the SSRI escitalopram. Escitalopram was initiated at 10 mg/day and increased at fortnightly intervals to 30 mg/day if needed. Gambling symptoms severity was significantly reduced at the end of treatment[208].
3. A 14-week open-label trial of N-acetylcysteine (NAC). NAC is thought to reduce reward-seeking behaviour by increasing extracellular concentrations of glutamate in the nucleus accumbens[209]. In this study, NAC was initiated at 600 mg/day for 2 weeks and increased

fortnightly to 1800 mg/day if needed. Gambling symptoms severity was significantly reduced at the end of treatment[209].

4. An 18-week randomised-controlled trial of the opioid antagonist naltrexone compared with placebo. Patients were randomised to either naltrexone (50 mg/day, 100 mg/day, or 150 mg/day) or placebo. Outcomes were equivalent between all doses of naltrexone. Compared with placebo, patients randomised to naltrexone showed significantly greater decreases in gambling severity, urges, and behaviour (according to scores on the Yale-Brown Obsessive Compulsive Scale adapted for Pathological Gambling [PG-YBOCS])[210].
5. A 10-week open-label trial of memantine. Similarly to NAC, memantine is thought to reduce gambling cravings by increasing extracellular glutamate in the nucleus accumbens via NMDA antagonism[211]. In this study, memantine was initiated at 10 mg/day for 2 weeks and was titrated fortnightly to 30 mg/day as needed. The retention rate in this study was unusually high, with 28 of 29 patients completing the entire 10-week study. Symptom severity significantly reduced[211].
6. A 12-week randomised-controlled trial of NAC compared with placebo in patients with co-occurring gambling disorder and nicotine dependence. Patients were randomised to either NAC 1200 mg/day (which was increased to 3000 mg/day based on clinical judgement) or placebo. In the first 6 weeks, patients received additional smoking cessation treatment, and in weeks 6-12, patients received motivational interviewing and imaginal desensitisation therapy for gambling disorder. For the current study, data from the 6-week period of treatment for gambling disorder are included. NAC showed no statistically significant benefits over placebo[212].

3.2 Method

3.2.1 Participants

In all trials, patients were adults (aged 18-75 years) with a diagnosis of gambling disorder according to DSM-IV criteria, which was confirmed through a semi-structured clinical interview[213]. Exclusion criteria were: current significant medical illness or abnormalities on physical examination; current pregnancy or breastfeeding; history of dementia, bipolar affective disorder, or any psychotic disorder; current substance misuse or dependence except nicotine; and recent (within 3 months) initiation of psychological or behavioural therapy.

All trials were carried out in accordance with the protocol and provisions of the Declaration of Helsinki. All study procedures and accompanying literature were approved by The Institutional

Review Boards of the University of Minnesota and the University of Chicago, USA. All patients provided informed, written consent to participate.

3.2.2 Study assessments

Data were gathered for all eligible patients regarding general demographics and problems due to gambling. Additionally, patients completed the following measures at baseline:

1. Structured Clinical Interview for Gambling Disorder (SCI-GD) – a clinician-administered, nine-item instrument that assesses gambling symptoms over the past 12 months according to the DSM criteria for gambling disorder (modified to reflect DSM-5)[213].
2. Structured Clinical Interview for DSM-IV (SCID-I) – to assess for the presence of psychiatric comorbidity[214].
3. Gambling Symptom Assessment Scale (G-SAS) – a validated, reliable self-report measure of gambling symptom severity over the past week. This scale consists of 12 items, each scoring 0-4, with a maximum score of 48[215].
4. Hamilton Rating Scale for Depression (HAM-D) – a validated, reliable clinician-administered measure of depression severity over the past month[216].
5. Hamilton Rating Scale for Anxiety (HAM-A) – a validated, reliable clinician-administered measure of anxiety symptom severity over the past month[217].

Improvement in G-SAS from baseline was an outcome measure in all included trials, and was chosen as the primary outcome measure for this analysis. For participants who withdrew before the end of the trial, the last available observation was used (last observation carried forward).

3.2.3 Statistical Analysis

All statistical analyses were carried out using Jamovi version 1.6.23.0 (https://www.jamovi.org)[218]. A one-way ANOVA demonstrated that there was no significant effect of study on mean change in G-SAS ($F_{(5,200)} = 1.65$, $p = 0.147$). Data were therefore pooled from all studies for subsequent analyses.

Potential predictors of treatment response were assessed through multiple linear regression analysis to reduce the risk of bias that can be introduced by dichotomising outcome and covariate variables[204,205]. I chose to enter the following predictors into a multiple linear regression model, based on reported relationships in the literature between predictor variables and treatment outcomes and *a priori* hypotheses, as detailed below:

- Demographic predictors: Age, sex, and ethnicity (dichotomised to Caucasian and non-Caucasian). Younger age has been associated with placebo response, and worse outcomes after psychological treatment, in gambling disorder[176,219,220]. Being male is a predictor of improved outcome after psychological therapy for gambling disorder[220]. Finally, Caucasians are less likely to be classed a placebo "responder" in randomised-controlled trials for gambling disorder[175].
- Predictors related to treatment: Whether the patient had previously received treatment for gambling disorder and the number of weeks the patient participated in the trial. Previous treatment experience can affect subsequent treatment outcomes[113-115]. Further, time spent in a clinical trial and adherence to treatment have been associated with placebo response and response to psychological therapy in gambling disorder[175,220].
- Clinical and severity predictors: Baseline G-SAS, HAM-A and HAM-D scores, whether the patient had any psychiatric co-morbidity, and whether the patient played strategic (e.g. poker) or non-strategic games (e.g. slots). Baseline severity of symptoms and psychiatric comorbidity has been associated with responsiveness to placebo in other conditions (e.g. depression[43]) and with outcome following psychological therapy in gambling disorder[220,221]. Finally, strategic gambling has also been linked to improved treatment outcome[221,222].

Since I had no hypotheses regarding whether one predictor would better explain the data than another, I entered all predictors simultaneously. I initially ran the model in all patients. Next, to identify whether predictors of treatment response differ for placebo or active medication, I ran the same models separately in patients randomised to active medication and randomised to placebo. The models were only applied to the pooled sample of patients in which all entered variables had been measured (i.e. patients with missing data were excluded via listwise deletion). Since the placebo response rate in gambling disorder trials is high[173], it is common practice to exclude patients with mild disorder from these trials to prevent floor or ceiling effects interfering with detection of medication effects[57]. Therefore, to ensure external validity of the current analysis, I excluded patients with a baseline G-SAS of less than 20 (classified as 'mild' severity of symptoms).

I assessed the robustness of my findings to choices made through two sensitivity analyses. First, I carried out the same regressions but included those with mild G-SAS scores at baseline to ensure that excluding these patients had not introduced selection bias. Second, I ran the model with percentage change in G-SAS as the dependent variable, rather than absolute change in G-SAS, to identify whether any predictors reached significance due to floor or ceiling effects.

3.3 Results

3.3.1 Baseline Characteristics

The final intent-to-treat sample size was n = 279 patients, which reduced to n = 235 patients after excluding those with a G-SAS of less than 20 at baseline. Of these, 168 patients (71%) were assigned to active medication arms and 67 (29%) were assigned to placebo. The patients' mean age was 47.76 ± 11.11 years. The majority of patients were single or divorced (n = 129, 55%), and 32 (18%) had a previous history of alcohol dependence. On average, patients started gambling aged 37.82 ± 12.52 years, and mean time to gambling becoming a problem was 10.07 ± 9.61 years. Forty-two percent of patients had previously sought treatment for gambling disorder. Across all studies, mean baseline G-SAS was 31.51 ± 6.42 (severe), which on average reduced to 19.28 ± 10.78 (mild). Baseline characteristics are summarised in Table 3.1.

Table 3.1 Baseline characteristics

Continuous variables	**Mean ± SD**
Age (yrs)	47.76 ± 11.11
G-SAS baseline	31.51 ± 6.42
HAM-A baseline	7.03 ± 4.37
HAM-D baseline	7.11 ± 4.08
Weeks completed	10.89 ± 5.15
Grant et al., 2003[207]	13.47 ± 4.30
Grant and Potenza, 2006[208]	8.80 ± 3.05
Grant et al., 2007[209]	7.77 ± 4.02
Grant et al., 2008a[210]	13.21 ± 6.06
Grant et al., 2010a[211]	9.78 ± 1.15
Grant et al., 2014[212]	6.00 ± 0.00
Categorical variables	***N* (%)**
Females	122 (52.0)
Caucasians	193 (86.0)
Previous gambling treatment	67 (42.0)
Psychiatric co-morbidity present	89 (51.0)
Strategic gambling	27 (12.0)
Education	
High school graduate or less	48 (21.0)
Some college	89 (40.0)
College graduate or more	87 (39.0)

Abbreviations: SD, standard deviation; G-SAS, Gambling Symptom Assessment Scale; HAM-A, Hamilton Rating Scale for Anxiety; HAM-D, Hamilton Rating Scale for Depression

3.3.2 Predictors of treatment response

3.3.2.1 All patients

When pooling all patients, the regression model was significant and explained 18% of the variance in change in G-SAS (adjusted $R^2 = 0.18$, $F_{(11,119)} = 3.60$, $p = 0.00021$). Positive predictors of treatment response included baseline G-SAS ($\beta = 0.37$, t = 4.26, $p < 0.0001$), number of weeks completed in the trial ($\beta = 0.19$, t = 2.33, $p = 0.0215$), and baseline depression severity ($\beta = 0.36$, t = 2.29, p = 0.0235). Baseline anxiety severity was the only negative predictor of treatment response ($\beta = -0.52$, t = -3.32, p = 0.0012).

3.3.2.2 Active arms only

For patients receiving active treatment, the regression model was significant and explained 22% of the variance in change in G-SAS (adjusted $R^2 = 0.22$, $F_{(11,85)} = 3.46$, p = 0.00051). The only positive predictors of treatment response in this model were baseline G-SAS ($\beta = 0.43$, t = 4.17, p < 0.0001) and number of weeks completed in the trial ($\beta = 0.30$, t = 3.20, p = 0.0020). Baseline anxiety severity approached significance as a negative predictor ($\beta = -0.35$, t = -1.87, p = 0.0643).

3.3.2.3 Placebo arms only

For patients receiving placebo, the regression model was significant and explained 30% of the variance in change in G-SAS (adjusted $R^2 = 0.30$, $F_{(11,22)} = 2.29$, $p = 0.04725$). Baseline anxiety severity was a negative predictor of placebo response ($\beta = -1.03$, t = -3.88, p = 0.0008), while baseline depression symptoms positively predicted placebo response ($\beta = 0.62$, t = 2.32, p = 0.0299). Further, non-Caucasian patients exhibited significantly increased change in G-SAS scores following placebo treatment ($\beta = 0.97$, t = 2.50, p = 0.0203).

Table 3.2 Results of multiple linear regression models calculated to predict treatment response (change in G-SAS). Separate models were calculated for all patients, and for those allocated to active medication or placebo.

	All patients		**Active arms only**		**Placebo arms only**	
Predictor	**β coeff.**	**95% CI**	**β coeff.**	**95% CI**	**β coeff.**	**95% CI**
Age	0.01	-0.17, 0.18	0.07	-0.14, 0.27	-0.18	-0.51, 0.15
Gender	0.17	-0.16, 0.51	0.18	-0.21, 0.58	-0.20	-0.90, 0.49
Non-Caucasian	0.11	-0.37, 0.58	-0.13	-0.75, 0.49	**0.97**	**0.17, 1.77***
Weeks completed	**0.19**	**0.03, 0.36***	**0.30**	**0.11, 0.49****	-0.22	-0.57, 0.12
Previous gambling treatment	-0.03	-0.36, 0.30	-0.01	-0.38, 0.37	0.16	-0.54, 0.85
Baseline G-SAS	**0.37**	**0.20, 0.55*****	**0.43**	**0.23, 0.64*****	0.27	-0.08, 0.62
Baseline HAM-A	**-0.52**	**-0.83, -0.21****	-0.35	-0.72, 0.02	**-1.03**	**-1.58, -0.48*****
Baseline HAM-D	**0.36**	**0.05, 0.67***	0.24	-0.13, 0.61	**0.62**	**0.07, 1.17***
Psychiatric comorbidity	-0.03	-0.37, 0.30	-0.04	-0.43, 0.34	0.40	-0.30, 1.11
Strategic gambling	-0.48	-1.03, 0.07	-0.43	-1.03, 0.17	0.13	-1.25, 1.52

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

β refers to standardised regression coefficients

3.3.3 Sensitivity analyses

To assess the robustness of my findings to choices I made during the analysis, the regression models detailed above were repeated, employing: 1. Patients with mild G-SAS scores (< 20) included; 2. Percentage reduction in G-SAS as the outcome variable.

3.3.3.1 Models including patients with mild G-SAS scores

The results of this analysis are summarised in Table 3.3. All models remained significant. In all patients, the model explained 24% of the variance in change in G-SAS (adjusted $R^2 = 0.24$, $F_{(11,127)} = 4.88$, $p < 0.00001$). The significant predictors were again baseline G-SAS, number of weeks completed in the trial, baseline depression severity, and baseline anxiety severity. In patients randomised to active medication, the model explained 25% of the variance in change in G-SAS (adjusted $R^2 = 0.25$, $F_{(11,91)} = 4.07$, $p < 0.0001$), and baseline HAM-A score was an additional significant negative predictor of treatment response ($\beta = -0.37$, $t = -2.11$, $p = 0.0379$). In patients assigned placebo, the model explained 31% of the variance in change in G-SAS (adjusted $R^2 = 0.31$, $F_{(11,24)} = 2.43$, $p = 0.0336$). In this group, baseline G-SAS was an additional positive predictor ($\beta = 0.50$, $t = 3.11$, $p = 0.0048$), while baseline HAM-D score was no longer a significant predictor.

3.3.3.2 Models with percentage change in G-SAS as dependent variable

These results are summarised in

Table 3.4. The models in all patients and in patients randomised to active medication remained significant, explaining 11% (adjusted $R^2 = 0.11$, $F_{(11,119)} = 2.42$, $p = 0.0093$) and 14% (adjusted $R^2 = 0.14$, $F_{(11,85)} = 2.41$, $p = 0.0117$) of the variance in percentage change in G-SAS, respectively. However, the model in patients randomised to placebo approached significance, explaining 28% of the variance in percentage change in G-SAS (adjusted $R^2 = 0.28$, $F_{(11,22)} = 2.16$, $p = 0.0599$). The only change in predictors in all 3 models was that baseline G-SAS was no longer significant in the model that included all patients, instead approaching significance (standardised $\beta = 0.17$, $t = 1.87$, $p = 0.0638$).

Table 3.3 Results of multiple linear regression models calculated to predict treatment response (change in G-SAS). These models included patients with mild symptoms (G-SAS < 20).

	All patients		**Active arms only**		**Placebo arms only**	
Predictor	**β coeff.**	**95% CI**	**β coeff.**	**95% CI**	**β coeff.**	**95% CI**
Age	0.02	-0.14, 0.18	0.07	-0.13, 0.26	-0.09	-0.40, 0.23
Gender	0.13	-0.18, 0.44	0.14	-0.23, 0.51	-0.24	-0.92, 0.45
Ethnicity	0.13	-0.32, 0.58	-0.11	-0.72, 0.50	**0.96**	**0.18, 1.73***
Weeks completed	**0.20**	**0.05, 0.35***	**0.30**	**0.12, 0.47****	-0.14	-0.48, 0.20
Previous gambling treatment	-0.04	-0.34, 0.26	-0.03	-0.38, 0.32	0.12	-0.56, 0.80
Baseline G-SAS	**0.45**	**0.29, 0.61*****	**0.48**	**0.28, 0.67*****	**0.50**	**0.17, 0.84****
Baseline HAM-A	**-0.52**	**-0.80, -0.23*****	**-0.37**	**-0.71, -0.02***	**-0.83**	**-1.36, -0.30****
Baseline HAM-D	**0.37**	**0.08, 0.66***	0.27	-0.07, 0.62	0.44	-0.08, 0.96
Psychiatric comorbidity	-0.00	-0.31, 0.31	-0.01	-0.37, 0.36	0.25	-0.41, 0.91
Strategic or non-strategic gambling	-0.45	-0.97, 0.08	-0.43	-1.01, 0.15	-0.03	-1.39, 1.34

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

β refers to standardised regression coefficients

Table 3.4 Results of multiple linear regression models calculated to predict treatment response (percentage change in G-SAS).

	All patients		**Active arms only**		**Placebo arms only**	
Predictor	**β coeff.**	**95% CI**	**β coeff.**	**95% CI**	**β coeff.**	**95% CI**
Age	0.02	-0.16, 0.20	0.08	-0.13, 0.30	-0.16	-0.49, 0.17
Gender	0.23	-0.11, 0.58	0.26	-0.15, 0.68	-0.19	-0.90, 0.51
Ethnicity	0.15	-0.34, 0.65	-0.08	-0.73, 0.58	**1.00**	**0.19, 1.82***
Weeks completed	**0.22**	**0.05, 0.39***	**0.35**	**0.15, 0.54*****	-0.21	-0.56, 0.15
Previous gambling treatment	-0.03	-0.37, 0.31	0.03	-0.37, 0.42	0.08	-0.63, 0.78
Baseline G-SAS	0.17	-0.01, 0.35	**0.25**	**0.03, 0.47***	0.00	-0.35, 0.36
Baseline HAM-A	**-0.55**	**-0.88, -0.23*****	-0.38	-0.77, 0.01	**-1.04**	**-1.60, -0.48*****
Baseline HAM-D	**0.38**	**0.05, 0.70***	0.26	-0.13, 0.65	**0.59**	**0.03, 1.15***
Psychiatric comorbidity	-0.04	-0.39, 0.31	-0.04	-0.45, 0.36	0.35	-0.36, 1.06
Strategic or non-strategic gambling	-0.44	-1.01, 0.13	-0.36	-0.99, 0.26	0.06	-1.35, 1.47

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

β refers to standardised regression coefficients

3.4 Discussion

In this analysis, I aimed to identify predictors of placebo and medication response in gambling disorder. Importantly, predictors of treatment response differed in those receiving placebo compared with those receiving active medication. Decreased baseline symptoms of anxiety, increased baseline symptoms of depression, and non-Caucasian ethnicity were associated with improved response to placebo, while baseline severity of gambling symptoms and number of weeks completed in the trial were predictors of medication response.

The associations of these variables were robust to choices made during the analysis. When including patients with mild severity of gambling symptoms, baseline anxiety and baseline severity of symptoms were additional predictors of medication and placebo response, respectively. All other predictors remained the same. I expected baseline severity would likely be an additional predictor in this case due to artifactual correlation (i.e. those with least severity at baseline have the least capacity to improve)[57]. When percentage change in G-SAS was the outcome variable, baseline severity of symptoms was less predictive of treatment response in the model with all patients, while remaining a significant predictor of medication response. This might have been driven by the loss of association between baseline severity and placebo response (β changed from 0.27 in the original model to 0.00 when percentage change in G-SAS was the outcome variable).

3.4.1 Predictors of placebo response

In those receiving placebo, baseline symptoms of anxiety and depression, and ethnicity were the most consistent predictors of treatment response.

Baseline anxiety symptoms negatively predicted response to placebo. Anxiety and gambling disorder frequently co-occur. Furthermore, there is evidence that anxiety disorders precede the onset of gambling disorder[202], and stress is predictive of relapse[201]. Therefore, it is possible that greater levels of anxiety symptoms at baseline convey a risk of diminished improvement or worsening of symptoms over time. Perhaps this is partially 'remediated' by medication, which could explain why anxiety symptoms were not a predictor of medication response. Another intriguing idea is that in these patients, anxiety lessens the placebo effect. Anxiety has been shown to decrease placebo effects on pain[109,148-150]. Furthermore, a recent machine learning study in patients with binge eating disorder, which can be thought of as a behavioural addiction similar to gambling disorder[223], showed that elevated anxiety symptoms mediated reductions in placebo response[224]. It is noteworthy that both gambling disorder and placebo effects have been linked to opioidergic and dopaminergic function[56,79,174]. More research into the neurobiology of placebo

and nocebo effects in gambling disorder, and how anxiety symptoms might be associated with these, is required.

Depressive symptoms at baseline positively predicted response in those allocated to placebo. By contrast, higher baseline depressive symptoms have been linked with *reduced* placebo response rate in treatment studies for depression[43], which makes the current finding perplexing. Gambling disorder is associated with depressive symptoms, which are thought to have a role in the condition's pathogenesis[225-227]. It should be noted that high levels of depressive symptoms and the presence of other axis I mental disorders were exclusion criteria in the treatment studies pooled for this analysis. Indeed, the average HAM-D score in this sample was 7.11 ± 4.08, indicating no or mild depressive symptoms. As a result, it is unclear whether the effect of baseline HAM-D score on treatment outcome is due to symptoms of depression, or to an epiphenomenon not directly measured. Nonetheless, the consequences of baseline depressive and anxious symptoms on treatment outcome in treatment studies for gambling disorder warrants further research.

Non-Caucasian ethnicity was also associated with greater reduction in gambling symptom severity in those randomised to placebo. This replicates a prior result that non-Caucasians receiving placebo in gambling disorder treatment studies were more likely to be classified as "responders"[175]. The reasons for this remain unknown. It has been suggested that non-Caucasians might experience *reduced* placebo effects because of healthcare disparities[228], but I am not aware of any empirical evidence supporting this. Interestingly, in a study exploring predictors of treatment outcome in a US outpatient problem gambling service, Asian Americans were shown to be more likely to benefit than Caucasians, while other ethnicities, such as Native Americans, were found to be less likely to benefit[229]. This suggests that ethnicity might be an important variable to consider in treatment studies for gambling disorder, and potentially as a factor in placebo response more widely.

3.4.2 Predictors of medication response

The most consistent predictors of treatment response in the active medication arms were baseline severity and weeks completed in the trial.

It is plausible that number of weeks completed in the trial represents a dose effect of medication, meaning that taking it for longer results in more benefit. But other explanations are also possible. Previous studies have demonstrated that symptom improvement with CBT for pathological gambling can be associated with 'treatment satisfaction'[221,230]. It is conceivable 'treatment satisfaction' in a medication trial could be represented by the number of weeks a participant

continues to take part. Patients could be more likely to withdraw if they are unsatisfied with the treatment. Importantly, number of weeks completed was not a predictor of symptom improvement in the placebo arms. Indeed, the beta coefficient for this predictor was negative in all analyses of the placebo arms. This might imply that longer-duration trials might have a lower placebo response rate. However, a previous analysis showed that placebo responders with gambling disorder continued significantly longer in treatment trials than non-responders[175]. This analysis used a dichotomous definition of response (more than 35% reduction in G-SAS) that possibly obscures some complexity in the data. Further, whether a 35% reduction in G-SAS is an appropriate cut-off for defining "response" is unknown[175]. More research is needed to determine whether the duration of a trial affects placebo response rates in gambling disorder.

I also found a positive association between baseline severity and medication response. In a previous systematic review, lower baseline symptom severity was predictive of improved outcome in gambling disorder[220]. However, all the studies included in that review involved psychosocial interventions and none involved medication. Instead, it is possible that medication response increases with greater baseline severity in gambling disorder. Supporting this, although baseline severity was always positively predictive of medication response in the current study, its association with placebo response was mixed. Baseline severity was non-significantly positively predictive of change in G-SAS in those randomised to placebo, but when the outcome variable was *percentage* change in G-SAS, the regression coefficient was zero. Gambling disorder might therefore be comparable to disorders such as depression or acute mania, where the benefit of medication over placebo increases with illness severity[45,59,62]. How best to account for baseline severity in the analysis of clinical trials has been the subject of much debate. The emerging consensus is that analysis of covariance should be used with the inclusion of baseline severity as a covariate[231-235]. Future research should explore whether including patients with more severe symptoms and/or the use of appropriate adjustment for baseline severity improves assay sensitivity in clinical trials for gambling disorder.

3.4.3 Limitations

There are several limitations to this study. First, the sample size is relatively small for a study of this nature. For example, a similar meta-analysis of individual patient data in antipsychotic trials of patients with acute mania included 1019 patients[45]. False positives are thus a possibility, even if the sensitivity analyses revealed that the results were robust to choices made during the analysis. Second, a disadvantage of pooling data in this way is that the studies chosen need to be relatively similar, to prevent bias in the statistical analyses. This can lead to loss of information about the effect of study-level variables on placebo response. Third, only the subset of individuals with data

for all measures were included in the regression model. I have not carried out an evaluation of the effects of missing data on the results. Fourth, the longest trial included in this analysis was 18 weeks in duration. It is uncertain whether the predictors found here would hold for outcomes beyond this timeframe. Fifth, in a linear analysis such as this, it could be questioned whether *statistically* significant predictors are *clinically* meaningful. In the current sample, the mean baseline G-SAS was severe at 31.51 and decreased to 19.28 on average (mild severity). On average therefore, there appears to be a clinically meaningful improvement, even though there is some debate about the optimum criteria for clinical improvement in gambling disorder[206]. As a result, it may be inferred that the present predictors are associated with clinically meaningful reductions in gambling disorder symptoms. Finally, a number of possible variables that might be linked to treatment response were either measured inconsistently or not at all in the present studies. These include expectations of therapeutic benefit[56,65,100], which probably influence both medication and placebo response[56], and triggers for gambling behaviour, which have been linked with placebo response in gambling disorder[175]. However, these were not measured consistently enough in the present studies to be incorporated into the regression model. Certain personality traits and being in the action stage of change are also predictors of response to psychological treatments for gambling disorder[220]. Moreover, the predictors of response may vary for different medication classes. I was unable to evaluate this in the current study due to the small sample sizes of the parent studies. Additional research is required to determine the effects of such variables in treatment studies for gambling disorder and whether they interact with the predictors found here.

3.5 Concluding comments

I conducted a pooled quasi-meta-analysis of medication studies to identify predictors of medication and placebo response in gambling disorder. I found that baseline symptoms of anxiety and depression, and non-Caucasian ethnicity, were significant predictors of placebo response. Predictors of medication response included baseline severity of gambling disorder symptoms and number of weeks completed in the trial. Further research is required to understand whether controlling for these variables improves signal detection of a medication or placebo effect in medical trials for gambling disorder. A similar approach should be considered to identify predictors of placebo response in other psychiatric conditions.

It is noteworthy that symptoms of anxiety and depression were predictors of placebo response in this patient group. Biomarkers that correlate with placebo effects on anxiety or depressive symptoms might be clinically relevant in psychiatric disorders generally. In the next chapter, I

report the results of a systematic review into the neuroimaging markers of placebo response in anxiety or depressive disorders.

Chapter 4 A Systematic Review of Functional Neuroimaging Correlates of Placebo Response in Patients with Anxiety or Depressive Disorders

4.1 Background and Aims

Depressive and anxiety disorders are the most common psychiatric conditions[3]. These disorders cause severe distress, limit daily function, and lower quality of life. Many patients do not improve or experience unwanted side effects with first-line treatments for these disorders. We therefore need new treatments, but, drug discovery is potentially hindered by the large placebo effect seen in psychotropic trials[28,56,236]. Despite the placebo effect being large and clinically relevant (see Chapter 1), the mechanisms that drive it in depressive and anxiety disorders remain little investigated[56].

By contrast, the psychoneurobiological mechanisms of placebo effects on pain have been investigated in detail. An interplay between prior expectations and learning cause measurable neurobiological changes associated with pain relief. Recent meta-analyses have verified biomarkers of placebo analgesia (particularly frontoparietal regions) leading to the hypothesis that these biomarkers might differentiate between placebo and drug response[101,129,183]. However, the neuroimaging correlates of placebo antidepressant and anxiolytic effects have not been ascertained. Identifying functional neuroimaging biomarkers of these effects might improve our understanding of the mechanisms involved.

I carried out a systematic review aimed at identifying neuroanatomical correlates of, and possible neurotransmitter systems important in, placebo antidepressant and anxiolytic effects. I aimed to understand current knowledge of potential neuroimaging correlates of these effects and identify hypotheses to be tested in future studies.

4.2 Method

The review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[237]. The protocol was registered prospectively on the PROSPERO database (CRD42019156911)[238]. I performed the systematic review and data extraction with four other reviewers (see Acknowledgements). All discrepancies were resolved by consensus.

4.2.1 Search Strategy and Terms

I systematically searched relevant mental and general health databases including Pubmed, PsycInfo, EMBASE and EMBASE classic, OVID Medline, and Web of Knowledge with no date or language restrictions. Searching this combination of databases retrieves 95% of the relevant literature in approximately 72% of systematic reviews[239]. To ensure maximum retrieval of relevant literature, I also searched the British Library's EThOS database of theses, and we hand-searched the reference lists of review articles found by the search for additional relevant records.

I included relevant terms to retrieve randomised studies, in which a placebo treatment was an intervention, and involving functional neuroimaging of patients with major depressive or anxiety disorders. The full search strategy is reproduced in Appendix A.

4.2.2 Inclusion criteria

Records were considered eligible if they met the following criteria:

1. Patients were adults (aged 18-65 years) with unipolar major depression or an anxiety disorder as defined in the International Classification of Diseases Problems 11th edition (ICD-11) (i.e. not obsessive-compulsive disorder or post-traumatic stress disorder)
2. The study involved a placebo intervention
3. Change in depressive or anxiety symptoms were an outcome measure
4. Patients underwent functional neuroimaging (positron emission tomography [PET], single-photon emission computed tomography [SPECT], functional magnetic resonance imaging [fMRI], or arterial spin labelling [ASL]) at baseline, as an intervention or as an outcome measure.
5. Imaging data were correlated with
    a. a clinical improvement, measured objectively, following placebo treatment, or
    b. placebo mechanisms such as learning or expectancies

4.2.3 Record Selection

At least two reviewers screened all titles and abstracts for potential eligibility against the inclusion criteria. The full-text for potentially eligible articles was then obtained. These were again screened by at least two reviewers and included in the final review if they met the inclusion criteria detailed above.

4.2.4 Quality Assessment

Risk of bias was assessed in line with the updated Cochrane Collaboration's tool for assessing risk of bias in randomised trials (ROB2)[240]. One reviewer recorded risk of bias for each record using a standardised form, and these assessments were independently checked by me. Risk of bias resulting from the randomisation protocol, deviations from the intended intervention, missing data, outcome measurement, and selective reporting was assessed. An overall assessment of risk of bias was also made for each record.

4.2.5 Data Extraction and Synthesis

One reviewer extracted data through the use of a piloted, standardized form. All extracted data were checked independently by me. Data concerning the patient population (including diagnosis, age, and gender), study design, imaging modality, key clinical and imaging results, amount of missing data, and authors' interpretations were extracted.

I was unable to appropriately pool a sufficient number of studies to carry out a formal meta-analysis. For Activation Likelihood Estimation (ALE), inclusion of at least 17-20 experiments are recommended to achieve adequate statistical power to detect moderately large effects[241]. Further, pooling across studies involving whole-brain, small volume correction and region-of-interest analyses violates the underlying assumptions of this meta-analytic model[242]. As I describe in the results, the included studies were few in number, and heterogeneous in imaging modality, analysis method and in outcome measures.

I instead undertook a narrative synthesis of the extracted data. I interrogated the data aiming to answer the following questions:

1. Activation or deactivation of which brain regions correlates with a placebo response in patients with depression or an anxiety disorder?
2. Which neurotransmitters or neurobiological systems might be important in placebo response in patients with depression or an anxiety disorder?

4.3 Results

An initial search was performed on 03/09/2019 and updated on 09/02/2021. In total, the searches identified 6,006 records. An additional 1 record was also identified through hand-searching of reference lists. After de-duplication, 3,286 titles and abstracts were screened for possible eligibility. From these, 234 full-text articles were sought. Sixteen articles met inclusion criteria and were included in the review (see Figure 4.1)[96-98,136,243-254].

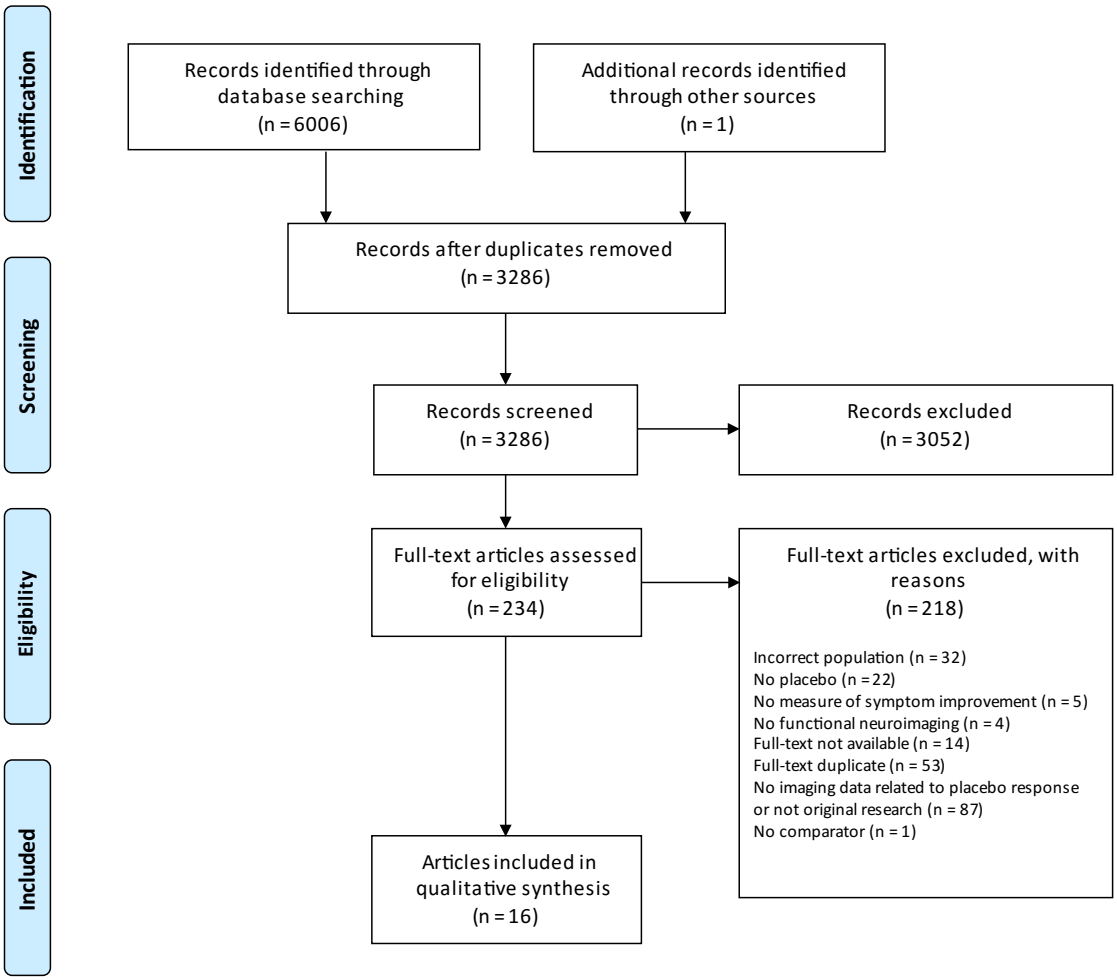


Figure 4.1 PRISMA flow diagram

4.3.1 Study characteristics

Twelve studies were of patients with depression and utilised the following imaging modalities: arterial spin labelling (ASL)[246], functional magnetic resonance imaging (fMRI)[97,136,243-245,247,251,253,254], or positron emission tomography (PET)[96,252]. Four studies investigated placebo effects in patients with social anxiety disorder and utilised PET[248-250] and fMRI[98]. Only two studies were published prior to 2012[250,252]. Half of the studies had samples of fewer than 50 patients, with sample sizes ranging from 8 to 279 patients. There was also overlap in patients per record with eight studies of patients with depression[96,243-247,251,253] and three of patients with social anxiety disorder[248-250] sharing some or all patients. Consequently, n = 503 is the maximum number of patients included in this review. The mean age of the patients in each study ranged from 28.8 ± 8.6 years to 49 ± 9.0 years. Most patients were female (67%) with only two studies reporting a majority of male patients[98,252].

4.3.2 Results of Quality Assessment

The results of the quality assessment are summarised in Figure 4.2 and Figure 4.3. The overall rating for the majority of studies (10 of 16, 62.5%) was 'some concerns'. Mostly this was because a preregistered analysis plan was absent, which meant that methods like dichotomising treatment groups into 'responders' and 'non-responders' or using 'small volume correction' were potentially indicative of selective reporting[96-98,136,248-250,252,253]. Additionally, there were concerns about missing data in three studies[96,244,253]. We rated two studies as high risk of bias. The first reported results from only 200 of 296 patients with no justification[247]. The second selectively reported results regarding the amygdala and not other regions of interest[254]. These potential sources of bias, and other key details of the included studies, are described further below.

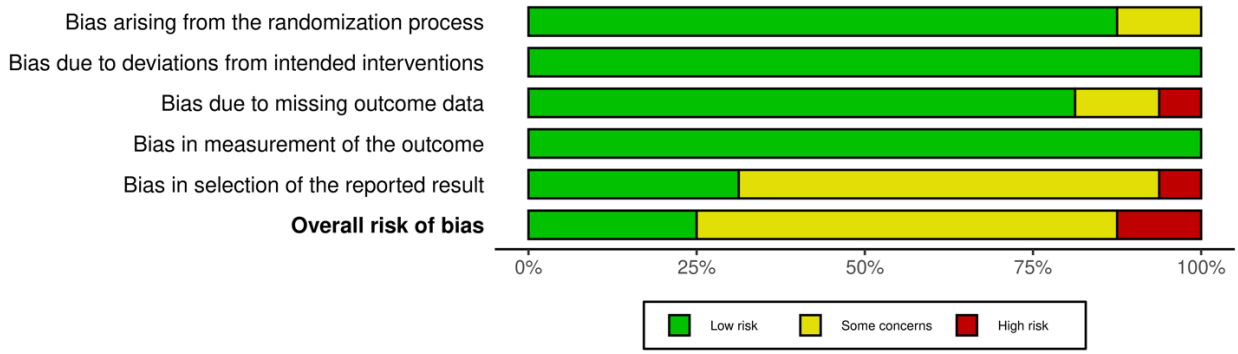


Figure 4.2 Summary of reviewers' judgements concerning risk of bias as a percentage of included records

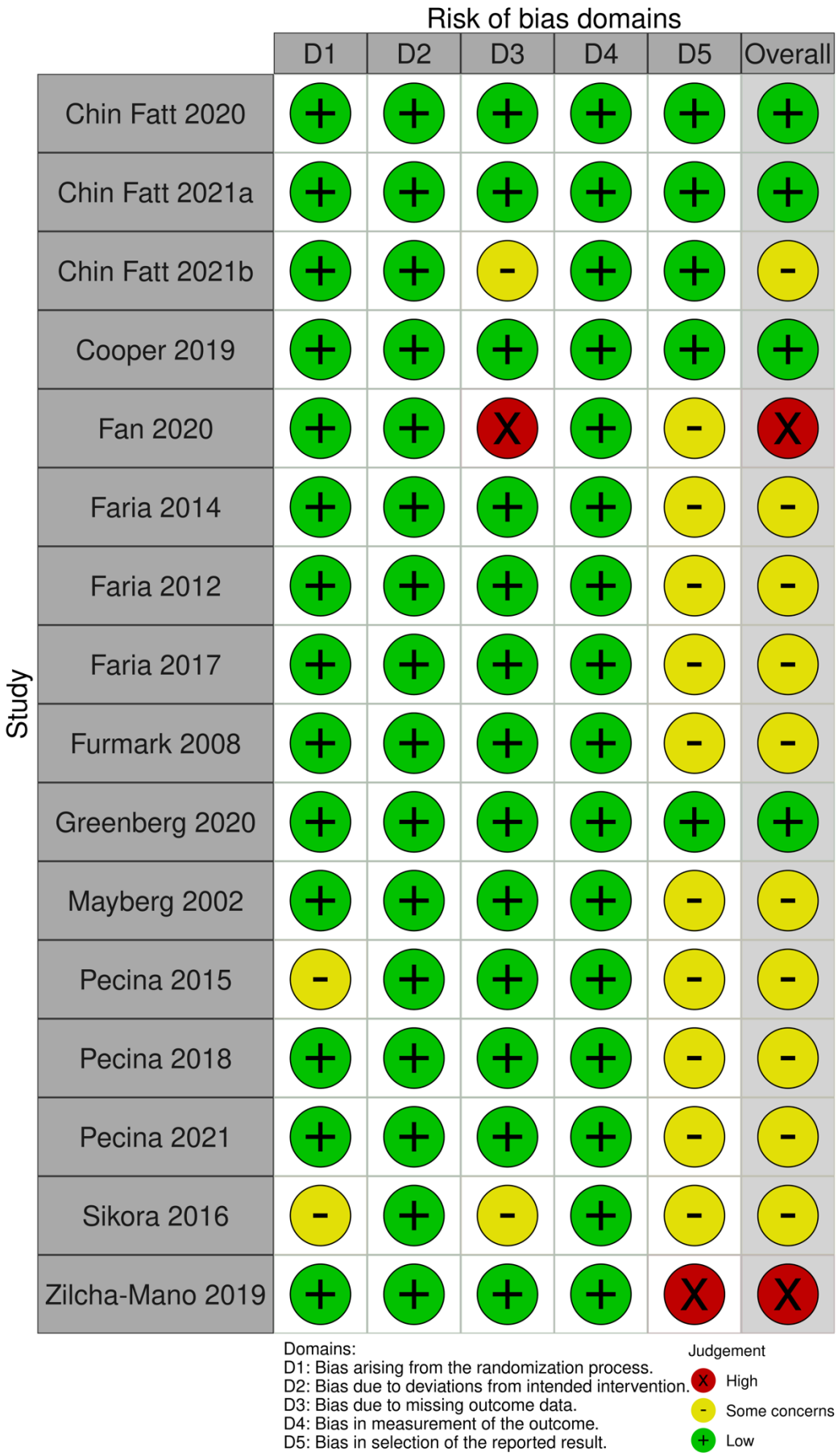


Figure 4.3 Traffic light plot showing results of risk of bias assessment for each included study.

4.3.3 Studies of Depression

Imaging markers of placebo antidepressant responses were explored in twelve of the included studies. The primary aim of these studies varied considerably:

1. Identifying possible functional neuroanatomical correlates of placebo antidepressant responses through a re-analysis of previously reported data[252]
2. To identify neural correlates of treatment response, either active medication or placebo[243-247,251]
3. To explore neural correlates of placebo mechanisms such as expectations[96,253,254]
4. To explore neural correlates of acute manipulation of expectations and reinforcement learning[97,136].

The important features and results of these studies are summarised in Table 4.1.

Table 4.1 Summary of data extracted from papers that explored functional neuroimaging correlates of placebo effects in patients with depression

Reference	*n*	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparator						
Chin Fatt et al. (2020)[245]	279	Sertraline	Placebo	8 weeks	HAMD-17	fMRI	Pre-treatment resting-state functional connectivity	32.2% (90/279) achieved remission (49 sertraline, 41 placebo)	• No within-network moderators • Higher connectivity of hippocampus with executive control network and lower connectivity of thalamus with visual and salience networks predicted better outcome with placebo • Higher connectivity of the hippocampus with the visual, dorsal attention, executive control networks, and thalamus, the limbic network with the salience and somatomotor networks, and the executive control network with the salience and somatomotor networks predicted greater improvement with placebo and worse outcome with sertraline.
Chin Fatt et al. (2021a)[243]	279	Sertraline	Placebo	8 weeks	HAMD-17	fMRI	Pre-treatment resting-state functional connectivity	As above	• As baseline connectivity between dorsolateral PFC and inferior parietal cortex increased, superiority of sertraline over placebo reduced. This was driven by a relative increase in efficacy of placebo. • A similar pattern was seen for baseline connectivity between subcallosal and posterior cingulate cortices.
Chin Fatt et al. (2021b)[244]	244	Sertraline	Placebo	8 weeks	HAMD-17	fMRI	Pre-treatment resting-state functional connectivity correlates of subgroups defined through principal component analysis	32% (79/244) achieved remission (39 sertraline, 40 placebo)	• Subgroups with greater improvement with placebo typified by increased connectivity within the limbic network, between hippocampus and visual network, and salience network with dorsal attention network

Reference	*n*	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparator						
Cooper et al. (2019)[246]	231	Sertraline	Placebo	8 weeks	HAMD-17	ASL	Pre-treatment resting-state cerebral perfusion	35% (81/231) achieved remission: 37% of patients on sertraline vs 33% of patients on placebo	• Relative perfusion in right posterior insula; left midbrain; right hippocampus; right inferior frontal; right middle and inferior frontal gyri (including the dorsolateral PFC); left precentral gyrus; left inferior frontal; left middle temporal gyrus; right caudate; left cerebellum; right middle, superior, and inferior frontal gyri; left middle frontal gyrus (and dorsolateral PFC); right middle temporal gyrus; left cuneus; left cingulate; left fusiform gyrus; and the left inferior frontal gyrus moderated response to placebo.
Fan et al. (2020)[247]	200	Sertraline	Placebo	8 weeks	HAMD-17	fMRI	Pre-treatment resting-state connectome fingerprints	No significant differences in clinical outcomes between groups	• Greater treatment response independent of modality predicted by decreased connectivity between executive, sensorimotor and salience networks, and increased connectivity between default mode network and the rest of the brain. • No connectome fingerprint specific to response to either sertraline or placebo was found
Greenberg et al. (2020)[251]	222	Sertraline	Placebo	8 weeks	HAMD-17	fMRI	Pre-treatment change in ventral striatal activity during a monetary reward task	Across groups, HAMD-17 scores significantly improved over time. No separate statistics for each group presented.	• Left ventral striatal 'reward index' moderated treatment effects. • Patients with greater increases in reward activity over time appeared more likely to benefit from placebo. Patients with lower increase in reward expectancy activity over time were more likely to benefit from sertraline.

Reference	*n*	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparator						
Mayberg et al. (2002)[252]	8	Fluoxetine	Placebo	6 weeks	HAMD-17	PET	Resting-state brain metabolism at baseline and after 6 weeks of treatment	8 of an original 17 responded to treatment (4 placebo, 4 fluoxetine)	• Increases seen in PFC (BA 9/46), premotor cortex (BA 6), inferior parietal cortex (BA 40), posterior insula, posterior cingulate (BA 23/31). • Decreases seen in sgACC (BA 25), hypothalamus, thalamus, supplementary sensory area, anterior insula, parahippocampus.
Pecina et al. (2015)[96]	35	'Active' placebo Open-label antidepressant	'Inactive' placebo None	2 weeks 10 weeks	QIDS-SR16	PET	MOR binding potential after 'active' vs 'inactive' placebo treatment	Symptom improvement significantly greater for 'active' vs 'inactive' placebo. Remission at study end significantly higher in placebo responders	• Placebo administration reduced MOR binding potential in nucleus accumbens • Degree of placebo-induced opioid release in the sgACC, nucleus accumbens, thalamus and amygdala explained 43% of the response to open-label antidepressant treatment.
Pecina et al. (2018)[97]	20	Placebo IV infusion Positive sham neurofeedback	No infusion Negative sham neurofeedback	Single session	Subjective expectation of mood improvement and subjective mood trial by trial	fMRI	Change in BOLD signal	Expectancy significantly higher during placebo infusion. Mood significantly improved following placebo infusion, following positive sham neurofeedback, and when expectancy was higher.	• Positive sham neurofeedback led to greater activity in bilateral ventro- and dorsolateral PFC, which was positively correlated with improved mood. • Increased activity in left ventro- and dorsolateral PFC associated with greater expectancy when mood was rated higher in previous trial. • However, activity in bilateral ventro- and dorsolateral PFC also negatively moderated the effect of higher expectation on subsequent mood improvement.

Reference	*n*	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	**Comparator**						
Pecina et al. (2021)[136]	20	Naltrexone 50mg Placebo IV infusion Positive sham neurofeedback	Placebo No infusion Negative sham neurofeedback	7-10 days	Subjective expectation of mood improvement and subjective mood trial by trial	fMRI	Change in BOLD signal	Expectancy significantly higher during placebo infusion. Mood significantly improved following positive sham neurofeedback, and this was greater when expectancy was higher.	• Higher activity in right ventro- and dorsolateral PFC associated with reduced expectancy*reinforcement condition effect on expectancy and mood ratings. • Naltrexone partially abolished the expectancy*reinforcement condition effect on expectancy and mood ratings. This was associated with reduced responses in right OFC during processing of positive reinforcement. • Participants with greater naltrexone-induced modulation of OFC activity during positive sham neurofeedback had higher expectancy and mood ratings
Sikora et al. (2016)[253]	29	'Active' placebo Open-label antidepressant	'Inactive' placebo None	2 weeks 10 weeks	QIDS-SR16	fMRI	Resting-state functional connectivity after 'active' and 'inactive' placebo	Symptom improvement significantly greater for 'active' vs 'inactive' placebo.	• Increased 'baseline' connectivity of the rACC with the salience network was significantly associated with greater placebo response. • Placebo-induced reduction in rACC with the salience network was also associated with greater placebo response. • 'Baseline' resting-state connectivity of the salience network was significantly predictive of placebo response.

Reference	*n*	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	**Comparator**						
Zilcha-Mano et al. (2019)[254]	23	High expectation (100% chance of receiving citalopram)	Low expectation (50% chance of receiving citalopram)	1 week	HAMD-24	fMRI	Change in BOLD signal during a masked emotional face task at baseline and 1 week after randomization to high or low expectation	Patients in the high expectation group demonstrated significantly greater outcome expectation.	• High expectation group showed a decrease in amygdala activation from scan 1 to 2 in the sad vs neutral face contrast, whereas low expectation group showed an increase. • Increases in outcome expectancy significantly correlated with reductions in left amygdala activity. • 63.41% of the effect of outcome expectancy on change in HAMD-24 was mediated by changes in amygdala activity.
		Citalopram	Placebo	8 weeks					

Abbreviations: HAMD, Hamilton rating scale for depression; QIDS-SR, Quick inventory of depressive symptomatology (self-report); ASL, Arterial spin labelling; fMRI, functional magnetic resonance imaging; PET, positron emission tomography; BOLD, blood-oxygen-level-dependent imaging; BA, Brodmann area; PFC, prefrontal cortex; sgACC, subgenual anterior cingulate cortex; MOR, mu-opioid receptor; OFC, orbitofrontal cortex; rACC, rostral anterior cingulate cortex.

4.3.3.1 Re-analysis of previously published trial data

Mayberg and colleagues conducted the first study to be published that reported imaging correlates of a placebo antidepressant response[252]. In this 6-week randomised trial of fluoxetine versus placebo, 8 of 15 depressed patients showed a treatment response, which was defined as a reduction of >50% on the 17-item Hamilton Depression Rating Scale (HAMD-17). Four of these patients were treated with placebo and 4 were treated with fluoxetine. The patients underwent PET imaging at baseline, one week and six weeks after treatment initiation. At each of these timepoints, changes in regional cerebral glucose metabolism were computed for the placebo responder and drug responder groups separately. Placebo response was associated with significant regional changes in metabolism (beta-$2_{(1972)}$ = 3.97, $p < 0.0001$). Metabolism increased in dorsolateral prefrontal cortex, premotor cortex, inferior parietal cortex, posterior insula, and posterior cingulate cortex. Metabolism decreased in subgenual anterior cingulate cortex, hypothalamus, thalamus, secondary somatosensory cortex, posterior insula and parahippocampus. Fluoxetine responders exhibited the same changes in metabolism, and some additional changes (decreased metabolism in caudate, putamen, hippocampus, and anterior insula). No activity was unique to placebo responders. It was not possible to make any inferences about the neurotransmitter systems that might be important in placebo antidepressant effects from this study.

4.3.3.2 Studies aiming to identify markers of treatment response

Six included papers were published from the Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care (EMBARC) trial[243-247,251]. The purpose of this trial was to find neural predictors or correlates of treatment response. Patients with major depressive disorder (n = 296) were scanned with MRI using sequences including ASL and fMRI at rest, and fMRI during a monetary reward task, before randomisation to either sertraline or placebo treatment. For the following eight weeks, the HAMD-17 was used to monitor treatment response.

Of the original 296 patients, 231 patients completed the baseline ASL scan, had good quality data, and attended at least 1 follow-up visit. Of these, 37% of the sertraline-treated patients and 33% of placebo-treated patients achieved remission (HAMD-17 < 7). Through a whole-brain, voxel-wise, linear mixed-effects model of the ASL and clinical data, thirty clusters of >100 voxels showed a significant treatment*time*relative cerebral perfusion interaction. Perfusion in regions including right putamen and insula, left inferior temporal gyrus, right orbital frontal gyrus, and left parahippocampal gyrus moderated response to sertraline. Conversely, moderators of placebo response included regions involved in cognitive control and the default mode networks, for

example right posterior insula, left midbrain, right hippocampus, right orbital frontal cortex, and right dorsolateral prefrontal cortex[246].

Analyses of the baseline resting-state fMRI data have been published in four papers[243-245,247]. In an exploration of pre-treatment resting-state data in 200 of the EMBARC patients, there was evidence that decreased connectivity between the executive, sensorimotor and salience networks, and increased connectivity between default mode network and the rest of the brain predicted greater response to treatment independent of modality. However, no 'connectome fingerprints' specific to placebo response were found[247]. In a larger sample of 244 of the EMBARC patients, subgroups of patients were identified through principal component analysis. Functional connectivity correlates of these groups were explored. In this sample, 39 sertraline-treated patients and 40 placebo-treated patients achieved remission. In subgroups who experienced greater improvement with placebo, there was increased connectivity within the limbic network, between hippocampus and visual network, and between salience network and dorsal attention network[244]. Finally, two analyses were carried out in a sample of 279 EMBARC patients, in whom 32.2% achieved remission (49 on sertraline and 41 on placebo). The first involved an exploratory analysis of baseline resting-state functional connectivity in seven networks and additional midbrain regions including hippocampus, striatum, thalamus and the amygdala. Higher connectivity of the hippocampus with the thalamus and the visual, dorsal attention, and executive control networks, and the limbic and the executive control networks with the salience and somatomotor networks, predicted better results with placebo and worse outcomes with sertraline[245]. In the second analysis, hypothesised seeds of interest (5mm spheres) were created and included dorsolateral prefrontal cortex, ventral striatum, amygdala and subcallosal cingulate cortex. A moderation analysis included a term for connectivity between these seeds and other regions of interest believed to be part of the same functional network. As baseline connectivity between dorsolateral prefrontal cortex and inferior parietal cortex increased, the relative efficacy of placebo increased compared with sertraline ($p = 0.05$). Baseline connectivity between the subcallosal and posterior cingulate cortices also showed a similar pattern ($p = 0.07$)[243].

Lastly, 222 of the EMBARC trial participants completed a monetary reward task at baseline[251]. The purpose of this task was to identify whether temporal changes in reward expectancy- and prediction error-related activity within the ventral striatum predicted or moderated response to sertraline or placebo. The authors created a 'reward index', calculated from the sum of the change in reward expectancy- and prediction error-related activity in the ventral striatum from the first half to the second half of the task. Treatment effects were significantly moderated by left ventral striatal reward index ($F_{(1,193)} = 12.93$, $p = 0.0004$). The threshold at which patients were more likely to benefit with sertraline was calculated from a Z-transformed distribution of each

patient's reward index. When left ventral striatal reward index was less than Z = -0.21, patients were more likely benefit from sertraline compared with placebo (raw HAMD-17 difference of ≥ 3, $t_{(193)} = 2.38$, p = 0.02, d = 0.32, $CI_{95\%}$ [0.06, 0.58]). The threshold at which patients were expected to benefit more greatly from placebo was not directly tested. However, based on results reported in the paper, placebo showed an advantage of approximately 0 to 4 points on the HAMD-17 over sertraline when reward index Z > 2 (i.e. a large increase in reward processing over time during the task)[251]. Dopamine is a major neurotransmitter in the ventral striatum, which suggests that dopamine may have a role in the antidepressant effects of placebo.

4.3.3.3 Studies exploring neural correlates of placebo mechanisms

Three of the included studies involved exploration of imaging markers of placebo mechanisms in patients with depression. First, Zilcha-Mano and colleagues[254] investigated the neural correlates of expectancy augmentation in an antidepressant trial using a variation of the 'open-hidden' paradigm. Twenty-three patients with depression underwent fMRI scanning while viewing masked emotional faces with neutral, fearful, sad, or happy expressions. The patients were then randomly assigned to either an open-label group with a 100% chance of receiving citalopram (*n* = 9) or a placebo-controlled group with a 50% chance of receiving either citalopram or placebo (*n* = 14). One week later, after being informed of their group assignment but before starting treatment, patients underwent a second fMRI scan while completing the same emotional face task. The patients then completed an 8-week clinical trial of citalopram versus placebo in which HAMD-24 was measured weekly. Compared with the placebo-controlled group, patients in the open-label group showed significantly improved expectations post-randomisation and a significant reduction in activity in the amygdala, bilateral dorsolateral prefrontal cortex and superior temporal gyrus when viewing sad compared with neutral faces. The amygdala was then chosen as a region of interest for further analysis, and a correlation was found between decreased left amygdala activity and increased expectancy score post-randomisation (r = -0.74, *p* = 0.006). Further, patients with higher expectancy scores showed more rapid reduction in HAMD-24 scores and this was mediated by greater reductions in amygdala activity post-randomisation (B = -0.09, *p* = 0.007). This finding remained present when repeating the analyses with only those who received active medication included. However, we had concerns about potential bias due to selective reporting in this study. The amygdala showed a significant difference in activity in the sad *vs*. neutral faces contrast only, whereas other regions demonstrated significant differences in activity in other relevant contrasts. The amygdala is then chosen as a region of interest for further analysis with little justification, and no further analyses regarding the other regions are reported[254]. Possible neurotransmitters involved in expectancy augmentation of antidepressant response could not be inferred from these data.

Peciña, Sikora and colleagues explored imaging markers of placebo mechanisms through a randomised crossover design comparing an 'active' and 'inactive' placebo antidepressant[96,253]. In brief, patients were given oral placebo for 1 week with instructions that it was an antidepressant ('active') followed by a 3-day washout and then 1 week of treatment with 'inactive' placebo, with disclosure that this was an inert control. After each condition, participants underwent neuroimaging. Once the patients had been imaged twice, they underwent a 10-week open-label trial with a licensed antidepressant.

In the former 2015 study, the relationship between placebo response and endogenous opioid release was explored through PET imaging with [^{11}C]carfentanil (a μ-opioid receptor-selective radiotracer) in 35 patients. After the "active" placebo treatment round the PET session included an additional intravenous infusion of 0.9% isotonic saline with instructions this was a 'rapid-acting antidepressant' as an acute placebo challenge. Placebo infusion during the PET scan decreased μ-opioid receptor binding potential in the nucleus accumbens (estimate = -0.43, Z = 4.72, $p < 0.001$). In addition, the degree of placebo-induced opioid release in the subgenual anterior cingulate cortex, nucleus accumbens, thalamus, and amygdala was related to both the decrease in depressive symptoms after 1 week of "active" placebo (estimates ≤ -0.38, Z's > 3.80, p's < 0.001) and with response to the open-label antidepressant at 10 weeks (estimates ≤ -0.60, Z's > 3.98, p's < 0.001). In the latter 2016 study, 29 of the 35 patients underwent a resting-state fMRI scan after each week of 'active' and 'inactive' oral placebo treatment. Compared with the 'inactive' placebo, there was a statistically significant reduction in depressive symptoms after 'active' placebo treatment ($F = 7.2$, $p = 0.012$). Increased baseline resting functional connectivity ($Z = 4.35$, adjust $R^2 = 0.65$, $p < 0.005$) and reduction in functional connectivity ($Z = 3.97$, $p < 0.05$) of the rostral anterior cingulate cortex within the salience network were significantly associated with placebo response. Finally, baseline resting state functional connectivity of the salience network as a whole was significantly predictive of placebo response ($r = 0.41$, $p = 0.018$), but not of response to antidepressant treatment[253].

We identified some potential sources of bias in this study. First, the 'baseline' scan was carried out after one week of 'inactive' placebo. However, this does not represent a true baseline, as the patients have experienced one week of an intervention, and some received 'active' placebo two weeks prior. It is known that placebo analgesia is reduced if participants have experienced a previously ineffective analgesic treatment[113]. Some of these patients will have experienced 'active' placebo first, and some 'inactive', and so such carryover effects could potentially confound any imaging results. Second, there is a discrepancy in the sample sizes of these papers (29 vs 35) with no explanation for the difference. Further, it was reported in the 2015 study that 10 of the 35 (29%) patients withdrew during the open-label antidepressant trial and no

compensatory intention-to-treat analysis was performed. It is unknown from the published data whether these patients were placebo responders[96].

4.3.3.4 Studies exploring neural correlates of acute manipulation of expectations and reinforcement

Two studies, both by Peciña and colleagues[97,136], explored the neural correlates of acute manipulation of antidepressant expectancies using a 'simulated neurofeedback task' in patients with depression. In brief, this task comprised six runs of 12 trials each, where each trial started with a timer cue representing an anticipation phase before either receiving or not receiving a 'rapid-acting antidepressant' (infusion/no infusion). In actuality, normal saline was administered in all 'infusion' trials. After the infusion cue, participants were shown sham neurofeedback with varying valence (either positive or negative). The purpose of this was to either negatively or positively reinforce the patients' expectations of benefit on a trial-by-trial basis. Patients rated their anticipated and actual mood improvements following the anticipation and simulated neurofeedback periods, respectively.

In the first of these studies, carried out in 20 patients, there was greater mood improvement during the infusion cue ($b = 0.12$, $p < 0.05$) and following the display of positive sham neurofeedback ($b = 0.32$, $p < 0.001$), and higher expectation of benefit predicted improved mood ($b = 0.22$, $p < 0.001$). Positive sham neurofeedback was associated with increased activity in bilateral ventro- and dorsolateral prefrontal cortices. Activity in these regions was positively correlated with improved mood ($b = 0.2$, $p < 0.001$). Greater expectancy was also associated with increased activity in the left ventro- and dorsolateral prefrontal cortices when mood improved in the preceding trial ($b = 0.05$, $p < 0.05$). However, the effect of increased expectancy on subsequent mood improvement was negatively moderated by activity in bilateral ventro- and dorsolateral prefrontal cortices ($b = -0.07$, $p < 0.05$). Finally, plasma β-endorphin concentration was also measured before and after the task in this study. Larger increases in plasma β-endorphin following the task were associated with both greater improvements in subjective mood in response to positive neurofeedback (estimate = 0.002, $p < 0.001$) and significantly increased expectancy ratings (estimate = 0.0007, $p = 0.02$)[97].

In a subsequent double-blind crossover study, 20 patients with depression carried out the same neurofeedback task twice: once following administration of naltrexone 50mg and once following matched placebo. As above, the task showed a behavioural effect of the placebo in that expectancy was increased during the 'antidepressant' infusion condition, and expectancy and mood were also increased following the positive sham neurofeedback. Interestingly, in this study higher activity in the right ventro- and dorsolateral prefrontal cortex was again associated with a

reduced placebo*neurofeedback condition interaction effect on expectancy and mood ratings. The placebo*neurofeedback condition effect on expectancy ($b = -1.00$, $p < 0.001$) and mood ratings ($b = -0.93$, $p = 0.003$) was also reduced by naltrexone. Naltrexone administration was further associated with reduced right orbitofrontal cortex activity during positive sham neurofeedback (max $t = 5.64$, cluster size = 334 voxels, $p < 0.001$). However, no significant differences in activity were seen during the expectancy manipulation. Greater naltrexone-induced reductions in orbitofrontal cortex activity during positive sham neurofeedback correlated with higher expectancy during the 'antidepressant' condition ($b = 0.40$, $p < 0.01$)[136].

Overall, these results suggest that the lateral prefrontal cortex, central orbitofrontal cortex, and the endogenous opioid system might be important in expectancy-related placebo antidepressant effects.

4.3.4 Social Anxiety Disorder

Four studies involving patients with social anxiety disorder met inclusion criteria. Three studies were re-analyses of clinical trials in which the primary aim was not to investigate the placebo response[248-250], while in the fourth study the primary aim was to examine the effects of expectations on SSRI treatment for social anxiety disorder[98]. The important features and results of these studies are summarised in Table 4.2.

The first published study exploring neuroimaging correlates of placebo response in patients with social anxiety disorder was a pooled re-analysis of the placebo arms of two randomised-controlled trials[250]. Twenty-five patients completed a public speaking task while undergoing PET imaging before and after 8 weeks of placebo treatment. The authors also obtained the patients' genotypes for the serotonin transporter-linked polymorphic region (5-HTTLPR) and the tryptophan hydroxylase-2 (TPH2) gene promoter. Ten of the 25 patients (40%) were classified as placebo responders based on improvement in clinical global impression scale. During the public speaking task, regional cerebral blood flow in the left amygdala reduced significantly more in placebo responders than in non-responders ($Z = 2.64$, $p = 0.048$). There was an additional effect of genotype. Only long allele of 5-HTTLPR and/or the G allele of the G-703T polymorphism in TPH2 homozygotes demonstrated a placebo response. Results from a mediation analysis indicated that the effect of the TPH2 polymorphism on placebo response was mediated by reduction in amygdala activity ($p = 0.029$)[250]. These findings suggest that the amygdala and serotonin might be important in placebo anxiolytic responses. However, this was a small sample size for this kind of statistical analysis, so there is a risk of false positives.

Table 4.2 Summary of data extracted from papers that explored functional neuroimaging correlates of placebo effects in patients with social anxiety disorder

		Interventions							
Reference	***n***	**Experimental**	**Comparator**	**Study duration**	**Treatment response definition**	**Imaging modality**	**Imaging measure**	**Clinical results**	**Imaging markers of placebo response or placebo mechanisms**
Faria et al. (2012)[249]	72	Citalopram or paroxetine	Placebo	6-8 weeks	CGI-I = 1 or 2	PET	rCBF during a public speaking task at baseline and study end	57% of SSRI group responded vs 30% of placebo group	• Both SSRI and placebo responders showed reductions in right ventrolateral amygdala and left basomedial/basolateral amygdala. The rCBF change correlated with clinical measures of anxiety. • Placebo responders additionally showed increased rCBF in right brainstem/pons compared with placebo non-responders.
Faria et al. (2014)[248]	72	Citalopram or paroxetine	Placebo	6-8 weeks	CGI-I = 1 or 2	PET	Functional connectivity during a public speaking task at baseline and study end	57% of SSRI group responded vs 30% of placebo group	• Placebo responders showed greater negative correlation between left amygdala and left dorsolateral PFC vs placebo non-responders. • Placebo responders showed greater negative correlation between left amygdala and right ventromedial and dorsolateral PFC, and greater positive correlation between left amygdala and right dorsomedial PFC vs SSRI responders.
Faria et al. (2017)[98]	46	'Overt' escitalopram	'Covert' escitalopram	9 weeks	LSAS-SR < 39	fMRI	BOLD signal change and functional connectivity during emotional face matching task	'Overt' treatment significantly superior (d=2.24 vs d=1.13)	• Increased reactivity to emotional faces in overt vs covert in bilateral posterior cingulate, left mid temporal gyrus, left inferior frontal gyrus. • Covert group showed increased connectivity between amygdala and right posterior cingulate and right insula when viewing emotional faces compared with overt group.

Reference	*n*	Interventions		Study duration	Treatment response definition	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparator						
Furmark et al. (2008)[250]	25	Placebo	None	8 weeks	CGI-I = 1 or 2	PET	rCBF during a public speaking task at baseline and study end. Changes in rCBF associated with genotype.	10 (40%) responded to placebo	• rCBF significantly reduced in left amygdala in placebo responders vs nonresponders. • Patients homozygous for the long allele of 5-HTTLPR and/or the G allele of the G-703T polymorphism in TPH2 exhibited a significantly greater reduction in amygdala activity vs heterozygotes. • Mediation analysis showed that the change in rCBF in the amygdala mediated the effect of G-703T polymorphism on CGI-I score.

Abbreviations: CGI-I, Clinical Global Impression-Improvement scale; LSAS-SR, Liebowitz Social Anxiety Scale; PET, positron emission tomography; fMRI, functional magnetic resonance imaging; rCBF, regional cerebral blood flow; BOLD, blood-oxygen-level-dependent imaging; SSRI, selective serotonin reuptake inhibitor; PFC, prefrontal cortex; 5-HTTLPR, serotonin transporter-linked polymorphic region; TPH2, tryptophan hydroxylase-2.

In two subsequent publications, involving identical samples, Faria and colleagues aimed to determine the roles of different amygdala subregions in placebo anxiolysis[248,249]. The sample for these studies included the twenty-five patients in the study above[250], and included further patients randomised to the placebo arms of trials of citalopram or paroxetine, yielding a total of 72 patients with social anxiety disorder. Again, patients carried out a public speaking task while undergoing PET imaging before and after 8 weeks of treatment. Twenty out of 35 patients were classified as SSRI responders (57%), and eleven out of 37 patients responded to placebo (30%)[249]. All treatment responders, whether they received SSRI or placebo, showed reduced cerebral blood flow in the left basomedial/basolateral ($Z = 2.49$, $p < 0.005$) and right ventrolateral amygdala ($Z = 2.95$, $p < 0.05$) during the second PET scan. The change in blood flow in these regions correlated significantly with reduced anxiety ($r's > 0.3$, $p's < 0.005$). There were no differences in regional cerebral blood flow between SSRI and placebo responders[249]. When the functional connectivity patterns of these patients were analysed, there was evidence that the correlation between left basomedial/basolateral amygdala activity and left dorsolateral prefrontal cortex was more negative in placebo responders than in non-responders ($Z = 3.42$, $p < 0.001$). Further, compared with SSRI responders, placebo responders showed more negative correlation between left amygdala and right ventromedial and dorsolateral prefrontal cortices, and more positive correlation with dorsomedial prefrontal cortex ($Z's > 3.00$, $p's = 0.001$)[248]. Inferences regarding the potential neurotransmitters involved cannot be made from these data.

Finally, whether expectations enhance the effects of antidepressant treatment was explored in 46 patients with social anxiety disorder[98]. All were treated with escitalopram 20 mg daily for 9 weeks (10 mg daily for the first week) but were randomised with respect to the instructions they received. Prior to treatment, 24 patients were informed that they would receive escitalopram while 22 patients were told they would receive an 'active placebo', likely to induce side-effects similar to escitalopram but with no expected symptom improvement. Before and after treatment, these patients underwent fMRI scanning while completing an emotional face-matching task. Outcomes on the Liebowitz social anxiety scale (LSAS) were significantly improved with overt compared with covert escitalopram ($d = 2.24$ and $d = 1.13$, respectively). The fMRI results demonstrated that overt treatment was associated with increased reactivity to emotional faces in bilateral posterior cingulate cortex, left mid temporal gyrus and left inferior frontal gyrus compared with covert treatment ($Z's > 3.60$, $p's \leq 0.0001$). A psychophysiological interaction (PPI) analysis demonstrated that the covert arm exhibited increased connectivity relative to the overt arm between the amygdala and right dorsal posterior cingulate cortex, and right insula, when viewing faces compared with shapes ($Z's \geq 2.85$, $p's \leq 0.002$). This was interpreted by the authors as evidence that the covert group processed fear more intensely. However, there was no

statistically significant difference in amygdala reactivity between groups, a key node in fear processing, when viewing emotional faces[98]. Inferences cannot be made about the neurotransmitters that might be involved from these data.

4.4 Discussion

This systematic review of the literature yielded 16 articles, of which 12 reported functional neuroimaging markers of placebo antidepressant responses and 4 reported markers of placebo anxiolytic responses. To my knowledge, this is the first systematic review summarising this literature. There was great heterogeneity between studies regarding sample size, imaging modality, whether patients were imaged at rest or during a task, whether imaging occurred at baseline or longitudinally, and whether the aim of the trial was to explore markers of treatment response or placebo mechanisms. Also coordinates of peak activity were not consistently reported, and so the reviewing team relied on authors' naming of brain regions, which could have added further inter-study variation. As a result, it is challenging to coherently synthesise the data to find pertinent patterns. Despite this, some signals in these data point to possible important functional neuroanatomical correlates of, and important neurotransmitter systems in, placebo antidepressant and anxiolytic effects.

4.4.1 Functional neuroanatomical correlates of placebo antidepressant and anxiolytic effects

A wide range of functional neuroanatomical regions were identified in the included studies. This is exemplified by two studies, in which exploratory analyses were undertaken with no *a priori* regions of interest[246,252]. They showed that cerebral blood flow in a wide range of regions at baseline[246] and after 6 weeks of placebo treatment[252] correlated with placebo response (see Table 4.1). Due to the lack of longitudinal imaging or manipulation of psychological mechanisms important in placebo effects, such as learning or expectations, it is not easy to infer from these data which regions might have greater causal importance. Nevertheless, the results from other included studies suggest that some regions might be important in mediating placebo antidepressant and anxiolytic effects. I discuss each of these regions in turn below.

4.4.1.1 Ventral striatum

As part of the EMBARC trial, reward processing in the ventral striatum at baseline was hypothesised *a priori* to predict treatment response to an antidepressant[251]. Reduced reward processing activity at baseline suggested patients were more likely to benefit from sertraline. Interestingly, increased activity suggested no advantage of medication, and a trend towards

superiority of placebo[251]. Significant superiority for placebo would likely be difficult to demonstrate in such a comparison as placebo effects operate in the medication arm as well[56,57]. Despite this, these results suggest that the ventral striatum might be an important neuroanatomical region in placebo antidepressant effects. This is supported by the finding that 'active' placebo treatment is associated with increased opioid release in the nucleus accumbens compared with an 'inactive' placebo[96]. The ventral striatum is reliably activated by placebo analgesia[129], suggesting that reward circuitry might be important in mediating placebo effects across domains.

4.4.1.2 Dorsolateral prefrontal cortex

Activity in dorsolateral prefrontal cortex was correlated with placebo response in many studies included in this review[97,136,243,245-248,252]. This region is reliably activated by placebo analgesia and is thought to play a role in generating placebo-related expectations/expectancies[101,129]. From the data in the current review, there was evidence that activity increased in the dorsolateral prefrontal cortex in placebo antidepressant responders after 6 weeks[252], and that baseline blood flow in this region moderated subsequent placebo response in an antidepressant trial[246]. In placebo responders with social anxiety disorder, there was decreased correlation between bilateral amygdala and dorsolateral prefrontal cortices during a public speaking task compared with SSRI responders[248]. In an analysis of the EMBARC trial data, as baseline resting-state connectivity between dorsolateral prefrontal and inferior parietal cortex increased, the relative efficacy of placebo also increased[243]. However, these results are all correlational and so causality cannot be inferred. Evidence for a causal role of dorsolateral prefrontal cortex activity in placebo antidepressant effects comes from the studies by Peciña et al., in which expectation of mood improvement led to greater activity in dorsolateral/ventrolateral prefrontal cortex[97,136]. However, activity in these regions also negatively moderated the effect of higher expectation and positive reinforcement on subsequent mood improvement[97,136]. This was interpreted by the authors as possibly reflecting a trade-off between expectation and mood experience[97]. It is also possible this finding was due to a 'ceiling' effect, as the lateral prefrontal cortex was only activated when mood had already improved[97]. In addition, a recent meta-analysis found that placebo analgesia-induced activation of the dorsolateral prefrontal cortex varies greatly between studies[183], further making its role in placebo effects difficult to interpret. The role the dorsolateral prefrontal cortex might play in placebo antidepressant or anxiolytic effects remains unclear.

4.4.1.3 Rostral anterior cingulate cortex and the default mode network

Activity in the rostral anterior cingulate cortex (rACC) was identified by only one study included in this review[253], however, this is a potentially important finding. Placebo analgesia consistently

activates the rACC[129]. In healthy volunteers, activity in this region correlated positively with placebo-induced reductions in "unpleasantness" when viewing aversive pictures[155], and a placebo anxiolytic increased activity in this region[159]. The rACC is a region within the default mode network[255]. The default mode network, and the rACC in particular, is hypothesised to be central in generating placebo effects[76]. Indeed, placebo response was correlated with increased activity in regions within the default mode network in a number of studies in this review[98,246,252].

It is thought that the default mode network is intrinsically anticorrelated with networks activated by performance of a task, such as the salience network[255,256]. This raises the question of whether reductions in activity in 'task-positive' networks play a role in placebo antidepressant or anxiolytic effects. Sikora and colleagues[253] found that resting-state functional connectivity between the rACC and salience network, and the *degree of reduction* in connectivity between the two following placebo treatment, was associated with placebo response. However, negative treatment experiences can reduce subsequent placebo effects[113-115], meaning these findings are confounded by the fact that half the patients in this trial experienced an 'inactive' placebo prior to 'active'. In a study in healthy volunteers, a placebo anxiolytic *increased* task-evoked connectivity between the rACC and salience network, and this was generalizable to the entire default mode network, i.e. the placebo reduced anticorrelation between the default mode network and the salience network[159]. Both anxiety and major depressive disorders have been linked to abnormal functional connectivity within the salience network, and between this network and others[257,258]. However, there is little evidence for the salience network playing a role in mediating placebo effects. Placebo analgesic treatment usually *reduces* activity in regions considered part of the salience network, particularly the dorsal anterior cingulate cortex and anterior insula[129]. Therefore, it could be hypothesised from the data in this review that an interaction between the default mode network and other 'task-positive' networks that mediate psychopathological mechanisms, such as the salience network, could be important in generating a placebo antidepressant or anxiolytic effect. Further research is needed to explore this hypothesis.

4.4.1.4 Orbitofrontal cortex

Orbitofrontal cortex activity was identified by a single study included in this review[136]. Placebo effects on disgust, unpleasantness, and placebo analgesia correlate with orbitofrontal cortex activity[76,101,154,155]. This region is also densely populated with μ-opioid receptors[259]. It is thought that the orbitofrontal cortex is important in judging value and encoding expectations regarding future events[101,259]. In line with this, when μ-opioid receptors were blocked by naltrexone 50mg, the expectancy effects of a placebo antidepressant and the reinforcement effects of positive

sham neurofeedback on subjective mood were reduced, and this was associated with reduced right central orbitofrontal cortex activity[136].

4.4.1.5 Amygdala

Activity in the amygdala was a correlate of placebo anxiolytic and antidepressant effects in a number of studies[96,98,248-250,254]. Three of these studies involved patients with social anxiety disorder, and change in amygdala activity was measured following 6-8 weeks of placebo administration[248-250]. The sample included in these studies overlapped, so the reduction in amygdala activity discussed in these papers could be considered one finding. In the fourth study of placebo anxiolysis that involved manipulation of expectation, there was no evidence of a significant difference in amygdala activity between 'overt' and 'covert' SSRI administration[98]. Indeed, change in amygdala activity in this study correlated with improvement in social anxiety symptoms, rather than with expectations[98]. It is therefore unclear whether changes in amygdala activity result from placebo mechanisms or represent a non-specific phenomenon. It is possible that any role the amygdala played in placebo mechanisms in that study was confounded by the fact all patients were given an SSRI, which could in itself have affected amygdala activity. In the two studies involving patients with depression, functional neuroimaging was carried out prior to administration of any active medication, removing this potential confound[96,254]. Both of these studies showed, although by slightly different means, that increased expectation of benefit correlated with either reduced activity or increased opioid binding in the amygdala, and that these effects correlated with subsequent response to antidepressants[96,254]. However, both of these studies were judged to be moderate risk of bias due to a large number of patients withdrawing which could have affected the results[96], and due to possible selective reporting of outcomes[254].

Placebo and expectancy-induced reductions in bilateral amygdala activity have been found during placebo analgesia[129] and in association with reduced feelings of "unpleasantness" when viewing aversive pictures[155]. In the latter study, this did not correlate with placebo response[155]. Further, the large EMBARC trial did not find a relationship between blood flow in the amygdala and placebo response[246]. Subgroups responsive to placebo in this trial did demonstrate increased resting connectivity within the limbic network (including bilateral amygdala) at baseline[244], and increased baseline connectivity between the limbic network and the salience and somatomotor networks predicted greater improvement with placebo and worse outcome with sertraline[245]. However, when looking at predictors of placebo response alone and not predictors of worse outcomes with sertraline there was no evidence of amygdala involvement[245]. It is possible instead

that reductions in amygdala activity represent a phenomenon non-specific to placebo, perhaps relating instead to treatment response or changes in affect. This needs further exploration.

4.4.1.6 Summary

In sum, from these data it appears that activity in the ventral striatum, rostral anterior cingulate cortex (and possibly other default mode network regions), orbitofrontal cortex and dorsolateral prefrontal cortex (and possibly other executive control and dorsal attention regions) correlates with placebo antidepressant effects. These regions' role in *causing* these effects is uncertain and needs further investigation. Studies of placebo anxiolysis focused on the amygdala. Reduced activity in this region correlated with symptom improvement, however, in the one study in which placebo mechanisms were specifically explored, there was no significant change in this region when comparing high and low expectancy. Instead, regions of the default mode network, such as posterior cingulate, appeared important. Whether change in amygdala activity reflects a specific or non-specific placebo mechanism in emotional processing needs further exploration.

4.4.2 Possible neurotransmitter systems involved in placebo antidepressant and anxiolytic effects

In the current review, there was direct evidence only for a role of the endogenous opioid system in placebo antidepressant effects. There was also some indirect evidence suggesting that dopamine and serotonin might be important in placebo antidepressant and placebo anxiolytic effects. Each of these neurotransmitter systems is discussed in turn below.

4.4.2.1 Endogenous opioids

The studies by Peciña and colleagues[96,136] suggest that the endogenous opioid system plays an important role in mediating placebo antidepressant effects. These studies showed that a placebo antidepressant caused opioid release, measured as a reduction in μ-opioid receptor binding potential, in the nucleus accumbens[96]. The administration of naltrexone, a μ-opioid antagonist also reduced the effects of expectancy and learning on antidepressant placebo effects[136]. There was also additional indirect evidence supporting a role for the endogenous opioid system in placebo antidepressant effects. For example, increased expectation of benefit and higher mood ratings from a placebo rapid acting antidepressant were associated with greater increases in plasma β-endorphin levels[97]. Further, connectivity of the rACC at rest, a key node in the endogenous opioid system[130,168], correlated with antidepressant response to an 'active' placebo[253]. The endogenous opioid system is important in placebo analgesia[79,130]. Although the present data are limited, they suggest the endogenous opioid system might be important in

placebo effects in multiple domains, including those involving emotions. This is supported by studies of placebo anxiolysis in healthy volunteers, which show overlap with regions important in placebo analgesia, particularly the rACC[155,159].

4.4.2.2 Dopamine

There was further indirect evidence for a role of dopamine in placebo antidepressant effects in this review. Activity in the ventral striatum was identified as a correlate of placebo antidepressant effects in two studies[96,251]. The ventral striatum is also reliably activated in placebo analgesia[129] and is an important centre of dopaminergic neurotransmission. There is direct evidence for dopamine mediating placebo effects in other domains, including pain[139] and Parkinson's disease[88,140]. Further work is required to understand whether dopamine plays a mediating role in placebo antidepressant effects.

4.4.2.3 Serotonin

Finally, one study in this review showed indirect evidence for a role for serotonin in placebo anxiolytic effects. The G allele of the G-703T polymorphism in TPH2 mediated placebo-induced reduction in CGI-I score in patients with social anxiety disorder via a reduction in amygdala activity[250]. As discussed above, it is unclear whether such a change in amygdala activity is specifically related to placebo mechanisms or whether this represents a non-specific treatment effect. Furthermore, this analysis involved a small sample size and so there is a possibility this is a false positive. There is no other evidence to my knowledge that serotonin plays a role in placebo anxiolysis or in other placebo effects. More studies are needed to understand whether the serotonergic system might be important in mediating placebo effects or whether it might interact with other neurotransmitter systems, such as the endogenous opioid system, in a placebo-specific process.

4.5 Concluding comments

Although limited by the heterogeneity of the studies included in this review, the results suggest that the endogenous opioid system, dopamine, and serotonin could be important neurotransmitter systems in placebo antidepressant and anxiolytic effects. Important neuroanatomical regions might include the rACC and default mode network, the ventral striatum, orbitofrontal cortex and dorsolateral prefrontal cortex. Of significance, there is evidence that activity in the rACC, default mode network, and orbitofrontal cortex is associated with placebo effects on unpleasantness, anxiety associated with threat of electric shock, and disgust in healthy volunteers[154,155,159]. The possible functions of these neurotransmitters and regions in placebo

antidepressant and anxiolytic effects needs further exploration in adequately powered studies designed with the aim of investigating the placebo effect, with consideration to possible confounds such as order effects, and involving longitudinal neuroimaging to begin to unpick causality.

It is noteworthy that I found a relative paucity of records reporting functional neuroimaging correlates of placebo anxiolysis compared with placebo antidepressant effects. This highlights the need for studies specifically exploring the mechanisms of placebo anxiolysis. It is possible that placebo anxiolysis has been little explored due to the lack of convenient experimental paradigms. In the next two chapters, I report studies in which I have attempted to validate two experimental placebo anxiolytic procedures.

Chapter 5 An Experimental Placebo Procedure using the 7.5% CO_2 Inhalational Model of Generalised Anxiety Disorder

5.1 Background

The placebo effect is a psychoneurobiological response resulting from the interplay between prior expectations and learning that leads to symptom reduction[81] (see section 1.4). We understand a great deal about the psychoneurobiology of the placebo effect, mostly through studies of placebo analgesia (as discussed in these reviews[76,100,101]). A placebo effect is thought to occur when incoming sensory signals are controlled via top-down systems recruited through the interplay of expectations and learning. Some of these top-down systems are also involved in regulation of affect[76,101], which suggests that it should be possible to manipulate affect through the use of placebos. However, only a few studies have explored this[95,152,154-156,158,159] and these have been influenced by confounders (see section 1.5). Further, these studies were carried out in healthy volunteers and so it is unclear whether any biomarkers identified in relation to induced placebo effects are clinically relevant. To test hypotheses regarding the psychoneurobiology of placebo effects on emotional states, a clinically relevant experimental paradigm is needed that reliably and reproducibly induces a placebo response.

An emotional domain that is both feasible to induce in healthy volunteers under experimental conditions and is clinically relevant is anxiety. One particularly well-studied model is the 7.5% CO_2 inhalational model of generalised anxiety (for a discussion of other models see Chapter 2). Inhalation of air enriched with 7.5% CO_2 for 20-30 minutes (or 'CO_2 challenge') mimics the subjective, autonomic and neurocognitive features of generalised anxiety disorder[191-193]. In addition, anxiety in this model is ameliorated by some standard pharmacological and psychological treatments for generalised anxiety disorder[194,195]. Since this model is treatment-responsive, has construct validity for clinical anxiety, and allows for control of the anxiogenic stimulus intensity, it is ideal for use in an experimental placebo procedure.

In the current study, I tested whether it was possible to induce a clinically relevant placebo response in the 7.5% CO_2 inhalational model of generalised anxiety. Placebo effects are maximal when verbal suggestions and learning are combined[76,92,111,112]. Therefore, I designed a paradigm that pairs verbal suggestions with learning. The design is similar to paradigms previously used to interrogate mechanisms of placebo analgesia[84,85,108].

5.2 Method

5.2.1 Ethics statement

This study was reviewed and approved by the Ethics and Research Governance Office at the University of Southampton (reference: 52726). Prior to starting the study, participants were informed that the aim was to explore a new 'lorazepam' anxiolytic nasal spray. Information regarding the aim to study the placebo effect was withheld initially; however, on completing the study participants were fully debriefed, and fully informed consent was sought a second time for use of their data. Participants were informed that they could decline to consent and their data would be destroyed. No participants withdrew consent.

5.2.2 Participants

Thirty-two healthy volunteers (aged 18-55) were recruited from the community via poster advert and through the e-folio research website. They were offered £15 or, if they were a psychology student, course credits as an incentive for participating. Exclusion criteria included: current or lifetime history of psychiatric illness as assessed by the Mini International Neuropsychiatric Interview for DSM 5 (MINI)[260]; body mass index <18 or >28 kg/m^2; chronic physical illness; regular smokers (>6 cigarettes per day); and use of medication in the previous 8 weeks. Participants with current alcohol intake >21 units per week or illicit drug misuse (used more than twice in the past 12 months) were also excluded. All inclusion and exclusion criteria were assessed via telephone screening interview.

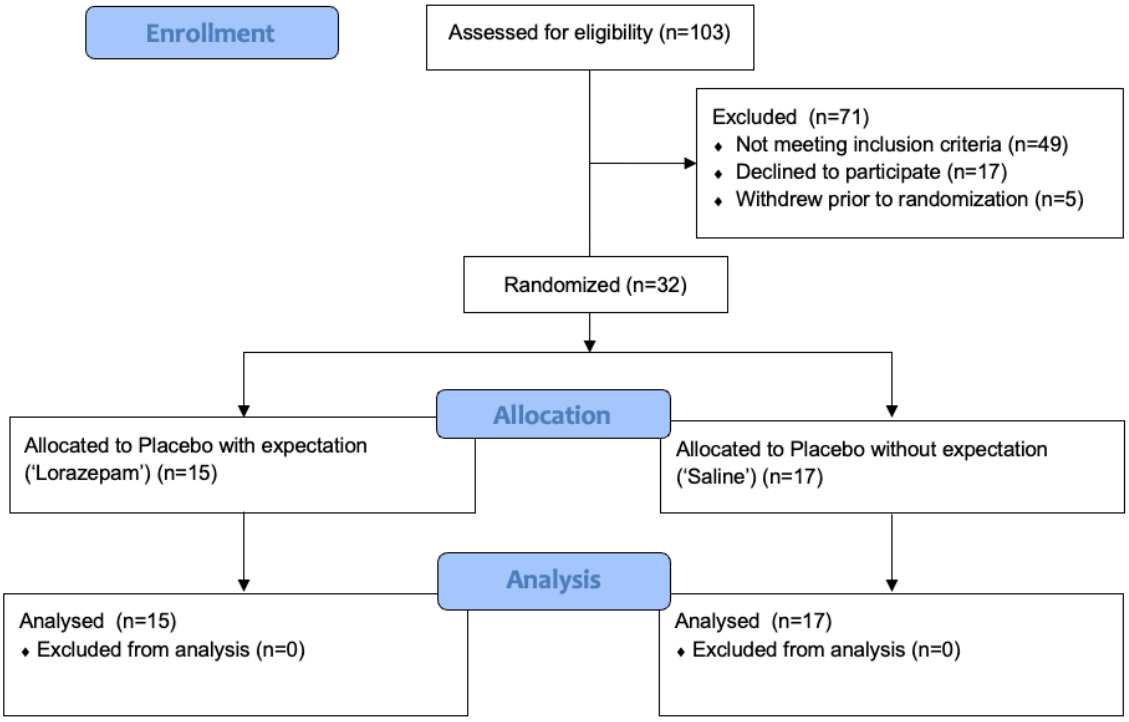


Figure 5.1 CONSORT diagram for the study.

5.2.3 Materials and procedures

5.2.3.1 The sham treatment

Potential participants were informed that the purpose of the study was to assess the effects of 'intranasal lorazepam' administered as a nasal spray. Lorazepam is a licensed treatment for anxiety disorders in the UK, but in reality is not available as a nasal spray. The spray administered to participants was instead a normal saline nasal spray with no active ingredients. I chose a placebo nasal spray over other modalities (e.g. a pill) for the following reasons. First, it is known that invasive procedures including sham injection and sham surgery show larger placebo effects than non-invasive alternatives such as a pill[261]. Second, so-called 'active' placebos that generate side-effects are more effective than 'inactive' placebos[160], possibly due to the expectations these side-effects engender[76]. For this study, it would perhaps have been possible to develop a rationale for sham injection that would have been believable for participants. However, injection is not without risks such as injury at the injection site or infection. On the other hand, a nasal spray is somewhat invasive (applicator needs to be inserted into the nostril) and causes 'side-effects' in terms of feeling the sprayed fluid enter the nostril accompanied by some itching or mild irritation. A placebo nasal spray has also been used to induce placebo effects in previous studies[95,152,156-158].

5.2.3.2 Experimental placebo procedure

Prior to participation, potential volunteers were given information about lorazepam, including that it is used as an anxiolytic and its possible side-effects. Following a screening telephone interview, eligible participants attended for an experimental session, comprising 3 segments: baseline pre-conditioning, conditioning and post-conditioning (see Figure 5.2). In the baseline pre-conditioning segment, participants underwent a 7.5% 'CO_2 challenge'. Air augmented with 7.5% CO_2 (21% O_2, balance N_2) was administered through an oronasal face mask for 20 minutes, as in previous studies[192,194,262,263]. Following this, participants underwent the conditioning phase of the experiment.

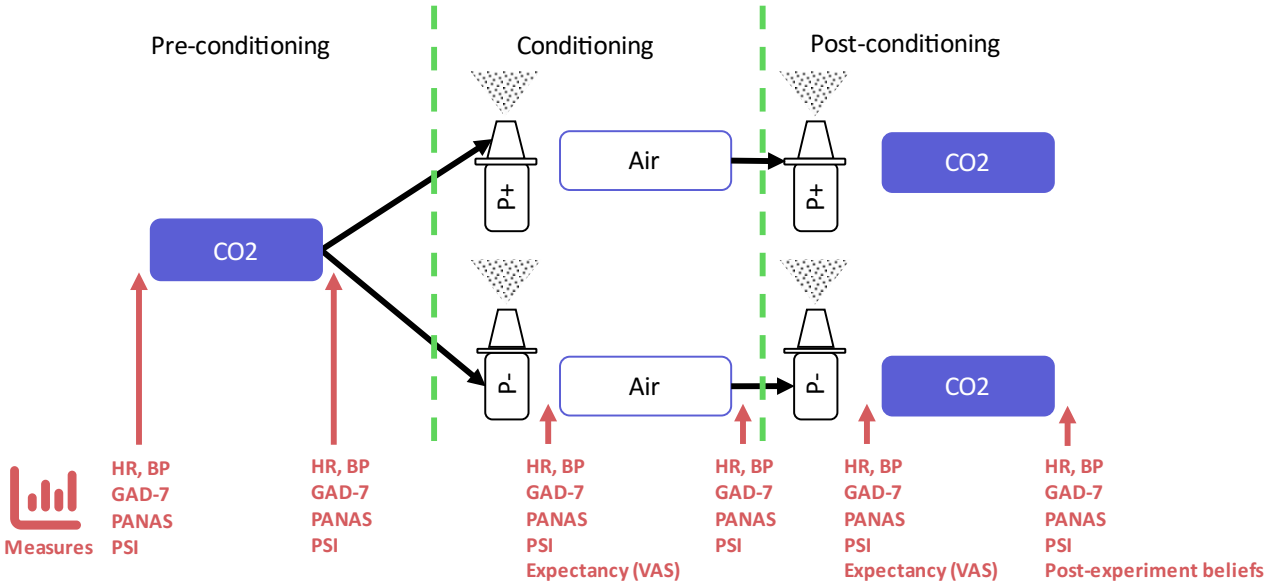


Figure 5.2 Schematic of novel experimental placebo anxiolysis paradigm. After baseline CO_2 challenge (pre-conditioning), participants are randomised to either the placebo with expectation (P+, 'lorazepam') or placebo without expectation group (P-, 'saline'). They then undergo a conditioning procedure, in which the participants inhale air in place of 7.5% CO_2 gas mixture. In the post-conditioning period participants again undergo CO_2 challenge. Outcome measures are taken before and after each inhalation period.

Abbreviations: HR, heart rate; BP, blood pressure; GAD-7, generalised anxiety disorder-7 questionnaire; PANAS, positive and negative affect schedule; PSI, panic symptom inventory; VAS, visual analogue scale.

Participants were randomised to one of two groups: placebo with expectation or placebo without expectation. I created a stratified (for gender) randomisation sequence in Microsoft Excel, and participants were randomised following the baseline CO_2 challenge. In the placebo with expectation group, participants were given a 'sham' anxiolytic treatment: a normal saline nasal spray labelled as 'intranasal lorazepam'. Participants took two doses intranasally. The spray was administered with information that it would work within seconds, as the nose is directly connected with the brain, and the spray would have a duration of action of 20-30 minutes. After receiving the spray, participants were told they would now repeat the CO_2 challenge for 20 minutes. Instead, for this inhalation period, the experimenter surreptitiously changed the inhaled gas to normal air. Normal air inhalation does not normally induce anxiety. The purpose of this deception was to condition the participants to believe the spray possessed anxiolytic properties. In the post-conditioning segment, participants again took two doses of the 'lorazepam' spray before undergoing another 20-minute CO_2 challenge. They were told that this was to investigate the effects of repeated doses of 'intranasal lorazepam'. For simplicity, this group is referred to as the 'lorazepam' group in the rest of this chapter.

The placebo without expectation group underwent exactly the same procedure, except they received truthful instructions throughout. After the pre-conditioning segment, they were given a nasal spray labelled 'saline'. This was accompanied by information that the spray contained normal saline only and should not have any effect on subjective anxiety. Participants were asked to administer two doses of this nasal spray intranasally. Following this, these participants also underwent normal air inhalation and were informed that the change to normal air would be the cause of any reductions in anxiety. In the post-conditioning segment, these participants again took two doses of the saline nasal spray, and were then told that the next inhalation would involve air enriched with 7.5% CO_2 and this would likely cause them to feel anxious. The purpose of this group was to control for the effects of repeated exposure to CO_2 challenge as well as any other non-specific 'apparent' placebo effects (such as natural relaxation, see section 1.3) that occurred during the experiment. Giving truthful instructions prevents attribution of reduced anxiety to the nasal spray and therefore a placebo effect post-conditioning[84,108]. For simplicity, this group is referred to as the 'saline' group in the rest of this chapter.

All treatments and instructions were given by an independent researcher while I was absent from the room. This was done so that I would be blind to treatment assignment. Previous experimental placebo studies utilising verbal suggestion or conditioning procedures have often not fully blinded the experimenter[84,90,92,108,112,155]. However, this can introduce responder bias through the

participant wanting to give the expected response, or wanting to please the researcher[162,163]. By blinding the experimenter, I hoped that the risk of responder bias would be reduced[163].

5.2.4 Measures

5.2.4.1 Baseline measures

Both placebo effectiveness and response to CO_2 challenge have been associated with trait variables. Effectiveness of a placebo has been linked with trait anxiety and affect[264,265], interoceptive awareness and openness[266], dispositional optimism[149,265], and locus of control[267]. However, it should be noted these predictors have failed to replicate in a recent meta-analysis[268]. Response to CO_2 challenge appears to be associated with trait anxiety[269] and anxiety sensitivity[269,270].

To ensure that the groups were balanced for potential predictors of placebo and CO_2 responsiveness, the following baseline measures were taken:

- Demographics including age, biological sex, and body mass index (BMI)
- Baseline heart rate and blood pressure
- Measures of trait anxiety including the Hospital Anxiety and Depression – Anxiety (HADS-A) sub-scale[271], the Penn State Worry Questionnaire (PSWQ)[272], the Intolerance of Uncertainty Scale (IUS)[273], and a modified version of the Generalised Anxiety Disorder 7-item (GAD-7) questionnaire[274] where each question is accompanied by a visual analogue scale ranging from *"Not at all"* to *"Nearly every day"*
- The HADS depression sub-scale (HADS-D)[271] – a measure of trait affect
- The Anxiety Sensitivity Index (ASI)[275] – a measure of anxiety sensitivity
- The Revised Life Orientation Test (LOTR)[276] – a measure of dispositional optimism
- The Locus of Control questionnaire (LOC)[277] – a measure of locus of control

5.2.4.2 CO_2 outcome measures

Outcome measures were taken before and after each inhalation. Subjective state anxiety was measured with a modified version of the GAD-7[274], where each question is represented by a visual analogue scale ranging from *"not at all"* to *"all of the time"*. This questionnaire is brief, highly applicable to diagnostic criteria for generalised anxiety disorder, and the modification to visual analogue scales means it is sensitive to change over time[192,262,263]. Psychological and somatic symptoms of anxiety were further measured through the panic symptom inventory (PSI)[278,279]. This is a 34-item scale that examines the psychological (e.g. feeling anxious, out of control, that they were dying) and somatic (e.g. heart pounding, breathlessness, muscle tension) symptoms of

panic attacks. The presence and severity of these symptoms are rated from 0 (not present) to 4 (very severe). Subjective changes in mood were assessed through the Positive and Negative Affect Schedule (PANAS)[280]. Autonomic stress was measured through heart rate and blood pressure measurements taken with an automated sphygmomanometer (Omron-M6, Medisave, UK) before and after each inhalation. These measures are known to differentiate between high and low anxiety states reliably[191-194].

5.2.4.3 Expectations

I measured conscious expectations of therapeutic benefit from the spray immediately before the conditioning period (air inhalation) and immediately before the post-conditioning CO_2 challenge. Increased expectations in the 'lorazepam' group would suggest that the conditioning procedure had been effective. Participants were instructed to rate their answer to the question *"how much do you expect this spray to reduce your anxiety by during the next inhalation?"* on a 10 cm visual analogue scale ranging from *"not at all"* to *"very much"*. To check whether this expectation remained present post-conditioning, I also assessed post-experiment beliefs about the 'sham' treatment using an adapted version of the therapy credibility questionnaire[281]. This questionnaire was originally designed to measure the credibility of the rationale for different therapies and the expected potential for benefit[281]. Three of the questions were adapted to measure beliefs about the effectiveness of the 'sham' treatment and read as follows:

1. *How confident are you that this treatment can successfully eliminate anxiety?*
2. *How confident would you be in recommending this treatment to a friend who suffers with anxiety in certain situations?*
3. *If you suffered with anxiety, would you be willing to have this treatment?*

Each item was scored using a Likert scale from 1 to 5, with higher scores indicating greater beliefs about effectiveness. Participants were asked to be honest in their answers. This questionnaire was completed in the presence of the unblinded independent researcher to avoid accidentally unblinding me.

5.2.5 Statistical analysis and power calculation

My outcome of interest was the change in subjective anxiety, mood and autonomic measures over the course of the procedure. I hypothesised that the 'lorazepam' group would exhibit a placebo effect, which would be measurable as a significant reduction in CO_2 outcome measures from pre- to post-conditioning, and a significant reduction in these measures compared with the 'saline' group post-conditioning.

Due to the Covid-19 pandemic, recruitment for this study was more difficult than anticipated (recruitment began in January 2020). However, the study is powered to detect meaningful placebo effects. The effect size of placebo treatment is known to be smaller in clinical trials compared with the effect sizes seen in experimental placebo studies[75]. Meta-analyses have shown that the average effect size of placebo analgesia in healthy volunteers is g = 1.24[184] and in studies combining verbal suggestion with behavioural conditioning it is d = 1.48[75]. To date, only one other study has combined verbal suggestion and behavioural conditioning to experimentally induce a placebo effect on affect. In this study, significant effects of the placebo were seen on 'unpleasantness' in a sample of 15 participants, suggesting a large effect size (although standard deviations were not reported)[155]. For this study, I recruited 32 participants, which provided 80% power to detect an effect size d > 1.03 with an alpha level of 0.05 (two-tailed). The study was therefore powered to detect a placebo effect size comparable to that seen in the literature for similar experimental placebo studies.

Statistical analysis was carried out using Jamovi version 1.6.23.0 (https://www.jamovi.org)[218]. Baseline characteristics were compared between groups with independent samples t-tests for continuous data or chi-squared tests for dichotomous data. Statistical analysis of subjective anxiety, mood, and autonomic CO_2 outcome measures, and change in expectations was carried out through mixed-model analysis of variance (ANOVA) with repeated measures. In all models time was the within-subject factor and group was the between-subject factor. Significant results were explored further through *post-hoc* t-tests. The post-experiment therapy credibility questionnaire was analysed through an independent samples t-test.

Exploratory analyses included a *post-hoc* power analysis and an analysis examining whether expectations regarding potential benefit influenced outcome. First, I explored the sample sizes needed to achieve 80% power to detect placebo anxiolysis given the effect sizes seen in this study. Second, to explore the effect of expectations, I calculated the 'CO_2 reactivity' for each participant in each inhalation by subtracting pre-inhalation values from post-inhalation values. Larger scores would indicate a greater reactivity to CO_2 challenge. I then calculated how this changed from pre-conditioning to post-conditioning by subtracting pre-conditioning reactivity from post-conditioning reactivity. Thus negative values represent a reduction in reactivity (potentially placebo anxiolysis) while positive values represent an increase in reactivity. I then correlated the change in CO_2 reactivity for each outcome measure with expectations post-conditioning for each group separately.

5.3 Results

5.3.1 Baseline characteristics

Baseline characteristics for each group are summarised in Table 5.1. The only difference between groups was a significant difference in HADS-D score. However, all participants scored within the normal range (maximum score was 6). Further, if correction for multiple comparisons is applied then this difference is not significant. There were no other significant differences between the groups in baseline characteristics or personality traits.

5.3.2 Expectations

Expectations were measured both prospectively and retrospectively. I measured expectation prospectively (from pre- to post-conditioning) using a visual analogue scale and analysed these data through mixed-model ANOVA with repeated measures. This revealed a significant effect of group ($F_{(1,30)} = 12.72$, $p = 0.001$, $\eta_p^2 = 0.30$) and a significant time*group interaction ($F_{(1,30)} = 4.91$, $p = 0.035$, $\eta_p^2 = 0.14$). Post-hoc tests showed these effects were driven by a significant increase in expectation from pre- to post-conditioning in the 'lorazepam' group only (mean difference = 1.68, $t_{(30)} = 2.66$, $p = 0.012$) and a significant difference in expectation between groups post-conditioning (mean difference = 3.22, $t_{(30)} = 4.46$, $p < 0.001$). See Figure 5.3.

I also measured expectations retrospectively using an adapted therapy credibility questionnaire which the participant completed at the end of the study. There was a significant difference in this measure between groups, with the 'lorazepam' group rating the 'sham' treatment as more credible (mean difference = 2.28, $t_{(30)} = 2.22$, $p = 0.034$, $d = 0.79$).

Overall, these results suggest that the conditioning paradigm induced a significant expectation of therapeutic benefit, which appeared to persist following the post-conditioning CO_2 challenge.

Table 5.1 Baseline characteristics. Values are mean ± SD for continuous variables and count (%) for dichotomous variables. Significant results are in bold.

	'Lorazepam'	**'Saline'**	**Test statistic**	**p value**
N	15	17		
Age	21.47 ± 1.96	19.71 ± 3.08	$t_{(30)} = 1.90$	0.067
Females	10 (67%)	11 (65%)	$\chi^2_{(1)} = 0.01$	0.907
BMI	23.03 ± 2.37	22.17 ± 2.50	$t_{(30)} = 0.99$	0.332
Modified GAD-7	12.14 ± 8.75	15.54 ± 10.95	$t_{(30)} = 0.96$	0.344
HADS-A	3.13 ± 1.92	3.29 ± 1.93	$t_{(30)} = 0.24$	0.815
HADS-D	**0.53 ± 0.74**	**1.82 ± 1.91**	**U = 72.50***	**0.030**
ASI	12.07 ± 7.16	12.82 ± 7.79	$t_{(30)} = 0.28$	0.778
IUS	50.87 ± 12.59	48.12 ± 12.05	$t_{(30)} = 0.63$	0.533
LOTR	17.33 ± 3.06	16.65 ± 3.86	$t_{(30)} = 0.55$	0.585
LOC	12.33 ± 3.96	11.29 ± 3.92	$t_{(30)} = 0.75$	0.462
PSWQ	39.20 ± 9.28	38.76 ± 10.10	$t_{(30)} = 0.13$	0.900
Systolic BP (mmHg)	120.47 ± 11.86	113.18 ± 9.89	$t_{(30)} = 1.90$	0.068
Diastolic BP (mmHg)	74.73 ± 9.54	71.35 ± 10.50	$t_{(30)} = 0.95$	0.351
Pulse rate (bpm)	70.33 ± 14.17	75.53 ± 14.04	$t_{(30)} = 1.04$	0.306

*Mann-Whitney U test used as assumption of equal variances violated
Abbreviations: BMI, Body mass index; GAD-7, Generalised Anxiety Disorder-7 questionnaire; HADS, Hospital Anxiety and Depression Scale; ASI, Anxiety Sensitivity Index; IUS, Intolerance of Uncertainty Scale; LOTR, Life Orientation Test Revised; LOC, Locus of Control questionnaire; PSWQ, Penn State Worry Questionnaire; BP, Blood pressure; mmHg, millimetres of mercury; bpm, beats per minute

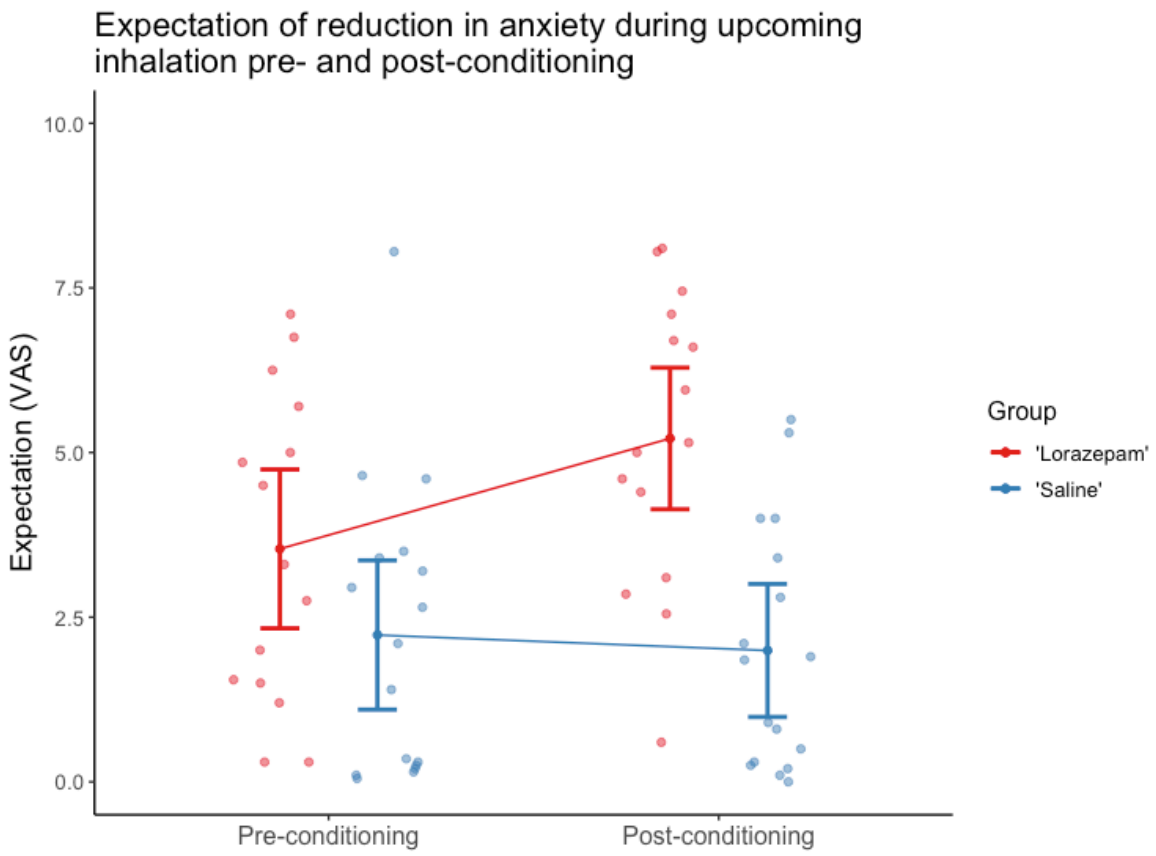


Figure 5.3 Graph showing expectation ratings pre- and post-conditioning in each group. Points represent estimated marginal means and error bars represent 95% confidence intervals. Expectation significantly increased in the 'lorazepam' group only, and there was a significant difference between groups post-conditioning.

5.3.3 Subjective and autonomic measures of anxiety

Subjective and autonomic measures of anxiety were analysed through mixed-model ANOVA. These results are summarised in Table 5.2. There was a significant effect of time in all outcome measures except diastolic blood pressure. Subjective anxiety, pulse rate, and negative affect were significantly increased, and positive affect significantly decreased, following each 7.5% CO_2 inhalation. There was an additional effect of group on systolic blood pressure. This appeared to be driven by a significant difference in systolic blood pressure between groups prior to the first inhalation (mean difference = 10.46, $CI_{95\%}$ [2.17, 18.75], $t_{(30)} = 2.58$, $p = 0.015$). Following the first inhalation, there were no significant differences in systolic blood pressure between the groups ($t_{(30)}$'s < 1.60, p's > 0.110). There were no significant time*group interactions in any outcome measure (F's < 1.08, p's > 0.350), suggesting that mean outcomes were similar in both groups across the experiment (see Figure 5.4 and Figure 5.5).

Table 5.2 Summary of mixed-model ANOVAs with repeated measures assessing CO_2 outcomes. Significant results in bold.

Outcome Measure	Effect	Test statistic	p value	η_p^2
Subjective Anxiety and Mood				
Modified GAD-7	**Time**	**$F_{(2.40,72.15)} = 28.90$**	**< 0.001**	**0.49**
	Group	$F_{(1,30)} = 0.10$	0.758	0.00
	Time*Group	$F_{(2.40,72.15)} = 0.40$	0.497	0.02
PANAS Positive Affect	**Time**	**$F_{(2.26,67.83)} = 38.72$**	**< 0.001**	**0.56**
	Group	$F_{(1,30)} = 0.10$	0.752	0.00
	Time*Group	$F_{(2.26,67.83)} = 1.07$	0.353	0.03
PANAS Negative Affect	**Time**	**$F_{(1.77,51.39)} = 15.25$**	**< 0.001**	**0.34**
	Group	$F_{(1,29)} = 0.24$	0.631	0.01
	Time*Group	$F_{(1.77,51.39)} = 0.46$	0.608	0.02
PSI	**Time**	**$F_{(1.80,54)} = 25.92$**	**< 0.001**	**0.46**
	Group	$F_{(1,30)} = 0.08$	0.785	0.00
	Time*Group	$F_{(1.80,54)} = 0.03$	0.959	0.00
Autonomic Measures				
Systolic BP (mmHg)	**Time**	**$F_{(3,90)} = 5.87$**	**0.001**	**0.16**
	Group	**$F_{(1,30)} = 5.24$**	**0.029**	**0.15**
	Time*Group	$F_{(3,90)} = 0.60$	0.620	0.02
Diastolic BP (mmHg)	Time	$F_{(3,90)} = 0.17$	0.914	0.01
	Group	$F_{(1,30)} = 0.86$	0.362	0.03
	Time*Group	$F_{(3,90)} = 1.03$	0.381	0.03
Pulse rate (bpm)	**Time**	**$F_{(1.70,50.87)} = 13.46$**	**< 0.001**	**0.31**
	Group	$F_{(1,30)} = 1.22$	0.278	0.04
	Time*Group	$F_{(1.70,50.87)} = 0.94$	0.383	0.03

Abbreviations: GAD-7, Generalised Anxiety Disorder-7 questionnaire; PANAS, Positive and Negative Affect Schedule; PSI, Panic Symptoms Inventory; BP, Blood pressure; mmHg, millimetres of mercury; bpm, beats per minute

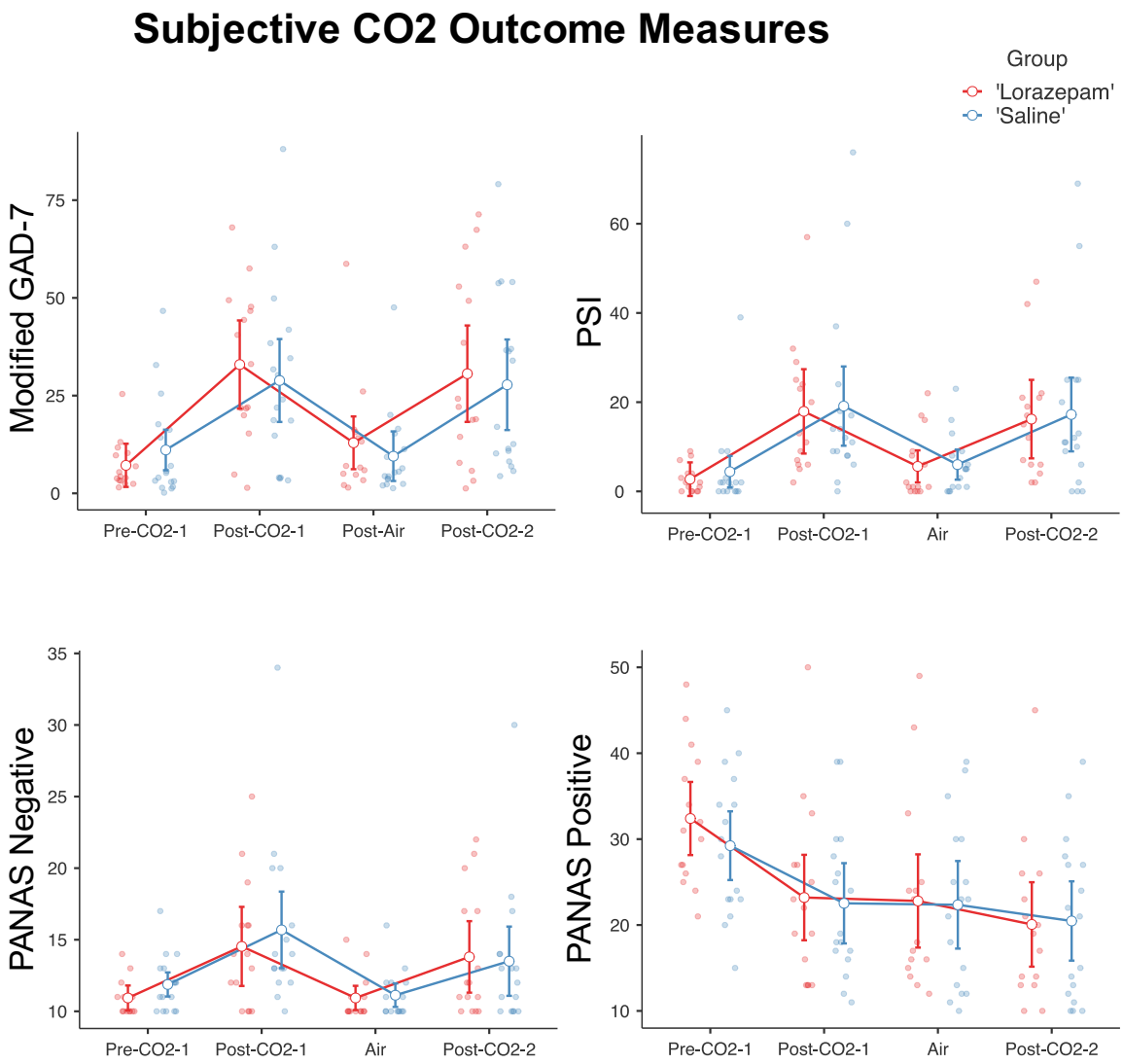


Figure 5.4 Graphs showing change in subjective anxiety and mood CO_2 outcome measures over the course of the testing session. Points represent estimated marginal means and error bars represent 95% confidence intervals.

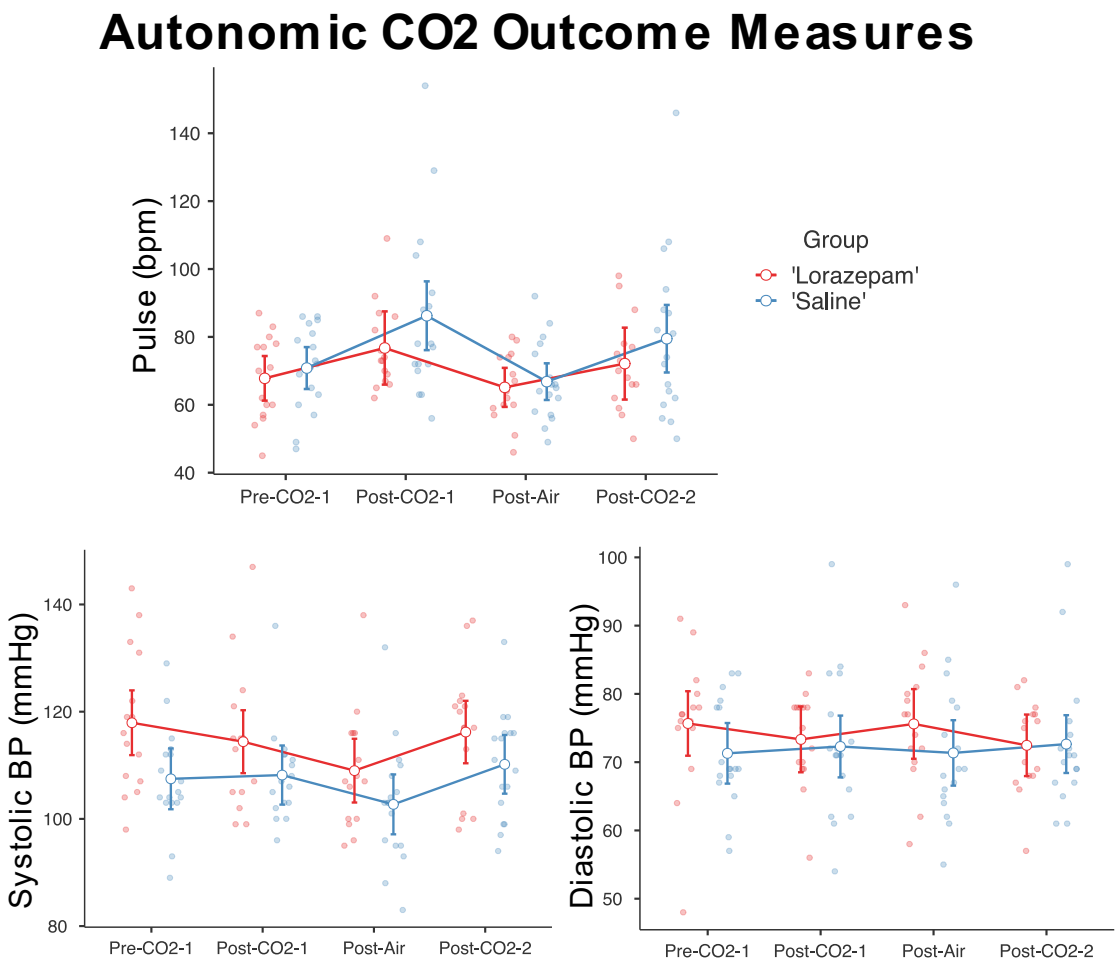


Figure 5.5 Graphs showing change in autonomic CO_2 outcome measures over the course of the testing session. Points represent estimated marginal means and error bars represent 95% confidence intervals.

5.3.4 Effect size of placebo anxiolysis

The effect sizes of 7.5% CO_2 inhalation on subjective and autonomic anxiety were large in this study (η_p^2 ranged from 0.16 to 0.56). However, the effect size of the time*group interaction was small in all outcome measures ($\eta_p^2 < 0.03$). As a result, large sample sizes would be needed to detect a placebo effect in this paradigm. In Table 5.3, I have summarised the effect sizes for *post-hoc* between-group comparisons following the post-conditioning CO_2 inhalation, and the sample size needed per group for 80% power to detect this effect. Minimum sample sizes ranged from 116 to 39246 per group.

Table 5.3 Summary of effect sizes for post-hoc between-group comparisons of subjective and autonomic outcome measures following the second CO_2 inhalation and sample size required for 80% power to detect the effect. Where mean difference is positive, this indicates a larger value for the 'lorazepam' group.

Variable	**Mean Difference**	**$CI_{95\%}$**	**Test result**	**Cohen's *d***	***N* per group required**
Modified GAD-7	2.83	-14.09, 19.75	$t_{(30)} = 0.34$, $p = 0.735$	0.12	1092
PANAS Positive Affect	-0.40	-7.15, 6.34	$t_{(30)} = 0.12$, $p = 0.903$	0.04	9813
PANAS Negative Affect	0.51	-2.89, 3.90	$t_{(30)} = 0.30$, $p = 0.763$	0.11	1299
PSI	-1.04	-13.11, 11.04	$t_{(30)} = 0.18$, $p = 0.862$	0.06	6281
Systolic BP (mmHg)*	1.92	-6.30, 10.14	$t_{(29)} = 0.48$, $p = 0.636$	0.19	436
Diastolic BP (mmHg)	-0.18	-6.36, 6.00	$t_{(30)} = 0.06$, $p = 0.953$	0.02	39246
Pulse rate (bpm)	-7.34	-21.87, 7.19	$t_{(30)} = 1.03$, $p = 0.311$	0.37	116

*Pre-inhalation 1 systolic BP included as a covariate, due to significant difference between groups
Abbreviations: $CI_{95\%}$, 95% confidence interval; GAD-7, Generalised Anxiety Disorder-7 questionnaire; PANAS, Positive and Negative Affect Schedule; PSI, Panic Symptoms Inventory; BP, Blood pressure; mmHg, millimetres of mercury; bpm, beats per minute

5.3.5 Exploratory analyses of effect of expectations on placebo anxiolysis

At the group level, average anxiety post-conditioning suggested no evidence for placebo effects. However, it was possible that subgroups of participants exhibited different responses. Indeed, on visual inspection of the data, some participants experienced reduced anxiety over time, while for others anxiety worsened. If expectation was associated with reduction in anxiety then this might suggest that some participants experienced placebo anxiolysis.

To explore this, I calculated change in CO_2 reactivity for each participant before and after placebo conditioning. There were no significant differences between the groups in change in CO_2 reactivity for any outcome measure (all $t_{(30)}$'s < 1.60, p's > 0.130). Next, I correlated change in CO_2 reactivity with expectation post-conditioning in the 'lorazepam' group and 'saline' group separately. In the 'lorazepam' group, and in subjective measures only, these correlations showed weak to moderate trend relationships between higher expectation post-conditioning and reduced responsiveness to CO_2 challenge (r's -0.37 to -0.47, p's < 0.180). In contrast, there was either no relationship or a relationship in the opposite direction in autonomic outcome measures and in all measures in the 'saline' group (see Figure 5.6).

It is additionally noteworthy that a number of participants in the 'lorazepam' group experienced increases in CO_2 reactivity, consistent with a nocebo response. This is illustrated in Figure 5.7.

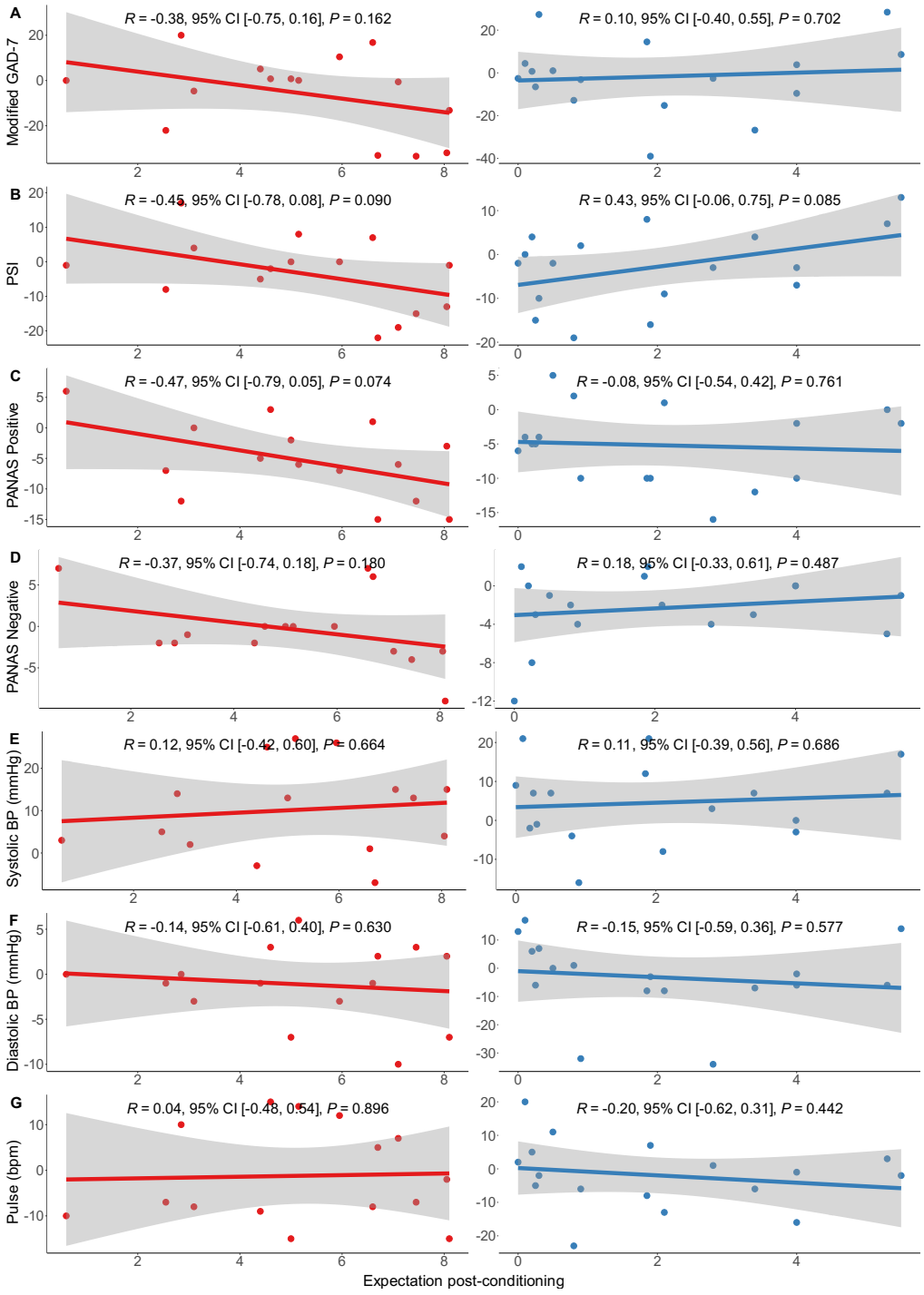


Figure 5.6 Correlations between change in CO_2 reactivity from pre- to post-conditioning in each outcome measure and expectation post-conditioning computed for each group separately (left: 'lorazepam'; right: 'saline'). Negative values on the y axis represent reduced CO_2 reactivity post-conditioning. There was a trend negative relationship between change in CO_2 reactivity in subjective measures of anxiety and expectation post-conditioning in the 'lorazepam' group only.

Abbreviations: GAD-7, Generalised Anxiety Disorder-7 questionnaire; PANAS, Positive and Negative Affect Schedule; PSI, Panic Symptoms Inventory; BP, Blood pressure; mmHg, millimetres of mercury; bpm, beats per minute.

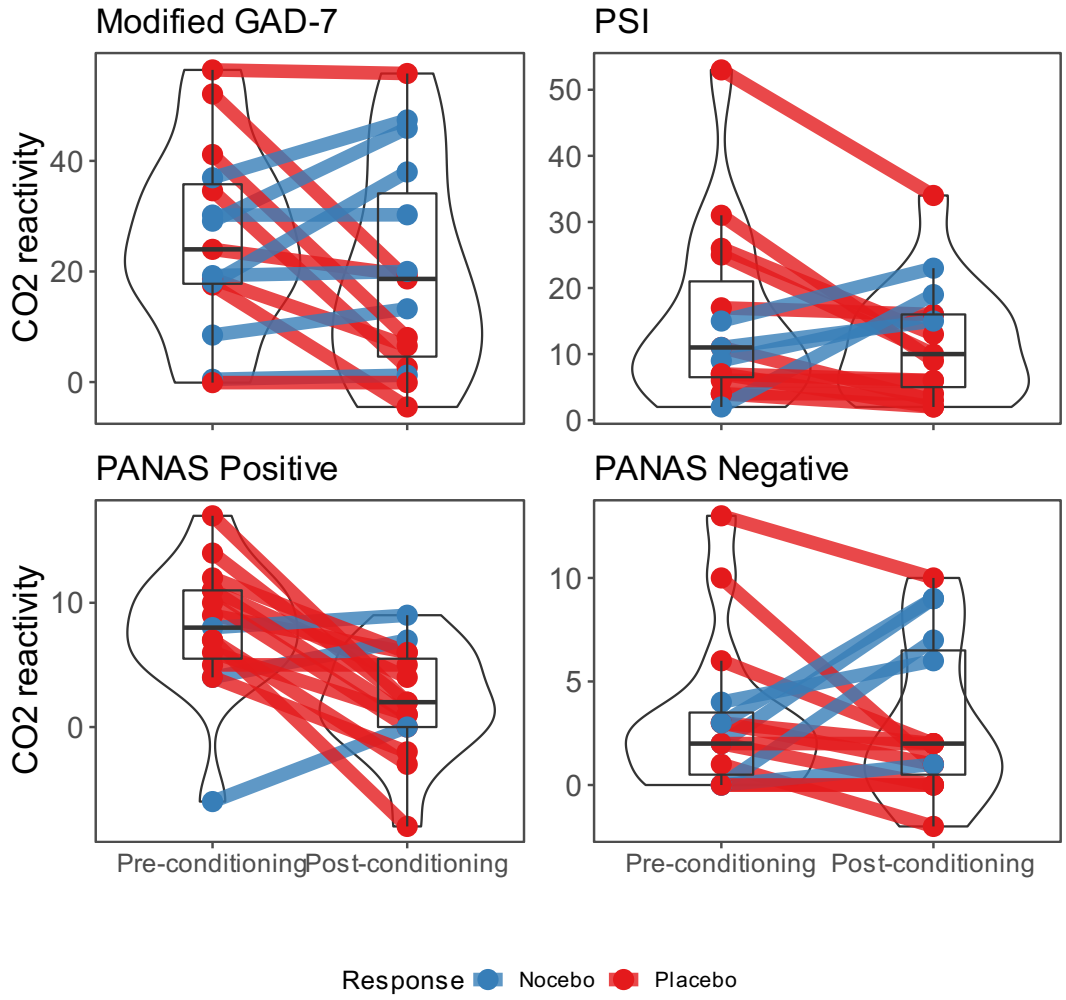


Figure 5.7 Change in CO_2 reactivity from pre- to post-conditioning in subjective CO_2 outcome measures for the 'lorazepam' group. Negative values on the y axis represent reduced CO_2 reactivity. In blue are participants who showed increases in CO_2 reactivity post-conditioning, consistent with a nocebo response. In red are participants who showed reductions, consistent with a placebo response.

Abbreviations: GAD-7, Generalised Anxiety Disorder-7 questionnaire; PANAS, Positive and Negative Affect Schedule; PSI, Panic Symptoms Inventory.

5.4 Discussion

The purpose of this study was to test whether a novel experimental placebo procedure reliably induced placebo anxiolysis in healthy volunteers. Contrary to my hypotheses, on average, both groups exhibited similar subjective and autonomic effects of CO_2 challenge during both inhalations. This occurred despite significantly increased expectations of benefit in the 'lorazepam' group following conditioning, which appeared to persist after the post-conditioning CO_2 challenge. Nevertheless, these expectations did not translate into a placebo effect at the group level. If there were placebo effects present, then the size of these effects were small[282]. *Post-hoc* comparisons revealed that the between-group effect size on all outcome measures ranged from d = 0.02 to 0.37. In contrast, standard anxiolytic treatments show large effects on measures of anxiety during 7.5% CO_2 inhalation in healthy volunteers. Seven days of treatment with lorazepam reduces fearfulness with an effect size of d = 0.81[195], 21 days of paroxetine treatment reduces nervousness with d = 0.92[195], and mindfulness techniques reduce state anxiety with partial $\eta^2 = 0.26$[194]. As described in the power analysis for this study (see section 5.2.5), previous experimental placebo conditioning paradigms demonstrate large placebo effects[75,155,184]. The placebo effects seen in the current study are unlikely to be meaningful or relevant when compared with placebo effects seen in other conditioning paradigms and with standard anxiolytic treatments. However, an exploratory analysis showed a trend-level negative relationship between expectations of benefit following conditioning and change in reactivity to CO_2 challenge in the 'lorazepam' group in subjective outcome measures only. The correlation coefficients suggested these were weak to moderate relationships[282,283]. Visual inspection of the graphs shows some participants, including some with high expectations, experienced an unexpected nocebo effect (worsening of anxiety following conditioning, see Figure 5.7). This is in contrast with studies of placebo analgesia using established paradigms, in which nearly all participants show improvements following conditioning (e.g.[109,148,284]). The result is a small average effect compared with control in the current study. The reasons for these unexpected nocebo responses could be the study design, the choice of anxiogenic stimulus, or these could possibly be consistent with a Bayesian predictive coding model of placebo effects. I discuss each in turn below.

5.4.1 Study design

Only one previous paradigm has been developed with the explicit aim of inducing placebo anxiolysis in healthy volunteers. Using an unpredictable threat of shock as the anxiogenic stimulus, a placebo accompanied by verbal suggestions it was an anxiolytic drug (either 'intranasal lorazepam' or 'laughing gas') reduced subjective fear and skin conductance responses in three

related studies, regardless of threat[158,159]. Since the effect was not specific to threat trials, it is possible that the results represent another phenomenon, such as reduced attention, rather than reduced anxiety. Relatedly, placebo effects on 'unpleasantness' of distressing images has been investigated in two studies[155,156]. Although 'unpleasantness' is a vague construct, it might have overlaps with anxiety or feelings of threat. In the first of these studies, placebo effects were seen following a conditioning procedure involving active medications (midazolam and flumazenil) on day 1, and then a testing session on day 2 with saline only[155]. In the second, an open-label placebo nasal spray reduced subjective distress in response to negative images but not neutral images in comparison with a control group[156].

The above studies share design features that differ from mine. First, in all the above studies, experimenters were unblinded and either involved in the deception of the participant or gave the placebo open-label. By contrast, in the current study the experimenter was blinded to the participant's treatment assignment. This should reduce the chance of responder bias influencing the results[163]. However, it should be noted that in the non-deceptive placebo study described above, effects were seen on the late positive potential (LPP, a neural biomarker of distress) even when participants were not asked to give feedback about their subjective feelings[156]. This suggests that perhaps responder bias perhaps plays less of a role in placebo effects than previously thought.

The other feature many of these studies share is a within-subjects design, in which the placebo condition was compared with a control condition in the same individual[155,158,159]. A within-subjects design can reduce the effect of inter-individual variability or confounders and increase signal to noise ratio[285]. The current study, by contrast, utilised a between-subjects design, in which a placebo group ('lorazepam') was compared with a control group ('saline'). Increased noise, for example due to factors other than placebo mechanisms influencing CO_2 reactivity, might have obscured the signal of the placebo effect. However, both groups were well balanced for potential confounders. Furthermore, between-group designs have been employed in many experimental placebo studies that demonstrated significant placebo effects[82,84,91,92,94,95,108,152,156].

Finally, the duration of the aversive stimulus was shorter, and outcomes were measured at multiple timepoints in the above studies. For example, in the threat of shock paradigm[158,159], the placebo was administered before each run of trials, each lasting 2-3 minutes, with a total of 12 runs. In the 2005 study of placebo effects on 'unpleasantness', the placebo and control were administered 3 times each, and ratings of 'unpleasantness' were taken after each block of images lasting approximately 5 minutes[155]. In the 2020 open-label placebo study, unpleasant images were presented for approximately 12 minutes, and ratings of 'unpleasantness' were taken after each

image (40 times)[156]. Outcomes are generally measured multiple times in the experimental placebo analgesia literature. In a paradigm similar to that employed in the current study, 10-15 moderately painful thermal laser stimuli were applied post-conditioning, with pain ratings taken after each stimulus[84,108]. By contrast, in the current study outcome measures were taken at a single timepoint post-conditioning after a relatively long duration aversive stimulus (20 minutes of 7.5% CO_2 inhalation). Both pain and anxiety are subject to the peak-end rule, which states that participants are inclined to recall events mostly by how they were perceived at the experience's peak and at its conclusion[286,287]. The single *post-hoc* measure used here might lack the sensitivity to detect early placebo effects as a result. Additionally, repeated applications of a successful placebo would theoretically enhance its effects by reinforcing expectations and conditioning[76]. Despite this, at least one study has shown that placebo effects can be induced on tonic pain (cold pressor test), and the onset of these effects corresponds with instructions about the expected duration of action of the placebo[284]. Perhaps more explicit instructions in the post-conditioning phase regarding expected effects of the sham 'lorazepam' would have enhanced its effects in the current study.

5.4.2 Choice of anxiogenic stimulus

Another possibility to consider is that 7.5% CO_2 inhalation is not conducive to inducing placebo effects. Inhalation of air 'enriched' with CO_2 is known to affect psychological, physiological and cognitive systems, inducing subjective feelings of anxiety, physical changes such as increased heart rate, blood pressure and skin conductance, and altering cognitive biases toward threatening information[39,192,193,288]. Results from a recent study suggest that the cognitive effects of CO_2 inhalation extend to frontal executive functions. Healthy participants completed an intra-extradimensional set shift (IDED) task and a spatial working memory task while undergoing 7.5% CO_2 challenge. Compared with air inhalation, participants made more extra-dimensional set shift errors in the IDED task, suggesting reduced cognitive flexibility, and more total errors (with the most at the hardest level) in the spatial working memory task[289]. This pattern of errors is consistent with reduced prefrontal cortical function: a similar profile is seen in patients with frontal lobe injury[290]. This is important as it is known that successful induction of placebo effects requires intact frontal cortical activity. For example, in patients with Alzheimer's disease, reduced Frontal Assessment Battery scores and reduced functional connectivity of the prefrontal lobes with the rest of the brain are associated with reductions in the placebo component of analgesia[291]. Additionally, when repetitive transcranial magnetic stimulation is used to transiently disrupt bilateral DLPFC function, placebo analgesia is completely blocked in healthy volunteers[292].

It is possible that prefrontal cortical disruption as a result of 7.5% CO_2 inhalation attenuated the placebo effect in the current study. Further studies are needed to understand this.

5.4.3 Expectations not realised: a possible explanation via Bayesian integration theory

I additionally found a non-significant trend association between increased expectations post-conditioning and reduced sensitivity to CO_2 challenge in subjective anxiety and mood measures only. A number of participants exhibited reasonably high expectation of benefit but did not experience a reduction in CO_2 reactivity, instead showing no change or an increase. These findings can potentially be explained through a Bayesian predictive coding framework, which has been offered as a potential model for understanding placebo effects[293].

In this framework, it is thought that predictions about sensory stimuli (the 'prior') are compared with noisy ascending sensory signals ('observation') and a decision is made to reconcile these that results in a perception (the 'posterior')[293,294]. This can be applied to a placebo experiment as follows. Previous experience and conditioning are combined to form a prior (in this case an expectation). If a subsequent stimulus does not match the prior this creates a 'prediction error'. The system operates to minimise prediction errors, and so a Bayesian statistical inference follows, where the outcome is inferred to be somewhere between the prior prediction and the stimulus intensity. The difference between this posterior and the actual stimulus intensity represents the placebo effect[293,294]. Crucially, in this framework, the prior expectations and ascending sensory signals are represented as probability density functions: meaning some level of certainty is encoded[293]. This is important, as it suggests that the 'weighting' given to prior expectations or to ascending sensory evidence is determined by the relative certainty of each[293,295]. For example, if the prior probability is uncertain compared with incoming sensory information, then the posterior will be more closely aligned with the more certain incoming sensory information. However, if the incoming sensory information is highly variable, then the posterior might be more closely aligned with expectations. In other words, the placebo effect could be considered as a type of 'perceptual illusion', like optical illusions[294]. Indeed, this framework has been demonstrated to accurately model the results of placebo conditioning behaviourally[294] and neural signals in the peri-aqueductal grey matter[295].

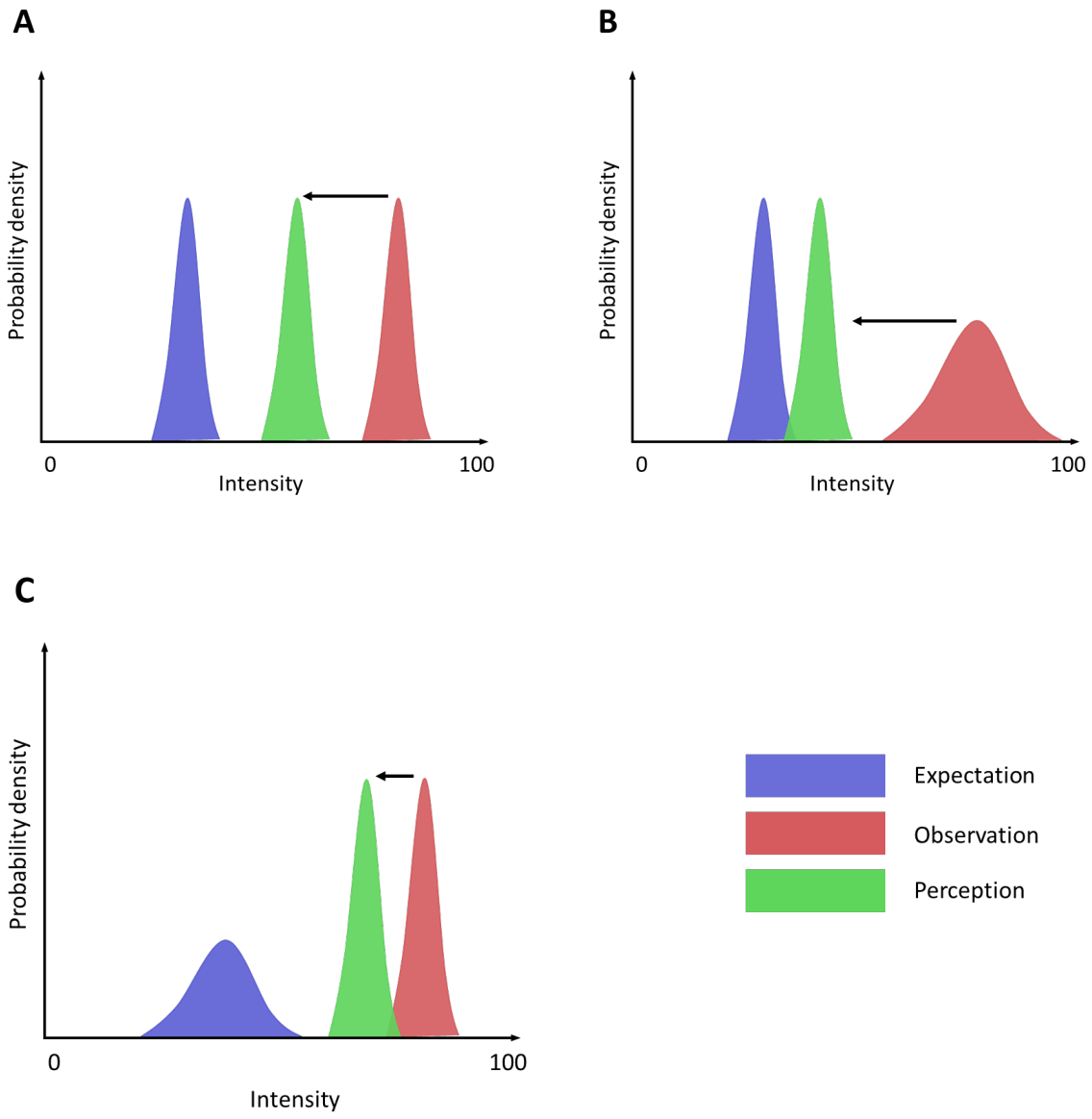


Figure 5.8 Cartoon demonstrating key concepts underpinning the Bayesian predictive coding framework of placebo effects. The 'prior' distribution (expectations) is compared with incoming sensory information (observation) and a decision is made that reconciles the two (perception) (A). Where incoming sensory information is noisy, then greater weight is given to the prior (B). However, if there is uncertainty regarding expectations for future stimuli, then greater weight is given to incoming sensory information (C).

The concepts described by the Bayesian predictive coding framework might explain the current results in two ways. First, this framework suggests that placebo effects are more pronounced when expectations are highly certain and when incoming sensory information is highly variable[293,295]. The effect of expectation certainty is evidenced by the finding that four conditioning sessions leads to more pronounced placebo effects than one conditioning session in healthy volunteers[148], as presumably more information leads to greater certainty in the predictive model. In the current study, I only carried out a single conditioning session. Mean expectancy post-conditioning in the 'lorazepam' group was 5.21 with the highest expectancy rating being 8.10 (maximum possible 10). This suggests that the group as a whole were only moderately certain about their beliefs. In addition, the variability regarding sensory information during CO_2 challenge is unknown. However, given that even 2 minutes of inhalation of 7.5% CO_2 can induce significant anxiety[288], and that test-retest reliability is high[296], it is likely the variability of this stimulus is low. Continuous subjective and autonomic measures of anxiety during CO_2 challenge would allow measurement of this variability directly. In sum, the paradigm as designed could have led to low certainty regarding the anxiolytic effect of the placebo and high certainty regarding anxiogenic effects of CO_2 challenge: a situation theorised to minimise placebo effects.

Second, if incoming sensory information is 'too different' from the prior model then it is possible this model will be abandoned[293]. The conditioning procedure in this study used a low intensity stimulus (air inhalation). In the subsequent testing phase participants again experienced a stimulus of high intensity (7.5% CO_2). This might have led participants to question whether a medication had been given at all. Against this argument is the post-experiment measure of beliefs, which suggests the 'lorazepam' group continued to hold positive expectations. Nevertheless, I did not ask participants whether they suspected any deception during or after the study. It is therefore unclear whether such shifts in explanatory models could have attenuated the placebo effect.

Although not sufficient to induce placebo effects at a group level, there was a trend between post-conditioning expectation and CO_2 challenge reactivity in subjective anxiety and mood measures only. This is a feature of placebo effects. For example, in patients with asthma, placebo treatment does not affect objective measures of respiratory function but does affect subjective reports of symptoms compared with no treatment[297]. Similarly, mindfulness techniques significantly reduce subjective anxiety during CO_2 challenge, but do not appear to affect autonomic measures in healthy volunteers[194]. This suggests there might be overlap between mechanisms mediating the effects of expectations and of mindfulness on CO_2-induced anxiety. Indeed, open monitoring was the most efficacious mindfulness technique, which involves active

reappraisal of incoming information[194], and functional imaging suggests overlap between regions involved in emotional reappraisal and placebo analgesia[298]. Given that this trend was seen, consideration should be given to measuring expectations and including these as a covariate in analyses of CO_2 challenge studies in future.

5.4.4 Limitations

This study had some limitations, most of which follow from the discussion above. First, the sample size of 32 was relatively small. Although this sample size should have been large enough to detect effect sizes seen in the experimental placebo literature, the small numbers precluded any exploratory analyses of demographic or personality trait predictors of CO_2 challenge outcome or of placebo effects. The study is also underpowered to detect smaller placebo effects, although it is unclear how relevant smaller effects would be. Further to this, there were more females than males in the study; however, proportions of females to males were similar in both groups minimising the risk of confounding. Second, there is no clear definition of what a 'treatment responder' is in the 7.5% CO_2 inhalational model of generalised anxiety. As a result, I could not assess whether there were different numbers of 'treatment responders' in each group, which could have been another way to assess for placebo effects. This approach has not been taken in other experimental placebo studies, so such an approach would not be consistent with the literature. Third, anxiety and mood was measured through a single questionnaire at the end of each inhalation. A continuous measure would have allowed quantification of the variation in subjective anxiety during a CO_2 challenge and might have revealed interesting expectancy effects. Finally, I did not collect subjective reports of how the participants experienced the placebo conditioning procedure. Reports regarding whether they suspected deception, and when, would have given valuable insights when appraising expectancy ratings and the post-experiment therapeutic credibility questionnaire.

5.5 Concluding comments

In summary, the 7.5% CO_2 inhalational model of generalised anxiety disorder does not appear conducive to inducing placebo effects. On average, both the placebo and control groups exhibited similar subjective and autonomic responses to CO_2 challenge pre- and post-conditioning. The effect size of placebo was small, smaller than the effect size of active pharmacological and psychological treatments. However, there was a trend association between expectation and change in reactivity to CO_2 challenge post-conditioning. Furthermore, there was an unexpected group of participants that appeared to exhibit nocebo responses, despite positive expectations. It is possible that aspects of the study design, the choice of anxiogenic stimulus, or behaviour in line

with a Bayesian predictive coding framework attenuated the effect of expectations on subsequent placebo response. Future studies should explore these possibilities.

Although placebo effects are maximal when verbal suggestions and learning are combined[76,92,111,112], placebo effects on emotions have been induced through verbal suggestions alone[95,156,157]. In the next chapter, I describe a second novel paradigm in which I explore whether placebo anxiolysis can be induced remotely through verbal suggestion alone in a behavioural anxiety challenge model.

Chapter 6 Placebo Response in a Modified Trier Social Stress Test

6.1 Background

As discussed in Chapter 5, few studies have explored placebo effects in experimentally-induced, clinically-relevant affective states[95,152,154-156,158,159]. Further, findings from these studies have been influenced by confounders (see section 1.5). There is a need for an experimental paradigm that both reliably and reproducibly elicits a placebo response and is clinically relevant. In Chapter 5, I tested a novel conditioning procedure designed to elicit placebo anxiolysis in the 7.5% CO_2 inhalational model of generalised anxiety. Although the conditioning procedure appeared to enhance expectations, this did not translate into placebo anxiolysis at the group level. This could be due to features of the CO_2-enriched air that mean it is not conducive to eliciting placebo effects. For example, inhalation of CO_2-enriched air might negatively impact prefrontal cortical function[289], which is necessary for producing placebo effects[291,292].

A number of paradigms exist for experimental induction of acute stress and anxiety (see Chapter 2). The Trier social stress test (TSST) is one of the most reliable, and does not involve a pharmacological or physiological challenge that could confound a placebo treatment[39,40,196]. Measures of stress and anxiety in the TSST are also ameliorated by standard anxiolytic treatments, e.g. the benzodiazepine alprazolam[197], and by experimental treatments, e.g. buprenorphine[198]. This suggests that the TSST has good construct validity for anxiety symptoms. The TSST has been used as an experimental model of social anxiety disorder (SAD)[39,40], however, there are some issues when the TSST is used for this purpose.

6.1.1 Rationale for a modified Trier social stress test

The original TSST was developed in 1993 by Kirschbaum and colleagues[299]. In this version, participants are asked to imagine they have applied for their dream job. They are then told they will shortly attend an interview for this position, and they need to convince three managers that they are the perfect applicant. Participants are given 10 minutes to prepare and then they give a 5-minute unstructured speech in front of 3 judges. The judges are briefed not to react. After 5 minutes the participant is asked to perform a surprise mental arithmetic task: a serial subtraction of 13 from 1022 for 5 minutes. Each time the participant makes an error they are asked to start again.

The emergence of SARS-Cov-2 and the subsequent Covid-19 pandemic meant it would not be feasible to run the TSST as described above. Due to social distancing requirements, it would not be possible to have four people in the same room. Further, all persons would need to wear facemasks, which could potentially be a confounder. Finally, the lack of social distancing might in itself be stressful in the context of a pandemic. I instead decided to modify the task for use online in a videoconferencing platform. While performing this modification I discovered other confounders in the original TSST that I could attempt to address, including:

- the test is designed to induce *stress* rather than social anxiety *symptoms*
- it is complex to set up
- it requires a large number of people to be available at the same time
- performance of confederates cannot be easily controlled for[300]
- the situation is extreme meaning its ecological validity for day-to-day experiences in patients with SAD is unclear

6.1.2 The InterneT-based Stress test for Social Anxiety Disorder (ITSSAD)

To address the issues detailed above, I created a modified version of the TSST that can be delivered via videoconferencing online: the InterneT-based Stress test for Social Anxiety Disorder (ITSSAD)[301]. I designed the ITSSAD to produce anxiety in participants through anticipation and then experience of a naturalistic "getting to know another" social interaction. Previous research has shown that similar social interaction tasks can induce significant anxiety in patients with SAD[302], and significant physiological arousal in healthy participants with high levels of social anxiety, with no difference between those completing the task face to face or via computer text chat[303]. In a recently published paper, I have shown that the anticipatory period and social interaction in the ITSSAD significantly increase anxiety and negative affect, and significantly reduce positive affect in volunteers with sub-clinical to clinical social anxiety symptoms[301]. Further, subjective anxiety following the social interaction is associated with burden of social anxiety symptoms, and not trait generalised anxiety, suggesting this paradigm has ecological validity for SAD[301] (see accompanying documents).

6.1.3 Aim of the current study

In the current study, I tested whether the ITSSAD paradigm could be used to induce placebo anxiolysis remotely in non-treatment-seeking volunteers with social anxiety symptoms. My hypothesis was that exposure to a 'sham' psychological treatment when accompanied by verbal

suggestions of 'feelings of relaxation' would reduce subjective anxiety following anticipation and experience of an online social interaction.

6.2 Methods

6.2.1 Ethics statement

This study was reviewed and approved by the Ethics and Research Governance Office at the University of Southampton (reference: 61411). Prior to starting the study, participants were informed that the aim was to explore social anxiety symptoms during videoconferencing. Information regarding aims to study the placebo effect was withheld initially; however, on completing the study participants were fully debriefed and fully informed consent was sought a second time for use of their data. Participants were informed that they could decline to consent and their data would be destroyed. No participants withdrew their consent.

6.2.2 ITSSAD procedure

The protocol for the ITSSAD is available online (https://www.researchsquare.com/article/pex-1649/latest)[304]. The paradigm begins with a 5-minute anticipation period. During this period, participants are shown the following instructions:

"In 5 minutes you will take part in a social interaction online using videoconferencing software. Your task will be to take some time to get to know the other person as you normally would. Just be yourself. You can talk about anything you want other than this experiment. You will be watched by 3 other experimenters who will be assessing your behaviour. We would like you to have your camera on during this interaction."

Following this period, participants enter a videoconference on Microsoft Teams (https://teams.microsoft.com)[305]. In the videoconference is an experimenter, and 3 other attendees: the 'judging panel' (Figure 6.1). The experimenter introduces themselves as the person the participant is tasked with 'getting to know'. They also describe the judging panel as a group of 'experts' who are there to monitor the actions of the participant and who will keep their cameras and microphones off for the duration of the social interaction. In truth, the judging panel are 'dummy' accounts that the experimenter signs into on several other devices/browser windows and sets to mute. In a previous study of healthy male volunteers, there was no significant difference in physiological stress between those who completed the task in front of a visible panel, and those who completed the task while the panel was behind a one-way mirror[306]. This shows that the *idea* of a panel is sufficient to create a social-evaluative context and consequent

anxiety. In this way, it was possible to maintain a social-evaluative context with a single experimenter[301]. Further, due to research showing that a panel with a mix of genders causes more stress than a single-gendered panel[307], the dummy accounts are named such that there are two apparently male and two apparently female 'experimenters' on the call.

The experimenter then asks the participant to begin the social interaction task and starts a 5-minute timer. To further reinforce social-evaluative threat, the experimenter also informs the participant that the interaction will be recorded for later review. The experimenter is briefed not to initiate conversation, instead allowing the participant to do so. If any silence lasts more than 30 seconds, then the experimenter can prompt the participant with a series of short, scripted statements, e.g. "I have a sister", or "I enjoy reading". The experimenter is also instructed to give minimal feedback and to give succinct verbal responses to questions posed by the participant, similar to the judging panel in the original TSST[196,299].

In this study, on completion of the 5-minute task and after completing outcome measures, participants took part in a coached mindfulness exercise. Mindfulness strategies reduce post-event processing in SAD, which is thought to be a maintenance factor in the disorder[308,309]. I therefore included this as a 'mood repair' for ethical reasons, in an attempt to prevent ongoing distress as a result of the ITSSAD task.

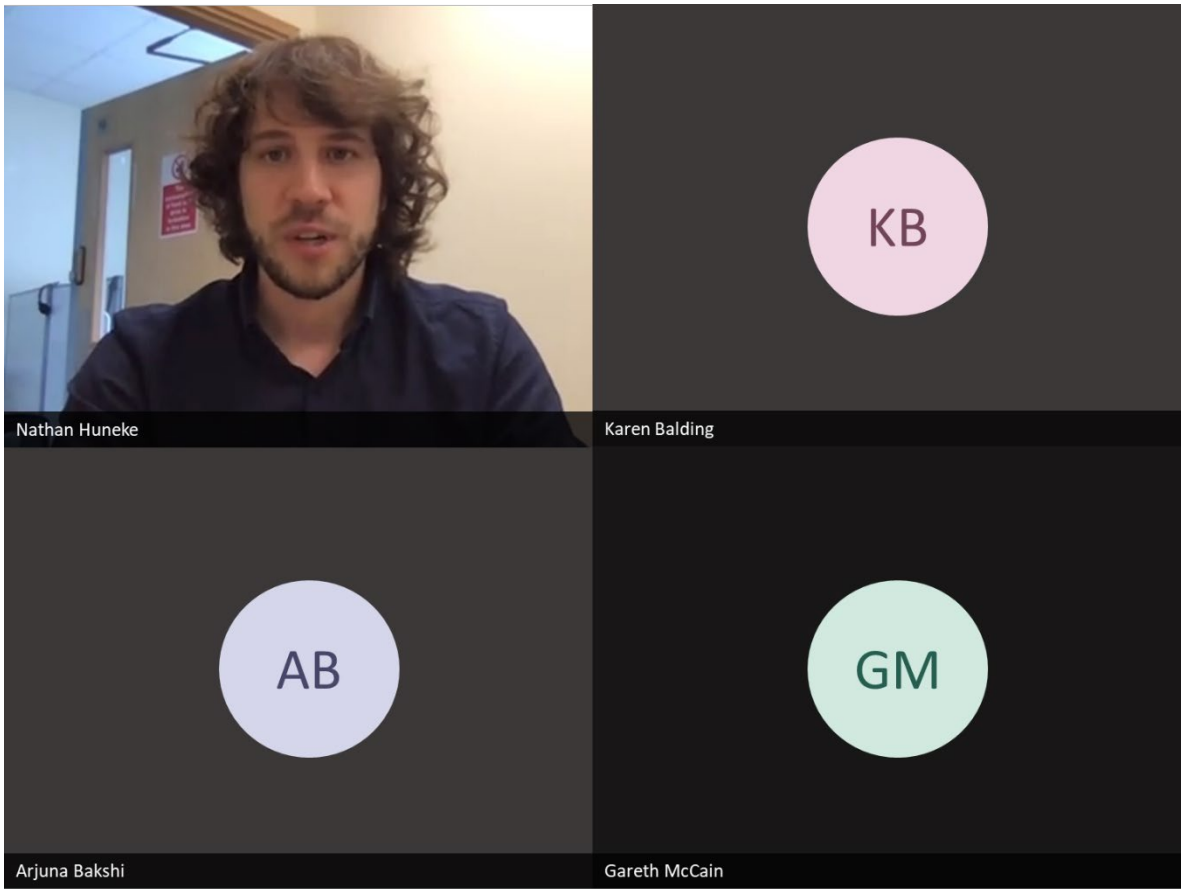


Figure 6.1 A mock-up of a videoconference in the ITSSAD as viewed by the participant. The experimenter and three ‘dummy’ accounts, introduced as a judging panel, are signed into the call.

6.2.3 Participants

Participants were recruited from the community via a mixture of poster, online and social media adverts. Participants who were psychology students at the University of Southampton were offered course credits for taking part, otherwise no incentives were offered for other participants. Inclusion criteria were age 18-45 years and presence of sub-clinical to clinical social anxiety symptoms. Social anxiety symptoms were assessed through the social phobia inventory (SPIN): a validated 17-item self-rated questionnaire[310]. Participants with a SPIN of greater than 14 were included, as this cut-off can differentiate between those with no social anxiety symptoms and those with social anxiety of varying intensity. Participants were excluded if they: had any current psychiatric disorder other than social phobia; any history of psychosis or bipolar affective disorder; any significant physical illness; any change in treatment (either medical or psychological) in the preceding 8 weeks; or regularly used illicit substances. Findings from 20 of these participants have already been reported to demonstrate proof-of-concept of the ITSSAD paradigm[301] (see accompanying documents).

6.2.4 Experimental placebo procedure

6.2.4.1 The sham treatment

Placebo effects are maximal when verbal suggestions and learning are combined[76,92,111,112]. A challenge with including a sham treatment in this study was that the ITSSAD is carried out remotely. A further challenge was that the ITSSAD was a new task, and so the effect of repeated exposure to the task was unknown, and potentially a large confounder when attempting to develop a conditioning manipulation. Previous placebo studies have shown that verbal suggestions alone can induce significant placebo effects[95,106,151,154]. In a particularly relevant group of studies, an attempt was made to create a placebo psychotherapy[311]. In these studies, participants were shown an abstract video, such as a green circle moving across a screen. Prior to viewing the video, some participants received a psychological rationale for why this video should alter their emotional state. This included suggestions that the video caused 'activation of early conditioned emotional schemata through the colour green'[311]. Participants who received this rationale exhibited significantly better mood and increased feelings of calmness compared with a control group who viewed the video with no suggestive rationale[311]. This demonstrates that verbal suggestions paired with an abstract stimulus can generate placebo effects on emotional state. For this study, I created a 'sham' placebo therapy by adapting a stock video (https://pixabay.com/videos/waves-smooth-gold-abstract-12916/) into a 5-minute abstract

animation showing a gold and black moving pattern (Figure 6.2). I used this ‘sham’ therapy to attempt to induce a placebo effect remotely via a webpage.

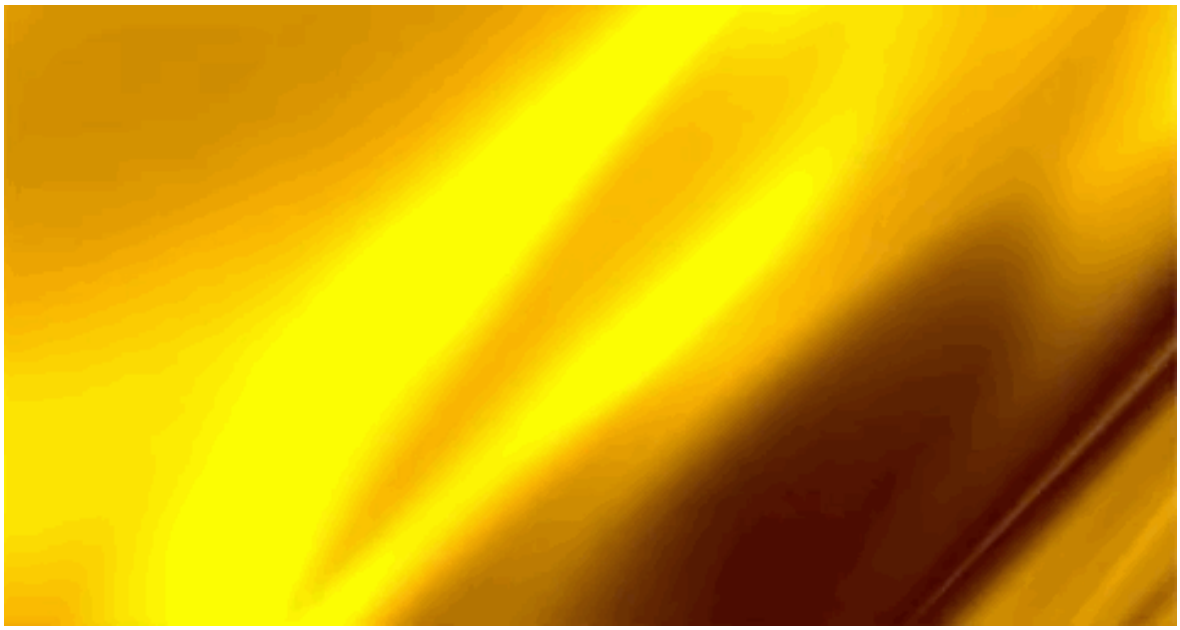


Figure 6.2 Abstract black and gold pattern shown to participants as a placebo treatment. Adapted from (https://pixabay.com/videos/waves-smooth-gold-abstract-12916/).

6.2.4.2 Study design

This was a parallel group randomised study assessing the effect of the 'sham' treatment (abstract video) given with a psychobiological rationale for improved anxiety compared with no rationale. The study design is summarised in Figure 6.3. I created a screening questionnaire and test session using the Qualtrics XM online survey software (https://www.qualtrics.com)[312]. Following screening, eligible participants were invited to attend a scheduled online test session. At the pre-arranged time, participants were sent a personalised link to join the test session. The test session began with baseline questionnaires (see section 6.2.5.1). After completing these measures participants underwent the placebo intervention.

Participants were randomised to one of two groups: placebo with psychobiological rationale for reduced anxiety (placebo group) or placebo with no rationale (control group). The randomisation sequence was generated by the Qualtrics software and applied at the relevant point in the survey flow. As a result, all experimenters were blind to allocation sequence. Participants randomised to the placebo group viewed a video of myself explaining that they were about to watch a video that is known to 'stimulate areas of the brain associated with feelings of relaxation and reductions in anxiety'. They were then told that we wanted to test whether this abstract video could reduce anxiety during a subsequent social interaction. Participants were asked to fully engage with the 'treatment' to derive the most benefit. In contrast, participants in the control group were told that they were in the control group, and that participants randomised to the other group were receiving an experimental intervention. They were then told they were about to view an abstract video as a placeholder to ensure the experiment was the same duration for both groups. The abstract animation depicted in Figure 6.2 was then shown on the screen for 5 minutes to all participants. See Appendix F for the full treatment instructions for both groups.

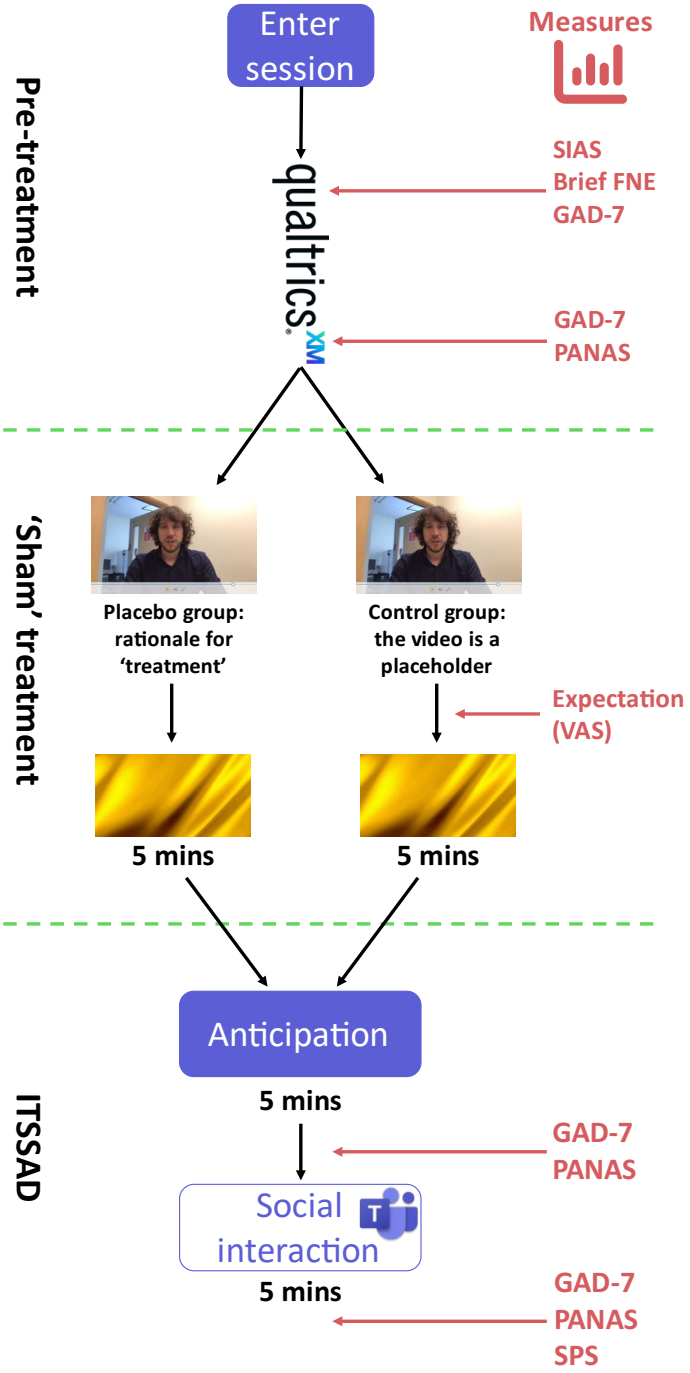


Figure 6.3 Schematic of the placebo anxiolysis paradigm. After baseline measures (pre-treatment), participants were randomised to either placebo (with treatment rationale) or control (video as a placeholder) groups. After viewing the abstract video for 5 minutes, participants underwent the ITSSAD.

Abbreviations: SIAS, social interaction anxiety scale; Brief FNE, brief fear of negative evaluation scale; GAD-7, generalised anxiety disorder 7-item; PANAS, positive and negative affect schedule; VAS, visual analogue scale; ITSSAD, InterneT-based Stress test for Social Anxiety Disorder; SPS, subjective perception of speech performance scale.

Following the placebo intervention, participants underwent the ITSSAD as described above. Since the participants had already seen a video that included me, my presence could have been a confounder during the social interaction. Therefore, an independent experimenter conducted the videoconference. To eliminate bias in the way the videoconference was executed, I blinded the experimenter to treatment assignment.

6.2.5 Measures

6.2.5.1 Baseline measures

To check whether groups were balanced for potential confounding trait characteristics, participants completed the following questionnaires to assess baseline anxiety and personality:

- Social Interaction Anxiety Scale (SIAS)[313]: A validated 20-item self-report questionnaire assessing anxiety of general social interaction.
- Brief Fear of Negative Evaluation Scale (Brief FNE)[314]: A validated 12-item self-report questionnaire assessing anxiety regarding evaluation by others.
- A modified version of the generalised anxiety disorder 7-item (GAD-7)[274], where each question was represented by a visual analogue scale ranging from *"not at all"* to *"nearly every day"*. This version of the GAD-7 has been shown to be sensitive to state changes in anxiety with high resolution[192,262,263]. Further, the GAD-7 questionnaire also captures social anxiety symptoms with good sensitivity[315].

6.2.5.2 Expectations

Expectations were assessed after receiving the rationale for the placebo (verbal suggestions) and before receiving the 'sham' treatment. Participants were asked to rate on a visual analogue scale (coded as 0 – 100) how effective they thought the video would be at reducing their anxiety. This scale ranged from *"not at all"* to *"very much"*.

6.2.5.3 ITSSAD outcome measures

Subjective anxiety and mood were measured at pre-treatment baseline, after the anticipatory period, and after the social interaction task. At all three timepoints, participants completed the modified GAD-7 with visual analogue scales as described above, with an additional instruction to consider their anxiety over the past 5 minutes. Additionally, participants completed the Positive and Negative Affect Schedule (PANAS)[280] as a measure of subjective mood at all three timepoints. Finally, after the social interaction task, participants also completed the subjective perception of

speech performance scale (SPS)[316]. This scale is a validated self-report questionnaire that allows measurement of subjective beliefs about performance during the social interaction.

6.2.6 Statistical analysis and power calculation

My outcome of interest was the change in subjective anxiety over the course of the experiment. I hypothesised that the placebo group would exhibit reduced anxiety following the anticipatory period and following the social interaction task compared with the control group.

Due to the novelty of the method, it was unknown what the effect size of the placebo in this paradigm might be. It is likely the effect size of a placebo manipulation based on verbal suggestion alone is lower than one that combines verbal suggestions and conditioning[112]. Therefore, the minimum meaningful effect to detect is likely to be lower than the effect sizes discussed in Chapter 5. In a previous study, placebo effects on anxiety were induced by verbal suggestion alone with an effect size of d = 0.91[159]. No other experimental placebo studies explicitly attempting to induce placebo anxiolysis have reported effect sizes for the placebo. However, in other relevant experimental placebo studies on emotion (e.g. sadness, unpleasantness), effect sizes ranged from d = 0.59 to 1.19, with only one study reporting an effect size less than d = 0.65[95,152,156,157,311]. I aimed for a minimum sample of 76 participants, which provides 80% power to detect d > 0.65 at an alpha of 0.05.

Statistical analyses were carried out using the afex, emmeans, and performance packages in R (https://CRAN.R-project.org/package=afex; https://CRAN.R-project.org/package=emmeans; https://CRAN.R-project.org/package=performance)[317-320]. Baseline characteristics were compared between groups with independent samples t-tests for continuous data or chi-squared tests for dichotomous data. Subjective perception of speech performance and expectations were assessed between groups through an independent samples t-test.

Change in anxiety and mood over time were assessed through linear mixed effects models. I chose to analyse the data through linear mixed effects modelling as this allows greater retention of data when repeated measures are unbalanced, e.g. due to dropouts during the study[205]. In this study, some participants dropped out before completing the social interaction task (see section 6.3.1). In a repeated measures ANOVA model, these participants' data would be excluded entirely. By contrast, in a linear mixed effects model these data points can be retained. In all models, time, group and their interaction were included as fixed effects, while participant was included as a random effect. The drivers of significant effects were assessed through *post-hoc* pairwise comparisons. Significance values for *post-hoc* comparisons were adjusted via the Tukey method.

I also fitted exploratory models that included expectation as an effect. The purpose was to assess whether participants' beliefs about the 'sham' treatment influenced subsequent anxiety. To do so, I fitted three models per outcome measure (modified GAD-7, PANAS positive and PANAS negative). All models included participant as a random effect, but fixed effects varied in complexity. The most complex model included all three-way interactions between time, group and expectation. The next most complex model included time*group and time*expectation interactions. Finally, the simplest model included no interaction terms: only time, group and expectation as fixed effects. I then compared each model to assess which explained the data best. Assessment criteria included change in Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), R^2, deviance, and the results of chi-squared tests assessing whether change in deviance was significant. I assessed the models from least complex to most complex: i.e. whether the addition of each interaction term provided significant improvement over the simpler model. If the more complex models provided significant improvement, this would suggest expectation might significantly influence the outcome at different timepoints, and this might differ between groups. Where the model containing interaction effects performed best, these interaction effects were further explored through *post-hoc* simple slope analysis to assess the covariate trend of expectation on the slope of outcome variable vs time. Significance values for *post-hoc* comparisons were adjusted via the Tukey method. Prior to fitting each model, expectation was centred on the mean to allow valid interpretation of interaction effects.

6.3 Results

6.3.1 Baseline Characteristics

I recruited 76 participants: 38 were randomised to placebo and 38 to the control group (Figure 6.4). Baseline characteristics for each group are summarised in Table 6.1. There were no significant differences between groups in baseline demographics or personality trait variables.

Two participants in the placebo group did not complete any outcome measures or the social interaction task and so could not be included in the analysis. One further participant dropped out before the anticipation period. In the control group, two participants withdrew before the anticipation period, one withdrew after the anticipation period, and one could not complete the social interaction task due to technical difficulties. Data recorded up until these participants withdrew were included in the linear mixed effects models reported below.

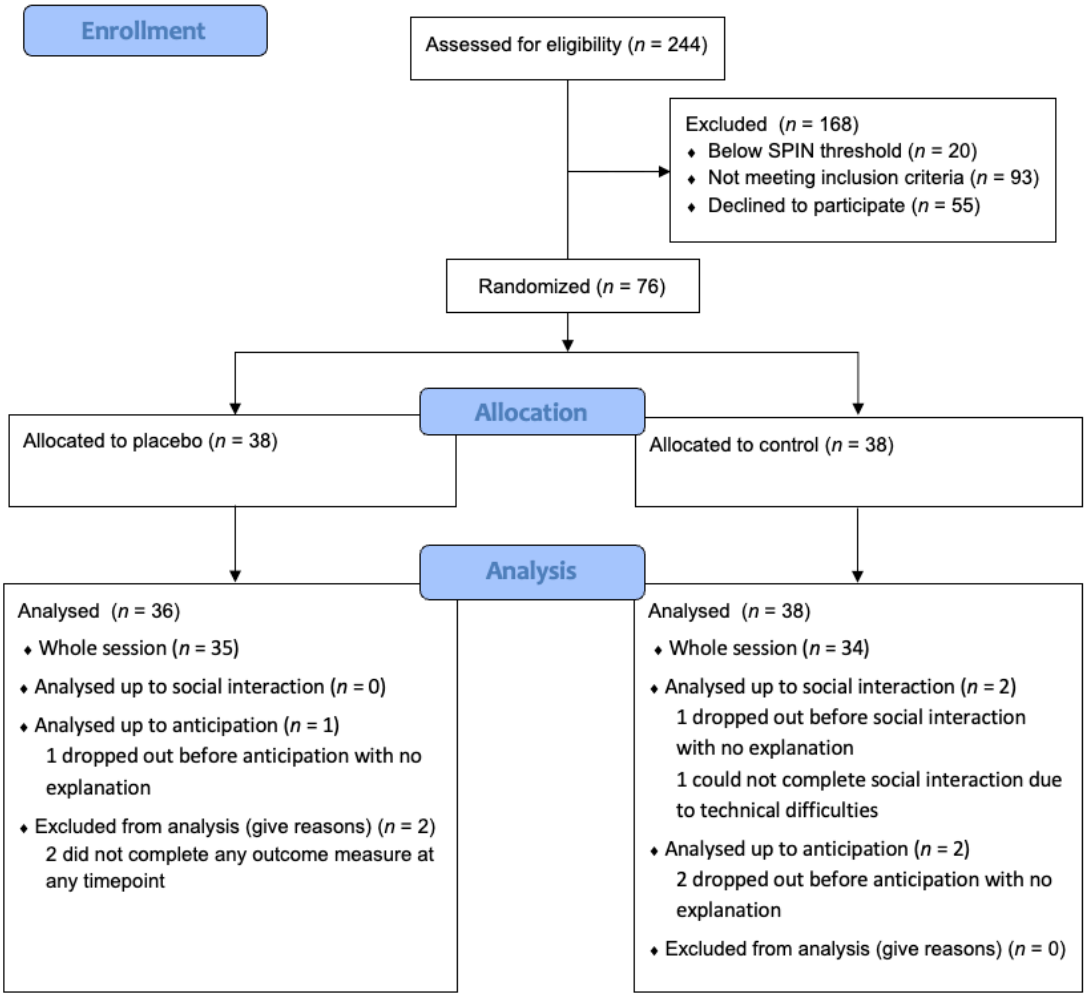


Figure 6.4 CONSORT flow diagram

Table 6.1 Baseline characteristics. Values are mean ± SD for continuous variables and count (%) for dichotomous variables.

	Placebo	**Control**	**Test statistic**	**p value**
N	38	38		
Age	19.05 ± 1.29	19.18 ± 1.61	$t_{(74)} = 0.39$	0.695
Females	34 (89.5%)	36 (94.7%)	Fisher's exact	0.674
SPIN	38.11 ± 12.56	37.61 ± 11.49	$t_{(74)} = 0.18$	0.857
Brief FNE	32.61 ± 5.94	34.18 ± 5.33	$t_{(72)} = 1.20$	0.234
SIAS	42.56 ± 12.94	41.55 ± 13.96	$t_{(72)} = 0.32$	0.750
Modified GAD-7	247.72 ± 133.64	259.39 ± 130.27	$t_{(72)} = 0.38$	0.705

Abbreviations: SPIN, Social Phobia Inventory; Brief FNE, Brief Fear of Negative Evaluation Scale; SIAS, Social Interaction Anxiety Scale; GAD-7, Generalised Anxiety Disorder 7-item.

6.3.2 Expectations

There was a significant difference in expectation between groups after receiving the verbal suggestions, with the placebo group reporting higher expectations (mean difference = 13.40, $t_{(72)}$ = 2.69, p = 0.009). However, it should be noted that although expectations were *statistically* different between groups, this was numerically small (13 out of 100), and expectations were generally low. Furthermore, there was one participant in the placebo group who reported a very high expectation in comparison to other participants. If the data are considered without this outlier then the distribution in both groups is similar, suggesting this datapoint might be skewing the results. See Figure 6.5.

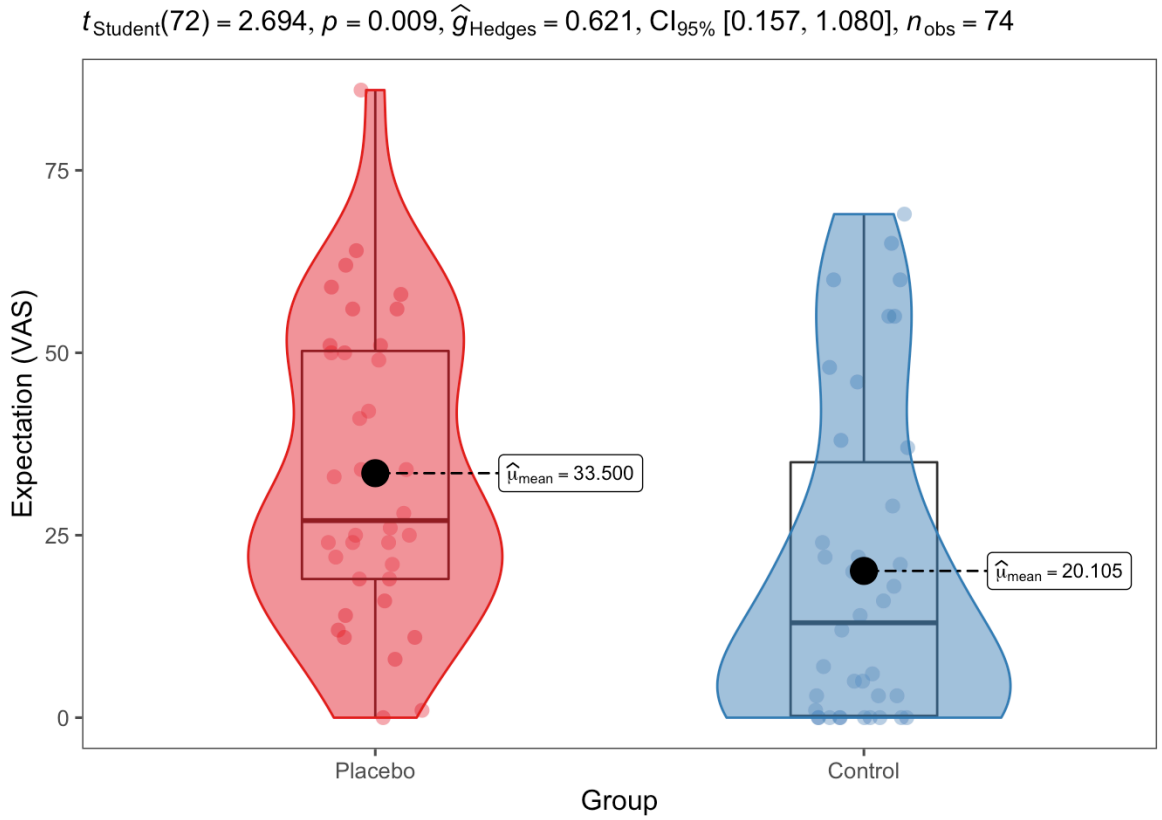


Figure 6.5 Violin and boxplots showing expectations in each group. Mean expectations were significantly higher in the placebo group.

6.3.3 ITSSAD outcome measures

Anxiety and mood measures were assessed through linear mixed effects models with time, group and time*group as fixed effects and participant as a random effect.

6.3.3.1 Anxiety

With modified GAD-7 as the dependent variable, the model's total explanatory power was substantial (conditional $R^2 = 0.59$) and the explanatory power of the fixed effects alone (marginal R^2) was 0.12. There was a significant effect of time ($F_{(2,138.15)} = 26.75$, $p < 0.001$), but no significant effect of group ($F_{(1,72)} = 1.43$, $p = 0.236$) or time*group interaction ($F_{(2,138.15)} = 0.70$, $p = 0.498$). The effect of time was driven by increased anxiety following the anticipatory period and following the social interaction task compared with pre-treatment in both groups (t's > 3.5, p_{tukey}'s ≤ 0.002). There were no significant differences between the groups at any timepoint. See Figure 6.6 and Table 6.2.

6.3.3.2 Mood

Separate models were calculated for positive affect and negative affect. For positive affect, the model's total explanatory power was substantial (conditional $R^2 = 0.67$) and the explanatory power of the fixed effects alone (marginal R^2) was 0.07. There was a significant effect of time ($F_{(2,135.63)} = 17.74$, $p < 0.001$), but no effect of group ($F_{(1,70.74)} = 1.24$, $p = 0.270$) or time*group interaction ($F_{(2,135.63)} = 0.19$, $p = 0.826$). In both groups positive affect decreased over the course of the experiment (t's > 2.86, p's ≤ 0.05). For negative affect, the model's explanatory power was also substantial (conditional $R^2 = 0.57$) and the explanatory power of the fixed effects alone (marginal R^2) was 0.06. There was a significant effect of time ($F_{(2,135.58)} = 15.72$, $p < 0.001$), but no effect of group ($F_{(1,70.03)} = 0.115$, $p = 0.735$) or time*group interaction ($F_{(2,135.58)} = 0.183$, $p = 0.833$). The effect of time was driven by a significant increase in negative affect from pre-treatment baseline to post-anticipation and remained elevated post-speaking task in both groups (t's > 2.9, p_{tukey}'s < 0.05). There were no differences between groups in either positive or negative affect at any timepoint.

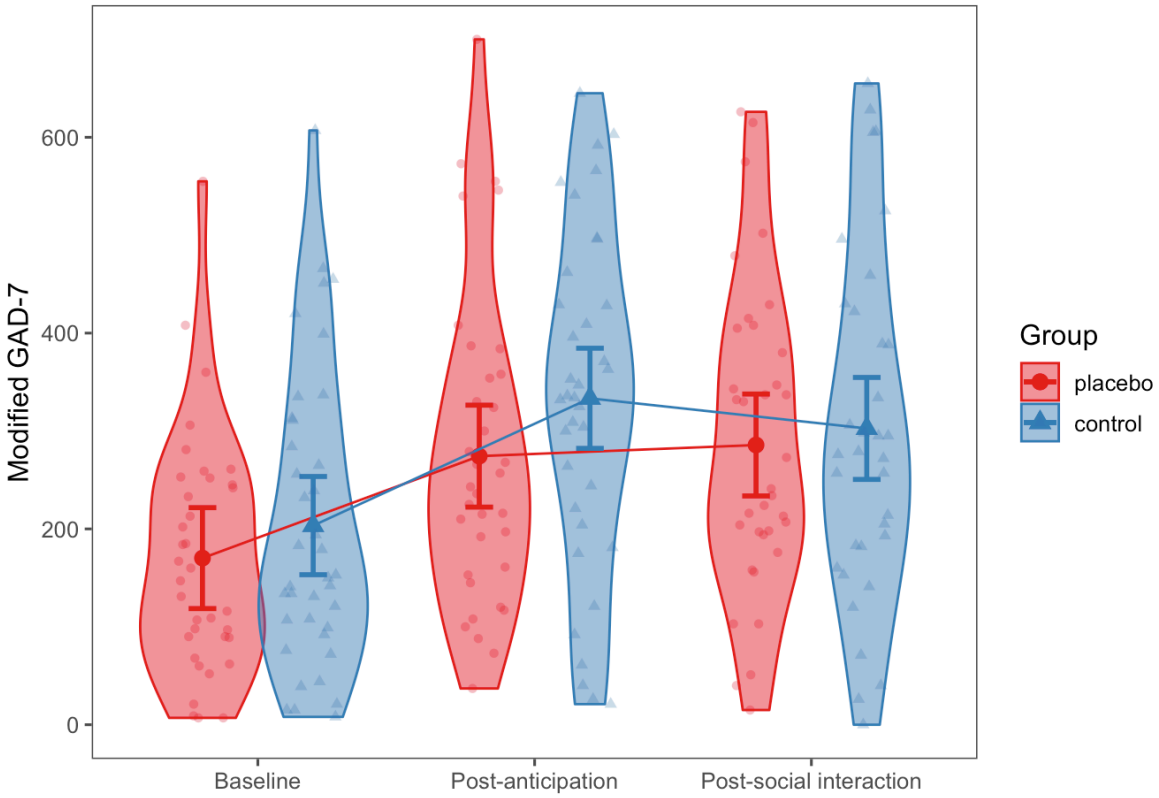


Figure 6.6 Estimated marginal means and 95% confidence intervals for modified GAD-7 scores over time in the placebo and control groups.

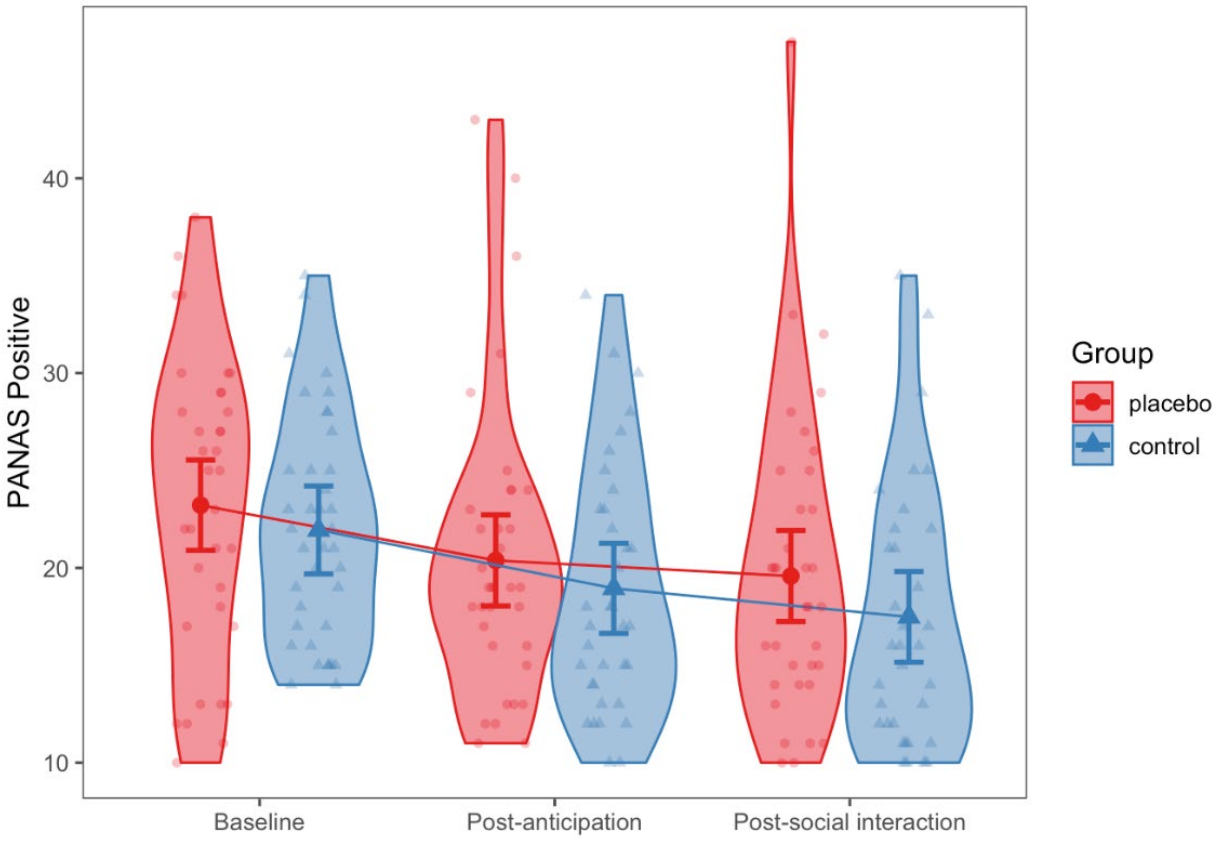


Figure 6.7 Estimated marginal means and 95% confidence intervals for PANAS positive affect scores over time in the placebo with and without expectation groups.

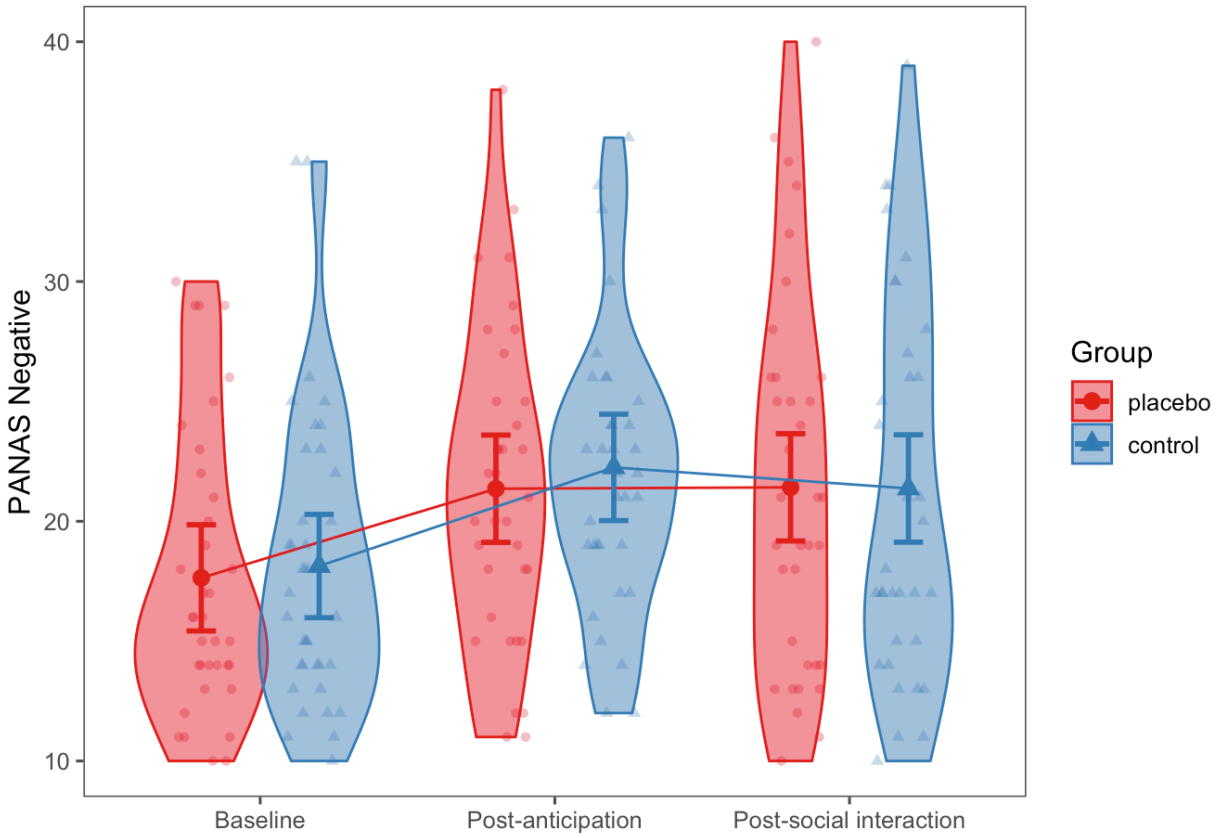


Figure 6.8 Estimated marginal means and 95% confidence intervals for PANAS negative affect scores over time in the placebo with and without expectation groups.

Table 6.2 Summary of post-hoc pairwise comparisons of ITSSAD outcomes. There were no significant differences between groups at any timepoint. Therefore, only changes within groups are presented. Values are estimated mean difference ± standard error.

		Placebo		Control	
	Comparison	**Estimate**	**Statistics**	**Estimate**	**Statistics**
Modified GAD-7	Post-anticipation – Pre-treatment	104.2 ± 25.3	$t_{(138)} = 4.11$, $p_{tukey} < 0.001$	130 ± 24.9	$t_{(139)} = 5.22$, $p_{tukey} < 0.001$
	Post-social interaction – Pre-treatment	115.5 ± 25.3	$t_{(138)} = 4.56$, $p_{tukey} < 0.001$	99.3 ± 25.4	$t_{(140)} = 3.91$, $p_{tukey} = 0.002$
PANAS Positive	Post-anticipation – Pre-treatment	-2.84 ± 0.99	$t_{(136)} = 2.86$, $p_{tukey} = 0.054$	-3.00 ± 0.99	$t_{(138)} = 3.03$, $p_{tukey} = 0.033$
	Post-social interaction – Pre-treatment	-3.64 ± 0.99	$t_{(136)} = 3.67$, $p_{tukey} = 0.005$	-4.46 ± 0.99	$t_{(139)} = 4.47$, $p_{tukey} < 0.001$
PANAS Negative	Post-anticipation – Pre-treatment	3.72 ± 1.09	$t_{(137)} = 3.41$, $p_{tukey} = 0.011$	4.12 ± 1.08	$t_{(139)} = 3.80$, $p_{tukey} = 0.003$
	Post-social interaction – Pre-treatment	3.78 ± 1.09	$t_{(137)} = 3.46$, $p_{tukey} = 0.009$	3.24 ± 1.09	$t_{(139)} = 2.96$, $p_{tukey} = 0.042$

Abbreviations: GAD-7, Generalised Anxiety Disorder 7-item; PANAS, Positive and Negative Affect Schedule

6.3.3.3 Subjective perception of speech performance

Subjective perception of performance during the social interaction task was assessed immediately after task completion. There was no significant difference in scores on this scale between the groups (mean difference = 0.576, $t_{(67)}$ = 0.225, p = 0.823).

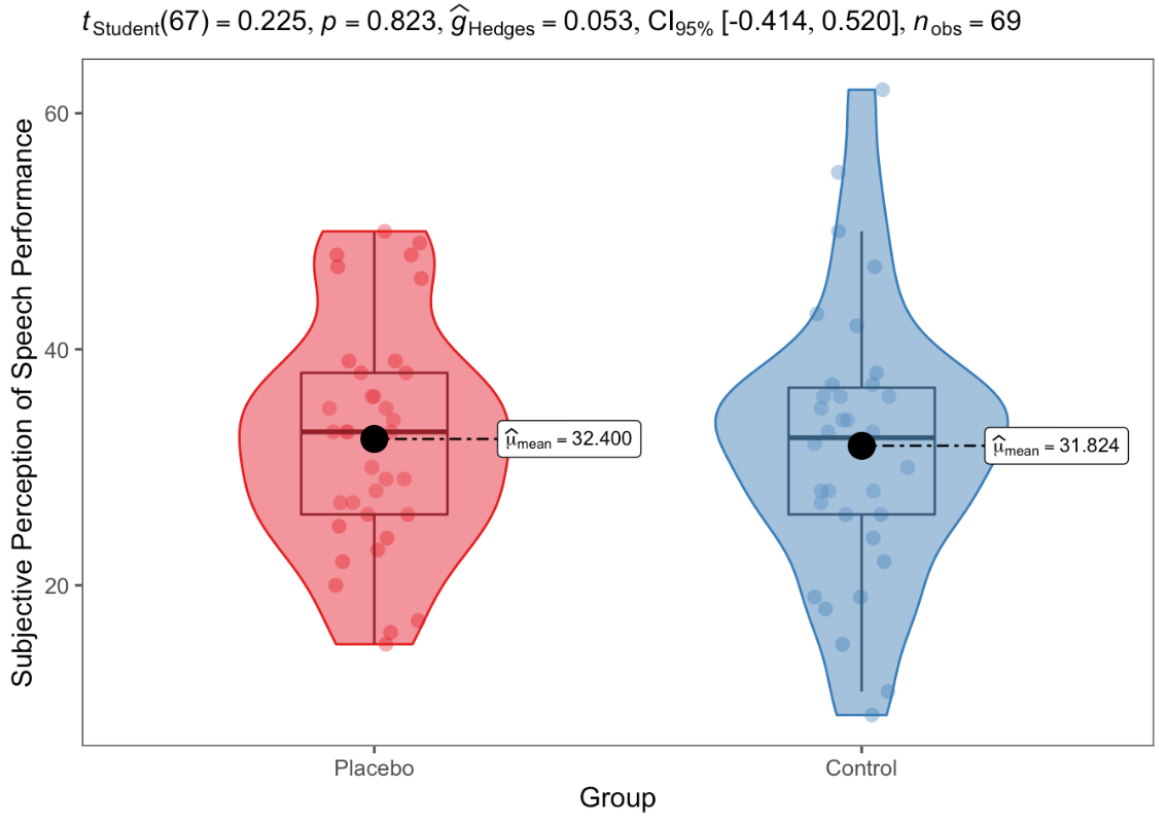


Figure 6.9 Violin and boxplots showing subjective perception of speech performance in each group. Higher values indicate better subjective performance. There was no significant difference between the groups.

6.3.4 Exploratory analysis

To explore whether expectations had any effect on ITSSAD outcomes, I fitted exploratory linear mixed effects models that included expectations as a fixed effect. For each outcome variable I calculated three models that included time, group and expectation as fixed effects and participant as a random effect with: all three-way interactions, two-way interactions, and no interactions. I compared each of these models to see which explained the data best (Table 6.3).

Table 6.3 Performance of exploratory linear mixed effects models to predict ITSSAD outcomes with expectations included as a predictor variable. Three models were compared for each outcome variable. Formulas for each model are included in the notes below. The winning model is highlighted in bold.

		AIC	BIC	R^2_c	R^2_m	Deviance	χ^2	df	p
Modified GAD-7	Model 1[a]	2683.479	2707.041	0.597	0.148	2669.479			
	Model 2[b]	2663.322	2700.347	0.595	0.152	2641.322	28.158	4	<0.001
	Model 3[c]	**2642.917**	**2690.040**	**0.610**	**0.166**	**2614.917**	**26.405**	**3**	**<0.001**
PANAS Positive	**Model 1[a]**	**1334.397**	**1357.926**	**0.679**	**0.226**	**1320.397**			
	Model 2[b]	1337.136	1374.110	0.685	0.233	1315.136	5.261	4	0.262
	Model 3[c]	1337.987	1385.045	0.688	0.249	1309.987	5.149	3	0.161
PANAS Negative	**Model 1[a]**	**1360.026**	**1383.555**	**0.576**	**0.109**	**1346.026**			
	Model 2[b]	1365.751	1402.726	0.573	0.112	1343.751	2.275	4	0.685
	Model 3[c]	1366.379	1413.437	0.578	0.132	1338.379	5.372	3	0.146

[a] time + expectation + group + (1|participant)
[b] time + expectation + group + time*group + time*expectation + (1|participant)
[c] time + expectation + group + time*group + time*expectation + time*expectation*group + (1|participant)
Abbreviations: GAD-7, Generalised Anxiety Disorder 7-item; PANAS, Positive and Negative Affect Schedule; AIC, Akaike information criterion; BIC, Bayesian information criterion; R^2_c, conditional R^2; R^2_m, marginal R^2; df, degrees of freedom

With modified GAD-7 as the dependent variable, the addition of all three-way interactions provided significant improvement when compared with the simpler model. Within this model, there was a significant effect of time ($F_{(2,134.67)} = 19.58$, $p < 0.001$) and of expectation ($F_{(1,69.35)} = 4.43$, $p = 0.039$). Interestingly, the relationship between modified GAD-7 and expectation was positive (estimate = 33.222 ± 15.777, $t_{(69.35)} = 2.11$, $p = 0.039$). Other effects were not significant, although group ($F_{(1,70.53)} = 3.29$, $p = 0.074$) and time*expectation*group ($F_{(2,133.59)} = 2.77$, $p = 0.066$) approached significance. Further *post-hoc* exploration showed that anxiety significantly increased from pre-treatment to post-anticipation and remained elevated following the social interaction (t's > 3.40, p_{tukey}'s < 0.010), with no significant differences between the groups at any timepoint. *Post-hoc* simple slope analysis revealed that estimates of the gradient of the slope of expectation versus modified GAD-7 did not significantly differ between groups at any timepoint (t's ≤ 1.60, p_{tukey}'s ≥ 0.599). See Table 6.4.

Table 6.4 Summary of post-hoc simple slope analysis of the slope of expectation versus modified GAD-7. There were no significant differences between groups at any timepoint. Values are estimated difference in gradient of the slope ± standard error between groups (with reference to the placebo group).

Timepoint	Estimated difference	Statistics
Pre-treatment	-22.08 ± 37.89	$t_{(130.56)} = 0.58$, $p_{tukey} = 0.992$
Post-anticipation	61.16 ± 38.20	$t_{(132.61)} = 1.60$, $p_{tukey} = 0.599$
Post-social interaction	41.45 ± 38.27	$t_{(133.15)} = 1.08$, $p_{tukey} = 0.887$

Abbreviations: GAD-7, Generalised Anxiety Disorder 7-item

With mood was the dependent variable, more complex models did not provide significant improvement over the simplest models with no interactions. In the model predicting positive affect, the effect of time was again significant ($F_{(2,137.87)} = 18.02$, $p < 0.001$), driven by a reduction in positive affect in both groups over the course of the experiment (t's > 4.19, p_{tukey}'s < 0.001). The effect of expectation was also significant and positively predictive of positive affect (estimate = 3.014 ± 0.669, $t_{(68.52)} = 4.51$, $p < 0.001$). In the model predicting negative affect, the effect of time was also significant ($F_{(2,138.03)} = 15.91$, $p < 0.001$), driven by increased negative affect in both groups over the course of the experiment (t's > 4.56, p_{tukey}'s < 0.001). The effect of expectation was again significant and positively predictive of negative affect (estimate = 1.498 ± 0.667, $t_{(68.23)} = 2.24$, $p = 0.028$).

All analysis code and full results are reproduced in Appendix G.

6.4 Discussion

The aim of this study was to determine whether placebo anxiolysis could be induced remotely in non-treatment-seeking volunteers with symptoms of social anxiety. I used a new paradigm that I developed (the ITSSAD) as the anxiogenic stimulus. The results confirmed that the ITSSAD significantly induces anxiety and negative affect, and reduces positive affect[301]. However, the placebo had little effect on the outcome, with both groups exhibiting similar scores and change in scores over time. Exploratory analyses showed that interactions between expectations, time and group were not significant. Where the effects of expectation were significant, these were *positively* predictive of outcome, regardless of positive or negative valence of the outcome variable. This suggests that participants who generally rated higher on the questionnaires also rated higher on expectation, possibly reflecting response bias such as extreme responding or acquiescence[321]. In sum, the 'sham' treatment seemed to have little effect on anxiety induced by the ITSSAD.

6.4.1 Potential explanations for a lack of placebo anxiolysis

6.4.1.1 Low pre-treatment expectations

Although mean pre-treatment expectation was statistically significantly higher in the placebo group compared with the control group, the numerical difference was small (13 out of 100). This mean difference between groups could potentially be even lower if the data are considered without an outlier present in the placebo group. Nevertheless, even if the verbal suggestions conferred some pre-treatment expectations, these were generally low: mean expectation was

33.50 out of 100. Pre-treatment expectations or expectancies of therapeutic benefit are known to be crucial in producing a placebo effect[76,100]. However, the data suggest that the treatment as presented in this study was not credible. Some previous studies exploring placebo effects on emotion have paired verbal suggestions with written information to strengthen the credibility of the 'sham' treatment[95,159]. By contrast, the information given to participants in the current study consisted only of a short video of myself presenting a putative psychobiological rationale for therapeutic benefit. Perhaps additionally providing (or displaying) written information sheets about the treatment would have strengthened its credibility for participants. I am not aware of any research comparing the capability of verbal or written information, or their combination, to induce pre-treatment expectations and subsequently placebo effects. This is a potential future research direction.

6.4.1.2 Aspects of the 'sham' therapy

An abstract video has been used as a 'sham' therapy previously in a series of three studies[311]. These studies demonstrated significant effects of the placebo on positive mood, calmness and perceived stress[311]. Importantly, these studies employed two placebo conditions: 'placebo only' and 'placebo plus'. The largest effects were seen in the 'placebo plus' condition[311]. The difference between these groups was the manner of the experimenter. In the 'placebo only' condition, the experimenter read the rationale for the treatment off a page, was impersonal, and appeared rushed. By contrast, in the 'placebo plus' condition, the experimenter came across as empathic, enthusiastic about the treatment, and keen to answer the participant's questions[311]. In the current study, I was concerned that a baseline 'social interaction' with an experimenter could confound the outcome of the social interaction that occurs in the ITSSAD. I instead chose to pre-record a video in which I gave the participants verbal suggestions of improvement. However, this delivery method likely mimicked the 'placebo only'-type experimenter described above[311]. The participant could not 'interact' with me in the video and could not ask questions. It is thought that the relationship with the practitioner is important for placebo effectiveness and health outcomes in general[322]. For example, placebo effects are larger when sham acupuncture is administered by an empathic and validating practitioner compared with a cold, rushed practitioner in patients with irritable bowel syndrome[323]. Interestingly, results from a recent study suggest that even a simulation of an empathic and interactive practitioner can promote a placebo response. A placebo analgesic treatment was introduced to healthy volunteers by a 'virtual experimenter', which comprised text on a screen accompanied by recorded audio. Importantly, participants *interacted* with this experimenter, by answering yes/no questions and having the opportunity to ask a question from a list of 'frequently asked questions'. The placebo led to significant reductions in pain resulting from hand immersion in hot water compared with pre-treatment and compared

with a 'no treatment' group[324]. It is possible that the efficacy of the placebo was reduced in the current study by the lack of opportunity for interaction with the practitioner. Further research is needed to explore whether interaction with a similar 'virtual experimenter' could promote placebo effects in a remote setting.

Another important consideration is dose. The 'sham' therapy was given as a single dose in this study. In the previous studies using a 'sham' abstract video, participants were encouraged to view the video as often as possible between assessment visits[311]. If participants had viewed the placebo at stressful times, and subsequently felt relaxed, this could have reinforced the verbal suggestions of efficacy through conditioning[76]. This notwithstanding, placebos show dose effects independent of conditioning[325]. For example, two placebo pills cause greater subjective feelings of drowsiness or alertness than a single pill administered to healthy volunteers before attending a one hour lecture[326]. It is possible that repeated dosing might have enhanced the efficacy of the placebo in the current study.

Finally, treatment adherence has been shown to be a predictor of placebo effects on 'wellbeing' in healthy volunteers[327]. However, whether participants were adherent with the 'therapy' in the current study is unknown. Participants were prompted with instructions that the more attention they paid to the stimulus the more benefit they would derive. However, I have no measure of engagement with the therapy or with the screen during the 5-minute treatment period. Adherence in the case of viewing a video on instruction could be appraised as a measure of therapeutic alliance. Therapeutic alliance is known to be related to treatment outcome, certainly with psychotherapy[328-331]. Further, at least one study has demonstrated that therapeutic alliance might be important in placebo antidepressant effects. In a double-blind RCT comparing escitalopram with placebo in 42 patients with depression, increased therapeutic alliance predicted reduced symptoms at week 4 in the placebo group but not in the medication group[332]. Speculatively, it is possible that poor therapeutic alliance was a factor in the current study. However, I do not have the data to confirm or refute this possibility.

6.4.1.3 The context

A treatment is administered not in isolation but within a given context. This context encompasses external and internal cues. External cues include verbal suggestions of improvement from the practitioner, treatment cues such as a pill or medical device, and place cues such as a clinical trials facility or doctor's office[101]. Internal cues include previous experiences of treatment, meaning schema, and emotional state[101]. It is thought that the interpretation of this context produces expectations that cause placebo effects: they are considered the 'active ingredients' of placebo effects[101].

Previous experimental placebo studies on affect have involved conditioning or giving suggestions about a treatment in a clinical or laboratory setting[91,94,95,152,155,156,158,159]. However, in the current study, participants were in their own home. A contextual cue that might be important for inducing placebo effects was not present as a result. I am not aware of any studies that have assessed whether placebos are less efficacious when administered in a non-clinical setting. If so, then this could have important implications for the efficacy of self-administered treatments, such as self-help or neurostimulation treatments (e.g. transcranial direct current stimulation[333]). How different settings might affect placebo efficacy should be explored in future studies.

6.4.1.4 Anxiety is predictive of reduced placebo effect or nocebo effect

A final consideration is that it might be challenging to reliably generate placebo effects in experimentally-induced anxiety. Only one paradigm has previously been developed with the aim of explicitly inducing placebo anxiolysis. Sham 'intranasal lorazepam' or 'laughing gas' reduced subjective fear and skin conductance responses to possible electric shock in three related studies, but this was regardless of threat cue[158,159]. It is therefore unclear whether this truly represents placebo anxiolysis or another phenomenon. Other experimental placebo studies on emotion have not involved experimental induction of anxiety[91,94,95,152,155,156]. However, increased state anxiety is known to reduce placebo, and increase nocebo, effects on pain and itch[109,148-151,334,335]. Pertinently, inducing stress via the TSST increases nocebo effects on experimental pain in healthy volunteers[336]. It has been suggested that changes in affect could mediate placebo effects: being given a placebo might reduce negative emotions, leading to reduced impact of bodily sensations[147,264]. Perhaps *inducing* high intensity negative emotions after administering a placebo interferes with this process. Supporting this, nocebo hyperalgesia is more resistant to extinction than placebo analgesia, and this resistance to extinction is mediated by heightened autonomic arousal[335]. The suggestion that it is not possible to reliably produce placebo effects during experimental induction of anxiety is additionally relevant to the previous chapter and is discussed further in Chapter 7.

6.4.2 Limitations

There are some limitations of this study. First, the majority of participants were female (92%). Since there were few males, it was not possible to assess for an effect of gender on placebo response or subjective anxiety. Compared with males, females tend to display higher subjective stress reactivity[337,338]. Both groups were well-balanced in numbers of females and males, but it is unknown whether my results would replicate in a more male-predominant sample. Second, I did not collect any physiological measures. It is possible that placebo effects might have been

detectable within physiological measures such as heart rate, blood pressure or skin conductance[158,159]. Due to the remote nature of the study, collecting such measures was challenging, but not impossible. For example, in recent studies of remotely delivered TSSTs, participants have been asked to use self-administered ECG monitors or their smartphone to measure heart rate[339,340]. Such additions should be considered for future studies employing the ITSSAD paradigm. Third, I did not include a low socially anxious group of participants. It is possible that in those with low levels of social anxiety the placebo might have been more or less efficacious. Finally, I did not measure natural recovery after the ITSSAD task, instead providing a 'mood repair' to prevent ongoing distress. Post-event processing is understood to be an important element in maintenance of SAD[341,342]. Since I did not measure post-event processing or natural recovery, it is unknown whether the placebo affected these processes. Further studies should explore this, as narratives regarding attribution of subjective symptom improvement to the 'treatment' or otherwise might partially explain placebo efficacy[343].

6.5 Concluding comments

In summary, there was no evidence that placebo effects could be induced in the ITSSAD paradigm. On average, changes in subjective anxiety and mood were similar in both groups at all timepoints. Exploratory analyses suggested that expectations did not interact with subjective measures of anxiety and mood. The lack of placebo effect might be due to a cold, impersonal 'practitioner' (video of me briefly giving rationale for the sham treatment) or other features of the treatment context such as single dose and the non-clinical setting. Another possibility is that experimental anxiety induction actively interferes with the placebo effect by preventing reductions in negative emotions. Further studies are needed to explore these possibilities.

Chapter 7 Overall Discussion

The central aim of this thesis was to extend understanding of the mechanisms of placebo effects in gambling, depressive, and anxiety disorders. The research presented demonstrates an attempt to study the mechanisms of placebo effects through an analysis of clinical trial data, a synthesis of the current literature, and through development of novel experimental paradigms in healthy volunteers. In this chapter, I summarise the principal findings from this research, their implications, and future research directions.

7.1 Thesis summary

First, I aimed to identify predictors of placebo response in gambling disorder, and whether these differed from predictors of medication response (Chapter 3). I carried out a secondary analysis of pooled patient-level data from six placebo-controlled trials of gambling disorder. Previous studies of this kind have dichotomised patients into 'responders' and 'non-responders', but doing so can result in bias. Instead, I analysed treatment response as a linear outcome variable to retain maximum information regarding its relationship with predictor variables. I found that predictors of placebo response differed from medication response. Placebo response was associated with decreased baseline symptoms of anxiety, increased baseline symptoms of depression, and non-Caucasian ethnicity. Conversely, medication response was associated with increased number of weeks completed in the trial and increased baseline severity of gambling symptoms.

Next, I synthesised current knowledge of functional neuroimaging correlates of placebo antidepressant and anxiolytic responses and identified hypotheses to be tested in future studies (Chapter 4). I carried out a systematic review of the neuroimaging literature, which yielded twelve and four articles that reported correlates of placebo antidepressant and anxiolytic responses, respectively. Qualitative synthesis of the research findings suggested that the rostral anterior cingulate cortex and default mode network, the ventral striatum, orbitofrontal cortex and dorsolateral prefrontal cortex might be important anatomical regions in these placebo responses. Findings regarding amygdala involvement were less consistent. Additionally, there was evidence for involvement of endogenous opioids, dopamine, and serotonin. Comparatively little research has been conducted in this field, as opposed to placebo analgesia, for example[129,183]. Indeed, lack of longitudinal imaging and poor control of potential confounds like order effects meant that conclusions regarding causality could not be made.

There is additionally only limited research exploring placebo mechanisms on emotion that has been carried out in healthy volunteers. Those studies that have been conducted have been affected by confounders, and it is unclear whether they induced placebo effects on clinically relevant features. In two studies, I sought to develop novel placebo paradigms within experimental models of clinical anxiety.

First, I created a novel placebo paradigm in the 7.5% CO_2 inhalational model of generalised anxiety (Chapter 5). This procedure combined verbal suggestions and conditioning to maximise the potential for a placebo effect. Although the sample size was small, group-level analysis demonstrated no statistically significant difference between the placebo group and a control group that did not undergo conditioning. *Post-hoc* computation of effect sizes showed that placebo conditioning conferred small effects on anxiety. Interestingly, an exploratory analysis revealed a trend association between increased expectations following conditioning and reduced reactivity to CO_2 inhalation in subjective measures only. Finally, there were a number of participants who exhibited *increased* CO_2 reactivity following conditioning (including some with high expectations), possibly reflecting a nocebo effect rather than placebo. Overall, the findings suggest that the 7.5% CO_2 inhalational model of generalised anxiety disorder cannot be relied upon for inducing placebo effects in healthy volunteers.

Second, I tested whether placebo effects could be remotely induced in volunteers with social anxiety symptoms in a modified TSST delivered online (the ITSSAD) (Chapter 6). Anxiety induced by the ITSSAD is associated with baseline levels of social anxiety symptoms[301], suggesting this is a clinically relevant paradigm. Participants were introduced to a 'sham' psychological treatment accompanied by verbal suggestions that this would lead to 'feelings of relaxation' and reduced anxiety. These suggestions conferred a numerically small but statistically significant increase in expectation in the placebo group compared with the control group that did not receive verbal suggestions. Despite this, there was no difference in subjective anxiety or mood between groups at any timepoint during the ITSSAD. Exploratory analyses suggested no interactions between expectations and group on the outcome. The findings suggest it is not possible to remotely induce placebo effects in the ITSSAD paradigm.

7.2 Implications for research

7.2.1 Research into placebo mechanisms in psychiatric disorders

The psychoneurobiological mechanisms of placebo effects in psychiatric disorders, in what situations they are activated, and in which individuals this occurs, remain poorly understood[56]. My

findings in this thesis highlight important gaps in the literature and challenges with addressing these. This has important implications for future research.

7.2.1.1 Neuroimaging of placebo effects

When systematically reviewing the literature, I found research highlighting neuroanatomical regions and neurotransmitter systems potentially involved in placebo antidepressant and anxiolytic effects (Chapter 4). To date, this neuroimaging research has been mostly correlational in nature rather than exploring causality. Furthermore, many papers comprised secondary analyses of already collected data, resulting in under-powered studies with potential for effects to appear more localised than they should and estimates of effect size to be biased[344]. Studies that did explore causality were affected by confounds such as order effects. Nevertheless, my findings from the review generate a number of important hypotheses for the field (see future research directions below), which if explored could lead to a detailed understanding of relevant biomarkers similar to our understanding of placebo analgesia[76,101]. Such understanding has led to hypothesis-driven identification of key brain and psychological determinants of placebo effects in patients with chronic pain[266].

In future, neuroimaging studies will need to be adequately powered and specifically designed to investigate the placebo effect in psychiatric disorders. This presents a challenge, as the effect size of placebo on putative biomarkers is largely unknown. Estimating potential effect sizes from thoroughly researched areas like placebo analgesia or basing power calculations on minimally clinically meaningful effect sizes are two strategies that potentially address this. Further study to identify the optimal approach is needed.

7.2.1.2 Discovery research using experimental models

Although placebo effects in clinical trials of psychiatric disorders are large, it is not feasible to carry out all placebo research in patients. To explore placebo effects, a placebo needs to be administered, usually in place of an active treatment. If the active treatment is known to be beneficial then this raises ethical questions regarding withholding of effective treatment. By contrast, if the efficacy of the active treatment is uncertain then use of a placebo control is more easily justified ethically, but could potentially lead to a reduction in observed placebo effects[75,184]. Experimental manipulation to maximise placebo effects in healthy volunteers is likely to increase the probability of detecting relevant mechanisms[75], potentially leading to improved understanding of the psychoneurobiology of placebo effects in psychiatric disorders.

Research to date has attempted to model placebo effects in relevant emotional states such as sadness[95,152] or anxiety via threat of shock[158,159]. However, whether findings from these models

are relevant in *patients* is unclear. Sadness alone probably does not encompass the totality of clinical major depression: for example, there is evidence that dysfunctional cognitions occur during sad mood only in those vulnerable to clinical depression[171]. Likewise, threat of shock induces *functional* anxiety as opposed to the *dysfunction* seen in anxiety disorder[172]. Experimental medicine models, by contrast, induce features resembling the dysfunctional symptoms seen in psychiatric disorder[39,40]. The use of such models in placebo research might allow discovery of clinically relevant mechanisms.

I attempted to induce placebo effects in two clinically relevant anxiety paradigms: the 7.5% CO_2 inhalational model of generalised anxiety and the ITSSAD. However, I found that these models were not conducive to reliable induction of placebo effects. A prospect that needs to be considered is that it is challenging to consistently elicit placebo effects on experimentally-induced, clinically-relevant anxiety. Placebo effects are reduced, and nocebo effects increased, on pain and itch by increased state anxiety[109,148-151,334-336]. Further, elevated autonomic arousal appears to be important in the persistence of nocebo hyperalgesia[335]. Unregulated autonomic arousal is a key marker of transition between functional and pathological anxiety[345]. Given this, it is noteworthy that the only successful induction of placebo effects on experimentally-induced anxiety has occurred in the threat of shock paradigm[158,159]. The effects of threat of shock on autonomic measures, such as heart rate and skin conductance responses, are mixed[165,346]. Conversely, inhalation of air enriched with 7.5% CO_2 reliably induces heightened autonomic arousal measurable via increased skin conductance levels, heart rate, and blood pressure[191,192,195,262,288]. The effects of the ITSSAD on autonomic arousal are unknown, but the TSST on which it is based also reliably induces autonomic arousal[196,347]. Perhaps these models' actions on prefrontal cortex lead to unregulated autonomic arousal[289]. If so, this might further explain the poor induction of placebo effects in these paradigms, as prefrontal cortex activity is necessary for generating a placebo effect[291,292]. The resulting hypothesis is that inducing autonomic arousal might hinder conversion of conditioning into a placebo effect. If so, this presents a challenge for future research into the mechanisms of placebo anxiolysis.

If the presence of autonomic arousal does inhibit this conversion, it might be possible to overcome this through maximising the effect of conditioning. Several methods could be employed to achieve this. For example, repeated conditioning sessions could be used, which is known to cause more pronounced placebo effects than a single conditioning session[148]. Second, allowing the participants to use the placebo in their daily lives could reinforce conditioning and exploit dosing effects of placebos before experimental testing[311,325]. Third, ensuring that the treatment is administered by a warm, empathic, and enthusiastic experimenter or health professional likely enhances placebo effects[311,322]. Finally, as per the Bayesian predictive coding framework[293],

ensuring high certainty of *anxiolysis* and low certainty of *anxiety* should enhance placebo effects. These hypotheses need further testing.

It is also worth contemplating that, since these experimental medicine models induce significant autonomic arousal, they could be utilised for investigation of nocebo effects in psychiatric disorder. Indeed, some participants in the study reported in the current thesis exhibited a nocebo response in the 7.5% CO_2 inhalational model, despite undergoing a *placebo* conditioning procedure. Tests of whether the Bayesian predictive coding framework predicts and explains placebo effects have been conducted[294,295], but to my knowledge no such experiment has examined this in nocebo effects. These experimental medicine models of anxiety could be used for a study of this nature.

7.2.2 Clinical trials in patients with psychiatric disorders

My findings also have implications for the design and interpretation of clinical trials for psychotropic medications. I found that predictors of placebo and medication response differed in clinical trials for gambling disorder (Chapter 3). This study adds to the literature exploring predictors of treatment response in gambling disorder[175,176], but also in other psychiatric disorders such as binge eating disorder[224], acute mania[45], social anxiety disorder[348], obsessive compulsive disorder[349], and depression[61,350].

Placebo response rate in psychotropic trials is variable but, when large, can reduce sensitivity of a trial to detect treatment efficacy as medication response rate is less variable[52,53,55]. Identifying predictors of treatment or placebo response could allow adjustment of analyses for these covariates, or enrichment of patient samples. For example, in a recently published trial comparing the antidepressant vortioxetine with placebo for depression, drug-placebo differences were increased in a subgroup of patients with increased baseline severity of depression and reduced baseline symptoms of anxiety[351]. The authors suggested that targeting this profile of patients for inclusion in clinical trials could increase the probability of success[351]. In the case of gambling disorder, my results suggest that targeting patients with increased severity of gambling symptoms and low symptoms of depression might maximise drug-placebo differences; but this needs empirical testing.

In addition, much of the research to date has aimed to identify predictors of placebo response in psychiatric disorders through meta-analyses or secondary analyses of previously collected data. Very often, the performance of identified predictors is not assessed in a novel external sample, which is an important limitation. Moreover, secondary data analysis involves scrutinising data collected for a different research question, which means the results and conclusions are

vulnerable to latent unmeasured variables[352]. Secondary analyses also do not allow measurement of some within-patient factors likely to be important in placebo response, such as expectations, although statistical *post-hoc* approximations of these have been attempted[353]. Some prospective research exploring determinants of placebo response in psychotropic trials has been carried out, but these studies often involve manipulation of expectations[65,98,254,354]. Therefore, the external validity of these findings for clinical trials in which expectations are not experimentally manipulated could be questioned. As with the neuroimaging literature, prospective studies designed to explore determinants of treatment and placebo response are needed.

Finally, treatment response is defined categorically in much of the research in this field, i.e. symptoms below a certain threshold equals treatment response. In contrast, I analysed treatment response as a continuous outcome measure. Some other researchers have also proposed modelling 'propensity to respond to placebo' as a continuous (and latent) variable, on the basis that placebo responsiveness is not binary but instead can vary along a continuum[355]. Modelling placebo responsiveness as a continuous variable potentially allows statistical estimation of the degree to which placebo effects explain improvements following active treatment, through infinite or finite mixture modelling[62,356], or mixed effects modelling[357]. Whether using such models improves assay sensitivity of clinical trials requires further study.

7.3 Possible implications for clinical practice

There is empirical evidence that placebo mechanisms can affect how successful our treatments are in the clinic. For example, higher expectations of benefit were associated with a greater chance of early response to the SSRI sertraline in patients with post-traumatic stress disorder, and better outcomes after 10 weeks[358]. This highlights the importance of giving clear treatment rationales and, within reason, working with patients to maximise their beliefs in the treatment. However, my findings in this thesis potentially highlight an additional consideration: communication style and patient anxiety during a consultation. As discussed above, it is possible that increased autonomic arousal prevented generation of placebo responses in the experimental models described in this thesis. If a patient were to experience increased autonomic arousal in the clinic, then this may reduce the effectiveness of a prescribed treatment. There is some evidence to support this speculation, albeit somewhat tangential. The findings of a systematic review of empirical studies suggest that a patient-centred clinician communication style (supportive, empathic, facilitative) is associated with reduced autonomic arousal in the patient[359]. Intriguingly, a similar communication style has been shown to enhance placebo response in patients with irritable bowel syndrome[323,360]. Perhaps reduction in autonomic arousal is the mechanism by which this occurs. Further research is needed to understand whether autonomic arousal

experienced in the clinic affects treatment outcomes. If so, this suggests that clinicians should adopt communication styles to limit autonomic arousal when introducing a new treatment. This might help to maximise the potential benefit of the treatment.

7.4 Future research directions

The discussion above highlights several future research directions. First, we need to test whether targeting patients with increased baseline severity of symptoms and reduced symptoms of depression for inclusion in clinical trials for gambling disorder improves assay sensitivity. We also need to identify predictors of placebo response in other disorders. Second, we need prospective studies that assess determinants of placebo response in psychotropic trials so that the impact of within-patient factors, such as expectations, can be explored in externally valid studies. Third, my systematic review highlighted important hypotheses regarding the role of brain regions (such as rostral anterior cingulate cortex and ventral striatum) and neurotransmitters (such as endogenous opioids and dopamine) in placebo antidepressant and anxiolytic responses. These hypotheses need further exploration. Finally, the role of autonomic arousal in prevention of placebo effects should be explored further, both within experimental paradigms in healthy volunteers and in clinical practice. Additionally, the potential for using experimental models of anxiety to investigate the nocebo effect requires further study.

7.5 Overall Conclusions

In this thesis I aimed to improve our understanding of the psychoneurobiology of placebo effects in gambling, depressive and anxiety disorders. My findings highlight several gaps in the literature for future study, as well as a few challenges. I identified novel predictors of placebo response in clinical trials of gambling disorder: reduced baseline symptoms of anxiety, increased baseline symptoms of depression, and non-Caucasian ethnicity. Further research is required to understand whether assay sensitivity of trials in gambling disorder can be improved through application of this knowledge. Data from the neuroimaging literature highlight brain regions and neurotransmitters that might be important in placebo antidepressant and anxiolytic effects. Challenges for further neuroimaging research of these placebo effects involves identifying how best to design studies to limit confounding and ensuring they are appropriately powered. Finally, I discovered challenges in inducing placebo anxiolysis in clinically relevant experimental medicine models. Further research is needed to understand how autonomic arousal interacts with placebo conditioning. Investigation of these challenges and future attempts to fill these gaps in the literature will improve our understanding of the mechanisms of placebo effects in psychiatric

disorders. This improved understanding might allow us to better design clinical trials, maximise the effectiveness of current treatments, and potentially highlight novel tractable therapeutic targets.

Appendix A Systematic Review Search Strategy (Chapter 4)

A.1 PubMed (MEDLINE)

Search terms (no limits, all fields and MeSH terms):

(Placebo OR placebos OR placebo effect OR "placebo response" OR nocebo OR nocebos)

AND

(Depression OR depressive OR Anxiety OR anxious OR major depressive disorder OR MDD OR generalized anxiety disorder OR GAD OR panic disorder OR simple phobia OR antidepressant OR fluoxetine OR Citalopram OR Escitalopram OR Fluvoxamine OR Paroxetine OR Sertraline OR anxiolytic OR anxiolytics OR benzodiazepine OR benzodiazepines OR alprazolam OR clonazepam OR diazepam OR lorazepam OR temazepam)

AND

(positron emission tomography OR PET OR Single-photon emission computed tomography OR SPECT OR functional magnetic resonance imaging OR functional MRI OR fMRI OR tomography OR cerebral blood flow)

A.2 OVID databases

PsycInfo, EMBASE + EMBASE classic, OVID Medline

Search terms (no limits):

(Placebo OR placebos OR placebo effect OR placebo response OR nocebo OR nocebos)

AND

(Depression OR depressive OR Anxiety OR anxious OR major depressive disorder OR MDD OR generalized anxiety disorder OR GAD OR panic disorder OR simple phobia OR antidepressant OR fluoxetine OR Citalopram OR Escitalopram OR Fluvoxamine OR Paroxetine OR Sertraline OR anxiolytic OR anxiolytics OR benzodiazepine OR benzodiazepines OR alprazolam OR clonazepam OR diazepam OR lorazepam OR temazepam)

AND

(positron emission tomography OR PET OR Single-photon emission computed tomography OR SPECT OR functional magnetic resonance imaging OR functional MRI OR fMRI OR tomography OR cerebral blood flow)

A.3 Web of Knowledge

Web of science (science citation index expanded), Biological abstracts, Biosis, Food science and technology abstracts

Search terms (no limits):

Placebo OR placebos OR placebo effect OR placebo response OR nocebo OR nocebos

AND

Depression OR depressive OR Anxiety OR anxious OR major depressive disorder OR MDD OR generalized anxiety disorder OR GAD OR panic disorder OR simple phobia OR antidepressant OR fluoxetine OR Citalopram OR Escitalopram OR Fluvoxamine OR Paroxetine OR Sertraline OR anxiolytic OR anxiolytics OR benzodiazepine OR benzodiazepines OR alprazolam OR clonazepam OR diazepam OR lorazepam OR temazepam

AND

positron emission tomography OR PET OR Single-photon emission computed tomography OR SPECT OR functional magnetic resonance imaging OR functional MRI OR fMRI OR tomography OR cerebral blood flow

A.4 EThOS, British Library

Search terms:

"Placebo" OR "placebos" OR "placebo effect" OR "placebo response" OR "nocebo" OR "nocebos"
AND

"Depression" OR "Anxiety" OR "major depressive disorder" OR "MDD" OR "generalized anxiety disorder" OR "GAD" OR "panic disorder" OR "simple phobia"

AND

"positron emission tomography" OR "PET" OR "SPECT" OR "functional magnetic resonance imaging" OR "functional MRI" OR "fMRI" OR "tomography"

Appendix B E-NASAL trial: study documents (Chapter 5)

B.1 Participant information sheet

Participant Information Sheet
26/10/21 Version 3.0

Study title: *Evaluation of a NAsal Spray in AnxioLytic treatment (E-NASAL) trial*

Project Lead: *Dr Nathan Huneke*
Supervisors*: Prof Matthew Garner, Prof David Baldwin, Prof Nic van der Wee*
Other researchers: *Dr Robert Gordon, Dr Naomi Phillips, Dr Hannah Rowlatt, Dr Harry Fagan, Dr Laura Molteni*
ERGO number*: 52726*

You are being invited to take part in the above research study. To help you decide whether you would like to take part or not, it is important that you understand why the research is being done and what it will involve. Please read the information below carefully and ask questions if anything is not clear or you would like more information before you decide to take part in this research. You may like to discuss it with others but it is up to you to decide whether or not to take part. If you are happy to participate you will be asked to sign a consent form.

What is the research about?
This study has been funded by the Medical Research Council. Dr Nathan Huneke is working towards a PhD in Clinical Neurosciences.

Anxiety disorders are the most common psychiatric disorder and because they typically result in impairment of daily life, are among the most burdensome of medical conditions. Generalised Anxiety Disorder (GAD) is a common anxiety disorder, yet it is often not diagnosed or treated properly. Patients who suffer from GAD experience excessive anxiety and worry, and a range of symptoms including muscular tension, restlessness, dizziness, difficulty in concentrating, and a feeling of being 'on edge', often with increases in heart rate, blood pressure and sweating.

If there was a human model of GAD, (that is, a way of temporarily producing some of the symptoms of GAD, but in a healthy person) it could be used to discover new and potentially more effective treatments and help us to understand what is happening in the body. We have been working on the development of a human model of GAD, using the inhalation of air enriched with 7.5% carbon dioxide (CO2) for 20 minutes. In healthy volunteers, this temporarily makes some people feel anxious and tense and reduces feelings of being relaxed and happy. It also temporarily increases blood pressure and heart rate as well as alters attention and emotion processing, which is commonly reported in anxious patients. The effects of CO2 are different from the inhalation of normal room air and we believe that this model could be used to explore how and whether treatments work in GAD.

In this study, we will examine the extent to which a widely used anti-anxiety drug, lorazepam, alters how healthy volunteers respond to inhalation of air enriched with 7.5% CO2 compared with a placebo. In particular, we will be testing a new way of delivering these anti-anxiety effects: a nasal spray. In a pilot study, this nasal spray was found to be safe and appeared to lower blood pressure and anxiety during inhalation of air enriched with 7.5% CO2.

We are recruiting healthy male and female volunteers, aged between 18 and 55 years, to be administered lorazepam nasal spray or placebo prior to inhaling 7.5% CO2.

Why have I been asked to participate?
You have been invited to participate since you have enquired about our advertised studies. We plan to recruit 60 participants for this study.

What will happen to me if I take part?
There are two parts to this study:

1. **10 minute telephone screening**
   Before entering the research study, the researchers will contact you and ask specific questions about your physical and mental health, to check that you are fit to take part in the study. This will take about 10 minutes, will be arranged at your convenience and can be completed over the phone. All information will remain confidential.

   You should be generally healthy, have no history of or current asthma, migraine, seizures, renal, cardiac or hepatic impairment or glaucoma, and have no present or past anxiety disorder or other mental health complaint. You should not take part if a close member of your family suffers from regular panic attacks or has been diagnosed with panic disorder.

   Your alcohol intake should not be more than 21 units per week. Note that one unit of alcohol equals one 25ml single measure of whisky (ABV 40%), or a third of a pint of beer (ABV 5-6%) or half a standard (175ml) glass of red wine (ABV 12%). You should not be a regular smoker (more than 6/day). You should not have used medication in the preceding 8 weeks, apart from occasional paracetamol, or local treatments. Females should be using adequate methods of contraception and should not be pregnant or breast feeding, or be considering becoming pregnant. If you meet our list of entry criteria, then you will be invited to the testing session.

2. **Testing Session (9.30-11.30: 2 hours)**
   Prior to the testing session you should refrain from alcohol for 36 hours. You also should not drink any caffeinated drinks after midnight prior to the test day. This is because alcohol and caffeine have effects on blood pressure and heart rate measurements; and alcohol may enhance the effects of the gas. However, the exception from this is if you regularly ingest caffeine in the morning, in which case you should have your usual caffeinated drink to avoid withdrawal effects during the study. You should not be a regular (i.e., daily) smoker and should not have smoked within 12 hours of the study session.

   At the beginning of the session, you will complete some questionnaires that ask about your physical and mental health. Throughout the session we will take several measures of your current mood (short questionnaires), blood pressure and heart rate (arm cuff).

   You will complete three 20-minute inhalations of air enriched with 7.5% CO2. The 7.5% CO2 gas is a mixture of carbon dioxide and air, with the air containing the usual amount of oxygen. The gas will be administered through a mask that covers your mouth and nose. This will be fitted prior to the inhalation of the gas to enable you to become accustomed to wearing it. You will wear the mask and remain seated in a comfortable position throughout the inhalation.

   During each inhalation you will complete a short (8 minute) computerised task that measures your attention. During this task you will be presented with a set of widely used experimental pictures of emotional faces. Before you agree to participate you will have the opportunity to view examples of these faces. Please remember that you are free to withdraw at any time. During the task we will measure your heart rate, skin conductance and eye movements through 6 electrodes placed on the surface of your skin (i.e. non-invasively).

You will be given a nasal spray, either lorazepam or placebo, prior to the second and third inhalations.

Are there any benefits in my taking part?
The study will last approximately 2 hours and you will be reimbursed £15 for your time when you complete the testing session. If you prefer, then you can ask to receive 24 course credits instead of the £15. You will also receive £15 towards travel costs to the test session site at College Keep. Beyond this you should not expect to directly benefit from taking part in this research study. However, the information we obtain from this study might help us to understand and treat patients with anxiety disorders in the future.

Are there any risks involved?
Participants who meet any of the exclusion criteria should not take part in the study. This includes pregnant women or women who are breastfeeding, and women who plan to become pregnant. All women will therefore be asked to answer some short questions about their use of contraception to exclude the possibility of pregnancy. The screening procedure will ask participants some questions about their physical and mental health. Information about local support services will be available to any participant that is concerned about their physical and mental wellbeing.

Since the testing session requires you to attend the department in person, there is a small risk of contracting covid-19. We have put in place a number of measures to minimise this risk. You will be given a fluid-repellant facemask to wear throughout the session. The experimenters will wear full personal protective equipment, and only one experimenter will be allowed in the room with you at any time. There is enough space to maintain 2 metres physical distancing. Finally, the department and all study equipment is thoroughly cleaned between testing sessions. You will be contacted the day after the study day to check your wellbeing and we will advise you about the actions you will need to take should you develop symptoms of covid-19.

During the testing session, you will receive either a lorazepam nasal spray or placebo nasal spray. You will also complete three 20-minute inhalations of air enriched with 7.5% CO2.

1. **Lorazepam**
   Lorazepam is a well-tolerated drug that is commonly used throughout the world, usually in multiple repeated doses rather than the two doses we will be using in this study. Lorazepam is usually available in tablet form, or as an injection. Administering lorazepam as a nasal spray is new. Despite this, we would expect possible side effects to be similar to those that occur with tablets. Commonly reported side effects include: drowsiness, dizziness, blurred vision, and nausea. The purpose of the medical screening at the start of the study is to make sure that it is safe for you to take the drug. It is unlikely that you will experience any side effects from two doses of lorazepam.

   Participants can discuss any concerns or side effects with the study medical team led by Professor David Baldwin (Consultant Psychiatrist). Contacted at dsb1@soton.ac.uk and 02382310764.

2. **Inhalation of air enriched with 7.5% CO2**
   Our research group has carried out in excess of 300 CO2 inhalation tests. Any effects of the gas inhalation are temporary and typically resolve within a minute after inhalation. Carbon dioxide inhalation may cause feelings of anxiety or unpleasantness. Other physiological effects that may occur include racing of heart, dizziness, pins and needles, and breathlessness. Some people also experience a mild headache afterwards.

   People experience and describe the effects of inhaling 7.5% CO2 gas in different ways, and there is no way of knowing in advance how you will respond. Some people do not notice it at all, and some experience more marked anxiety. Most people will notice some effects, and if you do not like the effects, you can ask to stop. These feelings should be short-lived (typically resolving within a couple of minutes) and

should not cause any lasting harm. The researchers will remain near you at all times and will offer reassurance if necessary. If you feel uncomfortable breathing the gas at any time during the procedure you may indicate that you wish the procedure to stop.

At the end of the study session you will remain in the testing room until you feel that any effects of the gas have worn off. We will contact you the day after the study day to check that you are healthy and well.

What data will be collected?
As part of the screening process, we will collect personally identifiable information including date of birth, ethnicity, gender identity, telephone number, address and medical history. We collect this information to ensure that the study groups are reasonably balanced in terms of demographics and to ensure that you are eligible to participate in the study. This information will be stored in an electronic database and on paper. We also collect your contact details so that we can maintain contact with you during the study, and to make any necessary follow up contacts. We will not retain your contact details for future studies.

During the testing session we will collect data on current mood (from questionnaires), blood pressure and heart rate, other psychophysiology measures including skin conductance, and measures of attention from the computerised task. These data will be stored in an electronic database and on paper.

All electronic databases containing personally identifiable information will be encrypted and password-protected. Where data has been collected using paper documents (e.g. questionnaires), all documents will be number-coded and stored in a locked filing cabinet. Personal data and consent forms will be stored separately from non-identifiable data.

Will my participation be confidential?
Your participation and the information we collect about you during the course of the research will be kept strictly confidential. However, if you were to provide the study team with any information that gives us concern about your safety or wellbeing, then we will have a duty to disclose this information. If we were to disclose this information, then we will discuss this with you at the time.

Only members of the research team (including supervisors) and responsible members of the University of Southampton may be given access to data about you for monitoring purposes and/or to carry out an audit of the study to ensure that the research is complying with applicable regulations. Individuals from regulatory authorities (people who check that we are carrying out the study correctly) may require access to your data. All these people have a duty to keep your information, as a research participant, strictly confidential.

Do I have to take part?
No, it is entirely up to you to decide whether or not to take part. If you decide you want to take part, you will need to sign a consent form to show you have agreed to take part.

What happens if I change my mind?
You have the right to change your mind and withdraw at any time without giving a reason and without your participant rights being affected.

If you withdraw from the study, you can request that we destroy the data we have gathered about you up until we have entered it into our secure database (approximately May 2021).

What will happen to the results of the research?
When the study has been completed, we shall analyse the data and report the findings. This will be reported in an appropriate scientific journal or presented at a scientific meeting. Your personal details will remain strictly confidential. Research findings made available in any reports or publications will not include information that can directly identify you without your specific consent. If you would like a copy of the final paper, you may request this.

Once the project is complete, all data will be transferred and stored in the University Research Repository. This will be held in an anonymised form and unlinked to any personal identifiable information. This allows other researchers to use the data for future research without being able to identify you. Data will be stored here for a minimum of 15 years.

Where can I get more information?
For further queries, please contact Dr Nathan Huneke (n.huneke@soton.ac.uk, tel: 02382 310 775).

What happens if there is a problem?
If you have a concern about any aspect of this study, you should speak to the researchers who will do their best to answer your questions.

If you remain unhappy or have a complaint about any aspect of this study, please contact the University of Southampton Research Integrity and Governance Manager (023 8059 5058, rgoinfo@soton.ac.uk).

Data Protection Privacy Notice
The University of Southampton conducts research to the highest standards of research integrity. As a publicly-funded organisation, the University has to ensure that it is in the public interest when we use personally-identifiable information about people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use information about you in the ways needed, and for the purposes specified, to conduct and complete the research project. Under data protection law, 'Personal data' means any information that relates to and is capable of identifying a living individual. The University's data protection policy governing the use of personal data by the University can be found on its website (https://www.southampton.ac.uk/legalservices/what-we-do/data-protection-and-foi.page).

This Participant Information Sheet tells you what data will be collected for this project and whether this includes any personal data. Please ask the research team if you have any questions or are unclear what data is being collected about you.

Our privacy notice for research participants provides more information on how the University of Southampton collects and uses your personal data when you take part in one of our research projects and can be found at http://www.southampton.ac.uk/assets/sharepoint/intranet/ls/Public/Research%20and%20Integrity%20Privacy%20Notice/Privacy%20Notice%20for%20Research%20Participants.pdf

Any personal data we collect in this study will be used only for the purposes of carrying out our research and will be handled according to the University's policies in line with data protection law. If any personal data is used from which you can be identified directly, it will not be disclosed to anyone else without your consent unless the University of Southampton is required by law to disclose it.

Data protection law requires us to have a valid legal reason ('lawful basis') to process and use your Personal data. The lawful basis for processing personal information in this research study is for the performance of a task carried out in the public interest. Personal data collected for research will not be used for any other purpose.

For the purposes of data protection law, the University of Southampton is the 'Data Controller' for this study, which means that we are responsible for looking after your information and using it properly. The University of Southampton will keep identifiable information about you for 15 years after the study has finished after which time any link between you and your information will be removed.

To safeguard your rights, we will use the minimum personal data necessary to achieve our research study objectives. Your data protection rights – such as to access, change, or transfer such information - may be limited, however, in order for the research output to be

reliable and accurate. The University will not do anything with your personal data that you would not reasonably expect.

If you have any questions about how your personal data is used, or wish to exercise any of your rights, please consult the University's data protection webpage (https://www.southampton.ac.uk/legalservices/what-we-do/data-protection-and-foi.page) where you can make a request using our online form. If you need further assistance, please contact the University's Data Protection Officer (data.protection@soton.ac.uk).

Thank you.

B.2 Debriefing statement

Study title: *Evaluation of a NAsal Spray in AnxioLytic treatment (E-NASAL) trial*

Debriefing Statement *(verbal and written) V2.0, 10/07/2020*
Project Lead: *Dr Nathan Huneke*
Supervisors*: Prof Matthew Garner, Prof David Baldwin, Prof Nic van der Wee*
Other researchers: *Dr Robert Gordon, Dr Naomi Phillips, Dr Hannah Rowlatt, Dr Harry Fagan, Dr Laura Molteni*
ERGO ID*: 52726*

Thank you for taking part in this experiment.

Study background: There is a need for improved treatments for patients with anxiety disorders, as many patients do not respond to first line psychological or pharmacological treatments, and drug treatments can cause unwanted side-effects. We normally test a new treatment by comparing it with a placebo; a 'dummy' treatment with no active ingredients. Sometimes, patients improve when given a placebo, and this is called 'the placebo response'. If many patients show this response in a clinical trial, it is difficult to establish whether a new treatment is effective. Learning more about how the placebo response works should make it easier to test potential new treatments.

The aim of this research was to examine the effects of a placebo treatment on subjective and autonomic responses to inhalation of air enriched with 7.5% carbon dioxide (CO_2) (in comparison to normal air inhalation). Your data will help us to understand whether healthy volunteers can have a placebo response in the 7.5% CO_2 model of anxiety. This experiment involved deception so that we could attempt to induce a placebo response.

You were not given active lorazepam at any point during this study. The nasal spray contained only salt water. You were randomized to one of two groups: 1) placebo with an expectation of it reducing anxiety or 2) placebo with no expectation of it reducing anxiety. If you were in group 1, then you were told that the nasal spray was 'lorazepam', which is known to have anti-anxiety effects and therefore would reduce your anxiety during the 7.5% CO_2 inhalation. This was reinforced by us surreptitiously turning off the supply of CO_2 during the second inhalation, so that you were breathing normal air. In the third inhalation, we re-started the supply of CO_2, so that you were again breathing air enriched with 7.5% CO_2. The purpose of this was to see whether your beliefs about the medication meant that you felt less anxious even when breathing air enriched with 7.5% CO_2. If you were in group 2, then you were told that the nasal spray contained salt water, and that you would be breathing normal air during the second inhalation. We did this to ensure that participants in group 2 had no expectation of a benefit from the spray. We can therefore compare the effects of learning and of expectation on anxiety during the CO_2 challenge between the two groups.

You may have a copy of this summary and a summary of the research findings once the project is completed if you wish. If you have any further questions please contact me, Dr Nathan Huneke, at n.huneke@soton.ac.uk, tel: 02382310775.

If you are interested in finding out more about the rationale behind this research and the methods used in this project, please see the references below:

1. *Bailey, J. E., Kendrick, A., Diaper, A., Potokar, J. P., Nutt, D. J. (2007). A validation of the 7.5% CO_2 model of GAD using paroxetine and lorazepam in healthy volunteers. Journal of Psychopharmacology, 21(1), 42-49.*
2. *Garner, M., Attwood, A., Baldwin, D. S., James, A., & Munafo, M. R. (2011). Inhalation of 7.5% Carbon Dioxide Increases Threat Processing in Humans. Neuropsychopharmacology, 36(8), 1557-1562.*
3. *Meyer B, et al. (2015) Neural mechanisms of placebo anxiolysis. J Neurosci, 35(19):7365-7373.*

A member of the research team will phone you tomorrow to check whether you have experienced any adverse events following your participation, and to discuss any additional queries that you might have.

In the event that you feel unwell please contact your General Practitioner or the NHS 111 service as per usual. In addition please feel able to contact the study medical doctors led by Dr Nathan Huneke, 02382310775.

During this study we asked you to reflect on certain aspects of your physical and mental health. If at any point during your studies you become concerned about your mental or physical health then please contact your General Practitioner.

As per current covid-19 guidance, you will need to self-isolate if:

- *you develop any symptoms of coronavirus (a high temperature, a new, continuous cough or a loss or change to your sense of smell or taste)*
- *you test positive for coronavirus – this means you have coronavirus*
- *you live with someone who develops symptoms or has tested positive*
- *someone in your support bubble has developed symptoms or has tested positive*
- *you're told by NHS Test and Trace that you've been in contact with a person with coronavirus*

If you test positive for coronavirus and are asked to provide details of the study team for contact tracing, then please contact the study medical doctors led by Dr Nathan Huneke, 02382310775, or email enasal@soton.ac.uk.

If you have more general worries during your time as a student in Southampton, then please also be aware that Enabling Services or your personal tutor are available to provide support and advice. To contact Enabling Services, please email enable@soton.ac.uk or telephone 02380 597 726. Skype appointments are also available.

Thank you again for your participation in this research.

Signature _____ Date _____

Name

If you have questions about your rights as a participant in this research, or if you feel that you have been placed at risk, you may contact the University of Southampton Research Integrity and Governance Manager (023 8059 5058, rgoinfo@soton.ac.uk).

B.3 Consent forms

B.3.1 Consent form 1

CONSENT FORM

Study title: *Evaluation of a NAsal Spray in AnxioLytic treatment (E-NASAL) trial*

Project Lead: *Dr Nathan Huneke*
Supervisors*: Prof Matthew Garner, Prof David Baldwin, Prof Nic van der Wee*
Other researchers: *Dr Robert Gordon, Dr Naomi Phillips, Dr Hannah Rowlatt, Dr Harry Fagan, Dr Laura Molteni*
ERGO number*: 52726*

Participant Identification Number:

Please initial the box(es) if you agree with the statement(s):

I have read and understood the information sheet (dated 10/07/20, V2.0) and have had the opportunity to ask questions about the study.	
I agree to take part in this research project and agree for my data to be used for the purpose of this study, and reports to be published in scientific journals and conferences.	
I understand my participation is voluntary and I may withdraw at any time for any reason without my participation rights being affected. I am aware that if I withdraw prior to completing the testing session then I will not be reimbursed for my time.	
I agree to have my contact details kept in a database, so that I can be contacted about this study.	
I understand that after the study has finished my data will be anonymised and held in the University Research Repository. I understand that other researchers might use this data for future research.	

Name of participant (print name)……

Signature of participant……

Date……

Name of researcher (print name)……

Signature of researcher ……

Date……

B.3.2 Consent form 2

CONSENT FORM 2

Study title: *Evaluation of a NAsal Spray in AnxioLytic treatment (E-NASAL) trial*

Project Lead: *Dr Nathan Huneke*
Supervisors*: Prof Matthew Garner, Prof David Baldwin, Prof Nic van der Wee*
Other researchers: *Dr Robert Gordon, Dr Naomi Phillips, Dr Hannah Rowlatt, Dr Harry Fagan, Dr Laura Molteni*
ERGO number*: 52726*

Participant Identification Number:

Please initial the box(es) if you agree with the statement(s):

I have read and understood the debrief sheet (dated 10/07/20, V2.0) and I am aware that the purpose of this study was to explore the placebo response in anxiety. I have had the opportunity to ask questions about the study.	
I agree to take part in this research project and agree for my data to be used for the purpose of this study, and reports to be published in scientific journals and conferences.	
I understand my participation is voluntary and I may withdraw at any time for any reason without my participation rights being affected. I am aware that if I withdraw prior to completing the testing session then I will not be reimbursed for my time.	
I agree to have my contact details kept in a database, so that I can be contacted about this study.	
I understand that after the study has finished my data will be anonymised and held in the University Research Repository. I understand that other researchers might use this data for future research.	

Name of participant (print name)……

Signature of participant……

Date……

Name of researcher (print name)……

Signature of researcher ……

Date……

Appendix C E-NASAL trial: psychometric and questionnaire measures (Chapter 5)

C.1 Trait Modified Generalised Anxiety Disorder 7-item (GAD-7)

GAD-7

Generally how often have you been bothered by the following problems? Rate each word by drawing a vertical line (or a cross) on the DASH-LINE scale below each statement to indicate the extent you have felt that way.

FEELING NERVOUS, ANXIOUS OR ON EDGE

Not at all Several days Over half the days Nearly every day

|.....|

NOT BEING ABLE TO STOP OR CONTROL WORRYING

Not at all Several days Over half the days Nearly every day

|.....|

WORRYING TOO MUCH ABOUT DIFFERENT THINGS

Not at all Several days Over half the days Nearly every day

|.....|

TROUBLE RELAXING

Not at all Several days Over half the days Nearly every day

|.....|

BEING SO RESTLESS THAT IT IS HARD TO SIT STILL

Not at all Several days Over half the days Nearly every day

|.....|

BECOMING EASILY ANNOYED OR IRRITABLE

Not at all Several days Over half the days Nearly every day

|.....|

FEELING AFRAID AS IF SOMETHING AWFUL MIGHT HAPPEN

Not at all Several days Over half the days Nearly every day

|.....|

C.2 Hospital Anxiety and Depression scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week. Don't take too long over you replies: your immediate is best.

I feel tense or 'wound up':

Most of the time
A lot of the time
From time to time, occasionally
Not at all

I still enjoy the things I used to enjoy:

Definitely as much
Not quite so much
Only a little
Hardly at all

I get a sort of frightened feeling as if something awful is about to happen:

Very definitely and quite badly
Yes, but not too badly
A little, but it doesn't worry me
Not at all

I can laugh and see the funny side of things:

As much as I always could
Not quite so much now
Definitely not so much now
Not at all

Worrying thoughts go through my mind:

A great deal of the time
A lot of the time
From time to time, but not too often
Only occasionally

I feel cheerful:

Not at all
Not often
Sometimes
Most of the time

I can sit at ease and feel relaxed:

Definitely
Usually
Not often
Not at all

I feel as if I am slowed down:

Nearly all the time
Very often
Sometimes
Not at all

I get a sort of frightened feeling like 'butterflies' in the stomach:

Not at all
Occasionally
Quite often
Very often

I have lost interest in my appearance:

Definitely
I don't take as much care as I should
I may not take quite as much care
I take just as much care as ever

I feel restless as I have to be on the move:

Very much indeed
Quite a lot
Not very much
Not at all

I look forward with enjoyment to things:

As much as I ever did
Rather less than I used to
Definitely less than I used to
Hardly at all

I get sudden feelings of panic:

Very often indeed
Quite often
Not very often
Not at all

I can enjoy a good book or radio or TV program:

Often
Sometimes
Not often
Very seldom

C.3 Anxiety sensitivity index (ASI)

Circle the *one* phrase that best represents the extent to which you agree with each item below. If any of the items concern something which is not part of your experience (e.g., "It scares me when I feel shaky" for someone who has never trembled or had the "shakes"), answer on the basis of how you think you might feel *if you had* such an experience. Otherwise, answer all items on the basis of your own experience.

1. It is important to me not to appear nervous.

   Very little A little Some Much Very much

2. When I cannot keep my mind on a task, I worry that I might be going crazy.

   Very little A little Some Much Very much

3. It scares me when I feel shaky (trembling).

   Very little A little Some Much Very much

4. It scares me when I feel faint.

   Very little A little Some Much Very much

5. It is important to me to stay in control of my emotions.

   Very little A little Some Much Very much

6. It scares me when my heart beats rapidly.

   Very little A little Some Much Very much

7. It embarrasses me when my stomach growls.

   Very little A little Some Much Very much

8. It scares me when I am nauseous.

Very little A little Some Much Very much

9. When I notice that my heart beats rapidly, I worry that I might have a heart attack..

Very little A little Some Much Very much

10. It scares me when I become short of breath.

Very little A little Some Much Very much

11. When my stomach is upset, I worry that I might be seriously ill.

Very little A little Some Much Very much

12. It scares me when I am unable to keep my mind on a task.

Very little A little Some Much Very much

13. Other people notice when I feel shaky.

Very little A little Some Much Very much

14. Unusual body sensations scare me.

Very little A little Some Much Very much

15. When I am nervous, I worry that I might be mentally ill.

Very little A little Some Much Very much

16. It scares me when I am nervous.

Very little A little Some Much Very much

C.4 Intolerance of uncertainty scale (IUS)

	Not at all characteristic of me		Somewhat characteristic of me		Entirely characteristic of me
1. Uncertainty stops me from having a firm opinion	1	2	3	4	5
2. Being uncertain means a person is disorganized	1	2	3	4	5
3. Uncertainty makes life intolerable	1	2	3	4	5
4. It's unfair not having any guarantees in life	1	2	3	4	5
5. My mind can't be relaxed if I don't know what will happen tomorrow	1	2	3	4	5
6. Uncertainty makes me uneasy, anxious, or stressed	1	2	3	4	5
7. Unforeseen events upset me greatly	1	2	3	4	5
8. It frustrates me not having all of the information I need	1	2	3	4	5
9. Uncertainty keeps me from living a full life	1	2	3	4	5
10. One should look ahead as to avoid surprises	1	2	3	4	5
11. A small unfortunate event can spoil everything even with the best of planning	1	2	3	4	5
12. When it's time to act, uncertainty paralyzes me	1	2	3	4	5
13. Being uncertain means that I am not first rate	1	2	3	4	5
14. When I am uncertain, I can't go forward	1	2	3	4	5
15. When I am uncertain, I can't function very well	1	2	3	4	5
16. Unlike me, others seem to know where they are going with their lives	1	2	3	4	5
17. Uncertainty makes me vulnerable, unhappy, or sad	1	2	3	4	5
18. I always want to know what the future has in store for me	1	2	3	4	5
19. I can't stand being taken by surprise	1	2	3	4	5
20. The smallest doubt can stop me from acting	1	2	3	4	5
21. I should be able to organise everything in advance	1	2	3	4	5
22. Being uncertain means I lack confidence	1	2	3	4	5
23. I think it's unfair that other people seem sure about their future	1	2	3	4	5
24. Uncertainty keeps me from sleeping soundly	1	2	3	4	5
25. I must get away from all uncertain situations	1	2	3	4	5
26. The ambiguities in life stress me	1	2	3	4	5
27. I can't stand being undecided about my future	1	2	3	4	5

C.5 Life orientation test – revised (LOTR)

Scale: Please be as honest and accurate as you can throughout. Try not to let your response to one statement influence your responses to other statements. There are no "correct" or "incorrect" answers. Answer according to your own feelings, rather than how you think "most people" would answer.
A = I agree a lot
B = I agree a little
C = I neither agree nor disagree
D = I disagree a little
E = I disagree a lot

1. In uncertain times, I usually expect the best. ____

2. It's easy for me to relax. ____

3. If something can go wrong for me, it will. (R) ____

4. I'm always optimistic about my future. ____

5. I enjoy my friends a lot. ____

6. It's important for me to keep busy. ____

7. I hardly ever expect things to go my way. (R) ____

8. I don't get upset too easily. ____

9. I rarely count on good things happening to me. (R) ____

10. Overall, I expect more good things to happen to me than bad. ____

C.6 Locus of control questionnaire (LOC)

For each question circle the letter indicating the statement that you agree with the most

1. a. Children get into trouble because their parents punish them too much.
   b. The trouble with most children nowadays is that their parents are too easy with them.

2. a. Many of the unhappy things in people's lives are partly due to bad luck.
   b. People's misfortunes result from the mistakes they make.

3. a. One of the major reasons why we have wars is because people don't take enough interest in politics.
   b. There will always be wars, no matter how hard people try to prevent them.

4. a. In the long run people get the respect they deserve in this world.
   b. Unfortunately, an individual's worth often passes unrecognized no matter how hard he tries.

5. a. The idea that teachers are unfair to students is nonsense.

b. Most students don't realize the extent to which their grades are influenced by accidental happenings.

6. a. Without the right breaks one cannot be an effective leader.
   b. Capable people who fail to become leaders have not taken advantage of their opportunities.

7. a. No matter how hard you try some people just don't like you.
   b. People who can't get others to like them don't understand how to get along with others.

8. a. Heredity plays the major role in determining one's personality.
   b. It is one's experiences in life which determine what they're like.

9. a. I have often found that what is going to happen will happen.
   b. Trusting to fate has never turned out as well for me as making a decision to take a definite course of action.

10. a. In the case of the well prepared student there is rarely if ever such a thing as an unfair test.
    b. Many times exam questions tend to be so unrelated to course work that studying is really useless

11. a. Becoming a success is a matter of hard work, luck has little or nothing to do with it.
    b. Getting a good job depends mainly on being in the right place at the right time.

12. a. The average citizen can have an influence in government decisions.
    b. This world is run by the few people in power, and there is not much the little guy can do about it.

13. a. When I make plans, I am almost certain that I can make them work.
    b. It is not always wise to plan too far ahead because many things turn out to be a matter of good or bad fortune anyhow.

14. a. There are certain people who are just no good.
    b. There is some good in everybody.

15. a. In my case getting what I want has little or nothing to do with luck.
    b. Many times we might just as well decide what to do by flipping a coin.

16. a. Who gets to be the boss often depends on who was lucky enough to be in the right place first.
    b. Getting people to do the right thing depends upon ability. Luck has little or nothing to do with it.

17. a. As far as world affairs are concerned, most of us are the victims of forces we can neither understand, nor control.
    b. By taking an active part in political and social affairs the people can control world events.

18. a. Most people don't realize the extent to which their lives are controlled by accidental happenings.
    b. There really is no such thing as "luck."

19. a. One should always be willing to admit mistakes.
    b. It is usually best to cover up one's mistakes.

20. a. It is hard to know whether or not a person really likes you.
    b. How many friends you have depends upon how nice a person you are.

21. a. In the long run the bad things that happen to us are balanced by the good ones.
    b. Most misfortunes are the result of lack of ability, ignorance, laziness, or all three.

22. a. With enough effort we can wipe out political corruption.
    b. It is difficult for people to have much control over the things politicians do in office.

23. a. Sometimes I can't understand how teachers arrive at the grades they give.
    b. There is a direct connection between how hard 1 study and the grades I get.

24. a. A good leader expects people to decide for themselves what they should do.
    b. A good leader makes it clear to everybody what their jobs are.

25. a. Many times I feel that I have little influence over the things that happen to me.
    b. It is impossible for me to believe that chance or luck plays an important role in my life.

26. a. People are lonely because they don't try to be friendly.
    b. There's not much use in trying too hard to please people, if they like you, they like you.

27. a. There is too much emphasis on athletics in high school.
    b. Team sports are an excellent way to build character.

28. a. What happens to me is my own doing.
    b. Sometimes I feel that I don't have enough control over the direction my life is taking.

29. a. Most of the time I can't understand why politicians behave the way they do.
    b. In the long run the people are responsible for bad government on a national as well as on a local level.

C.7 Penn state worry questionnaire (PSWQ)

Rate each of the following statements on a scale of 1 ("not at all typical of me") to 5 ("very typical of me"). Please do not leave any items blank.

Not at all typical of me				Very typical of me
1	2	3	4	5

1.	If I do not have enough time to do everything, I do not worry about it.	1	2	3	4	5
2.	My worries overwhelm me.	1	2	3	4	5
3.	I do not tend to worry about things.	1	2	3	4	5
4.	Many situations make me worry.	1	2	3	4	5
5.	I know I should not worry about things, but I just cannot help it.	1	2	3	4	5
6.	When I am under pressure I worry a lot.	1	2	3	4	5
7.	I am always worrying about something	1	2	3	4	5
8.	I find it easy to dismiss worrisome thoughts.	1	2	3	4	5
9.	As soon as I finish one task, I start to worry about everything else I have to do.	1	2	3	4	5
10.	I never worry about anything.	1	2	3	4	5
11.	When there is nothing more I can do about a concern, I do not worry about it anymore.	1	2	3	4	5
12.	I have been a worrier all my life.	1	2	3	4	5
13.	I notice that I have been worrying about things.	1	2	3	4	5
14.	Once I start worrying, I cannot stop.	1	2	3	4	5
15.	I worry all the time.	1	2	3	4	5
16.	I worry about projects until they are done.	1	2	3	4	5

C.8 Positive and Negative Affect Schedule (PANAS)

This scale consists of a number of words that describe different feelings and emotions. Read each item and circle the number that indicates to what extent you feel this way **RIGHT NOW**.

	Very slightly or Not at all	A Little	Moderately	Quite a Bit	Extremely
1. interested	1	2	3	4	5
2. distressed	1	2	3	4	5
3. excited	1	2	3	4	5
4. upset	1	2	3	4	5
5. strong	1	2	3	4	5
6. guilty	1	2	3	4	5
7. scared	1	2	3	4	5
8. hostile	1	2	3	4	5
9. enthusiastic	1	2	3	4	5
10. proud	1	2	3	4	5
11. irritable	1	2	3	4	5
12. alert	1	2	3	4	5
13. ashamed	1	2	3	4	5
14. inspired	1	2	3	4	5
15. nervous .....…	1	2	3	4	5
16. determined	1	2	3	4	5
17. attentive	1	2	3	4	5
18. jittery	1	2	3	4	5
19. active	1	2	3	4	5
20. afraid	1	2	3	4	5

C.9 State modified Generalised Anxiety Disorder 7-item (GAD-7)

During the last **20 minutes** how often have you been bothered by the following problems? Rate each word by drawing a vertical line on the scale below to indicate the extent you feel this way.

FEELING NERVOUS, ANXIOUS OR ON EDGE

Not at all Some of the time Most of the time All of the time

|.....|

NOT BEING ABLE TO STOP OR CONTROL WORRYING

Not at all Some of the time Most of the time All of the time

|.....|

WORRYING TOO MUCH ABOUT DIFFERENT THINGS

Not at all Some of the time Most of the time All of the time

|.....|

TROUBLE RELAXING

Not at all Some of the time Most of the time All of the time

|.....|

BEING SO RESTLESS THAT IT IS HARD TO SIT STILL

Not at all Some of the time Most of the time All of the time

|.....|

BECOMING EASILY ANNOYED OR IRRITABLE

Not at all Some of the time Most of the time All of the time

|.....|

FEELING AFRAID AS IF SOMETHING AWFUL MIGHT HAPPEN

Not at all Some of the time Most of the time All of the time

|.....|

C.10 Panic symptom inventory (PSI)

Please put a tick in the column which best describes the feelings that you had at the **AT THIS MOMENT**

	NOT AT ALL	SLIGHT	MODERATE	SEVERE	VERY SEVERE
MUSCLE PAIN	0	1	2	3	4
WEAKNESS	0	1	2	3	4
SWEATING	0	1	2	3	4
GOING MAD	0	1	2	3	4
TREMOR	0	1	2	3	4
DROWSINESS	0	1	2	3	4
NUMBNESS OF FINGERS AND TOES	0	1	2	3	4
DRY MOUTH	0	1	2	3	4
FEELINGS OF UNREALITY	0	1	2	3	4
CHURNING OF STOMACH/BUTTERFLIES	0	1	2	3	4
HEART RACING	0	1	2	3	4
NAUSEA	0	1	2	3	4
UNHAPPINESS	0	1	2	3	4
SHAKINESS	0	1	2	3	4
FEAR OF LOSS OF CONTROL	0	1	2	3	4
ANXIETY	0	1	2	3	4
CHOKING	0	1	2	3	4
HEADACHE	0	1	2	3	4
FEAR OF DYING	0	1	2	3	4
HEART POUNDING	0	1	2	3	4
FAINTNESS	0	1	2	3	4
TENSION	0	1	2	3	4
TINGLING	0	1	2	3	4
PINS AND NEEDLES	0	1	2	3	4
COLDNESS OF HANDS OR FEET	0	1	2	3	4
DIZZINESS	0	1	2	3	4
TIGHT MUSCLES	0	1	2	3	4
TEARFUL/SAD	0	1	2	3	4
BREATHLESSNESS	0	1	2	3	4
HEAVY HEADED	0	1	2	3	4
LEGS WOBBLY	0	1	2	3	4
APPREHENSION/FEAR	0	1	2	3	4
DISCOMFORT IN CHEST	0	1	2	3	4
HOT OR COLD ALL OVER	0	1	2	3	4
OTHER (PLEASE SPECIFY)	0	1	2	3	4

C.11 Expectations (Visual analogue scale)

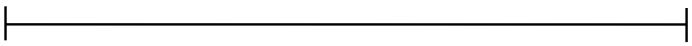
EXPECTATIONS

DIRECTIONS: Answer this question by putting a vertical line on the scale. For example:

Not at all *Extremely*

How much do you expect this spray to reduce your anxiety by during the next inhalation?

Not at all *Very much*



C.12 Post-experiment credibility questionnaire

Post Experiment Questionnaire (adapted from Barkovec and Nau, 1972)

How confident are you that this treatment can successfully eliminate anxiety?	Very slightly or not at all 1	A little 2	Moderately 3	Quite a bit 4	Extremely 5
How confident would you be in recommending this treatment to a friend who suffers with anxiety in certain situations?	1	2	3	4	5
If you suffered with anxiety, would you be willing to have this treatment?	1	2	3	4	5

Appendix D ITSSAD study: study documents (Chapter 6)

D.1 Participant information sheet

Participant Information Sheet
05/11/20 Version 1.2

Study title: *Investigating the role of perspective taking in social anxiety during videoconferencing*

Project Lead: *Dr Nathan Huneke*
Supervisors*: Prof Matthew Garner, Prof David Baldwin*
Other researchers: *Alex McEwan*
ERGO number*: 61411*

You are being invited to take part in the above research study. To help you decide whether you would like to take part or not, it is important that you understand why the research is being done and what it will involve. Please read the information below carefully and ask questions if anything is not clear or you would like more information before you decide to take part in this research. If you are happy to participate you will need to tick that you consent at the bottom of this page.

What is the research about?
Anxiety disorders are the most common psychiatric disorder and because they typically result in impairment of daily life, are among the most burdensome of medical conditions. Social Anxiety Disorder (SAD) is a common anxiety disorder, yet it is often not diagnosed or treated properly. Patients who suffer from SAD experience excessive anxiety and worry about social or performance situations, particularly where the person is exposed to potential scrutiny or unfamiliar people.

Since the covid-19 pandemic, many of our social interactions have moved online. Evidence suggests that even these online interactions can cause significant worry and anxiety for patients with SAD. In this study, we will explore how the use of videoconferencing software might influence social anxiety. We will also explore whether a newly developed psychological treatment for social anxiety disorder can be delivered quickly, remotely, and effectively. This study will take place entirely online.

We are recruiting male and female volunteers, aged between 18 and 45 years, who experience anxiety in social situations.

Why have I been asked to participate?
You have been invited to participate since you have enquired about our advertised studies.

What will happen to me if I take part?
After you have read this information sheet, you will be asked to give consent, so that we know you understand the study and wish to participate.

There are three parts to this study:

1. **Pre-screening**
   To check whether you meet our threshold for social anxiety, we will ask you to complete a pre-screening questionnaire that asks about your thoughts and feelings regarding social interaction.

2. **Screening**

If you meet our threshold for social anxiety, then we will ask you to complete an online screening questionnaire to check you are eligible for the remainder of the study. The questionnaire will ask specific questions about your physical and mental health. You should be generally healthy and have no present mental health complaints other than social anxiety disorder. You should not have received any new treatments, either medication or talking therapy, in the past 8 weeks. If you meet our list of entry criteria, then you will be invited to a testing session.

3. **Testing Session (Approximately 30 minutes)**
   This session will also take place entirely online.

   At the beginning of the session, you will complete some questionnaires that ask about your physical and mental health. You will then either receive our new therapy or you will watch a control video of the same length. After this you will engage in a brief social interaction using videoconferencing software. This interaction will be recorded. You will then complete some more questionnaires to assess your mood and anxiety following this interaction.

   Before you agree to participate you will have the opportunity to review this information.

Are there any benefits in my taking part?
Psychology students at the University of Southampton will receive 1 course credit for completing the pre-screening questionnaire. If eligible, psychology students at the University of Southampton will be given a further 9 course credits on completion of the rest of the study. Beyond this you should not expect to directly benefit from taking part in this research study. Participants not eligible to receive credits will not receive any direct benefits from participating in this research. However, the information we obtain from this study might help us to understand and treat patients with anxiety disorders in the future.

Are there any risks involved?
Participants who meet any of the exclusion criteria should not take part in the study. The screening procedure will ask participants some questions about their physical and mental health. Information about local support services will be available to any participant that is concerned about their physical and mental wellbeing.

We will be asking you to participate in a social interaction and this might cause you some mild anxiety. The interaction will be brief and we will check on your wellbeing on completion of this task. You will be able to stop at any time should you wish to end the conversation.

What data will be collected?
As part of the screening process, we will collect personally identifiable information including date of birth, gender identity, e-mail address and medical history. We collect this information to ensure that the study groups are reasonably balanced in terms of demographics and to ensure that you are eligible to participate in the study. This information will be stored in an electronic database. We also collect your contact details so that we can maintain contact with you during the study, and to make any necessary follow up contacts. We will not retain your contact details for future studies.

During the testing session we will collect data on current mood and anxiety from questionnaires. We will also record the social interaction for qualitative analyses. These data will be stored in an electronic database. All data will be anonymised prior to analysis.

All electronic databases containing personally identifiable information will be encrypted and password-protected.

Will my participation be confidential?
Your participation and the information we collect about you during the course of the research will be kept strictly confidential.

Only members of the research team (including supervisors) and responsible members of the University of Southampton may be given access to data about you for monitoring purposes and/or to carry out an audit of the study to ensure that the research is complying with applicable regulations. Individuals from regulatory authorities (people who check that we are carrying out the study correctly) may require access to your data. All these people have a duty to keep your information, as a research participant, strictly confidential.

Do I have to take part?
No, it is entirely up to you to decide whether or not to take part. If you decide you want to take part, you will need to give consent at the bottom of this page to show you have agreed to take part.

What happens if I change my mind?
You have the right to change your mind and withdraw at any time without giving a reason. If you withdraw before you complete the study then you will not receive the 9 course credits. If you withdraw from the study, you can request that we destroy the data we have gathered about you up until we have entered it into our secure database and begun analysis (approximately September 2021).

What will happen to the results of the research?
When the study has been completed, we shall analyse the data and report the findings. This will be reported in an appropriate scientific journal or presented at a scientific meeting. Your personal details will remain strictly confidential. Research findings made available in any reports or publications will not include information that can directly identify you without your specific consent. If you would like a copy of the final paper, you may request this.

Once the project is complete, all data will be transferred and stored in the University Research Repository. We will seek your consent to store the data openly under the open science framework. Your data will be held in an anonymised form and unlinked to any personal identifiable information. This allows other researchers to use the data for future research without being able to identify you. Data will be stored here for a minimum of 15 years.

Where can I get more information?
For further queries, please contact Dr Nathan Huneke (n.huneke@soton.ac.uk, tel: 02382 310 775).

What happens if there is a problem?
If you have a concern about any aspect of this study, you should speak to the researchers who will do their best to answer your questions.

If you remain unhappy or have a complaint about any aspect of this study, please contact the University of Southampton Research Integrity and Governance Manager (023 8059 5058, rgoinfo@soton.ac.uk).

Data Protection Privacy Notice
The University of Southampton conducts research to the highest standards of research integrity. As a publicly-funded organisation, the University has to ensure that it is in the public interest when we use personally-identifiable information about people who have agreed to take part in research. This means that when you agree to take part in a research study, we will use information about you in the ways needed, and for the purposes specified, to conduct and complete the research project. Under data protection law, 'Personal data' means any information that relates to and is capable of identifying a living individual. The University's data protection policy governing the use of personal data by the University can be found on its website (https://www.southampton.ac.uk/legalservices/what-we-do/data-protection-and-foi.page).

This Participant Information Sheet tells you what data will be collected for this project and whether this includes any personal data. Please ask the research team if you have any questions or are unclear what data is being collected about you.

Our privacy notice for research participants provides more information on how the University of Southampton collects and uses your personal data when you take part in one of our research projects and can be found at http://www.southampton.ac.uk/assets/sharepoint/intranet/ls/Public/Research%20and%20Integrity%20Privacy%20Notice/Privacy%20Notice%20for%20Research%20Participants.pdf

Any personal data we collect in this study will be used only for the purposes of carrying out our research and will be handled according to the University's policies in line with data protection law. If any personal data is used from which you can be identified directly, it will not be disclosed to anyone else without your consent unless the University of Southampton is required by law to disclose it.

Data protection law requires us to have a valid legal reason ('lawful basis') to process and use your Personal data. The lawful basis for processing personal information in this research study is for the performance of a task carried out in the public interest. Personal data collected for research will not be used for any other purpose.

For the purposes of data protection law, the University of Southampton is the 'Data Controller' for this study, which means that we are responsible for looking after your information and using it properly. The University of Southampton will keep identifiable information about you for 15 years after the study has finished after which time any link between you and your information will be removed.

To safeguard your rights, we will use the minimum personal data necessary to achieve our research study objectives. Your data protection rights – such as to access, change, or transfer such information - may be limited, however, in order for the research output to be reliable and accurate. The University will not do anything with your personal data that you would not reasonably expect.

If you have any questions about how your personal data is used, or wish to exercise any of your rights, please consult the University's data protection webpage (https://www.southampton.ac.uk/legalservices/what-we-do/data-protection-and-foi.page) where you can make a request using our online form. If you need further assistance, please contact the University's Data Protection Officer (data.protection@soton.ac.uk).

Thank you.

D.2 Debriefing statement

Study title: *Investigating the role of perspective taking in social anxiety during videoconferencing*

Debriefing statement: *V1, 17/09/2020*
Project Lead: *Dr Nathan Huneke*
Supervisors*: Prof Matthew Garner, Prof David Baldwin*
Other researchers: *Alex McEwan*
ERGO number*: 61411*

Thank you for taking part in this experiment. A secondary aim of this research was to examine the effects of a remotely delivered placebo treatment on anxiety experienced during a social interaction. Your data will help us to understand whether it is possible to induce a placebo response on anxiety remotely. This experiment involved deception so that we could attempt to induce a placebo response.

There is a need for improved treatments for patients with anxiety disorders, as many patients do not respond to first line psychological or pharmacological treatments, and drug treatments can cause unwanted side-effects. We normally test a new treatment by comparing it with a placebo; a 'dummy' treatment with no active ingredients. Sometimes, patients improve when given a placebo, and this is called 'the placebo response'. If many patients show this response in a clinical trial, it is difficult to establish whether a new treatment is effective. Learning more about how the placebo response works should make it easier to test potential new treatments.

You were not given an active treatment at any point during this study. The abstract pattern you were shown was a stock video. You were randomized to one of two groups: 1) placebo with an expectation of it reducing anxiety or 2) placebo with no expectation of it reducing anxiety. If you were in group 1, then you were told that the video combining the colour yellow with movement was a treatment we had developed to activate certain parts of your brain that reduce anxiety. In reality there is no evidence that combining the colour yellow with movement reduces anxiety. The purpose of this was to see whether your beliefs about the treatment meant that you felt less anxious when you subsequently completed the social interaction. If you were in group 2, then you were told that the video was a placeholder to fill time while the other group received a treatment. We did this to ensure that participants in group 2 had no expectation of a benefit from the video. We can therefore compare the effects of expectation on anxiety during the social interaction between the two groups.

In addition, to cause mild anxiety during the social interaction we informed you that three observers were watching you. In reality, some or all of these 'observers' were dummy accounts created to give the impression of a full observing panel.

You may have a copy of this summary and a summary of the research findings once the project is completed if you wish. If you have any further questions please contact me, Dr Nathan Huneke, at n.huneke@soton.ac.uk, tel: 02382310775.

If you are interested in finding out more about the rationale behind this research and the methods used in this project, please see the references below:

1. *Gaab et al. (2019). Effects and Components of Placebos with a Psychological Treatment Rationale – Three Randomized-Controlled Studies. Scientific Reports, 9:1421*

2. *Norton, A.R. and Abbot, M.J. (2016). Self-Focused Cognition in Social Anxiety: A Review of the Theoretical and Empirical Literature. Behaviour Change, 33(1): 44-64*

During this study we asked you to reflect on certain aspects of your physical and mental health. If at any point during your studies you become concerned about your mental or physical health then please contact your General Practitioner.

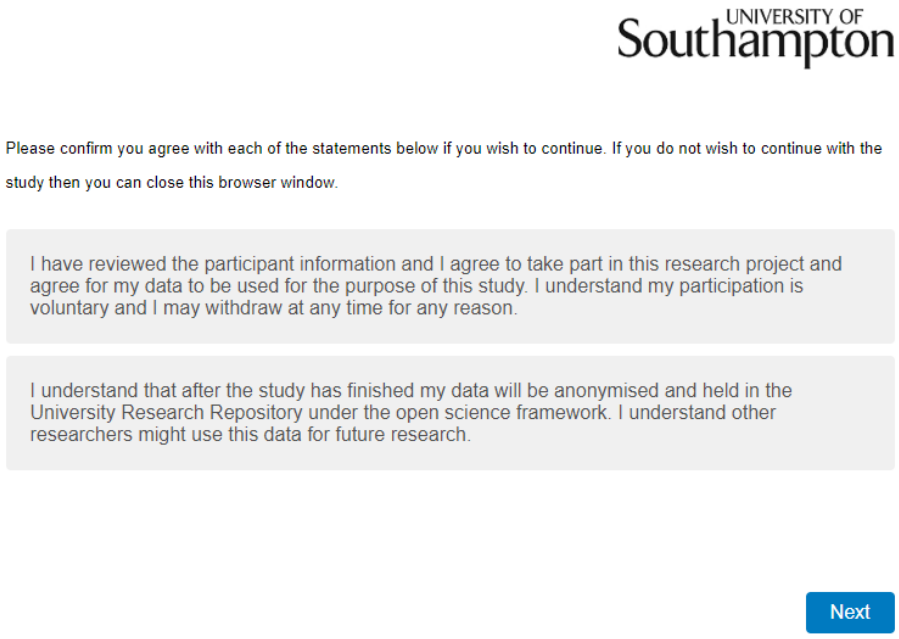
If you have more general worries during your time as a student in Southampton, then please also be aware that Enabling Services or your personal tutor are available to provide support and advice. To contact Enabling Services, please email enable@soton.ac.uk or telephone 02380 597 726. Skype appointments are also available.

Thank you again for your participation in this research.

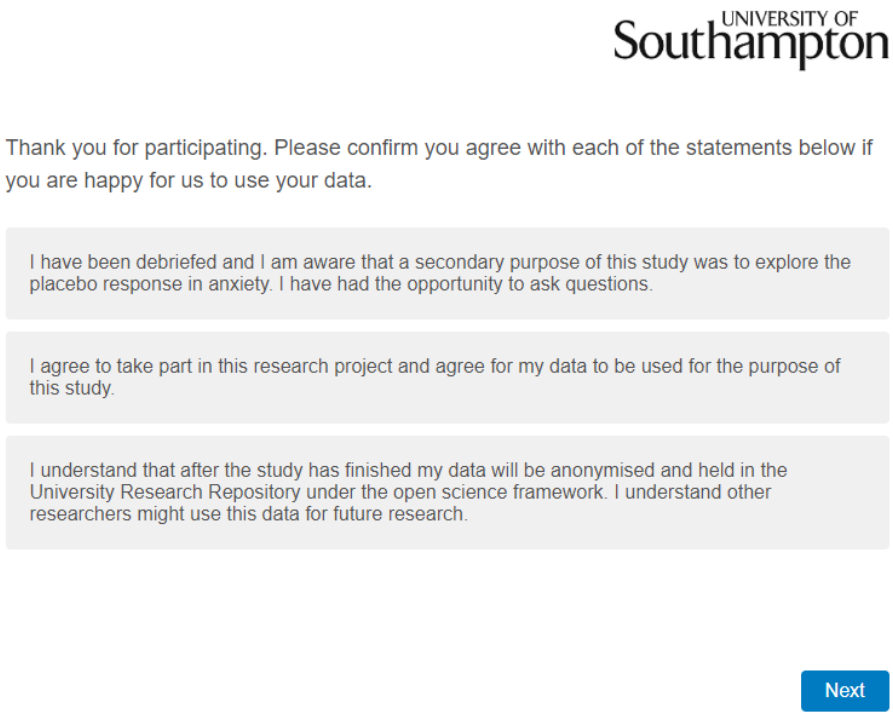
If you have questions about your rights as a participant in this research, or if you feel that you have been placed at risk, you may contact the University of Southampton Research Integrity and Governance Manager (023 8059 5058, rgoinfo@soton.ac.uk).

D.3 Consent forms

D.3.1 Consent form 1



D.3.2 Consent form 2



Appendix E ITSSAD study: psychometric and questionnaire measures (Chapter 6)

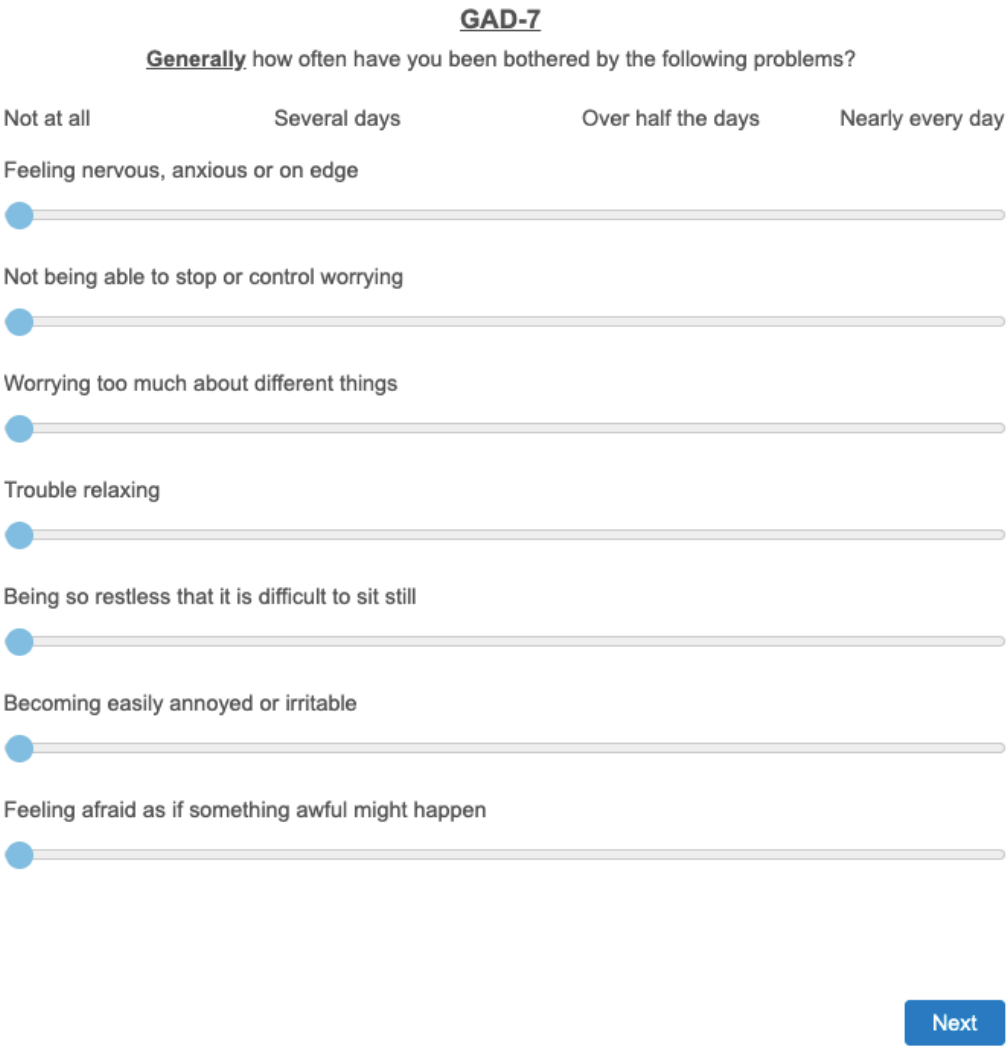
E.1 Social Phobia Inventory (SPIN)

Social Phobia Inventory (SPIN)

Please indicate how much the following problems have bothered you during the past week.

	Not at all	A little bit	Somewhat	Very much	Extremely
I am afraid of people in authority	○	○	○	○	○
I am bothered by blushing in front of people	○	○	○	○	○
Parties and social events scare me	○	○	○	○	○
I avoid talking to people I don't know	○	○	○	○	○
Being criticized scares me a lot	○	○	○	○	○
Fear of embarrassment causes me to avoid doing things or speaking to people	○	○	○	○	○
Sweating in front of people causes me distress	○	○	○	○	○
I avoid going to parties	○	○	○	○	○
I avoid activities in which I am the center of attention	○	○	○	○	○
Talking to strangers scares me	○	○	○	○	○
I avoid having to give speeches	○	○	○	○	○
I would do anything to avoid being criticized	○	○	○	○	○
Heart palpitations bother me when I am around people	○	○	○	○	○
I am afraid of doing things when people might be watching	○	○	○	○	○

E.2 Trait Modified Generalised Anxiety Disorder 7-item (GAD-7)



E.3 Social Interaction Anxiety Scale (SIAS)

Social Interaction Anxiety Scale (SIAS)

Instructions: For each item, please choose the column to indicate the degree to which you feel the statement is characteristic or true for you.

	Not at all characteristic or true of me	Slightly characteristic or true of me	Moderately characteristic or true of me	Very characteristic or true of me	Extremely characteristic or true of me
I get nervous if I have to speak with someone in authority (teacher, boss, etc.)	○	○	○	○	○
I have difficulty making eye contact with others	○	○	○	○	○
I become tense if I have to talk about myself or my feelings	○	○	○	○	○
I find it difficult to mix comfortably with the people I work with	○	○	○	○	○
I find it easy to make friends my own age	○	○	○	○	○
I tense up if I meet an acquaintance in the street	○	○	○	○	○
When mixing socially, I am uncomfortable	○	○	○	○	○

	Not at all characteristic or true of me	Slightly characteristic or true of me	Moderately characteristic or true of me	Very characteristic or true of me	Extremely characteristic or true of me
I feel tense if I am alone with just one other person	○	○	○	○	○
I am at ease meeting people at parties, etc.	○	○	○	○	○
I have difficulty talking with other people	○	○	○	○	○
I find it easy to think of things to talk about	○	○	○	○	○

I worry about expressing myself in case I appear awkward	○	○	○	○	○
I find it difficult to disagree with another's point of view	○	○	○	○	○
I have difficulty talking to attractive persons of the opposite sex	○	○	○	○	○

	Not at all characteristic or true of me	Slightly characteristic or true of me	Moderately characteristic or true of me	Very characteristic or true of me	Extremely characteristic or true of me
I find myself worrying that I won't know what to say in social situations	○	○	○	○	○
I am nervous mixing with people I don't know well	○	○	○	○	○
I feel I'll say something embarrassing when talking	○	○	○	○	○
When mixing in a group, I find myself worrying I will be ignored	○	○	○	○	○
I am tense mixing in a group	○	○	○	○	○
I am unsure whether to greet someone I know only slightly	○	○	○	○	○

E.4 Brief Fear of Negative Evaluation scale (BFNE)

Brief Fear of Negative Evaluation scale

Read each of the following statements carefully and indicate how characteristic it is of you

I worry about what other people will think of me even when I know it doesn't make any difference	✓ Not at all characteristic of me / Slightly characteristic of me / Moderately characteristic of me / Very characteristic of me / Extremely characteristic of me
I am unconcerned even if I know people are forming an unfavourable impression of me	
I am frequently afraid of other people noticing my shortcomings	
I rarely worry about what kind of impression I am making on someone	
I am afraid others will not approve of me	
I am afraid that people will find fault with me	
Other people's opinions of me do not bother me	
When I am talking to someone, I worry about what they may be thinking about me	
I am usually worried about what kind of impression I make	
If I know someone is judging me, it has little effect on me	
Sometimes I think I am too concerned with what other people think of me	
I often worry that I will say or do the wrong things	

Previous Next

E.5 Positive and Negative Affect Schedule (PANAS)

PANAS

This scale consists of a number of words that describe different feelings and emotions. Read each item and indicate to what extent you feel this way **RIGHT NOW**.

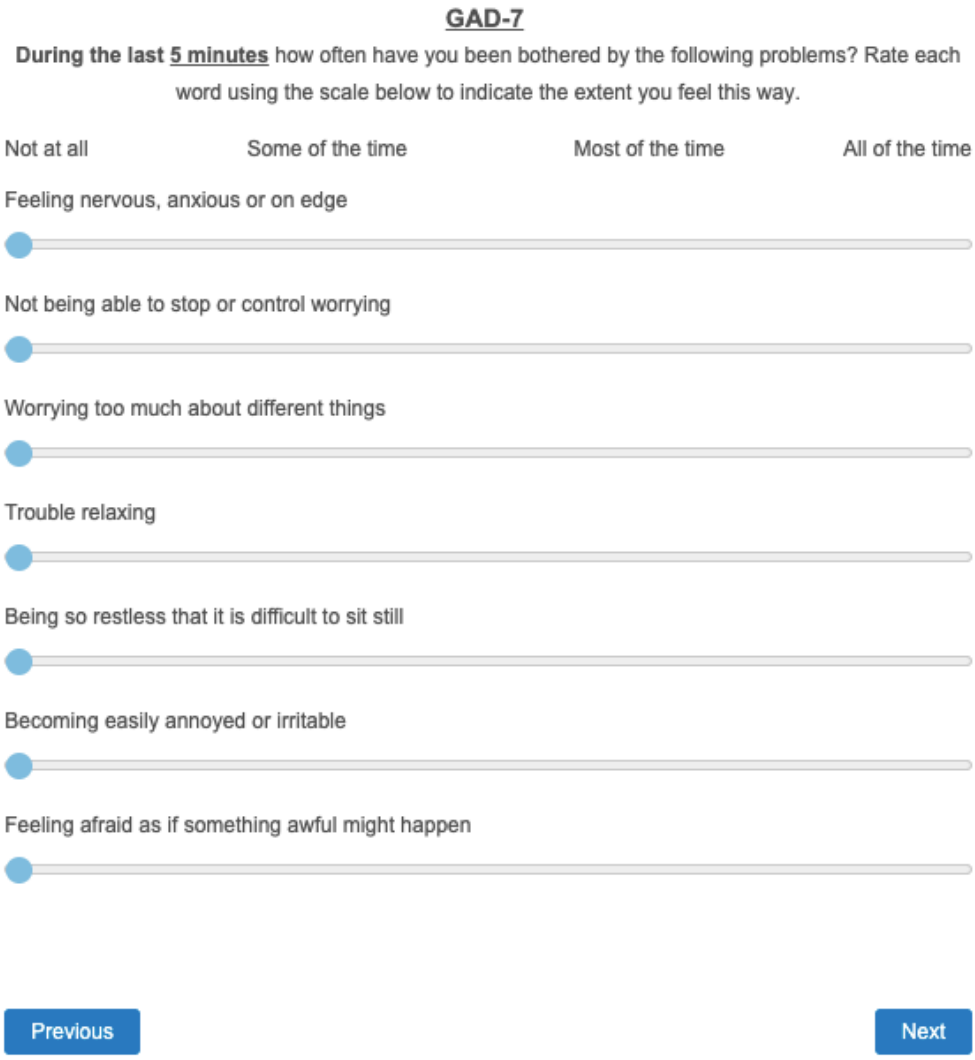
	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
Interested	○	○	○	○	○
Distressed	○	○	○	○	○
Excited	○	○	○	○	○
Upset	○	○	○	○	○
Strong	○	○	○	○	○
Guilty	○	○	○	○	○
Scared	○	○	○	○	○

	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
Hostile	○	○	○	○	○
Enthusiastic	○	○	○	○	○
Proud	○	○	○	○	○
Irritable	○	○	○	○	○
Alert	○	○	○	○	○
Ashamed	○	○	○	○	○
Inspired	○	○	○	○	○

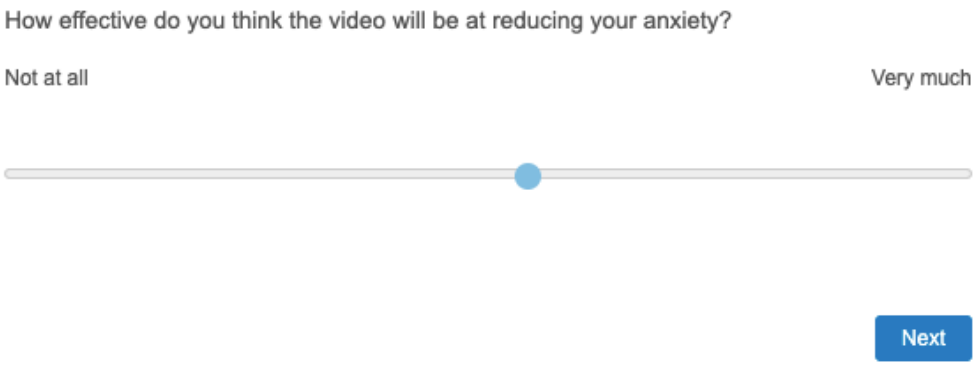
	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
Nervous	○	○	○	○	○
Determined	○	○	○	○	○
Attentive	○	○	○	○	○
Jittery	○	○	○	○	○
Active	○	○	○	○	○
Afraid	○	○	○	○	○

Next

E.6 State modified Generalised Anxiety Disorder 7-item (GAD-7)



E.7 Expectations (Visual analogue scale)



E.8 Perception of Speech Performance scale (SPS)

Perception of Speech Performance scale

We would like you to rate yourself on the features listed below. For each feature, choose the appropriate column to indicate how you felt you actually performed during the social interaction. Your evaluation will remain confidential.

	Not at all	Slightly	Moderately	Much	Very much
My statements were understandable	○	○	○	○	○
I kept eye contact	○	○	○	○	○
I stuttered	○	○	○	○	○
I had long pauses of more than 5 seconds	○	○	○	○	○
I fidgeted	○	○	○	○	○
I "um"ed and "ah"ed	○	○	○	○	○
	Not at all	Slightly	Moderately	Much	Very much
I had a clear voice	○	○	○	○	○
I seemed to tremble or shake	○	○	○	○	○
I sweated	○	○	○	○	○
I blushed	○	○	○	○	○
My face twitched	○	○	○	○	○
My voice quivered	○	○	○	○	○
	Not at all	Slightly	Moderately	Much	Very much
I appeared confident	○	○	○	○	○
I appeared nervous	○	○	○	○	○
I kept the other person interested	○	○	○	○	○
I generally spoke well	○	○	○	○	○
I made a good impression	○	○	○	○	○

Appendix F ITSSAD Treatment scripts

F.1 Placebo

Thank you very much for taking part in this project. I'm Dr Nathan Huneke, a psychiatrist and the lead for this project. You have been randomised to our treatment group. We have recently gathered data that suggests that perceiving the colour yellow combined with some movement appears to stimulate regions of your brain that lead to feelings of relaxation and reductions in anxiety. With so many of our interactions and treatments that we give moving online we are testing today whether giving a treatment that combines yellow colour and movement, delivered remotely, can help people who experience anxiety with online interactions. You are about to view a video that combines the colour yellow and some movement. Try to concentrate on the colour and allow it to fully impact you. Our research suggests that the more you engage, the more it activates the brain, and the more likely you are to benefit. The video will start shortly.

F.2 Control

Thank you very much for taking part in this project. I'm Dr Nathan Huneke, a psychiatrist and the lead for this project. You are in the control group for this study. At this point in the protocol, individuals in the other group receive a treatment that might reduce their anxiety. We need to ensure that the timings of the experiment match, so we are going to fill this time with a brief video. You are about to watch an abstract video that should not affect your experience during the rest of the experiment. The video will start shortly.

Appendix G ITSSAD Analysis Code and Outputs

```
library(tidyverse)

wide_data <- read.csv('../inputs/anonymised_dataset.csv')
wide_data$gender <- as.factor(wide_data$gender)
wide_data$placebogroup <- as.factor(wide_data$placebogroup)
wide_data$placebogroup <- relevel(wide_data$placebogroup, "placebo")
```

Baseline characteristics are summarised in Table 1.

```
library(tableone)

table1 <- CreateTableOne(
  vars = c("age", "gender", "spin", "brief_fne", "sias.total_score", "gad_baseline", "sps.total_sco
re"),
  strata = c("placebogroup"),
  data = wide_data
)

table1_for_kable <- print(table1, cramVars = "gender", exact = "gender")

kableExtra::kable(table1_for_kable, booktabs = T)
```

	placebo	control	p	test
n	38	38		
age (mean (SD))	19.05 (1.29)	19.18 (1.61)	0.695	
gender = Female/Male (%)	34/4 (89.5/10.5)	36/2 (94.7/5.3)	0.674	exact
spin (mean (SD))	38.11 (12.56)	37.61 (11.49)	0.857	
brief_fne (mean (SD))	32.61 (5.94)	34.18 (5.33)	0.234	
sias.total_score (mean (SD))	42.56 (12.94)	41.55 (13.96)	0.750	
gad_baseline (mean (SD))	247.72 (133.64)	259.39 (130.27)	0.705	
sps.total_score (mean (SD))	32.40 (9.60)	31.82 (11.62)	0.823	

Here are individual statistical tests

```
t.test(age ~ placebogroup, data = wide_data, var.equal = TRUE)


    Two Sample t-test

data:  age by placebogroup
t = -0.393, df = 74, p-value = 0.6954
alternative hypothesis: true difference in means between group placebo and group control is not equ
al to 0
95 percent confidence interval:
 -0.7986936  0.5355357
sample estimates:
mean in group placebo mean in group control
             19.05263              19.18421

t.test(spin ~ placebogroup, data = wide_data, var.equal = TRUE)


    Two Sample t-test

data:  spin by placebogroup
t = 0.18107, df = 74, p-value = 0.8568
alternative hypothesis: true difference in means between group placebo and group control is not equ
al to 0
95 percent confidence interval:
```

```
 -5.002067  6.002067
sample estimates:
mean in group placebo mean in group control
             38.10526              37.60526

t.test(sias.total_score ~ placebogroup, data = wide_data, var.equal = TRUE)


    Two Sample t-test

data:  sias.total_score by placebogroup
t = 0.32005, df = 72, p-value = 0.7499
alternative hypothesis: true difference in means between group placebo and group control is not equ
al to 0
95 percent confidence interval:
 -5.243873  7.249721
sample estimates:
mean in group placebo mean in group control
             42.55556              41.55263

t.test(brief_fne ~ placebogroup, data = wide_data, var.equal = TRUE)


    Two Sample t-test

data:  brief_fne by placebogroup
t = -1.1998, df = 72, p-value = 0.2342
alternative hypothesis: true difference in means between group placebo and group control is not equ
al to 0
95 percent confidence interval:
 -4.186874  1.040675
sample estimates:
mean in group placebo mean in group control
             32.61111              34.18421

t.test(gad_baseline ~ placebogroup, data = wide_data, var.equal = TRUE)


    Two Sample t-test

data:  gad_baseline by placebogroup
t = -0.38044, df = 72, p-value = 0.7047
alternative hypothesis: true difference in means between group placebo and group control is not equ
al to 0
95 percent confidence interval:
 -72.83546  49.49043
sample estimates:
mean in group placebo mean in group control
             247.7222              259.3947

fisher.test(wide_data$gender, wide_data$placebogroup)


    Fisher's Exact Test for Count Data

data:  wide_data$gender and wide_data$placebogroup
p-value = 0.6745
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
 0.04063127 3.57514408
sample estimates:
odds ratio
 0.4767756
```

G.1 Expectations

Were the verbal instructions successful?

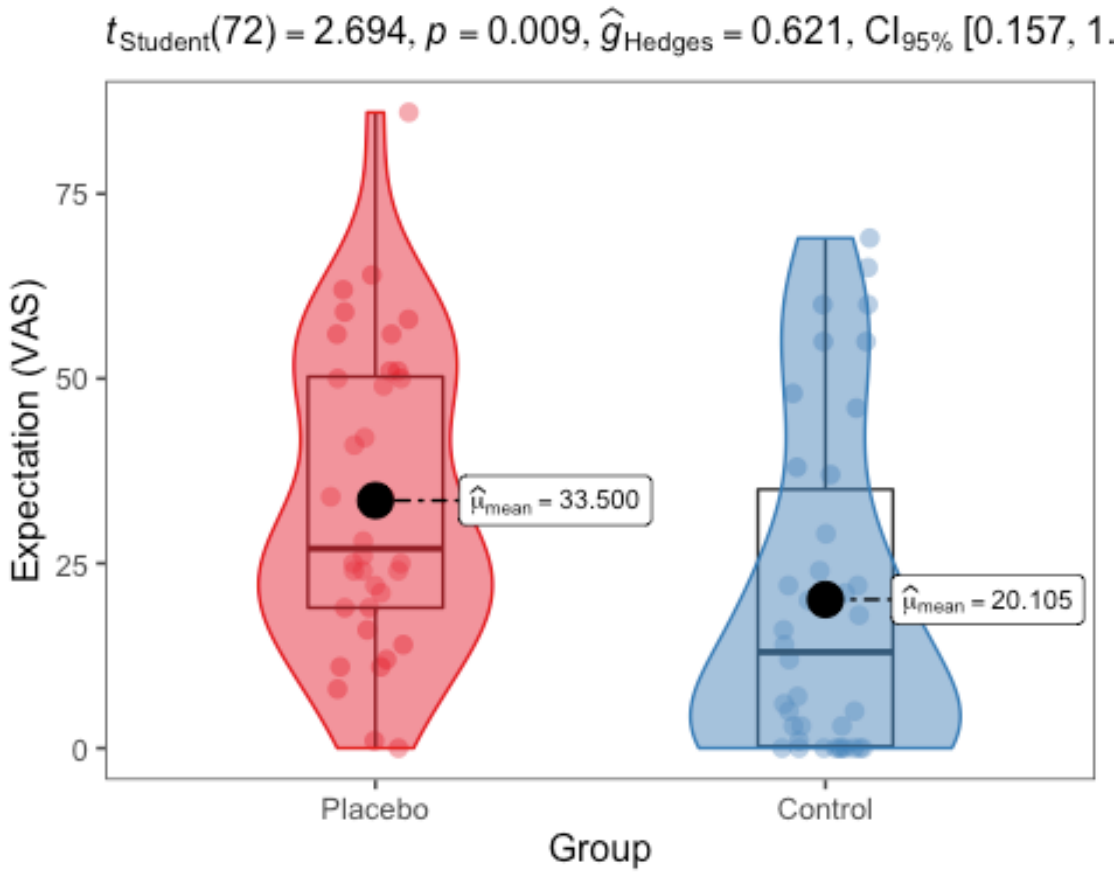
```
library(rstatix)
library(ggstatsplot)

levene_test(data = wide_data, expectancy ~ placebogroup)
```

```
# A tibble: 1 × 4
    df1   df2 statistic     p
  <int> <int>     <dbl> <dbl>
1     1    72    0.0957 0.758
```

```
kableExtra::kable(
  t_test(expectancy ~ placebogroup, data = wide_data, var.equal = TRUE, detailed = TRUE),
)
```

estimate	estimate1	estimate2	.y.	group1	group2	n1	n2	statistic	p	df	conf.low	conf.high
13.39474	33.5	20.10526	expectancy	placebo	control	38	38	2.69393	0.00878	72	3.482852	23.30662

```
ggbetweenstats(
  data = wide_data,
  x = placebogroup,
  y = expectancy,
  plot.type = "boxviolin",
  var.equal = TRUE,
  bf.message = FALSE,
  k = 3, # number of decimal places in all values
  xlab = "Group",
  ylab = "Expectation (VAS)",
  centrality.point.args = list(size = 5, color = "black"),
  violin.args = list(
    aes(
    fill = placebogroup,
    colour = placebogroup
    ),
    alpha = 0.5, width = 0.6),
  palette = "Set1",
  ggtheme = ggthemes::theme_few()
) +
  scale_x_discrete(labels = c("Placebo", "Control")) +
  scale_fill_manual(values = c("#E41A1C", "#377EB8"))
```



Placebo group significantly higher expectation, but mean still relatively low (only 33.50). Big range of expectation though, with some moderate to high (in both groups).

G.2 Mixed Effects Models

```
library(afex)
library(emmeans)
library(performance)

data <- read.csv('../inputs/dataset_long_format.csv')
data$time <- as.factor(data$time)
data$time <- relevel(data$time, "baseline")  # put baseline first
data$placebogroup <- as.factor(data$placebogroup)
data$placebogroup <- relevel(data$placebogroup, "placebo")
```

G.2.1 Anxiety

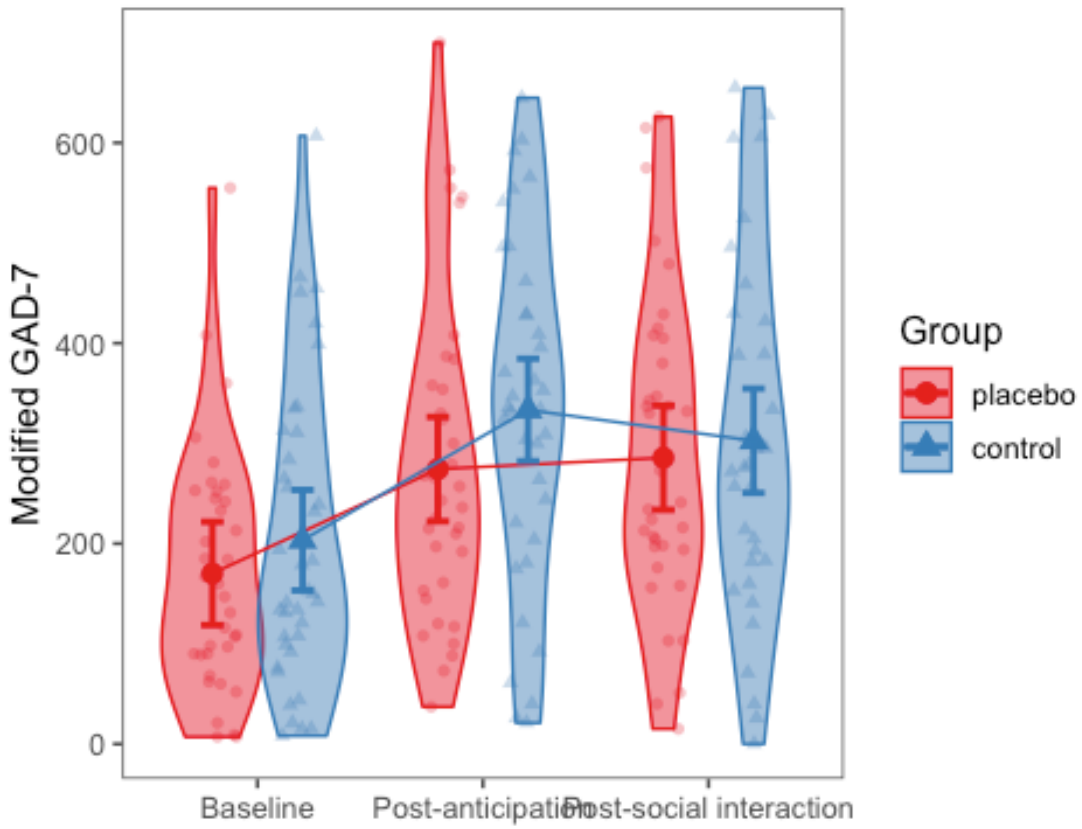
```
model_gad = mixed(
  gad7 ~ 1 + time + placebogroup + time*placebogroup + (1|participant_ID),
  data = data
  )

kableExtra::kable(model_gad$anova_table)
```

	num Df	den Df	F	Pr(>F)
time	2	138.14779	26.7518927	0.0000000
placebogroup	1	72.00792	1.4255511	0.2364104
time:placebogroup	2	138.14779	0.6986918	0.4989838

```
summary(model_gad)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula:
gad7 ~ 1 + time + placebogroup + time * placebogroup + (1 | participant_ID)
   Data: data

REML criterion at convergence: 2666.7

Scaled residuals:
     Min       1Q   Median       3Q      Max
-2.21291 -0.55056 -0.03111  0.47919  2.81383

Random effects:
 Groups         Name        Variance Std.Dev.
 participant_ID (Intercept) 13154    114.7
 Residual                   11290    106.3
Number of obs: 214, groups:  participant_ID, 74

Fixed effects:
                   Estimate Std. Error      df t value Pr(>|t|)
(Intercept)         261.599     15.245  72.008  17.160  < 2e-16 ***
time1               -74.831     10.267 139.171  -7.288 2.14e-11 ***
time2                42.265     10.315 137.382   4.097 7.11e-05 ***
placebogroup1       -18.202     15.245  72.008  -1.194    0.236
time1:placebogroup1   1.601     10.267 139.171   0.156    0.876
time2:placebogroup1 -11.335     10.315 137.382  -1.099    0.274
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) time1  time2  plcbg1 tm1:p1
time1       -0.023
time2        0.005 -0.488
placebogrp1  0.020  0.010  0.002
tm1:plcbgr1  0.010  0.011 -0.015 -0.023
tm2:plcbgr1  0.002 -0.015  0.008  0.005 -0.488

kableExtra::kable(model_performance(model_gad))
```

R2_conditional	R2_marginal	ICC	RMSE	Sigma
0.592742	0.1182714	0.5381141	NA	106.2557

```
afex_plot(
  model_gad,
  x = "time",
  trace = "placebogroup",
  mapping = c("shape", "color", "fill"),
  dodge = 0.8,
  data_geom = geom_violin,
  data_arg = list(dodge.width = 0.8),
  point_arg = list(size = 3),
  error_arg = list(size = 1, width = 0.2),
  legend_title = "Group"
) +
  geom_jitter(
    inherit.aes = FALSE,
    aes(
      x = time,
      y = gad7,
      colour = placebogroup,
      shape = placebogroup
    ),
    data = data,
    position = position_jitterdodge(),
    alpha = 0.3
    ) +
  scale_color_brewer(palette = "Set1") +
  scale_fill_brewer(palette = "Set1") +
  ggthemes::theme_few() +
  labs(y = "Modified GAD-7", x = "") +
  scale_x_discrete(labels = c("Baseline", "Post-anticipation", "Post-social interaction"))
```




```
gad_means <- emmeans(model_gad, list(pairwise ~ time*placebogroup), adjust = "Tukey")

kableExtra::kable(gad_means$`emmeans of time, placebogroup`)
```

time	placebogroup	emmean	SE	df	lower.CL	upper.CL
baseline	placebo	170.1667	26.05754	133.3961	118.6273	221.7061
anticipation	placebo	274.3261	26.32396	136.0353	222.2690	326.3832
end	placebo	285.6975	26.32396	136.0353	233.6404	337.7546
baseline	control	203.3684	25.36255	133.3961	153.2036	253.5332
anticipation	control	333.4003	25.86437	138.4582	282.2601	384.5405
end	control	302.6322	26.34508	144.2398	250.5599	354.7045

```
kableExtra::kable(gad_means$`pairwise differences of time, placebogroup`)
```

1	estimate	SE	df	t.ratio	p.value
baseline placebo - anticipation placebo	-104.15941	25.32177	137.5302	-4.1134333	0.0009294
baseline placebo - end placebo	-115.53084	25.32177	137.5302	-4.5625104	0.0001592
baseline placebo - baseline control	-33.20175	36.36282	133.3961	-0.9130688	0.9425747
baseline placebo - anticipation control	-163.23362	36.71459	135.8946	-4.4460149	0.0002569
baseline placebo - end control	-132.46549	37.05481	138.8229	-3.5748527	0.0063010
anticipation placebo - end placebo	-11.37143	25.39996	136.2949	-0.4476947	0.9976921
anticipation placebo - baseline control	70.95766	36.55420	134.7608	1.9411629	0.3819370
anticipation placebo - anticipation control	-59.07421	36.90415	137.2224	-1.6007470	0.5994835
anticipation placebo - end control	-28.30607	37.24264	140.1118	-0.7600448	0.9736427
end placebo - baseline control	82.32909	36.55420	134.7608	2.2522470	0.2212985
end placebo - anticipation control	-47.70278	36.90415	137.2224	-1.2926128	0.7886413
end placebo - end control	-16.93465	37.24264	140.1118	-0.4547112	0.9975160
baseline control - anticipation control	-130.03187	24.89842	138.6541	-5.2224952	0.0000093
baseline control - end control	-99.26373	25.39742	139.6405	-3.9084180	0.0019692
anticipation control - end control	30.76813	25.54085	137.3512	1.2046638	0.8339505

G.2.2 PANAS positive

```
model_panas_pos = mixed(
  panas_pos ~ 1 + time + placebogroup + time*placebogroup + (1|participant_ID),
  data = data
  )

kableExtra::kable(model_panas_pos$anova_table)
```

	num Df	den Df	F	Pr(>F)
time	2	135.62923	17.7392832	0.0000001
placebogroup	1	70.74355	1.2352464	0.2701522
time:placebogroup	2	135.62923	0.1912519	0.8261468

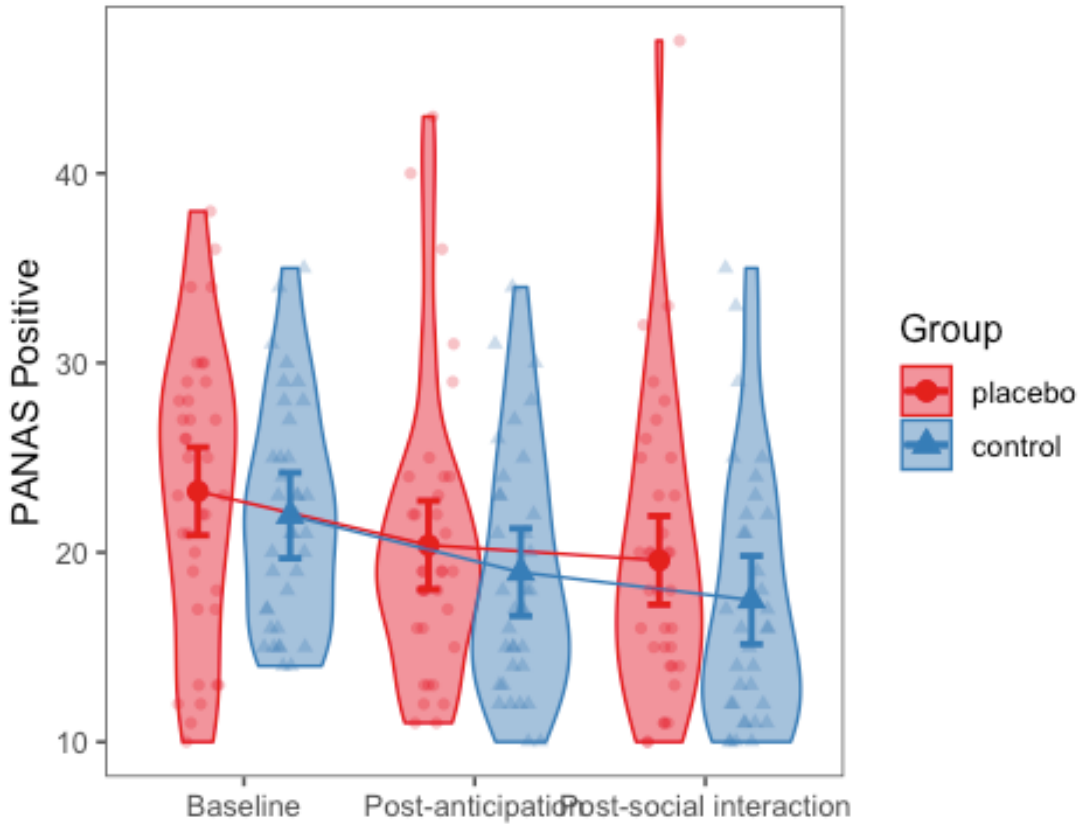
```
summary(model_panas_pos)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: panas_pos ~ 1 + time + placebogroup + time * placebogroup + (1 |
    participant_ID)
   Data: data

REML criterion at convergence: 1339.4

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.6153 -0.5450 -0.0757  0.4509  3.5469

Random effects:
 Groups         Name        Variance Std.Dev.
 participant_ID (Intercept) 32.07    5.663
```

```
 Residual                   17.31    4.161
Number of obs: 213, groups:  participant_ID, 74

Fixed effects:
                     Estimate Std. Error        df t value Pr(>|t|)
(Intercept)          20.26221    0.72042  70.74355  28.126  < 2e-16 ***
time1                 2.32258    0.40420 136.91175   5.746 5.67e-08 ***
time2                -0.59491    0.40610 134.89944  -1.465    0.145
placebogroup1         0.80068    0.72042  70.74355   1.111    0.270
time1:placebogroup1  -0.16326    0.40420 136.91175  -0.404    0.687
time2:placebogroup1  -0.08475    0.40610 134.89944  -0.209    0.835
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) time1  time2  plcbg1 tm1:p1
time1       -0.025
time2        0.010 -0.493
placebogrp1  0.019  0.013 -0.004
tm1:plcbgr1  0.013  0.003 -0.006 -0.025
tm2:plcbgr1 -0.004 -0.006 -0.002  0.010 -0.493
```

```
kableExtra::kable(model_performance(model_panas_pos))
```

R2_conditional	R2_marginal	ICC	RMSE	Sigma
0.6731969	0.0678232	0.6494194	NA	4.160676

```
afex_plot(
  model_panas_pos,
  x = "time",
  trace = "placebogroup",
  mapping = c("shape", "color", "fill"),
  dodge = 0.8,
  data_geom = geom_violin,
  data_arg = list(dodge.width = 0.8),
  point_arg = list(size = 3),
  error_arg = list(size = 1, width = 0.2),
  legend_title = "Group"
) +
  geom_jitter(
    inherit.aes = FALSE,
    aes(
      x = time,
      y = panas_pos,
      colour = placebogroup,
      shape = placebogroup
    ),
    data = data,
    position = position_jitterdodge(),
    alpha = 0.3
    ) +
  scale_color_brewer(palette = "Set1") +
  scale_fill_brewer(palette = "Set1") +
  ggthemes::theme_few() +
  labs(y = "PANAS Positive", x = "") +
  scale_x_discrete(labels = c("Baseline", "Post-anticipation", "Post-social interaction"))
```




```
panas_pos_means <- emmeans(model_panas_pos, list(pairwise ~ time*placebogroup),
                           adjust = "Tukey")

kableExtra::kable(panas_pos_means$`emmeans of time, placebogroup`)
```

time	placebogroup	emmean	SE	df	lower.CL	upper.CL
baseline	placebo	23.22222	1.171167	114.4423	20.90225	25.54220
anticipation	placebo	20.38324	1.180906	116.8349	18.04448	22.72199
end	placebo	19.58324	1.180906	116.8349	17.24448	21.92199
baseline	control	21.94737	1.139930	114.4423	19.68927	24.20547
anticipation	control	18.95137	1.168138	121.5109	16.63883	21.26391
end	control	17.48585	1.176834	124.0141	15.15657	19.81513

```
kableExtra::kable(panas_pos_means$`pairwise differences of time, placebogroup`)
```

1	estimate	SE	df	t.ratio	p.value
baseline placebo - anticipation placebo	2.8389870	0.9922917	136.2422	2.8610406	0.0541183
baseline placebo - end placebo	3.6389870	0.9922917	136.2422	3.6672552	0.0046369
baseline placebo - baseline control	1.2748538	1.6343414	114.4423	0.7800413	0.9703872
baseline placebo - anticipation control	4.2708538	1.6541400	117.9222	2.5819180	0.1099007
baseline placebo - end control	5.7363710	1.6602922	119.1710	3.4550369	0.0097086
anticipation placebo - end placebo	0.8000000	0.9945918	135.2148	0.8043501	0.9662952
anticipation placebo - baseline control	-1.5641332	1.6413348	115.6753	-0.9529642	0.9315432
anticipation placebo - anticipation control	1.4318668	1.6610500	119.1282	0.8620251	0.9546914
anticipation placebo - end control	2.8973839	1.6671768	120.3683	1.7378984	0.5097417
end placebo - baseline control	-2.3641332	1.6413348	115.6753	-1.4403723	0.7023732
end placebo - anticipation control	0.6318668	1.6610500	119.1282	0.3804020	0.9989413

1	estimate	SE	df	t.ratio	p.value
end placebo - end control	2.0973839	1.6671768	120.3683	1.2580453	0.8069627
baseline control - anticipation control	2.9960000	0.9880407	138.1724	3.0322636	0.0337754
baseline control - end control	4.4615172	0.9983065	138.5110	4.4690857	0.0002314
anticipation control - end control	1.4655172	1.0047906	135.6042	1.4585299	0.6910616

G.2.3 PANAS negative

```
model_panas_neg = mixed(
  panas_neg ~ 1 + time + placebogroup + time*placebogroup + (1|participant_ID),
  data = data
  )

kableExtra::kable(model_panas_neg$anova_table)
```

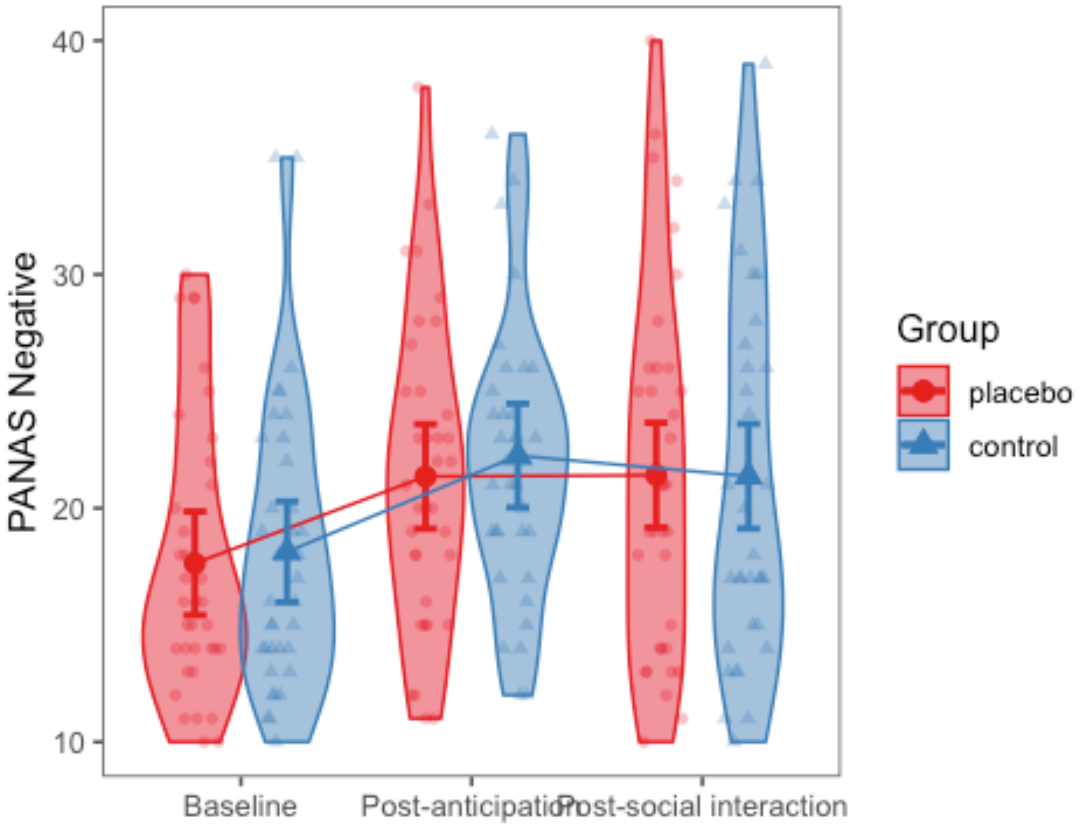
	num Df	den Df	F	Pr(>F)
time	2	135.57788	15.7247513	0.0000007
placebogroup	1	70.03258	0.1152439	0.7352671
time:placebogroup	2	135.57788	0.1832376	0.8327761

```
summary(model_panas_neg)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: panas_neg ~ 1 + time + placebogroup + time * placebogroup + (1 |
    participant_ID)
   Data: data

REML criterion at convergence: 1351.5

Scaled residuals:
    Min      1Q  Median      3Q     Max
-3.0044 -0.5382 -0.0426  0.4507  2.4736

Random effects:
 Groups         Name        Variance Std.Dev.
 participant_ID (Intercept) 24.21    4.920
 Residual                   20.92    4.574
Number of obs: 213, groups:  participant_ID, 74

Fixed effects:
                    Estimate Std. Error        df t value Pr(>|t|)
(Intercept)          20.35953    0.65552  70.03259  31.059  < 2e-16 ***
time1                -2.47429    0.44344 137.11778  -5.580 1.24e-07 ***
time2                 1.44295    0.44621 134.68471   3.234  0.00154 **
placebogroup1        -0.22253    0.65552  70.03259  -0.339  0.73527
time1:placebogroup1  -0.02381    0.44344 137.11778  -0.054  0.95725
time2:placebogroup1  -0.22247    0.44621 134.68471  -0.499  0.61889
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) time1  time2  plcbg1 tm1:p1
time1       -0.028
time2        0.010 -0.492
placebogrp1  0.017  0.014 -0.004
tm1:plcbgr1  0.014  0.005 -0.007 -0.028
tm2:plcbgr1 -0.004 -0.007 -0.001  0.010 -0.492

kableExtra::kable(model_performance(model_panas_neg))
```

R2_conditional	R2_marginal	ICC	RMSE	Sigma
0.5674885	0.0670533	0.5364027	NA	4.574161

```
afex_plot(
  model_panas_neg,
  x = "time",
  trace = "placebogroup",
  mapping = c("shape", "color", "fill"),
```

```
  dodge = 0.8,
  data_geom = geom_violin,
  data_arg = list(dodge.width = 0.8),
  point_arg = list(size = 3),
  error_arg = list(size = 1, width = 0.2),
  legend_title = "Group"
) +
  geom_jitter(
    inherit.aes = FALSE,
    aes(
      x = time,
      y = panas_neg,
      colour = placebogroup,
      shape = placebogroup
    ),
    data = data,
    position = position_jitterdodge(),
    alpha = 0.3
    ) +
  scale_color_brewer(palette = "Set1") +
  scale_fill_brewer(palette = "Set1") +
  ggthemes::theme_few() +
  labs(y = "PANAS Negative", x = "") +
  scale_x_discrete(labels = c("Baseline", "Post-anticipation", "Post-social interaction"))
```




```
panas_neg_means <- emmeans(model_panas_neg, list(pairwise ~ time*placebogroup),
                           adjust = "Tukey")

kableExtra::kable(panas_neg_means$`emmeans of time, placebogroup`)
```

time	placebogroup	emmean	SE	df	lower.CL	upper.CL
baseline	placebo	17.63889	1.119669	133.1399	15.42425	19.85353
anticipation	placebo	21.35748	1.131147	135.7702	19.12053	23.59442
end	placebo	21.41462	1.131147	135.7702	19.17767	23.65157
baseline	control	18.13158	1.089806	133.1399	15.97601	20.28715

time	placebogroup	emmean	SE	df	lower.CL	upper.CL
anticipation	control	22.24748	1.122988	140.8024	20.02739	24.46758
end	control	21.36712	1.133584	143.7236	19.12647	23.60777

```
kableExtra::kable(panas_neg_means$`pairwise differences of time, placebogroup`)
```

1	estimate	SE	df	t.ratio	p.value
baseline placebo - anticipation placebo	-3.7185879	1.090056	136.6112	-3.4113747	0.0107902
baseline placebo - end placebo	-3.7757308	1.090056	136.6112	-3.4637967	0.0091167
baseline placebo - baseline control	-0.4926901	1.562478	133.1399	-0.3153262	0.9995760
baseline placebo - anticipation control	-4.6085947	1.585800	136.9532	-2.9061635	0.0479323
baseline placebo - end control	-3.7282346	1.593321	138.4453	-2.3399145	0.1854103
anticipation placebo - end placebo	-0.0571429	1.093434	135.3794	-0.0522600	0.9999999
anticipation placebo - baseline control	3.2258978	1.570723	134.5002	2.0537660	0.3180339
anticipation placebo - anticipation control	-0.8900068	1.593925	138.2562	-0.5583743	0.9934607
anticipation placebo - end control	-0.0096467	1.601408	139.7282	-0.0060239	1.0000000
end placebo - baseline control	3.2830407	1.570723	134.5002	2.0901460	0.2987070
end placebo - anticipation control	-0.8328640	1.593925	138.2562	-0.5225239	0.9952038
end placebo - end control	0.0474962	1.601408	139.7282	0.0296590	1.0000000
baseline control - anticipation control	-4.1159047	1.083805	138.9384	-3.7976421	0.0029307
baseline control - end control	-3.2355445	1.094780	139.4221	-2.9554293	0.0418159
anticipation control - end control	0.8803601	1.104313	135.9170	0.7972020	0.9675667

Overall there is no evidence of any significant differences between the groups on anxiety or affect measures.

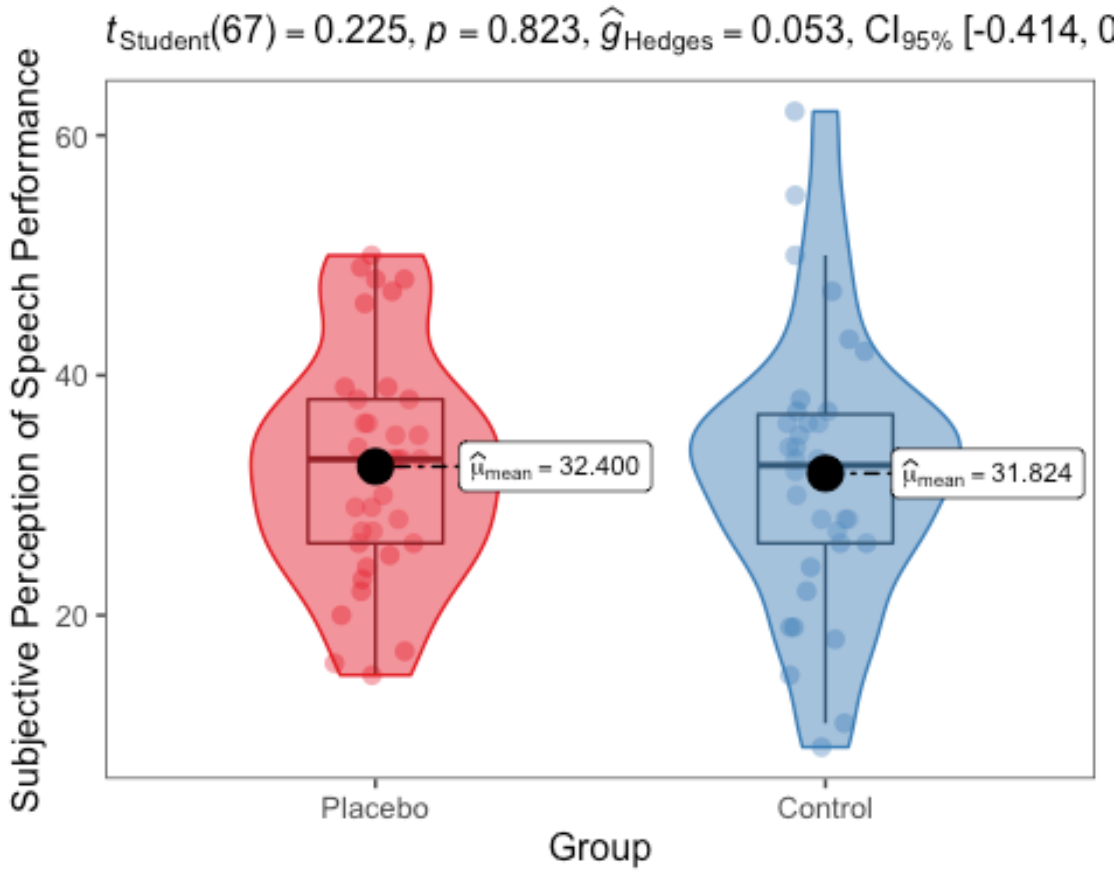
G.2.4 Perception of speech performance

```
levene_test(data = wide_data, sps.total_score ~ placebogroup)

# A tibble: 1 × 4
    df1   df2 statistic     p
  <int> <int>     <dbl> <dbl>
1     1    67     0.394 0.532

kableExtra::kable(
  t_test(sps.total_score ~ placebogroup,
         data = wide_data,
         var.equal = TRUE, detailed = TRUE),
)
```

estimate	estimate1	estimate2	.y.	group1	group2	n1	n2	statistic	p	df	conf.low	conf.high
0.5764706	32.4	31.82353	sps.total_score	placebo	control	38	38	0.224878	0.823	67	-4.54026	5.6932

```
ggbetweenstats(
  data = wide_data,
  x = placebogroup,
  y = sps.total_score,
  plot.type = "boxviolin",
  k = 3,
  var.equal = TRUE,
  bf.message = FALSE,
  xlab = "Group",
  ylab = "Subjective Perception of Speech Performance",
  centrality.point.args = list(size = 5, color = "black"),
  violin.args = list(
    aes(
    fill = placebogroup,
    colour = placebogroup
    ),
    alpha = 0.5, width = 0.6),
  palette = "Set1",
  ggtheme = ggthemes::theme_few()
) +
```

```
  scale_x_discrete(labels = c("Placebo", "Control")) +
  scale_fill_manual(values = c("#E41A1C", "#377EB8"))
```



G.3 Exploratory analyses

```
#placebo_long <- filter(data, placebogroup == "placebo")
#placebo_long$expectancy.centered <- scale(placebo_long$expectancy)

data$expectancy.centered <- scale(data$expectancy)
```

G.3.1 Anxiety

```
model_gad_exploratory1 = mixed(
  gad7 ~ time + expectancy.centered + placebogroup +
    time*placebogroup*expectancy.centered + (1|participant_ID),
  data = data,
  )

model_gad_exploratory2 = mixed(
  gad7 ~ time + expectancy.centered + placebogroup + time*placebogroup +
    time*expectancy.centered + (1|participant_ID),
  data = data,
)

model_gad_exploratory3 = mixed(
  gad7 ~ time + expectancy.centered + placebogroup + (1|participant_ID),
  data = data,
)

kableExtra::kable(
  anova(model_gad_exploratory3, model_gad_exploratory2,

model_gad_exploratory1)
)
```

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
model_gad_exploratory3	7	2683.479	2707.041	-1334.740	2669.479	NA	NA	NA
model_gad_exploratory2	11	2663.322	2700.347	-1320.661	2641.322	28.15785	4	1.16e-05
model_gad_exploratory1	14	2642.917	2690.040	-1307.458	2614.917	26.40478	3	7.80e-06

```
kableExtra::kable(
  performance::compare_performance(model_gad_exploratory3,
                                   model_gad_exploratory2,
                                   model_gad_exploratory1,
                                   rank = TRUE)
)
```

Name	Model	R2_conditional	R2_marginal	ICC	RMSE	Sigma	Performance_Score
model_gad_exploratory1	mixed	0.6099343	0.1656479	0.5324927	NA	105.2048	1.0000000
model_gad_exploratory3	mixed	0.5970661	0.1479873	0.5270800	NA	105.9598	0.2529543
model_gad_exploratory2	mixed	0.5951965	0.1516536	0.5228323	NA	106.5657	0.0519002

The best performing model is the most complex.

```
kableExtra::kable(anova(model_gad_exploratory1))
```

	num Df	den Df	F	Pr(>F)
time	2	134.66535	19.5805940	0.0000000
expectancy.centered	1	69.35130	4.4338545	0.0388556
placebogroup	1	70.53165	3.2864625	0.0741086
time:placebogroup	2	134.66535	0.3681055	0.6927383
time:expectancy.centered	2	133.59251	0.4027375	0.6692964
expectancy.centered:placebogroup	1	69.35130	0.7237629	0.3978402
time:expectancy.centered:placebogroup	2	133.59251	2.7740674	0.0660060

```
summary(model_gad_exploratory1)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula:
gad7 ~ time + expectancy.centered + placebogroup + time * placebogroup *
    expectancy.centered + (1 | participant_ID)
   Data: data

REML criterion at convergence: 2614.9

Scaled residuals:
     Min       1Q   Median       3Q      Max
-1.99579 -0.56769  0.00214  0.51676  2.67410

Random effects:
 Groups         Name        Variance Std.Dev.
 participant_ID (Intercept) 12607    112.3
 Residual                   11068    105.2
Number of obs: 214, groups:  participant_ID, 74

Fixed effects:
                                      Estimate Std. Error      df t value
(Intercept)                            256.733     15.743  70.532  16.307
time1                                  -66.921     10.717 136.104  -6.245
time2                                   36.807     10.784 133.772   3.413
expectancy.centered                     33.222     15.777  69.351   2.106
placebogroup1                          -28.541     15.743  70.532  -1.813
time1:placebogroup1                      1.293     10.717 136.104   0.121
time2:placebogroup1                     -8.624     10.784 133.772  -0.800
time1:expectancy.centered                2.327     10.665 134.205   0.218
time2:expectancy.centered               -9.238     10.693 133.239  -0.864
expectancy.centered:placebogroup1       13.422     15.777  69.351   0.851
time1:expectancy.centered:placebogroup1 -24.463     10.665 134.205  -2.294
```

```
time2:expectancy.centered:placebogroup1   17.159     10.693 133.239   1.605
                                        Pr(>|t|)
(Intercept)                              < 2e-16 ***
time1                                   5.06e-09 ***
time2                                    0.00085 ***
expectancy.centered                      0.03886 *
placebogroup1                            0.07411 .
time1:placebogroup1                      0.90414
time2:placebogroup1                      0.42530
time1:expectancy.centered                0.82760
time2:expectancy.centered                0.38919
expectancy.centered:placebogroup1        0.39784
time1:expectancy.centered:placebogroup1  0.02336 *
time2:expectancy.centered:placebogroup1  0.11094
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) time1  time2  expct. plcbg1 tm1:p1 tm2:p1 tm1:x. tm2:x.
time1       -0.028
time2        0.009 -0.490
expctncy.cn -0.047  0.004  0.000
placebogrp1  0.035  0.007  0.002 -0.310
tm1:plcbgr1  0.007  0.029 -0.022  0.013 -0.028
tm2:plcbgr1  0.002 -0.022  0.027 -0.008  0.009 -0.490
tm1:xpctnc.  0.004 -0.049  0.022 -0.011  0.013 -0.316  0.160
tm2:xpctnc.  0.000  0.022 -0.051  0.004 -0.008  0.160 -0.321 -0.497
expctncy.:1 -0.310  0.013 -0.008  0.120 -0.047  0.004  0.000 -0.003  0.003
tm1:xpct.:1  0.013 -0.316  0.160 -0.003  0.004 -0.049  0.022  0.122 -0.063
tm2:xpct.:1 -0.008  0.160 -0.321  0.003  0.000  0.022 -0.051 -0.063  0.123
            exp.:1 t1:.:1
time1
time2
expctncy.cn
placebogrp1
tm1:plcbgr1
tm2:plcbgr1
tm1:xpctnc.
tm2:xpctnc.
expctncy.:1
tm1:xpct.:1 -0.011
tm2:xpct.:1  0.004 -0.497
```

```
expl_gad_means <- emmeans(model_gad_exploratory1, list(pairwise ~ time*placebogroup),
                          adjust = "Tukey")

NOTE: Results may be misleading due to involvement in interactions

kableExtra::kable(expl_gad_means$`emmeans of time, placebogroup`)
```

time	placebogroup	emmean	SE	df	lower.CL	upper.CL
baseline	placebo	162.7914	27.02564	130.5596	109.3266	216.2563
anticipation	placebo	256.8820	27.47593	134.6774	202.5419	311.2222
end	placebo	266.2014	27.47593	134.6774	211.8613	320.5416
baseline	control	217.4911	26.09782	130.5596	165.8617	269.1204
anticipation	control	330.6437	26.71747	136.3872	277.8096	383.4777
end	control	308.2374	27.08019	140.4717	254.7000	361.7749

```
kableExtra::kable(expl_gad_means$`pairwise differences of time, placebogroup`)
```

1	estimate	SE	df	t.ratio	p.value
baseline placebo - anticipation placebo	-94.090613	26.59816	134.2363	-3.5374854	0.0071975
baseline placebo - end placebo	-103.410014	26.59816	134.2363	-3.8878631	0.0021530
baseline placebo - baseline control	-54.699645	37.56969	130.5596	-1.4559516	0.6926811
baseline placebo - anticipation control	-167.852236	38.00274	133.4224	-4.4168456	0.0002920
baseline placebo - end control	-145.446006	38.25861	135.4801	-3.8016539	0.0029150
anticipation placebo - end placebo	-9.319401	26.73086	132.3021	-0.3486383	0.9993082

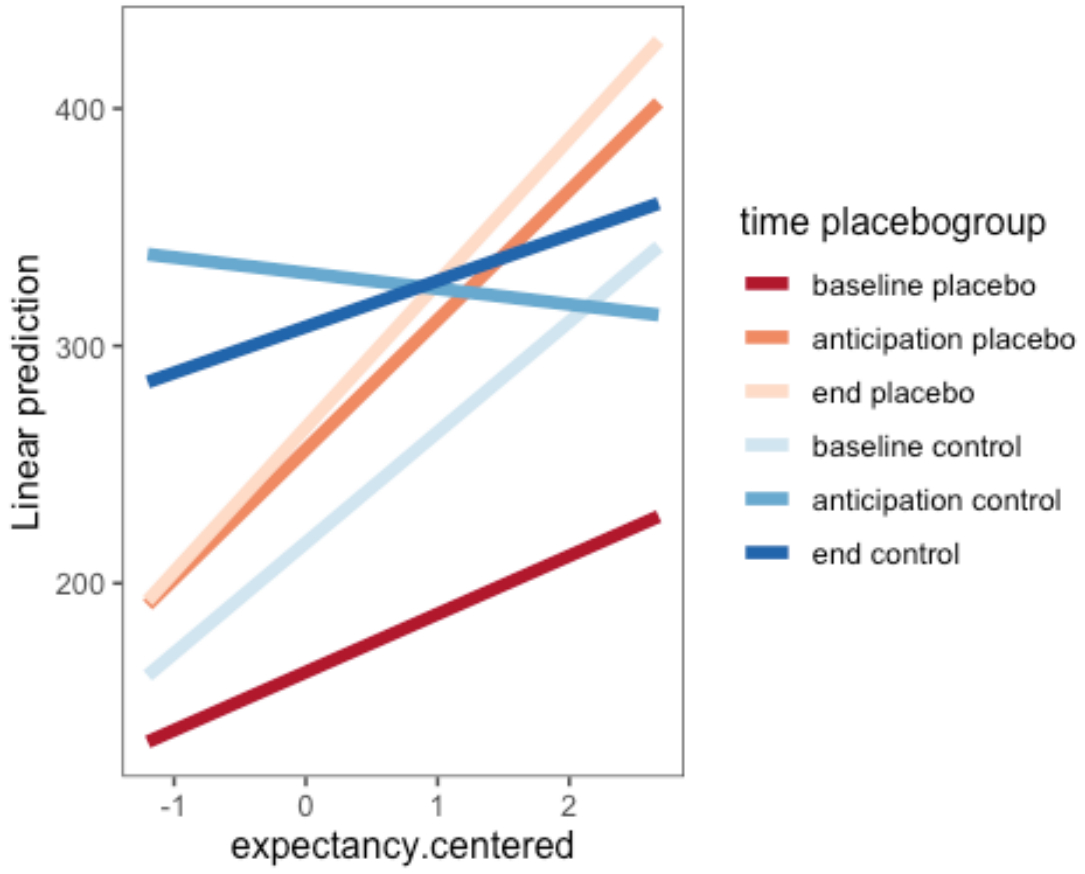
1	estimate	SE	df	t.ratio	p.value
anticipation placebo - baseline control	39.390968	37.89489	132.7153	1.0394796	0.9037051
anticipation placebo - anticipation control	-73.761623	38.32427	135.5070	-1.9246712	0.3917442
anticipation placebo - end control	-51.355393	38.57801	137.5188	-1.3312089	0.7672064
end placebo - baseline control	48.710369	37.89489	132.7153	1.2854072	0.7925222
end placebo - anticipation control	-64.442222	38.32427	135.5070	-1.6814989	0.5463559
end placebo - end control	-42.035992	38.57801	137.5188	-1.0896360	0.8847954
baseline control - anticipation control	-113.152591	25.87589	135.0322	-4.3728960	0.0003449
baseline control - end control	-90.746361	26.25024	135.7297	-3.4569726	0.0093347
anticipation control - end control	22.406230	26.42937	133.0580	0.8477775	0.9578297

```
gad.trends <- emtrends(model_gad_exploratory1, specs = pairwise ~ time:placebogroup,
var = "expectancy.centered")

kableExtra::kable(summary(gad.trends, infer = TRUE))
```

time	placebogroup	expectancy.centered.trend	SE	df	lower.CL	upper.CL	t.ratio	p.value
baseline	placebo	24.50871	28.34532	130.5596	-31.566860	80.58429	0.8646476	0.3888181
anticipation	placebo	54.56532	28.62926	133.0510	-2.062040	111.19268	1.9059287	0.0588164
end	placebo	60.85896	28.62926	133.0510	4.231596	117.48632	2.1257610	0.0353706
baseline	control	46.58942	25.14110	130.5596	-3.147241	96.32609	1.8531177	0.0661231
anticipation	control	-6.59745	25.29112	132.0493	-56.625615	43.43072	-0.2608603	0.7946066
end	control	19.40634	25.39139	133.2680	-30.815918	69.62860	0.7642882	0.4460466

contrast	estimate	SE	df	lower.CL	upper.CL	t.ratio	p.value
baseline placebo - anticipation placebo	-30.056607	27.70237	133.4755	-110.15952	50.04631	-1.0849832	0.8866089
baseline placebo - end placebo	-36.350243	27.70237	133.4755	-116.45316	43.75267	-1.3121709	0.7778733
baseline placebo - baseline control	-22.080710	37.88842	130.5596	-131.67288	87.51146	-0.5827825	0.9920169
baseline placebo - anticipation control	31.106165	37.98813	131.2187	-78.76618	140.97851	0.8188390	0.9636013
baseline placebo - end control	5.102375	38.05496	131.7614	-104.95652	115.16127	0.1340791	0.9999938
anticipation placebo - end placebo	-6.293636	27.78643	132.3021	-86.65003	74.06276	-0.2265003	0.9999166
anticipation placebo - baseline control	7.975898	38.10131	131.9628	-102.21455	118.16634	0.2093340	0.9999435
anticipation placebo - anticipation control	61.162773	38.20046	132.6114	-49.30645	171.63200	1.6011004	0.5993011
anticipation placebo - end control	35.158982	38.26692	133.1465	-75.49589	145.81386	0.9187827	0.9410864
end placebo - baseline control	14.269534	38.10131	131.9628	-95.92091	124.45998	0.3745156	0.9990209
end placebo - anticipation control	67.456408	38.20046	132.6114	-43.01282	177.92563	1.7658533	0.4912849
end placebo - end control	41.452618	38.26692	133.1465	-69.20226	152.10749	1.0832495	0.8872883
baseline control - anticipation control	53.186875	24.46559	133.0049	-17.56037	123.93412	2.1739460	0.2569501
baseline control - end control	27.183084	24.56923	133.2272	-43.86211	98.22828	1.1063874	0.8779946
anticipation control - end control	-26.003790	24.61358	132.5288	-97.18274	45.17516	-1.0564812	0.8975072

```
emmip(model_gad_exploratory1, time:placebogroup ~ expectancy.centered, cov.reduce = range, linearg
= list(size = 2)) + scale_color_brewer(palette = "RdBu") + ggthemes::theme_few()
```



G.3.2 PANAS Positive

```
model_panas_pos_exploratory1 = mixed(
  panas_pos ~ time + expectancy.centered + placebogroup +
    time*expectancy.centered*placebogroup + (1|participant_ID),
  data = data,
  )

model_panas_pos_exploratory2 = mixed(
  panas_pos ~ time + expectancy.centered + placebogroup + time*placebogroup +
    time*expectancy.centered + (1|participant_ID),
  data = data,
)

model_panas_pos_exploratory3 = mixed(
  panas_pos ~ time + expectancy.centered + placebogroup + (1|participant_ID),
  data = data,
)

kableExtra::kable(
  anova(model_panas_pos_exploratory3, model_panas_pos_exploratory2,
        model_panas_pos_exploratory1)
)
```

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
model_panas_pos_exploratory3	7	1334.397	1357.926	-660.1983	1320.397	NA	NA	NA
model_panas_pos_exploratory2	11	1337.136	1374.110	-657.5679	1315.136	5.260755	4	0.2615733
model_panas_pos_exploratory1	14	1337.987	1385.045	-654.9933	1309.987	5.149247	3	0.1611891

```
kableExtra::kable(
  performance::compare_performance(model_panas_pos_exploratory3,
                                   model_panas_pos_exploratory2,
                                   model_panas_pos_exploratory1,
                                   rank = TRUE)
)
```

Name	Model	R2_conditional	R2_marginal	ICC	RMSE	Sigma	Performance_Score
model_panas_pos_exploratory2	mixed	0.6849476	0.2333223	0.5890680	NA	4.108215	0.7585768
model_panas_pos_exploratory1	mixed	0.6874184	0.2491206	0.5837127	NA	4.108482	0.7475229
model_panas_pos_exploratory3	mixed	0.6785132	0.2261657	0.5845534	NA	4.135142	0.0392475

In this case, more complex models do not perform better than simpler ones.

```
kableExtra::kable(anova(model_panas_pos_exploratory3))
```

	num Df	den Df	F	Pr(>F)
time	2	137.86464	18.0221747	0.0000001
expectancy.centered	1	68.51960	20.2944183	0.0000266
placebogroup	1	69.76941	0.0322632	0.8579718

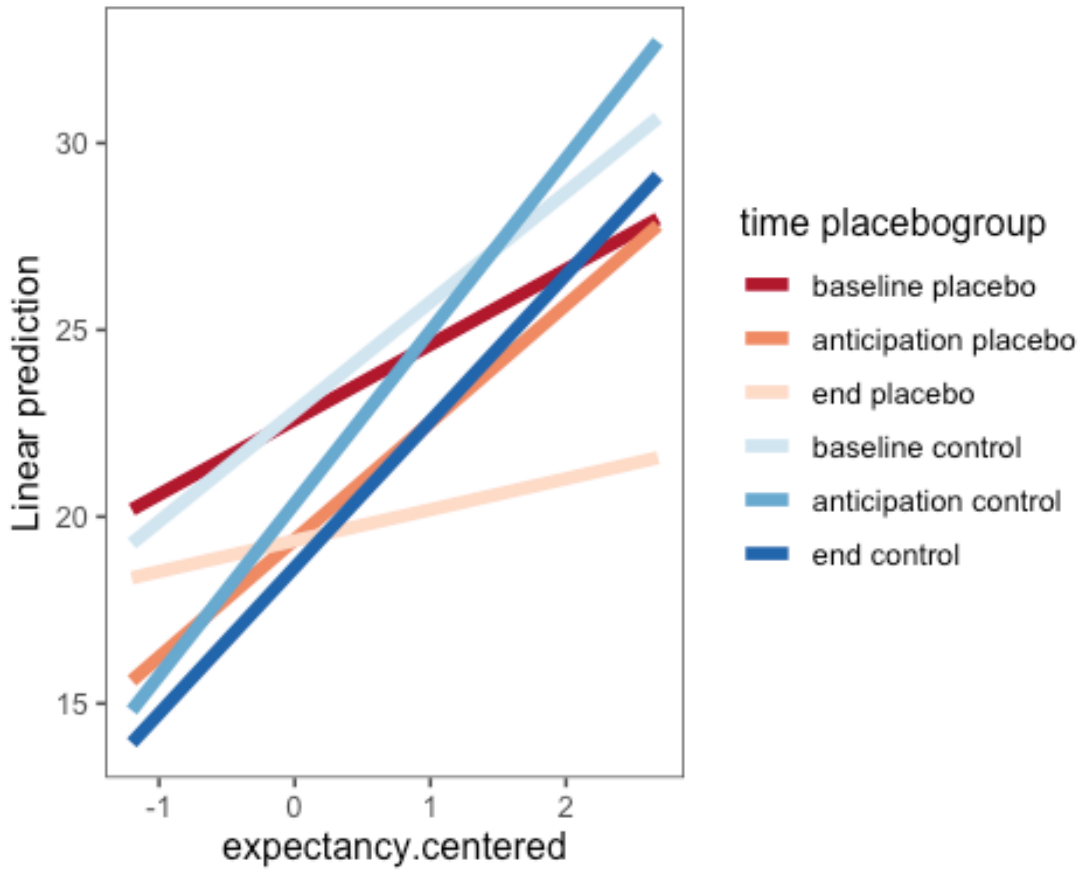
```
summary(model_panas_pos_exploratory3)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: panas_pos ~ time + expectancy.centered + placebogroup + (1 |
    participant_ID)
   Data: data

REML criterion at convergence: 1320.4

Scaled residuals:
    Min      1Q  Median      3Q     Max
-2.5329 -0.5136 -0.0754  0.4247  3.7676

Random effects:
 Groups         Name        Variance Std.Dev.
 participant_ID (Intercept) 24.06    4.905
 Residual                   17.10    4.135
Number of obs: 213, groups:  participant_ID, 74

Fixed effects:
                   Estimate Std. Error       df t value Pr(>|t|)
(Intercept)         20.2409     0.6397  69.5742  31.640  < 2e-16 ***
time1                2.3234     0.4012 139.3324   5.791 4.45e-08 ***
time2               -0.5934     0.4035 137.0323  -1.471    0.144
expectancy.centered  3.0137     0.6690  68.5196   4.505 2.66e-05 ***
placebogroup1       -0.1207     0.6718  69.7694  -0.180    0.858
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Correlation of Fixed Effects:
            (Intr) time1  time2  expct.
time1       -0.027
time2        0.010 -0.492
expctncy.cn -0.010  0.002  0.001
placebogrp1  0.020  0.013 -0.004 -0.306

expl_panas_pos_means <- emmeans(model_panas_pos_exploratory3,
                                list(pairwise ~ time*placebogroup),
                                adjust = "Tukey")

kableExtra::kable(expl_panas_pos_means$`emmeans of time, placebogroup`)
```

time	placebogroup	emmean	SE	df	lower.CL	upper.CL
baseline	placebo	22.47720	1.0145214	95.35930	20.46322	24.49118
anticipation	placebo	19.56042	1.0203324	97.01366	17.53535	21.58550
end	placebo	18.42373	1.0215166	97.37194	16.39640	20.45106
baseline	control	22.71853	0.9932686	96.62299	20.74707	24.68999
anticipation	control	19.80175	1.0083160	100.48418	17.80140	21.80210
end	control	18.66506	1.0119035	101.60989	16.65786	20.67226

```
kableExtra::kable(expl_panas_pos_means$`pairwise differences of time, placebogroup`)
```

1	estimate	SE	df	t.ratio	p.value
baseline placebo - anticipation placebo	2.9167798	0.6952496	139.57384	4.1952991	0.0006759
baseline placebo - end placebo	4.0534697	0.6987816	139.77657	5.8007673	0.0000006
baseline placebo - baseline control	-0.2413260	1.3437436	70.86445	-0.1795923	0.9999730
baseline placebo - anticipation control	2.6754539	1.5189842	110.32094	1.7613441	0.4948393
baseline placebo - end control	3.8121437	1.5221913	111.01056	2.5043789	0.1317131
anticipation placebo - end placebo	1.1366898	0.7024550	137.52965	1.6181676	0.5880301
anticipation placebo - baseline control	-3.1581058	1.5068925	108.42637	-2.0957738	0.2972872
anticipation placebo - anticipation control	-0.2413260	1.3437436	70.86445	-0.1795923	0.9999730
anticipation placebo - end control	0.8953639	1.5178667	110.90095	0.5898831	0.9915263
end placebo - baseline control	-4.2947956	1.5069238	108.43055	-2.8500418	0.0572061
end placebo - anticipation control	-1.3780158	1.5146816	110.21186	-0.9097726	0.9432661
end placebo - end control	-0.2413260	1.3437436	70.86445	-0.1795923	0.9999730
baseline control - anticipation control	2.9167798	0.6952496	139.57384	4.1952991	0.0006759
baseline control - end control	4.0534697	0.6987816	139.77657	5.8007673	0.0000006
anticipation control - end control	1.1366898	0.7024550	137.52965	1.6181676	0.5880301

```
emmip(model_panas_pos_exploratory1, time:placebogroup ~ expectancy.centered, cov.reduce = range, li
nearg = list(size = 2)) + scale_color_brewer(palette = "RdBu") + ggthemes::theme_few()
```



G.3.3 PANAS Negative

```
model_panas_neg_exploratory1 = mixed(
  panas_neg ~ time + expectancy.centered + placebogroup +
    time*expectancy.centered*placebogroup + (1|participant_ID),
  data = data,
  )

model_panas_neg_exploratory2 = mixed(
  panas_neg ~ time + expectancy.centered + placebogroup + time*placebogroup +
    time*expectancy.centered + (1|participant_ID),
```

```
  data = data,
)

model_panas_neg_exploratory3 = mixed(
  panas_neg ~ time + expectancy.centered + placebogroup + (1|participant_ID),
  data = data,
)

kableExtra::kable(
  anova(model_panas_neg_exploratory3,
        model_panas_neg_exploratory2,
        model_panas_neg_exploratory1)
)
```

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
model_panas_neg_exploratory3	7	1360.026	1383.555	-673.0130	1346.026	NA	NA	NA
model_panas_neg_exploratory2	11	1365.751	1402.726	-671.8757	1343.751	2.274631	4	0.6853916
model_panas_neg_exploratory1	14	1366.379	1413.437	-669.1895	1338.379	5.372322	3	0.1464778

```
kableExtra::kable(
  performance::compare_performance(model_panas_neg_exploratory3,
                                   model_panas_neg_exploratory2,
                                   model_panas_neg_exploratory1,
                                   rank = TRUE)
)
```

Name	Model	R2_conditional	R2_marginal	ICC	RMSE	Sigma	Performance_Score
model_panas_neg_exploratory3	mixed	0.5757771	0.1088796	0.5239443	NA	4.543051	0.7500000
model_panas_neg_exploratory1	mixed	0.5757622	0.1315393	0.5115060	NA	4.585040	0.4988294
model_panas_neg_exploratory2	mixed	0.5726059	0.1116015	0.5189163	NA	4.578786	0.2162070

As above, the best performing model is the simplest.

```
kableExtra::kable(anova(model_panas_neg_exploratory3))
```

	num Df	den Df	F	Pr(>F)
time	2	138.02587	15.908978	0.0000006
expectancy.centered	1	68.23144	5.034193	0.0281006
placebogroup	1	69.58586	1.037485	0.3119354

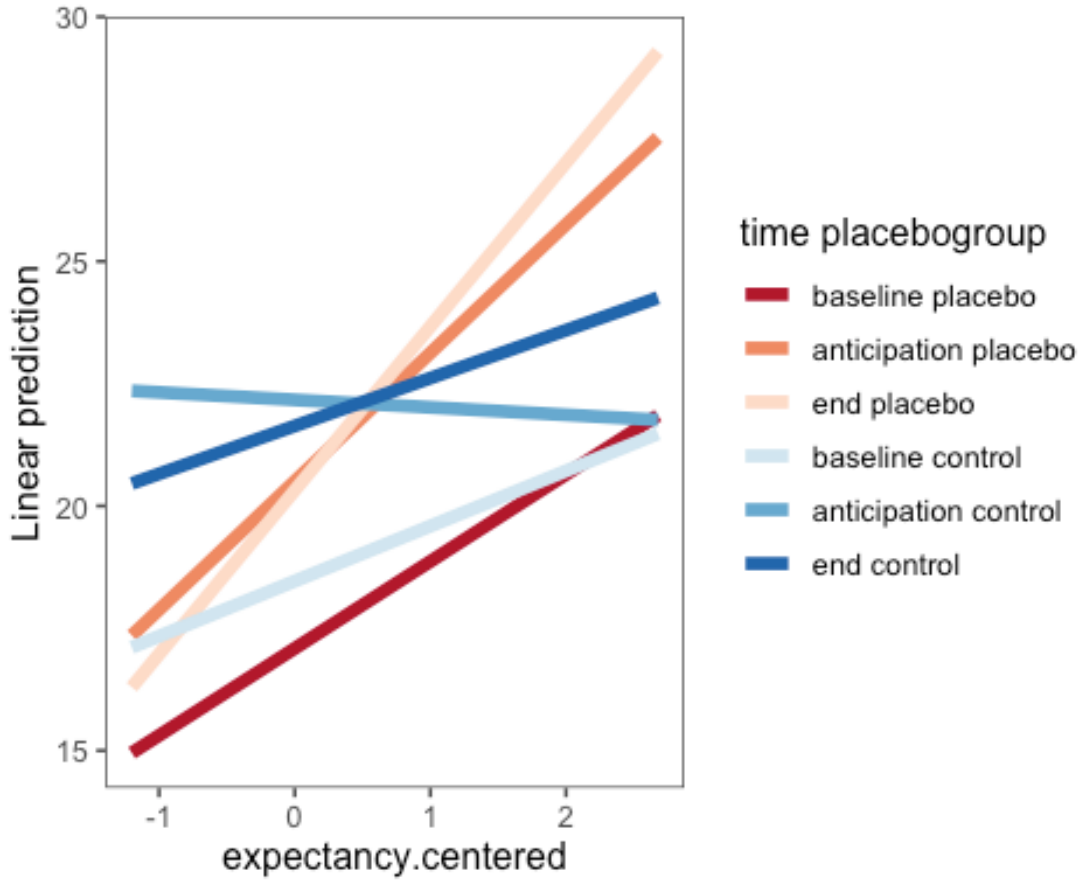
```
summary(model_panas_neg_exploratory3)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: panas_neg ~ time + expectancy.centered + placebogroup + (1 |
    participant_ID)
   Data: data

REML criterion at convergence: 1346

Scaled residuals:
     Min       1Q   Median       3Q      Max
-3.03436 -0.54812 -0.03595  0.51140  2.38349

Random effects:
 Groups         Name        Variance Std.Dev.
 participant_ID (Intercept) 22.72    4.766
 Residual                   20.64    4.543
Number of obs: 213, groups:  participant_ID, 74

Fixed effects:
                   Estimate Std. Error       df t value Pr(>|t|)
(Intercept)         20.3446     0.6387  69.3741  31.851  < 2e-16 ***
time1               -2.4712     0.4403 139.6191  -5.612 1.04e-07 ***
time2                1.4412     0.4431 137.1121   3.252  0.00144 **
expectancy.centered  1.4975     0.6674  68.2314   2.244  0.02810 *
placebogroup1       -0.6833     0.6708  69.5859  -1.019  0.31194
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Correlation of Fixed Effects:
            (Intr) time1  time2  expct.
time1       -0.028
time2        0.010 -0.492
expctncy.cn -0.010  0.002  0.001
placebogrp1  0.019  0.013 -0.004 -0.307
```

```
expl_panas_neg_means <- emmeans(model_panas_neg_exploratory3,
                                list(pairwise ~ time*placebogroup),
                                adjust = "Tukey")

kableExtra::kable(expl_panas_neg_means$`emmeans of time, placebogroup`)
```

time	placebogroup	emmean	SE	df	lower.CL	upper.CL
baseline	placebo	17.20681	1.028420	100.5665	15.16659	19.24702
anticipation	placebo	21.11919	1.035160	102.4578	19.06606	23.17231
end	placebo	20.70801	1.036545	102.8697	18.65224	22.76378
baseline	control	18.57339	1.007519	102.0947	16.57501	20.57178
anticipation	control	22.48577	1.024665	106.2730	20.45434	24.51721
end	control	22.07460	1.028860	107.5623	20.03513	24.11407

```
kableExtra::kable(expl_panas_neg_means$`pairwise differences of time,
                  placebogroup`)

emmip(model_panas_neg_exploratory1, time:placebogroup ~ expectancy.centered, cov.reduce = range, li
nearg = list(size = 2)) + scale_color_brewer(palette = "RdBu") + ggthemes::theme_few()
```



ACCOMPANYING DOCUMENTS

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Psychological Medicine

cambridge.org/psm

Review Article

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Author for correspondence:
Nathan T.M. Huneke,
E-mail: n.huneke@soton.ac.uk

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Why we need more research into the placebo response in psychiatry

Nathan T.M. Huneke[1,2], Nic van der Wee[3], Matthew Garner[1,4] and David S. Baldwin[1,2,5]

[1]Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK; [2]University Department of Psychiatry, Academic Centre, College Keep, 4-12 Terminus Terrace, Southampton, SO14 3DT, UK; [3]Department of Psychiatry, Leiden University Medical Center, Leiden, The Netherlands; [4]Academic Unit of Psychology, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, UK and [5]University Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

Abstract

Placebos are not inert, but exert measurable biological effects. The placebo response in psychiatric illness is important and clinically relevant, but remains poorly understood. In this paper, we review current knowledge about the placebo response in psychiatric medicine and identify research directions for the future. We argue that more research is needed into the placebo response in psychiatric medicine for three broad reasons. First, awareness of factors that cause placebo response, for whom, and when, within clinical trials will allow us to better evidence efficacy of new treatments. Second, by understanding how placebo mechanisms operate in the clinic, we can take advantage of these to optimise the effects of current treatments. Finally, exploring the biological mechanisms of placebo effects might reveal tractable targets for novel treatment development.

Introduction

A placebo is an inert substance or sham procedure given either as a form of psychological reassurance or to act as a control when testing the efficacy of active treatment. However, placebos are not inert in terms of outcomes. Since the advent of the placebo-controlled trial, it has been observed that patients in the placebo arm can show substantial improvements in symptoms (Beecher, 1955; McQueen, Cohen, St John-Smith, & Rampes, 2013). These observed improvements in symptoms are partly explained by non-specific effects, such as regression to the mean, epiphenomena related to the trial, or sampling bias from dropouts of the least improved (Ashar, Chang, & Wager, 2017; Ernst & Resch, 1995; Miller & Rosenstein, 2006). But, these improvements also result from specific placebo effects, which can be measured in a clinical trial by comparing a placebo arm with a 'natural history' or untreated arm (Ernst & Resch, 1995). These concepts have recently been captured in operational definitions reached by expert consensus. The 'placebo response' is defined as all within-group improvements that occur following administration of an inactive treatment and is attributable to both non-specific effects such as spontaneous improvement and specific placebo mechanisms (Evers et al., 2018). By contrast, the 'placebo effect' is the symptom improvement that is attributable to placebo mechanisms only (Evers et al., 2018). The placebo effect results from an interplay between expectations and learning that causes changes in biological systems including the immune system, hypothalamic–pituitary–adrenal axis and the endogenous opioid system (Benedetti, Carlino, & Pollo, 2011; Evers et al., 2018; Peciña & Zubieta, 2015). Placebos are not inert, but exert measurable biological effects.

The magnitude of the placebo response is not uniform across conditions. Conditions such as nausea or smoking seem to show relatively smaller placebo responses compared with insomnia or phobia (Krogsbøll, Hróbjartsson, & Gøtzsche, 2009). The placebo response in psychotropic drug trials has a relatively large effect size. Approximately 30% of patients in antidepressant and antipsychotic trials respond to placebo treatment (Furukawa et al., 2016; Leucht et al., 2018; Stein, Baldwin, Dolberg, Despiegel, & Bandelow, 2006; Walsh, Seidman, Sysko, & Gould, 2002). Two meta-analyses have shown that within-group pre-to-post effect size for placebo treatment ranges from 0.65 to 1.29 in anxiety disorders (Bandelow et al., 2015; De Vries, De Jonge, van den Heuvel, Turner, & Roest, 2016). These data demonstrate that the placebo response is an important and clinically relevant effect in psychiatry. However, it remains poorly understood.

In this paper, we argue that more research is needed into the placebo response in psychiatric medicine. We make the case that we need to understand this phenomenon for three broad reasons. First, we believe that improving our understanding of the placebo response within clinical trials will allow us to better evidence the efficacy of new treatments. Second,

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we feel that by understanding how to take advantage of placebo mechanisms operating in clinical settings, we will be able to maximise the effects of current treatments. Finally, exploring the biological mechanisms of placebo effects might reveal tractable targets for novel treatment development.

Although we focus on placebo in pharmacotherapeutic contexts, it should be noted that placebo responses also occur in psychotherapy. Furthermore, the factors leading to symptom improvement attributable to placebo may differ between pharmacotherapy and psychotherapy. For example, the quality of the clinician–patient interaction is not specific to drug efficacy and could be attributed to placebo, but this is potentially a factor more relevant to the efficacy of psychotherapy (Blease, 2018; Enck & Zipfel, 2019). Exploration of the placebo response in psychotherapy might improve our understanding of the specific mechanisms underlying its benefits, and potentially inform our understanding of the factors involved in placebo response in pharmacotherapy. A full discussion of these issues is outside the scope of the current paper, however, we refer the reader to a recent review in which this has been explored in detail (Enck & Zipfel, 2019).

Improving ability to evidence efficacy of new treatments

In recent years, many pharmaceutical companies have 'pulled out' of neuroscience research, including into neuropsychiatric disorders. One of the major factors behind this is the late-stage failure of potential treatments to show efficacy in phase II or III clinical trials (Skripka-Serry, 2013). Potential neuropsychiatric drugs show a large amount of attrition from phase I trials to approval. Of the 60% of compounds that progress to phase II trials, a third will progress to phase III, but less than half of these will be reviewed by regulatory bodies and only 8.2% will be approved (McArthur, 2017).

One factor that contributes to this high attrition rate is the placebo response. The placebo response in antidepressant trials has previously been reported to be substantial and growing (Walsh et al., 2002). In antipsychotic trials, the magnitude of the placebo response has increased over the past 40 years, while the effect sizes of medication have remained stable (Agid et al., 2013; Leucht et al., 2017). The result is that the clinical trial as an assay exhibits reduced sensitivity to detect separation between active medication and placebo (Enck, Bingel, Schedlowski, & Rief, 2013). Interestingly, a 2016 meta-analysis and meta-regression showed that the placebo response rate in antidepressant trials increased from 1978 to 1991, but from 1991 it has remained constant at 35–40% (Furukawa et al., 2016). The meta-regression performed in this study showed that trials lasting longer than 4 weeks, multi-centre trials, and trials with flexible dosing regimens were all associated with increased placebo response rates (Furukawa et al., 2016). The important finding here was that once certain methodological parameters became constant between the years 1990 and 2000 (duration of 8 weeks, multi-centre trials made up over 90% of all studies, fixed dosing became more common) the placebo response rate also became constant. Although it is likely impossible and might even be unhelpful to eliminate placebo response (Whitlock, Woodward, & Alexander, 2019), this result suggests that we can standardise certain factors in trial design and thus control placebo response rate. By reducing the variability of placebo response rate trial to trial, we can ensure that clinical trials are properly powered to detect an effect of active medication and thereby reduce the likelihood of 'failed trials'. However, it is probable that the important factors which need to be standardised will differ between diverse conditions. For instance, in antipsychotic trials it is increased sample size, shorter trial duration, shorter pre-trial washout, the rating scale used, studies outside the United States and shorter duration of illness that are associated with an increased placebo response rate (Leucht et al., 2018). We need to identify the important factors for all neuropsychiatric conditions and apply these insights in the design of psychotropic trials.

Consideration also needs to be given to the within-subject factors that increase placebo response rate. Placebo effects result from an interplay between prior expectations and subsequent learning (Ashar et al., 2017; Benedetti, Amanzio, Rosato, & Blanchard, 2011*a*, *b*). These mechanisms are known to be at play in clinical trials. For instance, the more active treatment arms there are in a trial, the higher the placebo response rate (Papakostas & Fava, 2009; Woods, Gueorguieva, Baker, & Makuch, 2005). This probably stems from an increased expectation from the patient that they will be randomised to receive an active medication. This is supported by the finding that the same drug produces larger effects in open trials compared with double-blind trials, i.e. when the patient is certain to receive active medication (Jensen et al., 2017; Rutherford et al., 2017). A patient's expectations will subsequently be updated through experience and learning (Ashar et al., 2017). For example, placebo analgesia is reduced if participants have experienced a previously ineffective analgesic treatment (Colloca & Benedetti, 2006; Kessner, Wiech, Forkmann, Ploner, & Bingel, 2013; Zunhammer et al., 2017). Such learning effects potentially confound crossover designs (Enck et al., 2013), and previous treatment experiences might influence a patient's expectations on entry to a clinical trial and their subsequent outcome (Benedetti, Carlino, & Piedimonte, 2016; Huneke & Baldwin, 2015). However, these possibilities are yet to be empirically tested, and the potential size of the effects is unknown. A simple first step would be to measure patients' expectations before and during clinical trials and include this parameter as a covariate in analysis of end-points (Benedetti et al., 2016). Another option to eliminate the influence of previous experience could be to prefer treatment-naïve patients, but it is currently unclear whether this reduces placebo response rate. It also remains unclear whether an individual who has responded to a placebo once is likely to do so again in future (Enck, Klosterhalfen, & Weimer, 2016). A full understanding of when, for whom, and to what degree placebo response can occur is needed.

Another suggestion to maximise assay sensitivity has been to measure placebo effect size through the inclusion of a 'no treatment' or 'natural history' control arm in clinical trials. In theory, patients in such a group would not be expected to improve, or if there was improvement then this would be the result of non-specific effects such as regression to the mean. Therefore, any difference between this control arm and the placebo arm would be due to a placebo effect. However, this design is not only ethically questionable (Enck et al., 2013) but is likely to be biased. A 2014 trial in patients with depression included such a 'no-treatment' arm, but the dropout rate in this arm was 40%, compared with 25% in the antidepressant arm and 10% in the placebo arm (Leuchter, Hunter, Tartter, & Cook, 2014). Such a large dropout rate will likely bias outcome measurements. One possibility to overcome this could be to use novel trial designs, such as a modified Zelen design, in which participants could be recruited to an observational study and a random sub-group then approached to participate in a clinical trial (Enck et al., 2013; Zelen, 1979). This would go some way to overcoming ethical issues and reduce the

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likelihood of dropouts. Such novel trial designs need to be tested, however, to ascertain their acceptability to potential participants, and to ensure that placebo response and effect of active medication can be accurately measured.

In summary, we need to understand which factors increase the chance for placebo response, for whom this occurs, and when. With this information, we could optimise trial designs to improve the chance of detecting efficacy of a novel treatment. We need to understand whether measuring expectations or using treatment-naïve patients would be beneficial. Finally, we need to test whether novel trial designs could improve assay sensitivity.

Maximising effects of current treatments

It is accepted among physicians and psychiatrists that placebo mechanisms including the patient's expectations and previous experience of treatment can affect the effectiveness of psychotropic drugs in clinical practice. A survey of 87 physicians in Germany showed that more than 60% agreed that patient expectations and prior experience mediates the effectiveness of antidepressants (Kampermann, Nestoriuc, & Shedden-Mora, 2017). If this is true, it would follow that understanding how to ensure experiences are positive and expectations are maximised would allow us to optimise the effectiveness of our treatments.

There is indeed empirical evidence that placebo mechanisms can affect the effectiveness of interventions in the clinic. This can be demonstrated by the 'open-hidden paradigm'. In these experiments, active medication is administered to a patient either in full view, or in a hidden fashion by a machine or through instructions that no medication is being given. Since the treatment is the same, the difference in effectiveness between the interventions is inferred to result from changes in the patient's belief and expectations (Wager & Atlas, 2015). A number of studies have shown that open administration of treatment in acute pain and Parkinson's disease is superior to hidden administration (Amanzio, Pollo, Maggi, & Benedetti, 2001; Atlas et al., 2012; Benedetti et al., 2003; Colloca, Lopiano, Lanotte, & Benedetti, 2004). This has also recently been demonstrated to be the case in social anxiety disorder. Patients openly given escitalopram improved with an effect size twice that of patients who received escitalopram but were told it was an 'active placebo' ($d = 2.24$ *v.* $d = 1.13$, respectively) (Faria et al., 2017). Furthermore, in patients with post-traumatic stress disorder, enhanced expectations of benefit was associated with higher likelihood of early response to sertraline, and improved outcomes after 10 weeks (Graham et al., 2018). The importance of expectations and beliefs for the effectiveness of treatments has led to the suggestion that we could develop interventions to improve pre-treatment expectations where they are particularly low (Enck et al., 2013). Such interventions and their effect on outcome have yet to be tested.

It has been argued that the doctor–patient relationship is key in activating beneficial placebo mechanisms in clinic (Thompson, Ritenbaugh, & Nichter, 2009), and perhaps we should be exploring this to understand how to maximise expectations. Although it would seemingly be clear that this should be the case, there is very little empirical supporting evidence. Some systematic review evidence shows that 'clinician warmth' and 'listening' are associated with patient satisfaction (Henry, Fuhrel-Forbis, Rogers, & Eggly, 2012), and that practitioners who are 'warm and friendly' are more effective than those who are 'impersonal or uncertain' for a range of conditions including hypertension, asthma and pain (Di Blasi, Harkness, Ernst, Georgiou, & Kleijnen, 2001). However, many studies included in these reviews were of poor quality and likely biased. Beyond this, there has been very little systematic exploration of how the doctor–patient interaction influences patient outcome, particularly in psychiatric medicine. We need more investigations that aim to understand whether the doctor–patient interaction matters, and if so, how doctors should interact with patients to maximise the benefits of any intervention.

Another important factor that influences treatment effects is adherence to medication. The more adherent a patient is, the more effective the treatment is likely to be. However, adherence with psychopharmacological treatments in those with severe mental illnesses is estimated to be only 40–50%, and a major contributor to poor medication adherence is the experience of side-effects (Velligan, Sajatovic, Hatch, Kramata, & Docherty, 2017). It is possible that placebo mechanisms could be utilised to improve adherence. The act of informing patients about possible side-effects of a medication engenders expectations that increase the chance the patient will report such side-effects (Neukirch & Colagiuri, 2015). This is an example of a 'nocebo effect': the experience of an adverse effect that is not attributable to the active ingredients of the treatment or therapy (Barsky, Saintfort, Rogers, & Borus, 2002; Petrie & Rief, 2019). Similar to how positive expectations and learning interact to produce placebo effects, it is thought that negative prior expectations and subsequent experience interact to produce nocebo effects (Petrie & Rief, 2019). For example, in a cohort of women receiving endocrine treatment for breast cancer, negative expectations at baseline increased the relative risk of side-effects over the course of 2 years, some of which were not attributable to the treatment (Nestoriuc et al., 2016). Additionally, changing the label of a placebo from branded to generic can reduce its effectiveness and increase reporting of side-effects in healthy volunteers, presumably because generic medicines are considered to be of 'poorer quality' (Colgan et al., 2015; Faasse, Cundy, Gamble, & Petrie, 2013; Petrie & Rief, 2019). Nocebo mechanisms are likely clinically relevant in the experience of side-effects and therefore adherence to medication. Theoretically it follows that to maximise current treatments we need to not only maximise placebo effects but also minimise the possibility of nocebo effects. Although it is necessary to fully inform patients of potential risks of treatment, framing this information positively, for example '90% of people will be unaffected', reduces the chance a patient will report side-effects (Webster, Weinman, & Rubin, 2018). Further research is required to understand how best to minimise nocebo effects in psychiatric medicine while maintaining informed consent, and whether this improves adherence to medication.

Another placebo mechanism that could be exploited to improve medication adherence is learning through classical conditioning. If a medication is paired with an unconditioned stimulus, such as a green drink, for a number of administrations then the green drink will eventually induce effects similar to active medication on its own. Such classical conditioning paradigms have successfully induced placebo immunosuppression and placebo analgesia (Babel et al., 2017; Goebel et al., 2002). One study has shown that such mechanisms could also be important in neuropsychiatric conditions. Ninety-nine children with attention-deficit hyperactivity disorder aged 6–12 years old were randomly assigned to 8 weeks of treatment in one of three arms: reduced-dose + placebo, reduced-dose only, or treatment as usual. All were treated with an optimal dose of mixed amphetamine salt for 4 weeks, but in the reduced-dose + placebo arm,

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treatment was paired with a visually distinctive placebo capsule. At 4 weeks, the dose of mixed amphetamine salt was reduced by 50% in the reduced-dose and reduced-dose + placebo arms. The reduced-dose only group showed a significant worsening of symptoms by week 8, while the reduced-dose + placebo and treatment as usual groups did not differ in symptom severity (Sandler, Glesne, & Bodfish, 2010). There are potential sources of bias in this study, including that the children's parents were the severity raters and were not blinded to the intervention: nevertheless, the results suggest that it might be possible to reduce side-effect burden and thus improve treatment adherence through placebo mechanisms.

In summary, further understanding is required regarding how pre-treatment expectations might influence patient outcome in the psychiatric clinic, and whether interventions to improve expectations are beneficial. We need further studies into the doctor–patient relationship, and how doctors could best interact with patients to maximise treatment effects. Finally, we need to explore whether placebo mechanisms could be used to improve medication adherence. All of these could lead to changes in practice that maximise the effectiveness of our current treatments.

Identification of novel treatment targets

As yet, research into the placebo effect has not led to identification of novel pharmacological targets in other fields. However, in psychiatry, it is possible that research into the placebo effect will directly lead to identification of tractable targets. It has been theorised that placebo effects are mediated by a 'relaxation' or reduction in negative emotions in anticipation that a distressing symptom might soon improve (Benedetti et al., 2011*a*, *b*; Flaten, Aslaksen, Lyby, & Bjørkedal, 2011). In psychiatry, the symptom targeted by treatment is often a patient's emotional state. Therefore, placebo mechanisms that act through changes in emotion might be directly clinically relevant. Placebo administration has indeed been shown capable of improving feelings of unpleasantness, disgust and negative mood through conditioning procedures or verbal suggestions (Glombiewski, Rheker, Wittkowksi, Rebstock, & Rief, 2019; Petrovic et al., 2005; Schienle, Ubel, Schongassner, Ille, & Scharmuller, 2014).

The question that follows is whether there are any neurobiological systems that could mediate the change in emotional state via placebo administration. There is a system that is common to placebo analgesia and placebo effects on emotion: the endogenous opioid system. Placebo-induced reductions of 'unpleasantness' are associated with increased rostral anterior cingulate cortex activity (Petrovic et al., 2005). This region is also known to be important in placebo analgesia (Atlas & Wager, 2014) and is a key node in the endogenous opioid system (Fields, 2004). Accumulating evidence suggests that the endogenous opioid system plays an important role in the experience of different affective states (Nummenmaa & Tuominen, 2018). It is therefore possible that, as is the case with placebo analgesia and opioid analgesics, recruitment of the endogenous opioid system in placebo effects on emotion would suggest that exogenous agents targeting this system could prove successful in treating affective symptoms. Recent evidence supports this argument. In a 2015 study, 35 depressed patients were scanned through positron emission tomography at baseline and following 1 week of a placebo treatment that was suggested would cause symptom improvement. This was followed by open-label antidepressant treatment for 10 weeks. The results showed that baseline μ-opioid binding potential in the nucleus accumbens, and degree of placebo-induced opioid release in nucleus accumbens, thalamus and subgenual anterior cingulate cortex, correlated with improvement in symptoms following antidepressant treatment (Pecina et al., 2015). Indeed, the endogenous opioid system is now being investigated as a possible therapeutic target in depression (Browne & Lucki, 2019).

Other neurobiological systems have been implicated in mediating placebo effects that might have relevance for placebo effects on emotion. For example, there is good evidence that the dopaminergic system is important in placebo effects. The ventral striatum, an important centre of dopaminergic neurotransmission, is reliably activated by placebo analgesia (Atlas & Wager, 2014) and degree of dopamine release in the nucleus accumbens explains 25% of the variance of placebo analgesic effects in healthy volunteers (Scott et al., 2008). Additionally, placebo effects in Parkinson's disease are mediated by the dopaminergic system (De La Fuente-Fernandez, 2001; Lidstone et al., 2010). Another example is the endocannabinoid system, which appears to mediate placebo analgesic effects conditioned by non-opioid analgesics such as ketorolac (Benedetti et al., 2011). Finally, hypothalamic–pituitary–adrenal axis activity and the cholecystokinin system have been linked to nocebo hyperalgesia (Benedetti, Amanzio, Vighetti, & Asteggiano, 2006). All of these systems are relevant to psychiatric symptoms and phenomena such as anxiety, anhedonia and psychosis. Indeed, both placebo analgesia and nocebo hyperalgesia involve activity in brain regions that process the affective component of pain, suggesting that the neurobiological systems that drive these responses might have direct effects on emotion (Atlas & Wager, 2014; Kong et al., 2008). However, it is presently unknown whether non-opioid systems are important in placebo effects on emotion. Understanding which of these other neurobiological systems are important might reveal additional tractable therapeutic targets for psychiatric medicine.

If we can identify biomarkers related to placebo effects under psychiatric conditions, then we might identify important neurotransmitter systems involved in symptom improvement and additional novel treatment targets. There has been some research exploring biomarkers of placebo response in depression and in anxiety, but these studies have often been re-analyses of data collected for other purposes and have only attempted to measure biomarkers at the end of treatment (Faria et al., 2012; Mayberg et al., 2002). To fully understand the neurobiological systems underpinning placebo effects in patients, we need to carry out prospective studies in which the primary aim is to identify placebo mechanisms. We also need longitudinal studies with measures at multiple time-points to understand how brain activity or other biomarkers change during the course of a placebo treatment. This might allow us to identify the activity, systems and time-points that are most important for therapeutic effects at the end of treatment.

Conclusion

In this paper, we have argued that more research is needed into the placebo response in psychiatry. We have shown that understanding the factors that cause placebo responses in clinical trials, and whether there are novel ways to measure this effect, is necessary for improving clinical trial design to better evidence the efficacy of new treatments. This is important as many novel treatments fail in the late stages of development, so any improvements in this area could allow more treatments to gain approval. Furthermore, we have shown that improved understanding of

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how placebo mechanisms including expectation and learning operate in the clinic, might allow us to maximise the effectiveness of our current treatment arsenal. Finally, early evidence in the field of depression has shown that research into the placebo response could lead to the identification of novel treatment targets in neuropsychiatric disease. For these reasons, it is vital that we pursue research into the placebo response.

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References

Agid, O., Siu, C. O., Potkin, S. G., Kapur, S., Watsky, E., Vanderburg, D., … Remington, G. (2013). Meta-regression analysis of placebo response in antipsychotic trials, 1970–2010. *American Journal of Psychiatry*, *170*(11), 1335–1344. doi:10.1176/appi.ajp.2013.12030315.

Amanzio, M., Pollo, A., Maggi, G., & Benedetti, F. (2001). Response variability to analgesics: A role for non-specific activation of endogenous opioids. *Pain*, *90*(3), 205–215. doi:10.1016/s0304-3959(00)00486-3.

Ashar, Y. K., Chang, L. J., & Wager, T. D. (2017). Brain mechanisms of the placebo effect: An affective appraisal account. *Annual Review of Clinical Psychology*, *13*(1), 73–98. doi:10.1146/annurev-clinpsy-021815-093015.

Atlas, L. Y., & Wager, T. D. (2014). A meta-analysis of brain mechanisms of placebo analgesia: Consistent findings and unanswered questions. In F. Benedetti, P. Enck, E. Frisaldi, & M. Schedlowski (Eds.), *Placebo* (pp. 37–69). Berlin, Heidelberg: Springer Berlin Heidelberg.

Atlas, L. Y., Whittington, R. A., Lindquist, M. A., Wielgosz, J., Sonty, N., & Wager, T. D. (2012). Dissociable influences of opiates and expectations on pain. *Journal of Neuroscience*, *32*(23), 8053–8064. doi:10.1523/jneurosci.0383-12.2012.

Babel, P., Bajcar, E. A., Adamczyk, W., Kicman, P., Lisińska, N., Świder, K., & Colloca, L. (2017). Classical conditioning without verbal suggestions elicits placebo analgesia and nocebo hyperalgesia. *PLoS One*, *12*(7), e0181856. doi:10.1371/journal.pone.0181856.

Bandelow, B., Reitt, M., Röver, C., Michaelis, S., Görlich, Y., & Wedekind, D. (2015). Efficacy of treatments for anxiety disorders. *International Clinical Psychopharmacology*, *30*(4), 183–192. doi:10.1097/yic.0000000000000078.

Barsky, A. J., Saintfort, R., Rogers, M. P., & Borus, J. F. (2002). Nonspecific medication side effects and the nocebo phenomenon. *JAMA*, *287*(5), 622–627. doi:10.1001/jama.287.5.622.

Beecher, H. K. (1955). The powerful placebo. *Journal of the American Medical Association*, *159*(17), 1602–1606. doi:10.1001/jama.1955.02960340022006.

Benedetti, F., Amanzio, M., Rosato, R., & Blanchard, C. (2011*a*). Nonopioid placebo analgesia is mediated by CB1 cannabinoid receptors. *Nature Medicine*, *17*(10), 1228–1230. doi:10.1038/nm.2435.

Benedetti, F., Amanzio, M., Vighetti, S., & Asteggiano, G. (2006). The biochemical and neuroendocrine bases of the hyperalgesic nocebo effect. *The Journal of Neuroscience*, *26*, 12014–12022. doi:10.1523/JNEUROSCI.2947-06.2006.

Benedetti, F., Carlino, E., & Piedimonte, A. (2016). Increasing uncertainty in CNS clinical trials: The role of placebo, nocebo, and Hawthorne effects. *Lancet Neurology*, *15*(7), 736–747. doi:10.1016/s1474-4422(16)00066-1.

Benedetti, F., Carlino, E., & Pollo, A. (2011b). How placebos change the patient's brain. *Neuropsychopharmacology*, *36*, 339–354. doi:10.1038/npp.2010.81.

Benedetti, F., Maggi, G., Lopiano, L., Lanotte, M., Rainero, I., Vighetti, S., … Pollo, A. (2003). Open versus hidden medical treatments: The patient's knowledge about a therapy affects the therapy outcome. *Prevention & Treatment*, *6*(1), Article 1a. doi:10.1037/1522-3736.6.1.61a.

Blease, C. R. (2018). Psychotherapy and placebos: Manifesto for conceptual clarity. *Frontiers in Psychiatry*, *9*, 379. doi:10.3389/fpsyt.2018.00379.

Browne, C. A., & Lucki, I. (2019). Targeting opioid dysregulation in depression for the development of novel therapeutics. *Pharmacology & Therapeutics*, *201*, 51–76. doi:10.1016/j.pharmthera.2019.04.009.

Colgan, S., Faasse, K., Martin, L. R., Stephens, M. H., Grey, A., & Petrie, K. J. (2015). Perceptions of generic medication in the general population, doctors and pharmacists: A systematic review. *BMJ Open*, *5*(12), e008915. doi:10.1136/bmjopen-2015-008915.

Colloca, L., & Benedetti, F. (2006). How prior experience shapes placebo analgesia. *Pain*, *124*(1), 126–133. doi:10.1016/j.pain.2006.04.005.

Colloca, L., Lopiano, L., Lanotte, M., & Benedetti, F. (2004). Overt versus covert treatment for pain, anxiety, and Parkinson's disease. *The Lancet Neurology*, *3*(11), 679–684. doi:10.1016/s1474-4422(04)00908-1.

De La Fuente-Fernandez, R. (2001). Expectation and dopamine release: Mechanism of the placebo effect in Parkinson's disease. *Science (New York, N.Y.)*, *293*(5532), 1164–1166. doi:10.1126/science.1060937.

De Vries, Y. A., De Jonge, P., van den Heuvel, E., Turner, E. H., & Roest, A. M. (2016). Influence of baseline severity on antidepressant efficacy for anxiety disorders: Meta-analysis and meta-regression. *The British Journal of Psychiatry*, *208*(6), 515–521, Retrieved from https://www.cambridge.org/core/services/aop-cambridge-core/content/view/8BC08080EE7A137EAA285B8D8EAFF488/S0007125000243973a.pdf/div-class-title-influence-of-baseline-severity-on-antidepressant-efficacy-for-anxiety-disorders-meta-analysis-and-meta-regression-div.pdf.

Di Blasi, Z., Harkness, E., Ernst, E., Georgiou, A., & Kleijnen, J. (2001). Influence of context effects on health outcomes: A systematic review. *The Lancet*, *357*(9258), 757–762. doi:10.1016/s0140-6736(00)04169-6.

Enck, P., Bingel, U., Schedlowski, M., & Rief, W. (2013). The placebo response in medicine: Minimize, maximize or personalize? *Nature Reviews Drug Discovery*, *12*(3), 191–204. doi:10.1038/nrd3923.

Enck, P., Klosterhalfen, S., & Weimer, K. (2016). Unsolved, forgotten, and ignored features of the placebo response in medicine. *Clinical Therapeutics*, *39*(3), 458–468. doi:10.1016/j.clinthera.2016.11.016.

Enck, P., & Zipfel, S. (2019). Placebo effects in psychotherapy: A framework. *Frontiers in Psychiatry*, *10*, 456. doi:10.3389/fpsyt.2019.00456.

Ernst, E., & Resch, K. L. (1995). Concept of true and perceived placebo effects. *BMJ*, *311*, 551–553. doi:10.1136/bmj.311.7004.551.

Evers, A. W. M., Colloca, L., Blease, C., Annoni, M., Atlas, L. Y., Benedetti, F., … Kelley, J. M. (2018). Implications of placebo and nocebo effects for clinical practice: Expert consensus. *Psychotherapy and Psychosomatics*, *87*(4), 204–210. doi:10.1159/000490354.

Faasse, K., Cundy, T., Gamble, G., & Petrie, K. J. (2013). The effect of an apparent change to a branded or generic medication on drug effectiveness and side effects. *Psychosomatic Medicine*, *75*(1), 90–96. doi:10.1097/PSY.0b013e3182738826.

Faria, V., Appel, L., Ahs, F., Linnman, C., Pissiota, A., Frans, O., … Furmark, T. (2012). Amygdala subregions tied to SSRI and placebo response in patients with social anxiety disorder. *Neuropsychopharmacology*, *37*(10), 2222–2232. doi:10.1038/npp.2012.72.

Faria, V., Gingnell, M., Hoppe, J. M., Hjorth, O., Alaie, I., Frick, A., … Furmark, T. (2017). Do you believe it? Verbal suggestions influence the clinical and neural effects of escitalopram in social anxiety disorder: A randomized trial. *EBioMedicine*, *24*, 179–188. doi:10.1016/j.ebiom.2017.09.031.

Fields, H. (2004). State-dependent opioid control of pain. *Nature Reviews. Neuroscience*, *5*, 565–575. doi:10.1038/nrn1431.

Flaten, M. A., Aslaksen, P. M., Lyby, P. S., & Bjørkedal, E. (2011). The relation of emotions to placebo responses. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *366*, 1818–1827. doi:10.1098/rstb.2010.0407.

Furukawa, T. A., Cipriani, A., Atkinson, L. Z., Leucht, S., Ogawa, Y., Takeshima, N., … Salanti, G. (2016). Placebo response rates in antidepressant trials: A systematic review of published and unpublished double-blind randomised controlled studies. *The Lancet Psychiatry*, *3*(11), 1059–1066, Retrieved from https://www.sciencedirect.com/science/article/abs/pii/S2215036616303078?via&per;3Dihub.

Glombiewski, J. A., Rheker, J., Wittkowksi, J., Rebstock, L., & Rief, W. (2019). Placebo mechanisms in depression: An experimental investigation of the impact of expectations on sadness in female participants. *Journal of Affective Disorders*, *256*, 658–667. doi:10.1016/j.jad.2019.06.070.

https://doi.org/10.1017/S0033291720003633 Published online by Cambridge University Press

Goebel, M. U., Trebst, A. E., Steiner, J., Xie, Y. F., Exton, M. S., Frede, S., … Schedlowski, M. (2002). Behavioral conditioning of immunosuppression is possible in humans. *FASEB Journal*, *16*, 1869–1873. doi:10.1096/fj.02-0389com.

Graham, B., Garcia, N. M., Burton, M. S., Cooper, A. A., Roy-Byrne, P. P., Mavissakalian, M. R., … Zoellner, L. A. (2018). High expectancy and early response produce optimal effects in sertraline treatment for post-traumatic stress disorder. *The British Journal of Psychiatry*, *213*(6), 704–708. doi:10.1192/bjp.2018.211.

Henry, S. G., Fuhrel-Forbis, A., Rogers, M. A. M., & Eggly, S. (2012). Association between nonverbal communication during clinical interactions and outcomes: A systematic review and meta-analysis. *Patient Education and Counseling*, *86*(3), 297–315. doi:10.1016/j.pec.2011.07.006.

Huneke, N. T., & Baldwin, D. S. (2015). Does previous experience of antidepressants form the expectations necessary for a placebo response? *The British Journal of Psychiatry*, *207*(6), 561. doi:10.1192/bjp.207.6.561a.

Jensen, K. B., Kirsch, I., Ponten, M., Rosen, A., Yang, K., Gollub, R. L., … Curie, A. (2017). Certainty of genuine treatment increases drug responses among intellectually disabled patients. *Neurology*, *88*(20), 1912–1918. doi:10.1212/WNL.0000000000003934.

Kampermann, L., Nestoriuc, Y., & Shedden-Mora, M. C. (2017). Physicians' beliefs about placebo and nocebo effects in antidepressants – an online survey among German practitioners. *PLoS One*, *12*(5), e0178719. doi:10.1371/journal.pone.0178719.

Kessner, S., Wiech, K., Forkmann, K., Ploner, M., & Bingel, U. (2013). The effect of treatment history on therapeutic outcome: An experimental approach. *JAMA Internal Medicine*, *173*(15), 1468. doi:10.1001/jamainternmed.2013.6705.

Kong, J., Gollub, R. L., Polich, G., Kirsch, I., Laviolette, P., Vangel, M., … Kaptchuk, T. J. (2008). A functional magnetic resonance imaging study on the neural mechanisms of hyperalgesic nocebo effect. *The Journal of Neuroscience*, *28*, 13354–13362. doi:10.1523/JNEUROSCI.2944-08.2008.

Krogsboll, L., Hróbjartsson, A., & Gøtzsche, P. C. (2009). Spontaneous improvement in randomised clinical trials: Meta-analysis of three-armed trials comparing no treatment, placebo and active intervention. *BMC Medical Research Methodology*, *9*(1), 1. doi:10.1186/1471-2288-9-1.

Leucht, S., Chaimani, A., Leucht, C., Huhn, M., Mavridis, D., Helfer, B., … Davis, J. M. (2018). 60 years Of placebo-controlled antipsychotic drug trials in acute schizophrenia: Meta-regression of predictors of placebo response. *Schizophrenia Research*, *201*, 315–323. doi:10.1016/j.schres.2018.05.009.

Leucht, S., Leucht, C., Huhn, M., Chaimani, A., Mavridis, D., Helfer, B., … Davis, J. M. (2017). Sixty years of placebo-controlled antipsychotic drug trials in acute schizophrenia: Systematic review, Bayesian meta-analysis, and meta-regression of efficacy predictors. *American Journal of Psychiatry*, *174*(10), 927–942. doi:10.1176/appi.ajp.2017.16121358.

Leuchter, A. F., Hunter, A. M., Tartter, M., & Cook, I. A. (2014). Role of pill-taking, expectation and therapeutic alliance in the placebo response in clinical trials for major depression. *The British Journal of Psychiatry*, *205*(6), 443–449. doi:10.1192/bjp.bp.113.140343.

Lidstone, S. C., Schulzer, M., Dinelle, K., Mak, E., Sossi, V., Ruth, T. J., … Stoessl, A. J. (2010). Effects of expectation on placebo-induced dopamine release in Parkinson disease. *Archives of General Psychiatry*, *67*(8), 857–865.

Mayberg, H. S., Silva, J. A., Brannan, S. K., Tekell, J. L., Mahurin, R. K., McGinnis, S., &, & Jerabek, P. A. (2002). The functional neuroanatomy of the placebo effect. *The American Journal of Psychiatry*, *159*, 728–737.

McArthur, R. A. (2017). Aligning physiology with psychology: Translational neuroscience in neuropsychiatric drug discovery. *Neuroscience & Biobehavioral Reviews*, *76*, 4–21. doi:10.1016/j.neubiorev.2017.02.004.

McQueen, D., Cohen, S., St John-Smith, P., & Rampes, H. (2013). Rethinking placebo in psychiatry: The range of placebo effects. *Advances in Psychiatric Treatment*, *19*(3), 162–170. doi:10.1192/apt.bp.112.010397.

Miller, F. G., & Rosenstein, D. L. (2006). The nature and power of the placebo effect. *Journal of Clinical Epidemiology*, *59*(4), 331–335. doi:10.1016/j.jclinepi.2005.12.001.

Nestoriuc, Y., von Blanckenburg, P., Schuricht, F., Barsky, A. J., Hadji, P., Albert, U. S., & Rief, W. (2016). Is it best to expect the worst? Influence of patients' side-effect expectations on endocrine treatment outcome in a 2-year prospective clinical cohort study. *Annals of Oncology*, *27*(10), 1909–1915. doi:10.1093/annonc/mdw266.

Neukirch, N., & Colagiuri, B. (2015). The placebo effect, sleep difficulty, and side effects: A balanced placebo model. *Journal of Behavioral Medicine*, *38*(2), 273–283. doi:10.1007/s10865-014-9590-5.

Nummenmaa, L., &, & Tuominen, L. (2018). Opioid system and human emotions. *British Journal of Pharmacology*, *175*(14), 2737–2749. doi:10.1111/bph.13812.

Papakostas, G. I., & Fava, M. (2009). Does the probability of receiving placebo influence clinical trial outcome? A meta-regression of double-blind, randomized clinical trials in MDD. *European Neuropsychopharmacology*, *19*(1), 34–40. doi:10.1016/j.euroneuro.2008.08.009.

Pecina, M., Bohnert, A. S., Sikora, M., Avery, E. T., Langenecker, S. A., Mickey, B. J., &, & Zubieta, J. K. (2015). Association between placebo-activated neural systems and antidepressant responses: Neurochemistry of placebo effects in major depression. *JAMA Psychiatry*, *72*(11), 1087–1094. doi:10.1001/jamapsychiatry.2015.1335.

Peciña, M., & Zubieta, J.-K. (2015). Molecular mechanisms of placebo responses in humans. *Molecular Psychiatry*, *20*(4), 416–423. doi:10.1038/mp.2014.164.

Petrie, K. J., & Rief, W. (2019). Psychobiological mechanisms of placebo and nocebo effects: Pathways to improve treatments and reduce side effects. *Annual Review of Psychology*, *70*(1), 599–625. doi:10.1146/annurev-psych-010418-102907.

Petrovic, P., Dietrich, T., Fransson, P., Andersson, J., Carlsson, K., & Ingvar, M. (2005). Placebo in emotional processing-induced expectations of anxiety relief activate a generalized modulatory network. *Neuron*, *46*, 957–969. doi:10.1016/j.neuron.2005.05.023.

Rutherford, B. R., Wall, M. M., Brown, P. J., Choo, T.-H., Wager, T. D., Peterson, B. S., … Roose, S. P. (2017). Patient expectancy as a mediator of placebo effects in antidepressant clinical trials. *American Journal of Psychiatry*, *174*(2), 135–142. doi:10.1176/appi.ajp.2016.16020225.

Sandler, A. D., Glesne, C. E., & Bodfish, J. W. (2010). Conditioned placebo dose reduction: A new treatment in attention-deficit hyperactivity disorder? *Journal of Developmental and Behavioral Pediatrics*, *31*(5), 369–375. doi:10.1097/DBP.0b013e3181e121ed.

Schienle, A., Ubel, S., Schongassner, F., Ille, R., & Scharmuller, W. (2014). Disgust regulation via placebo: An fMRI study. *Social Cognitive and Affective Neuroscience*, *9*(7), 985–990. doi:10.1093/scan/nst072.

Scott, D. J., Stohler, C. S., Egnatuk, C. M., Wang, H., Koeppe, R. A., & Zubieta, J.-K. (2008). Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. *Archives of General Psychiatry*, *65*, 220–231. doi:10.1001/archgenpsychiatry.2007.34.

Skripka-Serry, J. (2013). The great neuro-pipeline 'brain drain' (and why Big Pharma hasn't given up on CNS disorders). *Drug Discovery World*, *14*(4), 9–16.

Stein, D. J., Baldwin, D. S., Dolberg, O. T., Despiegel, N., & Bandelow, B. (2006). Which factors predict placebo response in anxiety disorders and major depression? *The Journal of Clinical Psychiatry*, *67*(11), 1741–1746.

Thompson, J. J., Ritenbaugh, C., & Nichter, M. (2009). Reconsidering the placebo response from a broad anthropological perspective. *Culture, Medicine, and Psychiatry*, *33*(1), 112–152. doi:10.1007/s11013-008-9122-2.

Velligan, D. I., Sajatovic, M., Hatch, A., Kramata, P., & Docherty, J. (2017). Why do psychiatric patients stop antipsychotic medication? A systematic review of reasons for nonadherence to medication in patients with serious mental illness. *Patient Preference and Adherence*, *11*, 449–468. doi:10.2147/ppa.s124658.

Wager, T. D., & Atlas, L. Y. (2015). The neuroscience of placebo effects: Connecting context, learning and health. *Nature Reviews Neuroscience*, *16*(7), 403–418. doi:10.1038/nrn3976.

Walsh, B. T., Seidman, S. N., Sysko, R., &, & Gould, M. (2002). Placebo response in studies of major depression: Variable, substantial, and growing. *JAMA: The Journal of the American Medical Association*, *287*, 1840–1847. Retrieved from http://jama.jamanetwork.com/article.aspx?articleid=194819.

Webster, R. K., Weinman, J., & Rubin, G. J. (2018). Positively framed risk information in patient information leaflets reduces side effect reporting: A double-blind randomized controlled trial. *Annals of Behavioral Medicine*, *52*(11), 920–929. doi:10.1093/abm/kax064.

https://doi.org/10.1017/S0033291720003633 Published online by Cambridge University Press

Whitlock, M., Woodward, P., & Alexander, R. (2019). Is high placebo response really a problem in depression trials? A critical Re-analysis of depression studies. *Innovations in Clinical Neuroscience*, *16*(07-08), 12–17.

Woods, S. W., Gueorguieva, R. V., Baker, C. B., & Makuch, R. W. (2005). Control group bias in randomized atypical antipsychotic medication trials for schizophrenia. *Archives of General Psychiatry*, *62*(9), 961. doi:10.1001/archpsyc.62.9.961.

Zelen, M. (1979). A new design for randomized clinical trials. *New England Journal of Medicine*, *300*(22), 1242–1245. doi:10.1056/NEJM197905313002203.

Zunhammer, M., Ploner, M., Engelbrecht, C., Bock, J., Kessner, S. S., & Bingel, U. (2017). The effects of treatment failure generalize across different routes of drug administration. *Science Translational Medicine*, *9*(393), eaal2999. doi:10.1126/scitranslmed.aal2999.

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Diverse predictors of treatment response to active medication and placebo in gambling disorder

Nathan T.M. Huneke [a,b,c,*], Samuel R. Chamberlain [a,b,c,d], David S. Baldwin [a,b,c,e], Jon E. Grant [f]

[a] Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, UK
[b] University Department of Psychiatry, University of Southampton, UK
[c] Southern Health National Health Service Foundation Trust, Southampton, UK
[d] Department of Psychiatry, University of Cambridge, UK
[e] University Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa
[f] Department of Psychiatry & Behavioral Neuroscience, University of Chicago, Pritzker School of Medicine, Chicago, IL, USA

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ABSTRACT

Gambling disorder creates a significant public health burden. Despite decades of clinical trials, there are no licensed pharmacological treatments for gambling disorder. Contributing factors to this are the high placebo response rates seen in clinical trials, the heterogeneity of the disorder and high rates of psychiatric comorbidities. Indeed, a number of demographic and clinical variables have previously been associated with altered responses to pharmacotherapy, psychotherapy and placebo. Which variables are likely to predict response to one modality over another remains uncertain. We carried out multiple linear regression analyses in a pooled dataset from six treatment studies in gambling disorder with the aim of identifying predictors of treatment response. Potential predictors were identified *a priori* through hypothesis and entered into models including all patients, and subsequently for those randomized to active medication or placebo separately. We found that baseline severity of gambling symptoms and number of weeks completed in a trial were predictors of active medication response, while decreased baseline symptoms of anxiety, increased baseline symptoms of depression, and non-Caucasian ethnicity were associated with placebo response. Sensitivity analyses showed that these associations were robust to choices made during the analysis. Further research is required to understand whether controlling for these variables, or using enriched samples, improves assay sensitivity in placebo-controlled clinical trials for gambling disorder.

1. Introduction

Gambling can be a normal recreational activity; however, in some individuals, gambling behavior can become persistent, recurrent and maladaptive, leading to problems with relationships, employment and financial difficulties (Grant et al., 2010b). The prevalence of subclinical problem gambling behaviors is approximately 3–4%, while a further 1% experience a gambling disorder (Black and Shaw, 2019; Buchanan et al., 2020). Patients with gambling disorder additionally often experience comorbid psychiatric disorders, such as substance use, depression or anxiety disorders (Black and Shaw, 2019; Kessler et al., 2008). Therefore, gambling disorder creates a significant public health burden.

At present there are no licensed pharmacological treatments for gambling disorder despite decades of clinical trials. One contributing factor to this is the large placebo response rate seen in clinical trials for gambling disorder, sometimes surpassing 70% (Kraus et al., 2020). As a result, it can be challenging to demonstrate medication benefits over placebo. Furthermore, gambling disorder is heterogeneous in nature, with differing behavioral subtypes and (as noted) high rates of comorbidity with other psychiatric disorders (Black and Shaw, 2019; Kessler et al., 2008). Which factors are more likely to predict response to one treatment modality or another remains uncertain. This could lead to poor control of confounders in clinical trials for gambling disorder, reducing the trials' assay sensitivities (Huneke et al., 2020). Understanding who is more or less likely to respond to treatment is therefore crucial.

Predictors of treatment response to pharmacotherapy, placebo, and psychotherapy have been explored in gambling disorder. A systematic

* Corresponding author. University Department of Psychiatry, Academic Centre, College Keep, 4-12 Terminus Terrace, Southampton, SO14 3DT, UK.
E-mail address: n.huneke@soton.ac.uk (N.T.M. Huneke).

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review of 33 studies of psychosocial interventions showed that male gender and low depression levels were consistent predictors of successful treatment (Merkouris et al., 2016). Other less consistent but nonetheless important predictors of positive outcomes included older age, being employed, Asian American ethnicity, lower pre-treatment gambling symptom severity, and being in the action stage of change (Merkouris et al., 2016). These findings are supported by similar associations seen in other studies. For example, younger age, lower education level, and higher baseline symptom severity have been linked with poorer treatment outcomes (Granero et al., 2020; Jimenez-Murcia et al., 2015). Conversely, a study conducted in a gambling service in Singapore found that increased seriousness of the patient's gambling problem at baseline was associated with *improved* outcome (Manning et al., 2014). Higher treatment satisfaction and playing strategic games (e.g. poker), as opposed to non-strategic games (e.g. slots), have also been associated with improved psychological treatment outcomes (Guo et al., 2014; Manning et al., 2014; Moragas et al., 2015).

Compared with this literature, predictors of improved outcomes with pharmacotherapy or placebo has received relatively little attention in the context of gambling disorder. In a pooled analysis of two double-blind placebo-controlled trials of opiate antagonists (naltrexone or nalmefene), younger age was associated with placebo response, while a family history of alcoholism and intensity of gambling urges were important in medication response (Grant et al., 2008b). In another study of 152 patients assigned to placebo in multiple randomized-controlled trials, placebo responders stayed in treatment significantly longer, were more likely to support 'enjoyment' and less likely to support 'loneliness' or 'boredom' as triggers for gambling, and were more likely to be non-Caucasian. Interestingly, there was no difference in age between placebo responders and non-responders in this study (Grant and Chamberlain, 2017). However, it is unknown whether these findings are specific to their respective studies or can be generalized to the gambling disorder population as a whole. The first study included only a single class of medication (opiate antagonists) (Grant et al., 2008b). It therefore remains to be determined whether family history of alcoholism and intensity of gambling urges are specific predictors of response to opiate antagonists. The second study did not include patients assigned to active medication, meaning it is unclear whether the predictive factors identified are specific to placebo response or are non-specific predictors of improvement in symptoms (Grant and Chamberlain, 2017; Huneke et al., 2020).

In this study, we aimed to identify predictors of treatment response in gambling disorder, and then to explore whether response predictors for pharmacotherapy and placebo differed. We pooled data from treatment studies carried out in patients meeting diagnostic criteria for gambling disorder by the same group of researchers. A number of different pharmacological agents were tested in these trials with either an open-label or double-blind, placebo-controlled design. We hypothesized that some variables measured in all studies would transcend study design and treatment modality to predict treatment response in gambling disorder. Specifically, based on the above reported relationships between predictor variables and treatment outcomes, we hypothesized that demographic (age, gender, and ethnicity) and clinical variables (baseline severity, symptoms of anxiety or depression, comorbid psychiatric illness, and non-strategic gambling) would predict response to treatment. Previous treatment experience is also known to affect subsequent treatment outcomes (Colloca and Benedetti, 2006; Kessner et al., 2013; Zunhammer et al., 2017). We therefore also hypothesized that previously seeking gambling treatment and number of weeks spent in the trial might be important. Our secondary hypothesis was that significant predictors of response to active medication and to placebo would differ. The information gleaned from this analysis might inform study design in future clinical trials for gambling disorder.

2. Method

2.1. Characteristics of included trials

We included data from six treatment studies in gambling disorder all carried out by the same group of researchers. In all trials, patients were adults (aged 18–75 years) with a diagnosis of gambling disorder according to DSM-5 criteria (studies conducted prior to DSM-5 used criteria for pathological gambling based on DSM-IV and all of these participants met DSM-5 criteria for gambling disorder when the criteria were reapplied), which was confirmed through a semi-structured clinical interview (Grant et al., 2004). Exclusion criteria were: current significant medical illness or abnormalities on physical examination; current pregnancy or breastfeeding; history of dementia, bipolar affective disorder, or any psychotic disorder; current substance abuse or dependence except nicotine; and recent (within 3 months) initiation of psychological or behavioral therapy. We included three double-blind, randomized, placebo-controlled trials:

1. A 16-week trial of paroxetine in 76 outpatients with gambling disorder in five centres across the USA and Spain. Patients who did not respond during a 1-week placebo run-in phase were randomized to paroxetine or placebo. Paroxetine was initiated at 10 mg/day, and increased to 20 mg/day during week 2 with flexible dosing up to 60 mg/day. Response rate did not significantly differ between groups (59% in the paroxetine group, 49% in the placebo group, $p = 0.390$) (Grant et al., 2003).
2. An 18-week trial of naltrexone in 77 patients. Patients who did not respond during a 1-week placebo run-in phase were randomized to one of four conditions: naltrexone 50 mg/day, 100 mg/day, 150 mg/day, or placebo. Outcomes did not differ significantly between the doses of naltrexone. Patients randomized to naltrexone exhibited significantly greater reductions in gambling severity ($p = 0.0094$), gambling urges ($p = 0.0053$), and gambling behavior ($p = 0.0134$) compared with patients on placebo (Grant et al., 2008a).
3. A 12-week trial of N-acetylcysteine in 28 patients with co-occurring nicotine dependence and gambling disorder. Patients were randomized to either N-acetylcysteine 1200 mg/day (increased to 3000 mg/day based on clinical judgement) or placebo. For the first 6 weeks, patients additionally received smoking cessation treatment, and in weeks 6–12, patients received imaginal desensitization plus motivational interviewing therapy for pathological gambling. Following this, all treatments ceased and patients were followed up 3 months later. There was no significant additional benefit of N-acetylcysteine over placebo during the treatment period. However, at the 3-month follow-up after treatment had ended, patients randomized to N-acetylcysteine exhibited significantly reduced severity of gambling symptoms ($p = 0.043$) (Grant et al., 2014). For the purposes of this study, data for the 6-week period of treatment for gambling disorder are included.

We also included three open-label treatment studies:

1. A 12-week open-label pilot study of escitalopram in 13 patients with co-occurring anxiety and gambling disorders. Patients completed a 1-week placebo run-in phase, after which escitalopram was initiated at 10 mg/day, increasing at fortnightly intervals to 30 mg/day (unless clinical improvement was seen at a lower dose). During the 12-week treatment period, there was a significant decrease in gambling symptom severity ($p = 0.002$) (Grant and Potenza, 2006).
2. A 14-week pilot study of N-acetylcysteine in 36 patients. N-acetylcysteine was initiated at 600 mg/day for 2 weeks and increased fortnightly to 1800 mg/day (unless clinical improvement was seen at a lower dose). There was a significant decrease in gambling symptom severity at the end of treatment ($p < 0.001$) (Grant et al., 2007).

3. A 10-week pilot study of open-label memantine in 29 patients. All eligible patients were started on 10 mg/day for 2 weeks, and the dose was increased fortnightly to 30 mg/day (unless clinical improvement was seen at a lower dose). There was an unusually high retention rate in this study, with 28 of 29 patients completing the entire 10-week treatment period. Gambling symptom severity significantly improved over the treatment period ($p < 0.001$) (Grant et al., 2010a).

An assessment of these trials' quality is available in supplementary material. All trials were carried out in accordance with the protocol and provisions of the Declaration of Helsinki. All study procedures and accompanying literature were approved by The Institutional Review Boards of the University of Minnesota and the University of Chicago. All patients provided informed, written consent to participate.

2.2. Study assessments

Data were gathered for all eligible patients regarding general demographics and problems due to gambling. Additionally, patients completed the following measures at baseline:

1. Structured Clinical Interview for Gambling Disorder (SCI-GD) – a clinician-administered, nine-item instrument that assesses gambling symptoms over the past 12 months according to the DSM criteria for gambling disorder (modified to reflect DSM-5) (Grant et al., 2004).
2. Structured Clinical Interview for DSM-IV (SCID-I) – to assess for the presence of psychiatric comorbidity (First et al., 1994).
3. Gambling Symptom Assessment Scale (G-SAS) – a validated, reliable self-report measure of gambling symptom severity over the past week. This scale consists of 12 items, each scoring 0–4, with a maximum score of 48 (Kim et al., 2009).
4. Hamilton Rating Scale for Depression (HAM-D) – a validated, reliable clinician-administered measure of depression severity over the past month (Hamilton, 1960).
5. Hamilton Rating Scale for Anxiety (HAM-A) – a validated, reliable clinician-administered measure of anxiety symptom severity over the past month (Hamilton, 1959).

In all trials, change in G-SAS (post-treatment G-SAS subtracted from baseline G-SAS) was an outcome measure of improvement in gambling disorder symptoms, and was therefore chosen as the primary outcome measure for this analysis.

2.3. Statistical analysis

All statistical analyses were carried out using Jamovi version 1.6.23.0 (https://www.jamovi.org) (The jamovi project, 2021). Descriptive statistics were calculated for demographic and baseline measures. A one-way ANOVA demonstrated that there were no significant differences between studies in mean change in G-SAS ($F_{(5,200)} = 1.65$, $p = 0.147$). Data were therefore pooled from all studies for subsequent analyses.

Potential predictors of treatment response were assessed through multiple linear regression analysis. We chose a linear analysis to reduce the risk of bias that can be introduced by transforming linear outcome and/or covariate variables into categorical groups (Ritz, 2021). Since the placebo response rate in gambling disorder trials is high (Kraus et al., 2020), it is common practice to exclude patients with mild disorder from these trials to prevent floor or ceiling effects interfering with detection of medication effects (Whitlock et al., 2019). Therefore, to ensure external validity of our analyses, we excluded patients with a baseline G-SAS of less than 20 (classified as 'mild' severity of symptoms). We entered the following predictors into the model, based on reported relationships in the literature between predictor variables and treatment outcomes and *a priori* hypotheses: age, gender, ethnicity (dichotomized to Caucasian and non-Caucasian), weeks completed in the trial, whether the patient had previously sought gambling treatment, baseline G-SAS, baseline HAM-D, baseline HAM-A, presence of psychiatric comorbidity, and whether the patient preferred strategic or non-strategic games. All predictors were entered simultaneously. We initially ran this model on all patients as a single group. Next, to identify whether predictors of treatment response differ for active medication or placebo, we ran the same models separately in patients randomized to active medication and in those randomized to placebo. Each model was applied only to the pooled sample of patients in which all entered variables had been measured. Where data were missing, these participants were excluded from the model via listwise deletion.

We assessed the robustness of our findings to choices made through two sensitivity analyses. First, we carried out the same regressions but included those with mild G-SAS scores at baseline to ensure we had not introduced selection bias by excluding these patients. Second, although patients with mild disorder were excluded, it is possible that in a regression analysis involving a change score as the dependent variable, that predictor variables could reach significance through floor or ceiling effects in the remaining sample. We therefore ran a sensitivity analysis with percentage reduction in G-SAS as the dependent variable, rather than absolute change in G-SAS, to identify whether any predictors reached significance due to remaining floor or ceiling effects. Percentage reduction in G-SAS was calculated as follows:

$$\left(\frac{Change\ in\ G-SAS}{Baseline\ G-SAS}\right) \times 100\%$$

3. Results

3.1. Baseline characteristics

The final intent-to-treat sample size was n = 279 patients, which reduced to n = 235 patients after excluding those with a G-SAS of less than 20 at baseline. Of these, 168 patients (71%) were assigned to active medication arms and 67 (29%) were assigned to placebo. The patients' mean age was 47.76 ± 11.11 years. The majority of patients were single or divorced (n = 129, 55%), and 32 (18%) had a previous history of alcohol dependence. On average, patients started gambling aged 37.82 ± 12.52 years, and mean time to gambling becoming a problem was 10.07 ± 9.61 years. Forty-two percent of patients had previously sought treatment for gambling disorder. Across all studies, mean baseline G-SAS was 31.51 ± 6.42 (severe), which on average reduced to 19.28 ± 10.78 (mild). Baseline characteristics are summarized in Table 1.

3.2. Predictors of treatment response

Predictor variables associated with response to treatment in all patients, and with active medication or placebo response, are summarized in Table 2.

3.2.1. All patients

When pooling all patients, the regression model was significant and explained 18% of the variance in change in G-SAS (adjusted $R^2 = 0.18$, $F_{(11,119)} = 3.60$, $p = 0.00021$). Significant positive predictors of treatment response included baseline G-SAS ($\beta = 0.37$, $t = 4.26$, $p < 0.0001$), number of weeks completed in the trial ($\beta = 0.19$, $t = 2.33$, $p = 0.0215$), and baseline HAM-D score ($\beta = 0.36$, $t = 2.29$, $p = 0.0235$). Baseline HAM-A score was the only significant negative predictor of treatment response ($\beta = -0.52$, $t = -3.32$, $p = 0.0012$).

3.2.2. Active arms only

For patients receiving active medication, the regression model was significant and explained 22% of the variance in change in G-SAS (adjusted $R^2 = 0.22$, $F_{(11,85)} = 3.46$, $p = 0.00051$). The only positive predictors of treatment response in this model were baseline G-SAS ($\beta =$

Table 1
Baseline characteristics.

Continuous variables	Mean (±SD)
Age (yrs)	47.76 (11.11)
G-SAS baseline	31.51 (6.42)
G-SAS final	19.28 (10.78)
HAM-A baseline	7.03 (4.37)
HAM-D baseline	7.11 (4.08)
Weeks completed	10.89 (5.15)
Grant et al. (2003)	13.47 (4.30)
Grant and Potenza (2006)	8.80 (3.05)
Grant et al. (2007)	7.77 (4.02)
Grant et al. (2008a)	13.21 (6.06)
Grant et al. (2010a)	9.78 (1.15)
Grant et al. (2014)	6.00 (0.00)
Categorical variables	*N* (%)
Females	122 (52.0)
Caucasians	193 (86.0)
Previous gambling treatment	67 (42.0)
Psychiatric co-morbidity present	89 (51.0)
Strategic gambling	27 (12.0)
Education	
High school graduate or less	48 (21.0)
Some college	89 (40.0)
College graduate or more	87 (39.0)

Abbreviations: SD, standard deviation; G-SAS, Gambling Symptom Assessment Scale; HAM-A, Hamilton Rating Scale for Anxiety; HAM-D, Hamilton Rating Scale for Depression.

Table 2
Results of multiple linear regression models calculated to predict treatment response (change in G-SAS). Separate models were calculated for all patients, and for those allocated to active medication or placebo.

	All patients		Active medication		Placebo	
Predictor	β coeff.	95% CI	β coeff.	95% CI	β coeff.	95% CI
Age	0.01	−0.17, 0.18	0.07	−0.14, 0.27	−0.18	−0.51, 0.15
Gender	0.17	−0.16, 0.51	0.18	−0.21, 0.58	−0.20	−0.90, 0.49
Non-Caucasian	0.11	−0.37, 0.58	−0.13	−0.75, 0.49	**0.97**	**0.17, 1.77**[a]
Weeks completed	**0.19**	**0.03, 0.36**[a]	**0.30**	**0.11, 0.49**[b]	−0.22	−0.57, 0.12
Previous gambling treatment	−0.03	−0.36, 0.30	−0.01	−0.38, 0.37	0.16	−0.54, 0.85
Baseline G-SAS	**0.37**	**0.20, 0.55**[d]	**0.43**	**0.23, 0.64**[d]	0.27	−0.08, 0.62
Baseline HAM-A	**−0.52**	**−0.83, -0.21**[b]	−0.35	−0.72, 0.02	**−1.03**	**−1.58, -0.48**[c]
Baseline HAM-D	**0.36**	**0.05, 0.67**[a]	0.24	−0.13, 0.61	**0.62**	**0.07, 1.17**[a]
Psychiatric comorbidity	−0.03	−0.37, 0.30	−0.04	−0.43, 0.34	0.40	−0.30, 1.11
Strategic gambling	−0.48	−1.03, 0.07	−0.43	−1.03, 0.17	0.13	−1.25, 1.52

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, [d] $p < 0.0001$.
β refers to standardised regression coefficients.

0.43, t = 4.17, p < 0.0001) and number of weeks completed in the trial (β = 0.30, t = 3.20, p = 0.0020). Baseline HAM-A score approached significance as a negative predictor (β = −0.35, t = −1.87, p = 0.0643).

3.2.3. Placebo arms only

For patients receiving placebo, the regression model was significant and explained 30% of the variance in change in G-SAS (adjusted R^2 = 0.30, $F_{(11,22)} = 2.29$, $p = 0.04725$). Baseline HAM-A score was a negative predictor of placebo response (β = −1.03, t = −3.88, p = 0.0008), while baseline HAM-D score positively predicted placebo response (β = 0.62, t = 2.32, p = 0.0299). Finally, being non-Caucasian was a significant positive predictor of change in G-SAS scores following placebo treatment (β = 0.97, t = 2.50, p = 0.0203).

3.3. Sensitivity analyses

To assess the robustness of our findings against choices made during analysis, we modified the regression models detailed above with: 1. Patients with mild G-SAS scores (<20) included and 2. Percentage reduction in G-SAS as the dependent variable. The results of these sensitivity analyses are summarized in Supplementary Tables S1 and S2.

All models including patients with baseline G-SAS score of less than 20 remained significant. In patients assigned active medication, baseline HAM-A score was an additional significant negative predictor of treatment response (β = −0.37, t = −2.11, p = 0.0379). In patients assigned placebo, baseline G-SAS score was an additional positive predictor (β = 0.50, t = 3.11, p = 0.0048), while baseline HAM-D score was no longer a significant predictor.

When percentage reduction in G-SAS was the dependent variable, the only change in predictors in all 3 models was that baseline G-SAS score was no longer significant in the model that included all patients, instead approaching significance (β = 0.17, t = 1.87, p = 0.0638). Baseline G-SAS remained a significant predictor of response to active medication (β = 0.25, t = 2.30, p = 0.0240).

4. Discussion

We aimed to identify predictors of treatment response, and whether the predictors for pharmacotherapy and placebo response differed, in gambling disorder. Through multiple regression analyses, we identified that baseline severity of gambling symptoms, number of weeks completed in a trial, and baseline anxiety and depressive symptoms were significant predictors of subsequent response to treatment. Importantly, these associations appeared to be driven by opposing effects in patients assigned to active medication and those assigned to placebo. In those randomized to active medication, higher baseline severity of gambling symptoms and number of weeks completed in the trial were predictors of greater treatment response, while decreased baseline symptoms of anxiety, increased baseline symptoms of depression, and non-Caucasian ethnicity were associated with improved response to placebo. Our sensitivity analyses showed that these associations were robust to choices made during the analysis.

4.1. Predictors of response to active medication

We found that the number of weeks a patient spent in a trial was positively predictive of response to active medication. It is plausible this represents a dose-effect of medication, i.e. taking it for longer leads to greater improvement. However, there could be other explanations for this association. For example, previous studies have shown that 'treatment satisfaction' can be associated with symptom improvement following cognitive behavioral therapy (CBT) for pathological gambling (Guo et al., 2014; Manning et al., 2014). It is conceivable the effect of weeks completed might represent 'treatment satisfaction' in that patients could be more likely to withdraw from a clinical trial if they are unsatisfied. Counter to this, number of weeks completed was not a predictor of reduction in G-SAS in the placebo arms. Indeed, the beta coefficient for this predictor was negative in the placebo arms. Not only does this suggest that the effect of number of weeks in the trial is not a 'treatment satisfaction' effect, this also potentially suggests that trials of longer duration might reduce placebo response rate. In a previous analysis of placebo response in gambling disorder, patients who responded to placebo had, on average, spent a greater number of weeks in the trial compared with those who did not respond (Grant and Chamberlain, 2017). This analysis involved a dichotomous definition of response (greater than 35% reduction in G-SAS) that possibly obscures

some nuance in the data. Further, it is not known whether a 35% reduction in G-SAS is an appropriate cut-off for defining "response" (Grant and Chamberlain, 2017). The effect of study duration on both active medication and placebo response requires further study.

We also found a positive association between baseline severity and response to active medication. This remained significant when percentage reduction in G-SAS was the dependent variable, suggesting this was not the result of a ceiling effect. A previous systematic review exploring predictors of treatment response in gambling disorder found that lower baseline symptom severity was predictive of improved outcome (Merkouris et al., 2016). However, all the studies included in that review involved psychosocial interventions, and none involved medication. Instead, increased baseline severity might be predictive of improved outcome with medication. Supporting this, baseline G-SAS score was not a predictor of placebo response in this sample. This is potentially similar to conditions such as depression where increasing severity is associated with greater benefit of medication over placebo (Fournier et al., 2010). Baseline symptom severity could be a useful clinical indicator to help identify who might derive benefit from medication or psychotherapeutic interventions in those with gambling disorder.

4.2. Predictors of placebo response

Increased anxiety symptoms at baseline was associated with lower change in G-SAS over time. Anxiety is often comorbid with gambling disorder, and there is evidence that anxiety disorders precede the onset of gambling disorder (Kessler et al., 2008), and stress is predictive of relapse (Buchanan et al., 2020). It is possible therefore that those entering a trial with increased anxiety symptoms at baseline are at risk of deteriorating, or minimally improving, over the course of a trial. The effect of baseline anxiety on the progression of gambling disorder symptoms within a clinical trial should be considered during analysis.

Another intriguing possibility is that anxiety reduces the placebo effect in these patients. Anxiety is known to reduce placebo and increase nocebo effects on pain (Colloca et al., 2010; Corsi and Colloca, 2017; Morton et al., 2009; Staats et al., 2001), and has also been correlated with nocebo effects on itch (Bartels et al., 2016). It is noteworthy that the opioidergic and dopaminergic systems have been implicated in both gambling disorder and placebo effects (Benedetti et al., 2011; Grant et al., 2016; Huneke et al., 2020). Further investigation of the neurobiology of placebo and nocebo effects in gambling disorder, and how anxiety symptoms might interact with these, is needed.

HAM-D scores at baseline positively predicted placebo response. Gambling disorder is associated with symptoms of depression, and these have been hypothesized to be important in the pathogenesis of the disorder (Hodgins et al., 2005; Kim et al., 2006; Valleur et al., 2016). However, in treatment studies of depression, higher baseline depressive symptoms have been linked to *reduced* placebo response rates (Stein et al., 2006), which makes the present result puzzling. It should be noted that in the treatment studies included in the present analysis, high levels of depressive symptoms and the presence of other axis I psychiatric disorders were exclusion criteria. Indeed, in this sample the average HAM-D score was 7.11 (±4.08) suggesting no or mild depressive symptoms. It is unclear therefore whether the effect of baseline HAM-D score on treatment outcome is the result of depressive symptoms or an epiphenomenon not directly measured. Nevertheless, the effects of baseline depressive and anxious symptoms on treatment outcome in studies of medication for gambling disorder warrants further research.

We also found that non-Caucasian ethnicity was associated with greater reduction in G-SAS in the placebo groups. This replicates a previous finding that non-Caucasians receiving placebo in treatment studies for gambling disorder were more likely to be categorized as "responders" (Grant and Chamberlain, 2017). The reasons for this are unclear. It has been argued that non-Caucasians might experience *reduced* placebo effects that normally result from a clinical encounter due to inequalities in health services (Friesen and Blease, 2018), although we are not aware of any empirical data showing this to be the case. Interestingly, a study exploring predictors of treatment outcome in a US outpatient problem gambling service found that Asian Americans were more likely to benefit than Caucasians, while other ethnicities such as Native Americans were less likely to demonstrate a favorable outcome (Ingle et al., 2008). Ethnicity might be an important variable to consider in treatment studies of gambling disorder.

4.3. Limitations

There are several limitations that need to be discussed. First, for a study of this kind, the sample size is relatively small. There is a risk therefore of false positives, although the findings were robust to different analysis choices as shown by our sensitivity analyses. Second, due to the nature of the linear regression model chosen, we could only include those individuals with data for all measures. The effect of missing data on the outcome has not been assessed. Third, the longest trial duration in this analysis was 18 weeks. It is unclear whether the predictors identified here are also associated with outcomes beyond this time. Fourth, in a linear analysis such as this, it could be argued whether statistically significant predictors are also predictive of clinically significant outcomes. In the present sample, mean baseline G-SAS was 31.51 (severe), which on average reduced to 19.28 (mild). Although the ideal assessment and threshold for clinical improvement in gambling disorder remains in doubt to some extent (Walker et al., 2006), this would generally be regarded as a clinically significant improvement. This suggests that the identified predictors are associated with clinically significant improvements in gambling symptoms. Finally, there are a number of potential factors that might be associated with treatment response that were not measured consistently or at all in these studies. For example, expectations of therapeutic benefit, which likely play a role in both active medication response and placebo effects (Huneke et al., 2020; Petrie and Rief, 2019; Rutherford et al., 2017). Triggers for gambling behavior have also been associated with placebo response in gambling disorder (Grant and Chamberlain, 2017), but were not measured consistently enough in the present studies to be included in the regression model. Predictors of response to psychological treatments for gambling disorder also include certain personality traits and being in the action stage of change (Merkouris et al., 2016). Additionally, predictors of response might differ for different classes of medication. Due to the small sample sizes of parent studies we were unable to assess this within this dataset. Further studies are needed to assess the effects of such factors in medication studies for gambling disorder, and whether they interact with the predictors identified here.

5. Conclusion

We carried out a pooled analysis of medication studies in gambling disorder. We found that baseline severity of symptoms and number of weeks completed were significant predictors of active medication response. Conversely, baseline anxiety and depressive symptoms, and non-Caucasian ethnicity were significant predictors of placebo response. Further research is required to understand whether controlling for these variables, or using enriched samples, improves assay sensitivity in placebo-controlled clinical trials for gambling disorder.

CRediT author statement

Nathan Huneke: Conceptualization, Formal Analysis, Writing – Original Draft, Writing – Review & Editing **Samuel Chamberlain:** Conceptualization, Resources, Data curation, Writing – Review & Editing **David Baldwin:** Supervision, Writing – Review & Editing **Jon Grant:** Conceptualization, Investigation, Resources, Data curation, Supervision, Writing – Review & Editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Huneke's role in this study was funded by a Medical Research Council Clinical Research Training Fellowship (MR/T000902/1). Dr Huneke has also consulted for Emteq Ltd. Prof. Grant has received research grants from the TLC Foundation for Body-Focused Repetitive Behaviors, and Otsuka, Biohaven, and Avanir Pharmaceuticals. He receives yearly compensation for acting as editor-in-chief of the Journal of Gambling Studies and has received royalties from Oxford University Press, American Psychiatric Publishing, Inc., Norton Press, and McGraw Hill. Prof. Chamberlain's role in this study was funded by a Wellcome Trust Clinical Fellowship (110049/Z/15/Z & 110049/Z/15/A). Prof. Chamberlain receives honoraria from Elsevier for journal editorial work. He previously consulted for Promentis. Prof. Baldwin receives honoraria from Wiley for journal editorial work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2021.09.053.

References

Bartels, D.J.P., Van Laarhoven, A.I.M., Van De Kerkhof, P.C.M., Evers, A.W.M., 2016. Placebo and nocebo effects on itch: effects, mechanisms, and predictors. Eur. J. Pain 20 (1), 8–13.

Benedetti, F., Carlino, E., Pollo, A., 2011. How placebos change the patient's brain. Neuropsychopharmacology 36, 339–354.

Black, D.W., Shaw, M., 2019. The Epidemiology of Gambling Disorder. Springer International Publishing, pp. 29–48.

Buchanan, T.W., McMullin, S.D., Baxley, C., Weinstock, J., 2020. Stress and gambling. Curr. Opin. Behav. Sci. 31, 8–12.

Colloca, L., Benedetti, F., 2006. How prior experience shapes placebo analgesia. Pain 124 (1), 126–133.

Colloca, L., Petrovic, P., Wager, T.D., Ingvar, M., Benedetti, F., 2010. How the number of learning trials affects placebo and nocebo responses. Pain 151 (2), 430–439.

Corsi, N., Colloca, L., 2017. Placebo and nocebo effects: the advantage of measuring expectations and psychological factors. Front. Psychol. 8.

First, M., Spitzer, R., Gibbon, M., Williams, J., 1994. Structured Clinical Interview for DSM-IV Patient Edition. Biometrics Research Department, New York, Version 2.0.

Fournier, J.C., Derubeis, R.J., Hollon, S.D., Dimidjian, S., Amsterdam, J.D., Shelton, R.C., Fawcett, J., 2010. Antidepressant drug effects and depression severity. J. Am. Med. Assoc. 303 (1), 47.

Friesen, P., Blease, C., 2018. Placebo effects and racial and ethnic health disparities: an unjust and underexplored connection. J. Med. Ethics 44 (11), 774–781.

Granero, R., Valero-Solis, S., Fernández-Aranda, F., Gómez-Peña, M., Moragas, L., Mena-Moreno, T., Pino-Gutierrez, A.D., Codina, E., Martín-Romera, V., Casalé, G., Agüera, Z., Baenas-Soto, I., Valenciano-Mendoza, E., Mora-Maltas, B., Sánchez, I., Lozano-Madrid, M., Menchón, J.M., Murcia, S.J., 2020. Response trajectories of gambling severity after cognitive behavioral therapy in young-adult pathological gamblers. J. Behav. Addict. 9 (1), 140–152.

Grant, J.E., Chamberlain, S.R., 2017. The placebo effect and its clinical associations in gambling disorder. Ann. Clin. Psychiatr. 29 (3), 167–172.

Grant, J.E., Chamberlain, S.R., Odlaug, B.L., Potenza, M.N., Kim, S.W., 2010a. Memantine shows promise in reducing gambling severity and cognitive inflexibility in pathological gambling: a pilot study. Psychopharmacology (Berl.) 212 (4), 603–612.

Grant, J.E., Kim, S.W., Hartman, B.K., 2008a. A double-blind, placebo-controlled study of the opiate antagonist naltrexone in the treatment of pathological gambling urges. J. Clin. Psychiatr. 69 (5), 783–789.

Grant, J.E., Kim, S.W., Hollander, E., Potenza, M.N., 2008b. Predicting response to opiate antagonists and placebo in the treatment of pathological gambling. Psychopharmacology 200 (4), 521–527.

Grant, J.E., Kim, S.W., Odlaug, B.L., 2007. N-acetyl cysteine, a glutamate-modulating agent, in the treatment of pathological gambling: a pilot study. Biol. Psychiatr. 62 (6), 652–657.

Grant, J.E., Kim, S.W., Potenza, M.N., Blanco, C., Ibanez, A., Stevens, L., Hektner, J.M., Zaninelli, R., 2003. Paroxetine treatment of pathological gambling: a multi-centre randomized controlled trial. Int. Clin. Psychopharmacol. 18 (4), 243–249.

Grant, J.E., Odlaug, B.L., Chamberlain, S.R., 2016. Neural and psychological underpinnings of gambling disorder: a review. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 65, 188–193.

Grant, J.E., Odlaug, B.L., Chamberlain, S.R., Potenza, M.N., Schreiber, L.R., Donahue, C. B., Kim, S.W., 2014. A randomized, placebo-controlled trial of N-acetylcysteine plus imaginal desensitization for nicotine-dependent pathological gamblers. J. Clin. Psychiatr. 75 (1), 39–45.

Grant, J.E., Potenza, M.N., 2006. Escitalopram treatment of pathological gambling with co-occurring anxiety: an open-label pilot study with double-blind discontinuation. Int. Clin. Psychopharmacol. 21 (4), 203–209.

Grant, J.E., Schreiber, L., Odlaug, B.L., Kim, S.W., 2010b. Pathologic gambling and bankruptcy. Compr. Psychiatr. 51 (2), 115–120.

Grant, J.E., Steinberg, M.A., Kim, S.W., Rounsaville, B.J., Potenza, M.N., 2004. Preliminary validity and reliability testing of a structured clinical interview for pathological gambling. Psychiatr. Res. 128 (1), 79–88.

Guo, S., Manning, V., Thane, K.K.W., Ng, A., Abdin, E., Wong, K.E., 2014. Predictors of treatment outcome among asian pathological gamblers (PGs): clinical, behavioural, demographic, and treatment process factors. J. Gambl. Stud. 30 (1), 89–103.

Hamilton, M., 1959. The assessment of anxiety states by rating. Br. J. Med. Psychol. 32 (1), 50–55.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatr. 23 (1), 56.

Hodgins, D.C., Peden, N., Cassidy, E., 2005. The association between comorbidity and outcome in pathological gambling: a prospective follow-up of recent quitters. J. Gambl. Stud. 21 (3), 255–271.

Huneke, N.T.M., van der Wee, N., Garner, M., Baldwin, D.S., 2020. Why we need more research into the placebo response in psychiatry. Psychol. Med. 50 (14), 2317–2323.

Ingle, P.J., Marotta, J., McMillan, G., Wisdom, J.P., 2008. Significant others and gambling treatment outcomes. J. Gambl. Stud. 24 (3), 381–392.

Jimenez-Murcia, S., Granero, R., Fernandez-Aranda, F., Arcelus, J., Aymami, M.N., Gomez-Pena, M., Tarrega, S., Moragas, L., Del Pino-Gutierrez, A., Sauchelli, S., Fagundo, A.B., Brewin, N., Menchon, J.M., 2015. Predictors of outcome among pathological gamblers receiving cognitive behavioral group therapy. Eur. Addiction Res. 21 (4), 169–178.

Kessler, R.C., Hwang, I., LaBrie, R., Petukhova, M., Sampson, N.A., Winters, K.C., Shaffer, H.J., 2008. DSM-IV pathological gambling in the national comorbidity survey replication. Psychol. Med. 38 (9), 1351–1360.

Kessner, S., Wiech, K., Forkmann, K., Ploner, M., Bingel, U., 2013. The effect of treatment history on therapeutic outcome: an experimental approach. JAMA Int. Med. 173 (15), 1468.

Kim, S.W., Grant, J.E., Eckert, E.D., Faris, P.L., Hartman, B.K., 2006. Pathological gambling and mood disorders: clinical associations and treatment implications. J. Affect. Disord. 92 (1), 109–116.

Kim, S.W., Grant, J.E., Potenza, M.N., Blanco, C., Hollander, E., 2009. The gambling symptom assessment scale (G-SAS): a reliability and validity study. Psychiatr. Res. 166 (1), 76–84.

Kraus, S.W., Etuk, R., Potenza, M.N., 2020. Current pharmacotherapy for gambling disorder: a systematic review. Expet Opin. Pharmacother. 21 (3), 287–296.

Manning, V., Ng, A., Koh, P.K., Guo, S., Gomathinayagam, K., Wong, K.E., 2014. Pathological gamblers in Singapore: treatment response at 3 months. J. Addiction Med. 8 (6), 462–469.

Merkouris, S.S., Thomas, S.A., Browning, C.J., Dowling, N.A., 2016. Predictors of outcomes of psychological treatments for disordered gambling: a systematic review. Clin. Psychol. Rev. 48, 7–31.

Moragas, L., Granero, R., Stinchfield, R., Fernández-Aranda, F., Fröberg, F., Aymamí, N., Gómez-Peña, M., Fagundo, A.B., Islam, M.A., Del Pino-Gutiérrez, A., Agüera, Z., Savvidou, L.G., Arcelus, J., Witcomb, G.L., Sauchelli, S., Menchón, J.M., Jiménez-Murcia, S., 2015. Comparative analysis of distinct phenotypes in gambling disorder based on gambling preferences. BMC Psychiatr. 15 (1).

Morton, D.L., Watson, A., El-Deredy, W., Jones, A.K.P., 2009. Reproducibility of placebo analgesia: effect of dispositional optimism. Pain 146 (1), 194–198.

Petrie, K.J., Rief, W., 2019. Psychobiological mechanisms of placebo and nocebo effects: pathways to improve treatments and reduce side effects. Annu. Rev. Psychol. 70 (1), 599–625.

Ritz, C., 2021. Statistical analysis of continuous outcomes from parallel-arm randomized controlled trials in nutrition—a tutorial. Eur. J. Clin. Nutr. 75 (1), 160–171.

Rutherford, B.R., Wall, M.M., Brown, P.J., Choo, T.-H., Wager, T.D., Peterson, B.S., Chung, S., Kirsch, I., Roose, S.P., 2017. Patient expectancy as a mediator of placebo effects in antidepressant clinical trials. Am. J. Psychiatr. 174 (2), 135–142.

Staats, P.S., Staats, A., Hekmat, H., 2001. The additive impact of anxiety and a placebo on pain. Pain Med. 2 (4), 267–279.

Stein, D.J., Baldwin, D.S., Dolberg, O.T., Despiegel, N., Bandelow, B., 2006. Which factors predict placebo response in anxiety disorders and major depression? J. Clin. Psychiatr. 67 (11), 1741–1746.

The jamovi project, 2021. jamovi. https://www.jamovi.org. Version 1.6.23.0.

Valleur, M., Codina, I., Venisse, J.L., Romo, L., Magalon, D., Fatseas, M., Chereau-Boudet, I., Gorsane, M.A., Guilleux, A., Groupe, J.E.U., Grall-Bronnec, M., Challet-Bouju, G., 2016. Towards a validation of the three pathways model of pathological gambling. J. Gambl. Stud. 32 (2), 757–771.

Walker, M., Toneatto, T., Potenza, M.N., Petry, N., Ladouceur, R., Hodgins, D.C., El-Guebaly, N., Echeburua, E., Blaszczynski, A., 2006. A framework for reporting outcomes in problem gambling treatment research: the Banff, Alberta Consensus. Addiction 101 (4), 504–511.

Whitlock, M.E., Woodward, P.W., Alexander, R.C., 2019. Is high placebo response really a problem in depression trials? A critical Re-analysis of depression studies. Innov. Clin. Neurosci. 12–17.

Zunhammer, M., Ploner, M., Engelbrecht, C., Bock, J., Kessner, S.S., Bingel, U., 2017. The effects of treatment failure generalize across different routes of drug administration. Sci. Transl. Med. 9 (393), eaal2999.

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REGULAR RESEARCH ARTICLE

Functional Neuroimaging Correlates of Placebo Response in Patients With Depressive or Anxiety Disorders: A Systematic Review

Nathan T.M. Huneke, Ibrahim H. Aslan, Harry Fagan, Naomi Phillips, Rhea Tanna, Samuele Cortese, Matthew Garner, David S. Baldwin

Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK (Dr Huneke, Dr Aslan, Prof Garner, and Prof Baldwin); Southern Health National Health Service Foundation Trust, Southampton, UK (Dr Huneke, Dr Fagan, Dr Tanna, and Prof Baldwin); Solent National Health Service Trust, Southampton, UK (Dr Phillips and Prof Cortese); University Department of Psychiatry, Academic Centre, Southampton, UK (Dr Huneke, Dr Aslan, Dr Fagan, and Prof Baldwin); University Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa (Prof Baldwin); School of Psychology, Faculty of Environmental and Life Sciences (Prof Garner), and Center for Innovation in Mental Health, School of Psychology, Faculty of Environmental and Life Sciences (Prof Cortese), University of Southampton, Southampton, UK; Hassenfeld Children's Hospital at NYU Langone, New York University Child Study Center, New York City, New York, USA (Prof Cortese); Division of Psychiatry and Applied Psychology, School of Medicine, University of Nottingham, Nottingham, UK (Prof Cortese).

Correspondence: Nathan T. M. Huneke, University Department of Psychiatry, Academic Centre, College Keep, 4-12 Terminus Terrace, Southampton, SO14 3DT, UK (n.huneke@soton.ac.uk).

Abstract

Background: The mechanisms underlying placebo effects of psychotropic drugs remain poorly understood. We carried out the first, to our knowledge, systematic review of functional neuroimaging correlates of placebo response in adults with anxiety/depressive disorders.
Methods: We systematically searched a large set of databases up to February 2021 based on a pre-registered protocol (PROSPERO CRD42019156911). We extracted neuroimaging data related to clinical improvement following placebo or related to placebo mechanisms. We did not perform a meta-analysis due to the small number of included studies and significant heterogeneity in study design and outcome measures.
Results: We found 12 relevant studies for depressive disorders and 4 for anxiety disorders. Activity in the ventral striatum, rostral anterior cingulate cortex and other default mode network regions, orbitofrontal cortex, and dorsolateral prefrontal cortex correlated with placebo antidepressant responses. Activity in regions of the default mode network, including posterior cingulate cortex, was associated with placebo anxiolysis. There was also evidence for possible involvement of the endogenous opioid, dopamine, and serotonin systems in placebo antidepressant and anxiolytic effects.
Conclusions: Several brain regions and molecular systems may be involved in these placebo effects. Further adequately powered studies exploring causality and controlling for confounders are required.

Keywords: Placebo response, depression, anxiety, functional neuroimaging

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Introduction

Anxiety and depression are the most common psychiatric conditions (Wittchen et al., 2011) and cause significant distress, impair function, and reduce quality of life. There is a need to improve treatments for these conditions, because many patients do not respond or experience unwanted side effects. Placebo-controlled trials are the gold-standard method for assessing efficacy of medications. However, the placebo response in psychotropic trials is a large effect. Approximately 30% of patients in antidepressant trials demonstrate a placebo response (Walsh et al., 2002; Stein et al., 2006; Furukawa et al., 2016), and in anxiety disorders the effect size of placebo ranges from .65 to 1.29 (Bandelow et al., 2015; De Vries et al., 2016). This has implications for the design and interpretation of psychotropic drug trials. However, the mechanisms underlying placebo effects in depression and anxiety are poorly understood (Huneke et al., 2020).

Symptom improvement in the placebo arm of a trial can be partly explained by nonspecific phenomena, such as regression to the mean or sampling bias due to dropouts of the least improved patients (Ernst and Resch, 1995; Ashar et al., 2017; Evers et al., 2018). However, improvements can also result from specific placebo effects in which an interplay between learning and expectations causes biological changes in the immune system, hypothalamic-pituitary-adrenal axis, and the endogenous opioid system (Ernst and Resch, 1995; Benedetti et al., 2011; Peciña and Zubieta, 2015; Evers et al., 2018). The neuroimaging correlates of placebo effects in particular domains are well understood, such as in placebo analgesia (Atlas and Wager, 2014; Wager and Atlas, 2015; Zunhammer et al., 2021). However, neuroimaging correlates of placebo antidepressant and anxiolytic effects have not been delineated. Identifying these markers might help us understand the mechanisms involved in placebo effects in these conditions. This might allow us to improve clinical trial design or identify novel therapeutic targets (Huneke et al., 2020).

We carried out a systematic review to identify functional neuroimaging correlates of the placebo effect in adults with anxiety or depression. We aimed to understand current knowledge of the neuroanatomy and neurotransmitter systems important in these effects and identify hypotheses to be tested in future studies.

METHODS

The review was carried out according to PRISMA guidelines (Page et al., 2021). Five authors (N.H., I.A., H.F., N.P., R.T.) performed the systematic review and data extraction independently in pairs. All discrepancies were resolved by consensus. The protocol was registered prospectively with PROSPERO (CRD42019156911).

Literature Search

Our full search strategy is reported in the supplemental material. We performed the search, with no date or language restrictions, on March 9, 2019, and updated on September 2, 2021. We also reviewed reference lists of relevant review articles for additional records.

At least 2 reviewers screened all titles and abstracts against the following inclusion criteria: the study was a randomized trial involving a placebo intervention; patients were aged 18–65 years with a unipolar depressive or anxiety disorder; patients underwent functional neuroimaging (positron emission tomography [PET], single-photon emission computed tomography, functional magnetic resonance imaging [fMRI]), and change in depressive or anxiety symptoms was an outcome measure. Although not prespecified, we chose to also include arterial spin labelling (ASL) imaging on reviewing our search results to avoid excluding potentially informative studies. We obtained full texts for potentially eligible articles, which were then screened by at least 2 reviewers. Articles were included if they presented neuroimaging data associated with an objective clinical improvement following placebo treatment or with placebo mechanisms such as learning or expectancy.

Quality Assessment

We assessed for risk of bias with the Cochrane Collaboration's risk of bias 2 tool for randomized trials (Sterne et al., 2019). One reviewer (I.A., H.F., or R.T.) recorded risk of bias for each record using a standardized form, and these assessments were independently checked by a second reviewer (N.H.). We assessed the risk of bias due to randomization, deviations from the intended intervention, missing data, outcome measurement, and selective reporting.

Data Extraction and Synthesis

One reviewer (I.A., H.F., or R.T.) extracted data by using a piloted, standardized form. All extracted data were checked independently by a second reviewer (N.H.). We extracted data regarding the patient population, study design, imaging modality, missing data, and key clinical and imaging results.

Due to the small number of included articles, of which only approximately one-half included whole-brain analyses, and the significant heterogeneity in study design and outcome measures, we were unable to conduct a formal meta-analysis. We therefore undertook a narrative synthesis of the data.

RESULTS

Our search initially identified 6006 records. We identified 1 additional record through hand-searching of reference lists. After de-duplication, we screened 3286 titles and abstracts, 234 full-text articles were obtained, and 16 records met inclusion criteria (Figure 1). Twelve studies were of patients with depression utilizing the following imaging modalities: ASL (Cooper et al., 2019), fMRI (Sikora et al., 2016; Pecina et al., 2018; Zilcha-Mano et al., 2019; Chin Fatt et al., 2020; Fan et al., 2020; Greenberg et al., 2020; Chin Fatt et al., 2021b; Chin Fatt et al., 2021a; Peciña et al., 2021), and PET (Mayberg et al., 2002; Pecina et al., 2015). Four studies were in patients with social anxiety disorder (SAD) utilizing PET (Furmark et al., 2008; Faria et al., 2012; Faria et al., 2014) and fMRI (Faria et al., 2017). Sample sizes ranged from 8 to 279 patients, and one-half of the studies were carried out in samples of fewer than 50 patients. There was overlap in these samples with 8 studies of patients with depression (Peciña et al., 2015; Sikora et al., 2016; Cooper et al., 2019; Chin Fatt et al., 2020; Fan et al., 2020; Greenberg et al., 2020; Chin Fatt et al., 2021b; Chin Fatt et al., 2021a) and 3 of patients with SAD (Furmark et al., 2008; Faria et al., 2012, 2014) sharing similar or identical samples. Therefore, the maximum total number of patients included in this review is n = 503. The mean age of the patients in each study ranged from 28.8 years (SD ± 8.6) to 49 years (SD ± 9.0). Most patients were female (67%), with only 2 studies reporting a majority of male patients (Mayberg et al., 2002; Faria et al., 2017).

We report here the key details of the included studies, which are summarized in Tables 1 and 2.

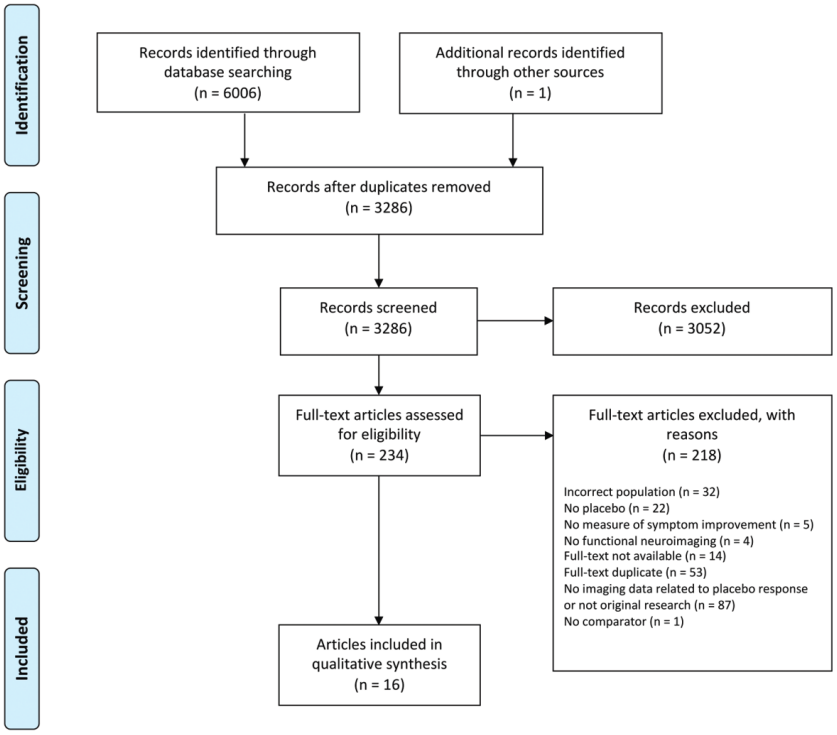

Figure 1. Study selection flow diagram.

Depression

Twelve studies described imaging markers of placebo antidepressant responses. The first published study to report imaging markers of placebo antidepressant responses was carried out by Mayberg et al. (2002). In this 6-week randomized trial of fluoxetine compared with placebo, 8 of 15 patients with depression responded to treatment (fluoxetine n = 4, placebo n = 4). The patients underwent PET imaging at baseline and at 1 week and 6 weeks after commencing treatment, and changes in regional cerebral glucose metabolism at these timepoints were computed separately for the placebo and drug responder groups. Placebo response was associated with significant regional changes in metabolism (beta-$2_{(1972)}$ = 3.97, $P < .0001$). Increased cerebral glucose metabolism was seen in regions including dorsolateral prefrontal cortex, posterior insula and posterior cingulate cortex; and decreased metabolism in subgenual anterior cingulate cortex, thalamus, anterior insula, and parahippocampus. These regions overlapped with those seen in patients who responded to fluoxetine.

Six relevant papers for this review have been published from the Establishing Moderators and Biosignatures of Antidepressant Response in Clinical Care (EMBARC) trial (Cooper et al., 2019; Chin Fatt et al., 2020, 2021a, 2021b; Fan et al., 2020; Greenberg et al., 2020). The aim of this trial was to identify neural predictors or correlates of response to treatment. Patients with major depressive disorder (n = 296) underwent ASL and fMRI at rest, and fMRI during a monetary reward task, before randomization to treatment with either sertraline or placebo. The 17-item Hamilton Rating Scale for Depression (HAMD-17) was used to monitor response to treatment.

Of the original 296 patients, 231 completed the baseline ASL scan. Of these, 37% of patients who received sertraline and 33% of patients who received placebo achieved remission (HAMD-17 < 7). Through a whole-brain, voxel-wise, linear mixed-effects model of the ASL and clinical data, 30 clusters of >100 voxels were found to be significant moderators of treatment response, that is, these brain regions showed a significant treatment×time×relative cerebral perfusion interaction. Perfusion in regions including right putamen and insula, left inferior temporal gyrus, right orbital frontal gyrus, and left parahippocampal gyrus moderated response to sertraline. Conversely, moderators of placebo response included regions involved in cognitive control and the default mode networks, such as right posterior insula, right orbital frontal cortex, and right dorsolateral prefrontal cortex (Cooper et al., 2019).

A number of analyses of the baseline resting-state fMRI data have been conducted (Chin Fatt et al., 2020, 2021a, 2021b; Fan et al., 2020). In an analysis exploring pretreatment resting-state connectome fingerprints of treatment response in 200 of the EMBARC patients, no connectome fingerprints specific to placebo response were found (Fan et al., 2020). In a larger sample

Table 1. Summary of Papers Examining Functional Neuroimaging Markers of Placebo Effects in Patients With Depression

Reference	n	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Analysis	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparator							
Chin Patt et al. (2020)	279	Sertraline	Placebo	8 wk	HAMD-17	fMRI	Pretreatment resting-state functional connectivity	ROI	Total of 32.2% (90/279) achieved remission (49 sertraline, 41 placebo)	No within-network moderators Higher connectivity of hippocampus with executive control network and lower connectivity of thalamus with visual and salience networks predicted better outcome with placebo Higher connectivity of the hippocampus with the visual, dorsal attention, executive control networks, and thalamus, the limbic network with the salience and somatomotor networks, and the executive control network with the salience and somatomotor networks predicted greater improvement with placebo and worse outcome with sertraline.
Chin Patt et al. (2021a)	244	Sertraline	Placebo	8 wk	HAMD-17	fMRI	Pretreatment resting-state functional connectivity correlates of subgroups defined through principal component analysis	ROI	Total of 32% (79/244) achieved remission (39 sertraline, 40 placebo)	Subgroups with greater improvement with placebo typified by increased connectivity within the limbic network, between hippocampus and visual network, and salience network with dorsal attention network
Chin Patt et al. (2021b)	279	Sertraline	Placebo	8 wk	HAMD-17	fMRI	Pretreatment resting-state functional connectivity	ROI	As above	As baseline connectivity between dorsolateral PFC and inferior parietal cortex increased, superiority of sertraline over placebo reduced. This was driven by a relative increase in efficacy of placebo. A similar pattern was seen for baseline connectivity between subcallosal and posterior cingulate cortices.

Table 1. Continued

Reference	n	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Analysis	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparator							
Cooper et al. (2019)	231	Sertraline	Placebo	8 wk	HAMD-17	ASL	Pretreatment resting-state, relative cerebral perfusion	Whole brain	Total of 35% (81/231) achieved remission: 37% of patients on sertraline vs 33% of patients on placebo	Relative perfusion in right posterior insula; left midbrain; right hippocampus; right inferior frontal; right middle and inferior frontal gyri (including the dorsolateral PFC); left precentral gyrus; left inferior frontal; left middle temporal gyrus; right caudate; left cerebellum; right middle, superior, and inferior frontal gyri; left middle frontal gyrus (and dorsolateral PFC); right middle temporal gyrus; left cuneus; left cingulate; left fusiform gyrus; and the left inferior frontal gyrus moderated response to placebo.
Fan et al. (2020)	200	Sertraline	Placebo	8 wk	HAMD-17	fMRI	Pre-treatment resting-state connectome fingerprints	Whole brain	No significant differences in clinical outcomes between groups	Greater treatment response independent of modality predicted by decreased connectivity between executive, sensorimotor and salience networks, and increased connectivity between default mode network and the rest of the brain. No connectome fingerprint specific to response to either sertraline or placebo was found
Greenberg et al. (2020)	222	Sertraline	Placebo	8 wk	HAMD-17	fMRI	Pre-treatment change in ventral striatal activity during a monetary reward task	ROI	Across groups, HAMD-17 scores significantly improved over time. No separate statistics for each group presented.	Left ventral striatal "reward index" moderated treatment effects. Patients with greater increases in reward activity over time appeared more likely to benefit from placebo. Patients with lower increase in reward expectancy activity over time were more likely to benefit from sertraline.

Table 1. Continued

Reference	n	Interventions		Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Analysis	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparator							
Mayberg et al. (2002)	8	Fluoxetine	Placebo	6 wk	HAMD-17	PET	Resting-state brain metabolism at baseline and after 6 wk of treatment	Whole brain	Total of 8 of an original 17 responded to treatment (4 placebo, 4 fluoxetine)	Increases seen in PFC (BA 9/46), premotor cortex (BA 6), inferior parietal cortex (BA 40), posterior insula, posterior cingulate (BA 23/31). Decreases seen in sgACC (BA 25), hypothalamus, thalamus, supplementary sensory area, anterior insula, parahippocampus.
Pecina et al. (2015)	35	"Active" placebo Open-label antidepressant	"Inactive" placebo None	2 wk 10 wk	QIDS-SR16	PET	MOR binding potential after "active" vs "inactive" placebo treatment	ROI	Symptom improvement significantly greater for "active" vs "inactive" placebo. Remission at study end significantly higher in placebo responders	Placebo administration reduced MOR binding potential in nucleus accumbens Degree of placebo-induced opioid release in the sgACC, nucleus accumbens, thalamus and amygdala explained 43% of the response to open-label antidepressant treatment.
Pecina et al. (2018)	20	Placebo IV infusion Positive sham neurofeedback	No infusion Negative sham neurofeedback	Single session	Subjective expectation of mood improvement and subjective mood trial by trial	fMRI	Change in BOLD signal	Whole brain	Expectancy significantly higher during placebo infusion. Mood significantly improved following placebo infusion, following positive sham neurofeedback, and when expectancy was higher.	Positive sham neurofeedback led to greater activity in bilateral ventro- and dorsolateral PFC, which was positively correlated with improved mood. Increased activity in left ventro- and dorsolateral PFC associated with greater expectancy when mood was rated higher in previous trial. However, activity in bilateral ventro- and dorsolateral PFC also negatively moderated the effect of higher expectation on subsequent mood improvement.
Pecina et al. (2021)	20	Naltrexone 50 mg Placebo IV infusion Positive sham neurofeedback	Placebo No infusion Negative sham neurofeedback	7–10 d	Subjective expectation of mood improvement and subjective mood trial by trial	fMRI	Change in BOLD signal	Whole brain	Expectancy significantly higher during placebo infusion. Mood significantly improved following positive sham neurofeedback, and this was greater when expectancy was higher.	Higher activity in right ventro- and dorsolateral PFC associated with reduced expectancy*reinforcement condition effect on expectancy and mood ratings.

Table 1. Continued

Reference	n	Interventions: Experimental	Interventions: Comparator	Study duration	Symptom outcome measure	Imaging modality	Imaging measure	Analysis	Clinical results	Imaging markers of placebo response or placebo mechanisms
										Naltrexone partially abolished the expectancy*reinforcement condition effect on expectancy and mood ratings. This was associated with reduced responses in right OFC during processing of positive reinforcement. Participants with greater naltrexone-induced modulation of OFC activity during positive sham neurofeedback had higher expectancy and mood ratings
Sikora et al. (2016)	29	"Active" placebo	"Inactive" placebo	2 wk	QIDS-SR16	fMRI	Resting-state functional connectivity after "active" and "inactive" placebo	Whole-brain and exploratory ROI	Symptom improvement significantly greater for "active" vs "inactive" placebo.	Increased "baseline" connectivity of the rACC with the salience network was significantly associated with greater placebo response. Placebo-induced reduction in rACC with the salience network was also associated with greater placebo response. "Baseline" resting-state connectivity of the salience network was significantly predictive of placebo response.
		Open-label antidepressant	None	10 wk						
Zilcha-Mano et al. (2019)	23	High expectation (100% chance of receiving citalopram) Citalopram	Low expectation (50% chance of receiving citalopram) Placebo	1 wk 8 wk	HAMD-24	fMRI	Change in BOLD signal during a masked emotional face task at baseline and 1 wk after randomization to high or low expectation	Whole-brain and ROI	Patients in the high expectation group demonstrated significantly greater outcome expectation.	High expectation group showed a decrease in amygdala activation from scan 1 to 2 in the and vs neutral face contrast, whereas low expectation group showed an increase. Increases in outcome expectancy significantly correlated with reductions in left amygdala activity. Total of 63.41% of the effect of outcome expectancy on change in HAMD-24 was mediated by changes in amygdala activity.

Abbreviations: ASL, Arterial spin labelling; BA, Brodmann area; BOLD, blood-oxygen-level-dependent imaging; fMRI, functional magnetic resonance imaging; HAMD, Hamilton rating scale for depression; MOR, mu-opioid receptor; OFC, orbitofrontal cortex; PET, positron emission tomography; PFC, prefrontal cortex; QIDS-SR, Quick inventory of depressive symptomatology (self-report); rACC, rostral anterior cingulate cortex; ROI, region of interest; sgACC, subgenual anterior cingulate cortex.

Table 2. Summary of papers examining functional neuroimaging markers of placebo effects in patients with social anxiety disorder

Reference	n	Interventions		Study duration	Treatment response definition	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparison						
Faria et al. (2012)	72	Citalopram or paroxetine	Placebo	6-8 wk	CGI-I = 1 or 2	PET	rCBF during a public speaking task at baseline and study end	Total of 57% of SSRI group responded vs 30% of placebo group	Both SSRI and placebo responders showed reductions in right ventrolateral amygdala and left basomedial/basolateral amygdala. The rCBF change correlated with clinical measures of anxiety. Placebo responders additionally showed increased rCBF in right brainstem/pons compared with placebo nonresponders.
Faria et al. (2014)	72	Citalopram or paroxetine	Placebo	6-8 wk	CGI-I = 1 or 2	PET	Functional connectivity during a public speaking task at baseline and study end	Total of 57% of SSRI group responded vs 30% of placebo group	Placebo responders showed greater negative correlation between left amygdala and left dorsolateral PFC vs placebo nonresponders. Placebo responders showed greater negative correlation between left amygdala and right ventromedial and dorsolateral PFC, and greater positive correlation between left amygdala and right dorsomedial PFC vs SSRI responders.
Faria et al. (2017)	46	"Overt" escitalopram	"Covert" escitalopram	9 wk	LSAS-SR < 39	fMRI	BOLD signal change and functional connectivity during emotional face matching task	"Overt" treatment significantly superior ($d = .24$ vs $d = 1.13$)	Increased reactivity to emotional faces in overt vs covert in bilateral posterior cingulate, left mid temporal gyrus, left inferior frontal gyrus. Covert group showed increased connectivity between amygdala and right posterior cingulate and right insula when viewing emotional faces compared with overt group.

Table 2. Continued

Reference	n	Interventions		Study duration	Treatment response definition	Imaging modality	Imaging measure	Clinical results	Imaging markers of placebo response or placebo mechanisms
		Experimental	Comparison						
Furmark et al. (2008)	25	Placebo	None	8 wk	CGI-I = 1 or 2	PET	rCBF during a public speaking task at baseline and study end. Changes in rCBF associated with genotype.	Total of 10 (40%) responded to placebo	rCBF significantly reduced in left amygdala in placebo responders vs nonresponders. Patients homozygous for the long allele of 5-HTTLPR and/or the G allele of the G-703T polymorphism in TPH2 exhibited a significantly greater reduction in amygdala activity vs heterozygotes. Mediation analysis showed that the change in rCBF in the amygdala mediated the effect of G-703T polymorphism on CGI-I score.

Abbreviations: 5-HTTLPR, serotonin transporter-linked polymorphic region; BOLD, blood-oxygen-level-dependent imaging; CGI-I, Clinical Global Impression–Improvement scale; fMRI, functional magnetic resonance imaging; LSAS-SR, Liebowitz Social Anxiety scale; PET, positron emission tomography; PFC, prefrontal cortex; rCBF, regional cerebral blood flow; SSRI, selective serotonin reuptake inhibitor; TPH2, tryptophan hydroxylase-2.

of 244 patients, connectivity correlates of subgroups identified through principal component analysis were explored. Increased connectivity within the limbic network, between hippocampus and visual network, and salience network with dorsal attention network was associated with subgroups who experienced greater improvement with placebo (Chin Fatt et al., 2021a). Finally, 2 analyses were conducted in 279 EMBARC patients. The first was an exploratory seed-based analysis of 7 networks and some midbrain regions including hippocampus, striatum, thalamus, and amygdala, in a moderation model. Higher connectivity of the hippocampus with the thalamus and the visual, dorsal attention, and executive control networks, and the limbic and the executive control networks with the salience and somatomotor networks predicted improved outcomes with placebo and worse outcomes with sertraline (Chin Fatt et al., 2020). In the second analysis, connectivity between 5-mm-sphere seeds of interest thought to be within the same functional network was included as a term in a moderation analysis. As baseline connectivity between dorsolateral prefrontal cortex and inferior parietal cortex increased, the superiority of sertraline over placebo treatment reduced ($P = .05$). The reduced superiority of sertraline was driven by a relative increase in efficacy of placebo (Chin Fatt et al., 2021b). The differing sample sizes in these analyses were not fully explained, and so there is a risk of bias in these findings.

Finally, 222 EMBARC patients completed a monetary reward task at baseline (Greenberg et al., 2020) to identify whether temporal changes in reward processing within the ventral striatum predicted or moderated treatment response. The authors calculated a "reward index" from the sum of the increase in reward expectancy and the decrease in prediction error-related activity in the ventral striatum from the first half to the second half of the task. The left ventral striatal reward index significantly moderated treatment effects ($F_{(1,193)} = 12.93$, $P = .0004$). Reduced left ventral striatal reward index at baseline conferred greater likelihood of deriving benefit from treatment with sertraline compared with placebo (threshold $Z = -.21$, raw HAMD-17 difference of ≥3, $t_{(193)} = 2.38$, $P = .02$, $d = .32$, 95% CI = 0.06 to 0.58). The threshold at which patients were expected to benefit more greatly from placebo was not directly tested, but from data presented in the paper, when reward index $Z > 2$, placebo treatment showed an advantage of approximately 0 to 4 points on the HAMD-17 over sertraline (Greenberg et al., 2020).

In a version of the "open-hidden" paradigm, Zilcha-Mano et al. (2019) explored the neural correlates of expectancy augmentation in an antidepressant trial. Twenty-three patients with depression underwent fMRI scanning while they viewed masked emotional faces displaying fearful, sad, happy, or neutral expressions. The patients were then randomized to 1 of 2 groups: an open-label group that had 100% chance of receiving citalopram (n = 9) or a placebo-controlled group that had a 50% chance of receiving either citalopram or placebo (n = 14). One week later, after being told which group they had been allocated to but before they received treatment, patients underwent a second fMRI scan while completing the same emotional face task. Following this, the patients completed an 8-week clinical trial of citalopram compared with placebo. Patients in the open group showed significantly improved outcome expectancy post-randomization compared with the placebo-controlled group ($W = 31.5$, $P = .007$). Further, the open-label group showed a significant reduction in activity in the amygdala, bilateral dorsolateral prefrontal cortex, and superior temporal gyrus following randomization compared with the placebo-controlled group in the sad vs neutral faces contrast. The amygdala was then chosen as a region of interest, and a linear association was found between reduction in left amygdala activity and increase in expectancy score post-randomization ($r = -.74$, $P = .006$). A mediation analysis showed that HAMD-24 scores decreased at a faster rate for patients with increased expectancy scores, and this was mediated by greater reductions in amygdala activity post-randomization ($B = -.09$, $P = .007$). However, we noted possible selective reporting in this trial. The amygdala only showed a significant difference in activity in the sad vs neutral faces contrast, whereas other regions demonstrated significant differences in activity in other relevant contrasts. The amygdala is then chosen as a region of interest with little justification, and no further analyses regarding other significant regions are reported (Zilcha-Mano et al., 2019).

Peciña et al. (2015) and Sikora et al. (2016) explored whether imaging correlates of placebo mechanisms can predict antidepressant treatment outcomes using an experimental placebo lead-in phase followed by a 10-week open-label antidepressant trial. Patients were given oral placebo with instructions that this was an antidepressant for 1 week ("active") followed by a 3-day washout and then 1 week of treatment with "inactive" placebo, with disclosure that this was an inert control. After each placebo condition, participants underwent neuroimaging. Results from PET imaging with the μ-opioid receptor-selective radiotracer [^{11}C]carfentanil were reported in 35 patients. After the "active" placebo condition, the PET session additionally included an i.v. infusion of .9% isotonic saline with instructions this was a "rapid-acting antidepressant" as an acute placebo challenge to induce endogenous opioid release. Placebo administration during the PET scan reduced μ-opioid receptor binding potential in the nucleus accumbens (estimate = −.43, $Z = 4.72$, $P < .001$). Further, degree of placebo-induced opioid release in the subgenual anterior cingulate cortex, nucleus accumens, thamalus, and amygdala was associated with reduction in depressive symptoms after 1 week of "active" placebo (estimates ≤ −.38, $Z > 3.80$, $P < .001$) and with response to open-label antidepressant at 10 weeks (estimates ≤ −.60, $Z > 3.98$, $P < .001$). Results from resting-state fMRI scans after each placebo condition were reported in 29 patients. Reduction in depressive symptoms was significantly greater after 1 week of the "active" placebo than after "inactive" placebo ($F = 7.2$, $P = .012$). Increased baseline resting functional connectivity ($Z = 4.35$, adjust $R^2 = .65$, $P < .005$) and reduction in connectivity following "active" placebo of the rostral anterior cingulate cortex within the salience network ($Z = 3.97$, $P < .05$) were associated with greater placebo response (Sikora et al., 2016). However, we identified some potential risks of bias. First, the "baseline" was the scan carried out after 1 week of "inactive" placebo. This does not represent a true baseline due to the crossover design. Placebo analgesia is reduced if participants have experienced a previously ineffective analgesic treatment (Colloca and Benedetti, 2006). Such effects could potentially confound these results. Second, no explanation is given for the discrepancy in sample size in these papers.

Two studies by Pecina et al. (2018, 2021) attempted to manipulate trial-by-trial antidepressant expectancies through a "simulated neurofeedback" task. In brief, this task involved 6 runs of 12 trials, where each trial began with a timer cue reflecting an anticipation period prior to either receiving or not receiving a "rapid-acting antidepressant" infusion (in reality, normal saline). After the infusion cue, participants were shown sham neurofeedback with differing valence (either positive or negative). After both the anticipation and simulated neurofeedback periods, participants rated their expected and actual mood improvements, respectively. In the 2018 study involving 20 patients, there was

greater mood improvement during the infusion cue (b = .12, $P < .05$) and following the display of positive sham neurofeedback (b = .32, $P < .001$), and higher expectation of benefit predicted improved mood (b = .22, $P < .001$). Positive sham neurofeedback led to greater activity in bilateral ventro- and dorsolateral prefrontal cortices, which was positively correlated with improved mood (b = .2, $P < .001$). Increased activity in left ventro- and dorsolateral prefrontal cortices was also associated with greater expectancy when mood improved in the previous trial (b = .05, $P < .05$). However, activity in bilateral ventro- and dorsolateral prefrontal cortices also negatively moderated the effect of higher expectation on subsequent mood improvement (b = –.07, $P < .05$). Finally, β-endorphin plasma levels were also measured before and after the task. Greater increases in β-endorphins were associated with increased expectancy ratings (estimate = .0007, $P = .02$) and greater subjective mood improvement in response to positive neurofeedback (estimate = .002, $P < .001$) (Peciña et al., 2018). In a subsequent double-blind crossover study, 20 patients with depression carried out the same neurofeedback task twice: once following treatment with naltrexone 50 mg and once following matched placebo. In this study, higher activity in the right ventro- and dorsolateral prefrontal cortex was again associated with a reduced placebo×neurofeedback condition effect on expectancy and mood ratings. Naltrexone reduced the effect of the placebo×neurofeedback condition interaction on expectancy (b = –1.00, $P < .001$) and mood ratings (b = –.93, $P = .003$). Naltrexone was also associated with reduced activity in the right orbitofrontal cortex during positive sham neurofeedback (max t = 5.64, cluster size = 334 voxels, $P < .001$). Greater naltrexone-induced reductions in orbitofrontal cortex activity during positive sham neurofeedback correlated with higher expectancy during the "antidepressant" condition (b = .40, $P < .01$) (Peciña et al., 2021).

Social Anxiety Disorder

Four studies involving patients with SAD were included in this review. First, in a pooled secondary analysis of 2 randomized-controlled trials, 25 patients with SAD completed a public speaking task while undergoing PET imaging before and after 8 weeks of placebo treatment. The patients' genotypes for the serotonin transporter-linked polymorphic region (5-HTTLPR) and the tryptophan hydroxylase-2 (TPH2) gene promoter were also obtained. Ten of the 25 patients (40%) were classified as placebo responders based on reduction in clinical global impression scale at study end. Regional cerebral blood flow in the left amygdala during the public speaking task decreased significantly more in placebo responders compared with nonresponders ($Z = 2.64$, $P = .048$). There was an additional effect of genotype in that only patients homozygous for the long allele of 5-HTTLPR and/or the G allele of the G-703T polymorphism in TPH2 exhibited a placebo response. A mediation analysis suggested that reduction in amygdala activity mediated the effect of the TPH2 polymorphism on placebo response ($P = .029$) (Furmark et al., 2008). These results raise the possibility that the amygdala and serotonin are important in placebo anxiolytic responses. However, this was a small sample size for this analysis, so there is a risk of false positives.

In a subsequent study, Faria et al. (2012, 2014) aimed to delineate the roles of different amygdala subregions in placebo anxiolysis. The 72 patients included in this study were pooled from 3 placebo-controlled trials of citalopram or paroxetine vs placebo, some of whom were also in the sample described above (Furmark et al., 2008), again undergoing PET imaging during a public speaking task before and after treatment. Twenty of 35 patients were classified as selective serotonin reuptake inhibitor (SSRI) responders (57%), and 11 of 37 patients responded to placebo (30%) (Faria et al., 2012). All treatment responders exhibited reduced cerebral blood flow in the left basomedial/basolateral ($Z = 2.49$, $P < .005$) and right ventrolateral amygdala ($Z = 2.95$, $P < .05$) subregions during the second PET scan. Moreover, the change in blood flow in these regions correlated significantly with reduced anxiety ($r > 0.3$, $P < .005$). There were no differences between SSRI and placebo responders (Faria et al., 2012). An analysis of functional connectivity patterns in these patients showed that placebo responders exhibited increased negative correlation between left basomedial/basolateral amygdala activity and left dorsolateral prefrontal cortex compared with nonresponders ($Z = 3.42$, $P < .001$). Compared with SSRI responders, placebo responders showed greater negative correlation between left amygdala and right ventromedial and dorsolateral prefrontal cortices and more positive correlation with dorsomedial prefrontal cortex ($Z > 3.00$, $P = .001$) (Faria et al., 2014). Inferences regarding potential neurotransmitters involved cannot be made from these data.

Finally, the role of expectations in augmenting antidepressant treatment was explored in 46 patients (Faria et al., 2017). All were treated with escitalopram for 9 weeks but were randomized regarding their instructions. Prior to treatment, 24 patients were informed that they would receive escitalopram while 22 patients were told they would receive an "active placebo" likely to induce side-effects like escitalopram but with no expected symptom improvement. At the beginning and end of treatment, these patients underwent fMRI scanning while they completed an emotional face-matching task. Overt escitalopram treatment caused significantly improved outcomes on the Liebowitz social anxiety scale ($d = 2.24$ vs $d = 1.13$ for covert treatment). The fMRI results showed that overt treatment was associated with increased activity to emotional faces in the bilateral posterior cingulate cortex, left mid temporal gyrus, and left inferior frontal gyrus compared with covert treatment at the end of the study ($Z > 3.60$, $P \leq .0001$). A psychophysiological interaction analysis demonstrated that the covert arm exhibited increased connectivity relative to the overt arm between the amygdala and right dorsal posterior cingulate cortex, and right insula, when viewing faces compared with shapes ($Z \geq 2.85$, $P \leq .002$). This was interpreted by the authors as evidence of greater fear processing in the covert group. However, there was no statistically significant difference in amygdala reactivity between groups when viewing emotional faces.

Results of Quality Assessment

The results of our quality assessment are summarized in Figures 2 and 3. Most studies (10, 62.5%) were rated as "some concerns." For most, this was due to the lack of a preregistered analysis plan, which meant practices such as dichotomizing treatment groups or the use of "small volume correction" might represent selective reporting (Mayberg et al., 2002; Furmark et al., 2008; Faria et al., 2012, 2014, 2017; Peciña et al., 2015, 2018, 2021; Sikora et al., 2016). In addition, we had concerns regarding missing data for 3 studies (Pecina et al., 2015; Sikora et al., 2016; Chin Fatt et al., 2021a). Two studies were rated as high risk of bias: Fan et al. (2020) due to missing data as results are reported for only 200 participants from the EMBARC trial (as opposed to the 296 reported in other papers) with no justification for this difference; and Zilcha-Mano et al. (2019) due to apparent selective reporting of results as detailed above. See supplemental material for full details of how each risk of bias rating was reached.

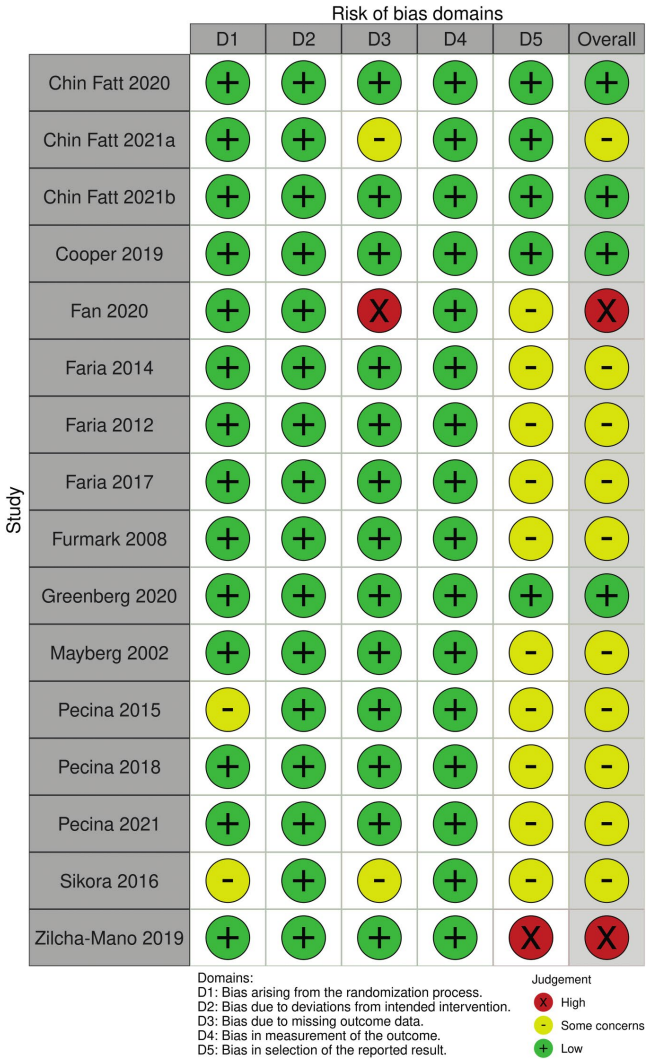

Figure 2. Traffic light plot summarizing review authors' judgements regarding risk of bias for each included study.

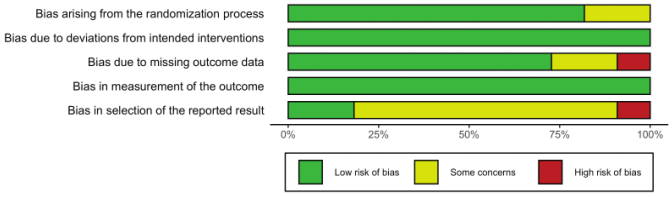

Figure 3. Plot showing review authors' judgements regarding risk of bias by percentage.

DISCUSSION

To our knowledge, this is the first systematic review of the functional neuroanatomy of placebo response in patients with anxiety or depression. We identified 12 articles reporting functional neuroimaging markers of placebo antidepressant responses and 4 reporting markers of placebo anxiolytic responses. There was substantial heterogeneity in terms of sample size, imaging modality, whether patients were imaged at rest or during a task, at baseline or longitudinally, the type of task, and the primary aim of the study. Further, coordinates of peak activity related to placebo responses were not consistently reported. We instead relied on authors' naming of brain regions, possibly introducing further inter-study variation. It is therefore challenging to coherently synthesize the data to identify relevant patterns. Nevertheless, there are signals suggesting possible neuroanatomical correlates of, and important neurotransmitter systems in, placebo antidepressant and anxiolytic effects.

Functional Neuroanatomical Correlates of Placebo Antidepressant and Anxiolytic Effects

Data from the EMBARC trial suggest that the ventral striatum (VS) might be important in placebo antidepressant effects. Reduced reward-related activity in the VS suggested patients were more likely to benefit from sertraline. Conversely, increased activity suggested no advantage of medication and a trend towards superiority of placebo (Greenberg et al., 2020). Significant superiority for placebo would likely be difficult to demonstrate in such a comparison because placebo effects operate in the medication arm (Huneke et al., 2020) as well as other nonspecific effects such as regression to the mean. A role for the VS in placebo antidepressant effects is further supported by the finding that "active" placebo treatment is associated with increased opioid release in the nucleus accumbens compared with an "inactive" placebo (Peciña et al., 2015). The VS is activated by placebo analgesia (Atlas and Wager, 2014), suggesting that reward circuitry might be important in placebo effects across domains.

Activity in dorsolateral prefrontal cortex (dlPFC) correlated with placebo response in many studies (Mayberg et al., 2002; Faria et al., 2014; Peciña et al., 2018, 2021; Cooper et al., 2019; Chin Fatt et al., 2020, 2021b; Fan et al., 2020). This region is reliably activated by placebo analgesia and is thought to be important in generating placebo-related expectancies (Atlas and Wager, 2014; Wager and Atlas, 2015). From the current data, activity increased in the dlPFC in placebo antidepressant responders after 6 weeks (Mayberg et al., 2002), and baseline blood flow in this region moderated subsequent placebo response (Cooper et al., 2019). In placebo responders with SAD, there was decreased correlation between bilateral amygdala and dorsolateral prefrontal cortices during a public speaking task compared with SSRI responders (Faria et al., 2014). Importantly, expectation of mood improvement led to greater dorsolateral/ventrolateral prefrontal cortex activity (Peciña et al., 2018, 2021). This suggests that lateral prefrontal cortex is important in maintaining antidepressant expectancies. However, activity here also negatively moderated the effect of higher expectation and positive reinforcement on subsequent mood improvement (Peciña et al., 2018, 2021). It is possible this finding was due to a "ceiling" effect, because lateral prefrontal cortex was activated when mood had already improved (Peciña et al., 2018). A recent meta-analysis found that placebo analgesia-induced activation of the dlPFC varies greatly between studies (Zunhammer et al., 2021), further making its role in placebo effects difficult to interpret.

Activity in the rostral anterior cingulate cortex (rACC) was identified as important by only 1 study in this review (Sikora et al., 2016). The rACC is activated in placebo analgesia (Atlas and Wager, 2014) and in placebo anxiolysis in healthy volunteers (Petrovic et al., 2005; Meyer et al., 2019). The rACC is in the default mode network, and this network is potentially crucial in generating placebo effects (Ashar et al., 2017). Indeed, placebo response was correlated with increased activity in regions within the default mode network in a number of studies (Mayberg et al., 2002; Faria et al., 2017; Cooper et al., 2019). Further studies are needed investigating the role of the default mode network in placebo antidepressant or anxiolytic effects.

Orbitofrontal cortex (OFC) activity was identified by a single study (Peciña et al., 2021). Placebo analgesia correlates with increased activity in centro-lateral OFC (Wager and Atlas, 2015; Ashar et al., 2017), and this region is densely populated with μ-opioid receptors (Van Steenbergen et al., 2019). The OFC is considered to be important in judging value and encoding expectations regarding outcomes or future events (Wager and Atlas, 2015; Van Steenbergen et al., 2019). Consistently, when μ-opioid receptors were blocked by naltrexone, antidepressant expectancies and the effects of positive reinforcement on mood were reduced, and this was associated with reduced right central orbitofrontal cortex activity (Peciña et al., 2021).

Activity in the amygdala was correlated with placebo anxiolytic and antidepressant effects in a number of studies (Furmark et al., 2008; Faria et al., 2012, 2014, 2017; Peciña et al., 2015; Zilcha-Mano et al., 2019). Three of these studies involved an overlapping sample of patients with SAD, so the reduction in amygdala activity seen could be considered a single finding (Furmark et al., 2008; Faria et al., 2012, 2014). In the fourth study of placebo anxiolysis, there was no evidence of a significant difference in amygdala activity between "overt" and "covert" SSRI administration (Faria et al., 2017). Change in amygdala activity instead correlated with improvement in social anxiety symptoms rather than expectations (Faria et al., 2017). It is therefore unclear whether changes in amygdala activity are due to placebo mechanisms or represent a non-specific phenomenon. Determining this is difficult owing to no "no treatment" arms for comparison, although such arms can be problematic in themselves (Huneke et al., 2020). The 2 studies involving patients with depression carried out functional neuroimaging prior to administration of any active medication (Peciña et al., 2015; Zilcha-Mano et al., 2019). Both studies showed that increased expectation of benefit related to either reduced activity or increased opioid binding in the amygdala (Peciña et al., 2015; Zilcha-Mano et al., 2019). However, both studies were judged to be at risk of bias due to lack of blinding (Peciña et al., 2015) and selective reporting of outcomes (Zilcha-Mano et al., 2019). Placebo and expectancy-induced reductions in bilateral amygdala activity have been found during placebo analgesia (Atlas and Wager, 2014) and in association with reduced feelings of "unpleasantness" when viewing aversive pictures (Petrovic et al., 2005). In the latter study, this did not correlate with placebo response (Petrovic et al., 2005). Further, the large EMBARC trial did not find a relationship between blood flow in the amygdala and placebo response (Cooper et al., 2019). Subgroups responsive to placebo in this trial did have increased resting connectivity within the limbic network (including bilateral amygdala) at baseline (Chin Fatt et al., 2021a); however, when looking at predictors of placebo response alone and not predictors of worse outcomes with sertraline, there was no evidence of amygdala involvement (Chin Fatt et al., 2020). It is possible instead that reductions in amygdala activity represent a phenomenon nonspecific to placebo, perhaps relating instead

to treatment response or changes in affect. This needs further exploration.

Possible Neurotransmitter Systems Involved in Placebo Antidepressant and Anxiolytic Effects

The current data show direct evidence only for a role of the endogenous opioid system in placebo antidepressant effects. A placebo antidepressant caused opioid release in the nucleus accumbens (Peciña et al., 2015), and the administration of naltrexone reduced the effects of expectancy and learning on antidepressant placebo effects (Peciña et al., 2021). There was also additional indirect evidence: increased expectation of benefit and higher mood ratings from a placebo antidepressant were associated with greater increases in plasma β-endorphin levels (Peciña et al., 2018). The endogenous opioid system is important in placebo analgesia (Fields, 2004; Benedetti et al., 2011). Although the present data are limited, they suggest endogenous opioids might be important in placebo effects in other domains, including those involving affect. This is supported by studies of placebo anxiolysis in healthy volunteers, which show overlap with regions important in placebo analgesia (Petrovic et al., 2005; Meyer et al., 2019).

There was further indirect evidence for a role of dopamine in placebo antidepressant effects. The VS was identified as a neuroanatomical correlate of placebo antidepressant effects in 2 studies (Peciña et al., 2015; Greenberg et al., 2020). The VS is also reliably activated in placebo analgesia (Atlas and Wager, 2014) and is an important center of dopaminergic neurotransmission. There is direct evidence for dopamine mediating placebo effects in other domains, including pain (Scott et al., 2008) and Parkinson's disease (De La Fuente-Fernandez, 2001; Lidstone et al., 2010). Further work is required to understand whether dopamine plays a mediating role in placebo antidepressant effects.

Finally, 1 study in this review showed indirect evidence for a role for serotonin in placebo anxiolytic effects. Presence of the G allele of the G-703T polymorphism in TPH2 mediated placebo-induced reduction in CGI-I score in patients with SAD via a reduction in amygdala activity. As discussed above, it is unclear whether this result is specific to placebo effects or whether this represents another non-specific treatment effect. Furthermore, this analysis involved a small sample size and so there is a possibility this is a false positive. There is no other evidence to our knowledge that serotonin plays a role in placebo anxiolysis or in other placebo effects.

Limitations

This review has some limitations. First, as with all systematic reviews, we are limited by the quality of the component studies we included. The results of our quality analysis suggest that there was potential for false positives and selective reporting. Where applicable, those findings should be considered with caution. Second, we did not carry out formal meta-analysis for several reasons: the small number of included studies, substantial overlap of study samples, a small number of whole-brain analyses, and the considerable heterogeneity between studies. Therefore, we can only make limited inferences about the relative importance of findings between studies.

CONCLUSION

We carried out the first systematic review of functional neuroimaging correlates of placebo response in patients with depressive or anxiety disorders. Although limited by the heterogeneity of the studies included in this review, our results suggest that activity in the rACC and default mode network, the VS, OFC, and dlPFC might be central in placebo antidepressant and anxiolytic effects. These regions' role in causing these effects is less certain and needs further investigation. Meanwhile activity in the amygdala might represent a nonspecific treatment effect. Important neurotransmitter systems could include the endogenous opioid system, dopamine, and serotonin. These hypotheses need further exploration in adequately powered studies designed with the primary aim of exploring the placebo effect, with consideration to possible confounds such as order effects, and involving longitudinal neuroimaging to begin to unpick causality.

Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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Interest Statement

N.T.M.H. has consulted for Emteq Ltd with no compensation. All other authors declare no potential conflict of interest.

Data Availability

Data accompanying this systematic review are available on the Open Science Framework (https://osf.io/fvb3a/).

References

Ashar YK, Chang LJ, Wager TD (2017) Brain mechanisms of the placebo effect: an affective appraisal account. Annu Rev Clin Psychol 13:73–98.

Atlas LY, Wager TD (2014) A Meta-analysis of brain mechanisms of placebo analgesia: consistent findings and unanswered questions. In: Placebo (Benedetti F, Enck P, Frisaldi E, Schedlowski M, eds), pp37–69. Berlin, Heidelberg: Springer Berlin Heidelberg.

Bandelow B, Reitt M, Röver C, Michaelis S, Görlich Y, Wedekind D (2015) Efficacy of treatments for anxiety disorders: a meta-analysis. Int Clin Psychopharmacol 30:183–192.

Benedetti F, Carlino E, Pollo A (2011) How placebos change the patient's brain. Neuropsychopharmacology 36:339–354.

Chin Fatt CR, Jha MK, Cooper CM, Fonzo G, South C, Grannemann B, Carmody T, Greer TL, Kurian B, Fava M, McGrath PJ, Adams P, McInnis M, Parsey RV, Weissman M, Phillips ML, Etkin A, Trivedi MH (2020) Effect of intrinsic patterns of functional brain connectivity in moderating antidepressant treatment response in major depression. Am J Psychiatry 177:143–154.

Chin Fatt CR, Cooper CM, Jha MK, Minhajuddin A, Rush AJ, Trombello JM, Fava M, McInnis M, Weissman M, Trivedi MH (2021a) Differential response to SSRI versus Placebo and distinct neural signatures among data-driven subgroups of patients with major depressive disorder. J Affect Disord 282:602–610.

Chin Fatt CR, Cooper C, Jha MK, Aslan S, Grannemann B, Kurian B, Greer TL, Fava M, Weissman M, McGrath PJ, Parsey RV, Etkin A,

Phillips ML, Trivedi MH (2021b) Dorsolateral prefrontal cortex and subcallosal cingulate connectivity show preferential antidepressant response in major depressive disorder. Biol Psychiatry Cogn Neurosci Neuroimaging 6:20–28.
Colloca L, Benedetti F (2006) How prior experience shapes placebo analgesia. Pain 124:126–133.
Cooper CM, Chin Fatt CR, Jha M, Fonzo GA, Grannemann BD, Carmody T, Ali A, Aslan S, Almeida JRC, Deckersbach T, Fava M, Kurian BT, McGrath PJ, McInnis M, Parsey RV, Weissman M, Phillips ML, Lu H, Etkin A, Trivedi MH (2019) Cerebral Blood perfusion predicts response to sertraline versus placebo for major depressive disorder in the EMBARC trial. Eclinicalmedicine 10:32–41.
De La Fuente-Fernandez R (2001) Expectation and dopamine release: mechanism of the placebo effect in Parkinson's disease. Science 293:1164–1166.
de Vries YA, de Jonge P, van den Heuvel E, Turner EH, Roest AM (2016) Influence of baseline severity on antidepressant efficacy for anxiety disorders: meta-analysis and meta-regression. Br J Psychiatry 208:515–521.
Ernst E, Resch KL (1995) Concept of true and perceived placebo effects. Bmj 311:551–553.
Evers AWM, et al. (2018) Implications of placebo and nocebo effects for clinical practice: expert consensus. Psychother Psychosom 87:204–210.
Fan S, Nemati S, Akiki TJ, Roscoe J, Averill CL, Fouda S, Averill LA, Abdallah CG (2020) Pretreatment brain connectome fingerprint predicts treatment response in major depressive disorder. Chronic Stress (Thousand Oaks) 4:2470547020984726.
Faria V, Appel L, Åhs F, Linnman C, Pissiota A, Frans Ö, Bani M, Bettica P, Pich EM, Jacobsson E, Wahlstedt K, Fredrikson M, Furmark T (2012) Amygdala subregions tied to SSRI and placebo response in patients with social anxiety disorder. Neuropsychopharmacology 37:2222–2232.
Faria V, Ahs F, Appel L, Linnman C, Bani M, Bettica P, Pich EM, Wahlstedt K, Fredrikson M, Furmark T (2014) Amygdala-frontal couplings characterizing SSRI and placebo response in social anxiety disorder. Int J Neuropsychopharmacol 17:1149–1157.
Faria V, Gingnell M, Hoppe JM, Hjorth O, Alaie I, Frick A, Hultberg S, Wahlstedt K, Engman J, Månsson KNT, Carlbring P, Andersson G, Reis M, Larsson EM, Fredrikson M, Furmark T (2017) Do you believe it? verbal suggestions influence the clinical and neural effects of escitalopram in social anxiety disorder: a randomized trial. Ebiomedicine 24:179–188.
Fields H (2004) State-dependent opioid control of pain. Nat Rev Neurosci 5:565–575.
Furmark T, Appel L, Henningsson S, Ahs F, Faria V, Linnman C, Pissiota A, Frans O, Bani M, Bettica P, Pich EM, Jacobsson E, Wahlstedt K, Oreland L, Långström B, Eriksson E, Fredrikson M (2008) A link between serotonin-related gene polymorphisms, amygdala activity, and placebo-induced relief from social anxiety. J Neurosci 28:13066–13074.
Furukawa TA, Cipriani A, Atkinson LZ, Leucht S, Ogawa Y, Takeshima N, Hayasaka Y, Chaimani A, Salanti G (2016) Placebo response rates in antidepressant trials: a systematic review of published and unpublished double-blind randomised controlled studies. Lancet Psychiatry 3:1059–1066.
Greenberg T, et al. (2020) Reward related ventral striatal activity and differential response to sertraline versus placebo in depressed individuals. Mol Psychiatry 25:1526–1536.
Huneke NTM, van der Wee N, Garner M, Baldwin DS (2020) Why we need more research into the placebo response in psychiatry. Psychol Med 50:2317–2323.
Lidstone SC, Schulzer M, Dinelle K, Mak E, Sossi V, Ruth TJ, de la Fuente-Fernández R, Phillips AG, Stoessl AJ (2010) Effects of expectation on placebo-induced dopamine release in Parkinson disease. Arch Gen Psychiatry 67:857–865.
Mayberg HS, Silva JA, Brannan SK, Tekell JL, Mahurin RK, McGinnis S, Jerabek PA (2002) The functional neuroanatomy of the placebo effect. Am J Psychiatry 159:728–737.
Meyer B, Yuen KSL, Saase V, Kalisch R (2019) The functional role of large-scale brain network coordination in placebo-induced anxiolysis. Cereb Cortex 29:3201–3210.
Page MJ et al. (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Syst Rev 10:89.
Peciña M, Bohnert AS, Sikora M, Avery ET, Langenecker SA, Mickey BJ, Zubieta JK (2015) Association between placebo-activated neural systems and antidepressant responses: neurochemistry of placebo effects in major depression. JAMA Psychiatry 72:1087–1094.
Peciña M, Chen J, Lyew T, Karp JF, Dombrovski AY (2021) μ-Opioid antagonist naltrexone partially abolishes the antidepressant placebo effect and reduces OFC encoding of reinforcement. Biol Psychiatry Cogn Neurosci Neuroimag. 6:1002–1012.
Peciña M, Heffernan J, Wilson J, Zubieta JK, Dombrovski AY (2018) Prefrontal expectancy and reinforcement-driven antidepressant placebo effects. Transl Psychiatry 8:222.
Peciña M, Zubieta JK (2015) Molecular mechanisms of placebo responses in humans. Mol Psychiatry 20:416–423.
Petrovic P, Dietrich T, Fransson P, Andersson J, Carlsson K, Ingvar M (2005) Placebo in emotional processing–induced expectations of anxiety relief activate a generalized modulatory network. Neuron 46:957–969.
Scott DJ, Stohler CS, Egnatuk CM, Wang H, Koeppe RA, Zubieta JK (2008) Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. Arch Gen Psychiatry 65:220–231.
Sikora M, Heffernan J, Avery ET, Mickey BJ, Zubieta JK, Peciña M (2016) Salience network functional connectivity predicts placebo effects in major depression. Biol Psychiatry Cogn Neurosci Neuroimaging 1:68–76.
Stein DJ, Baldwin DS, Dolberg OT, Despiegel N, Bandelow B (2006) Which factors predict placebo response in anxiety disorders and major depression? An analysis of placebo-controlled studies of escitalopram. J Clin Psychiatry 67:1741–1746.
Sterne JAC, et al. (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. Bmj 366:l4898.
van Steenbergen H, Eikemo M, Leknes S (2019) The role of the opioid system in decision making and cognitive control: a review. Cogn Affect Behav Neurosci 19:435–458.
Wager TD, Atlas LY (2015) The neuroscience of placebo effects: connecting context, learning and health. Nat Rev Neurosci 16:403–418.
Walsh BT, Seidman SN, Sysko R, Gould M (2002) Placebo response in studies of major depression: variable, substantial, and growing. Jama 287:1840–1847.
Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B, Olesen J, Allgulander C, Alonso J, Faravelli C, Fratiglioni L, Jennum P, Lieb R, Maercker A, van Os J, Preisig M, Salvador-Carulla L, Simon R, Steinhausen HC (2011) The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur Neuropsychopharmacol 21:655–679.
Zilcha-Mano S, Wang Z, Peterson BS, Wall MM, Chen Y, Wager TD, Brown PJ, Roose SP, Rutherford BR (2019) Neural mechanisms of expectancy-based placebo effects in antidepressant clinical trials. J Psychiatr Res 116:19–25.
Zunhammer M, Spisák T, Wager TD, Bingel U; Placebo Imaging Consortium (2021) Meta-analysis of neural systems underlying placebo analgesia from individual participant fMRI data. Nat Commun 12:1391.

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A novel procedure to investigate social anxiety using videoconferencing software: A proof-of-concept study

Nathan T.M. Huneke[a,b,*], Hannah Rowlatt[a], Joshua Hyde[c], Alexander McEwan[a], Louise Maryan[c], David S. Baldwin[a,b,d], Matthew Garner[a,c]

[a] Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK
[b] University Department of Psychiatry, Academic Centre, College Keep, 4-12 Terminus Terrace, Southampton, SO14 3DT, UK
[c] School of Psychology, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, UK
[d] University Department of Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

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ABSTRACT

Social anxiety disorder (SAD) is very common and can be significantly disabling. New treatments are needed as the remission rate for SAD is the lowest of all the anxiety disorders. Experimental medicine models, in which features resembling a clinical disorder are experimentally induced, are a cost-effective and timely approach to explore potential novel treatments for psychiatric disorders. Following the emergence of SARS-CoV-2, there is a need to develop experimental medicine models that can be carried out remotely. We developed a novel procedure to investigate SAD (the InterneT-based Stress test for Social Anxiety Disorder; ITSSAD) that can be carried out entirely online by a single investigator, potentially reducing costs and maximising internal reliability. The procedure involves an anticipatory period followed by a naturalistic social interaction task. In a sample of 20 non-treatment-seeking volunteers with symptoms of SAD, the ITSSAD induced significant subjective anxiety and reduced positive affect. Further, increased social anxiety symptoms at baseline predicted increased anxiety during the social interaction task. This protocol needs further validation with physiological measures. The ITSSAD is a new tool for researchers to investigate mechanisms underlying social anxiety disorder.

1. Introduction

Social anxiety disorder (SAD) is one of the most common mental disorders, with an estimated lifetime prevalence of more than 6% in Europe (Fehm et al., 2005). SAD can be significantly disabling due to excessive apprehension regarding social situations, leading to avoidance and an impairment in functioning (Hendriks et al., 2016). New treatments for SAD are needed, as only 64.9% of patients remit after 4 years - the lowest remission rate of all the anxiety disorders (Hendriks et al., 2016). Experimental medicine models, in which important resembling features of a clinical disorder are experimentally induced, can be a cost-effective and timely approach to explore potential novel treatments for psychiatric disorders (Baldwin et al., 2017). Following the emergence of the SARS-CoV-2 pandemic, in-person research and social contacts have been restricted in many parts of the World. This has highlighted the need for tasks and experimental procedures that can be conducted virtually or online to allow research into anxiety disorders to continue (Kirschbaum, 2021).

A key element in the development of SAD is social-evaluative threat (Clark and Wells, 1995; Wong et al., 2020; Wong and Rapee, 2016). Social-evaluative stimuli are those that implicitly or explicitly communicate judgement of a person, for example facial expressions, eye contact or behaviours such as applauding or leaving a room (Wong and Rapee, 2016). It is thought that a combination of trait factors such as inherited temperament, culture, parent behaviour and previous life events lead to these social-evaluative stimuli being appraised as threatening (Wong and Rapee, 2016). Resultant changes in neurobiology, cognition and behaviours designed to detect and eliminate threatening social-evaluative situations (e.g. amygdala overactivity, anticipatory and post-event processing, avoidance behaviour) might be important in maintaining that high level of threat (Nelemans et al., 2017; Wong and Rapee, 2016).

A number of tasks have employed social-evaluative situations to investigate stress. Possibly the most widely used paradigm that involves the induction of social-evaluative stress in laboratory conditions is the Trier Social Stress Test (TSST) (Dickerson and Kemeny, 2004; Frisch

* Corresponding author at: University Department of Psychiatry, Academic Centre, College Keep, 4-12 Terminus Terrace, Southampton, SO14 3DT, UK.
E-mail address: n.huneke@soton.ac.uk (N.T.M. Huneke).

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et al., 2015; Kirschbaum et al., 1993). The TSST involves a short preparation period followed by a public speaking task and surprise mental arithmetic task performed in front of an observing panel of two or more experimenters (Kirschbaum et al., 1993). This task reliably induces subjective stress and anxiety, and worsens negative mood (Allen et al., 2014). Two studies in adults (Eagle et al., 2021; Harvie et al., 2021) and one in adolescents (Gunnar et al., 2021) have shown that a TSST administered via videoconferencing platforms can induce as robust a stress response as an in-person version. Variations on the TSST include giving a short speech observed by one or more judges (Kocovski et al., 2011), performing mental arithmetic while being shown feedback about 'expected performance' (Dedovic et al., 2005), and singing in front of an audience (Brouwer and Hogervorst, 2014). Challenges with carrying out such tasks include the logistics of organising an observing panel and controlling for potential confounds such as the gender composition of the panel and their behaviour (Frisch et al., 2015; Narvaez Linares et al., 2020). In addition, although these tasks induce considerable stress, it is unclear whether this stress is consistent with symptoms of SAD. For example, cortisol reactivity is usually an outcome measure of the TSST, and heightened cortisol responses to the TSST (on a population level) have been associated with the prevalence of stress-related disorders generally, not SAD specifically (Miller and Kirschbaum, 2019). Further, those with social anxiety have demonstrated both increased (Roelofs et al., 2009) and reduced (Crişan et al., 2016; Shirotsuki et al., 2009) cortisol responses to the TSST compared with healthy volunteers. Task design has been highlighted as a potential reason for these inconsistent results (Crişan et al., 2016). Furthermore, while patients with SAD do experience fear during public speaking, this is not a specific feature, as many individuals might experience this, without having SAD (Panayiotou et al., 2017).

Here we report a proof-of-concept study to highlight a novel social interaction paradigm designed to induce social anxiety employing a naturalistic social interaction and videoconferencing software (the InterneT-based Stress test for Social Anxiety Disorder; ITSSAD). The ITSSAD includes a simple task involving 'getting to know' another person, which can induce significant anxiety in those with SAD and significant physiological arousal in healthy volunteers with high levels of social anxiety (Nordahl et al., 2016; Shalom et al., 2015). We hypothesised that this naturalistic task, likely to be encountered in daily life by those with SAD and easily reproducible online, would induce detectable anxiety in an online experimental setting. We focused on measuring subjective anxiety as experimentally-induced subjective stress is positively associated with sub-clinical and clinical social anxiety symptoms (Panayiotou et al., 2017; Taylor et al., 2020). Further, there is evidence that subjective stress reactivity in social situations is an important factor in the maintenance of SAD (Nelemans et al., 2017). If subjective stress/anxiety induced by an experimental model of SAD were associated with trait social anxiety symptoms, rather than a more generalized measure of trait anxiety, this would suggest that subjective acute anxiety is specific and relevant for SAD. We therefore hypothesised that the anxiety induced by the ITSSAD task would be associated with trait social anxiety symptoms specifically and not with a generalized trait anxiety measure.

2. Method

2.1. The ITSSAD

The ITSSAD (Fig. 1) can be carried out entirely online. We designed the ITSSAD to induce anxiety through anticipation, and subsequent experience of, a naturalistic social-evaluative situation (Allen et al., 2017; Dickerson and Kemeny, 2004). The ITSSAD begins with a 5-minute anticipatory period. During this, we showed participants task instructions as follows:

"*In 5 minutes you will take part in a social interaction online using videoconferencing software. Your task will be to take some time to get to know the other person as you normally would. Just be yourself. You can talk about anything you want other than this experiment. You will be watched by 3 other experimenters who will be assessing your behaviour. We would like you to have your camera on during this interaction.*"

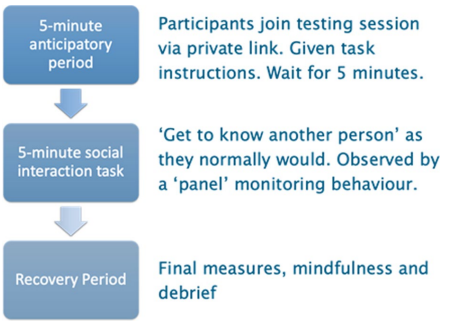

Fig. 1. Summary of the protocol for our modified Trier Social Stress Test, the ITSSAD.

After the anticipatory period, participants enter a videoconference. Present in the videoconference is an experimenter who introduces themselves as the person the participant is tasked with 'getting to know' and introduces a (mock) observing panel of 'experts' who are present to monitor the participants' behaviour. These appeared to be attendees to the videoconference who had turned their cameras off, but in reality were 'dummy' accounts logged into by the experimenter on other devices/browser windows and placed on mute. This allowed us to maintain a social-evaluative context whilst only having one experimenter. A previous study investigated whether a judging panel needed to be visible to induce stress during a public speaking task in healthy male volunteers: there was no significant difference in physiological stress between those who completed the task in front of a visible panel, and those who completed the task while the panel was behind a one-way mirror (Andrews et al., 2007). This indicates that the suggestion of the presence of a panel is adequate to induce a social-evaluative context and subsequent anxiety. We also named the dummy accounts to indicate the panel contained a mix of genders, as this has been shown to induce greater stress than a single-gendered panel (Narvaez Linares et al., 2020). We ensured that, along with the experimenter, two apparently male and two apparently female 'experimenters' were visible to the participant. To reinforce the social-evaluative context, the experimenter also informs the participant that the interaction will be recorded for review later.

The experimenter then begins a 5-minute timer and participants are asked to begin the social interaction task. The experimenter was briefed not to initiate conversation, instead allowing the participant to sit in silence if they did not initiate conversation. Experimenters were briefed to respond with non-elaborate verbal answers to questions posed by a participant whilst maintaining as neutral a facial expression as possible, much like the judging panel in the original TSST (Allen et al., 2017; Kirschbaum et al., 1993). If a silence lasted more than 30 seconds, the experimenter could prompt the participant with a short statement, for example, "I am a student at the university".

On completion of the 5 minutes, participants enter a recovery period. During this time, participants complete a coached mindfulness exercise. Mindfulness strategies are known to reduce post-event processing in SAD (Cassin and Rector, 2011; Shikatani et al., 2014) and so this was included as a 'mood repair'. Following the mindfulness task participants are fully debriefed.

2.2. Design of proof-of-concept study

2.2.1. Ethics statement

This study was reviewed and approved by the Ethics and Research Governance Office at the University of Southampton (reference: 61411) and performed in accordance with relevant local guidelines and regulations and the Declaration of Helsinki. Prior to starting the study, participants were informed that the aim was to explore social anxiety symptoms during videoconferencing. As the protocol involved some deception (described above), participants were fully debriefed at the end of the study and informed consent was sought a second time for us to retain their data. No participants withdrew consent for their data to be used.

2.2.2. Participants

For this proof-of-concept study, we recruited 20 participants aged 18-45 years with sub-clinical to clinical social anxiety symptoms. We felt a practical paradigm for exploring social-evaluative threat should induce anxiety with a large effect size. In a within-subjects design, a sample size of 20 participants will detect an effect of at least $d = 0.66$ with 80% power. Therefore, if subjective anxiety was significantly induced in this study, the effect would likely be moderate to large and suggest proof-of-concept of the paradigm. Social anxiety symptoms were assessed through the social phobia inventory (SPIN): a validated 17-item self-rated questionnaire (Connor et al., 2000). Participants with a SPIN of greater than 14 were included. This cut-off can differentiate between those with SAD of varying intensity and those with no social anxiety symptoms (Connor et al., 2000). We excluded participants via a self-report questionnaire if they reported: any current psychiatric disorder other than SAD; any history of psychosis or bipolar affective disorder; any significant physical illness; any recent treatment (either psychological or any systemic medication excluding paracetamol in the preceding 8 weeks); regularly using illicit substances; consuming more than 21 units of alcohol per week; or consuming more than 8 caffeinated drinks a day.

2.2.3. Study procedure

The study was carried out using both Qualtrics XM online survey software (https://www.qualtrics.com) (Qualtrics, 2021) and Microsoft Teams (https://teams.microsoft.com) (Microsoft, 2021). Participants initially completed a screening questionnaire that included the SPIN. Those who were eligible were then invited to attend a test session. Participants entered the test session via a private, personalised link sent to them by e-mail. The participants completed the test session from a private space of their choosing.

To fully characterize this non-treatment seeking sample, on entry into the session, participants completed the following questionnaires to assess trait anxiety and personality characteristics: Social Interaction Anxiety Scale (SIAS, (Mattick and Clarke, 1998)), Brief Fear of Negative Evaluation Scale (Brief FNE, (Leary, 1983)), and a modified version of the generalised anxiety disorder 7-item (GAD-7, (Spitzer et al., 2006)), where each question was represented by a visual analogue scale ranging from "not at all" to "nearly every day". After these assessments, participants completed the ITSSAD as described above.

2.2.4. Outcome measures

We measured subjective anxiety and mood before (at session baseline) and after the anticipatory period, and after the social interaction task. At all three timepoints, participants were asked to complete the modified GAD-7 with visual analogue scales ranging from "not at all" to "all of the time". Each item on this version of the GAD-7 was scored between 0 and 100. All items were then summed to give a total score (maximum 700). This version of the GAD-7 has been shown to be sensitive to state changes in anxiety with high resolution (Huneke et al., 2020). The GAD-7 questionnaire also captures social anxiety symptoms with good sensitivity (Kroenke et al., 2007). Subjective mood was assessed at all three timepoints through the Positive and Negative Affect Schedule (PANAS, (Watson et al., 1988)).

2.2.5. Statistical analysis

We carried out statistical analysis using the afex package in R (https://CRAN.R-project.org/package=afex) (Singmann et al., 2021). We assessed change in anxiety and mood over time through linear mixed-effects models (estimated using restricted maximum likelihood). Time was entered as a fixed effect while participant was included as a random effect. We chose to analyse the data through linear mixed-effects modelling as this allows greater retention of data when repeated measures are unbalanced, e.g. due to dropouts during the study. In this study, one participant dropped out prior to completing the anticipatory period and a further participant dropped out prior to completing the social interaction task. Linear mixed-effects modelling allowed us to retain datapoints already collected for these participants in the analysis. Where there was a significant effect of time (degrees of freedom calculated via Satterthwaite's method), we carried out *post-hoc* pairwise comparisons (t-tests) to assess for significant differences between timepoints. All statistical hypotheses were two-tailed and significance values for post-hoc comparisons were adjusted using the Tukey method.

To assess whether anxiety during the social interaction task was related to social anxiety symptoms, as opposed to trait generalized anxiety, we created an exploratory linear mixed-effects model including interaction terms of time*SPIN and time*trait GAD-7 as fixed effects, with participant included as a random effect. Both SPIN and GAD-7 variables were centered on the mean prior to carrying out the analysis. The significance of the interactions was tested through two-tailed F tests (degrees of freedom calculated via Satterthwaite's method). We explored the direction of significant interactions through analysis of simple main effects.

3. Results

Baseline characteristics of the participants are summarised in Table 1. On average, the participants exhibited moderate to severe social anxiety symptoms, with a mean SPIN of 38.95 ± 11.63. The majority (85%) were female.

Linear mixed-effects modelling showed a significant effect of time on anxiety ($F_{(2,35)} = 5.96$, $p = 0.0059$), negative affect ($F_{(2,35)} = 4.41$, $p = 0.0196$), and positive affect ($F_{(2,35)} = 10.15$, $p = 0.0003$). *Post-hoc* t-tests revealed that anxiety and negative affect increased following anticipation, and anxiety remained elevated after the speaking task. Positive affect decreased following anticipation, and remained decreased after the speaking task (Table 2 and Fig. 2).

We also carried out an exploratory linear-mixed effects analysis examining the effect of trait generalized anxiety and social anxiety on subjective anxiety experienced during the ITSSAD. Both time*trait GAD-7 ($F_{(2,33.8)} = 12.06$, $p = 0.0001$) and time*SPIN ($F_{(2,32.3)} = 5.13$, $p = 0.0116$) interactions were significant. *Post-hoc* analysis of simple effects

Table 1
Sample characteristics

Variable	Value
Age (yrs)	19.10 ± 1.21
Females	17 (85%)
SPIN	38.95 ± 11.63
Brief FNE	33.05 ± 6.36
SIAS	38.15 ± 12.86
GAD-7	260.90 ± 161.41

Note: values are reported as mean ± standard deviation for continuous variables, and count (%) for categorical variables. Abbreviations: SPIN, Social Phobia Inventory; Brief FNE, Brief Fear of Negative Evaluation Scale; SIAS, Social Interaction Anxiety Scale; GAD-7, Generalised Anxiety Disorder 7-item.

Table 2
Summary of post-hoc pairwise comparisons.

Comparison	GAD-7		PANAS Negative		PANAS Positive	
	Estimated Mean Difference	t-test results	Estimated Mean Difference	t-test results	Estimated Mean Difference	t-test results
Pre- vs post-anticipation	-124.6 ± 38.2	$t_{(35)}$=3.26, p=0.0068	-4.61 ± 1.56	$t_{(35)}$=2.95, p=0.0151	4.51 ± 1.19	$t_{(35)}$=3.80, p=0.0016
Pre-anticipation vs Post-speaking task	-100.0 ± 38.9	$t_{(35)}$=2.57, p=0.0380	-2.71 ± 1.59	$t_{(35)}$=1.70, p=0.2174	4.81 ± 1.21	$t_{(35)}$=3.97, p=0.0010
Post-anticipation vs Post-speaking task	24.6 ± 39.1	$t_{(35)}$=0.63, p=0.8050	1.90 ± 1.60	$t_{(35)}$=1.19, p=0.4695	0.30 ± 1.22	$t_{(35)}$=0.24, p=0.9678

Note: values are reported as estimated mean difference ± standard error. All significance values are Tukey-adjusted for multiple comparisons. Abbreviations: GAD-7, Generalised Anxiety Disorder 7-item; PANAS, Positive and Negative Affect Schedule.

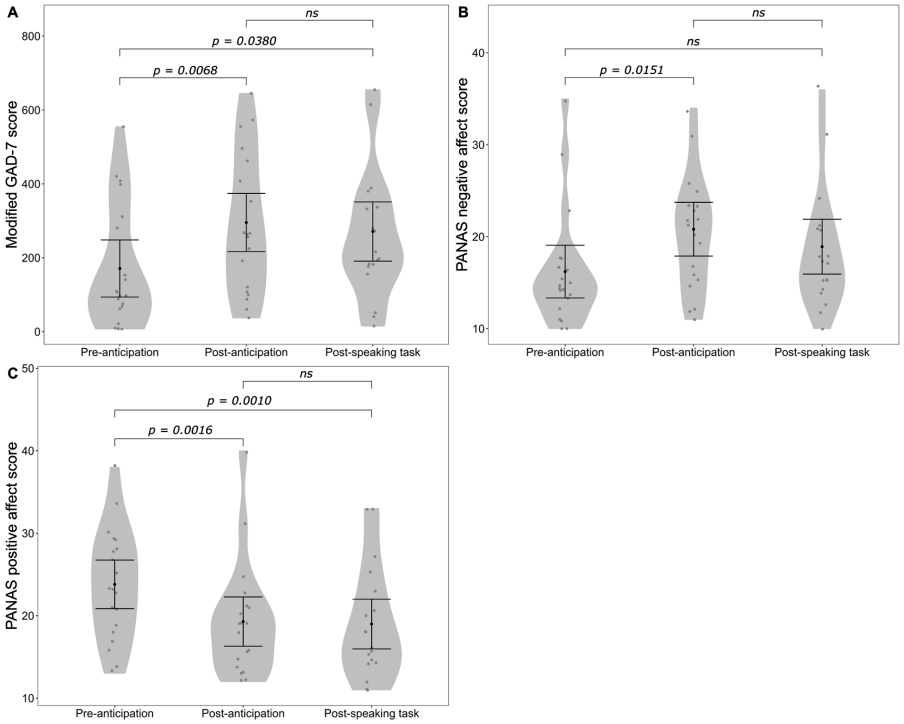

Fig. 2. Violin plots showing modified GAD-7 (A), PANAS negative affect (B) and PANAS positive affect (C) scores over time. Anxiety and negative affect increased (vs. pre-anticipation session baseline), while positive affect decreased. Points represent estimated marginal means, and error bars represent 95% confidence interval. Significance values shown originate from post-hoc pairwise t-tests with Tukey adjustment for multiple comparisons. Abbreviations: GAD-7, Generalised Anxiety Disorder screener; PANAS, Positive and Negative Affect Schedule.

Table 3
Summary of simple effects of trait generalized anxiety and social anxiety symptoms on subjective anxiety experienced during the ITSSAD protocol.

Time	GAD-7					SPIN				
	Estimate	**95% CI**	**df**	**t**	**$p_{(tukey)}$**	**Estimate**	**95% CI**	**df**	**t**	**$p_{(tukey)}$**
Pre-anticipation	**0.896**	**0.55, 1.24**	**42.6**	**5.29**	**<0.0001**	1.64	-3.10, 6.38	42.6	0.70	0.4891
Post-anticipation	**0.978**	**0.60, 1.36**	**44.7**	**5.17**	**<0.0001**	1.51	-3.29, 6.30	42.9	0.63	0.5294
Post-speaking task	-0.034	-0.42, 0.35	44.8	-0.18	0.8583	**9.66**	**4.83, 14.50**	**43.2**	**4.03**	**0.0002**

Note: Significant results in bold. Abbreviations: GAD-7, Generalised Anxiety Disorder 7-item; SPIN, Social Phobia Inventory; CI, Confidence Interval; df, degrees of freedom.

demonstrated that SPIN score positively predicted anxiety during the speaking task, but was not significantly related to anxiety beforehand. Conversely, there was a significant positive effect of trait GAD-7 on anxiety during anticipation, but no significant effect on anxiety during the speaking task (Table 3 and Fig. 3).

4. Discussion

In this proof-of-concept study, we showed that it is possible to induce social anxiety symptoms through a novel procedure using videoconferencing software (ITSSAD). Subjective anxiety was increased by a pre-task anticipation period, and anxiety remained elevated following a naturalistic social interaction task. In addition, positive affect decreased during the pre-task anticipation period, and positive affect was not significantly different following the social interaction task. Finally, increased baseline SPIN scores predicted increased anxiety during the social interaction, while trait GAD-7 did not, suggesting this task specifically induces features of social anxiety disorder.

Recently, a number of online versions of social stress tests have been developed (Eagle et al., 2021; Gunnar et al., 2021; Harvie et al., 2021). However, these protocols are designed to induce *stress* and do not necessarily induce social anxiety symptoms specifically.

The ITSSAD utilizes a naturalistic social interaction task that is easy to administer and is likely to be ecologically valid. Subjective anxiety increased following both the anticipation period and social interaction task. In addition, anxiety during the social interaction was predicted specifically by SPIN scores at baseline. This suggests the social interaction activated cognitive factors important in SAD. Social interaction tasks are known to induce social anxiety symptoms and can activate psychological mechanisms, such as negative self-evaluation (Nordahl et al., 2016). Exploration of the factors involved in the ITSSAD are outside the scope of the current proof-of-concept study, but this warrants further investigation.

Our novel approach potentially possesses other advantages over previously developed social stress tests. A limitation of the original TSST and its offline and online variants is the logistical challenge and human resources cost involved in setting up the test sessions. The TSST and its variants require laboratory space and multiple individuals (at least 2) to be available contemporaneously for approximately 30 minutes to test a single participant (Allen et al., 2017; Kirschbaum et al., 1993). In comparison, the ITSSAD can be carried out by a single investigator with only a laptop in any private space. In addition, an important potential confounder of the original TSST and online variants is the characteristics and behaviour of confederates. Variations in acting between different confederates, or within confederates, can affect the internal reliability of the TSST (Allen et al., 2017; Frisch et al., 2015; Wallergård et al., 2011). There is also evidence that committee members can empathically mirror the stress of the participant (Buchanan et al., 2012), potentially leading to distorted interactions. The gender composition of the panel is also known to be an important determinant of stress in the participant (Narvaez Linares et al., 2020). A number of virtual reality adaptations of the TSST have been developed to attempt to mitigate against this: however, the type of virtual reality seems to be relevant. Immersive environments, defined as completely replacing audio-visual cues with virtual reality, demonstrated significantly greater cortisol reactivity than non-immersive environments (Helminen et al., 2019). These kinds of immersive environments require costly headsets and other equipment, as well as resources to build the virtual world. By comparison, in the ITSSAD the observing panel can be 'dummy' accounts controlled by a single experimenter. This allows complete control over confederate behaviour and characteristics of the panel such as gender composition. This is likely to provide high internal reliability of the protocol for minimal cost. Further studies are needed to determine internal reliability and costs of this protocol in comparison to other tests of social-evaluative threat.

There are some limitations of this proof-of-concept study. Firstly, the sample size of 20 is small and our findings should accordingly be interpreted with caution. We also did not recruit a low socially anxious sample, and so it is unclear whether anxiety induced in the ITSSAD only occurs in those with high social anxiety symptoms. Future studies should determine how low socially anxious individuals behave when completing the ITSSAD. In addition, we did not collect physiological measures. The TSST is known to increase cortisol levels as well as activate the sympathetic nervous system, which are important features for its validity in investigating stress-related disorders (Allen et al., 2017; Narvaez Linares et al., 2020). Administering the TSST via videoconference also increases both heart rate (Eagle et al., 2021; Harvie et al., 2021) and salivary cortisol concentrations (Gunnar et al., 2021). For the ITSSAD, we were interested in subjective stress response as this is positively associated with trait social anxiety and might be a factor in the maintenance of SAD (Nelemans et al., 2017; Panayiotou et al., 2017; Taylor et al., 2020). Nevertheless, if the ITSSAD were to induce

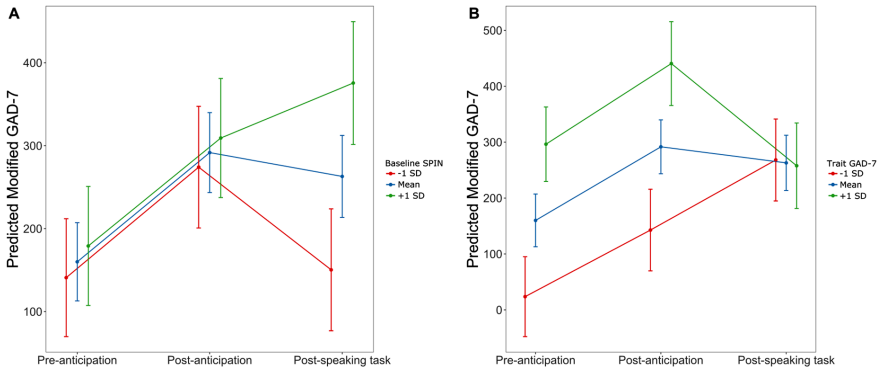

Fig. 3. Line plots showing predicted modified GAD-7 score over time during the ITSSAD protocol. Points represent predicted means, and error bars represent 95% confidence interval. The effect of SPIN score when trait GAD-7 is held constant is shown in (A). This shows that participants with increased SPIN scores are predicted to experience increased anxiety during the speaking task. SPIN scores are predicted to have little effect on anxiety prior to the speaking task. Conversely, the effect of trait GAD-7 when SPIN is held constant is shown in (B). Trait GAD-7 is predicted to affect anxiety experienced during anticipation, but has little effect on anxiety during the speaking task. Abbreviations: GAD-7, Generalised Anxiety Disorder screener; SPIN, Social Phobia Inventory.

autonomic anxiety responses then this paradigm could be useful for investigating other potential psychopathophysiological mechanisms of SAD, for example anxiety sensitivity and interoception (Dixon et al., 2015). Brief social interactions are known to induce cardiovascular responses consistent with threat in those with high trait social anxiety (Shalom et al., 2015; Shimizu et al., 2011). It is therefore likely that our protocol would induce similar physiological responses, but this needs testing empirically. Additionally, our participants were also mostly female and due to the small sample size we could not assess for an effect of gender on subjective anxiety. Women tend to exhibit higher subjective stress reactivity than men (Kelly et al., 2008; Rausch et al., 2008). It is unknown whether our results would replicate in a more male-predominant sample. We also did not measure natural recovery following the task, opting instead for a mood repair after the task. We did this to ensure safety and stabilisation of volunteers who were participating in the study remotely. However, post-event processing is thought to be an important factor in the aetiology and maintenance of SAD (Wong et al., 2020; Wong and Rapee, 2016). Furthermore, we did not measure anxiety and mood following the mood repair, so we cannot be sure how quickly induced anxiety 'washes out' following aided recovery. Future studies exploring natural and aided recovery, and post-event processing, following the ITSSAD are warranted. Lastly, we did not control for activities participants undertook, or substances ingested, prior to the testing session. Activities as diverse as brushing teeth, engaging in physical exercise, and eating can affect cortisol responses in the TSST (Narvaez Linares et al., 2020). However, the impact of these behaviours on subjective anxiety is less clear. Regardless, we observed a robust anxiety response without such rigorous control. Further studies are needed to determine whether controlling activities for a period of time before the testing session can improve signal to noise ratio.

Our novel InterneT-based Stress test for Social Anxiety Disorder (ITSSAD) induced significant anxiety in volunteers with subclinical to clinical social anxiety. Subjective anxiety during the social interaction task correlated with trait symptoms of social anxiety disorder. The ITSSAD possesses many advantages for investigating social anxiety including that it is low-cost, easy to carry out, has high internal validity due to complete control of confederates, and involves a naturalistic social interaction task. The ITSSAD is a new tool for researchers to investigate the mechanisms of social anxiety disorder.

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CRediT authorship contribution statement

Nathan T.M. Huneke: Conceptualization, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Hannah Rowlatt:** Investigation, Writing – review & editing. **Joshua Hyde:** Investigation, Writing – review & editing. **Alexander McEwan:** Methodology, Investigation, Writing – review & editing. **Louise Maryan:** Investigation, Writing – review & editing. **David S. Baldwin:** Supervision, Writing – review & editing. **Matthew Garner:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

All authors declare no conflict of interest.

References

Allen, A.P., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., 2014. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. Neurosci. Biobehav. Rev. 38, 94–124.

Allen, A.P., Kennedy, P.J., Dockray, S., Cryan, J.F., Dinan, T.G., Clarke, G., 2017. The Trier Social Stress Test: principles and practice. Neurobiol. Stress 6, 113–126.

Andrews, J., Wadiwalla, M., Juster, R.P., Lord, C., Lupien, S.J., Pruessner, J.C., 2007. Effects of manipulating the amount of social-evaluative threat on the cortisol stress response in young healthy men. Behav. Neurosci. 121 (5), 871–876.

Baldwin, D.S., Hou, R., Gordon, R., Huneke, N.T., Garner, M., 2017. Pharmacotherapy in generalized anxiety disorder: novel experimental medicine models and emerging drug targets. CNS Drugs 31 (4), 307–317.

Brouwer, A.-M., Hogervorst, M.A., 2014. A new paradigm to induce mental stress: the Sing-a-Song Stress Test (SSST). Front. Neurosci. 8, 224.

Buchanan, T.W., Bagley, S.L., Stansfield, R.B., Preston, S.D., 2012. The empathic, physiological resonance of stress. Soc. Neurosci. 7 (2), 191–201.

Cassin, S.E., Rector, N.A., 2011. Mindfulness and the attenuation of post-event processing in social phobia: an experimental investigation. Cogn. Behav. Ther. 40 (4), 267–278.

Clark, D.M., Wells, A., 1995. A cognitive model of social phobia, Social phobia: Diagnosis, assessment, and treatment. The Guilford Press, New York, NY, US, pp. 69–93.

Connor, K.M., Davidson, J.R.T., Churchill, L.E., Sherwood, A., Foa, E., Weisler, R.H., 2000. Psychometric properties of the Social Phobia Inventory (SPIN) - New self-rating scale. Br. J. Psychiatry 176, 379–386.

Crișan, L.G., Vulturar, R., Miclea, M., Miu, A.C., 2016. Reactivity to Social Stress in Subclinical Social Anxiety: emotional Experience. Cogn. Appraisals, Behav., Physiol. Front. Psychiatry 7.

Dedovic, K., Renwick, R., Mahani, N.K., Engert, V., Lupien, S.J., Pruessner, J.C., 2005. The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. J. Psychiatry Neurosci. 30 (5), 319–325.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130 (3), 355.

Dixon, L.J., Kemp, J.J., Farrell, N.R., Blakey, S.M., Deacon, B.J., 2015. Interoceptive exposure exercises for social anxiety. J. Anxiety Disord. 33, 25–34.

Eagle, D.E., Rash, J.A., Tice, L., Proeschold-Bell, R.J., 2021. Evaluation of a remote, internet-delivered version of the Trier Social Stress Test. Int. J. Psychophysiol. 165, 137–144.

Fehm, L., Pelissolo, A., Furmark, T., Wittchen, H.-U., 2005. Size and burden of social phobia in Europe. Eur. Neuropsychopharmacol. 15 (4), 453–462.

Frisch, J.U., Hausser, J.A., Mojzisch, A., 2015. The Trier Social Stress Test as a paradigm to study how people respond to threat in social interactions. Front. Psychol. 6, 14.

Gunnar, M.R., Reid, B.M., Donzella, B., Miller, Z.R., Gardow, S., Tsakonas, N.C., Thomas, K.M., DeJoseph, M., Bendezu, J.J., 2021. Validation of an online version of the Trier Social Stress Test in a study of adolescents. Psychoneuroendocrinology 125, 105111.

Harvie, H., Jain, B., Nelson, B., Knight, E., Roos, L.E., Giuliano, R.J., 2021. Induction of acute stress through an internet-delivered Trier Social Stress Test as assessed by photoplethysmography on a smartphone.

Helminen, E.C., Morton, M.L., Wang, Q., Felver, J.C., 2019. A meta-analysis of cortisol reactivity to the Trier Social Stress Test in virtual environments. Psychoneuroendocrinology 110, 104437.

Hendriks, S.M., Spijker, J., Licht, C.M.M., Hardeveld, F., De Graaf, R., Batelaan, N.M., Penninx, B.W.J.H., Beekman, A.T.F., 2016. Long-term disability in anxiety disorders. BMC Psychiatry 16 (1).

Huneke, N.T.M., Broulidakis, M.J., Darekar, A., Baldwin, D.S., Garner, M., 2020. Brain Functional Connectivity Correlates of Response in the 7.5% CO2 Inhalational Model of Generalized Anxiety Disorder: a Pilot Study. Int. J. Neuropsychopharmacolog. 23 (4), 268–273.

Kelly, M.M., Tyrka, A.R., Anderson, G.M., Price, L.H., Carpenter, L.L., 2008. Sex differences in emotional and physiological responses to the Trier Social Stress Test. J. Behav. Ther. Exp. Psychiatry 39 (1), 87–98.

Kirschbaum, C., 2021. Why we need an online version of the Trier Social Stress Test. Psychoneuroendocrinology 125, 105129.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28 (1-2), 76–81.

Kocovski, N.L., MacKenzie, M.B., Rector, N.A., 2011. Rumination and distraction periods immediately following a speech task: effect on postevent processing in social anxiety. Cogn. Behav. Ther. 40 (1), 45–56.

Kroenke, K., Spitzer, R.L., Williams, J.B.W., Monahan, P.O., Löwe, B., 2007. Anxiety Disorders in Primary Care: prevalence, Impairment, Comorbidity, and Detection. Ann. Intern. Med. 146 (5), 317.

Leary, M.R., 1983. A brief version of the Fear of Negative Evaluation Scale. Personal. Soc. Psychol. Bull. 9 (3), 371–375.

Mattick, R.P., Clarke, J.C., 1998. Development and validation of measures of social phobia scrutiny fear and social interaction anxiety. Behav. Res. Ther. 36 (4), 455–470.

Microsoft, 2021. Microsoft Teams (Version 1.4.00.11161). https://teams.microsoft.com.

Miller, R., Kirschbaum, C., 2019. Cultures under stress: a cross-national meta-analysis of cortisol responses to the Trier Social Stress Test and their association with anxiety-related value orientations and internalizing mental disorders. Psychoneuroendocrinology 105, 147–154.

Narvaez Linares, N.F., Charron, V., Ouimet, A.J., Labelle, P.R., Plamondon, H., 2020. A systematic review of the Trier Social Stress Test methodology: issues in promoting study comparison and replicable research. Neurobiol. Stress 13, 100235.

Nelemans, S.A., Hale Iii, W.W., Branje, S.J.T., van Lier, P.A.C., Koot, H.M., Meeus, W.H. J., 2017. The role of stress reactivity in the long-term persistence of adolescent social anxiety symptoms. Biol. Psychol. 125, 91–104.

Nordahl, H.M., Nordahl, H., Wells, A., 2016. Metacognition and perspective taking predict negative self-evaluation of social performance in patients with social anxiety disorder. J. Exp. Psychopathol. 7 (4), 601–607.
Panayiotou, G., Karekla, M., Georgiou, D., Constantinou, E., Paraskeva-Siamata, M., 2017. Psychophysiological and self-reported reactivity associated with social anxiety and public speaking fear symptoms: effects of fear versus distress. Psychiatry Res. 255, 278–286.
Qualtrics, 2021. Qualtrics XM Platform. https://www.qualtrics.com.
Rausch, S.M., Auerbach, S.M., Gramling, S.E., 2008. Gender and ethnic differences in stress reduction, reactivity, and recovery. Sex Roles 59 (9-10), 726–737.
Roelofs, K., van Peer, J., Berretty, E., Jong, P.d., Spinhoven, P., Elzinga, B.M., 2009. Hypothalamus–pituitary–adrenal axis hyperresponsiveness is associated with increased social avoidance behavior in social phobia. Biol. Psychiatry 65 (4), 336–343.
Shalom, J.G., Israeli, H., Markovitzky, O., Lipsitz, J.D., 2015. Social anxiety and physiological arousal during computer mediated vs. face to face communication. Comput. Hum. Behav. 44, 202–208.
Shikatani, B., Antony, M.M., Kuo, J.R., Cassin, S.E., 2014. The impact of cognitive restructuring and mindfulness strategies on postevent processing and affect in social anxiety disorder. J. Anxiety Disord. 28 (6), 570–579.
Shimizu, M., Seery, M.D., Weisbuch, M., Lupien, S.P., 2011. Trait social anxiety and physiological activation: cardiovascular threat during social interaction. Pers. Soc. Psychol. Bull. 37 (1), 94–106.
Shirotsuki, K., Izawa, S., Sugaya, N., Yamada, K.C., Ogawa, N., Ouchi, Y., Nagano, Y., Nomura, S., 2009. Salivary cortisol and DHEA reactivity to psychosocial stress in socially anxious males. Int. J. Psychophysiol. 72 (2), 198–203.
Singmann, H., Bolker, B., Westfall, J., Aust, F., Ben-Shachar, M.S., 2021. afex: analysis of factorial experiments. R package version 1 1, 0-.
Spitzer, R.L., Kroenke, K., Williams, J.B., Löwe, B., 2006. A brief measure for assessing generalized anxiety disorder: the GAD-7. Arch. Intern. Med. 166 (10), 1092–1097.
Taylor, C.T., Tsai, T.C., Smith, T.R., 2020. Examining the link between positive affectivity and anxiety reactivity to social stress in individuals with and without social anxiety disorder. J. Anxiety Disord. 74, 102264.
Wallergård, M., Jönsson, P., Johansson, G., Karlson, B., 2011. A Virtual Reality Version of the Trier Social Stress Test: a Pilot Study. Presence 20 (4), 325–336.
Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. J. Pers. Soc. Psychol. 54 (6), 1063–1070.
Wong, Q.J., McEvoy, P.M., Rapee, R.M., 2020. The structure of social-evaluative threat detection in social anxiety disorder. J. Anxiety Disord. 74, 102273.
Wong, Q.J., Rapee, R.M., 2016. The aetiology and maintenance of social anxiety disorder: a synthesis of complementary theoretical models and formulation of a new integrated model. J. Affect. Disord. 203, 84–100.

List of References

1. Rehm J, Shield KD. Global burden of disease and the impact of mental and addictive disorders. *Current psychiatry reports* 2019;21(2):1-7.

2. Eaton WW, Bienvenu OJ, Nestadt G, et al. The Burden of Mental Disorders. In: Eaton WW, Fallin D (eds.) *Public Mental Health*; 2019 p3-32.

3. Wittchen H-U, Jacobi F, Rehm J, et al. The size and burden of mental disorders and other disorders of the brain in Europe 2010. *European Neuropsychopharmacology* 2011;21(9):655-79.

4. Mojtabai R, Stuart EA, Hwang I, et al. Long-term effects of mental disorders on employment in the National Comorbidity Survey ten-year follow-up. *Social Psychiatry and Psychiatric Epidemiology* 2015;50(11):1657-68.

5. Knudsen AK, Øverland S, Hotopf M, et al. Lost working years due to mental disorders: an analysis of the Norwegian disability pension registry. 2012.

6. De Graaf R, Tuithof M, Van Dorsselaer S, et al. Comparing the effects on work performance of mental and physical disorders. *Social Psychiatry and Psychiatric Epidemiology* 2012;47(11):1873-83.

7. Vos T, Lim SS, Abbafati C, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet* 2020;396(10258):1204-22.

8. Whiteford HA, Degenhardt L, Rehm J, et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *The Lancet* 2013;382(9904):1575-86.

9. Walker ER, McGee RE, Druss BG. Mortality in Mental Disorders and Global Disease Burden Implications. *JAMA psychiatry* 2015;72(4):334.

10. Bloom DE, Cafiero E, Jané-Llopis E, et al. The global economic burden of noncommunicable diseases: Program on the Global Demography of Aging, 2012.

11. Mueser KT, Deavers F, Penn DL, et al. Psychosocial treatments for schizophrenia. *Annual review of clinical psychology* 2013;9:465-97.

12. Crowe M, Whitehead L, Wilson L, et al. Disorder-specific psychosocial interventions for bipolar disorder—A systematic review of the evidence for mental health nursing practice. *International Journal of Nursing Studies* 2010;47(7):896-908.

13. National Institute for Health and Care Excellence. *Depression in adults: treatment and management (update)*. https://www.nice.org.uk/guidance/ng222.

14. Nathan PE, Gorman JM. *A guide to treatments that work*. 4th ed: Oxford University Press; 2015.

15. Mashour GA, Walker EE, Martuza RL. Psychosurgery: past, present, and future. *Brain Research Reviews* 2005;48(3):409-19.

16. Millan MJ, Goodwin GM, Meyer-Lindenberg A, et al. Learning from the past and looking to the future: emerging perspectives for improving the treatment of psychiatric disorders. *European Neuropsychopharmacology* 2015;25(5):599-656.

17. Koek RJ, Roach J, Athanasiou N, et al. Novel Neurostimulation therapeutic approaches for treatment-resistant psychiatric disorders. *Treatment Resistance in Psychiatry* 2019:325-47.

18. Kazdin AE. Addressing the treatment gap: A key challenge for extending evidence-based psychosocial interventions. *Behaviour research and therapy* 2017;88:7-18.

19. Holmes EA, Ghaderi A, Harmer CJ, et al. The Lancet Psychiatry Commission on psychological treatments research in tomorrow's science. *The Lancet Psychiatry* 2018;5(3):237-86.

20. Bereza BG, Machado M, Ravindran AV, et al. Evidence-based review of clinical outcomes of guideline-recommended pharmacotherapies for generalized anxiety disorder. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie* 2012;57(8):470-8.

21. Springer KS, Levy HC, Tolin DF. Remission in CBT for adult anxiety disorders: A meta-analysis. *Clinical Psychology Review* 2018;61:1-8.

22. Cuijpers P, Karyotaki E, Weitz E, et al. The effects of psychotherapies for major depression in adults on remission, recovery and improvement: a meta-analysis. *Journal of Affective Disorders* 2014;159:118-26.

23. Gaynes BN, Warden D, Trivedi MH, et al. What did STAR* D teach us? Results from a large-scale, practical, clinical trial for patients with depression. *Psychiatric Services* 2009;60(11):1439-45.

24. Linden M. How to define, find and classify side effects in psychotherapy: from unwanted events to adverse treatment reactions. *Clinical Psychology & Psychotherapy* 2013;20(4):286-96.

25. Reynolds GP, Kirk SL. Metabolic side effects of antipsychotic drug treatment–pharmacological mechanisms. *Pharmacology & Therapeutics* 2010;125(1):169-79.

26. Chandler DJ. Something's got to give: psychiatric disease on the rise and novel drug development on the decline. *Drug Discovery Today* 2013;18(3-4):202-06.

27. Nutt D, Goodwin G. ECNP Summit on the future of CNS drug research in Europe 2011: report prepared for ECNP by David Nutt and Guy Goodwin. *European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology* 2011;21(7):495-99.

28. Skripka-Serry J. The great neuro-pipeline ‘brain drain’ (and why Big Pharma hasn’t given up on CNS disorders). *Drug Discovery World* 2013;14(4):9-16.

29. McArthur RA. Aligning physiology with psychology: Translational neuroscience in neuropsychiatric drug discovery. *Neuroscience & Biobehavioral Reviews* 2017;76:4-21.

30. Hashimoto K. Rapid-acting antidepressant ketamine, its metabolites and other candidates: A historical overview and future perspective. *Psychiatry and clinical neurosciences* 2019;73(10):613-27.

31. Tabuteau H, Jones A, Anderson A, et al. Effect of AXS-05 (Dextromethorphan-Bupropion) in Major Depressive Disorder: A Randomized Double-Blind Controlled Trial. *American Journal of Psychiatry* 2022:appi. ajp. 21080800.

32. Owen DR, Rupprecht R, Nutt DJ. Stratified medicine in psychiatry: a worrying example or new opportunity in the treatment of anxiety? *Journal of Psychopharmacology* 2013;27(2):119-22.

33. Pollak TA, Lennox BR, Müller S, et al. Autoimmune psychosis: an international consensus on an approach to the diagnosis and management of psychosis of suspected autoimmune origin. *The Lancet Psychiatry* 2020;7(1):93-108.

34. Stewart AM, Nguyen M, Poudel MK, et al. The failure of anxiolytic therapies in early clinical trials: what needs to be done. *Expert opinion on investigational drugs* 2015;24(4):543-56.

35. Griebel G, Holmes A. 50 years of hurdles and hope in anxiolytic drug discovery. *Nature reviews Drug discovery* 2013;12(9):667-87.

36. Monteggia LM, Heimer H, Nestler EJ. Meeting report: can we make animal models of human mental illness? *Biological Psychiatry* 2018;84(7):542-45.

37. Scarr E, Millan MJ, Bahn S, et al. Biomarkers for psychiatry: the journey from fantasy to fact, a report of the 2013 CINP think tank. *International Journal of Neuropsychopharmacology* 2015;18(10):pyv042.

38. Leiser SC, Dunlop J, Bowlby MR, et al. Aligning strategies for using EEG as a surrogate biomarker: a review of preclinical and clinical research. *Biochemical Pharmacology* 2011;81(12):1408-21.

39. Bailey JE, Dawson GR, Dourish CT, et al. Validating the inhalation of 7.5% CO2 in healthy volunteers as a human experimental medicine: a model of generalized anxiety disorder (GAD). *Journal of Psychopharmacology* 2011;25(9):1192-98.

40. Baldwin DS, Hou R, Gordon R, et al. Pharmacotherapy in generalized anxiety disorder: novel experimental medicine models and emerging drug targets. *CNS Drugs* 2017;31(4):307-17.

41. Grillon C, Robinson OJ, Cornwell B, et al. Modeling anxiety in healthy humans: a key intermediate bridge between basic and clinical sciences. *Neuropsychopharmacology* 2019;44(12):1999-2010.

42. Furukawa TA, Cipriani A, Atkinson LZ, et al. Placebo response rates in antidepressant trials: a systematic review of published and unpublished double-blind randomised controlled studies. *The Lancet Psychiatry* 2016;3(11):1059-66.

43. Stein DJ, Baldwin DS, Dolberg OT, et al. Which Factors Predict Placebo Response in Anxiety Disorders and Major Depression? *The Journal of clinical psychiatry* 2006;67(11):1741-46.

44. Walsh BT, Seidman SN, Sysko R, et al. Placebo response in studies of major depression: variable, substantial, and growing. *JAMA : the journal of the American Medical Association* 2002;287:1840-7.

45. Welten C, Koeter M, Wohlfarth T, et al. Placebo response in antipsychotic trials of patients with acute mania: results of an individual patient data meta-analysis. *European Neuropsychopharmacology* 2015;25(7):1018-26.

46. Nierenberg AA, Ostergaard SD, Iovieno N, et al. Predictors of placebo response in bipolar depression. *International Clinical Psychopharmacology* 2015;30(2):59-66.

47. De Vries YA, De Jonge P, van den Heuvel E, et al. Influence of baseline severity on antidepressant efficacy for anxiety disorders: meta-analysis and meta-regression. *The British Journal of Psychiatry* 2016;208(6):515-21.

48. Bandelow B, Reitt M, Röver C, et al. Efficacy of treatments for anxiety disorders. *International Clinical Psychopharmacology* 2015;30(4):183-92.

49. Agid O, Siu CO, Potkin SG, et al. Meta-regression analysis of placebo response in antipsychotic trials, 1970-2010. *American Journal of Psychiatry* 2013;170(11):1335-44.

50. Leucht S, Leucht C, Huhn M, et al. Sixty Years of Placebo-Controlled Antipsychotic Drug Trials in Acute Schizophrenia: Systematic Review, Bayesian Meta-Analysis, and Meta-Regression of Efficacy Predictors. *American Journal of Psychiatry* 2017;174(10):927-42.

51. Faraone SV, Newcorn JH, Cipriani A, et al. Placebo and nocebo responses in randomised, controlled trials of medications for ADHD: a systematic review and meta-analysis. *Molecular Psychiatry* 2022;27(1):212-19.

52. Iovieno N, Nierenberg AA, Parkin SR, et al. Relationship between placebo response rate and clinical trial outcome in bipolar depression. *Journal of Psychiatric Research* 2016;74:38-44.

53. Iovieno N, Papakostas GI. Correlation between different levels of placebo response rate and clinical trial outcome in major depressive disorder: a meta-analysis. *Journal of Clinical Psychiatry* 2012;73(10):1300-6.

54. Enck P, Bingel U, Schedlowski M, et al. The placebo response in medicine: minimize, maximize or personalize? *Nature Reviews: Drug Discovery* 2013;12(3):191-204.

55. Huneke NT. Is superiority to placebo the most appropriate measure of efficacy in trials of novel psychotropic medications? *European neuropsychopharmacology: the journal of the European College of Neuropsychopharmacology* 2022;62:7-9.

56. Huneke NTM, van der Wee N, Garner M, et al. Why we need more research into the placebo response in psychiatry. *Psychological Medicine* 2020;50(14):2317-23.

57. Whitlock ME, Woodward PW, Alexander RC. Is High Placebo Response Really a Problem in Depression Trials? A Critical Re-analysis of Depression Studies. *Innovations in Clinical Neuroscience* 2019;16(7-08):12-17. (accessed 2019 Jul-Aug).

58. Leucht S, Chaimani A, Leucht C, et al. 60 years of placebo-controlled antipsychotic drug trials in acute schizophrenia: Meta-regression of predictors of placebo response. *Schizophrenia Research* 2018;201:315-23.

59. Fournier JC, Derubeis RJ, Hollon SD, et al. Antidepressant Drug Effects and Depression Severity. *JAMA* 2010;303(1):47.

60. Papakostas GI, Fava M. Does the probability of receiving placebo influence clinical trial outcome? A meta-regression of double-blind, randomized clinical trials in MDD. *European Neuropsychopharmacology* 2009;19(1):34-40.

61. Trivedi MH, South C, Jha MK, et al. A novel strategy to identify placebo responders: prediction index of clinical and biological markers in the EMBARC trial. *Psychotherapy and Psychosomatics* 2018;87(5):285-95.

62. Stone MB, Yaseen ZS, Miller BJ, et al. Response to acute monotherapy for major depressive disorder in randomized, placebo controlled trials submitted to the US Food and Drug Administration: individual participant data analysis. *BMJ* 2022;378.

63. Woods SW, Gueorguieva RV, Baker CB, et al. Control Group Bias in Randomized Atypical Antipsychotic Medication Trials for Schizophrenia. *Archives of General Psychiatry* 2005;62(9):961.

64. Jensen KB, Kirsch I, Ponten M, et al. Certainty of genuine treatment increases drug responses among intellectually disabled patients. *Neurology* 2017;88(20):1912-18.

65. Rutherford BR, Wall MM, Brown PJ, et al. Patient Expectancy as a Mediator of Placebo Effects in Antidepressant Clinical Trials. *American Journal of Psychiatry* 2017;174(2):135-42.

66. Jütte R. The early history of the placebo. 2013;21(2):94-97.

67. McQueen D, Cohen S, St John-Smith P, et al. Rethinking placebo in psychiatry: the range of placebo effects. *Advances in psychiatric treatment* 2013;19(3):162-70.

68. Beecher HK. The powerful placebo. *Journal of the American Medical Association* 1955;159(17):1602-6.

69. Ernst E, Resch KL. Concept of true and perceived placebo effects. *BMJ* 1995;311:551-53.

70. Hróbjartsson A, Gøtzsche PC. Is the Placebo Powerless? *New England Journal of Medicine* 2001;344(21):1594-602.

71. Hrobjartsson A, Gotzsche PC. Is the placebo powerless? Update of a systematic review with 52 new randomized trials comparing placebo with no treatment. *Journal of Internal Medicine* 2004;256(2):91-100.

72. Krogsbøll L, Hróbjartsson A, Gøtzsche PC. Spontaneous improvement in randomised clinical trials: meta-analysis of three-armed trials comparing no treatment, placebo and active intervention. *BMC Medical Research Methodology* 2009;9(1):1.

73. Kienle GS, Kiene H. The Powerful Placebo Effect: Fact or Fiction? *Journal of Clinical Epidemiology* 1997;50(12):1311-18.

74. Miller FG, Rosenstein DL. The nature and power of the placebo effect. *Journal of Clinical Epidemiology* 2006;59(4):331-35.

75. Vase L, Riley JL, Price DD. A comparison of placebo effects in clinical analgesic trials versus studies of placebo analgesia. *Pain* 2002;99(3):443-52.

76. Ashar YK, Chang LJ, Wager TD. Brain Mechanisms of the Placebo Effect: An Affective Appraisal Account. *Annual Review of Clinical Psychology* 2017;13(1):73-98.

77. Albring A, Wendt L, Benson S, et al. Placebo effects on the immune response in humans: the role of learning and expectation. *PloS One* 2012;7:e49477.

78. Goebel MU, Trebst AE, Steiner J, et al. Behavioral conditioning of immunosuppression is possible in humans. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2002;16:1869-73.

79. Benedetti F, Carlino E, Pollo A. How placebos change the patient's brain. *Neuropsychopharmacology* 2011;36:339-54.

80. Pecina M, Zubieta JK. Molecular mechanisms of placebo responses in humans. *Molecular Psychiatry* 2015;20(4):416-23.

81. Evers AWM, Colloca L, Blease C, et al. Implications of Placebo and Nocebo Effects for Clinical Practice: Expert Consensus. *Psychotherapy and Psychosomatics* 2018;87(4):204-10.

82. Colloca L, Benedetti F. Placebo analgesia induced by social observational learning. *Pain* 2009;144(1):28-34.

83. Levine JD, Gordon NC, Fields HL. The mechanism of placebo analgesia. *Lancet* 1978;2:654-7.

84. Huneke NT, Brown CA, Burford E, et al. Experimental placebo analgesia changes resting-state alpha oscillations. *PloS One* 2013;8(10):e78278.

85. Price DD, Milling LS, Kirsch I, et al. An analysis of factors that contribute to the magnitude of placebo analgesia in an experimental paradigm. *Pain* 1999;83(2):147-56.

86. Carvalho C, Caetano JM, Cunha L, et al. Open-label placebo treatment in chronic low back pain. *Pain* 2016;157(12):2766-72.

87. Benedetti F, Colloca L, Torre E, et al. Placebo-responsive Parkinson patients show decreased activity in single neurons of subthalamic nucleus. *Nature Neuroscience* 2004;7(6):587-88.

88. De La Fuente-Fernandez R. Expectation and Dopamine Release: Mechanism of the Placebo Effect in Parkinson's Disease. *Science* 2001;293(5532):1164-66.

89. Sandler AD, Glesne CE, Bodfish JW. Conditioned placebo dose reduction: a new treatment in attention-deficit hyperactivity disorder? *Journal of Developmental and Behavioral Pediatrics* 2010;31(5):369-75.

90. Levine ME, Stern RM, Koch KL. The Effects of Manipulating Expectations Through Placebo and Nocebo Administration on Gastric Tachyarrhythmia and Motion-Induced Nausea. *Psychosomatic Medicine* 2006;68(3):478-86.

91. Quinn VF, Colagiuri B. Sources of Placebo-Induced Relief From Nausea: The Role of Instruction and Conditioning. *Psychosomatic Medicine* 2016;78(3):365-72.

92. Bartels DJP, Van Laarhoven AIM, Haverkamp EA, et al. Role of Conditioning and Verbal Suggestion in Placebo and Nocebo Effects on Itch. *PloS One* 2014;9(3):e91727.

93. Kaptchuk TJ, Friedlander E, Kelley JM, et al. Placebos without Deception: A Randomized Controlled Trial in Irritable Bowel Syndrome. *PloS One* 2010;5(12):e15591.

94. Stern J, Candia V, Porchet RI, et al. Placebo-mediated, Naloxone-sensitive suggestibility of short-term memory performance. *Neurobiol Learn Mem* 2011;95(3):326-34.

95. Glombiewski JA, Rheker J, Wittkowksi J, et al. Placebo mechanisms in depression: An experimental investigation of the impact of expectations on sadness in female participants. *Journal of Affective Disorders* 2019;256:658-67.

96. Pecina M, Bohnert AS, Sikora M, et al. Association Between Placebo-Activated Neural Systems and Antidepressant Responses: Neurochemistry of Placebo Effects in Major Depression. *JAMA psychiatry* 2015;72(11):1087-94.

97. Pecina M, Heffernan J, Wilson J, et al. Prefrontal expectancy and reinforcement-driven antidepressant placebo effects. *Transl Psychiatry* 2018;8(1):222.

98. Faria V, Gingnell M, Hoppe JM, et al. Do You Believe It? Verbal Suggestions Influence the Clinical and Neural Effects of Escitalopram in Social Anxiety Disorder: A Randomized Trial. *EBioMedicine* 2017;24:179-88.

99. Geuter S, Koban L, Wager TD. The Cognitive Neuroscience of Placebo Effects: Concepts, Predictions, and Physiology. *Annual Review of Neuroscience* 2017;40(1):167-88.

100. Petrie KJ, Rief W. Psychobiological Mechanisms of Placebo and Nocebo Effects: Pathways to Improve Treatments and Reduce Side Effects. *Annual Review of Psychology* 2019;70(1):599-625.

101. Wager TD, Atlas LY. The neuroscience of placebo effects: connecting context, learning and health. *Nature Reviews Neuroscience* 2015;16(7):403-18.

102. Amanzio M, Pollo A, Maggi G, et al. Response variability to analgesics: a role for non-specific activation of endogenous opioids. *Pain* 2001;90(3):205-15.

103. Atlas LY, Whittington RA, Lindquist MA, et al. Dissociable Influences of Opiates and Expectations on Pain. *Journal of Neuroscience* 2012;32(23):8053-64.

104. Benedetti F, Maggi G, Lopiano L, et al. Open versus hidden medical treatments: The patient's knowledge about a therapy affects the therapy outcome. *Prevention & Treatment* 2003;6(1):Article 1a.

105. Colloca L, Lopiano L, Lanotte M, et al. Overt versus covert treatment for pain, anxiety, and Parkinson's disease. *The Lancet Neurology* 2004;3(11):679-84.

106. Swider K, Bąbel P, Wronka E, et al. Placebo analgesia induced by verbal suggestion in the context of experimentally induced fear and anxiety. *PloS One* 2019;14(9):e0222805.

107. Babel P, Bajcar EA, Adamczyk W, et al. Classical conditioning without verbal suggestions elicits placebo analgesia and nocebo hyperalgesia. *PloS One* 2017;12(7):e0181856.

108. Watson A, El-Deredy W, Iannetti GD, et al. Placebo conditioning and placebo analgesia modulate a common brain network during pain anticipation and perception. *Pain* 2009;145:24-30.

109. Corsi N, Colloca L. Placebo and Nocebo Effects: The Advantage of Measuring Expectations and Psychological Factors. *Frontiers in Psychology* 2017;8.

110. Kube T, Rief W. Are placebo and drug-specific effects additive? Questioning basic assumptions of double-blinded randomized clinical trials and presenting novel study designs. *Drug Discovery Today* 2017;22(4):729-35.

111. Carlino E, Torta DM, Piedimonte A, et al. Role of explicit verbal information in conditioned analgesia. *European Journal of Pain (London, England)* 2015;19(4):546-53.

112. Colloca L, Tinazzi M, Recchia S, et al. Learning potentiates neurophysiological and behavioral placebo analgesic responses. *Pain* 2008;139(2):306-14.

113. Colloca L, Benedetti F. How prior experience shapes placebo analgesia. *Pain* 2006;124(1):126-33.

114. Kessner S, Wiech K, Forkmann K, et al. The Effect of Treatment History on Therapeutic Outcome: An Experimental Approach. *JAMA Internal Medicine* 2013;173(15):1468.

115. Zunhammer M, Ploner M, Engelbrecht C, et al. The effects of treatment failure generalize across different routes of drug administration. *Science Translational Medicine* 2017;9(393):eaal2999.

116. Tasso AF, Pérez NA, Moore M, et al. Hypnotic responsiveness and nonhypnotic suggestibility: Disparate, similar, or the same? *International Journal of Clinical and Experimental Hypnosis* 2020;68(1):38-67.

117. De Pascalis V, Chiaradia C, Carotenuto E. The contribution of suggestibility and expectation to placebo analgesia phenomenon in an experimental setting. *Pain* 2002;96(3):393-402.

118. Oakley DA, Walsh E, Mehta MA, et al. Direct verbal suggestibility: Measurement and significance. *Consciousness and Cognition* 2021;89:103036.

119. Parsons RD, Bergmann S, Wiech K, et al. Direct verbal suggestibility as a predictor of placebo hypoalgesia responsiveness. *Psychosomatic Medicine* 2021;83(9):1041-49.

120. Shor RE, Orne EC. Harvard group scale of hypnotic susceptibility, form A. *International Journal of Clinical and Experimental Hypnosis* 1962.

121. Weitzenhoffer AM, Hilgard ER. *Stanford hypnotic susceptibility scale, form C*: Palo Alto, CA: Consulting Psychologists Press; 1962.

122. Gudjonsson GH, Clare IC. The relationship between confabulation and intellectual ability, memory, interrogative suggestibility and acquiescence. *Personality and Individual Differences* 1995;19(3):333-38.

123. Asch SE. Effects of group pressure upon the modification and distortion of judgments. *Organizational influence processes* 1951;58:295-303.

124. Loftus EF, Palmer JC. Reconstruction of automobile destruction: An example of the interaction between language and memory. *Journal of verbal learning and verbal behavior* 1974;13(5):585-89.

125. Mehling WE, Price C, Daubenmier JJ, et al. The multidimensional assessment of interoceptive awareness (MAIA). *PloS One* 2012;7(11):e48230.

126. Kleckner IR, Wormwood JB, Simmons WK, et al. Methodological recommendations for a heartbeat detection-based measure of interoceptive sensitivity. *Psychophysiology* 2015;52(11):1432-40.

127. Derbyshire SW, Oakley DA. A role for suggestion in differences in brain responses after placebo conditioning in high and low hypnotizable subjects. *Pain* 2013;154(9):1487-88.

128. Benedetti F, Carlino E, Piedimonte A. Increasing uncertainty in CNS clinical trials: the role of placebo, nocebo, and Hawthorne effects. *Lancet Neurology* 2016;15(7):736-47.

129. Atlas LY, Wager TD. A Meta-analysis of Brain Mechanisms of Placebo Analgesia: Consistent Findings and Unanswered Questions. In: Benedetti F, Enck P, Frisaldi E, et al. (eds.) *Placebo*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014 p37-69.

130. Fields H. State-dependent opioid control of pain. *Nature reviews. Neuroscience* 2004;5:565-75.

131. Amanzio M, Benedetti F. Neuropharmacological dissection of placebo analgesia: expectation-activated opioid systems versus conditioning-activated specific subsystems. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 1999;19:484-94.

132. Petrovic P, Kalso E, Petersson KM, et al. Placebo and opioid analgesia-- imaging a shared neuronal network. *Science (New York, N.Y.)* 2002;295:1737-40.

133. Zubieta J-K, Bueller JA, Jackson LR, et al. Placebo effects mediated by endogenous opioid activity on mu-opioid receptors. *The Journal of neuroscience* 2005;25:7754-62.

134. Eippert F, Bingel U, Schoell ED, et al. Activation of the opioidergic descending pain control system underlies placebo analgesia. *Neuron* 2009;63:533-43.

135. Wager TD, Scott DJ, Zubieta J-K. Placebo effects on human mu-opioid activity during pain. *Proceedings of the National Academy of Sciences of the United States of America* 2007;104:11056-61.

136. Peciña M, Chen J, Lyew T, et al. μ-Opioid antagonist naltrexone partially abolishes the antidepressant placebo effect and reduces OFC encoding of reinforcement. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* 2021.

137. Benedetti F, Amanzio M, Rosato R, et al. Nonopioid placebo analgesia is mediated by CB1 cannabinoid receptors. *Nature Medicine* 2011;17(10):1228-30.

138. Haber SN. 11 Neuroanatomy of Reward: A View from the Ventral Striatum. In: Gottfried JA (ed.) *Neurobiology of sensation and reward*; 2011 p235-61.

139. Scott DJ, Stohler CS, Egnatuk CM, et al. Placebo and nocebo effects are defined by opposite opioid and dopaminergic responses. *Archives of General Psychiatry* 2008;65:220-31.

140. Lidstone SC, Schulzer M, Dinelle K, et al. Effects of expectation on placebo-induced dopamine release in Parkinson disease. *Archives of General Psychiatry* 2010;67(8):857-65.

141. Zhou P, Critchley H, Garfinkel S, et al. The conceptualization of emotions across cultures: a model based on interoceptive neuroscience. *Neuroscience & Biobehavioral Reviews* 2021;125:314-27.

142. Ekman P. An argument for basic emotions. *Cognition & Emotion* 1992;6(3-4):169-200.

143. Barrett LF. Are emotions natural kinds? *Perspectives on Psychological Science* 2006;1(1):28-58.

144. Barrett LF. Solving the emotion paradox: Categorization and the experience of emotion. *Personality and Social Psychology Review* 2006;10(1):20-46.

145. Lindquist KA, Gendron M, Satpute AB, et al. Language and emotion: Putting words into feelings and feelings into words *Handbook of Emotions*. 4th ed. New York, NY: The Guilford Press; 2016.

146. Barrett LF, Bar M. See it with feeling: affective predictions during object perception. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2009;364(1521):1325-34.

147. Flaten MA, Aslaksen PM, Lyby PS, et al. The relation of emotions to placebo responses. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 2011;366:1818-27.

148. Colloca L, Petrovic P, Wager TD, et al. How the number of learning trials affects placebo and nocebo responses. *Pain* 2010;151(2):430-39.

149. Morton DL, Watson A, El-Deredy W, et al. Reproducibility of placebo analgesia: Effect of dispositional optimism. *Pain* 2009;146(1):194-98.

150. Staats PS, Staats A, Hekmat H. The Additive Impact of Anxiety and a Placebo on Pain. *Pain Medicine* 2001;2(4):267-79.

151. Bartels DJP, Van Laarhoven AIM, Van De Kerkhof PCM, et al. Placebo and nocebo effects on itch: effects, mechanisms, and predictors. *European Journal of Pain* 2016;20(1):8-13.

152. Göhler AC, Haas JW, Sperl MFJ, et al. Placebo nasal spray protects female participants from experimentally induced sadness and concomitant changes in autonomic arousal. *Journal of Affective Disorders* 2021.

153. Friehs T, Rief W, Glombiewski JA, et al. Deceptive and non-deceptive placebos to reduce sadness: A five-armed experimental study. *Journal of Affective Disorders Reports* 2022;9:100349.

154. Schienle A, Ubel S, Schongassner F, et al. Disgust regulation via placebo: an fMRI study. *Social Cognitive and Affective Neuroscience* 2014;9(7):985-90.

155. Petrovic P, Dietrich T, Fransson P, et al. Placebo in emotional processing--induced expectations of anxiety relief activate a generalized modulatory network. *Neuron* 2005;46:957-69.

156. Guevarra DA, Moser JS, Wager TD, et al. Placebos without deception reduce self-report and neural measures of emotional distress. *Nature Communications* 2020;11(1).

157. Rebstock L, Schafer LN, Kube T, et al. Placebo prevents rumination: An experimental study. *Journal of Affective Disorders* 2020;274:1152-60.

158. Meyer B, Yuen KS, Ertl M, et al. Neural mechanisms of placebo anxiolysis. *Journal of Neuroscience* 2015;35(19):7365-73.

159. Meyer B, Yuen KSL, Saase V, et al. The Functional Role of Large-scale Brain Network Coordination in Placebo-induced Anxiolysis. *Cerebral Cortex* 2019;29(8):3201-10.

160. Rief W, Glombiewski JA. The hidden effects of blinded, placebo-controlled randomized trials: An experimental investigation. *Pain* 2012;153(12):2473-77.

161. von Wernsdorff M, Loef M, Tuschen-Caffier B, et al. Effects of open-label placebos in clinical trials: a systematic review and meta-analysis. *Scientific Reports* 2021;11(1):1-14.

162. Allan LG, Siegel S. A Signal Detection Theory Analysis of the Placebo Effect. *Evaluation & the Health Professions* 2002;25(4):410-20.

163. Hróbjartsson A, Kaptchuk TJ, Miller FG. Placebo effect studies are susceptible to response bias and to other types of biases. *Journal of Clinical Epidemiology* 2011;64(11):1223-29.

164. Schmitz A, Grillon C. Assessing fear and anxiety in humans using the threat of predictable and unpredictable aversive events (the NPU-threat test). *Nature Protocols* 2012;7(3):527-32.

165. Davies CD, Craske MG. Psychophysiological responses to unpredictable threat: effects of cue and temporal unpredictability. *Emotion* 2015;15(2):195-200.

166. Phillips ML, Ladouceur CD, Drevets WC. A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. *Molecular Psychiatry* 2008;13(9):833-57.

167. Rive MM, Mocking RJ, Koeter MW, et al. State-dependent differences in emotion regulation between unmedicated bipolar disorder and major depressive disorder. *JAMA psychiatry* 2015;72(7):687-96.

168. Nummenmaa L, Tuominen L. Opioid system and human emotions. *British Journal of Pharmacology* 2018;175(14):2737-49.

169. Colasanti A, Rabiner E, Lingford-Hughes A, et al. Opioids and anxiety. *Journal of Psychopharmacology* 2011;25(11):1415-33.

170. Browne CA, Lucki I. Targeting opioid dysregulation in depression for the development of novel therapeutics. *Pharmacol Ther* 2019;201:51-76.

171. Van der Does W. Different types of experimentally induced sad mood? *Behavior Therapy* 2002;33(4):551-61.

172. Robinson OJ, Vytal K, Cornwell BR, et al. The impact of anxiety upon cognition: perspectives from human threat of shock studies. *Frontiers in Human Neuroscience* 2013;7:203.

173. Kraus SW, Etuk R, Potenza MN. Current pharmacotherapy for gambling disorder: a systematic review. *Expert Opinion on Pharmacotherapy* 2020;21(3):287-96.

174. Grant JE, Odlaug BL, Chamberlain SR. Neural and psychological underpinnings of gambling disorder: A review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2016;65:188-93.

175. Grant JE, Chamberlain SR. The placebo effect and its clinical associations in gambling disorder. *Annals of Clinical Psychiatry* 2017;29(3):167-72.

176. Grant JE, Kim SW, Hollander E, et al. Predicting response to opiate antagonists and placebo in the treatment of pathological gambling. *Psychopharmacology* 2008;200(4):521-27.

177. Simmonds MC, Higgins JP. Covariate heterogeneity in meta-analysis: criteria for deciding between meta-regression and individual patient data. *Statistics in Medicine* 2007;26(15):2982-99.

178. Teramukai S, Matsuyama Y, Mizuno S, et al. Individual patient-level and study-level meta-analysis for investigating modifiers of treatment effect. *Japanese journal of clinical oncology* 2004;34(12):717-21.

179. Lambert PC, Sutton AJ, Abrams KR, et al. A comparison of summary patient-level covariates in meta-regression with individual patient data meta-analysis. *Journal of Clinical Epidemiology* 2002;55(1):86-94.

180. Prater KE, Hosanagar A, Klumpp H, et al. Aberrant amygdala–frontal cortex connectivity during perception of fearful faces and at rest in generalized social anxiety disorder. *Depression and Anxiety* 2013;30(3):234-41.

181. Groenewold NA, Opmeer EM, De Jonge P, et al. Emotional valence modulates brain functional abnormalities in depression: Evidence from a meta-analysis of fMRI studies. *Neuroscience & Biobehavioral Reviews* 2013;37(2):152-63.

182. Gopalakrishnan S, Ganeshkumar P. Systematic Reviews and Meta-analysis: Understanding the Best Evidence in Primary Healthcare. *Journal of family medicine and primary care* 2013;2(1):9-14.

183. Zunhammer M, Spisak T, Wager TD, et al. Meta-analysis of neural systems underlying placebo analgesia from individual participant fMRI data. *Nat Commun* 2021;12(1):1391.

184. Forsberg JT, Martinussen M, Flaten MA. The placebo analgesic effect in healthy individuals and patients: a meta-analysis. *Psychosomatic Medicine* 2017;79(4):388-94.

185. Gilles C, Schunck T, Erb G, et al. Human models as tools in the development of psychotropic drugs. *Dialogues in Clinical Neuroscience* 2002;4(4):377-87.

186. Gouzoulis-Mayfrank E, Heekeren K, Neukirch A, et al. Inhibition of return in the human 5HT2A agonist and NMDA antagonist model of psychosis. *Neuropsychopharmacology* 2006;31(2):431-41.

187. DellaGioia N, Hannestad J. A critical review of human endotoxin administration as an experimental paradigm of depression. *Neuroscience & Biobehavioral Reviews* 2010;34(1):130-43.

188. Pringle A, Harmer CJ. The effects of drugs on human models of emotional processing: an account of antidepressant drug treatment. *Dialogues in Clinical Neuroscience* 2022.

189. Harmer CJ, Shelley NC, Cowen PJ, et al. Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *American Journal of Psychiatry* 2004;161(7):1256-63.

190. Huneke NT, Walsh AE, Brown R, et al. No evidence for an acute placebo effect on emotional processing in healthy volunteers. *Journal of Psychopharmacology* 2017;31(12):1578-87.

191. Bailey JE, Argyropoulos SV, Kendrick AH, et al. Behavioral and cardiovascular effects of 7.5% CO2 in human volunteers. *Depression and Anxiety* 2005;21(1):18-25.

192. Garner M, Attwood A, Baldwin DS, et al. Inhalation of 7.5% carbon dioxide increases threat processing in humans. *Neuropsychopharmacology* 2011;36(8):1557-62.

193. Garner M, Attwood A, Baldwin DS, et al. Inhalation of 7.5% carbon dioxide increases alerting and orienting attention network function. *Psychopharmacology* 2012;223(1):67-73.

194. Ainsworth B, Marshall JE, Meron D, et al. Evaluating psychological interventions in a novel experimental human model of anxiety. *Journal of Psychiatric Research* 2015;63:117-22.

195. Bailey JE, Kendrick A, Diaper A, et al. A validation of the 7.5% CO2 model of GAD using paroxetine and lorazepam in healthy volunteers. *Journal of Psychopharmacology* 2007;21(1):42-49.

196. Allen AP, Kennedy PJ, Dockray S, et al. The Trier Social Stress Test: Principles and practice. *Neurobiol Stress* 2017;6:113-26.

197. Fries E, Hellhammer DH, Hellhammer J. Attenuation of the hypothalamic-pituitary-adrenal axis responsivity to the Trier Social Stress Test by the benzodiazepine alprazolam. *Psychoneuroendocrinology* 2006;31(10):1278-88.

198. Bershad AK, Jaffe JH, Childs E, et al. Opioid partial agonist buprenorphine dampens responses to psychosocial stress in humans. *Psychoneuroendocrinology* 2015;52:281-88.

199. Grant JE, Schreiber L, Odlaug BL, et al. Pathologic gambling and bankruptcy. *Comprehensive Psychiatry* 2010;51(2):115-20.

200. Black DW, Shaw M. The Epidemiology of Gambling Disorder: Springer International Publishing; 2019 p29-48.

201. Buchanan TW, McMullin SD, Baxley C, et al. Stress and gambling. *Current Opinion in Behavioral Sciences* 2020;31:8-12.

202. Kessler RC, Hwang I, LaBrie R, et al. DSM-IV pathological gambling in the National Comorbidity Survey Replication. *Psychological Medicine* 2008;38(9):1351-60.

203. Ober K, Benson S, Vogelsang M, et al. Plasma noradrenaline and state anxiety levels predict placebo response in learned immunosuppression. *Clinical Pharmacology and Therapeutics* 2012;91(2):220-6.

204. Altman DG, Royston P. The cost of dichotomising continuous variables. *BMJ* 2006;332(7549):1080.1.

205. Ritz C. Statistical analysis of continuous outcomes from parallel-arm randomized controlled trials in nutrition—a tutorial. *European Journal of Clinical Nutrition* 2021;75(1):160-71.

206. Walker M, Toneatto T, Potenza MN, et al. A framework for reporting outcomes in problem gambling treatment research: the Banff, Alberta Consensus. *Addiction* 2006;101(4):504-11.

207. Grant JE, Kim SW, Potenza MN, et al. Paroxetine treatment of pathological gambling: a multi-centre randomized controlled trial. *International Clinical Psychopharmacology* 2003;18(4):243-9.

208. Grant JE, Potenza MN. Escitalopram treatment of pathological gambling with co-occurring anxiety: an open-label pilot study with double-blind discontinuation. *International Clinical Psychopharmacology* 2006;21(4):203-9.

209. Grant JE, Kim SW, Odlaug BL. N-acetyl cysteine, a glutamate-modulating agent, in the treatment of pathological gambling: a pilot study. *Biological Psychiatry* 2007;62(6):652-7.

210. Grant JE, Kim SW, Hartman BK. A double-blind, placebo-controlled study of the opiate antagonist naltrexone in the treatment of pathological gambling urges. *Journal of Clinical Psychiatry* 2008;69(5):783-9.

211. Grant JE, Chamberlain SR, Odlaug BL, et al. Memantine shows promise in reducing gambling severity and cognitive inflexibility in pathological gambling: a pilot study. *Psychopharmacology* 2010;212(4):603-12.

212. Grant JE, Odlaug BL, Chamberlain SR, et al. A randomized, placebo-controlled trial of N-acetylcysteine plus imaginal desensitization for nicotine-dependent pathological gamblers. *Journal of Clinical Psychiatry* 2014;75(1):39-45.

213. Grant JE, Steinberg MA, Kim SW, et al. Preliminary validity and reliability testing of a structured clinical interview for pathological gambling. *Psychiatry Research* 2004;128(1):79-88.

214. First M, Spitzer R, Gibbon M, et al. Structured clinical interview for DSM-IV Patient Edition.(Version 2.0). *New York: Biometrics Research Department* 1994.

215. Kim SW, Grant JE, Potenza MN, et al. The Gambling Symptom Assessment Scale (G-SAS): a reliability and validity study. *Psychiatry Research* 2009;166(1):76-84.

216. Hamilton M. A rating scale for depression. *Journal of neurology, neurosurgery, and psychiatry* 1960;23(1):56.

217. Hamilton M. The assessment of anxiety states by rating. *British Journal of Medical Psychology* 1959;32(1):50-55.

218. The jamovi project. *jamovi (Version 1.6.23.0). https://www.jamovi.org* 2021.

219. Jimenez-Murcia S, Granero R, Fernandez-Aranda F, et al. Predictors of Outcome among Pathological Gamblers Receiving Cognitive Behavioral Group Therapy. *European Addiction Research* 2015;21(4):169-78.

220. Merkouris SS, Thomas SA, Browning CJ, et al. Predictors of outcomes of psychological treatments for disordered gambling: A systematic review. *Clinical Psychology Review* 2016;48:7-31.

221. Manning V, Ng A, Koh PK, et al. Pathological gamblers in Singapore: treatment response at 3 months. *Journal of Addiction Medicine* 2014;8(6):462-9.

222. Moragas L, Granero R, Stinchfield R, et al. Comparative analysis of distinct phenotypes in gambling disorder based on gambling preferences. *BMC Psychiatry* 2015;15(1).

223. Farstad SM, von Ranson KM, Hodgins DC, et al. The influence of impulsiveness on binge eating and problem gambling: A prospective study of gender differences in Canadian adults. *Psychology of Addictive Behaviors* 2015;29(3):805.

224. Goyal RK, Kalaria SN, McElroy SL, et al. An exploratory machine learning approach to identify placebo responders in pharmacological binge eating disorder trials. *Clinical and Translational Science* 2022.

225. Hodgins DC, Peden N, Cassidy E. The association between comorbidity and outcome in pathological gambling: a prospective follow-up of recent quitters. *Journal of Gambling Studies* 2005;21(3):255-71.

226. Kim SW, Grant JE, Eckert ED, et al. Pathological gambling and mood disorders: clinical associations and treatment implications. *Journal of Affective Disorders* 2006;92(1):109-16.

227. Valleur M, Codina I, Venisse JL, et al. Towards a Validation of the Three Pathways Model of Pathological Gambling. *Journal of Gambling Studies* 2016;32(2):757-71.

228. Friesen P, Blease C. Placebo effects and racial and ethnic health disparities: an unjust and underexplored connection. *Journal of Medical Ethics* 2018;44(11):774-81.

229. Ingle PJ, Marotta J, McMillan G, et al. Significant Others and Gambling Treatment Outcomes. *Journal of Gambling Studies* 2008;24(3):381-92.

230. Guo S, Manning V, Thane KKW, et al. Predictors of Treatment Outcome Among Asian Pathological Gamblers (PGs): Clinical, Behavioural, Demographic, and Treatment Process Factors. *Journal of Gambling Studies* 2014;30(1):89-103.

231. Deyoreo M, Smith BP. Reducing Costs and Improving Fit for Clinical Trials that Have Positive-Valued Data. *Statistics in Biopharmaceutical Research* 2017;9(2):234-42.

232. Vickers AJ. The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. *BMC Medical Research Methodology* 2001;1(1):1-4.

233. Vickers AJ, Altman DG. Analysing controlled trials with baseline and follow up measurements. *BMJ* 2001;323(7321):1123-24.

234. Egbewale BE, Lewis M, Sim J. Bias, precision and statistical power of analysis of covariance in the analysis of randomized trials with baseline imbalance: a simulation study. *BMC Medical Research Methodology* 2014;14(1):1-12.

235. Van Breukelen GJ. ANCOVA versus change from baseline had more power in randomized studies and more bias in nonrandomized studies. *Journal of Clinical Epidemiology* 2006;59(9):920-25.

236. Stahl SM, Greenberg GD. Placebo response rate is ruining drug development in psychiatry: why is this happening and what can we do about it? *Acta Psychiatrica Scandinavica* 2019;139(2):105-07.

237. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Systematic Reviews* 2021;10(1).

238. Huneke N, Fagan H, Cortese S, et al. A systematic review exploring the functional neuroanatomy of the placebo effect in patients with anxiety or depressive disorders. *PROSPERO* 2019;CRD42019156911.

239. Bramer WM, Rethlefsen ML, Kleijnen J, et al. Optimal database combinations for literature searches in systematic reviews: a prospective exploratory study. *Systematic Reviews* 2017;6(1).

240. Sterne JAC, Savovic J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* 2019;366:l4898.

241. Eickhoff SB, Nichols TE, Laird AR, et al. Behavior, sensitivity, and power of activation likelihood estimation characterized by massive empirical simulation. *Neuroimage* 2016;137:70-85.

242. Müller VI, Cieslik EC, Laird AR, et al. Ten simple rules for neuroimaging meta-analysis. *Neuroscience & Biobehavioral Reviews* 2018;84:151-61.

243. Chin Fatt CR, Cooper C, Jha MK, et al. Dorsolateral Prefrontal Cortex and Subcallosal Cingulate Connectivity Show Preferential Antidepressant Response in Major Depressive Disorder. *Biological Psychiatry-Cognitive Neuroscience and Neuroimaging* 2021;6(1):20-28.

244. Chin Fatt CR, Cooper CM, Jha MK, et al. Differential response to SSRI versus Placebo and distinct neural signatures among data-driven subgroups of patients with major depressive disorder. *Journal of Affective Disorders* 2021;282:602-10.

245. Chin Fatt CR, Jha MK, Cooper CM, et al. Effect of intrinsic patterns of functional brain connectivity in moderating antidepressant. *American Journal of Psychiatry* 2020;177(2):143-54.

246. Cooper CM, Chin Fatt CR, Jha M, et al. Cerebral Blood Perfusion Predicts Response to Sertraline versus Placebo for Major Depressive Disorder in the EMBARC Trial. *EClinicalMedicine* 2019;10:32-41.

247. Fan S, Nemati S, Akiki TJ, et al. Pretreatment Brain Connectome Fingerprint Predicts Treatment Response in Major Depressive Disorder. *Chronic Stress (Thousand Oaks)* 2020;4:247054702098472.

248. Faria V, Åhs F, Appel L, et al. Amygdala-frontal couplings characterizing SSRI and placebo response in social anxiety disorder. *International Journal of Neuropsychopharmacology* 2014;17(8):1149-57.

249. Faria V, Appel L, Ahs F, et al. Amygdala subregions tied to SSRI and placebo response in patients with social anxiety disorder. *Neuropsychopharmacology* 2012;37(10):2222-32.

250. Furmark T, Appel L, Henningsson S, et al. A Link between Serotonin-Related Gene Polymorphisms, Amygdala Activity, and Placebo-Induced Relief from Social Anxiety. *The Journal of Neuroscience* 2008;28(49):13066-74.

251. Greenberg T, Fournier JC, Stiffler R, et al. Reward related ventral striatal activity and differential response to sertraline versus placebo in depressed individuals. *Molecular Psychiatry* 2020;25(7):1526-36.

252. Mayberg HS, Silva J, Brannan SK, et al. The functional neuroanatomy of the placebo effect. *The American Journal of Psychiatry* 2002;159(5):728-37.

253. Sikora M, Heffernan J, Avery ET, et al. Salience Network Functional Connectivity Predicts Placebo Effects in Major Depression. *Biological psychiatry. Cognitive neuroscience and neuroimaging* 2016;1(1):68-76.

254. Zilcha-Mano S, Wang Z, Peterson BS, et al. Neural mechanisms of expectancy-based placebo effects in antidepressant clinical trials. *Journal of Psychiatric Research* 2019;116:19-25.

255. Raichle ME. The brain's default mode network. *Annual Review of Neuroscience* 2015;38:433-47.

256. Fox MD, Snyder AZ, Vincent JL, et al. From The Cover: The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proceedings of the National Academy of Sciences* 2005;102(27):9673-78.

257. Hamilton JP, Chen MC, Gotlib IH. Neural systems approaches to understanding major depressive disorder: An intrinsic functional organization perspective. *Neurobiology of Disease* 2013;52:4-11.

258. Pannekoek JN, Veer IM, Van Tol M-J, et al. Resting-state functional connectivity abnormalities in limbic and salience networks in social anxiety disorder without comorbidity. *European Neuropsychopharmacology* 2013;23(3):186-95.

259. Van Steenbergen H, Eikemo M, Leknes S. The role of the opioid system in decision making and cognitive control: A review. *Cognitive, Affective, & Behavioral Neuroscience* 2019;19(3):435-58.

260. Sheehan DV, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of clinical psychiatry* 1998;59 Suppl 20:22-33.

261. Liu T. Route of placebo administration: Robust placebo effects in laboratory and clinical settings. *Neuroscience & Biobehavioral Reviews* 2017;83:451-57.

262. Huneke NTM, Broulidakis MJ, Darekar A, et al. Brain Functional Connectivity Correlates of Response in the 7.5% CO2 Inhalational Model of Generalized Anxiety Disorder: A Pilot Study. *International Journal of Neuropsychopharmacology* 2020;23(4):268-73.

263. Pinkney V. *7.5% carbon dioxide inhalation: modelling anxiety and assessing pharmacological effects*. PhD thesis. University of Southampton, 2016.

264. Geers AL, Faasse K, Guevarra DA, et al. Affect and emotions in placebo and nocebo effects: What do we know so far? *Social and Personality Psychology Compass* 2020.

265. Kern A, Kramm C, Witt CM, et al. The influence of personality traits on the placebo/nocebo response: A systematic review. *Journal of Psychosomatic Research* 2020;128:109866.

266. Vachon-Presseau E, Berger SE, Abdullah TB, et al. Brain and psychological determinants of placebo pill response in chronic pain patients. *Nature Communications* 2018;9(1).

267. Horing B, Weimer K, Muth ER, et al. Prediction of placebo responses: a systematic review of the literature. *Frontiers in Psychology* 2014;5:1079.

268. Kang H, Miksche MS, Ellingsen D-M. The association between personality traits and placebo effects: A preregistered systematic review and meta-analysis. 2022.

269. Eke M, McNally RJ. Anxiety sensitivity, suffocation fear, trait anxiety, and breath-holding duration as predictors of response to carbon dioxide challenge. *Behaviour Research and Therapy* 1996;34(8):603-07.

270. Olatunji BO, Wolitzky-Taylor KB, Babson KA, et al. Anxiety sensitivity and CO2 challenge anxiety during recovery: differential correspondence of arousal and perceived control. *Journal of Anxiety Disorders* 2009;23(4):420-8.

271. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica* 1983;67(6):361-70.

272. Meyer TJ, Miller ML, Metzger RL, et al. Development and validation of the penn state worry questionnaire. *Behaviour research and therapy* 1990;28(6):487-95.

273. Buhr K, Dugas MJ. The Intolerance of Uncertainty Scale: psychometric properties of the English version. *Behav Res Ther* 2002;40(8):931-45.

274. Spitzer RL, Kroenke K, Williams JB, et al. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Archives of Internal Medicine* 2006;166(10):1092-97.

275. Peterson R, Reiss S. Anxiety sensitivity index manual (2nd ed.). *International Diagnostic Systems, Worthington, OH* 1992.

276. Scheier MF, Carver CS, Bridges MW. Distinguishing optimism from neuroticism (and trait anxiety, self-mastery, and self-esteem): a reevaluation of the Life Orientation Test. *Journal of Personality and Social Psychology* 1994;67(6):1063-78.

277. Rotter JB. Generalized expectancies for internal versus external control of reinforcement. *Psychological Monographs* 1966;80(1):1-28.

278. Clark D, Hemsley D. The effects of hyperventilation; individual variability and its relation to personality. *Journal of Behavior Therapy and Experimental Psychiatry* 1982;13(1):41-47.

279. Nutt DJ, Glue P, Lawson C, et al. Flumazenil provocation of panic attacks: evidence for altered benzodiazepine receptor sensitivity in panic disorder. *Archives of General Psychiatry* 1990;47(10):917-25.

280. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology* 1988;54(6):1063-70.

281. Borkovec TD, Nau SD. Credibility of analogue therapy rationales. *Journal of Behavior Therapy and Experimental Psychiatry* 1972;3(4):257-60.

282. Cohen J. *Statistical power analysis for the behavioral sciences.* 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.

283. Schober P, Boer C, Schwarte LA. Correlation coefficients: appropriate use and interpretation. *Anesthesia & Analgesia* 2018;126(5):1763-68.

284. Camerone EM, Wiech K, Benedetti F, et al. 'External timing'of placebo analgesia in an experimental model of sustained pain. *European Journal of Pain* 2021;25(6):1303-15.

285. Sedgwick P. Randomised controlled trials:"within subject" versus "between subject" designs. *BMJ* 2014;349.

286. Kahneman D, Fredrickson BL, Schreiber CA, et al. When more pain is preferred to less: Adding a better end. *Psychological Science* 1993;4(6):401-05.

287. Müller UW, Witteman CL, Spijker J, et al. All's bad that ends bad: there is a peak-end memory bias in anxiety. *Frontiers in Psychology* 2019;10:1272.

288. Pappens M, De Peuter S, Vansteenwegen D, et al. Psychophysiological responses to CO2 inhalation. *International Journal of Psychophysiology* 2012;84(1):45-50.

289. Savulich G, Hezemans FH, van Ghesel Grothe S, et al. Acute anxiety and autonomic arousal induced by CO2 inhalation impairs prefrontal executive functions in healthy humans. *Translational Psychiatry* 2019;9(1):1-10.

290. Owen AM, Roberts AC, Polkey CE, et al. Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. *Neuropsychologia* 1991;29(10):993-1006.

291. Benedetti F, Arduino C, Costa S, et al. Loss of expectation-related mechanisms in Alzheimer's disease makes analgesic therapies less effective. *Pain* 2006;121(1-2):133-44.

292. Krummenacher P, Candia V, Folkers G, et al. Prefrontal cortex modulates placebo analgesia. *PAIN®* 2010;148(3):368-74.

293. Büchel C, Geuter S, Sprenger C, et al. Placebo analgesia: a predictive coding perspective. *Neuron* 2014;81(6):1223-39.

294. Anchisi D, Zanon M. A Bayesian perspective on sensory and cognitive integration in pain perception and placebo analgesia. *PloS One* 2015;10(2):e0117270.

295. Grahl A, Onat S, Büchel C. The periaqueductal gray and Bayesian integration in placebo analgesia. *Elife* 2018;7:e32930.

296. Poma SZ, Milleri S, Squassante L, et al. Characterization of a 7% carbon dioxide (CO2) inhalation paradigm to evoke anxiety symptoms in healthy subjects. *Journal of Psychopharmacology* 2005;19(5):494-503.

297. Wechsler ME, Kelley JM, Boyd IO, et al. Active albuterol or placebo, sham acupuncture, or no intervention in asthma. *New England journal of medicine* 2011;365(2):119-26.

298. Zhang W, Guo J, Zhang J, et al. Neural mechanism of placebo effects and cognitive reappraisal in emotion regulation. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2013;40:364-73.

299. Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 1993;28(1-2):76-81.

300. Wallergård M, Jönsson P, Johansson G, et al. A Virtual Reality Version of the Trier Social Stress Test: A Pilot Study. *Presence* 2011;20(4):325-36.

301. Huneke NTM, Rowlatt H, Hyde J, et al. A novel procedure to investigate social anxiety using videoconferencing software: A proof-of-concept study. *Psychiatry Research* 2022;316:114770.

302. Nordahl HM, Nordahl H, Wells A. Metacognition and Perspective Taking Predict Negative Self-Evaluation of Social Performance in Patients with Social Anxiety Disorder. *Journal of Experimental Psychopathology* 2016;7(4):601-07.

303. Shalom JG, Israeli H, Markovitzky O, et al. Social anxiety and physiological arousal during computer mediated vs. face to face communication. *Computers in Human Behavior* 2015;44:202-08.

304. Huneke NT, Baldwin DS, Garner M. Protocol for Conducting the InterneT-based Stress test for Social Anxiety Disorder (ITSSAD), 26 August 2022, PROTOCOL (Version 1). *Protocol Exchange* 2022.

305. Microsoft. *Microsoft Teams (Version 1.4.00.11161), https://teams.microsoft.com* 2021.

306. Andrews J, Wadiwalla M, Juster RP, et al. Effects of manipulating the amount of social-evaluative threat on the cortisol stress response in young healthy men. *Behavioral Neuroscience* 2007;121(5):871-6.

307. Narvaez Linares NF, Charron V, Ouimet AJ, et al. A systematic review of the Trier Social Stress Test methodology: Issues in promoting study comparison and replicable research. *Neurobiology of Stress* 2020;13:100235.

308. Cassin SE, Rector NA. Mindfulness and the attenuation of post-event processing in social phobia: An experimental investigation. *Cognitive Behaviour Therapy* 2011;40(4):267-78.

309. Shikatani B, Antony MM, Kuo JR, et al. The impact of cognitive restructuring and mindfulness strategies on postevent processing and affect in social anxiety disorder. *Journal of Anxiety Disorders* 2014;28(6):570-79.

310. Connor KM, Davidson JRT, Churchill LE, et al. Psychometric properties of the Social Phobia Inventory (SPIN) - New self-rating scale. *British Journal of Psychiatry* 2000;176:379-86.

311. Gaab J, Kossowsky J, Ehlert U, et al. Effects and Components of Placebos with a Psychological Treatment Rationale - Three Randomized-Controlled Studies. *Scientific Reports* 2019;9(1):1421.

312. Qualtrics. *Qualtrics XM Platform, https://www.qualtrics.com* 2021.

313. Mattick RP, Clarke JC. Development and validation of measures of social phobia scrutiny fear and social interaction anxiety. *Behav Res Ther* 1998;36(4):455-70.

314. Leary MR. A brief version of the Fear of Negative Evaluation Scale. *Personality and Social Psychology Bulletin* 1983;9(3):371-75.

315. Kroenke K, Spitzer RL, Williams JBW, et al. Anxiety Disorders in Primary Care: Prevalence, Impairment, Comorbidity, and Detection. *Annals of Internal Medicine* 2007;146(5):317.

316. Rapee RM, Lim L. Discrepancy between self-and observer ratings of performance in social phobics. *Journal of Abnormal Psychology* 1992;101(4):728.

317. Lüdecke D, Ben-Shachar MS, Patil I, et al. performance: An R package for assessment, comparison and testing of statistical models. *Journal of Open Source Software* 2021;6(60).

318. Singmann H, Bolker B, Westfall J, et al. afex: Analysis of factorial experiments. *R package version 1.0-1* 2021.

319. emmeans: Estimated Marginal Means, aka Least-Squares Means [program]. R package version 1.8.0 version, 2022.

320. R: A Language and Environment for Statistical Computing [program]. Vienna, Austria: R Foundation for Statistical Computing, 2022.

321. Silber H, Johnson TP. Culture and Response Behavior: An Overview of Cultural Mechanisms Explaining Survey Error: Springer International Publishing; 2020 p67-86.

322. Van Dulmen A, Bensing J. Health promoting effects of the physician-patient encounter. *Psychology, Health & Medicine* 2002;7(3):289-300.

323. Kaptchuk TJ, Kelley JM, Conboy LA, et al. Components of placebo effect: randomised controlled trial in patients with irritable bowel syndrome. *BMJ* 2008;336(7651):999-1003.

324. Horing B, Beadle SC, Inks Z, et al. A virtual experimenter does not increase placebo hypoalgesia when delivering an interactive expectancy manipulation. *Scientific Reports* 2020;10(1):20353.

325. Meissner K, Linde K. Are blue pills better than green? How treatment features modulate placebo effects. *International Review of Neurobiology* 2018;139:357-78.

326. Blackwell B, Bloomfield S, Buncher CR. Demonstration to medical students of placebo responses and non-drug factors. *The Lancet* 1972;299(7763):1279-82.

327. El Brihi J, Horne R, Faasse K. Prescribing placebos: an experimental examination of the role of dose, expectancies, and adherence in open-label placebo effects. *Annals of Behavioral Medicine* 2019;53(1):16-28.

328. Martin DJ, Garske JP, Davis MK. Relation of the therapeutic alliance with outcome and other variables: a meta-analytic review. *Journal of Consulting and Clinical Psychology* 2000;68(3):438.

329. Iacoviello BM, McCarthy KS, Barrett MS, et al. Treatment preferences affect the therapeutic alliance: implications for randomized controlled trials. *Journal of Consulting and Clinical Psychology* 2007;75(1):194.

330. Vîslă A, Constantino MJ, Newkirk K, et al. The relation between outcome expectation, therapeutic alliance, and outcome among depressed patients in group cognitive-behavioral therapy. *Psychotherapy Research* 2018;28(3):446-56.

331. Marziali E, Marmar C, Krupnick J. Therapeutic alliance scales: development and relationship to psychotherapy outcome. *The American Journal of Psychiatry* 1981.

332. Zilcha-Mano S, Roose SP, Barber JP, et al. Therapeutic alliance in antidepressant treatment: cause or effect of symptomatic levels? *Psychotherapy and Psychosomatics* 2015;84(3):177-82.

333. Alonzo A, Fong J, Ball N, et al. Pilot trial of home-administered transcranial direct current stimulation for the treatment of depression. *Journal of Affective Disorders* 2019;252:475-83.

334. Colloca L, Benedetti F. Nocebo hyperalgesia: how anxiety is turned into pain. *Current Opinion in Anesthesiology* 2007;20(5):435-39.

335. Colagiuri B, Quinn VF. Autonomic arousal as a mechanism of the persistence of nocebo hyperalgesia. *The Journal of Pain* 2018;19(5):476-86.

336. Roderigo T, Benson S, Schols M, et al. Effects of acute psychological stress on placebo and nocebo responses in a clinically relevant model of visceroception. *Pain* 2017;158(8):1489-98.

337. Kelly MM, Tyrka AR, Anderson GM, et al. Sex differences in emotional and physiological responses to the Trier Social Stress Test. *Journal of Behavior Therapy and Experimental Psychiatry* 2008;39(1):87-98.

338. Rausch SM, Auerbach SM, Gramling SE. Gender and Ethnic Differences in Stress Reduction, Reactivity, and Recovery. *Sex Roles* 2008;59(9-10):726-37.

339. Eagle DE, Rash JA, Tice L, et al. Evaluation of a remote, internet-delivered version of the Trier Social Stress Test. *International Journal of Psychophysiology* 2021;165:137-44.

340. Harvie H, Jain B, Nelson B, et al. Induction of acute stress through an internet-delivered Trier Social Stress Test as assessed by photoplethysmography on a smartphone. 2021.

341. Wong QJ, McEvoy PM, Rapee RM. The structure of social-evaluative threat detection in social anxiety disorder. *Journal of Anxiety Disorders* 2020;74:102273.

342. Wong QJ, Rapee RM. The aetiology and maintenance of social anxiety disorder: A synthesis of complementary theoretical models and formulation of a new integrated model. *Journal of Affective Disorders* 2016;203:84-100.

343. Haas JW, Ongaro G, Jacobson E, et al. Patients' experiences treated with open-label placebo versus double-blind placebo: a mixed methods qualitative study. *BMC psychology* 2022;10(1):1-12.

344. Cremers HR, Wager TD, Yarkoni T. The relation between statistical power and inference in fMRI. *PloS One* 2017;12(11):e0184923.

345. Kenwood MM, Kalin NH, Barbas H. The prefrontal cortex, pathological anxiety, and anxiety disorders. *Neuropsychopharmacology* 2022;47(1):260-75.

346. Kreibig SD. Autonomic nervous system activity in emotion: A review. *Biological Psychology* 2010;84(3):394-421.

347. Montero-López E, Santos-Ruiz A, García-Ríos MC, et al. A virtual reality approach to the Trier Social Stress Test: Contrasting two distinct protocols. *Behavior Research Methods* 2016;48(1):223-32.

348. Frick A, Engman J, Alaie I, et al. Neuroimaging, genetic, clinical, and demographic predictors of treatment response in patients with social anxiety disorder. *Journal of Affective Disorders* 2020;261:230-37.

349. Storch EA, Tendler A, Schneider SC, et al. Moderators and predictors of response to deep transcranial magnetic stimulation for obsessive-compulsive disorder. *Journal of Psychiatric Research* 2021;136:508-14.

350. Wathra RA, Mulsant BH, Reynolds III CF, et al. Differential Placebo Responses for Pharmacotherapy and Neurostimulation in Late-Life Depression. *Neuromodulation: Technology at the Neural Interface* 2022.

351. Watanabe Y, Nishimura A, Kikuchi T, et al. Central monitoring of depression and anxiety symptoms reduces placebo responses in depression clinical trials: A post hoc exploratory analysis of data from the phase III CCT-004 trial of vortioxetine. *Neuropsychopharmacology Reports* 2022.

352. Cheng HG, Phillips MR. Secondary analysis of existing data: opportunities and implementation. *Shanghai archives of psychiatry* 2014;26(6):371.

353. Jamshidian F, Hubbard AE, Jewell NP. Accounting for perception, placebo and unmasking effects in estimating treatment effects in randomised clinical trials. *Statistical Methods in Medical Research* 2014;23(3):293-307.

354. Zilcha-Mano S, Brown PJ, Roose SP, et al. Optimizing patient expectancy in the pharmacologic treatment of major depressive disorder. *Psychological Medicine* 2019;49(14):2414-20.

355. Kessels R, Mozer R, Bloemers J. Methods for assessing and controlling placebo effects. *Statistical Methods in Medical Research* 2019;28(4):1141-56.

356. Tarpey T, Petkova E. Modelling placebo response via infinite mixtures. *JP Journal of Biostatistics* 2010;4(2):161.

357. Petkova E, Tarpey T, Govindarajulu U. Predicting potential placebo effect in drug treated subjects. *The International Journal of Biostatistics* 2009;5(1).

358. Graham B, Garcia NM, Burton MS, et al. High expectancy and early response produce optimal effects in sertraline treatment for post-traumatic stress disorder. *The British Journal of Psychiatry* 2018;213(6):704-08.

359. Del Piccolo L, Finset A. Patients' autonomic activation during clinical interaction: A review of empirical studies. *Patient Education and Counseling* 2018;101(2):195-208.

360. Kelley JM, Lembo AJ, Ablon JS, et al. Patient and practitioner influences on the placebo effect in irritable bowel syndrome. *Psychosomatic Medicine* 2009;71(7):789.