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Understanding the experience of Anxiety in young people with Autism Spectrum Disorder (ASD)

by

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ABSTRACT

FACULTY OF SOCIAL, HUMAN AND MATHEMATICAL SCIENCES

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Thesis for the degree of Doctorate in Clinical Psychology

UNDERSTANDING THE EXPERIENCE OF ANXIETY IN YOUNG PEOPLE WITH AUTISM SPECTRUM DISORDER (ASD)

Emma Victoria Lee

A systematic review and meta-analysis was conducted to explore the Cortisol Awakening Response (CAR) in children with Autism Spectrum Disorder (ASD), in comparison to their typically developing (TD) peers. The CAR, characterised by an increase in cortisol in the 30-60 minutes after waking, is widely becoming used as a physiological marker of stress in the general population. Given the prevalence of anxiety and stress reported in people with ASD, this review aimed to explore whether children with ASD show the expected pattern of CAR seen in the general population, and therefore, whether this would be a useful way of measuring stress in this population. Results from the meta-analysis indicated no significant differences in CAR demonstrated by children with or without ASD. However, the review highlighted limitations in the current research; a lack of adherence to recommended CAR sampling protocol, few studies with a matched control group, and poor control of potentially confounding variables. The review highlighted wide variability in results shown by ASD groups across studies, indicating the need for further exploration of the CAR in this population to be confident of its use as a reliable marker of physiological stress in this group.

The subsequent empirical paper extended the current literature regarding the CAR in children with ASD, and contributes to the understanding of the experience of anxiety in this population. Using a mixed methods design incorporating questionnaires, interviews, physiological data (cortisol) and experience sampling data, the study explored the experience of anxiety and CAR compared to a matched control group. Results indicate a significantly smaller CAR in ASD group compared to TD, but overall comparable daily levels of cortisol (AUC). Results also show discrepancies between informants for both groups. ASD adolescents self-reported significantly fewer symptoms of anxiety than their TD peers, whilst parents and teachers of ASD adolescents reported significantly more symptoms in this group compared with the TD group. Self-reported day-to-day anxiety was also lower in the ASD group. TD adolescents self-reported higher attentional control than ASD peers. Experience sampling data indicated no significant association between cortisol levels and rated emotions at each time point. For both groups, experience sampling data indicated higher cortisol levels were associated with lower noise, unstructured activity and large group sizes. The study highlights the need for further, controlled exploration of the CAR in ASD to understand the differences in patterns shown, and further exploration of appropriate ways of assessing anxiety in this population.

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DECLARATION OF AUTHORSHIP

I, Emma Lee declare that this thesis, entitled **'Understanding the experience of Anxiety in Young People with ASD'** 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:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. 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;

3. Where I have consulted the published work of others, this is always clearly attributed;

4. 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;

5. I have acknowledged all main sources of help;

6. 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;

7. None of this work has been published before submission

Signed: ……

Date: …19.05.17

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Definitions and Abbreviations

ASD	Autism Spectrum Disorders
TD	Typically developing
GAD	Generalised Anxiety Disorder
OCD	Obsessive Compulsive Disorder
PTSD	Post Traumatic Stress Disorder
SAD	Social Anxiety Disorder
IU	Intolerance of Uncertainty
SCAS	Spence Children's Anxiety Scale
SDQ	Strengths and Difficulties Questionnaire
SAS-TR	School Anxiety Scale, Teacher Report
AQ	Autism Quotient Questionnaire
DISC-IV	Diagnostic Interview Schedule for Children, Fourth Edition
DSM 5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
WASI-II	Wechsler Abbreviated Scale of Intelligence, Second Edition
ACS	Attentional Control Scale
HPA-axis	Hypothalamic–Pituitary–Adrenal axis
CAR	Cortisol Awakening Response
AUC	Area under the curve
DR	Diurnal Rhythm
EHCP	Education Health Care Plan
30+Wake	30 minutes after wakening

Chapter 1: An exploration of the Cortisol Awakening Response (CAR) in children and adolescents with Autism Spectrum Disorder; A systematic review and meta-analysis

1.1 Introduction

1.1.1 Autism Spectrum Disorder (ASD)

Autism Spectrum Disorder (ASD) is characterised by persistent impairment in social communication and social interaction (American Psychiatric Association (APA), 2013). The Diagnostic and Statistical Manual (DSM) 5 criteria requires deficits in each of the following areas of social communication; non-verbal communication, social-emotional reciprocity and establishing and maintaining relationships (APA, 2013). Alongside deficits in social communication and interaction, there must be evident impairment in the repertoire of a persons' behaviours, activities or interests (APA, 2013). This must include two of the following; having a restricted range of behaviours, engaging in highly repetitive behaviours/speech, having intense/fixated interests or rigid adherence to routines (APA, 2013).

For ASD diagnosis, these difficulties should cause significant impairment to at least one area of social/occupational functioning, and be present in the person's early developmental stages of life (APA, 2013). DSM 5 criteria acknowledges that symptoms may appear hidden or masked until after the early developmental period, as some people can learn strategies to hide their difficulties. These strategies may become problematic as the child gets older and where they no longer meet the more complex social and communication demands of the environment (APA, 2013).

ASD diagnosis is categorised by level of severity in DSM 5, using levels 1-3, where higher scores indicate more severe symptoms of ASD and more impairment resulting from these symptoms. Level 3 severity would indicate very severe deficits in social communication and severe difficulties in changing behaviour/extremely restricted behaviours (APA, 2013). Individuals diagnosed with ASD who are considered to have level 3 severity may be non-verbal or have very restricted language or interaction skills. Quantifying level of severity of ASD has been proposed as a method of indicating how much support an individual requires in their daily life (APA, 2013).

The diagnosis of ASD in DSM 5 has changed significantly from DSM-IV-TR (APA, 2000), which included multiple types of Autism Spectrum Disorders, including Asperger's Syndrome and Pervasive Developmental Disorder, not otherwise specified (PDD-NOS). The removal of different types of ASD and the addition of categorisation of severity reflects current conceptualisations of ASD as impairment in two mains areas, but where these sit along a dimensional trait categorisation, to capture variation in severity in each domain. This variability is reflected in the heterogeneity of ASD presentation across social communication, interaction and repetitive/restricted behaviour domains. The requirement for symptoms to be present in the early developmental period reflects current conceptualisations of ASD as a lifelong, neurodevelopmental condition, with a mixed aetiology, combining environmental, genetic and neurological risk factors (Chaste & Leboyer, 2012).

It is estimated that around 1% of the UK population meet the criteria for ASD (Baird et al., 2006), with prevalence rates for the ratio of male to females varying from 3.3:1 to 5:1 (Baird et al., 2006; Fombonne, Quirke, & Hagen, 2011). In addition to deficits in social communication and language, studies have shown that people with ASD experience sensory difficulties more frequently than those without ASD (Leekam, Nieto, Libby, Wing, & Gould, 2007); either showing oversensitivity or under-sensitivity to sensory stimuli (Ben-Sasson et al., 2009). It has been widely acknowledged that people with ASD also struggle to adapt or cope with change or novel situations, showing a strong preference for 'sameness' (Kanner, 1943; World Health Organisation, 2006). Furthermore, research has found that up to 70% of young people with ASD show symptoms of at least one co-morbid psychiatric disorder, such as social anxiety disorder (SAD) or attention deficit hyperactivity disorder (ADHD) (Simonoff et al., 2006; White, Oswald, Ollendick, & Scahill, 2009).

1.1.2 Anxiety Disorders

Anxiety disorders are amongst the most common mental health disorders in young people in the UK, with an estimated 3.3% of children aged 5-16 years reported to have at least one type of anxiety disorder (Green, McGinnity, & Meltzer, 2005). In their large cross sectional epidemiological study, Green et al., (2005) reported a higher incidence of anxiety disorders in females (3.8%) compared to males (2.9%), and in adolescents (4.4%) versus children aged 5-10 years (2.2%). Anxiety disorders have been linked with poorer academic performance (Essau, Conradt, & Petermann, 2000), early school withdrawal (Van Ameringen, Mancini, & Farvolden, 2003), and poorer social functioning (La Greca & Lopez, 1998). In addition, sleep related problems were found in 88% of adolescents in a large randomised control trial, which also highlighted positive associations between anxiety severity and number of sleep-related difficulties (Alfano, Ginsburg, & Kingery, 2007).

DSM 5 outlines several anxiety disorders that reflect specific and circumscribed fears about objects and situations that are avoided in an attempt to reduce anxiety, including separation anxiety disorder (SpAD), social anxiety disorder (SAD), generalised anxiety disorder (GAD), panic disorder and agoraphobia (APA, 2013). SAD is characterised by a persistent fear of social situations, where there is a possibility of evaluation by others (APA, 2013) and in children, can present as excessive clinginess or prolonged crying in social situations. This diagnosis is commonly associated with withdrawal and social avoidance, along with negative self-evaluation about one's performance (Wells, 1997). In contrast, specific phobias are fears of a particular object, place or situation that reflects a marked and disproportionate fearful response. The person usually recognises this anxiety response is out of proportion with the amount of risk presented by that specific object/place/situation (APA, 2013). Specific phobia also incorporates persistent avoidance of the object of fear, and avoidance of situations in which it might be encountered (APA, 2013).

SpAD is the earliest emerging disorder and it reflects fears and concerns about being separated from or worries that something bad will happen to the primary caregiver, where these feelings are described as developmentally inappropriate (APA, 2013, p. 190). Children who are diagnosed with SpAD are at increased risk of developing panic disorder (PD; see review by Kossowsky et al., 2013). PD is characterised by panic attacks and the fear of future panic attacks, which are defined as an intense and sudden sense of fear and dread, accompanied by physiological symptoms of anxiety such as heart palpitations, dizziness, shaking and nausea (APA, 2013). Agoraphobia, commonly associated with panic disorder, incorporates a specific fear of going places where the person has experienced, or believes they will experience a panic attack, and therefore these places are avoided (APA, 2013).

Common across these anxiety disorders, reflecting fears and avoidance of specific objects or events are physiological symptoms of anxiety, behavioural change in relation to feared objects, places or situations, and cognitive symptoms of excessive worry regarding the specific feared object or event. In contrast, GAD involves a broad, extensive pattern of worry across all situations and a preoccupation with anticipated and everyday events (APA, 2013). GAD represents a more chronic and persistent pattern of worry, independent of the situational or environmental context, which the person has difficulty controlling (APA, 2013).

Whilst medical professionals outline the differences in anxiety disorders and their symptoms, anxiety literature also highlights key differences in the way anxiety can be conceptualised. Most notably, Speilberger (1996) first introduced the concept of differences between 'state' and 'trait' anxiety. Trait anxiety refers to an individual's characteristic predisposition to react to situations in a similar way, consistent across lifespan. This influences their likelihood of responding with state anxiety when confronted with situations perceived to be threatening. State anxiety, therefore, captures the transitory emotional and physiological response

an individual displays when anticipating or faced with a threatening situation. Both these sub types of anxiety (state and trait) are thought to be multi-faceted (Endler & Kocovski, 2001); trait anxiety includes social evaluation, physical danger, ambiguity, daily routines, and state anxiety incorporates a cognitive appraisal of the situation, with an autonomic physiological response. Given this broad conceptualisation of anxiety, capturing both state and trait components, it has most conceptual links to GAD.

Theoretical frameworks in anxiety highlight a complex set of factors linked to its development and that reflect within individual (e.g., temperament, genetic and cognitive risk) and external risk factors (e.g., parenting and peer relationships). For example, Murray, Creswell, & Cooper (2009) presented an integrative review of factors influencing the development of anxiety in childhood, concluding a complex interaction between parental anxiety, environmental and genetic factors. Figure 1 shows how genetic risk and family aggregation, particularly parental anxiety can influence child vulnerability to anxiety. In support, in a recent large community based sample, the presence of an anxiety disorder in adolescence was predicted by presence of anxiety disorder in the parent when the child was preschool age (Ranøyen, Stenseng, Klöckner, Wallander, & Jozefiak, 2015). This association was mediated by current parental symptoms of anxiety, which suggests that parental anxiety and family aggregation of symptoms is a persistent risk throughout childhood for the development of childhood anxiety in offspring.

Behavioural inhibition refers to a pattern of responding to situations in a fearful or restrained way when faced with unfamiliar situations. This temperamental pattern has been associated with higher parental anxiety, and increased vulnerability to childhood anxiety. Higher parental anxiety may also predispose children to biased information processing of threat information, although the exact pathway for this vulnerability is unclear (Hadwin, Garner, & Perez-Olivas, 2006). Figure 1 highlights the bidirectional association between parenting and the child's behaviours. It suggests that a child's temperament may influence a parent's behaviour towards them, especially in anxious parents, and this in turn may lead to unhelpful parenting behaviours, such as overprotection, maintaining the child's anxiety (Murray et al., 2009). Overprotective or over-controlled parenting, as a manifestation of the parent's anxiety, may restrict a child's wider experiences or sense of control/autonomy. This in turn may influence their own cognitive biases about their ability to cope with unfamiliar situations, or lead to avoidance of novel situations, thereby increasing the risk of anxiety (Murray et al., 2009).

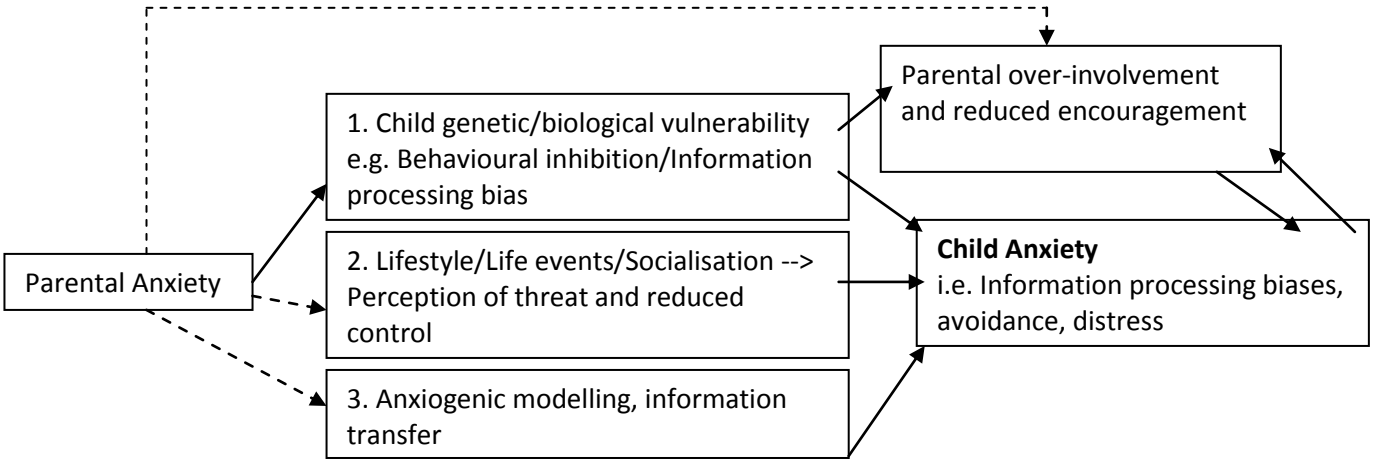

Figure 1. Pathways to child anxiety. ⟶ Parental anxiety accounts for the factor; - - -▸ Parental anxiety raises the risk of the factor (Murray et al., 2009)

The third component of Murray et al.'s (2009) anxiety framework highlights the pathway of modelling on a child's predisposition to anxiety. Avoidance or overestimation of threat by parents, as a function of their own anxiety, may be modelled by their offspring. This pathway echoes the observational learning pathway, proposed by Rachman (1977) as of one his three pathways to acquisition of fear. This framework suggested fears are acquired by direct conditioning experiences (exposure to negative life events), vicarious learning (learning through observation and modelling) and verbal threat information. These frameworks suggest a pivotal role of parental anxiety in the potential development and maintenance of childhood anxiety.

1.1.3 Anxiety in children with ASD

Young people with ASD experience higher levels of anxiety than their typically developing peers (Boulter, Freeston, South, & Rodgers, 2014; Kerns & Kendall, 2012). Several studies have highlighted that up to half of individuals diagnosed with ASD also meet the diagnostic criteria for an anxiety disorder (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Simonoff et al., 2008). For example, in a large systematic review of the literature concerning anxiety disorders as co-morbidities in ASD, Steensel, Bögels, and Perrin, (2011) found that across studies 39.6% of children with ASD met the DSM-IV-TR (APA, 2000) criteria for an anxiety disorder.

Diagnosing anxiety disorders in children with ASD has proven difficult, due to a range of factors, one of which includes difficulties establishing which symptoms are core features of ASD, and which are standalone symptoms of anxiety (MacNeil, Lopes, & Minnes, 2009). Diagnostic

overshadowing, whereby symptoms are attributed to the developmental disorder, ASD, may reduce recognition of anxiety symptoms (MacNeil et al., 2009). Grondhuis and Aman (2012) highlighted difficulties in identification as symptoms of both disorders such as avoidance and irritability overlap, further confounding difficulties in accurate categorisation of symptoms.

There are further difficulties diagnosing anxiety in ASD, due to the nature of ASD itself. For example, language difficulties are common in ASD (APA, 2013) and may influence ability to accurately describe subjective feelings of anxiety, highlighting the importance of finding alternative ways of capturing anxiety in this population. Furthermore, in recent reviews of the assessment of anxiety in ASD, difficulties in accurate measurement of anxiety have been highlighted, due to lack of validated measures specific to ASD and varying levels of verbal ability linked with symptom expression (Grondhuis & Aman, 2012; MacNeil, Lopes, & Minnes, 2009).

These difficulties are further exacerbated by generic difficulties in measuring psychopathology in any population, including lack of consistency across informants (see De Los Reyes & Kazdin, 2005 for review) and relying on informants to accurately identify, interpret, and self-report on their own emotional responses. Researchers found that in a sample of 665 children and adolescents, increased ability to differentiate emotions and speak about them was associated with fewer internalising difficulties such as anxiety, depression and rumination (Rieffe, Oosterveld, Miers, Terwogt, & Ly, 2008). Research has also suggested this self-reflection process, of identifying one's own emotions and differentiating between them, may be especially difficult for people with ASD (Hill, Berthoz, & Frith, 2004). This is further supported by Rieffe and colleagues who concluded that children with high functioning autism show "more fragmented understanding of emotions and their own emotion experience" (Rieffe et al., 2011, pp. 667).

It is crucial to consider difficulties identifying anxiety in ASD due to its impact on daily life. Research comparing anxious children, with or without an ASD diagnosis, suggests that higher number of anxiety symptoms and higher ASD symptoms were associated with lower quality of life scores, when rated on parent outcome measures (Steensel, Bögels, & Dirksen, 2012). Wood & Gadow (2010) highlighted the bi-directional relationship between anxiety severity and ASD symptom severity, such that when anxiety symptoms reduced, so did parent report of ASD symptoms. This finding suggests that anxiety is likely to compound and negatively impact daily life as it is linked to more severe ASD symptoms and therefore is likely to increase impairment in everyday activities associated with social interactions, and ability to cope with change.

These difficulties in recognising and diagnosing anxiety disorders in ASD make it problematic to identify individuals who would benefit from targeted interventions. This is increasingly important given that a recent meta-analysis exploring Cognitive Behaviour Therapy (CBT) suggests it to be effective in anxiety symptom reduction on clinician or parent rated

outcome measures in an ASD population and when delivered across different contexts (Kreslins, Robertson, & Melville, 2015; Luxford, Hadwin & Kovshoff, 2016).

Research has increasingly focused on measuring objective physiological measures of anxiety and stress in people with ASD. This increase is in part due to an interest in capturing the recognised physiological response to stress or threat in anxiety disorders in the context of actual or perceived threat (Rapee & Heimburg, 1997). This measurement is of particular interest in research involving people with ASD, as it would provide an objective marker of stress/anxiety independent of the person's ability to recognise their own emotional response in situations and that would allow consideration of the validation of anxiety reports across different sources. Interest in cortisol as an objective measure of stress/anxiety in children with ASD has also been growing due to additional difficulties in measuring symptoms of anxiety individuals with ASD in the context of language abilities and discrepancies between informants (APA, 2013; De Los Reyes & Kazdin, 2005).

1.1.4 Cortisol Awakening Response (CAR)

The hypothalamic-pituitary-adrenal (HPA) axis is the core physiological response system involved in the body's response to stress. Cortisol is the stress hormone that can be measured, to identify the pattern of individuals' HPA axis activity over the course of the day, whereby greater HPA axis activity is indicative of elevated stress. This activity pattern (evident in children over the age of 1 year) follows a diurnal cycle, with higher cortisol levels in the morning, falling throughout the day to be lowest in the evening (Gröschl, Rauh, & Dörr, 2003). There is now a wealth of research which suggests individuals show a specific peak in cortisol levels in the hour after awakening, known as the Cortisol Awakening Response (CAR) (for review see Fries, Dettenborn, & Kirschbaum, 2009). It is widely accepted that around 75% of the healthy population display this initial peak in cortisol (Wust et al., 2000b), which is defined as a rise of 2.49nmol/l or more of cortisol in adults, in the 30-60 minutes after wakening (Wust et al., 2000b). It is important to note however, that in children, the criteria to display a CAR is simply to show any increase in cortisol levels in the 30-60 minutes after waking (Rosmalen et al., 2005), as a level of more than 2.49nmol/l is not often reached.

The Cortisol Awakening Response (CAR) is thought to be separate from the normal circadian rhythm of cortisol rising and falling, and was first described by Pruessner et al. (1997), who suggested this as a "reliable biological marker of adrenocortical activity" (p. 2546). Subsequent research has identified the CAR as an accurate measure of HPA-axis reactivity and it shows a robust response over time (Schmidt-Reinwald et al., 1999). Intra-individual responses in CAR have also been shown to be stable, demonstrating the pattern of an individual's CAR does not vary significantly when measured across a number of days (Hucklebridge, Hussain, Evans, & Clow, 2005).

Further research has explored how social, biological and environmental factors moderate the CAR. Researchers have hypothesised that this specific peak in cortisol, 30-60 minutes following awakening, could link to an individual's anticipation of daily stress (Chida & Steptoe, 2009; Fries et al., 2009). This would hypothetically be linked to an increase in CAR in populations experiencing or anticipating more stress. There are further suggestions that the CAR may be a preparatory mechanism, to help individuals manage their anticipated daily stress (Powell & Schlotz, 2012), by increasing the cortisol levels and therefore the readiness to cope with the day's events. In contrast, it is also hypothesised that a blunting of the CAR may reflect the HPA-axis response to overstimulation, or hypercortisolism, causing it to be less reactive to daily stress and therefore showing a smaller CAR (Chida & Steptoe, 2009; Fries et al., 2009). It has therefore been argued that the influence of specific factors including psychosocial and physiological problems may depend on the chronicity and severity of the symptoms.

Since establishing the CAR, research has focused on identifying environmental, biological and psychosocial factors associated with an increased or flattening of the morning peak. In their review of the CAR, Fries et al., (2009) concluded that in healthy populations, age, menstrual cycle and sleep duration do not affect the size of an individual's CAR, although earlier research suggested small effect sizes for the influence of gender on the CAR, with females showing a slightly increased CAR (Pruessner et al, 1997).

In a large meta-analysis reviewing the psychosocial factors influencing the magnitude of the CAR, Chida and Steptoe (2009) found that an increase in CAR was positively associated with work-related and general stress. As the HPA-axis is closely linked to stress sensitivity, research has focused on stress, or perceived stress factors and their influence on the CAR, with increasing evidence for an increased CAR for those reporting higher levels of chronic worry (Schlotz, Hellhammer, Schulz, & Stone, 2004). Similarly, Wust et al., (2000a) concluded that the CAR is increased in community based populations with chronic stress and higher worry, as measured by self-report on the Trier Inventory for the Assessment of Chronic Stress (TICS) (Schulz & Schlotz, 1999).

Research has also begun exploring the CAR's variability and reactivity in response to daily stressors, investigating whether anticipation of a stressful event or exposure to a single stressor can influence an individual's pattern of CAR on the day or the following day. One study, looking at the effects of perceived stressful events on the CAR, indicated that in workers reporting higher chronic workload and stress, their CAR peak was significantly increased on work days compared with weekends, demonstrating the CAR can be affected by anticipation of upcoming events (Schlotz et al., 2004). Levels of cortisol have also been found to be higher in an adult population (n = 2981) with anxiety disorder, and these individuals showed a higher peak in the CAR than individuals without an anxiety disorder (Vreeburg et al., 2010). This finding is similar to the relative increase

in CAR seen in other stress related psycho-social factors, such as general stress (Chida & Steptoe, 2009).

These findings provide support for the hypothesis that the CAR is associated with an individuals' anticipated stress, and would therefore explain why this peak may be higher in clinical populations of anxious individuals, whose negative bias in thinking may lead them to anticipate and experience higher stress than healthy controls in the day. Powell and Schlotz, (2012) demonstrated that increased CARs were associated with attenuated responses to daily life stressors, meaning participants showed less distress to stressful events on the same day, if they showed an increase in CAR in the morning. This further provides support for the hypothesis that the CAR is a preparatory mechanism for anticipating the daily stresses.

However, Fries et al., (2009) concluded that certain physical and psychiatric conditions such as cardiovascular problems and chronic pain have been associated with a flattened CAR. Similarly, Chida and Steptoe (2009) concluded that fatigue, exhaustion and burnout were associated with a reduced CAR. This is consistent with the finding that Post Traumatic Stress Disorder (PTSD) is associated with a flattening of the CAR peak (Wessa, Rohleder, Kirschbaum, & Flor, 2006), which further suggests that severe and chronic stress may cause the HPA-axis response to become blunted after repeated exposure to high levels of cortisol. In addition to literature focusing on PTSD, research regarding anxiety suggested that trait anxiety was negatively associated with CAR peak, and this influence of trait anxiety on the CAR was mediated by anticipatory anxiety, (their levels of worry regarding an upcoming laboratory test they were informed would be unpleasant) (Walker, O'Connor, Schaefer, Talbot, & Hendrickx, 2011). Consistently, when exploring variability in CAR responses to daily situations, Gartland, O'Connor, Lawton, and Bristow, (2014) found that when daily hassles were appraised as more stressful, this had a subsequent negative effect on the increase of the CAR the next day, meaning participants who perceived their hassles as more stressful showed a decrease in the size of their CAR the next day.

These findings, demonstrating a flattening of the CAR, especially when associated with chronic and persistent stress, such as in PTSD and trait anxiety, would provide support for the hypothesis that the CAR is reactive to daily stresses, but can become blunted in response to overstimulation. This would help explain why there is inconsistency in the research as to the influence of symptoms such as anxiety, stress and other lifestyle factors on the size of the CAR, and it suggests that the effect on the CAR of these factors is associated with the severity and chronicity of these symptoms. Given the links to psychosocial factors including stress with an increased CAR, these findings indicate that the CAR may be a useful way of measuring an individual's reactivity to stress, via their HPA-axis activity, as an objective measure of stress, as the research suggests that the CAR will be higher in those with anxiety but lower in those with persistent/chronic stress.

1.1.5 Exploring the CAR in typical development

Traditional methods of measuring childhood stress and anxiety rely on self-report or parent/ teacher report of behavioural and cognitive symptoms of anxiety, including questionnaire measures such as the Revised Children's Anxiety and Depression Scale (Chorpita, Yim, Moffitt, Umemoto, & Francis, 2000) and the Spence Children's Anxiety Scale (Spence, 1998). Whilst these measures have been shown to have good psychometric properties, (Chorpita, Moffitt, & Gray, 2005; Essau, Sasagawa, Anastassiou-Hadjicharalambous, Guzmán, & Ollendick, 2011; Spence, Barrett, & Turner, 2003), they rely on the participant's accurate self-reported or parental recognition of symptoms associated with elevated anxious affect. In addition, several studies have found that the correspondence between different reports is low (review by De Los Reyes & Kazdin, 2005). The measurement of anxiety via cortisol levels provides an opportunity to more reliably capture stress and anxiety in children. In addition, this measure provides an index of individual differences in anxiety that is independent of verbalisation abilities in children and adolescents themselves, or parent and teacher ability to recognise symptoms.

Research has explored the relationship between anxiety in young people, and their CAR, to establish whether this may be a useful index of stress in this population. Research has also demonstrated an inverse relationship between the increase in the CAR and mental wellbeing, indicating that positive functioning was linked to a reduced CAR in adolescents, (Rickard, Chin, & Vella-Brodrick, 2015). In addition, given the extensive co-morbidity of anxiety disorders in children with ASD, further research has started to investigate the CAR in this population, (for review see Taylor & Corbett, 2014).

There have typically been two research designs associated with the collection of salivary cortisol in children; those that focus on collection of samples before and after a specific stressor, and those that take multiple samples throughout the day, to chart the child's circadian rhythm of cortisol. Studies investigating the CAR tend to be of the latter design, taking multiple saliva samples throughout the day, including at least one sample in the first hour of wakening, to establish the child's individual pattern of cortisol levels and to compare this with others in the population. This approach is argued to reflect measurement of a child's stable anxiety levels (akin to trait anxiety). The research showing robust, stable intra-individual patterns of CAR across a period of time (Hucklebridge et al., 2005) would indicate that the influence of stress and worry on the CAR may reflect a conceptualisation of trait anxiety as being the mechanism influencing the magnitude of the CAR. Trait anxiety, most widely conceptualised as closely associated with GAD (APA, 2013) reflects an individual's characteristic pattern of responding to multiple situations with a state anxiety response. Therefore, given the robust pattern of the CAR, changes in the CAR due to psychosocial factors such as stress and worry would hypothetically be assumed to be representative of trait anxiety. In contrast, methodology utilising exposure to stressors with pre-

and post-samples of cortisol to measure for reactivity in cortisol, focuses instead on a child's state anxiety reactions. This type of design may likely capture a child's anxiety responses associated with specific anxiety disorders, such as SAD, SpAD or Specific phobia (APA, 2013).

As cortisol is the stress hormone used to measure HPA-axis reactivity, one would expect that moment-to-moment experiences of state anxiety (when a person is in an actual or perceived threatening situation) would lead to increased levels of cortisol at that specific time. The literature also then suggests that repeated exposure to stress and anxiety then influences the overall pattern of an individual's cortisol responses; the CAR and DR (Chida & Steptoe, 2009; Fries et al., 2009). Researchers have suggested that trait anxiety influences the magnitude of the CAR in a healthy adult population, and further analysis indicated that this influence was mediated by anticipatory anxiety (regarding exposure to a stressor) (Walker, O'Connor, Schaefer, Talbot & Hendrickx, 2011). This indicates that the CAR may be influenced by trait anxiety, and therefore associated levels of GAD, but also by state anxiety, more frequently conceptualised as specific anxiety disorders such as SAD, SpAD, Panic Disorder.

In this review, we will focus on research studies that have measured the CAR and related diurnal rhythm in children with ASD, using multiple samples of saliva collected throughout the day.

1.1.6 Diurnal Rhythm

In contrast to the CAR, other researchers have focused on measuring the general diurnal rhythm of cortisol across the day. This diurnal rhythm is a widely-established pattern of cortisol, as part of the HPA axis, displayed by the general population, whereby people show their highest levels of cortisol in the morning, and these levels gradually decline throughout the day (Kiess et al., 1995; Tsigos & Chrousos, 2002).

This diurnal rhythm is separate from the CAR as it does not measure the specific levels of cortisol in the 60 minutes after waking, but measures the general daily pattern of cortisol. This is usually achieved by taking at least two saliva samples across the day (morning and night). Research suggests the CAR is distinct from the diurnal rhythm of cortisol, and is an additional biological process, rather than part of a natural rise and fall of cortisol throughout the day (Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2007).

Whilst the diurnal rhythm is separate from the CAR, it has been used in research as a potential measure or predictor of psychiatric symptoms, in the healthy population, (Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005; Shirtcliff & Essex, 2008). We have therefore included studies in this review that measure diurnal pattern of cortisol in children with ASD, as, similar to the CAR, this may be a useful predictor of HPA-axis dysregulation.

1.1.7 Rationale and objectives

This review explored the current literature with regards to the evidence of the CAR in a specific population; children and young people with ASD, given the commonality of anxiety in this population, and the link between anxiety disorders and stress.

Review aims:

1. To present a systematic overview to evaluate the utility of the CAR in children and young people with ASD

2. To utilise a meta-analytic approach to explore whether children and young people with ASD show the same patterns of CAR as the typical population.

1.2 Method

1.2.1 Protocol Registration

The search protocol for this systematic review and meta-analysis was registered on the International Prospective Register of Systematic Reviews PROSPERO, (http://www.crd.york.ac.uk/ PROSPERO, protocol number: CRD42017051187).

1.2.2 Data sources

Searches were completed by the author and an independent researcher in parallel, on 5 electronic databases; Web of Science via Web of Knowledge and PsychInfo, EMBASE, PubMed and Medline via EBSCO. Initial searches and later data extraction and quality assessment were completed by the first author and an independent researcher, to increase inter-rater reliability. Following the initial searches there were four disagreements between the two researchers, which were resolved by consensus, with the addition of one paper, and exclusion of the other three due to lack of further information available (2), and inappropriate study design. In total, 109 papers were identified, and of these, 42 were assessed for eligibility for this review. After retrieving and examining these papers, 8 were included in this review. A further 7 articles were identified and screened using reference lists of identified papers, and assessing papers those identified had been cited in. A diagram of this process can be seen in Figure 2.

The following search terms were used: Autis*, ASD, Asperger* or "Pervasive Developmental Disorder", and "Cortisol Awakening" or "Awakening Cortisol". The search included papers identifying these words in their key words, Title or Abstract.

A final search was run again in February 2017, using the same search strategy before analysis, to check for additional papers published since the initial search in November 2016. No further papers were added after this search.

1.2.3 Data extraction and Synthesis

A variety of information was extracted from the identified papers, including participant demographics (age, gender, locality), participant information (diagnosis), study design (instructions regarding cortisol sampling) and outcome measures used (cortisol levels and time of samples), as well as key findings and limitations. Pre-agreed data extraction tables were used to

extract objective data (see appendix A). Three disagreements were resolved by consensus and a re-examination of the papers.

1.2.4 Inclusion Criteria

Papers were included if the studies involved children aged 18 or under, with a diagnosis of ASD (APA, 2013). Studies were included if they used a quantitative methodology, and were included if they had a control group or not. Studies were also included if they were intervention studies, measuring the effect of specific interventions on the CAR.

Studies were included if the outcome measure was salivary cortisol levels, and if they took at least two measurements of salivary cortisol, with at least one of these samples being within 30 minutes of the participants waking. This has been widely used to capture the peak in an individual's cortisol levels, as part of the diurnal rhythm (see Taylor & Corbett, 2014 for review). Studies were included if they evidenced quantitative analysis of cortisol levels at different time points and analysed the pattern of these cortisol levels.

1.2.5 Exclusion Criteria

Papers were excluded if they focused on the caregivers, parents or siblings of the child with ASD. Dissertations, conference presentations and other unpublished works were excluded if further data could not be obtained. Review papers were also excluded.

1.2.6 Meta-analysis process

For inclusion in the meta-analysis component of the review, additional inclusion/exclusion criteria were applied to the 15 selected papers identified from the data search above (see figure 2).

1.2.6.1 Meta-analysis inclusion criteria

Studies needed to use best practice guidelines for measuring the CAR; taking samples immediately upon waking, then 30 minutes after waking (Stalder et al., 2016). Studies were also required to use a case control study design, comparing cortisol levels and the CAR between a group of children with ASD to a matched control group of children without ASD. Papers were required to confirm ASD status by formal diagnosis according to DSM-IV or ICD-10. Five studies identified from the initial systematic search above met these eligibility criteria for inclusion in the meta-analysis.

1.2.6.2 Meta-analysis exclusion criteria

Papers were excluded from the meta-analysis if, after unsuccessful contact with authors, no further information could be obtained regarding specific cortisol values (see appendix B for details). One study (from the 5 that met the additional meta-analysis inclusion criteria) was excluded from the meta-analysis for this reason. Therefore, in total, 4 studies identified from the 15 papers retrieved by the initial systematic search were used in the meta-analysis (see Table 1).

1.2.6.3 Meta-analysis data extraction and analysis

Data extracted from these papers was entered into Review Manager 5.3 (Cochrane Collaboration, 2014). Standard mean differences (SMD) in CAR were combined for each study, using the inverse variance method. A random effects model was used, given the inherent heterogeneity of the studies, and an I^2 statistic was calculated to allow an estimate of between study heterogeneity in SMD. An I^2 value of more than 50% indicates substantial heterogeneity between the studies used in the meta-analysis (Higgins, Thompson, Deeks, & Altman, 2003).

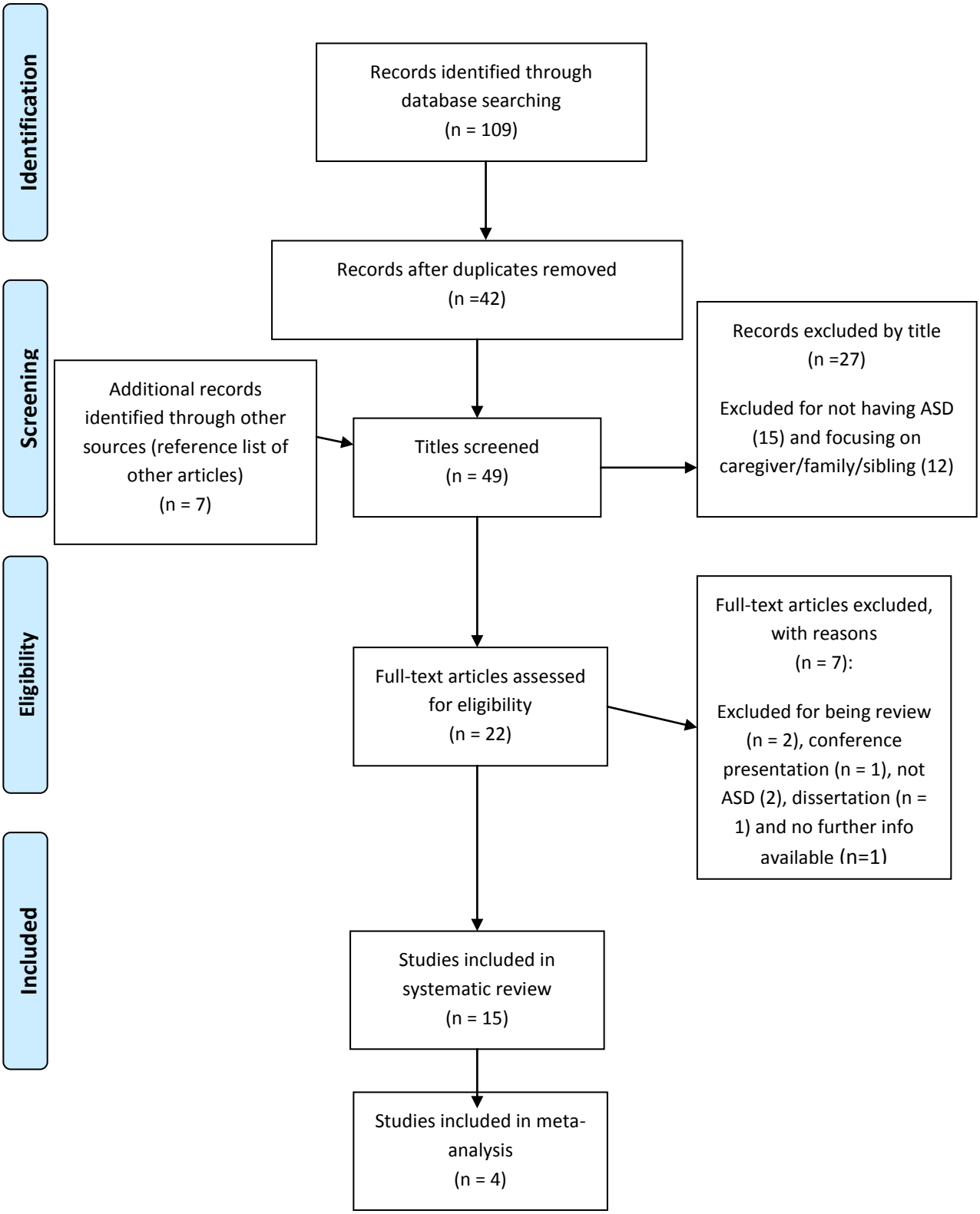

Figure 2. PRISMA flow chart, showing study selection process

Table 1:

Characteristics of studies included in meta-analysis (identified from the studies included in the systematic review)

Study information					**ASD**			**Control group**			**Results**
First author and year	**Country data collected from**	**Outcome measure**	**Group definition**	**Exclusion**	**N (f/m)**	**Mean age (SD) or range**	**Mean CAR (SD)**	**N (f/m)**	**Mean age (SD) or range**	**Mean CAR (SD)**	
Corbett & Schupp, 2014	USA	Salivary cortisol at immediate wake, 30 mins + wake, afternoon, evening. 3 days	DSM-IV, ADOS, formal diagnosis by clinician	IQ below 70. Pubertal. Female. Medications. Control: neuro-developmental conditions	46 male	10.3 8-12.1yr	0.21 (0.50)	48 male	9.9 8-12.1yr	0.23 (0.28)	No sig. dif. in CAR
Brosnan et al., 2009	UK	Salivary cortisol at immediate wake, 30mins + wake, 8pm. 2 days	DSM-IV, formal diagnosis by clinician	Learning or physical disability. Medication. Female. Comorbidities	20 male	12.8 (1.91) 11-16yr	0.29 (1.21)	28 male	13.3 (1.91) 11-16yr	1.63 (2.58)	Absence of CAR in ASD, sig. dif from control
Tomarken, Han &	USA	Salivary cortisol at	DSM-IV, ADOS,	IQ below 70. Medication.	36 (6/30)	10.2 (1.96)	0.64 (2.00)	27 (4/23)	9.71 (1.54)	0.68 (1.44)	No sig. dif. in

Corbett, 2015		immediate wake, 30mins + wake, afternoon, evening. 3 days	formal diagnosis by clinician								CAR
Zinke et al., 2010	Germany	Salivary cortisol at immediate wake, 30mins + wake. 2 days	ICD-10, ADI & ADOS	Insufficient verbal abilities, IQ below 78. Genetic, infectious or metabolic disorders.	15 (2/13)	9.1 6-12yr	5.16 (5.17)	25 (4/21)	9.0 6-12yr	7.59 (5.89)	No sig. dif. in CAR

Table 2:

Characteristics of the remaining studies included in the review but excluded from meta-analysis

Study information					**ASD**		**Control group**		**Results**
First author and year	**Country data collected from**	**Outcome measure**	**Group definition**	**Exclusion**	**N (f/m)**	**Mean age (SD) or range**	**N (f/m)**	**Mean age (SD) or range**	
Marinovic-Curin et al., 2008	Croatia	Salivary cortisol at immediate wake, 30+ wake, before lunch, 60 after lunch, 6pm and just before sleep. 1 day	DSM-IV and ICD-10 criteria, determined by child psychiatrist. ADI and Childhood autism rating scale	Psychiatric or endocrinology disorders Female	9 male	11.9 (2.97)	7 male	11.6 (4.27)	No sig dif. in CAR or cortisol circadian rhythm between groups
Tordjman et al., 2014	France	8:00, 11:00, 16:00, 24:00, 8:00 next day. 8:00 samples were collected 15-60 minutes after	DSM-IV and ICD-10, observations by 2 child psychiatrists,	Medication History of encephalopathy or neuro-endocrinological disease or obesity. Control group: history of	55 (19/36)	11.3 ± 4.1years	32 (10/22)	11.7 ± 4.9 years	Sig higher cortisol at all time points in ASD group. Sig variability in

		awakening and therefore assumed to represent the CAR	confirmed by ADI and ADOS (delivered by psychiatrist).	psychopathological, developmental or neurological disorder, family history of Autistic Disorder.					ASD group
Corbett et al., 2006	US	Mid-afternoon, evening, morning sample. Morning sample within 30 mins of waking. 2 days	Previous records and concurrent ADOS. Clinical judgement based on DSM-IV	Female History of cardiovascular, endocrine, pulmonary, liver or kidney disease, or neurological conditions e.g. epilepsy. Control group: history of neurodevelopmental disorders including autism	12 male	8.5 years	10 male	9.2 years	No sig dif in overall levels of cortisol or daily variation. Sig variability in ASD.
Kidd et al., 2012	US	Within 30 mins of waking, mid-afternoon (2pm approx.), evening 30 mins before bed. 2 days.	DSM-IV, ADOS and ADI	Genetic or chromosomal disorder, chronic illness, significant visual impairment, Tourette's, seizure disorder, OCD, anxiety, depression, other psychiatric disorders. Parents seeking treatment	26 (4/22)	2 - 5.5 years	26 (3/23)	2 - 5.5 years	Higher cortisol levels at morning sample in ASD.

				for sleep disorder, use of oral or inhalant cortico-steroids during study. Control group excluded if any evidence of cognitive or developmental delay.					
Viau et al., 2010	Canada	Wake, 30 mins after wake, and bedtime. 1 day a week. Averages then computed for each time point for 2 weeks' pre-dog, 4 weeks during dog, and 2 weeks after dog	Diagnostic evaluation by a team of independent professionals	Allergic to dogs or taking oral steroids	42 (5/37)	7.1 +/- 3.1 years	N/A	N/A	Normal diurnal rhythms in salivary cortisol at each experimental condition. Sig effect of intervention on CAR - reduction in CAR when service dog introduced
Gabriels et al., 2013	US	Wake, 30+ wake, before lunch and 4pm. 3 days	Met or exceeded cut off on SCQ	IQ below 40. Female. Reached puberty	21	3-9 years	N/A	N/A	Compared Low and High repetitive

			(above 15), and ADOS. Diagnosed confirmed by psychologist, using clinical records, SCQ and ADOS						behaviours groups. All showed expected diurnal rhythm. Levels of cortisol sig lower in High RB group.
Sharpley et al., 2016	Australia	Wake, 30 mins after wake and between 2-4pm. 1 day	DSM-IV criteria, confirmed by clinical psychologist. ADOS-2.	Male Genetic or neurological conditions, or previous or comorbid psychiatric disorder. IQ below 70 on WASI	39	10.1 (SD 2.7)	N/A	N/A	Expected increase from waking to 30 min later (i.e., the CAR) then a decrease to the afternoon sampling.
Bitsika et al., 2015	Australia	30 mins after waking, and between 2-4pm. 1 day	DSM-5 and ADOS confirmed by Clinical Psychologist	Concurrent genetic or neurological conditions, or previous DSM psychiatric disorders or current comorbidities, or taking	150	11.1 (3.3)	N/A	N/A	Expected pattern of higher cortisol in morning

				anxiety medication. Female. IQ below 70 on WISC-IV					
Putnam et al., 2015	US	Morning upon waking (between 7-9am), Midday and evening (7-9pm). 2 consecutive weekends.	ASD diagnosis by physician or psychologist. Standardized checklist documenting cognitive ability and ASD symptoms	Female Control group: prior or current psychiatric diagnosis or special education classification, or history of significant physical illness or medical condition	29 male	7-12 years	14 male	9.36 (1.55)	No sig dif in cortisol levels between control and High functioning ASD group, but sig higher cortisol in Low functioning ASD group compared to HFA or control.
Corbett et al., 2008	US	Within 30 mins of waking, afternoon between 1-4pm and evening 30 mins before bed. 3 days for 2 consecutive weekends	ADOS and ADI Diagnosis confirmed according to DSM-IV, SCQ score	ASD group: Children with Autism Spectrum disorders - PDD-NOS and Asperger's. Control group: history of neurodevelopmental disorders and history of	22 (1/21)	8.81 (1.90)	22 (3/19)	9.35 (1.75)	No sig dif in diurnal rhythm of cortisol. Sig variability in ASD group.

				serious physical illness (endocrine, cardiovascular, neurological). Scores above cut off on SCQ					
Corbett et al., 2009	US	Within 30 mins of waking, afternoon between 1-4pm and evening 30 mins before bed. 3 days; 2 consecutive weekends	DSM-IV, ADOS, SCQ score	Children with PDD-NOS and Asperger's. Control group: ASD traits measured on SCQ	22 (1/21)	8.81 (1.90)	22 (3/19)	9.35 (1.75)	Shallower slope from morning to night in ASD; greater variability in daily cortisol

1.3 Results

1.3.1 Sample Characteristics

In the 15 studies included, the age range of participants varied from 2 to 18 years, with an average age of M = 9.1 years across 795 participants (Tables 1 and 2). All studies included a sample with a diagnosis of an Autism Spectrum Disorder; Autism, Asperger's or PDD-NOS.

The majority of participants across the studies were male (87%); nearly half of the studies (7) using all male participants and only 1 study with only female participants. Most studies (80%) confirmed diagnoses of ASD with validated and widely accepted diagnostic measures for assessing ASD; Autism Diagnostic Interview Revised (ADI-R, Lord, Rutter & LeCouteur, 1994) or Autism Diagnostic Observation Schedule (ADOS, Lord, Rutter, DiLavore, & Risi, 1999). The remaining studies relied on prior formal diagnoses by paediatric clinicians, or diagnostic evaluation by appropriately qualified professionals (psychologist, paediatrician, psychiatrist).

The majority of studies (11) included a matched control comparison group, but of these, only 5 used the preferred sampling method for accurate CAR, taking samples at immediate wakening and 30 minutes after (Stalder et al., 2016). Of the remaining 4 studies, 3 used the preferred sampling method for CAR but had no control sample, and 1 had neither a control sample, nor the preferred CAR sampling method. For studies included in the review that did not use the preferred CAR method (n = 7), these studies collected saliva samples only at 30 minutes after wakening, and at least one other time point in the day.

For example, Tordjman et al., (2014) have used this approach (collecting saliva 30 minutes after waking), arguing that it is sufficient to reflect some element of the CAR because it captures the rise in cortisol 30-60 minutes after wakening (see also Pruessner et al., 1997; Wust et al., 2000b). However, it is important to note that these studies cannot accurately measure the true CAR, which requires calculation of the increase in cortisol from immediate wakening to 30-60 minutes later. However, studies that only use one sample after awakening have been included in this review, as they can still provide useful comparisons when looking at the overall pattern of cortisol rhythms in children and adolescents with ASD.

One study (Viau et al., 2010) used an experimental design to measure the effect of an intervention (introduction of service dogs) on the size of the CAR, whereas all other studies focused on establishing and comparing the CAR or diurnal pattern of cortisol in children with ASD.

1.3.2 Presence of similar CAR and Diurnal Pattern in both populations

Four studies included in the meta-analysis, plus one additional study (Marinovic-Curin et al., 2008) did measure the CAR using cortisol samples at immediate wakening and 30 minutes after, and had a control comparison group of typically developing peers, matched on age. Of these 5 studies, 80% indicated the presence of a CAR in children with ASD that did not differ significantly from that seen in the typically developing control group. These results provide some evidence to suggest that the CAR in children with ASD is similar to that seen in the general population (Corbett & Schupp, 2014; Marinovic-Curin et al., 2008; Tomarken, Han & Corbett, 2015; Zinke, Fries, Kliegel, Kirschbaum, & Dettenborn, 2010).

Similarly, Corbett, Mendoza, Abdullah, Wegelin and Levine (2006) found no significant difference in the daily pattern of cortisol levels between ASD groups (n =12) and typically developing controls (n = 10), with the highest cortisol levels seen in the morning, 30 minutes after waking, and then declining throughout the day, in the same pattern for both groups. However, this study did not measure the CAR directly, so only represents the overall diurnal pattern of cortisol.

Whilst Gabriels et al., (2013) did not include a control comparison group, their results indicate that their participants with ASD (n = 21) also displayed a diurnal rhythm and CAR as you would expect in the general population, with an initial rise followed by a fall in cortisol levels throughout the day. However, the increase in cortisol from immediate wake and 30 minutes after wake did not reach statistical significance, although these two morning samples were significantly higher than the afternoon or evening samples.

In the only intervention study included in this review, Viau et al., (2010) found children with ASD (n = 42) showed the expected rise in cortisol, 30 minutes after waking, in comparison to their immediate waking samples, indicative of the CAR seen in the general population. This study also reported that the proportion of the increase in cortisol after waking, (the CAR) decreased during the intervention stage (introduction of a therapy dog), providing evidence that the CAR may be linked to stress and can be moderated by intervention. Viau et al., (2010) further showed that when the intervention ceased, the CAR's proportionate increase rose again.

1.3.3 Differences in observed and expected CAR

In contrast to studies above, which highlight the lack of group differences in CAR, Tordjman et al. (2014) reported evidence that contrasts the notion of comparable CAR and diurnal patterns in children with ASD with that of the general population. This study (n = 87*)* found a significant effect of group on cortisol levels, (F(1,71) = 16.28, p = 0.0001), indicating that the ASD group (n = 55) showed significantly higher levels of cortisol at all time points and flatter

slopes, indicating less of a decline throughout the day than the typically developing group (n = 32). This finding suggests the ASD group did not show as much of an increase in cortisol in the 30 minutes after wakening as would be expected, but this cannot be accurately asserted due to not having a measurement of cortisol at immediate awakening, and only at 30 minutes after waking.

In addition, Kidd et al. (2012) found that young children with ASD showed waking values of cortisol to be 1.34 units higher than a typically developing control group, but stated that there was uncertainty regarding this result due to wide confidence intervals. The study further concluded there were no statistically significant differences in cortisol levels between the autism group (n = 26*)* and typically developing group (n = 26) at any time point across the day.

A study including all-female sample showed the expected CAR and diurnal pattern, however, Sharpley et al., (2016) found that 15 of their 39 showed a decrease in cortisol levels 30 minutes after waking, compared with their immediate waking levels. A further 6 participants showed an increase in cortisol of less than 10% in the 30 minutes after waking, which is much less than typically reported in the literature (Pruessner et al., 1997; Wust et al., 2000b). The researchers reported that this lack of increase in cortisol was unlikely to be due to significant arousal before waking which may have prompted the CAR in pre-wake period, as there was no significant difference in waking cortisol levels between participants that showed the CAR and those that did not (Sharpley et al., 2016).

Similarly, Bitsika et al., (2015) found the presence of a typical diurnal rhythm, with cortisol levels highest in the 30 minutes after waking, in participants aged between 6 - 18 years in the overall sample (n =150). However, 14.7% of their sample showed a reverse diurnal rhythm, with lower levels of cortisol in the morning compared with the afternoon. No differences were found across age.

Putnam et al., (2015) explored the differences in diurnal rhythm across high and low functioning ASD groups compared with a typically developing control group (n = 33). High functioning ASD was defined as those without cognitive or language impairment and average or above IQ, and low functioning ASD was defined as those with cognitive impairment and significantly below average IQ. All groups showed significantly higher levels of cortisol in the 30 minutes after waking than in the afternoon or evening. However, this study also found a significant main effect of group on cortisol levels at all time points, (F(2, 40) =7.231, p =.002), with post hoc Bonferroni corrected comparisons indicating marginal mean scores to be significantly higher in the low functioning ASD group compared with the high functioning ASD group (0.19 and 0.12, p = .002) and between low functioning ASD group and the typically developing group (0.19 and 0.14, p = .021). This finding indicates that lower levels of functioning in ASD may alter the pattern of the diurnal rhythm, and potentially the CAR, in comparison to control groups or higher functioning ASD groups.

1.3.4 Variability

An important finding from many of the studies in this review is that children with ASD showed significantly more variability in their cortisol levels and patterns of cortisol than the typically developing groups (Corbett et al., 2006, 2008, 2009; Kidd et al., 2012; Tordjman et al., 2014). Bitsika et al., (2015) found that within their ASD sample, there was a sub-sample of boys who did not show the same diurnal pattern of cortisol as expected (i.e. their cortisol levels did not show a decline from morning to evening), and this notion of sub-samples might explain why other studies found significant variation in the ASD groups.

In addition, Corbett et al., (2006) found that whilst their ASD group showed the expected pattern of diurnal rhythm of cortisol, this group also showed a pattern of gradual decline in the levels of morning cortisol over the course of the 6 days sampling, which may indicate that longer term studies assessing the stability of the CAR and diurnal rhythm are needed.

1.3.5 Quality Assessment

Papers included in the review were assessed for methodological quality based on meeting predefined criteria; (1) control of confounding variables affecting CAR; reporting age, gender, IQ, medication (Wust et al., Fries et al., 2009), (2) presence of matched control sample, (3) measures taken to increase participant compliance with best practice guidelines for collection of CAR data (Stalder et al., 2016), (4) clear procedural instructions regarding saliva samples, (5) presence of sampling at immediate wakening and 30 minutes after waking.

Table 3 provides definitions for the quality assessment criteria used. Studies were given an overall score, based on how fully they met each of the criteria; 0 = not met/no information available, 1 = partially met, 2 = fully met. Assessment of quality was completed by the first author and an independent researcher to improve inter-rater reliability. There were 9 1-point disagreements between the researchers, which were resolved by consensus and clarification of the assessment criteria.

Table 3:

Quality Assessment criteria and definition

Criteria	Definition
1. Control	Presence of a control sample
2. Age	Reporting of age and/or pubertal stage
3. Gender	Reporting of gender and potential influence on results (e.g. was gender controlled for)
4. Medications	Reporting of medications taken/exclusion criteria if medication taken, and report of potential effect
5. IQ	Appropriate and valid measures of cognitive ability, was this controlled for and matched across groups
6. Accurate CAR recording	Did the study take saliva samples at immediate waking then 30 minutes after?
7. ASD diagnosis	Did the study use validated and reliable measures of assessment of ASD - e.g. ADOS, ADI, according to DSM-IV-TR or 5 criteria?
8. Quality of instructions	Were instructions on saliva sampling procedure clear, accurate (including refraining from eating, drinking, brushing teeth)
9. Improving compliance with sampling protocol	Were measures used to help increase compliance with correct saliva sampling techniques, e.g. TrackCap electronic monitoring, was adherence assessed?
10. Completeness of data	Were missing data reported and accounted for? Did the study have low levels of missing data?

Table 4 shows that studies included in the review scored between 7 and 17 out of 20, with the 4 studies identified as suitable for inclusion in the meta-analysis, scoring an average of 15 points, suggesting the risk of bias in the studies included to be acceptable. Whilst 2 studies scored below 10 they were included in the review, as they both used best practice methods of collecting CAR (immediate wake and 30 minutes after).

Whilst 11 of the studies included a matched control group, 4 studies did not, and these absences makes it difficult to ascertain whether the cortisol patterns displayed by participants with ASD are similar or distinct from those of the general population. The majority of studies (13) reported adequate information about their participant sampling and recruitment techniques, however due to study designs, none of the studies used randomised or blinded conditions. All studies reported adequate cortisol analysis techniques, with sufficient detail of cortisol extraction and assay techniques.

There was variation in instructions given to participants regarding protocols for collecting saliva samples, and variations in salivary cortisol collection kits used. Nearly half of the studies (7) did not use any methods for increasing participant compliance with cortisol sampling procedures. This lack of rigour makes it difficult to ascertain how accurately participants complied with sampling times, which has been shown to be particularly important for measuring the CAR (Stalder et al., 2016), as delays in sampling compared to reported time of sampling may skew the reported pattern of cortisol levels.

Two studies employed TrackCap systems, which are electronic monitoring systems that track when test tubes are removed and returned to the collection kits, to help provide a more accurate time stamp for the sample. Both studies found there was no significant difference between reported sample times and TrackCap registered times (Corbett & Schupp, 2014; Kidd et al., 2012), which helps improve the likelihood that the reported patterns in cortisol in this study do reflect the CAR, as the timing of the samples matched best practice guidelines (Stalder et al., 2016).

Other studies aimed to increase compliance with sampling times by asking parents to use a diary to record sampling times, or used researchers within the participants' environment to ensure samples were collected at the correct time. However, in Brosnan et al.'s (2009) study where the researcher collected samples from the ASD group, this in turn created unequal research procedures, as the control group samples were collected by parents. The presence of the researcher introduces an artificial component to the participant's normal waking routine, and this may have influenced their stress levels and potential cortisol levels.

It is important for studies to consider the effect of confounding variables such as cognitive functioning on cortisol levels, as specific areas of cognition involving the hippocampus and prefrontal cortex have been shown to correlate with increased/decreased magnitude of CAR. For example, Almela, van der Meij, Hidalgo, Villada, and Salvador, (2012) found that an increase in

the magnitude of the CAR seen in participants correlated to a poorer declarative memory. Similarly, poorer overall cognitive functioning, as measured by a comprehensive selection of cognitive tests, was associated with an attenuated CAR (Evans et al., 2011). A further variable to consider is age, specifically pubertal stage, as Kiess et al., (1995) found significant correlations between pubertal stage and cortisol levels, with higher cortisol being associated with adolescence and increasing age.

Controlling for the effect of cognitive functioning on cortisol levels, 6 studies had exclusion criteria of participants with IQs below 70, one study excluded those with IQs below 40. 5 studies used validated measures of IQ (WASI; Wechsler, 1999) and included this in their analysis, showing no significant effect or correlation of IQ with cortisol levels. Interestingly, Kidd et al., (2012) found correlations between cognitive functioning, estimated on the Mullen Scale of Early Learning (Mullen, 1997) and cortisol levels, with higher cortisol levels associated with lower cognitive functioning. Three studies reported using inconsistent measures of IQ across their samples and did not report the effect of IQ on their results, making it hard to conclude that variation in cognitive functioning did not influence their reported patterns of cortisol. Furthermore, 3 studies did not report IQ levels at all for participants.

To further reduce the influence of confounding variables, 4 studies excluded participants taking any medication, and 4 excluded all participants with current or historic co-morbid psychiatric disorders. Zinke et al., (2010) included additional analyses comparing medicated and non-medicated participants, finding no significant effects on cortisol levels, and Sharpley et al., (2016) reported that whilst 54% of participants were taking anxiety medications, these were not reported to affect cortisol secretion, according to manufacturers.

Controlling for the effects of medication allows the researchers to have increased confidence that their reported cortisol patterns are not influenced by this and it also acts to reduce the external validity of these studies to the wider ASD population, as research has established psychiatric disorders, especially anxiety disorders can be prevalent in up to half of individuals with ASD (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Simonoff et al., 2008). Therefore, by excluding participants with co-morbid psychiatric conditions, the ASD sample included in these studies may not accurately reflect the true ASD population.

Table 4:

Quality assessment scores for studies included in review. Criteria Score: 0 = not met/no information available, 1 = partially met, 2 = fully met

Study	**Criteria**										
	1: Control	2: Age	3: Gender	4: Meds	5: IQ	6: CAR	7: ASD Diagnosis	8: Instructions	9: Compliance	10: Attrition	**Total**
Studies included in meta-analysis											
Corbett & Schupp, (2014)	2	2	2	2	2	2	2	2	1	0	**17**
Brosnan et al., (2009)	2	1	2	2	0	2	1	2	1	0	**13**
Tomarken et al., (2015)	2	1	1	2	2	2	2	2	0	2	**16**
Zinke et al., (2010)	2	1	1	2	2	2	2	0	1	1	**14**
Studies not in meta-analysis											
Marinovic-Curin et al., (2008)	2	1	2	0	0	2	2	0	0	0	**9**
Tordjman et al., (2014)	2	2	2	2	1	0	2	2	0	1	**14**
Corbett et al., (2006)	2	2	2	0	0	0	2	2	1	1	**12**
Kidd et al., (2012)	2	2	1	2	1	0	2	2	2	1	**15**

Viau et al., (2010)	0	1	2	1	0	2	0	0	0	1	**7**
Gabriels et al., (2013)	0	2	2	0	1	2	2	2	0	2	**13**
Sharpley et al., (2016)	0	1	2	2	2	2	2	1	1	0	**13**
Bitsika et al., (2015)	0	1	2	1	2	0	2	1	1	0	**10**
Putnam et al., (2015)	2	1	2	0	2	0	0	2	0	2	**11**
Corbett et al., (2008)	2	1	1	0	1	0	2	2	2	0	**11**
Corbett et al., (2009)	2	1	1	0	2	0	2	2	0	0	**10**

1.3.6 Meta-analysis results

Results from the meta-analysis indicated no significant difference between ASD and TD groups in their mean CAR; $z = 1.52$, $p = .13$, suggesting that both groups show the same pattern of a rise in cortisol in the 30 minutes after wakening. The I^2 test for between study heterogeneity indicates that only 6% of the variability in effect estimate is likely to be due to the heterogeneity of the studies. Figure 3 shows that whilst two studies report higher mean CAR in the typically developing group, this did not reach statistical significance, and the overall pooled effect showed no significant difference in CAR between the groups.

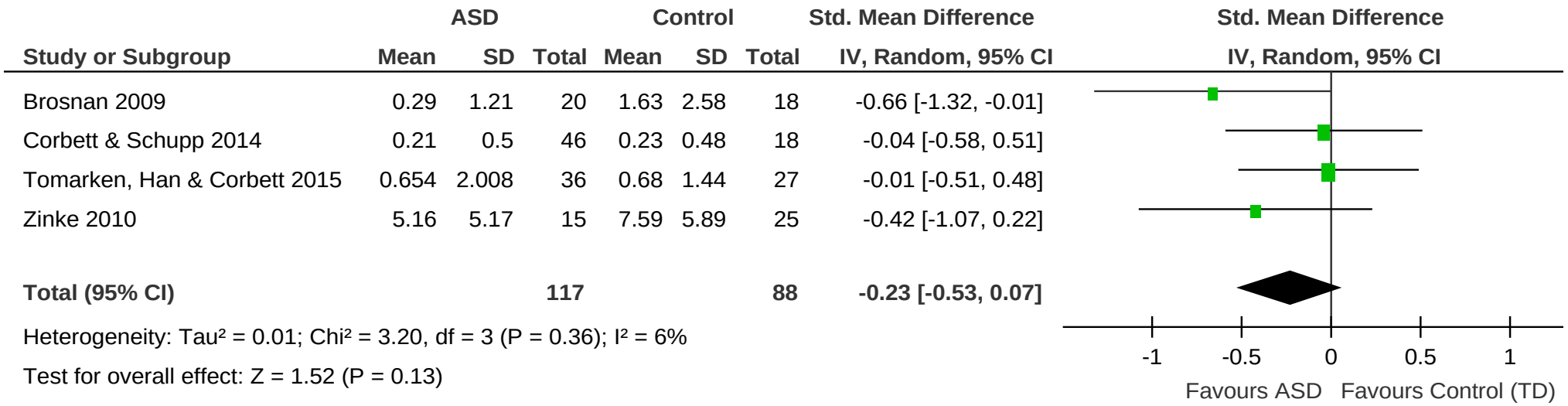

Figure 3: Forest plot for meta-analysis of differences in CAR between ASD and control groups

1.4 Discussion

The CAR is a widely established biological phenomenon, involving a peak in cortisol levels in the 30-60 minutes after waking that has been shown to be influenced by psychosocial factors such as stress, anxiety, depression, fatigue and cognitive ability (Chida & Steptoe, 2009; Fries et al., 2009). The prevalence of anxiety in ASD is estimated to be up to 50% (de Bruin, Ferdinand, Meester, de Nijs, & Verheij, 2007; Simonoff et al., 2008), therefore this review sought to explore whether the CAR can be seen consistently in populations with ASD, to help establish whether this may be a useful biological marker of stress and anxiety for this population.

Overall, results suggest that children with ASD showed a comparable CAR to typically developing children. This comprehensive, narrative synthesis of the literature indicated similar CAR and diurnal patterns of cortisol across the groups. These results indicate that the CAR is a useful objective indicator of physiological stress and could prove to be a useful index with which to monitor stress in developing populations, including ASD.

However, it is important to note that this review also highlighted several key studies that did not find the expected CAR or diurnal rhythm pattern as expected for the general population, and therefore interpretation of this result must be taken with some caution. Sharpley et al., (2016) demonstrated that their overall mean results found the expected CAR and cortisol diurnal rhythm. However, this study also highlighted that over half of their participants did not show this expected pattern, indicating that future studies should be mindful of inter-individual variability. Bitsika et al., (2015) also found that 14.7% of their sample showed a pattern in contrast of the expected diurnal rise and fall of cortisol levels throughout the day.

This variability may reflect individual differences in the chronicity of stress. Previous research suggests that anxiety and stress can increase the CAR in the typical population (Vreeburg et al., 2010). Alternatively, further studies indicate that chronic stress or prolonged exposure to stressors such as in PTSD or burnout is associated with a flattened CAR (Chida & Steptoe, 2009). It may be plausible to hypothesise that some children in these samples experienced anxiety, given the prevalence of anxiety (up to 84% in a recent review) in children with ASD (White et al., 2009), and that this led to an increase in their CAR.

It may also be plausible however, that a proportion of these children, given the neurodevelopmental nature and lifelong course of ASD (APA, 2000), may have experienced anxiety or stress for a prolonged period of time, therefore, in line with previous research on chronic exposure to stress, we would expect them to show an attenuated CAR. The variation in individual exposure to stress throughout their lifetime may impact on the effect of the CAR and may account for the variation in results reported by the studies included in this review. Future

studies exploring the CAR should consider the moderating impact of anxiety and previous exposure to chronic stress.

The clinical implications for the findings of this review suggest that measuring cortisol in children with ASD, specifically using controlled measurement of the CAR, using samples at immediate waking and 30 minutes after, may provide a useful insight into an individual's physiological levels of stress, compared to that of the general population. Given the documented difficulties with language and emotion recognition in people with ASD (APA, 2000; Hill, Berthoz, & Frith, 2004), the pattern of CAR may provide clinicians with information regarding the person's daily anticipated stress, if they have difficulties with reporting this themselves. Based on the results of this meta-analysis and systematic review, one could expect individuals with ASD to show similar patterns of CAR to the general population, and therefore deviations from this pattern may indicate psychopathology, for example elevated anxiety.

1.4.1 Limitations and Future Research

A significant limitation of the research in this review is the lack of studies using best practice recommendations for the calculation of an accurate CAR, and using a control comparison group, controlling for IQ, gender and age, to compare these results to. A consistent finding throughout this review is the need for more controlled studies into the CAR in children with ASD, controlling for medication use, gender, lifetime stress, and impairment related to ASD symptoms and IQ. This review highlights the variation in controlling for these potential confounding variables in the existing literature, making it challenging to draw firm conclusions from the results, especially given the contrasting findings amongst the studies. Of the 15 studies included in the review, only 5 used a matched control group and took saliva samples at immediate wakening and 30 minutes after. This highlights how limited the research is in this area, and emphasises the need for more comprehensive investigations of the CAR in children with ASD to ascertain whether the CAR can be used as a reliable biological indicator of physiological and psychosocial stress.

This review also highlights the paucity of research involving females with ASD, as the majority of participants were male. Previous research has provided contrasting evidence for the effect of gender on the CAR, with some studies suggesting a slightly increased CAR in females (Fries et al., 2009). The inclusion of more males in research may reflect the demographics of the ASD population (Baird et al., 2006; Fombonne, Quirke, & Hagen, 2011), however, further research could usefully work to include more females with a view to using the CAR as a marker of physiological stress in the ASD population as a whole.

In summary, this paper incorporates the first meta-analysis amalgamating the existing data in the field measuring the CAR in young people with ASD, alongside comprehensive narrative

synthesis of additional research in this area. Collectively, the reviewed studies demonstrate the potential validity in using the CAR as an objective, physiological marker of stress. This recommendation is strengthened by the results presented in the meta-analysis which across four high quality studies indicate no significant difference in CAR between the ASD group and typically matched controls. The meta-analysis and review also highlight the importance of further, more controlled studies, to enable some understanding of the evident variability presented within individual studies. A wider and more robust research base will allow clearer conclusions to be drawn about the exact pattern of CAR in children with ASD, to more conclusively establish whether this index can be used as a reliable and valid measure of stress/anxiety in young people with ASD.

Chapter 2: Understanding the experience of anxiety in young people with Autism Spectrum Disorder (ASD)

2. 1 Introduction

2.1.1 Anxiety

It is estimated that anxiety disorders are one of the most common mental health disorders in young people, affecting over 3% of children aged 5-16 years in the UK (Green, McGinnity, & Meltzer, 2005). Green et al., reported rates of diagnosis to be higher in females (3.8%) compared to males (2.9%), and in adolescents (~4%) versus children (~ 2%) (Green et al., 2005). In a large-scale survey of over 10,000 adolescents in the US, anxiety disorders were the most common mental disorders, with lifetime prevalence rates of 31.9% (Merikangas et al., 2010).

Anxiety is linked with poorer academic performance (Essau, Conradt, & Petermann, 2000), early school withdrawal (Van Ameringen, Mancini, & Farvolden, 2003), and poorer social functioning (La Greca & Lopez, 1998). Furthermore, research conducted using a longitudinal study of over 1000 children, found that anxiety disorders in childhood (under 16 years) were significantly associated with risk in adulthood of subsequent anxiety disorders, academic underachievement, and onset of additional disorders such as depression and substance misuse (Woodward & Fergusson, 2001).

The onset of anxiety in children is argued to reflect a complex mix of interacting factors, including environmental, biological, genetic and cognitive influence (Murray, Cresswell, & Cooper, 2009; Rapee et al., 2009). For example, Murray et al., (2009) proposed a framework for understanding the development of anxiety in childhood, centring on the role of parental anxiety and how this influences three pathways; biological/genetic vulnerability, life events and modelling of anxious behaviours. Further frameworks have focused specifically on the core role of attentional processes in the development of and vulnerability to anxiety in childhood, highlighting that attentional biases towards threat information, and reduced attentional control are linked with increased disposition to anxiety in childhood (review by Hadwin, Visu-Petra, Muris, Derakshan & Macleod, 2016).

2.1.2 Autism Spectrum Disorder and Anxiety

Autism Spectrum Disorder is characterised by significant deficits in social communication, interaction and a restrictive repertoire of behaviours (APA, 2013), that often cause significant impact on social functioning (Bellini, 2004) and academic achievement (see Fleury et al., 2014 for review). Characteristic of ASD is the 'need for sameness' (WHO, 2016) and difficulties in adapting to change. Research has increasingly focused on mental health challenges in ASD, highlighting that amongst the most common comorbid conditions are anxiety and depression (Simonoff et al., 2008). Some studies have indicated lifetime prevalence rates of anxiety of nearly 40% in individuals diagnosed with ASD (van Steensen et al., 2011), highlighting increased prevalence compared with a general population.

2.1.3 Theoretical Models of Anxiety in ASD

Difficulties highlighted by researchers associated with the measurement of anxiety in ASD raise questions about whether anxiety experienced by people with ASD is etiologically and phenotypically similar to the anxiety experienced by the typically developing population. Researchers have considered, for example, whether anxiety in ASD is phenotypically altered, by the presence of ASD and its presence is associated with distinct causal pathways (Wood & Gadow, 2010). Wood and Gadow's (2010) review of research into common genetic markers of anxiety in TD and ASD populations includes several studies that indicate common factors to suggest that anxiety may be phenotypically similar in both populations. While the presentation of anxiety may be similar, several theoretical frameworks have proposed that pathways to anxiety in individuals with ASD include both distinctive and unique features, compared with typically developing populations.

Wood and Gadow (2010) proposed a framework incorporating common risk factors of anxiety from the TD literature alongside proposed risk factors specific to ASD. For example, ASD-related stressors such as social confusion and the unpredictability of social situations were proposed to increase social anxiety, providing some explanation for why social anxiety disorder is common in young people with ASD, with prevalence rates between 29% - 49 % in studies of 10-16 year olds (Bellini, 2004; Simonoff et al., 2008). The notion of social confusion and difficulties predicting social situations and outcomes links to a recent conceptualisation of anxiety in ASD. Boulter, Freeston, South and Rodgers (2014) explored the potential role of 'Intolerance of Uncertainty' (IU) in the development of anxiety in people with ASD. IU has been defined as a 'broad dispositional risk factor for the development and maintenance of clinically significant anxiety' (Carleton, 2012, p. 939). In typically developing models of anxiety, IU has been theorised as most closely linked to GAD and worry (Comer et al., 2009; Dugas, Gagnon, Ladouceur, & Freeston, 1998), but recent research with adult populations comparing clinical, community and control samples, indicates IU to be linked to symptoms of multiple anxiety

disorders such as GAD, SAD and panic (Carleton et al., 2012). Boulter et al., (2014) argued that IU is a useful construct to explain anxiety in children with ASD, due to its overlap with core features of ASD, such as preference for sameness and resistance to change (Kanner (1943).

Conceptual frameworks of anxiety in ASD incorporate specific risk factors linked to social situations, raising the question of how young people diagnosed with ASD experience anxiety in school. School settings are associated with fluid social situations and unstructured activity and associated difficulties (e.g., increased noise levels) and collectively these factors may potentially increase feelings of anxiety in children with ASD. Reviews of the literature have suggested that people with ASD report increased sensitivity to noise (Stiegler & Davis, 2010), and that certain types of noise/sensory input (uncontrollable, unpredictable and unexpected) are more distressing (Ashburner, Bennett, Rodger, & Ziviani, 2013). School environments provide opportunity for frequent exposure to these types of stressors, especially in break or transition times (between lessons). These difficulties may be further confounded by additional stressors of the school day, such as multiple social interactions and periods of unstructured time. Moving between lessons or having breaks creates uncertainty in expected behaviour, and researchers have theorised that intolerance of this uncertainty is associated with anxiety (Boulter et al., 2014). A preference for sameness, routine and predictability is inherently symptomatic of people with ASD (Kanner, 1943; WHO, 2006), so unstructured and unpredictable times throughout a school day, especially with multiple, uncertain social situations, could be hypothesised as being stress/anxiety provoking for individuals with ASD.

This increased risk of anxiety in ASD, associated with school specific factors may be more evident for young people who also report recognised predispositions to experiencing anxious affect (i.e., those with elevated trait anxiety). Trait anxiety reflects a characteristic predisposition to experience increased and transitory (state) anxiety in situations perceived as potentially threatening (Speilberger, 1996).

2.1.4 Assessment of Anxiety in ASD

Anxiety in TD is assessed via parent and teacher report, and via self-report from middle childhood. In clinical settings, clinicians would also aim to obtain information from multiple sources using semi-structured standardised interview techniques, as well as questionnaires (Silverman & Ollendick, 2005). Findings in TD (review by De Los Reyes & Kazdin, 2005) and ASD populations (e.g., White et al., 2009) highlighted that questionnaire measures used to assess anxiety show poor inter-rater reliability, following the pattern of multiple informant discrepancies established in the general population. For example, a large community sample of school-aged children without ASD (n = 1039), all participating in a randomised control trial of an anxiety prevention CBT school-based programme, highlighted agreement between parent and teacher

report of child anxiety to be acceptable but poor between parent-child or teacher-child agreement (Miller, Martinex, Shumka, & Baker, 2014). Similarly, in a review of multi-informant models in the general population, Smith (2007) advises that the most accurate informant for psychiatric disorder will depend on the child's age, setting and type of problem, suggesting that for older children with internalising disorders such as anxiety disorders, child, then parent, then teacher report is most accurate. De Los Reyes & Kazdin, (2005) highlighted that discrepancies in the rating of internalising versus externalising disorders (e.g. conduct disorder) may reflect their less observable behavioural features. Similarly, further research has found adolescents with ASD endorsed more symptoms of anxiety compared with parents and teachers (Hurtig et al., 2009). While White, Schry, and Maddox, (2012) reported that adolescents with high functioning autism underreported their symptoms of anxiety, compared to parent and clinician reports.

In a recent large systematic review, De Los Reyes et al., (2015) highlight that across a range of mental health disorders, spanning from early childhood to adulthood, there are consistently discrepancies in multiple informant reporting of disorders such as depression, social anxiety, conduct disorder and attention/hyperactivity difficulties. The review highlights the relevance of context; emphasising that multiple informant discrepancies may reflect the varying contexts in which symptoms are displayed (home vs school) and how these contexts may influence what symptoms are recorded by particular informants (parent, child, teacher, clinician). This review highlights the ongoing difficulties with discrepancies between informants in the general population, not specific to an ASD population, or anxiety difficulties, which highlights the importance and continued value of assessing for all mental health difficulties in childhood using multiple informants from different contexts.

In a systematic review of literature regarding assessment of anxiety in ASD, MacNeil, Lopes, and Minnes, (2009) highlighted further difficulties clinicians face determining which symptoms are inextricably linked to ASD symptomatology, and which distinctively reflect comorbid anxiety symptoms. Of note, diagnostic overshadowing, whereby symptoms are attributed to the neurodevelopmental disorder of ASD rather than an anxiety disorder, has been argued to hinder accurate recognition of anxiety in this population (MacNeil et al., 2009). Moreover, this challenge may be linked to the difficulties around discriminant validity of existing measures and poor inter-rater reliability (White et al., 2009).

The difficulties discriminating ASD symptoms from anxiety symptoms are further exacerbated when examining specific social anxiety disorder symptoms. Research has increasingly found symptoms of SAD and ASD overlap, including avoidance of social situations and preference for aloneness (White, Bray & Ollendick, 2011). Similarly, Cath, Ran, Smit, Van Balkom and Comijs, (2008) found that in people with SAD, without co-morbid ASD, these participants scored as highly on the domains for social communication/interactions difficulties of the Autism Quotient Questionnaire (assessing for traits of ASD) as participants with ASD and

SAD. This further highlights the overlap between the two disorders, and therefore the difficulties in correctly distinguishes unique features of either (White et al., 2011).

Researchers have also noted that the assessment of anxiety disorders relies on individuals with ASD accurately identifying and interpreting their own emotional responses, and that this process might be specifically problematic for this population (Hill, Berthoz, & Frith, 2004; Rieffe et al., 2011). Furthermore, assessment of self-reported anxiety symptoms relies on the language abilities of the informant. A recent study evaluating psychometric properties of two self-reported anxiety questionnaires in an ASD population suggests that parent-child agreement in anxiety symptoms correlated with the child having higher IQ and less severe ASD symptoms (Kaat & Lecavalier, 2015). Consistently, in a recent review of the literature regarding accurate assessment of anxiety in children with ASD, Grondhuis and Aman, (2012) highlighted the link between verbal ability and reported anxiety symptoms, suggesting that the number of anxiety symptoms reported is positively correlated to communication abilities. The authors argued that this relationship may reflect a lack of insight in individuals with less cognitive ability into their own emotional experiences, or a limited ability to communicate feelings of anxiety. This argument fits with research highlighting positive links between verbal ability and an ability to understand mental states in themselves and others in children diagnosed with ASD (Happé, 1995; review by Baron-Cohen, Lombardo & Tager-Flusberg, 2013).

2.1.5 Triangulation of data from different sources

Research that aims to understand risk factors linked to the development of anxious affect needs to ensure that the measurement of anxiety in ASD is reliable and valid. In line with recommendations from MacNeil et al., (2009), it is important to use multiple methods of assessment, including standardised questionnaires and clinical interviews, from multiple sources of informants (parent, child, and teacher). A further issue highlighted by current research is that studies use questionnaires or clinical interviews that have been established using typically developing populations. MacNeil et al., (2009) indicated there are only two specific measures designed for assessing psychiatric symptoms in ASD, but these need further testing to establish reliability and validity. Instead, research to date focuses on using standardised questionnaire methods and interviews for assessing anxiety. One way of exploring the validity of anxiety measures is to look at associations between these more traditional measures with a more objective index of stress; Cortisol.

2.1.6 Cortisol Awakening Response

The HPA-axis is a central system, largely associated with the regulation of stress, and has been long since established as a useful index of stress in the general population (Fries et al., 2009). Cortisol, a stress hormone, can be measured to identify the pattern of individuals' HPA-axis activity over the course of the day, with greater HPA-axis activity indicating elevated stress. Cortisol levels, as part of the HPA-axis, follow a widely established diurnal rhythm, with increased levels in the morning and a gradual decrease throughout the day (Gröschl, Rauh, & Dörr, 2003). A unique feature of the HPA-axis, in addition to the diurnal rhythm of cortisol, is the Cortisol Awakening Response (CAR); a sharp peak in cortisol levels in the 30 - 60 minutes after wakening (Pruessner et al., 1997), which is estimated to be found in 75% of the healthy population (Wust et al., 2000b).

Since being first systematically reviewed and used by Pruessner et al., (1997), there has been a large expansion in the research focusing on CAR (Stalder et al., 2016) as an index of stress in typically developing and atypical populations. The CAR has been found to be moderated by a wide range of psychosocial factors, including job stress, fatigue, burnout, psychiatric disorders and physical ill-health (for review see Fries et al., 2009). An increase in CAR has also been linked to anticipation of daily stresses; hence research has found it to be increased in populations who experience elevated anxiety (Vreeburg et al., 2010). For example, an increased CAR was positively associated with stable (trait-like) measures of anxiety in a community sample of typically developing 10-12 year olds (n = 1768) (Greaves-Lord et al., 2007). The study aimed to test whether HPA-axis activity, including CAR, was associated with current self-reported anxiety symptoms and/or historical anxiety (measured by parents retrospectively reporting their child's anxious behaviours from aged 4). The study found higher cortisol levels and a higher CAR were associated with persistent and historical (and not current) symptoms of anxiety at preschool age, thereby emphasising its link to chronic anxiety (Greaves-Lord et al., 2007).

Given the high proportion of individuals with ASD who also experience anxiety, research has begun to explore the diurnal rhythm of cortisol in this population (Fries et al, 2009; Pruessner et al., 1997). The existing research exploring the CAR in children with ASD versus a typically developing population is however limited. More research is also needed to explore the nature of the diurnal rhythm of cortisol in children with ASD, and associations with reported levels of anxiety symptoms. Given the extensive research indicating discrepancies between multiple informants when rating childhood symptoms of mental health disorders in TD and ASD populations (De Los Reyes et al., 2015; White et al., 2009), the inclusion of an objective measure of stress, which could be theorised as linked to an individuals' experience of anxiety in a certain situation, is a valuable addition to this field of research. Measuring self and other – reported levels

of anxiety, and assessing for their correlation to levels of cortisol at each point in time, and general patterns of anxiety and cortisol rhythms could help establish which informant report is most closely linked to the individual's physiological experience of stress. This could therefore indicate for each group (TD and ASD), which informants' rating of anxiety would be most useful to rely on in having a more accurate reflection of that individuals' experience of anxiety.

2.1.7 Aims of the current research

The current study aimed to explore anxiety in adolescents diagnosed with ASD in a school setting, according to multiple informants. While capturing trait-like symptoms of anxiety (via questionnaires and interview), the study aimed to extend current findings to explore self-reported levels of anxiety and emotions more generally across the school day using an experiencing sampling technique. This allows researchers to consider contextual factors that might moderate feelings of anxious affect at any point in time. The study considered three factors that might be associated with anxiety in ASD in school; noise, group size and activity. In order to validate reported anxiety symptoms we measured the CAR, and cortisol level at each time point across the school day, to have an objective, physiological marker of stress, exploring whether this was associated with reported levels of affect and contextual factors within the school day, and might therefore be a useful way of monitoring and identifying stress and anxiety in this population.

2.1.7.1 Research hypotheses

(1) Parent, teacher and self-reported measures of anxiety may not correlate for either group, similar to previous research. Correlations between specific informants' ratings of higher anxiety and higher cortisol levels would indicate that this informant may be the most useful source of information about the physiologically experienced anxiety in each group.
(2) School specific factors such as noise, group size and activity would correlate with self-reported levels of anxiety, happiness and anger, in the ASD group. Higher levels of noise, bigger group sizes and unstructured activity would be expected to correlate with higher anxiety, lower happiness and higher anger in the ASD group.
(3) Individual differences such as trait anxiety and attentional control would be correlated with experience sampling anxiety (state anxiety) in each group. Higher levels of (trait) anxiety and less attentional control as rated by self-reported questionnaire measures would be expected to correlate with higher reported levels of anxiety on experience sampling data, in each group.
(4) There will be no significant difference in CAR between groups, and CAR will correlate with reported measures of anxiety in each group.

(5) Cortisol levels for each time point would correlate with reported self-rated emotion, for each group. Higher cortisol would be expected to correlate with higher anxiety, lower happiness and higher anger, as a physiological measure of stress.

2.2 Method

2.2.1 Analysis plan from hypotheses

(1) Explore group differences in reported anxiety across multiple informants
(2) Assess associations between anxiety as rated by multiple informants for each group using correlational analysis
(3) Explore associations between school specific factors and experience sampling anxiety in each group, using correlational analysis
(4) Explore correlations between general factors of anxiety (anxiety measured by questionnaires) and experience sampling emotion in each group
(5) Establish pattern of CAR for each group and association with anxiety measures
(6) Explore correlations of cortisol with questionnaire rated anxiety from multiple informants and self-reported experience sampling emotions

2.2.2 Participants

2.2.2.1 Inclusion Criteria

Participants were required to be in Years 7-10 (aged 11-15), attending mainstream secondary school, or special educational needs school. For inclusion in the ASD group, participants needed a diagnosis of ASD listed on their EHCP. To have a diagnosis of ASD on their EHCP, participants will have historically been assessed by a Child Psychiatrist or Psychologist, using the Autism Diagnostic Observation Schedule (ADOS, Lord, Rutter, DiLavore, & Risi, 1999) and Autism Diagnostic Interview (ADI, Rutter, Le Couteur, & Lord, 2003) and/or clinical judgement. Participants were required to have English as their first language, due to the number of written questionnaires to complete. Participants were required to have an IQ level above 70.

All participants were required to show elevated levels of worry at screening, as measured by the Spence Children's Anxiety Scale (SCAS) (Generalised anxiety items) (Spence, 1998).

2.2.2.2 Exclusion Criteria

All participants were excluded if, after screening they scored below 8 on the screening task for worry. Participants in the TD group were excluded if they had a diagnosis of ASD in their EHCP. TD participants were also excluded if they scored above 30 on the Autism Quotient

Questionnaire: Adolescent version (AQ-Adol) (Baron-Cohen, 2006). In the TD group, all scores were < 30 (so no participant was excluded).

Following advice from expert consensus panel guidelines (Stalder et al., 2016), which suggest case-by-case evaluation and exclusion of participants on glucocorticoid medication or with endocrine disorders, no participants were excluded.

2.2.3 Screening Procedure

102 typically developing (TD) male pupils were sent opt-out consent forms to their home address, informing them of the 6-item screening questionnaire they would be asked to complete as part of a study looking at anxiety in ASD. One parent contacted the school to opt-out of participation; the remaining 101 pupils completed initial screening.

Adolescents diagnosed with ASD were recruited from three schools in the South of England, all of which are commissioned to provide specific provisions for pupils with Autism Spectrum Disorder, within close proximity to each other, in the researcher's geographical location. Schools were selected as they had previously been involved in research and expressed a wish to continue this involvement. Thirty male pupils in Years 7-10 with ASD were identified from a Special Educational Needs School, and 13 from two mainstream secondary schools. The parents of these pupils were sent opt-in consent forms, requesting permission for their son to complete the 6-item screening questionnaire. Of these 43, 29 returned consent and completed the screening, 12 were not returned, and 2 declined, stating their child was "too anxious".

Twenty-three (/101; 23%) TD (see below for details) and 16 (/29; 55%) adolescents with ASD were eligible to take part in the further study (see below). Of the 16 eligible adolescents with ASD, 12 adolescents and families took part in the final study[1]. Of the 23 TD pupils identified, 14 agreed to further participation[2].

2.2.3.1 Sample characteristics after screening

After screening, the final sample included 12 pupils (M = 13.1 years, SD = 1.24) with ASD and 14 TD pupils (M = 13.07 years, SD = 1.22). A Mann-Whitney test indicated there were no significant differences between scores from ASD participants (*Mdn* = 9.50) and TD participants (*Mdn* = 9.50) on the 6-item screening questionnaire (SCAS); $U = 73.00$, $z = -.57$, $p = .57$. This suggests participants were self-reporting similar levels of worry at initial screening

[1]The families of two adolescents with ASD identified as eligible declined further participation. A further two eligible participants with ASD completed the screening at school after the designated end of data collection date were contacted to inform them that they had reported elevated anxiety scores but would not be invited to take further part in the study. Parents were given information about anxiety if they had further concerns.

[2] Three pupils declined participation due to personal reasons, two declined as they were moving out of area, and four could not be contacted by the researcher.

(mean scores can be seen in Table 5). There was a significant difference in scores on the AQ, between the ASD group (M = 38.67, SD = 6.98) and the TD group (M = 18.68, SD = 8.62) ($t(24) = 6.42$, $p < .001$; see Table 5).

Participants in each group were matched for age and IQ. An independent t-test showed there were no significant differences in estimated full-scale IQ between the ASD group (M = 95.00, SD = 11.59) and TD group (M = 102.07, SD = 8.86); $t(24) = 1.76$, $p = .09$). However, the TD group scored significantly higher on the Vocabulary task (M = 52.57, SD = 5.12), measuring verbal ability compared with the ASD group (M = 46.59, SD = 6.18); $t(24) = 2.70$, $p = .012$. Full sample characteristics are shown in Table 5.

Four participants reported to taking medication (methylphenidates) for ADHD, and one participant took anti-rejection medication following a liver transplant. All participants taking medication orally did so at least one hour prior to or after taking their saliva samples.

2.2.4 Design

The study consisted of an initial screening procedure to identify participants for the main study. The subsequent main study comprised of 2 parts; the experience sampling component of the project was a between subjects repeated measures 2 (Typically developing and ASD group) x 5 (measurements across 5 time points) design, comparing emotional responses and associated factors (i.e., affect, noise, social size) in young people with or without ASD, across 2 consecutive school days.

2.2.4.1 Screening measures

Anxiety symptoms. The Spence Children's Anxiety Scale (SCAS) (Spence, 1998) was used to screen all participants for self-reported symptoms of generalised anxiety disorder (GAD). This subscale is appropriate for children aged 7 to 16 years and consists of 6 items on which participants rate the frequency they experience the symptom; never (0), sometimes (1), often (2), always (3), which yields a total score between 0–18. Pupils scoring 8 or above on the 6 item SCAS screening task were invited to take part in further measures. Scores above 8 indicate one standard deviation above the mean score for males aged 8-15 on this measure (Spence, 1998), indicating higher than average scores of worry.

Cognitive ability. Two subtests of the Wechsler Abbreviated Scale of Intelligence Second Edition (WASI - II) (Wechsler, 1999) were used to assess cognitive ability. The two subtests associated with verbal ability and perceptual reasoning (i.e., vocabulary and matrix reasoning) were completed by the first author (EL) at the adolescent's home or in school and can be used to

estimate general cognitive ability via a full-scale IQ score. This short form assessment has good test-retest reliability, α = .88 (Wechsler, 1999).

Autism traits. In order to ensure that no TD adolescent met criteria for elevated ASD symptoms, parents of all participants completed the Autism Quotient Questionnaire: Adolescent version (AQ-Adol) (Baron-Cohen, 2006); a 50-item scale to assess for symptoms of ASD designed for 12-15-year-olds to assess for traits of autism. Parents rate items by the extent to which they agree with the statements; definitely agree, slightly agree, slightly disagree and definitely disagree to generate a score range from 0 to 50 and where scores > 30 are considered to be elevated (Baron-Cohen, Hoekstra, Knickmeyer, & Wheelwright, 2006). This measure shows good internal consistency, with Cronbach's α =.79 for the whole scale, with test re-test reliability also being high (*r* =.92) (Baron-Cohen et al., 2006). In the current study, the measure also had good internal consistency (α =.94).

2.2.4.2 Main study Questionnaire Measures

Anxiety symptoms. We used the parent- and self-report versions of the SCAS (Spence, 1998) to measure anxiety symptoms. The current study used the SCAS total score, and this is made up of sub-scales measuring social phobia, separation anxiety, obsessive/compulsive, panic/agoraphobia and physical injury fears. Each item requires participants to judge how often they experience specific symptoms, from: never (0), sometimes (1), often (2) or always (3), and total anxiety scores range from 0 - 114. Research has found the SCAS to have good convergent validity and test-re-test reliability (Essau, Sasagawa, Anastassiou-Hadjicharalambous, Guzmán, & Ollendick, 2011; Spence, Barrett, & Turner, 2003). Self-reported and parent-report internal consistency is good (αs > .8); Essau et al., 2011; Nauta et al., 2004). Further research has demonstrated that the SCAS parent version also demonstrated good sensitivity and specificity when used to assess symptoms of anxiety in a sample with ASD, compared with a standardised clinical interview (Zainal et al., 2014). Internal consistency in the current study for parent and adolescent report was very good; α = .95 and .91 respectively.

Anxiety in school. We used the School Anxiety Scale - Teacher Report (SAS-TR) to measure feelings of anxiety in a school setting. The teacher rates how the child has been over the last 6 months across 16 items using a Likert scale of 0 to 3 (never = 0, sometimes = 1, often = 2 and always = 3). Total anxiety scores were calculated (possible score range = 0-48). The psychometric properties of the SAS-TR were assessed using a large sample of children aged 5-12 and results suggest good internal reliability (α = .93), along with good convergent and discriminant validity (Lyneham, Street, Abbott, & Rapee, 2008). The measure in the current study had high internal consistency, α =.96.

Anxiety Disorders. The Diagnostic Interview Schedule for Children Version 4 (DISC IV)-Anxiety Module, (Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000) is a structured interview assessment appropriate for use with parents of 6-18 year olds, to assess for diagnoses of psychiatric disorders, using criteria listed in DSM-IV-TR (APA, 2000) and ICD-10 (WHO, 2006). Questions regarding specific symptoms are followed in a stem/contingent structure by questions specifying frequency, duration and onset to establish diagnoses. The DISC-IV Anxiety module contains subsections of various types of anxiety disorders. For this study, we administered the Generalised Anxiety, Obsessive-Compulsive, Panic disorder, Separation Anxiety, Social Phobia and Specific Phobia modules. This structured interview was completed by parents, administered electronically, on an encrypted laptop, using an automatic scoring software, in either the adolescent's home or school environment and when the young person was not present. Total number of diagnoses met by each adolescent was recorded, along with total number of symptoms met across the 6 modules of anxiety disorders. The DISC-IV has been shown to have good test-retest reliability in comparison to earlier versions (Shaffer et al., 2000). Previous versions of the DISC have been shown to have good criterion validity, K = .62 for anxiety disorders (Schwab-Stone et al., 1996).

Attention Control. We measured adolescents' ability to shift and focus attention using the Attentional Control Scale (ACS) (Derryberry & Reed, 2002). This self-reported measure includes 20 items and uses a 4 point Likert scale from 1 = almost never, 2 = sometimes, 3 = often and 4 = always, to assess symptoms of attentional control (score range = 20-80). Judah, Grant, Mills and Lechner, (2014), demonstrated the measure to have good validity, correlating with other cognitive tasks that require attention control, such as switch trial performance tasks, and antisaccade performance. Higher scores indicate increased ability to control and sustain attention. Internal consistency in this study was good, with Cronbach's α =.81

Behavioural symptoms. We used the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997) to measure behavioural symptoms in each group. The SDQ is a 25-item questionnaire that is used to assess symptoms of common psychiatric difficulties in young people aged 4-17 years. The self-report, parent and teacher report versions were used in this study, asking participants to rate how true statements are (0 = not true, 1 = somewhat true, 2 = certainly true). The current study used the internalising difficulties (made up of emotional symptoms and peer relationship difficulties subscales) scale score. The possible range of scores in each scale is 0 to 20, with a maximum of 40 for total scores. Total scores between 14-16 (parent), 15-17 (self-report) or 12-15 (teacher) indicate slightly higher/lowered scores. Total scores between 17-19 (parent), 18-19 (self-report) or 16-18 (teacher) indicate high/low behaviours and scores between 20-40 (parent and self-report) or 19-40 (teacher) suggest very high/very low number of behaviours. The scale has been found to have good psychometric properties (Goodman, 2001). In the current study, Cronbach's alphas in the TD group were: parent report α = .67, teacher report α = .66 and self-

report α = .69, and in the ASD group: parent report α = .65, and above .7 for self and teacher report. This is similar to reported internal consistency of the measure in other research using similar populations (Salomone et al., 2014).

2.2.4.3 Experience Sampling

Hewlett Packard iPaq electronic palm-pilot devices were used to collect experience sampling measures throughout the day and at the same time participants were asked to provide a cortisol sample (see below). The palm-pilot prompted participants to answer a series of questions 5 times a day (wake, 30 minutes after wake, 11am, 3pm, and 7pm). Questions included a rating of current emotion, (anxiety, happiness and anger on a sliding scale of 0-10 and where increased scores indicate more intense emotion). They were also asked to indicate their activity, environment, noise level and number of other people present (0, 1-5, 6-10 or more than 10). The researcher demonstrated to each participant and their parent how to use the sliding scale to rate their emotions. Each emotion scale was accompanied by visual images depicting the varying ends the scale, e.g. happy/sad faces for 'happiness' rating. The researcher also discussed with each participant various examples of mood states, allowing them to practice rating their emotion on the scale. Participants were prompted to answer a series of yes/no questions in relation to their saliva sample (e.g. had they exercised in the last 30 minutes). At the end of each day, participants were prompted to indicate whether anything unusual had occurred that day, and if so, to detail it in a diary-like entry. Data was stored on the device as an electronic file.

2.2.4.4 Cortisol measures

Participants provided cortisol samples using Salivette® Code Blue Cortisol sample collection kits (saliva sampling device, Sarstedt Ltd, Leicester, UK). The researcher demonstrated to participants and their parents how to collect their sample, by chewing the dental swab for 30 seconds, then returning to the Salivette® plastic container. Participants were instructed not to eat, drink (other than water), exercise or brush their teeth in the 30 minutes before taking their saliva sample, as these factors have been suggested to influence cortisol secretion (Clow et al., 2004; Hill et al., 2008; Rosmond, Holm, & Björntorp, 2000; Stalder et al., 2016). Participants were given ziplock bags, labelled according to Day 1 or Day 2, each containing 5 Salivette® containers, each labelled with the time of sample, day, and participant ID number. Participants and parents were informed of the importance of adhering to sampling at the correct time, using the correct Salivette®, as variations in actual sampling time from procedural sampling time have been showed to reduce the accuracy of plotting cortisol patterns, especially the CAR (Stalder et al., 2016).

Participants provided these samples at each time point of the experience sampling, and were prompted by the electronic palm-pilot device to indicate whether they had taken their sample,

and whether or not they had eaten, drunk, exercised or brushed their teeth in the 30 minutes before the sample.

Participants stored their samples in a small pouch, containing the electronic device, for the duration of the 2 days' experience sampling procedure. In reviewing CAR procedures, Stalder et al. (2016) acknowledge that salivary cortisol will remain relatively stable for up to 5 days at room temperature. Samples were then collected by the researcher the day after the participants completed the study, and stored in a locked freezer, below -20C. Saliva samples were sent for analysis of free cortisol levels at the Molecular Biology and Genetics Laboratory, Ruhr-University Bochum, Germany. Each cortisol assay was analysed twice, to calculate a mean cortisol level for each sample given.

2.2.5 Procedure

Headteachers from three schools in the South of England gave consent for their school's participation in this study. Male pupils in a mainstream and secondary schools completed initial screening as detailed above. Following screening, written consent for participation was obtained by the researcher from the pupil, their parent and their form tutor. The researcher met with each participant and their parent, to administer the two items of the WASI-II with the participant, complete the DISC-IV interview with the parent, the series of questionnaire measures, and outline the protocol for the experience sampling procedure and cortisol sampling. These sessions took place at the participant's home or at school, and lasted between 60-90 minutes.

Participants were visited by the researcher the day prior to commencing the experience sampling part of the study. The researcher provided the palm-pilot electronic devices and Salivette® cortisol collection kits, and reiterated procedural instructions with the adolescent and parent. Each participant completed 2 consecutive school days of experience sampling at five time points (making a maximum of 10 data points for each participant), including the provision of saliva samples. The researcher then met with the participant again the day after experience sampling, to collect the palm-pilot and saliva samples.

Study adherence rates for the experience sampling procedure were 96.9%; out of a possible 260 data points, data was collected at 252 time points. Two participants (1 ASD, 1 TD) missed one time sample, one participant with ASD missed two time samples, and one TD participant missed the first four time samples. Two cortisol samples were unable to be processed; therefore 0.8% of cortisol data was missing. Completion rate of questionnaire measures from participants, parents and teachers was 100%, with missing data within questionnaires ranging from 0 - 1.2% and with no one participant having more than 10% of missing data across all

questionnaires. Due to the equal spread of missing data points across questionnaire measures, missing data values were replaced with the series mean before scores were calculated.

2.2.6 Ethics

Ethical approval was obtained from the University of Southampton Research Governance Office, and the Hampshire Research Ethics Committee (IRAS Number 203085).

2.3 Results

2.3.1 Approach to cortisol analysis

The CAR and area under the curve (AUC) across the five cortisol samples (i.e., the mean of each time point for each day) were the two main indices of cortisol levels. The CAR was calculated by subtracting the immediate wake level of cortisol from the 30 minutes after wake level (Time 2 - Time 1). Preliminary analysis compared the CAR between days and because this was not significantly different the days were collapsed to provide an overall CAR for each group.

Cortisol measurements for each time point and day were positively skewed and non-normally distributed, therefore we log transformed the data (base 10) (see Tomarken et al., 2015). Further analysis showed the log transformed data was normally distributed, so all analyses were carried out using this data and non-transformed data is reported in tables and figures. Cortisol at each time point was compared across the two days using a repeated measures ANOVA for 2 Day (Day 1, Day 2) by 5 Time (wake, 30 minutes +wake, 11am, 3pm, 7pm) repeated for day and time. As the main effect of Day was non-significant and there was no interaction between Day and time, the cortisol measures were collapsed for each time point to provide 5 values. AUC was calculated for the mean sample (Day 1 and 2 combined) across all five time points (figure 4).

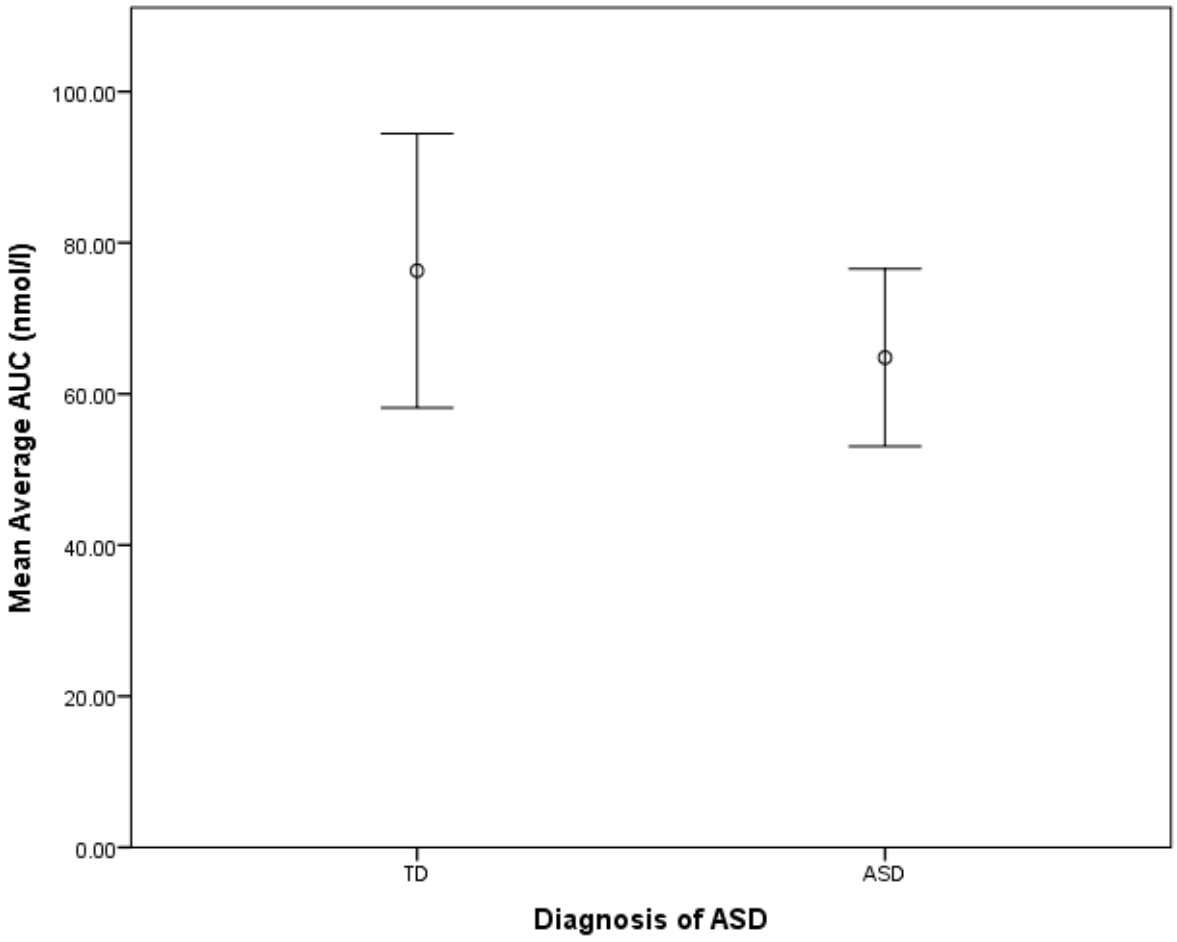

Figure 4. Mean AUC for each group, ASD and TD, calculated by combining the average cortisol levels at each time point across the two days

Normal distribution was assessed separately according to group (TD or ASD) for each scale used. The majority of data was found to be normally distributed and have homogeneity of variance. However, teacher-reported data from the SAS-TR, and SDQ (Internalising scale) were not normally distributed, nor was the total number of diagnoses met on the DISC-IV. The 6-item SCAS screening questionnaire and self-report experience sampling data scores for anxiety, happiness, anger and noise were also found not to be normally distributed (in all cases Shapiro-Wilk <.9, $p < .05$), therefore non-parametric tests were used. As sample sizes were unequal, when using parametric tests, pooled variance estimate t-tests were calculated, to account for unequal sample sizes by weighting each sample's variance. When performing correlational analysis, Bonferroni corrections were applied to account for performing multiple correlations, by dividing the significance value (p = .05) by number of analyses run. Correlations reaching statistical significance without Bonferroni adjustments are also reported, and their interpretation is made with caution, due to the recognised potential effect of multiple comparisons.

Table 5:

The mean, SD and range for ASD, TD and total group scores on; age, cognitive ability (IQ and subscales), self-reported anxiety and school anxiety, parent and teacher reported anxiety symptoms and self-reported attention

Group		**ASD**			**TD**		**Total**		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Sample characteristics									
Age	13.10	1.24	11.3-15	13.07	1.22	11.4-14.8	13.08	1.20	11.5-15.0
Full Scale IQ	95.00	11.59	72-109	102.07	8.86	84-117	98.81	10.62	72-117
Vocabulary	46.58	6.18	36-54	52.57	5.12	44-62	49.81	6.30	36-62
Matrix reasoning	47.50	9.57	31-58	50.00	7.55	33-62	48.85	8.46	31-62
SCAS anxiety									
GAD screen	10.25	2.92	8-18	11.14	3.25	8-17	10.73	3.08	8-18
Parent	45.20	18.06	15-68	21.05	13.66	3-42	32.20	19.78	3-68
Adolescent	31.30	13.97	6-53	45.53	17.68	18-81	38.96	17.34	6-81
SDQ internalising									
Parent	14.62	2.90	10-20	6.57	5.01	0-17	10.29	5.79	0-20
Adolescent	9.75	3.91	3-17	7.14	3.03	2-11	8.35	3.64	2-17
Teacher	10.42	4.88	4-17	1.71	2.46	0-8	5.73	5.76	0-17
Other measures									
Teacher report School anxiety	18.89	8.26	10-35	6.38	4.07	0-14	12.15	8.89	0-35
Self report Attentional control	41.71	9.22	21-54	48.38	6.00	36-61	45.30	8.22	21-61

2.3.2 Group differences

Pooled variance t-tests showed that according to the SCAS, parents reported significantly more symptoms for the ASD group (M = 45.20, SD = 18.06) compared with the TD group (M = 21.05, SD = 13.66; $t(24) = 3.88$, $p < .001$. In contrast, the TD group rated themselves as more anxious (M = 45.53, SD = 17.68) compared with the ASD group (M = 31.30, SD = 13.97), and this difference was significant ($t(24) = 2.25$, $p = .03$) (see table 5).

Consistent with the SCAS, on the DISC-IV, parents of adolescents with ASD reported significantly more symptoms of anxiety (M = 26.75, SD = 7.20) than parents of TD adolescents (M = 15.29, SD = 10.04; $t(24) = 3.29$, $p < .001$). The total number of diagnoses met on the DISC-IV was significantly lower in the TD group (*Mdn* = 2.00) than in the ASD group (*Mdn* = 3.50), $U = 43.00$; z = -2.19, $p = .03$, $r = -.43$).

Parents reported significantly more internalising difficulties for adolescents with ASD (M = 14.62, SD = 2.90) than TD peers (M = 6.57, SD = 5.01), $t(24) = 4.89$, $p < .001$, see Table 5). Similarly, teachers rated participants with ASD as having significantly more internalising difficulties (*Mdn* = 11.00) than the TD group (*Mdn* = 0.50); $U = 8.00$, z = -3.95, $p < .001$. Teacher reported School Anxiety for the ASD group (*Mdn* = 15.50) was also significantly higher than the TD group (*Mdn* = 6.00; $U = 7.00$, z = -3.96, $p < .001$). However, self-reported symptoms for the ASD group (M = 9.75, SD = 3.91) and TD group (M = 7.14, SD = 3.03) on the SDQ internalising scale were not significantly different ($t(24) = 1.92$, $p = .07$).

On the self-reported measure of attentional control, the TD group showed significantly higher scores (M = 48.38, SD = 6.00) than the ASD group (M = 41.71, SD = 9.22); $t(24) = 2.20$, $p = .04$). This suggests that TD pupils reported having higher levels of control over their attention than their peers with ASD.

2.3.2.1 Experience sampling data[3]

Average emotion levels were calculated for each participant using their scores for each of the 10 time points across the 2 days, to give average anxiety, anger/frustration and happiness scores (possible range = 0 – 10; see Table 6). The Mann Whitney test indicated significantly lower average level of self-reported anxiety in the ASD group (*Mdn* = 1.25), compared to the typically developing group (*Mdn* = 4.05); $U = 16.50$, z = -2.87, $p = .003$). Participants with ASD showed significantly higher self-reported levels of happiness (*Mdn* = 7.65) than the TD group (*Mdn* = 6.10); $U = 18.50$, z = -2.7, $p = .004$, and significantly lower levels of anger (*Mdn* = 0.80) than the TD group (*Mdn* = 3.70); $U = 18.00$, z = -2.77, $p = .004$.

Additional information related to the context in which emotions were made include noise levels, activity (structured or unstructured) and number of people in the social group. The average noise level (possible range = 0-10) was significantly higher for the TD group (*Mdn* = 2.85), compared with the ASD group (*Mdn* = 1.40); $U = 19.00$, z = -3.34, $p < .001$).

[3] Experience sampling data also assessed compliance with recommended best practice guidelines (Stalder et al., 2016), regarding refraining from eating or drinking, brushing teeth or exercising in the 30 minutes before taking the salivary cortisol sample. At each time point, participants were asked to indicate yes/no as to whether they had complied with this advice. Overall, 79% of participants indicated they had not eaten/drunk anything in the 30 minutes prior to their sample, 91.7% had not exercised, and 95.6% had not brushed their teeth.

There was no significant difference in activity (structured or unstructured) between the groups, $U = 7654.5$, $z = -.57$, $p = .64$ at each time point through the day. There was a significant difference in social group size between the TD group ($Mdn = 2.00$) and the ASD group ($Mdn = 2.00$); $U = 6849.00$, $z = -2.01$, $p = .04$, with ASD adolescents being in significantly larger social groups than the TD participants (table 6).

Table 6:

Mean, SD and range for experience sampling data (emotional measures and noise) and CAR, for ASD (117 data points) and TD groups (135 data points), and frequency responses for activity and social status from experience sampling

Measure	**ASD (n=12)**			**TD (n=14)**		
	Mean	SD	Range (0-10)	Mean	SD	Range (0-10)
Emotional Measures						
Anxiety	1.36	1.19	0-3	3.85	1.921	1-7
Positive Affect	7.92	1.17	6-9.80	6.23	1.20	4.50-8.60
Anger	1.15	1.13	0-2.90	3.25	1.32	1.40-5.20
Noise	1.62	0.65	0.67-2.50	2.89	0.93	1.50-4.10
Cortisol						
CAR	-2.42nmol/l	9.46	-22.31 - 11.35	8.04nmol/l	12.20	-7.45 - 32.27
AUC	64.80nmol/l	18.49	38.01 - 93.75	76.29nmol/l	31.41	45.90 - 117.24
Activity	Structured	Unstructured		Structured	Unstructured	
	27 (23.1%)	90 (76.9%)		27 (20%)	108 (80%)	
Social Status	Alone	Small group	Large group	Alone	Small group	Large group
	22 (18.9%)	66 (56.4%)	29 (24.8%)	39 (28.9%)	72 (53.3%)	24 (17.8%)

CAR = Cortisol Awakening Response, measuring increasing in cortisol from wake to 30 minutes after wake, AUC = Area under the curve, measuring total cortisol level in the day. Social Status = Alone (1), Small group (2), Large group (3). Activity = Structured (1), Unstructured (2)

2.3.3 Hypothesis (1); Association between multiple informants

Agreement between informants (parent, adolescent and teacher) was generally poor both within groups and for the two groups collapsed and between questionnaire measures and experience sampling data, with most correlations between informants being non-significant.

For the ASD group, there was a significant association between self-reported anxiety on SCAS with total number of anxiety symptoms on the DISC-IV and total diagnoses met on the DISC-IV, as rated by parents (r_s = .64, p < .05*;* r_s = .59, p < .05). This pattern was also replicated in the TD group; r_s = .72, p < .01*;* r_s = .64, p < .05, indicating that in both groups, higher total self-reported anxiety was associated with more parent-reported symptoms of anxiety and diagnoses met on the DISC-IV.

2.3.4 Hypothesis (2); Association between school specific factors and anxiety

Data from the experience sampling component of the study was analysed using correlations to look for associations between reported mood, noise, activity, social status and cortisol at each specific time point. Associations were explored separately for each group (see Appendix AB) and for both groups combined (see Table 7). Noise, activity and social status were not significantly associated with self-reported anxiety, happiness or anger at each time point, from the experience sampling data for either group. This indicates that these school specific factors were no more influential in the ASD group than the TD group, as previously hypothesised.

Cortisol level was significantly associated with reported noise level, where lower noise was associated with higher cortisol levels for both groups. In addition, across both groups higher cortisol levels were associated with unstructured activity and smaller social groups were associated with higher cortisol levels (r_s*s* >.22, *ps* <.008, adjusted using Bonferroni corrections to account for multiple comparisons*).* Considering associations between contextual factors, the results showed that higher noise levels were significantly associated with structured activities (such as being in lesson or playing games) and larger social groups (r_s*s* >.33, *ps* <.008, Bonferroni adjusted), in both groups.

Table 7:

Spearman's rho correlations between mood, noise, cortisol, activity and social status from experience sampling data for both groups. Significant associations are highlighted.

Measure	1	2	3	4	5	6	7
1. Anxiety	-	**.66*****	**-.62*****	**.23*****	-0.02	-0.01	0.06
2. Anger		-	**-.55*****	**.24*****	-0.04	0.00	-0.02
3. Positive Affect			-	**-.12***	-0.09	-0.04	0.04
4. Noise				-	**-.22*****	**-.26*****	**.33*****
5. Cortisol					-	**.22*****	**-.20*****
6. Activity						-	**-.39*****
7. Social Status							-

** p < .05, ** p < .01, *** p < .008 (Bonferroni corrections for multiple comparisons)*

2.3.5 Hypothesis (3); Association between individual factors and self-reported experience sampling data

2.3.5.1 ASD

Attention control was negatively associated with mean anger and anxiety ratings from experience sampling data, indicating higher levels of attentional control were associated with lower day-to-day anger and anxiety. This association did not meet significance when Bonferroni adjustments were applied.

2.3.5.2 TD

Total anxiety on SCAS self-report was significantly correlated with mean anxiety ratings from experience sampling data, suggesting that higher day-to-day anxiety ratings were associated with anxiety scores on self-report SCAS.

Attention control was negatively associated with anxiety ratings from experience sampling data, suggesting those with higher attentional control reported less day-to-day anxiety. Attention control was positively correlated with positive affect ratings from experience sampling, suggesting higher attention control was linked to higher happiness (table 9). As with the ASD group, these associations met statistical significance at $p < .05$, but did not meet Bonferroni adjusted significance.

2.3.6 Hypothesis (4); Pattern of CAR and association with anxiety measures

Analysis exploring CAR between groups highlighted a significant difference in CAR between the ASD group (M = -2.42, SD = 9.46) and TD group (M = 8.04, SD = 12.20), $t(24) = 2.41$, $p = .02$). The TD group displayed a significantly larger CAR than the ASD group.

A two-way repeated measures ANOVA was used to explore effects of group (ASD, TD) and 5 times of sampling (wake, 30+wake, 11am, 3pm and 7pm) on cortisol levels. Mauchly's test for sphericity was not significant for the effect of time, $\chi^2(9) = 11.38$, $p = .25$, therefore sphericity was assumed. There was a significant main effect of time on cortisol levels $F(4, 96) = 155.73$, $p < .001$ (figure 5). Post-hoc tests using the Bonferroni corrections indicate cortisol was significantly higher at Time 1 (wake) (M = 24.26, SD = 7.97) than at Time 3 (11am) (M = 7.37, SD = 3.64), Time 4 (3pm) (M = 7.70, SD = 6. 21) or Time 5 (7pm) (M = 3.99, SD = 3.26), but not significantly higher than Time 2 (30+wake) (M = 27.48, SD = 12.22). Cortisol was also significantly higher at Time 2 than at Time 3, 4 or 5. Post hoc comparisons also indicate that Time 5 cortisol levels were significantly lower than Time 3 or 4 levels. There was no significant effect of group (ASD vs TD); $F(1, 24) = 1.07$, $p = .31$, indicating cortisol levels were similar across the groups collapsed across each time point. There was also no significant group and Time interaction; $F(4, 96) = 1.31$, $p = .27$ (figure 5).

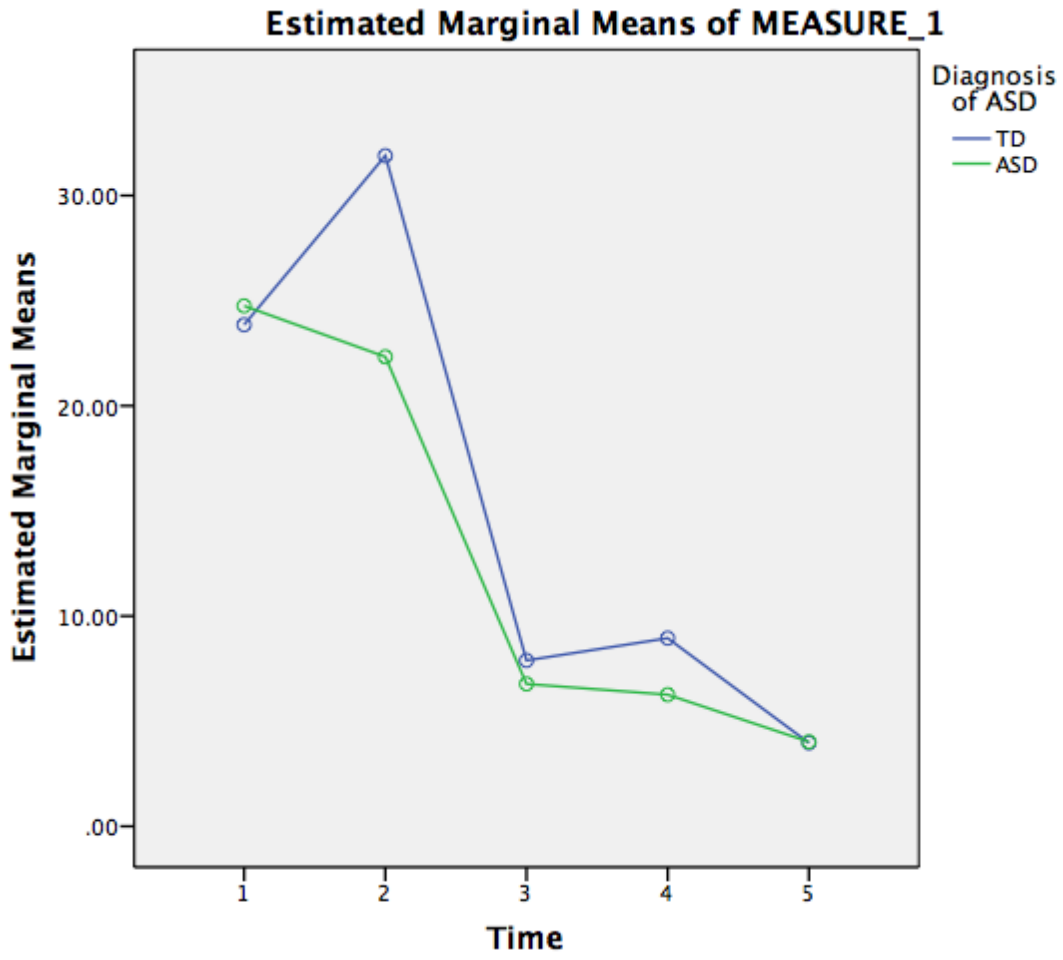

Figure 5: Estimated marginal mean cortisol levels (nmol/l) at each of the time points, collapsed across the two days, showing significant effect of time on cortisol levels, but no effect of group and no group x time interaction.

2.3.6.1 ASD

With respect to associations within groups and the CAR, teacher-reported internalising difficulties was positively correlated with mean CAR, (r_s = .71, p = .009), suggesting more internalising difficulties were associated with a higher CAR.

2.3.6.2 TD

Scores on teacher-reported internalising difficulties were negatively associated with CAR, (r_s = .66, p = .009), suggesting fewer internalising difficulties were associated with a higher CAR.

2.3.7 Hypothesis (5); Association of cortisol with experience sampling data and questionnaire measures

2.3.7.1 ASD

AUC was significantly positively correlated with attention control, and negatively associated with self-reported total anxiety score on the SCAS, self-reported internalising difficulties and mean anger scores from experience sampling; indicating higher total levels of cortisol were associated with higher reported attentional control, lower self-reported anxiety and lower day to day anger ratings (table 8).

2.3.7.2 TD

There were no significant correlations between cortisol or total AUC and any anxiety measures.

Table 8

Correlations between measures for ASD group. Significant correlations are highlighted. Non-parametric correlations are reported in italics for non-normal data

Measure	1	2	3	4	5	6	7	8	9	10	11
Anxiety											
1. SCAS Parent	-	0.36	**.60***	-0.06	*0.31*	-0.13	*0.47*	*-0.40*	*0.50*	0.34	-0.36
2. SCAS Child		-	0.24	**.67***	*0.32*	**-.64***	***.64****	*-0.48*	*0.55*	0.21	**-0.84****
3. SDQ Internalising scale - Parent			-	0.28	*0.30*	-.11	*-0.00*	*-0.16*	*0.09*	0.52	-0.17
4. SDQ Internalising scale -Self-report				-	*-0.04*	-0.30	*0.15*	*-0.13*	*0.15*	-0.06	-0.43
5. SDQ Internalising scale -Teacher					-	*-.24*	*-0.00*	*-0.18*	*-0.04*	***0.71****	*-0.43*
Attention											
6. ACS							***-.73****	*0.57*	***-0.66****	-0.35	**0.87****
Experience Sampling											
7. Mean anxiety							-	***-.80*****	***0.84****	*0.22*	***-0.79****
8. Mean happiness								-	***-0.70****	*-0.45*	*0.60*
9. Mean anger									-	*0.26*	***-0.76****
Cortisol											
10. Mean CAR										-	-0.30
11. Mean AUC											-

** p < .05, ** p < .01, *** p < .005 (Bonferroni adjustment for multiple comparisons)*

Table 9:

Correlations between measures for TD group. Significant correlations are highlighted. Non-parametric correlations are reported in italics for non-normal data

Measure	**1**	**2**	**3**	**4**	***5***	**6**	**7**	**8**	**9**	**10**	**11**
Anxiety											
1. SCAS Parent	-	0.48	**0.83*** **	0.52	*0.08*	-0.18	*0.02*	*0.02*	*-0.04*	-0.01	0.21
2. SCAS Child		-	0.44	**0.87*** **	*-0.11*	-0.47	***.72*** **	*-0.40*	*0.38*	0.10	0.07
3. SDQ Internalising scale - Parent			-	0.51	*0.06*	-0.22	*0.20*	*-0.19*	*-0.00*	-0.19	0.08
4. SDQ Internalising scale -Self-report				-	*-0.35*	-0.42	***.71*** **	*-0.46*	*0.47*	0.21	0.25
5. SDQ Internalising scale -Teacher					-	*0.32*	*-0.10*	*-0.00*	*-0.10*	***-0.66****	*-0.41*
Attention											
6. ACS						-	***-.73*** **	***.62****	*-0.42*	-0.05	-0.39
Experience Sampling											
7. Mean anxiety							-	***-.76*** **	***0.79*****	*-0.02*	*-0.03*
8. Mean happiness								-	***-0.87*****	*-0.01*	*0.17*
9. Mean anger									-	*0.10*	*-0.30*
Cortisol											
10. Mean CAR										-	**0.60***
11. Mean AUC											-

** p < .05, ** p < .01, *** p < .005 (Bonferroni adjustment for multiple comparisons)*

2.4 Discussion

2.4.1 Summary of findings

This study aimed to develop a more comprehensive understanding of the CAR in adolescents with ASD, compared to their typically developing peers, and explored factors that may be associated with this response (i.e. reported affect, social group and activity). We explored whether CAR and cortisol levels were associated with multiple informants reports of anxiety. Furthermore, we hoped to develop a wider understanding of self-reported affect across the school day, using experience sampling methods, and whether this was associated with cortisol levels. The study explored whether school specific factors (e.g. group work) or more general factors (e.g. attentional control) were associated with affect in each group.

The findings suggested that this group of adolescents with ASD did not show the same pattern of CAR as the TD group. The TD group displayed the expected pattern of increase in cortisol 30 minutes after waking (Pruessner et al., 1997), however, the ASD group showed a significantly flattened CAR in comparison. This is in contrast to the findings of the meta-analysis in Chapter 1 and based on existing findings, but does support findings from other similar studies that have suggested an absence of CAR in ASD populations (Brosnan et al., 2009).

Findings of a flattened CAR in the ASD group may support previous research suggesting chronic stress and anxiety is associated with a reduced CAR (Chida & Steptoe, 2009). Using models of anxiety in ASD (Boulter et al., 2014; Wood & Gadow, 2010), it could be conceptualised that stable, trait factors associated with the pathway to anxiety in ASD, such as rigid thought, social confusion, and sensory difficulties, lead to chronic vulnerability and experience of anxiety, as people with ASD may always experience these ASD-related stressors, given the neurodevelopmental nature of the disorder. This is also consistent with previous research that indicates trait anxiety was negatively associated with a peak in CAR (Walker et al., 2011).

Whilst the study found significant differences in CAR between the groups, there was no significant difference in AUC; the total volume of cortisol released by an individual each day. This suggests that both groups experienced the same overall levels of physiological arousal. This contrasts to the experience sampling data which showed significantly higher levels of self-reported negative affect for the TD group. These findings could support previous research suggesting participants with ASD have difficulties recognising and interpreting their own emotions (Hill et al., 2004), as the difference in experience sampling data but not overall cortisol levels could represent deficits in recognising the body's emotional experience of anxiety. However, there may have been alternative sources of physiological arousal for participants. It may have been that other factors,

such as exposures to stressors at times other than the specified sampling time, led to an increase in cortisol, which could be reflected in participants samples but might not link with their rated anxiety.

AUC, whilst not being significantly different between the groups, was significantly correlated with self-reported anxiety on the SCAS and on the experience sampling data, with higher AUC associated with lower anxiety reports, only in the ASD group. This is the opposite of what might be expected, as AUC represents total cortisol levels in the day, and with cortisol being a stress hormone, one would expect higher levels to be associated with higher stress.

In both groups, experience sampling reports of anxiety did not correlate with cortisol levels at the same time point. Experience sampling levels of anxiety did however significantly correlate with social status in the TD group, suggesting that higher anxiety was associated with bigger group sizes. This pattern was not true for the ASD group, which might have been expected, given the conceptualisation of anxiety in ASD incorporating IU and the prediction that greater social size might lead to more unpredictable social situations and therefore increase the likelihood of anxiety (Boulter et al., 2014).

For both groups, the experience sampling data suggests higher cortisol levels were associated with lower noise, unstructured activity and large group sizes. It was anticipated, given the Wood & Gadow (2010) model, that these 3 factors would be specifically associated with anxiety in the ASD group, and therefore associations in both groups suggests these factors may not have affected anxiety in ASD more than they did in the TD group. Noise, which could contribute to sensory difficulties in ASD, was not associated with felt negative affect in the ASD, but was significantly correlated with it in TD group. The finding that higher noise levels were associated with lower cortisol levels is somewhat unexpected, as, especially in the ASD group, it would be expected that higher noise may lead to more sensory difficulties, which may lead to higher stress and therefore higher cortisol levels. More research is needed to explore the relationship between noise and cortisol. One hypothesis may be that higher cortisol levels were found in the morning, as expected by the diurnal rhythm, and this would also be the time when participants were at home/in bed and therefore noise levels were reduced in comparison to later time samples where noise is higher due to school environment, but cortisol is lower. However, noise was measured subjectively, and individuals will vary in their sensitivity and rating of noise. Therefore, differences in noise may not reflect actual variation in noise level, which would need to be measured objectively, before conclusions regarding noise and its association with cortisol could be drawn.

Data from the experience sampling procedure does not indicate any school-specific factors that were associated with anxiety in ASD, but instead suggested larger group size, higher noise levels and unstructured activities were associated with higher anxiety in the TD group.

Whilst there were no significant differences in estimated overall cognitive ability, the ASD participants had significantly lower verbal ability than the TD group. Increased verbal ability has previously been positively correlated to higher self-reported anxiety symptoms (Grondhuis & Aman, 2012). Having lower verbal ability in the ASD group may have led to difficulties understanding the questionnaire and experience sampling questions fully. This may explain the significantly lower levels of anxiety self-reported by the ASD group compared to their peers, across both questionnaire and experience sampling methods. This also makes it hard to draw conclusions on the lack of school-specific factors found to be associated with anxiety in ASD which one might expect (Boulter et al., 2014; Wood & Gadow, 2010).

Results show that for both groups, self-reported total anxiety on SCAS was positively associated with number of anxiety symptoms reported on the DISC-IV by parents, which may indicate that the semi-structured interview with parents is a useful way of measuring anxiety in both populations. However, aside from this, agreement between multiple informants was poor. The study consistently found that parent and teacher reports of anxiety in ASD were higher than the adolescents' ratings of their own anxiety. In reversal, the TD group rated themselves higher for anxiety across questionnaire and experience sampling measures. These informant discrepancies are consistent with current literature regarding multiple informants (De Los Reyes & Kazdin, 2005). The contrasting pattern of rated anxiety symptoms between the ASD and TD group may be conceptualised by De Los Reyes and Kazdin's (2005) Attribution Bias Context Model, which suggests that the informant's perspective and attribution of the behaviour will influence their report. As awareness of one's own psychological state is commonly impaired in ASD (Williams, 2010), the tendency for parents and teachers to report higher anxiety symptoms than the individual themselves might reflect this difficulty. Similarly, in the TD group, their own perspective and ability to recognise their own internal emotional state may have led to higher self-report of anxiety, and supports research findings that internalising disorders, being less observable in terms of behaviour, may produce higher multiple informant discrepancies (De Los Reyes & Kazdin, 2005; Smith, 2007). Our results are consistent with Smith (2007), who predicted that for TD adolescents with internalising disorders, the best source of informant would be the adolescent themselves, then parent and teacher. Our findings, potentially indicating under-reporting of anxiety symptoms by adolescents with ASD are consistent with previous research by White, Schry and Maddox (2012) who found that adolescents with ASD under-reported symptoms of anxiety compared to parent and clinician report.

Whilst the discrepancies in informant report of anxiety symptoms in both groups are consistent with previous research, it is crucial to consider the lack of validated measures for assessing anxiety in ASD (MacNeil et al., 2009). Lower self-reported symptoms of anxiety in the ASD group may reflect our use of questionnaire measures designed for the general population.

Previous reviewers summarise current literature regarding the role of attentional control in the disposition to anxiety (Hadwin et al., 2016). This review highlights consistent findings of higher attentional control being associated with lower levels of anxiety or vulnerability to anxiety, which was replicated in our study, in both groups.

2.4.2 Methodological limitations

The experience sampling procedure relied on participants and their families reporting what time they awoke on average in the mornings, so that the devices used could be pre-programmed to alarm at this time for each participant. Whilst this ensures the procedure was individualised for participant's personal sleep/wake cycle, it relies on participants waking at the time they stated. If participants woke naturally before their alarm, this would initiate the CAR, and therefore samples taken at recorded 'wake' time, may instead reflect the already rising levels of cortisol. Errors in recorded wake time and natural wake time have been reported to affect reliability of CAR data (Stalder et al., 2016); delays in immediate wake sampling can significantly reduce the estimate of the CAR. In future, research procedures should seek to use devices that allow participants to input measures manually as soon as they wake each morning, rather than specified alarm wake time.

Whilst having an all-male sample restricts the findings' generalisability to the wider population, our findings of a flattened CAR do support findings from research involving a female sample of adolescents with ASD who also showed a dysregulated CAR and HPA-axis activity (Sharpley, Bitsika, Andronicos, & Agnew, 2016). However, more research is needed with larger mixed gender samples, as the evidence is still tentative and mixed regarding the exact pattern of the CAR in ASD.

A further limitation is the potential for the experience sampling and cortisol sampling procedures to have raised the participant's anxiety, as they were completing measures in school, where they could potentially be observed by peers. By completing measures in school, this may have led to fears of social evaluation, known to contribute to the experience of social anxiety (Clark & Wells, 1995).

2.4.3 Future Research

Future research should include a controlled comparison group without anxiety, for each group (TD and ASD) to compare the pattern of the CAR, to draw firmer conclusion about the applicability of the pattern of CAR seen in our groups. This would help understand the proportionate flattening of the CAR in our ASD group when compared to a non-anxious population, to explore whether presence of anxiety altered the CAR in this group compared to a non-anxious group.

Research should also look to assess the chronicity of anxiety symptoms from a developmental perspective, to assess for differences between groups in history and stability of anxiety symptoms. This is needed before conclusions can be drawn from potential hypotheses regarding the chronicity of ASD-related stressors linked to the development of anxiety, and their link with potentially attenuated CAR due to this chronic exposure to stress (Chida & Steptoe, 2009; Fries et al., 2009).

Research could also consider assessing levels of parental anxiety, exploring whether this is associated with reported anxiety in their children, given the pivotal role parenting is thought to play in conceptualisations of the development of anxiety (Murray et al., 2009). It would be helpful to explore whether differences in parental levels of anxiety might mediate anxiety differences reported by parents of participants, seen between the groups in our sample, or whether this differs according to child diagnosis (ASD or not).

In addition, future research should also consider the differences between DSM-IV-TR (APA, 2000) and DSM 5 (APA, 2013) in the categorisation of anxiety disorders. DSM 5 no longer categorises OCD or PTSD as anxiety disorders, therefore future research needs to utilise new measures that reflect this, as more generalised measures such as the SCAS include indices of OCD, which would no longer contribute to total scores of anxiety, according to DSM 5 (APA, 2013). Changes to the classification of SAD should also be taken into consideration, as symptoms now must be present for at least 6 months in groups of all ages, whereas previously in children presence of symptoms was required for 4 weeks. This may reduce frequency of participants meeting SAD criteria in future research studies.

2.4.4 Clinical implications

Our findings that there were no school-specific factors associated with anxiety in the ASD group may suggest that interventions used for anxiety in TD populations could be as beneficial for this group, as the study would suggest there were no significant differences in factors in the school day that influenced anxiety. This is useful in identifying appropriate treatment interventions, as school based CBT interventions are already beginning to demonstrate effectiveness in reducing symptoms of anxiety in young people with ASD (Luxford, Hadwin, & Kovshoff, 2016).

The findings that the TD group report significantly higher levels of anxiety when using self-report measures than are reported by their parents or teachers is worth considering for clinical practice. It may indicate that choice of informant for anxiety symptoms may depend on diagnosis, and that more screening using self-report measures in the TD population may be needed to identify a potential group of pupils with anxiety symptoms that are being missed.

Our findings, potentially indicate the under-reporting of anxiety symptoms by adolescents with ASD and highlight the need for focused work in schools regarding emotion recognition and emotional literacy, given that research suggests recognition and interpretation of self-emotion may be impaired in this population (Hill, Berthoz, & Frith, 2004). Our finding that AUC was comparable across groups may suggest that overall physiological stress levels were similar across groups, but intervention may be most useful when focused on helping aid recognition of stress and anxiety in the ASD population.

The findings of higher internalising difficulties, according to parent/teacher report SDQ for the ASD group could indicate that clinically, this group could be more at risk of other psychiatric disorders, in addition to showing signs of anxiety. This needs to be taken into account when planning effective interventions, to consider the effects of co-morbid psychiatric symptoms on overall anxiety severity and intervention effectiveness. In addition, Wood & Gadow (2010) argue that increases in anxiety symptoms increases severity of ASD symptoms, and it may be that this in turn leads to an increase in ASD related difficulties and behaviours that may be categorised as externalising difficulties (e.g. restless, hyperactivity) when measured on standardised questionnaires designed for typically developing populations. This further highlights the importance of researching validated measures specifically designed for people with ASD.

2.4.5 Conclusion

This study utilised multiple informants and multiple methods of assessment (questionnaires, interviews, experience sampling and physiological measures) to develop a broader understanding of the experience of anxiety in adolescents with ASD, in comparison to a typically developing matched control group. The study highlights ongoing difficulties in the accurate assessment of anxiety in the general population as well as in ASD, with mixed patterns of discrepancies between informants according to diagnoses. The study also highlights the need for extended research into the CAR in ASD populations in relation to chronicity of anxiety, and in comparison to a non-anxious population.

Appendices

Literature Review

Appendix A: Data extraction table template

Appendix B: Table of Studies excluded from meta-analysis

Appendix C: Table of Studies excluded from systematic review

Empirical Paper

Appendix D: Research Protocol

Appendix E: NHS NRES REC approval letter

Study Consent and information sheets

Appendix F: Headteacher consent form

Appendix G: Headteacher letter sent out with consent to parents

Appendix H: Opt-out consent form for initial screening

Appendix I: Opt-in consent form for screening, for ASD participants

Appendix J: Parent information and consent form

Appendix K: Young person's information and consent form (11-13 years)

Appendix L: Young person's information and consent form (14-15 years)

Appendix M: Teacher information and consent form

Appendix N: Anxiety information for parents of children scoring highly at screening

Questionnaire measures used in study

Appendix O:6 items from Spence Anxiety Scale, used for screening

Appendix P: Autism Quotient Questionnaire

Appendix Q: Spence Children's Anxiety Scale - Self-report

Appendix R: Strengths and Difficulties Questionnaire - Self-report

Study Data analysis

Appendix A: Data extraction table template

Category	Information
Study Title	
Authors	
Country	
Date Published	
Study objective	
Study design	
Control Participants	
ASD participants	
Age range (and Means)	
Inclusion criteria	
Exclusion Criteria	
ASD diagnosis	
Duration of cortisol collection	
Exclusion from data analysis	
Total number of participants	
Number of cortisol samples	
Time of cortisol samples	
How was CAR calculated	
Validity of instructions used for cortisol	
Data analysis	
Differences in sampling times	
Control group mean wake cortisol	
ASD wake mean cortisol	
Control CAR peak cortisol	
ASD 30+ cortisol	
Control CAR	
ASD CAR	
Difference in CAR between groups	
Proportion showing CAR	
Number of missing samples	
Confounding variables controlled for	
Possibility of compliance issues	
External validity	
Selection bias	

Appendix B: Studies excluded from meta-analysis, after meta-analysis inclusion criteria applied, then subsequent exclusion criteria applied to 15 papers from initial search

Reference	Reason for exclusion
Marinović-Ćurin, J., Marinović-Terzić, I., Bujas-Petković, Z., Zekan, L., Škrabić, V., Đogaš, Z., & Terzić, J. (2007). Slower cortisol response during ACTH stimulation test in autistic children. *European Child & Adolescent Psychiatry*, *17*(1), 39–43. https://doi.org/10.1007/s00787-007-0632-1	Contact with author requesting specific CAR data unsuccessful

Appendix C: Table of Studies excluded from systematic review

Reference	Reason for exclusion
Hoshino, Y., Yokoyama, F., Watanabe, M., Murata, S., Kaneko, M., & Kumashiro, H. (1987). The diurnal variation and response to dexamethasone suppression test of saliva cortisol level in autistic children. *Psychiatry and Clinical Neurosciences*, *41*(2), 227-235.	No further information available. Contact with authors not successful
Taylor, J. L., & Corbett, B. A. (2014). A review of rhythm and responsiveness of cortisol in individuals with autism spectrum disorders. *Psychoneuroendocrinology*, *49*, 207–228. https://doi.org/10.1016/j.psyneuen.2014.07.015	Review paper presenting multiple studies
Corbett, B. A., & Schupp, C. W. (2013). Comprehensive Investigation of the Cortisol Awakening Response in Children with Autism. *Biological Psychiatry*, *73*(9), 96S–97S. Meeting Abstract: 298. Conference Presentation: 68th Annual Scientific Meeting of the Society-of-Biological-Psychiatry. Location: San Francisco, CA. MAY 16-18, 2013	Conference presentation title. Data later published in Corbett & Schupp 2014, included in the systematic review
Ocklenburg, S., Korte, S. M., Peterburs, J., Wolf, O. T., & Gunturkun, O. (2016). Stress and laterality - The comparative perspective. *Physiology & Behavior*, *164*, 321–329. https://doi.org/10.1016/j.physbeh.2016.06.020	Not ASD population
Gustafsson, P. A., Gustafsson, P. E., Anckarsäter, H., Lichtenstein, P., Ljung, T., Nelson, N., & Larsson, H. (2011). Heritability of cortisol regulation in children. *Twin Research and Human Genetics*, *14*(6), 553–561. https://doi.org/10.1375/twin.14.6.553	Not ASD population
Fung, L. K. (2016). Cortisol in individuals with autism spectrum disorder: Meta-analysis and systematic review. *Journal of the American Academy of Child and Adolescent Psychiatry*, *55* , Issue 10 , S325 https://doi.org/10.1016/j.jaac.2016.07.367	Conference presentation title and review presentation
Kidd, S. A. (2012). *Sleep and cortisol in preschool-aged children with autism and typically developing children*. ProQuest Information & Learning, US.	Dissertation abstract - data published in Kidd et al. (2012), included in systematic review

Appendix D: Research Protocol for Ethics application

IRAS: [redacted]
ERGO Study ID: [redacted] Version 2, 01.07.16

Research Proposal

Rationale:

This study aims to explore the physiological and subjective experience of anxiety in young people, and whether this differs between peers with or without ASD. We aim to increase understanding of anxiety in young people with ASD, and whether their subjective rating of anxiety matches their physiological response (cortisol sample). We also aim to explore whether parent, teacher and self-report levels of anxiety correlate, and whether this differs across the groups (ASD vs Typically Developing).

Autistic Spectrum Disorder (ASD) includes a pattern of deficits in social communication and interaction, as well as restricted or repetitive behaviours and interests (American Psychiatric Association (APA), 2013) that occurs in around 1% of individuals in the UK (Baird et al., 2006).

Anxiety disorders include Social Anxiety, Generalised Anxiety, Panic Disorder and Agoraphobia (APA, 2013). Social Anxiety is characterised by a persistent fear of social situations, where there is a possibility of evaluation by others (APA, 2013) and in children, can present as excessive clinginess or prolonged crying in social situations. This commonly leads to withdrawal and social avoidance, along with negative self-evaluation about one's performance (Wells, 1997).Generalised Anxiety involves a more extensive pattern of worry across all situations and a preoccupation with anticipated and everyday events (APA, 2013). In contrast, Panic Disorder is characterised by panic attacks and the fear of future panic attacks, which are defined as an intense and sudden sense of fear and dread, accompanied by physiological symptoms of anxiety such as heart palpitations, dizziness, shaking and nausea (APA, 2013). Agoraphobia, commonly associated with panic disorder, incorporates a specific fear of going places where the person has experienced, or believes they will experience a panic attack, and therefore these places are avoided (APA, 2013). Common across all anxiety disorders are physiological symptoms of anxiety, behavioural change in relation to feared objects, places or situations, and cognitive symptoms of excessive worry.

The hypothalamic-pituitary-adrenal (HPA) axis is a physiological response involved in the body's response to stress. Cortisol is the stress hormone that can be measured, to plot the pattern on an individuals' HPA axis activity over the course of the day, and research suggests individuals show a peak in cortisol levels in the hour after awakening (Fries, Dettenborn & Kirschbaum, 2009), known as the Cortisol Awakening Response (CAR). Research has also suggested that levels of cortisol are higher in individuals with anxiety, and these individuals show a higher peak in the CAR than individuals without anxiety disorders (Vreeburg et al., 2010). The study aims to build on previous research identifying a specific rise in cortisol levels 30 minutes after wakening, the cortisol awakening response (CAR) and we hope to explore whether this occurs in young people with ASD, how this is affected by their subjective experience of anxiety, and whether the response differs to young people without ASD.

Research consistently suggests that young people with ASD experience higher levels of anxiety than their peers (Boulter, Freeston, South & Rodgers, 2014), so the study also aims to build on previous research which debates whether there are ASD specific stressors of anxiety, and explore what might account for raised anxiety levels in this population group. Several studies have highlighted that up to half of individuals diagnosed with ASD also meet the diagnostic criteria for an anxiety disorder (De Bruin, Ferdinand, Meester, de Nijs & Verheij, 2007, & Simonoff et al., 2008). It is important therefore to understand how anxiety presents in children with ASD.

By collecting subjective measures of anxiety through self-report via palm pilots throughout the day, this allows us to compare this with physiological measures of anxiety, and allows us to assess anxiety reactivity throughout the school day.

IRAS: [redacted]
ERGO Study ID: [redacted] Version 2, 01.07.16

The research questions are: (1) How does the experience of anxiety in young people with ASD differ from typically developing matched comparisons, (2) do self-reported measures of anxiety correlate with objective, physiological measures of anxiety, (3) does this correlation vary across the groups, (4) are there ASD specific factors associated with higher levels of reported anxiety, (5) do young people with ASD show the same pattern of cortisol increase 30 minutes after waking as peers without ASD?

Procedure:

The study is a between subjects repeated measures design, comparing anxiety responses in young people with ASD, compared to anxiety responses in peers without ASD. Data will be gathered in home and school environment, across 2 consecutive school days.

Pupils will be recruited from up to 4 schools in the [redacted] area, that are resourced to provide ASD provision. One school will be approached and screened initially, before further schools are contacted, if necessary for recruitment.

For typically developing (TD) group: All parents of male pupils in Years 7-10 will be sent an opt out letter via ParentMail email, informing them that their son will be screened for worry, as part of a screening measure for the main study. The email will include a 'read receipt' asking parents to indicate that they have read the email and opt-out consent form. Parents will be asked to return an opt-out slip if they do not wish their son to be involved in the screening. Pupils in Years 7-10 will be asked to complete the 6 item generalised anxiety sub scale, from the Spence Children's Anxiety Scale (SCAS) to screen for anxiety.

For ASD group: Teachers will be asked to identify pupils with an ASD diagnosis, by reviewing pupil's Education and Healthcare Plans (EHCP). All pupil with a diagnosis of ASD that the school are aware of will have an EHCP listing the diagnosis, and outlining their needs according to this diagnosis. We believe that in the schools we wish to recruit from, the pupils with a formal diagnosis will be known to teachers, as schools are normally involved in the process of ASD assessment and diagnosis. We will contact parents of these children to ask for opt-in consent for screening, using the same 6 items from the SCAS as in the typically developing group. We will ask parents of children with ASD diagnosis detailed in their EHCP to confirm this is a diagnosis their child has. We have chosen to ask teachers look at pupil's EHCP's to identify pupils with ASD as we felt this would be a more timely way to identify appropriate pupils to approach for the study.

For TD group: All pupils scoring highly on the Spence Children's anxiety scale will be screened for ASD symptoms using the Autism Quotient Questionnaire (AQ). If pupils without a formal diagnosis of ASD score highly on the AQ they will be excluded from the TD group.

We aim to select 15 pupils for each group; those with ASD and those without, all with above clinical levels of anxiety when screened on the Spence. These groups will be matched on anxiety level and academic achievement. Parents will be informed if their child scores highly for worry on the screening task by means of inviting them to participate in the main study, and explaining that they have been invited as a result of these elevated levels of worry.

Pupils will be asked to sign consent for the main study.

Pupils will be asked to complete 3 questionnaires and 2 tasks from the WASI to assess IQ.

Parents will be asked to sign their consent for their child's participation, and their own participation, to complete a 20-30 minute semi structured interview about their child, along with 3 questionaires.

Teachers of identified pupils will be asked complete a consent form to participate in the study, then asked to complete 2 questionnaires.

During the main study, pupils will be given a palm pilot device, programmed to prompt them at 5 times a day to take a saliva sample, answer questions relating to their mood (using sliding scales) and identify what is going on around them (noise level, number of people, environment they are in). The palm pilot will go off at wakening, 30 minutes after wakening, 11am, 3 pm and 7pm. Participants will be asked to provide a sample of salivary cortisol. This will be achieved by having participants chew on a dental salivette for approximately 30 seconds, then put the swab in a plastic vial, labelled with their unique ID number, and the correct date and time of the sample. Samples will be kept in the palm pilot pouch, accompanying the child all day, and collected at the end of the 2 days by the researcher. The researcher will arrange to collect the samples and palm-pilot device from the child the day after the study, either at their home or school, depending on their preference. The researcher will transport the samples to a locked fridge at University of Southampton, and in accordance with the Human Tissue act, samples will be sent for analysis within 7 days of collection.

Saliva samples will be analysed by the Molecular and Genetics Laboratory at the following address:

Molecular Biology and Genetics Laboratory
Department of Genetic Psychology
Faculty of Psychology
Ruhr-University Bochum
GAFO 04/620
Universitätsstr. 150
44780 Bochum
Germany

Pupils will be asked to complete palm pilot questions for 2 consecutive school days.

At the end of each day, pupils will be asked to record whether anything unusual had happened that day, and if so to describe it.

All participants will be met by the researcher and debriefed when samples and palm-pilots are collected. Participants and their parents will have the researcher's contact details should they wish to ask questions about the project, and the researcher will provide feedback to the school regarding the outcome of the study. The researcher will provide information on support services for anxiety to the parents and teachers invovled in the study, should they wish to gain more information or support for their child's worry. The researcher will feed back the results to pupils during school hours.

Measurement:

Please see attachments for copies of the SDQ (parent, teacher and self report versions) (Goodman, 1997), the Spence Children's Anxiety Scale (Spence, 1998) full questionnaire, and the 6 items that will be used from this measure as a screening, the School anxiety scale (teacher report) and the Autism Quotient Scale. The questions/prompts asked by the palm pilot device are also attached.

Copies of the Attentional control scale (Derryberry & Reed, 2002), Diagnostic Interview Schedule for Children Anxiety Module (DISC) (Shaffer, Fisher, Lucas, Dulcan & Schwab-Stone, 2000) and the WASI (Wechsler, 1999) vocabulary and Matrix reasoning tasks are unavailable as attachments due to copyright, but are available from the university resource room.

6 items from the Spence Anxiety Scale will be used as a screening measure to identify high levels of generalised anxiety. These items have been validated as a stand alone sub scale in measuring general worry, as detailed on http://scaswebsite.com/docs/normssubscales.pdf. (Retrieved on 1st July 2016).

The 6 items are as followed:

I worry about things..... Never Sometimes Often Always

When I have a problem, I get a funny feeling in my stomach..... ...Never Sometimes Often Always

I feel afraid..... Never Sometimes Often Always

When I have a problem, my heart beats really fast..... Never Sometimes Often Always

I worry that something bad will happen to me..... Never Sometimes Often Always

When I have a problem, I feel shaky..... Never Sometimes Often Always

All pupils will also be screened using the Autism Quotient Questionnaire (Baron-Cohen, 2006) which measures symptoms of ASD.

The WASI is a set of subtests designed to establish an IQ score for children. 2 sub tests of the WASI, the Vocabulary and Matrix Reasoning tasks, can be used as a mini form of the WASI to estimate full scale IQ. Vocabulary involves asking children what specific words mean, and matrix reasoning asks children to identify which picture matches those shown in the task.

The Attentional Control Scale is a 20 item measure of young people's ability to maintain and control their attention. Items are rated on a 1-4 scale between almost never and almost always. Some items are reversed scored. Example items are:

It's very hard for me to concentrate on a difficult task when there are noises around. (R)

When I need to concentrate and solve a problem, I have trouble focusing my attention. (R)

When I am working hard on something, I still get distracted by events around me. (R)

My concentration is good even if there is music in the room around me.

The Spence Children's anxiety scale (SCAS) self and parent report will also be used. This is a 45 item scale comprised of scales measuring various anxiety disorders; separation anxiety, generalised anxiety, social phobia, obsessive/compulsive, and panic/agoraphobia.

The Diagnostic Interview Schedule for Children, Anxiety module is a set of semi-structured interviews completed by the parent, administered by the researcher. It assesses symptoms of various anxiety disorders according to DSM-IV-TR criteria. This is administered via an encrypted laptop which then computes a score to assess whether answers meet diagnostic criteria for separation anxiety, social phobia, generalised anxiety, panic/agoraphobia or obsessive compulsive disorder. The interview also assesses duration of symptoms as well as degree of impairment due to symptoms.

The Strengths and Difficulties questionnaire (SDQ) will be completed by parents, teachers and participants, and consists of 25 items measuring behavioural problems on 5 scales; conduct, hyperactivity, peer relationship problems, emotional symptoms and prosocial behaviour.

Participants will also be prompted (through the use of palm pilots) to rate their anxiety, affect, current activity and social status at 5 specified intervals across the day (see attached document for palm-pilot prompts and questions). They will also be asked to take a salivary cortisol sample.

IRAS: [redacted]
ERGO Study ID: [redacted] Version 2, 01.07.16

Participants:

Male pupils in Years 7-10 from up to 4 schools in the [redacted]. Pupils will be screened initially for levels of anxiety. For the ASD group, teachers will be asked to identify pupils with a clinical diagnosis of ASD, by checking the child's EHCP. We will only be recruiting male participants due to extremely limited number of suitable female participants with ASD diagnosis in identified schools. We will use pupils in year 7-10 as we have a restricted number of participants, and wanted to exclude those who may be under additional stress due to GCSE exam preparation. Participants with below average academic achievement will be excluded as so to match pupils in both groups on academic levels. For pupils without ASD diagnosis, selected to participate in the typically developing group, if they score highly on the AQ (screen for ASD) they will be excluded from the TD group.

The sample size will be restricted to 15 in each group, 30 in total, due to cost of resources, small target population and the intensive nature of the study.

Requests for participation shall first be made to schools by approaching the headteacher and gaining appropriate consent for school participation.

Once approved, opt-out letter emails will be sent to the parents of all male pupils in the identified schools, in years 7-10, informing them of the study, and that their son will be asked to complete a short questionnaire on worry to see whether or not they may be eligible to take part in a research study looking to explore the experience of anxiety in young people. Parents will be informed that as part of this, their son's academic information will be reviewed by the researcher. Parents will be asked to sign an opt-out form if they do not wish their son to complete screening. There will be a 'read receipt' attached to the email to ensure parents have received the information.

All pupils with a diagnosis of ASD will be identified by asking teachers to look at a pupil's Education Healthcare plan, which should list this if they have a diagnosis. For children with ASD, the parents will be sent an opt-in consent form to consent to the screening.

TD Male pupils who have not opted out will be screened using a short 6 item anxiety scale. Academic information will be obtained to ascertain achievement levels, to exclude those with below average attainment.

As participants will be under 16 years of age, parents of pupils identified as scoring highly for anxiety will be asked for their consent in the main study.

The researcher will meet with children in school or at home to provide information about the study and request their participation and consent.

All parents consenting to involvement in the main study will complete the Autism Quotient Questionnaire (AQ) to screen for ASD symptoms. Pupils in the typically developing group, identified as not having an ASD diagnosis will be excluded from the study if they score highly on the AQ. Pupils with a diagnosis of ASD will be excluded if they do not score above clinical cut off for ASD symptoms on the AQ.

Headteacher permission with be obtained from all schools. Parents and the young person will be asked to sign the attached consent form for the main section of the study. Consent for screening will be inferred if parents do not return the opt-out form. Teachers will be asked to sign a consent form for their participation.

We have chosen to use opt-out consent for initial screening of TD children as the task required of the pupils is very short and it is not anticipated this should cause any significant distress to pupils completing it. Opt-out consent for screening has also been used in previous similar studies in this area.

IRAS: [redacted]
ERGO Study ID: [redacted] Version 2, 01.07.16

Risk management:

Participants may experience inconvenience caused by the palm pilot prompting them to answer questions throughout the day, and may cause slight disruption to school lessons and morning routine. To minimise this disruption, data collection will be restricted to 2 days, and only 2 of the samples each day will be within school hours. Pupils may also be uncomfortable with providing a saliva sample in front of peers, and this will be managed by ensuring support staff are aware of the study and available for reassurance.

To protect confidentiality of data on the palm-pilot device, the devices will be protected with a password that is given to the participant, to ensure other people cannot access the device. The data on the device does not contain any personally identifiable information, it is numerical data that would not make sense to people other than the researcher, instead of text answers, and all data is stored according to participant number, not name.

It is also recognised that pupils with ASD may experience anxiety in the change of their usual routine when completing the research. This will be managed by providing pupils with ASD time to consider their decision to participate, and an opportunity to discuss with the researcher or teacher any anxiety they may have about this change in routine. We hope this anxiety is minimised due to the short duration of the research.

There is a possibility that young people may become anxious by the measurement of anxiety itself, and this will be addressed by debriefing participants at the end of the study, and reminding them throughout of their right to withdraw from the study. The researcher will also give the participants an opportunity to feedback about their experience of being part of the study. The researcher will provide informtion on support services for anxiety, to the child, parent and teachers.

While it is hoped that this study should not cause undue discomfort for participants, it is possible that data provided may identify participants with high levels of recorded anxiety. If participants are identified as clinically anxious by the DISC parent interview, or score above clinical cut off in the full version of the Spence Anxiety Scale (completed by parents), that school SENCo or teacher shall be notified, as will the child's parent. They can then be signposted to the appropriate service. Similarly, if any participant's cortisol levels are above normal levels (as reported by laboratory), these results will be shared with the school SENCo or teacher and given to the parent or guardian and they will be advised to take this to their GP.

Data Storage:

Cortisol samples will be collected by the researcher and transferred immediately to a locked fridge at University of Southampton. Upon completion of measures, participants will be coded by age and number. All names will be removed from questionnaires and pupils will be assigned a numeric code. Only the researcher will have access to the key to this code, in order from them to identify pupils if measures indicate clinically significant high levels of anxiety or unusual cortisol samples. Participants will be made aware that there data is not fully confidential due to this. Data will be stored in a locked cabinet or password protected computer in accordance with Data Protection Act. Cortisol samples will be coded by age and number.

References

American Psychiatric Association, APA. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *The Lancet*, *368*(9531), 210-215

Baron-Cohen, S., Hoekstra, R. A., Knickmeyer, R. & Wheelwright, S. (2006). The Autism-Spectrum Quotient (AQ) - Adolescent Version. *Journal of Autism and Developmental Disorders, 36,* 343-350

Boulter, C., Freeston, M., South, M., & Rodgers, J. (2014). Intolerance of uncertainty as a framework for understanding anxiety in children and adolescents with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 44*(6), 1391–1402. doi: 10.1007/s10803-013-2001-x

de Bruin E.I., Ferdinand R.F., Meesters S., de Nijs P.F.A. &, Verheij F. (2007).High rates of psychiatric co-morbidity in PDD-NOS. *Journal for Autism and Developmental Disorders, 37,*877–886

Derryberry, D., & Reed, M. A. (2002). Anxiety-related attentional biases and their regulation by attentional control. *Journal of Abnormal Psychology, 111,* 225– 236. doi:10.1037/0021-843X.111.2.225

Fries, E., Dettenborn, L., &Kirschbaum, C. (2009). The cortisol awakening response (CAR): facts and future directions. *International Journal of Psychophysiology, 72* (1), 67-73. doi: 10.1016/j.ijpsycho.2008.03.014

Goodman, R. (1997). The Strengths and Difficulties Questionnaire: A Research Note. *Journal of Child Psychology and Psychiatry*, 38, 581-586

Shaffer D., Fisher P., Lucas C., Dulcan M., & Schwab-Stone M. (2000). NIMH Diagnostic Interview Schedule for Children, Version IV (NIMH DISC-IV): description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child and Adolescent Psychiatry*, 39, 28-38.

Simonoff, E., Pickles, A., Charman, T., Chandler, S. Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 47* (8), 921-29.doi:10.1097/CHI.0b013e318179964f

Spence, S.H. (1998). A measure of anxiety symptoms among children. *Behaviour Research and Therapy, 36* (5), 545-566

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. The Psychological Corporation: San Antonio.

Wells, A. (1997). *Cognitive Therapy of Anxiety Disorders: A Practice Manual and Conceptual Guide*. Wiley: UK

Vreeburg, S.A., Zitman, F.G., van Pelt, J., DeRijk, R.H., Verhagen, J.C.M., van Dyck, R., Hoogendijk,

Appendix E: NHS NRES REC approval letter

NHS
Health Research Authority

South Central - Hampshire A Research Ethics Committee
Level 3, Block B
Whitefriars
Lewins Mead
Bristol
BS1 2NT

Telephone: 0117 3421328

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

22 September 2016

[redacted]
Trainee Clinical Psychologist
[redacted]
Building 44a, Doctorate in Clinical Psychology, Psychology Department
University of Southampton
Highfield, Southampton
SO17 1BJ

Dear [redacted]

Study title: **Understanding the experience of anxiety in young people with Autistic Spectrum Disorders**

REC reference: [redacted]
IRAS project ID: [redacted]

Thank you for your submission responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Miss Elizabeth Hearn, nrescommittee.southcentral-hampshirea@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Southampton University insurance]	1	23 May 2016
Letters of invitation to participant [Letter sent with opt-out consent]	2	01 July 2016
Letters of invitation to participant [Letter sent with opt-in consent for ASD pupils]	1	01 July 2016
Letters of invitation to participant [Letter sent to headteachers inviting participation]	4	01 July 2016
Non-validated questionnaire [Palm pilot questions]	3	22 April 2016
Other [REC review, unfavourable opinion decision letter]	1	24 June 2016
Other [Letter addressing REC issues from previous review 24.06.16]	1	01 July 2016
Other [redacted research CV]	1	27 July 2016
Other [Published paper evidencing proof of concept for whole school screening, used in schools in same area]	1	21 August 2016
Other [Power Calculation as requested, demonstrating sample size needed]	1	06 September 2016
Other [Information for parents of children identified as anxious from initial screening (TRACKED CHANGES)]	2	13 September 2016
Participant consent form [Headteacher consent form]	4	01 July 2016
Participant consent form [Opt-out consent form]	1	01 July 2016
Participant consent form [Opt-in consent for screening for ASD children]	1	01 July 2016
Participant consent form [Parent info and consent form]	5	21 August 2016
Participant consent form [Teacher info and consent form]	5	21 August 2016
Participant consent form [Young person's information sheet and consent form (Age 11-13)]	5	21 August 2016
Participant consent form [Young person's information sheet and consent form (14-15 years)]	5	21 August 2016

REC Application Form [REC_Form_06092016]		06 September 2016
Research protocol or project proposal [Project proposal]	2	01 July 2016
Summary CV for Chief Investigator (CI) [Brief CV [redacted]	1	18 May 2016
Summary CV for supervisor (student research) [Brief CV [redacted]	1	18 May 2016
Validated questionnaire [Autism Quotient Questionnaire]	1	18 May 2016
Validated questionnaire [School Anxiety scale]	1	18 May 2016
Validated questionnaire [SDQ self report]	1	18 May 2016
Validated questionnaire [SDQ teacher version]	1	18 May 2016
Validated questionnaire [SDQ Parent version]	1	18 May 2016
Validated questionnaire [Spence Anxiety Scale]	1	18 May 2016
Validated questionnaire [6 items for Screening questionnaire]	1	01 July 2016

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at
http://www.hra.nhs.uk/hra-training/

16/SC/0413	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

pp [redacted]

Dr Simon Kolstoe
Chair

Email:nrescommittee.southcentral-hampshirea@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Ms Diana Galpin*

Appendix F: Headteacher consent form

IRAS: [redacted]

UNIVERSITY OF Southampton

HEADTEACHER CONSENT FORM
Version 4, 01.07.2016

Study Title: Understanding the experience of anxiety in young people with Autistic Spectrum Disorders
Researcher names: [redacted]
ERGO study ID number: [redacted]

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

I understand that my school's participation in this study will involve assisting [redacted] with the following:

- Selection of students
- Obtaining consent from parents
- Identification of a named staff members in school whom students could approach for support if required
- Group session in school to complete standardised questionnaires
- Data collection in school during academic year 2016/2017
- Providing staff members to monitor data collection
- Provide a separate activity/task for any students who do not wish to participate in the initial screening questionnaires (to be decided by the class teacher, for example short quiz or puzzle) ☐
- Identification of pupils with a diagnosis of ASD indicated on their EHCP

I have read and understood the following documents in connection with this study:

- Opt-out consent email/letter to parents
- Letter to parents requesting full participation of their children
- Participant information sheet
- Student consent form
- University of Southampton School of Psychology Ethics Committee code of practice ☐

Further to reading the above documents, I have had the opportunity to ask questions about the study. I understand that I remain free to direct any future questions, comments or concerns about the study as they arise, to [redacted]. ☐

I understand that I am at liberty to contact the Southampton University Ethics Committee to discuss any complaints I may have pertaining to this research. ☐

I understand that names will be removed from data collected, and will only be accessible by the University researcher. ☐

I consent to [redacted] conducting this study to explore the nature of anxiety in children with ASD. ☐

Name of school:.....

Name of Headteacher: .....

Signature of Headteacher: .....
Date: .....

Appendix G: Headteacher letter sent out with consent to parents

01.07.16, Version 4

ERGO Study ID: [redacted]
IRAS: [redacted]

UNIVERSITY OF Southampton

Parent Name

Parent Address

Study Title: Understanding the experience of anxiety in young people with Autistic Spectrum Disorders
Researcher names: [redacted]
ERGO study ID number: [redacted]

Dear Parent

Please find enclosed information regarding a research project that the school is involved with, and which your child may be eligible to participate in. This project has been agreed by the Headteacher of the school, following discussion with the researcher and approval from relevant ethical approval boards. Teachers within the school are aware of the research project and named teachers will be available to discuss this. The information enclosed details an initial screening test that your child will be asked to complete, and asking you to opt-out of this if you do not wish him to participate. Please read this information carefully, as it provides information on how to contact the researcher if you have any questions regarding this research.

I have given my permission for the pupils at this school to be contacted by this researcher, and am confident that the project will be of value to the school going forward.

Yours sincerely

Headteacher of ....

Appendix H: Opt-out consent form for initial screening

01.07.16, Version 4

Study ID: [redacted]

IRAS: [redacted]

Parent Name

Parent Address

UNIVERSITY OF Southampton

Study Title: Understanding the experience of anxiety in young people with Autistic Spectrum Disorders
Researcher names: [redacted]
ERGO study ID number: 1[redacted]

Dear Parent

I am contacting you to inform you of a research study that is taking place, which will be asking local school pupils for their involvement. I am a trainee clinical psychologist, conducting a study to explore the experience of anxiety in young people with Autism Spectrum Disorders (ASD) and as part of this, I hope to recruit a number of pupils without ASD to compare my results to.

In order to find participants for my research, the Headteacher at your child's school has agreed that I may ask all male pupils in Years 7 – 10 to complete a short screening questionnaire, asking them about different things they may worry about, and how often they worry. This screening questionnaire will take no longer than 3 minutes to complete, and will be done in tutor time, to avoid disruption to your child's learning.

Your child may be invited to take part in the main research study if their questionnaire results indicate that they have higher levels of worry than some of their peers. Participation in this further study is voluntary and you will be contacted again with more information if your child is invited to take part. We will inform you if your child scores highly for worry, by inviting you to take part in the main research study.

If you do not wish for your child to complete this short initial screening questionnaire, then please complete the opt-out form below and return to your child's teacher.

If you have any questions, please email [redacted]. If you have any concerns regarding this research, please contact the Chair of the Ethics Committee, Psychology, University of Southampton, Southampton, SO17 1BJ. Phone: +44 (0)23 8059 3856, email fshs-rso@soton.ac.uk

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I do not give permission for my child to take part in the screening questionnaire for the above study. On behalf of my child, I opt-out of their participation in this research.

Signed.....

Date.....

Parent Name.....

Child's name.....

Please return to xxxxx at your child's school.

Appendix I: Opt-in consent form for screening, for ASD participants

01.07.16, [redacted]

[redacted]

Parent Name

Parent Address

[redacted]

UNIVERSITY OF Southampton

Study Title: Understanding the experience of anxiety in young people with Autistic Spectrum Disorders
Researcher names: [redacted]
ERGO study ID number: [redacted]

Dear Parent

I am contacting you to inform you of a research study that is taking place, which will be asking local school pupils for their involvement. I am a trainee clinical psychologist, conducting a study to explore the experience of anxiety in young people with Autism Spectrum Disorders (ASD).

In order to find participants for my research, the Headteacher at your child's school identified children with a listed diagnosis of ASD, in their EHCP, and has allowed me to contact you.

I wish to ask your child to complete a short screening questionnaire, asking them about different things they may worry about, and how often they worry. This 6 item screening questionnaire will take no longer than 3 minutes to complete, and will be done in tutor time, to avoid disruption to your child's learning. I am also asking pupils within the school that do not have a diagnosis of ASD to complete this screening questionnaire, to identify pupils with elevated levels of worry, to compare my results to.

Your child may be invited to take part in the main research study if their questionnaire results indicate that they have higher levels of worry than some of their peers. Participation in this further study is voluntary and you will be contacted again with more information if your child is invited to take part. We will inform you if your child scores highly for worry, by inviting you to take part in the main research study. We will also provide information on support services you can access if you have concerns.

If you are happy for your child to complete this short initial screening questionnaire, then please complete the consent form below and return to your child's teacher. If your child does not wish to complete the questionnaire at the time, he does not have to. If he chooses to complete the questionnaire, following your consent, we will infer his consent from this, by the fact that he is completing the questions.

If you have any questions, please email [redacted] at [redacted] If you have any concerns regarding this research, please contact the Chair of the Ethics Committee, Psychology, University of Southampton, Southampton, SO17 1BJ. Phone: +44 (0)23 8059 3856, email fshs-rso@soton.ac.uk

01.07.16, Version 1

Study ID: [redacted]

IRAS: [redacted]

UNIVERSITY OF Southampton

I give permission for my child to take part in the screening questionnaire for the above study. On behalf of my child, I consent to their participation in this research.

Signed.....

Date.....

Parent Name.....

Child's name.....

Please return to xxxxx at your child's school.

Appendix J: Parent information and consent form

UNIVERSITY OF Southampton

Parent or Guardian

Home or School address

<Date>

Dear Parent or Guardian

Invitation to take part in a research project: Understanding the experience of anxiety in young people with Autism Spectrum Disorders (ASD).

Researcher: [redacted] **Ethics number** [redacted]

We would like to invite your child to take part in our research study. Before you decide, it is important to understand why the research is being done and what it will involve for your child. Please take time to read the following information carefully. Please ask us if there is anything that is not clear or if you would like more information. If you are happy to participate you will be asked to sign a consent form.

What is the research about?

This project is being carried out by a trainee clinical psychologist as part of their doctoral training and is funded by the University of Southampton.

The aim of this research is to explore how young people with ASD experience anxiety, and whether this differs to their peers.

The project has three main aims: (1) To explore what anxiety looks like across the school day for children with ASD; (2) to examine differences in anxiety between children with ASD and their typically developing (TD) peers, and (3) to compare the self-reported experience of anxiety with physiological experience.

We hope that this project will help us to understand whether children with ASD experience anxiety differently to their peers, and what sort of things make them worried or concerned. It will also allow us to identify any differences between self-reported worries or concerns and physiological arousal. This information should help develop our understanding of anxiety in ASD and could be used to help support children in schools.

[21.08.16] [Version 5] Study ID: [redacted] IRAS: [redacted]

UNIVERSITY OF Southampton

Why has my child been chosen?

The study aims to increase our understanding of anxiety in young people with ASD. To be able to do this, we need to compare how young people with ASD experience anxiety to a group of their peers who are anxious but do not have ASD.

We previously wrote to you to explain that we would be conducting a screening test at your child's school to look for a group of children who show higher levels of worry than some of their peers. Your child has been selected to take part in our study as his results indicated higher levels of worry than some of his other peers, and he attends a mainstream school. If he has a diagnosis of ASD, he will be part of the main research group to help us understand anxiety in young people with ASD. If he does not have ASD he will be part of a comparison group, to compare our results to children with ASD who are anxious.

What will happen if my child takes part?

The researcher will come to meet you and your child to explain the study in detail and answer any questions you may have.

If you choose to take part your child will first be asked to complete three questionnaires and complete a 30-60 activity involving a vocabulary task and abstract reasoning picture task.

You will be asked to complete a 20-30 minute semi-structured interview about your child, and complete 3 questionnaires. This interview will take place at your child's school, or at your home, whichever is more convenient for you.

Your child's teacher will also be asked to complete 2 questionnaires about your child.

The main project will then take place on 2 consecutive school days. For the duration of the study your child will be given access to a palm-pilot device. This is a small electronic portable device that is programmed with our research questions. This will prompt them at 5 specific intervals to answer several questions about their mood and what is going on around them. They will also be asked to provide a sample of cortisol (which is found in saliva, and is an indicator of stress). This will involve chewing on a dental roll for 30 seconds. The palm-pilot will ask prompts when your child wakes up, 30 minutes after waking, then at 11am, 3pm and 7pm. The researcher will demonstrate the procedure to you and your son before the study. The researcher will collect all the samples and the palm-pilot device the day after the study, at home or school.

Are there any benefits in my taking part?

When the project has been completed, [redacted] (Trainee Clinical Psychologist) will feedback findings and implications to school staff. This should help provide school staff with a more in depth understanding of how anxiety affects children with ASD, and how they can use this information to assist pupils in the school day.

If we find that factors relating to school are linked with anxiety in young people, we will let the school know this, and advise on ways in which they can help their pupils, or let them know who else could help. The researcher will present the results of the research to the school and teachers, and be available to discuss the potential implications of the findings with the teachers.

UNIVERSITY OF Southampton

Are there any risks involved?
We do not perceive there to be any risk involved with this research. However we recognise that your child may experience slight disruption to their morning routine and school day, and may feel uncomfortable providing a saliva sample in public. We will ensure support staff are available to assist with this and reassure the young person. You will also be able to discuss any concerns with these staff or the researcher.

If, during the course of the study, it is identified that your child has above clinical levels of anxiety, we will inform you and the SENCo at your child's school, and provide information on support services your child could access.

Will my child's participation be confidential?
Anonymity cannot be confirmed as participants will be using palm pilots during the school day. Once collected, data will be coded and all names removed. This information will then be stored in a locked cabinet or a password protected computer in accordance with the Data Protection Act. Data will not be fully confidential as the researcher will have access to a key identifying which code each pupil is assign, this will be in order to identify pupils that score significantly highly on anxiety measures, in order for the researcher to inform you about this.

What happens if I change my mind?
You are free to withdraw from the research at anytime, without providing an explanation.

What happens if something goes wrong?
In the unlikely case of concern or complaint, please contact:
Chair of the Ethics Committee, Psychology,
University of Southampton
Southampton
SO17 1BJ.
Phone: +44 (0)23 8059 4663, email fshs-rso@soton.ac.uk

Where can I get more information?
If you would like any more information please feel free to get in contact:

[redacted] (Trainee Clinical Psychologist), email [redacted]

[21.08.16] [Version 5] Study ID: [redacted] IRAS: [redacted]

UNIVERSITY OF Southampton

PARENT CONSENT FORM (21.08.16, Version 5)

Study title: Understanding the experience of anxiety in young people with ASD

Researchers' names: [redacted]
Ethics reference: [redacted]

What do I have to do now?

If you are happy for your child to take part in the study then please initial the box(es) if you agree with the statement(s):

1. I confirm that I have read and understood the information sheet (21.08.16, Version 5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered. ☐

2. I confirm that I understand I have the option to deny giving personal information asked for if I wish to do so. ☐

3. I understand my child's participation is voluntary and they can withdraw at any time without giving any reason, without my legal rights being affected. ☐

4. I agree for my child to take part in this research project and agree for the data to be used for the purpose of this study ☐

5. I agree to the collection and storage of tissue samples (saliva) from my child ☐

Name of child _____Child's date of birth _____

Your name .....Your signature.....

Your relationship to the child .....Date.....

PLEASE RETURN THE COMPLETED CONSENT FORM TO YOUR CHILD'S SCHOOL

[21.08.16] [Version 5] Study ID: [redacted] IRAS [redacted]

Appendix K: Young person's information and consent form (11-13 years)

UNIVERSITY OF Southampton

Young person's information sheet (21.08.16 Version 5) (Ages 11-13)

Ethics number: [redacted] IRAS: [redacted]

Understanding the experience of anxiety in young people with Autism Spectrum Disorders

We are asking if you would like to take part in a research project to help us understand what sorts of things make young people worried in school.

Before you decide, you need to understand what the research is and what you might be asked to do.

Please read this with your parents or teacher and think carefully about it, and ask any questions you can think of about it.

What is research?

Research is a careful experiment to try to find out an answer to an important question.

Why are we doing the research?

We want to understand what happens when young people who have Autism get worried. We want to see if how a person says they feels matches up to what their body feels. We are interested in trying to understand how it feels for young people who worry.

Why me?

When you answered some questions about worry, you said that you sometimes worry. If you have ASD, we would like you to take part, to help us understand what makes you worried.

If you don't have ASD, we would like you to take part so that we can understand what makes you worried and see if this is different to people who have Autism.

Everybody we ask is a boy in years 7-10.

Study [redacted] Version 5, 21.08.1601.07.16

UNIVERSITY OF Southampton

Do I have to take part?

No! It is up to you.

You can ask the researcher if you have any questions about the project before you take part.

Your parents will be asked to give permission for you to take part, and we will also be asking your teachers. BUT it is your decision if you want to take part.

If you do decide to take part, we need you to sign the consent form at the bottom of this information sheet, to say that you have understood the project and would like to take part.

If you change your mind about taking part at any time during the project, that is ok, you can stop taking part if you want.

What will happen if I decide to take part?

You will answer some questionnaires and complete some tasks. The tasks involves some puzzles and word games to see how well you understand things. It will take about 30-60 minutes.

Your parents will answer some questions about you too, and so will one of your teachers.

After this, we will ask you to choose 2 school days to do the main project in. You will be given a palm-pilot device, which is a small electronic device, like a mobile phone. This will go off 5 times each day and ask you to complete certain questions and to take a saliva sample.

The palm-pilot device will go off at 5 times in the day; when you wake up, 30 minutes after you wake up, then at 11am, 3pm and 7pm. You will be asked to give a saliva sample by chewing on a dental swab (like a piece of cloth) and spitting it into a small pot. You will be given a pouch to carry the equipment with you throughout the day.

Study ID[redacted] Version 5, 21.08.1601.07.16

UNIVERSITY OF Southampton

There will be teachers in your school that you can talk to about the research at any time, and the researcher will also be available. At the end of each day, we will ask you to write down whether anything unusual happened that day, and if it did, to describe it.

At the end of the 2 days, the researcher will collect the samples and the palm-pilot device, and will send off your samples to be analysed. All of your data will be confidential – this means that other people will not be able to know that it is your data.

What are the benefits of taking part?

You will be helping us to understand how young people experience worry, and this may help us to develop ways of helping people manage this. We are hoping that our research will help the school to understand more about when young people with ASD worry, and what they might be able to do to help with this.

If we find out things about school that make young people worry, we will let the school know this, so they can find ways to help. The researcher will come to the school to talk to the teachers about ways they might be able to help.

What happens when the study finishes?

When the study finishes, we will look at all the information gathered to see what it can tell us. The researcher will come back to the school to tell pupils what they have learned from the project, and they will also talk to the teachers about how they might be able to help people with ASD who worry. Sometimes, once we have finished a project we will publish this information so other researchers can find out about what we have been doing and what we found.

We will never put your name in any information about the project.

What happens if there is a problem?

You can talk to your parents or teacher. And remember, you can stop taking part in the project at any time, and you can speak to us if you are worried.

Study ID[redacted] Version 5, 21.08.1601.07.16

UNIVERSITY OF Southampton

Who is running the research?

The research is organised by [redacted] a trainee clinical psychologist, and supervised by two other researchers, [redacted]

Who has checked this study?

This project has been reviewed by several other people who work at the University of Southampton and the NHS, who check research to make sure it is ok.

Where can I find out more?

If you have any questions about the study, you can ask me now or at any time, or you can ask your teacher or parents to ask me. My name is [redacted] and my email is [redacted]

What happens if I find the questions upsetting?

You can speak to your parent/guardian or your class teacher. If you are still worried or upset, then remember, you do not have to take part.

Study ID: [redacted] Version 5, 21.08.1601.07.16

UNIVERSITY OF Southampton

If you are happy to help us with this study, then answer the questions below and sign your name.

I have read and understood the information sheet (21.08.16, Version 5) 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 ☐

I agree to the collection and storage of tissue samples (saliva) as part of this study ☐

I understand my participation is voluntary and I may withdraw at any time without my legal rights being affected ☐

Name of participant (print name).....

Signature of participant.....

Date.....

Parent Name:.....

Study ID[redacted] Version 5, 21.08.1601.07.16

Appendix L: Young person's information and consent form (14-15 years)

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Young person's information sheet (21.08.16 Version 5) (Ages 14-15)

Ethics number: [redacted] **IRAS:** [redacted]

Understanding the experience of anxiety in young people with Autism Spectrum Disorders

We would like you to help us with our research study. Please read this information carefully and talk to your mum, dad or teacher about the study. Ask us if there is anything that is not clear or if you want to know more. Take time to decide if you want to take part. It is up to you if you want to do this. If you don't then that's fine.

Why are we doing the research?

We want to understand what it is like for young people who have Autism Spectrum Disorders (ASD) when they get worried. We are interested in how people describe their worries, and whether this matches what happens in your body when you worry.

Why have I been invited to take part?

When you did the short questionnaire about worry, it showed that you worry a bit more than some other young people.

If you have ASD, you have been invited to take part in the study because we are interested in learning how young people with ASD experience anxiety.

If you don't have ASD, we are interested in what happens when you worry, and whether this is different to people who have autism.

Everyone we ask to be involved in the study is a male pupil in years 7-10.

Do I have to take part?

No, it is completely up to you.

Your parents will also be asked to give their permission for you to take part, and we have given them some information about the project. **But you have the final decision as to whether or not you want to take part.**

If you do decide to take part, we need you to sign the consent form at the bottom of this information sheet, to say that you have understood the project and would like to take part. We'll give you a copy of this information sheet to keep.

Study ID: [redacted] Version 5, 21.08.16

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If you change your mind about taking part at any time during the project, that is ok, you can stop taking part if you want.

What will happen if I decide to take part?

You will be asked to complete several questionnaires and a short task, which will take about 30-60 minutes. The task involves some puzzles and word games to give us an idea of how you understand things.

Your parents will also be asked to complete a short questionnaire about your worries, and we will ask one of your teachers to complete a questionnaire too.

After this, the researcher will agree with you two days where you will complete the main study. The researcher will give you a palm-pilot device and this will be programmed to prompt you to answer several questions and ask you to take a saliva sample. A palm-pilot device is a small, portable electronic device that you can carry with you. It is small, not heavy, and will only go off when we programme it to, it is very similar to a mobile phone.

The palm-pilot device will go off at 5 times in the day; when you wake up, 30 minutes after you wake up, then at 11am, 3pm and 7pm. You will be asked to give a saliva sample by chewing on a dental swab and spitting it into a small pot. You will be given a pouch to carry the equipment with you throughout the day.

There will be teachers in your school that you can talk to about the research at any time, and the researcher will also be available. At the end of each day, we will ask you to write down whether anything unusual happened that day, and if it did, to describe it.

At the end of the 2 days, the researcher will collect the samples and the palm-pilot device, and will send off your samples to be analysed. All of your data will be confidential – this means that other people will not be able to know that it is your data.

What are the benefits of taking part?

You will be helping us to understand how young people experience worry, and this may help us to develop ways of helping people manage this. We are hoping that our research will help the school to understand more about when young people with ASD worry, and what they might be able to do to help with this.

If we find out there are things about school that make young people worried, we will let the school know so they can try to help. We will also let them know ways of helping pupils who worry about things to do with school. The researcher will come to the school to talk to the teachers about ways they might be able to help.

Study ID:[redacted] Version 5, 21.08.16

UNIVERSITY OF Southampton

What happens when the study finishes?

When the study finishes, we will look at all the information gathered to see what it can tell us. The researcher will come back to the school to tell pupils what they have learned from the project, and they will also talk to the teachers about how they might be able to help people with ASD who worry. Sometimes, once we have finished a project we will publish this information so other researchers can find out about what we have been doing and what we found.

We will never publish your name or any other information that will let people know who you are.

What happens if there is a problem or something goes wrong?

You can talk to your parents or teachers if you have any worries, or you can contact the researcher. Remember, you can stop taking part if you want to.

Who is organising the research?

The research is organised by [redacted] a trainee clinical psychologist, and supervised by two other researchers, [redacted]

Who has reviewed this study?

This project has been reviewed by several other people who work at the University of Southampton and the NHS, who check research to make sure it is ok.

Where can I find out more?

If you have any questions about the study, you can ask me now or at any time, or you can ask your teacher or parents to ask me. My name is [redacted] and my email is [redacted]

What happens if I find some of the questions upsetting?

If you need any advice or help on how you feel about the questionnaires or anything else we ask you to do you can speak to a number of different people. This could be someone you know, like your parent/guardian or your class teacher. We have given them information about support services to help young people with lots of worries. If you are still worried or upset, then remember, you do not have to take part.

Study ID: [redacted] Version 5, 21.08.16

UNIVERSITY OF Southampton

If you are happy to help us with this study, then answer the questions below and sign your name.

I have read and understood the information sheet (21.08.16, Version 5) 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 ☐

I agree to the collection and storage of tissue samples (saliva) as part of this study ☐

I understand my participation is voluntary and I may withdraw at any time without my legal rights being affected ☐

Name of participant (print name)……

Signature of participant……

Date……

Parent Name:……

Study ID:[redacted] Version 5, 21.08.16

Appendix M: Teacher information and consent form

UNIVERSITY OF Southampton

Teacher

School address

<Date>

Dear Teacher

Invitation to take part in a research project: Understanding the experience of anxiety in young people with Autism Spectrum Disorders (ASD).

Researcher: [redacted] **Ethics number** [redacted]

We would like to invite to take part in our research study. Before you decide, it is important to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Please ask us if there is anything that is not clear or if you would like more information. If you are happy to participate you will be asked to sign a consent form.

What is the research about?

This project is being carried out by a trainee clinical psychologist as part of their doctoral training and is funded by the University of Southampton.

The aim of this research is to explore how young people with ASD experience anxiety, and whether this differs to their peers.

The project has three main aims: (1) To explore what anxiety looks like across the school day for children with ASD; (2) to examine differences in anxiety between children with ASD and their typically developing (TD) peers, and (3) to compare the self-reported experience of anxiety with physiological experience.

We hope that this project will help us to understand whether children with ASD experience anxiety differently to their peers, and what sort of things make them worried or concerned. It will also allow us to identify any differences between self-reported worries or concerns and physiological arousal. This information should help develop our understanding of anxiety in ASD and could be used to help support children in schools.

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Why have I been chosen?

The study aims to increase our understanding of anxiety in young people with ASD. To be able to do this, we need to compare how young people with ASD experience anxiety to a group of their peers who are anxious but do not have ASD.

An important part of our research is gathering information from teachers of the pupils who have been invited to take part. You have been invited to take part in the research as your school has agreed to be part of this research, and some of the pupils you teach have been invited to participate in the study.

What will happen if I take part?

You will be asked to complete two questionnaires about pupils that you teach who are participating in our main research study. You will also be asked to support the child to complete the research project, and direct them to the researcher should they have any concerns or questions regarding the research. You will be fully briefed about the project by the researcher, and given information regarding services to contact if the pupil becomes anxious about the research.

For the duration of the study the pupils participating will be given access to a palm-pilot device. This is a small electronic portable device that is programmed with our research questions. This will prompt them at 5 specific intervals to answer several questions about their mood and what is going on around them. They will also be asked to provide a sample of cortisol (which is found in saliva). This will involve chewing on a dental roll for 30 seconds, then placing this in a plastic vial. The palm-pilot will ask prompts when the child wakes up, 30 minutes after waking, then at 11am, 3pm and 7pm. You will be asked to support the child if they need assistance in taking 2 saliva samples that occur in the school day (11am & 3pm) over 2 consecutive days, when they are prompted.

Are there any benefits in my taking part?

When the project has been completed, [redacted] (Trainee Clinical Psychologist) will feedback findings and implications to school staff. This should help provide school staff with a more in depth understanding of how anxiety affects children with ASD, and how they can use this information to assist pupils in the school day.

If we find factors indicating aspects of the school day or environment that are linked to anxiety in young people, the researcher will feed this back to the school, and offer advice on ways to help with this, and information on further support services. The researcher will visit the school after the research has finished, to discuss the results and any implications following this.

Are there any risks involved?

We do not perceive there to be any risk involved with this research. However we recognise that pupils may experience slight disruption to their morning routine and school day, and may feel uncomfortable providing a saliva sample in public. We hope that by having well informed teachers participating in the research, you will be able

assist and reassure the young person. You will also be able to discuss any concerns with the researcher.

If, during the course of the study, it is identified that a pupil has above clinical levels of anxiety, we will inform the SENCo at the school, and provide information on support services the pupil could access.

Will my participation be confidential?
Anonymity cannot be confirmed as we will need to identify specific teachers that can provide specific information on the pupils involved. Once collected, data will be coded and all names removed. This information will then be stored in a locked cabinet or a password protected computer in accordance with the Data Protection Act. Data is not fully confidential, as if results indicate significantly high levels of anxiety in pupils, the researcher will be able to identify that pupil by their code, and inform the SENCo and pupil's parents.

What happens if I change my mind?
You are free to withdraw from the research at anytime, without providing an explanation.

What happens if something goes wrong?
In the unlikely case of concern or complaint, please contact:
Chair of the Ethics Committee, Psychology,
University of Southampton
Southampton
SO17 1BJ.
Phone: +44 (0)23 8059 4663, email fshs-rso@soton.ac.uk

Where can I get more information?
If you would like any more information please feel free to get in contact:

[redacted] (Trainee Clinical Psychologist), email [redacted]

UNIVERSITY OF Southampton

TEACHER CONSENT FORM (21.08.16, Version 5)

Study title: Understanding the experience of anxiety in young people with ASD

Researchers' names: [redacted]
Ethics reference [redacted]

What do I have to do now?

If you are happy to take part in the study then please initial the box(es) if you agree with the statement(s):

1. I confirm that I have read and understood the information sheet (21.08.16, Version 5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered. ☐

2. I confirm that I understand I have the option to deny giving personal information asked for if I wish to do so. ☐

3. I understand my participation is voluntary and I can withdraw at any time without giving any reason, without my legal rights being affected. ☐

5. I agree to take part in this research project and agree for the data to be used for the purpose of this study ☐

Your name .....Your signature.....

Your school .....Date.....

PLEASE RETURN THE COMPLETED CONSENT FORM
TO THE RESEARCHER

[21.08.16] [Version 5] Study ID: [redacted] IRAS: [redacted]

Appendix N: Anxiety information sheet given to parents of children scoring highly for worry at screening

Anxiety

Your child, along with many of his peers, recently completed a short questionnaire at school, asking them about worry. The results from this screening questionnaire indicate that your child's score was higher on this measure of worry than their peers. We have therefore provided this information sheet about anxiety if you have concerns about your child's level of worry.

What if my child's screening questionnaire indicates higher levels of worry?

We have used a short, 6 item questionnaire that is used to help identify young people who might be showing signs of worrying more than their classmates. This measure is not a diagnostic tool - it DOES NOT mean your child has an anxiety disorder, it just shows us that they may be worrying more than other children their age.

There are lots of different things that could have affected your child's score on the questionnaire that day - it may be that there are lots of things going on for them at the moment, or they were feeling particularly worried about something.

We would recommend that you discuss this with your child, to see whether they feel they worry more, and whether or not this is causing them any difficulties. You might also like to discuss this with your child's teacher.

What is anxiety?

Anxiety, or worry, is a relatively common mental health difficulty, that 1 in 4 people in the UK experience.

13.09.16, Version 2, ERGO No: 1[redacted] Study No:[redacted]

When anxiety or worry begins to impact how well a person can function e.g. it interferes with their social functioning or performance at school, or in daily living activities, this is when it may be time to talk to someone about getting help to manage this worry.

Many young people may get worried at school, especially during exam times, or when things change, for example at the beginning of a new term. For most young people, this worry does not stay around long. If your child has been feeling worried for more than a few weeks, this is also when you might like to seek advice from a professional about it.

Anxiety, especially in young people, can often be displayed in physical symptoms - getting frequent headaches/stomach aches, feeling dizzy or sick, getting hot, sweaty and clammy or getting 'butterflies in the stomach'.

What to do if you are worried about your child's anxiety?

Talk to your child about this, to see if they are also concerned. Discuss with your child's teacher, to see if they have noticed that your child may be anxious. If you have concerns, the school will be able to support you to access professional help for your child, and give them an opportunity to discuss their worries further.

Who else can help?

If you are still concerned that your child may have higher levels of anxiety or worry than their peers, and this appears to be

causing them difficulties, we would advise that you discuss this with your GP about the help that may be available in your area.

Here are some useful websites that you might like to look at with your child:

http://www.youngminds.org.uk/

https://www.anxietyuk.org.uk/our-services/anxiety-information/young-people-and-anxiety/

http://youth.anxietybc.com/

If you would like more information from the researcher, please contact [redacted]

Appendix O:6 items from Spence Anxiety Scale, used for screening

Spence Children's Anxiety Scale, Generalised Anxiety Sub Scale

I worry about things..... Never Sometimes Often Always

When I have a problem, I get a funny feeling in my stomach..... ...Never Sometimes Often Always

I feel afraid..... Never Sometimes Often Always

When I have a problem, my heart beats really fast..... Never Sometimes Often Always

I worry that something bad will happen to me..... Never Sometimes Often Always

When I have a problem, I feel shaky..... Never Sometimes Often Always

Appendix P: Autism Quotient Questionnaire

The Adolescent Autism Spectrum Quotient (AQ)
Ages 12-15 years

SPECIMEN, FOR RESEARCH USE ONLY.

For full details, please see:

S. Baron-Cohen, R. Hoekstra, R. Knickmeyer, S. Wheelwright, (2006)
The Autism Spectrum Quotient (AQ) – Adolescent Version
Journal of Autism and Developmental Disorders.

Name:..... Sex:.....

Date of birth:..... Today's Date.....

How to fill out the questionnaire

Below is a list of statements about your child. Please read each statement very carefully and rate how strongly you agree or disagree by selecting the appropriate option opposite each question.

DO NOT MISS ANY STATEMENT OUT.

Examples

E1. S/he is willing to take risks.	definitely agree	slightly agree	slightly disagree	definitely disagree
E2. S/he likes playing board games.	definitely agree	slightly agree	slightly disagree	definitely disagree
E3. S/he finds learning to play musical instruments easy.	definitely agree	slightly agree	slightly disagree	definitely disagree
E4. S/he is fascinated by other cultures.	definitely agree	slightly agree	slightly disagree	definitely disagree

	Definitely Agree	**Slightly Agree**	**Slightly Disagree**	**Definitely Disagree**
1. S/he prefers to do things with others rather than on her/his own.				
2. S/he prefers to do things the same way over and over again.				
3. If s/he tries to imagine something, s/he finds it very easy to create a picture in her/his mind.				
4. S/he frequently gets so strongly absorbed in one thing that s/he loses sight of other things.				
5. S/he often notices small sounds when others do not.				
6. S/he usually notices car number plates or similar strings of information.				
7. Other people frequently tell her/him that what s/he has said is impolite, even though s/he thinks it is polite.				
8. When s/he is reading a story, s/he can easily imagine what the characters might look like.				
9. S/he is fascinated by dates.				
10. In a social group, s/he can easily keep track of several different people's conversations.				
11. S/he finds social situations easy.				
12. S/he tends to notice details that others do not.				
13. S/he would rather go to a library than a party.				
14. S/he finds making up stories easy.				
15. S/he finds her/himself drawn more strongly to people than to things.				
16. S/he tends to have very strong interests, which s/he gets upset about if s/he can't pursue.				
17. S/he enjoys social chit-chat.				

	Definitely Agree	Slightly Agree	Slightly Disagree	Definitely Disagree
18. When s/he talks, it isn't always easy for others to get a word in edgeways.				
19. S/he is fascinated by numbers.				
20. When s/he is reading a story, s/he finds it difficult to work out the characters' intentions.				
21. S/he doesn't particularly enjoy reading fiction.				
22. S/he finds it hard to make new friends.				
23. S/he notices patterns in things all the time.				
24. S/he would rather go to the theatre than a museum.				
25. It does not upset him/her if his/her daily routine is disturbed.				
26. S/he frequently finds that s/he doesn't know how to keep a conversation going.				
27. S/he finds it easy to "read between the lines" when someone is talking to her/him.				
28. S/he usually concentrates more on the whole picture, rather than the small details.				
29. S/he is not very good at remembering phone numbers.				
30. S/he doesn't usually notice small changes in a situation, or a person's appearance.				
31. S/he knows how to tell if someone listening to him/her is getting bored.				
32. S/he finds it easy to do more than one thing at once.				
33. When s/he talks on the phone, s/he is not sure when it's her/his turn to speak.				
34. S/he enjoys doing things spontaneously.				
35. S/he is often the last to understand the point of a joke.				

	Definitely Agree	Slightly Agree	Slightly Disagree	Definitely Disagree
36. S/he finds it easy to work out what someone is thinking or feeling just by looking at their face.				
37. If there is an interruption, s/he can switch back to what s/he was doing very quickly.				
38. S/he is good at social chit-chat.				
39. People often tell her/him that s/he keeps going on and on about the same thing.				
40. When s/he was younger, s/he used to enjoy playing games involving pretending with other children.				
41. S/he likes to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).				
42. S/he finds it difficult to imagine what it would be like to be someone else.				
43. S/he likes to plan any activities s/he participates in carefully.				
44. S/he enjoys social occasions.				
45. S/he finds it difficult to work out people's intentions.				
46. New situations make him/her anxious.				
47. S/he enjoys meeting new people.				
48. S/he is a good diplomat.				
49. S/he is not very good at remembering people's date of birth.				
50. S/he finds it very to easy to play games with children that involve pretending.				

Appendix Q: Spence Children's Anxiety Scale - Self-report

SPENCE CHILDREN'S ANXIETY SCALE

Your Name: _____ **Date:** _____

PLEASE PUT A CIRCLE AROUND THE WORD THAT SHOWS HOW OFTEN EACH OF THESE THINGS HAPPEN TO YOU. THERE ARE NO RIGHT OR WRONG ANSWERS.

1.	I worry about things	Never	Sometimes	Often	Always
2.	I am scared of the dark	Never	Sometimes	Often	Always
3.	When I have a problem, I get a funny feeling in my stomach	Never	Sometimes	Often	Always
4.	I feel afraid	Never	Sometimes	Often	Always
5.	I would feel afraid of being on my own at home	Never	Sometimes	Often	Always
6.	I feel scared when I have to take a test	Never	Sometimes	Often	Always
7.	I feel afraid if I have to use public toilets or bathrooms	Never	Sometimes	Often	Always
8.	I worry about being away from my parents	Never	Sometimes	Often	Always
9.	I feel afraid that I will make a fool of myself in front of people	Never	Sometimes	Often	Always
10.	I worry that I will do badly at my school work	Never	Sometimes	Often	Always
11.	I am popular amongst other kids my own age	Never	Sometimes	Often	Always
12.	I worry that something awful will happen to someone in my family	Never	Sometimes	Often	Always
13.	I suddenly feel as if I can't breathe when there is no reason for this	Never	Sometimes	Often	Always
14.	I have to keep checking that I have done things right (like the switch is off, or the door is locked)	Never	Sometimes	Often	Always
15.	I feel scared if I have to sleep on my own	Never	Sometimes	Often	Always
16.	I have trouble going to school in the mornings because I feel nervous or afraid	Never	Sometimes	Often	Always
17.	I am good at sports	Never	Sometimes	Often	Always
18.	I am scared of dogs	Never	Sometimes	Often	Always
19.	I can't seem to get bad or silly thoughts out of my head	Never	Sometimes	Often	Always
20.	When I have a problem, my heart beats really fast	Never	Sometimes	Often	Always
21.	I suddenly start to tremble or shake when there is no reason for this	Never	Sometimes	Often	Always
22.	I worry that something bad will happen to me	Never	Sometimes	Often	Always
23.	I am scared of going to the doctors or dentists	Never	Sometimes	Often	Always
24.	When I have a problem, I feel shaky	Never	Sometimes	Often	Always
25.	I am scared of being in high places or lifts (elevators)	Never	Sometimes	Often	Always

26.	I am a good person	Never	Sometimes	Often	Always
27.	I have to think of special thoughts to stop bad things from happening (like numbers or words)	Never	Sometimes	Often	Always
28	I feel scared if I have to travel in the car, or on a Bus or a train	Never	Sometimes	Often	Always
29.	I worry what other people think of me	Never	Sometimes	Often	Always
30.	I am afraid of being in crowded places (like shopping centres, the movies, buses, busy playgrounds)	Never	Sometimes	Often	Always
31.	I feel happy	Never	Sometimes	Often	Always
32.	All of a sudden I feel really scared for no reason at all	Never	Sometimes	Often	Always
33.	I am scared of insects or spiders	Never	Sometimes	Often	Always
34.	I suddenly become dizzy or faint when there is no reason for this	Never	Sometimes	Often	Always
35.	I feel afraid if I have to talk in front of my class	Never	Sometimes	Often	Always
36.	My heart suddenly starts to beat too quickly for no reason	Never	Sometimes	Often	Always
37.	I worry that I will suddenly get a scared feeling when there is nothing to be afraid of	Never	Sometimes	Often	Always
38.	I like myself	Never	Sometimes	Often	Always
39.	I am afraid of being in small closed places, like tunnels or small rooms.	Never	Sometimes	Often	Always
40.	I have to do some things over and over again (like washing my hands, cleaning or putting things in a certain order)	Never	Sometimes	Often	Always
41.	I get bothered by bad or silly thoughts or pictures in my mind	Never	Sometimes	Often	Always
42.	I have to do some things in just the right way to stop bad things happening	Never	Sometimes	Often	Always
43.	I am proud of my school work	Never	Sometimes	Often	Always
44.	I would feel scared if I had to stay away from home overnight	Never	Sometimes	Often	Always
45.	Is there something else that you are really afraid of?	YES	NO		
	Please write down what it is_____				
	_____				
	_____				
	How often are you afraid of this thing?	Never	Sometimes	Often	Always

Appendix R: Strengths and Difficulties Questionnaire - Self-report

Strengths and Difficulties Questionnaire S 11-17

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of how things have been for you over the last six months.

Your Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
I try to be nice to other people. I care about their feelings	☐	☐	☐
I am restless, I cannot stay still for long	☐	☐	☐
I get a lot of headaches, stomach-aches or sickness	☐	☐	☐
I usually share with others (food, games, pens etc.)	☐	☐	☐
I get very angry and often lose my temper	☐	☐	☐
I am usually on my own. I generally play alone or keep to myself	☐	☐	☐
I usually do as I am told	☐	☐	☐
I worry a lot	☐	☐	☐
I am helpful if someone is hurt, upset or feeling ill	☐	☐	☐
I am constantly fidgeting or squirming	☐	☐	☐
I have one good friend or more	☐	☐	☐
I fight a lot. I can make other people do what I want	☐	☐	☐
I am often unhappy, down-hearted or tearful	☐	☐	☐
Other people my age generally like me	☐	☐	☐
I am easily distracted, I find it difficult to concentrate	☐	☐	☐
I am nervous in new situations. I easily lose confidence	☐	☐	☐
I am kind to younger children	☐	☐	☐
I am often accused of lying or cheating	☐	☐	☐
Other children or young people pick on me or bully me	☐	☐	☐
I often volunteer to help others (parents, teachers, children)	☐	☐	☐
I think before I do things	☐	☐	☐
I take things that are not mine from home, school or elsewhere	☐	☐	☐
I get on better with adults than with people my own age	☐	☐	☐
I have many fears, I am easily scared	☐	☐	☐
I finish the work I'm doing. My attention is good	☐	☐	☐

Do you have any other comments or concerns?

Appendix S: School Anxiety Scale - Teacher report (SAS-TR)

School Anxiety Scale – Teacher Report

For each item please fill in the circle that best describes how this child has been **over the last three months or this school year**. Please answer all of the items.

	Never	Sometimes	Often	Always
1. This child is afraid of asking questions in class	○	○	○	○
2. This child speaks only when someone asks a question of them	○	○	○	○
3. This child worries what other people think of him/her	○	○	○	○
4. This child does not volunteer answers or comments during class	○	○	○	○
5. This child is afraid of making mistakes	○	○	○	○
6. This child hates being the centre of attention	○	○	○	○
7. This child hesitates in starting tasks or asks whether they understood the task before starting	○	○	○	○
8. This child worries about things	○	○	○	○
9. This child worries that (s)he will do badly at school	○	○	○	○
10. This child worries that something bad will happen to him/her	○	○	○	○
11. This child seems very shy	○	○	○	○
12. This child complains of headaches, stomach aches or feeling sick	○	○	○	○
13. This child feels afraid when (s)he has to talk in front of the class	○	○	○	○
14. This child hesitates to speak when in group situations	○	○	○	○
15. When this child has a problem, (s)he feels shaky	○	○	○	○
16. This child appears nervous when approached by other children or adults	○	○	○	○

Appendix T: Attentional Control Scale (ACS)

IRAS[redacted] ERGO[redacted] Version 1, 01.07.16
Participant Number Date

Attentional Control Scale

Please rate each question as to how much this applies to you

1 = *almost never* 2 = *sometimes* 3 = *often* 4 = *always*

	1	2	3	4
It's very hard for me to concentrate on a difficult task when there are noises around				
When I need to concentrate and solve a problem, I have trouble focusing my attention				
When I am working hard on something, I still get distracted by events around me				
My concentration is good even if there is music in the room around me				
When concentrating, I can focus my attention so that I become unaware of what's going on in the room around me				
When I am reading or studying, I am easily distracted if there are people talking in the same room				
When trying to focus my attention on something, I have difficulty blocking out distracting thoughts				
I have a hard time concentrating when I'm excited about something				
When concentrating I ignore feelings of hunger or thirst				
I can quickly switch from one task to another				
It takes me a while to get really involved in a new task				
It is difficult for me to coordinate my attention between the listening and writing required when taking notes during lectures				
I can become interested in a new topic very quickly when I need to				
It is easy for me to read or write while I'm also talking on the phone				
I have trouble carrying on two conversations at once				
I have a hard time coming up with new ideas quickly				
After being interrupted or distracted, I can easily shift my attention back to what I was doing before				
When a distracting thought comes to mind, it is easy for me to shift my attention away from it				
It is easy for me to alternate between two different tasks				
It is hard for me to break from one way of thinking about something and look at it from another point of view				

Appendix U: Palm-pilot questions

1. Please take your saliva sample.
2. Out of 10 with 10 being the most and 1 being the least, how worried or concerned are you feeling right now?

1 2 3 4 5 6 7 8 9 10

3. Out of 10 with 10 being the most and 1 being the least, how happy are you feeling right now?

1 2 3 4 5 6 7 8 9 10

4. Out of 10 with 10 being the most and 1 being the least, how angry or frustrated are you feeling right now?

1 2 3 4 5 6 7 8 9 10

5. Out of 10 with 10 being the most and 1 being the least, rate how noisy it is right now?

1 2 3 4 5 6 7 8 9 10

6. What are you doing at the moment?

- In lesson
- Break time
- Lunch time
- In between lessons
- At home
- Travelling
- Outside
- In bedroom
- Eating dinner
- Watching TV
- Playing games
- Other

3a. (if in lesson) What lesson are you in?

- English
- Maths
- Science
- Geography
- History
- Art
- Drama

- Music
- P.E

3b (if in lesson) Are you working alone or in a group?

Alone In a group

7. How many people are with you?

I'm alone 0-5 5-10 over 10

8. Who are you with?
   - Family
   - Friends
   - Teachers
   - Nobody
   - People I don't like

9. Have you done any exercise in the last 30 minutes?

Yes No

10. Have you eaten any food or drank anything in the last 30 minutes?

Yes No

10. Have you brushed your teeth in the last 30 minutes?

Yes No

11. Have you taken your saliva sample?

Yes No

End of the day prompt:

Has anything unusual happened during the day?

Yes No

If yes, please describe what happened:

Appendix V: Power Calculation for sample size

Version 1, 06.09.16, IRAS Number[redacted]

Power Calculation for Cortisol samples

An *a priori* sample size calculation to determine the required N to detect differences between groups in diurnal cortisol secretion was carried out using GPower (Faul et al. 2007). Based on previous research (Spratt et al., 2012), a small to medium effect can be expected. With α set at .05, and in order to achieve a power of .80 to detect an group x time effect of f= .25 (using General Linear Model with 2 groups and 5 repeated measures), a total sample size of N=24 is required.

Faul et al., G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences *Behav Res Methods***39**, 175 (2007).

Spratt, E. G., Nicholas, J. S., Brady, K. T., Carpenter, L. A., Hatcher, C. R., Meekins, K. A., ... & Charles, J. M. (2012). Enhanced cortisol response to stress in children in autism. *Journal of autism and developmental disorders*,*42*(1), 75-81.

G*Power output:

Analysis: A priori: Compute required sample size

Input:	Effect size f	= 0.25
	α err prob	= 0.05
	Power (1-β err prob)	= 0.80
	Number of groups	= 2
	Number of measurements	= 5
	Corr among rep measures	= 0.5
	Nonsphericity correction ε	= 0.8
Output:	Noncentrality parameter λ	= 12.0000000
	Critical F	= 2.6793943
	Numerator df	= 3.2000000
	Denominator df	= 70.4000000
	Total sample size	= 24
	Actual power =	0.8077801

Appendix W: Compliance data for experience sampling procedure

	ASD (120 possible data points, 3 missing)		TD (140 possible data points, 5 missing)		Total	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Food/Drink 30 mins before sample?	17 (14.5%)	100 (85.5%)	36 (26.7%)	99 (73.3%)	53 (21%)	199 (79%)
Exercise 30 mins before sample?	11 (9.4%)	106 (90.6%)	10 (7.4%)	125 (92.6%)	21 (8.3%)	231 (91.7%)
Brushed teeth 30 mins before sample?	4 (3.4%)	113 (96.6%)	7 (5.2%)	128 (94.8%)	11 (4.4%)	241 (95.6%)

Appendix X: Proportion of participants meeting clinical cut off on questionnaire measures, and cut off points required

SCAS: Percentage meeting cut off (to indicate elevated anxiety: above 84th percentile), (Spence, n.d)

Parent Total SCAS cut off = above 23 (based on T-scores)

Self-report Total SCAS cut off = above 32 (based on T-scores)

Separation Anxiety Parent cut off: >4, and Self Report cut off: >3

Social Anxiety Parent cut off: >6, Self Report cut off: >7

Panic Parent cut off: >1, Self Report cut off: > 4

Physical Injury parent cut off: > 4, Self Report cut off: >3

OCD parent cut off: >1, Self Report cut off > 6

GAD parent cut off: > 4, Self Report cut off > 8

Percentage of participants showing elevated levels of anxiety, according to t-scores on SCAS						
	TD		ASD		Total	
	Parent report	Self report	Parent report	Self report	Parent report	Self report
Separation anxiety	42.8%	71.4%	83.3%	75%	61.5%	73.0%
Social Anx	21.4%	71.4%	83.3%	16.6%	50%	46.1%
Panic	57.1%	71.4%	91.6%	33.3%	73.0%	53.8%
Physical Injury	35.7%	85.7%	50%	58.3%	42.3%	73.0%
OCD	42.8%	50%	83.3%	33.3%	61.5%	42.3%
GAD	42.8%	50%	100%	33.3%	69.2%	42.3%
Total SCAS score	50%	78.5%	91.6%	50%	73.0%	65.3%

Table 3: Categorising SDQ scores for 4-17 year olds (not validated for 18+)

	Original 3-band categorisation			Newer 4-band categorisation			
	Normal	Borderline	Abnormal	Close to average	Slightly raised (/slightly lowered)	High (/Low)	Very high (very low)
Parent completed SDQ							
Total difficulties score	0-13	14-16	17-40	0-13	14-16	17-19	20-40
Emotional problems score	0-3	4	5-10	0-3	4	5-6	7-10
Conduct problems score	0-2	3	4-10	0-2	3	4-5	6-10
Hyperactivity score	0-5	6	7-10	0-5	6-7	8	9-10
Peer problems score	0-2	3	4-10	0-2	3	4	5-10
Prosocial score	6-10	5	0-4	8-10	7	6	0-5
Impact score	0	1	2-10	0	1	2	3-10
Teacher completed SDQ							
Total difficulties score	0-11	12-15	16-40	0-11	12-15	16-18	19-40
Emotional problems score	0-4	5	6-10	0-3	4	5	6-10
Conduct problems score	0-2	3	4-10	0-2	3	4	5-10
Hyperactivity score	0-5	6	7-10	0-5	6-7	8	9-10
Peer problems score	0-3	4	5-10	0-2	3-4	5	6-10
Prosocial score	6-10	5	0-4	6-10	5	4	0-3
Impact score	0	1	2-6	0	1	2	3-6
Self-completed SDQ							
Total difficulties score	0-15	16-19	20-40	0-14	15-17	18-19	20-40
Emotional problems score	0-5	6	7-10	0-4	5	6	7-10
Conduct problems score	0-3	4	5-10	0-3	4	5	6-10
Hyperactivity score	0-5	6	7-10	0-5	6	7	8-10
Peer problems score	0-3	4-5	6-10	0-2	3	4	5-10
Prosocial score	6-10	5	0-4	7-10	6	5	0-4
Impact score	0	1	2-10	0	1	2	3-10

Note that both these systems only provide a rough-and-ready way of screening for disorders; combining information from SDQ symptom and impact scores from multiple informants is better, but still far from perfect.

SDQ cut offs (YouthinMind, 2016)

Percentage of participants meeting criteria for significant difficulties on SDQ (Abnormal or High levels)									
	TD			ASD			Total		
	Parent report	Self report	Teacher report	Parent report	Self report	Teacher report	Parent report	Self report	Teacher report
Total difficulties	35.7%	21.4%	7.1%	100%	50%	66.6%	65.3%	34.6%	34.6%
Emotional problems	35.7%	21.4%	0%	83.3%	41.6%	41.6%	57.6%	30.7%	19.2%
Conduct problems	21.4%	7.1%	0%	58.3%	25%	41.6%	38.4%	15.3%	19.2%
Hyperactivity	14.2%	28.5%	21.4%	83.3%	50%	50%	46.1%	38.4%	34.6%
Peer problems	28.5%	7.1%	7.1%	100%	16.6%	66.6%	61.5%	11.5%	34.6%
Prosocial	14.2%	0%	7.1%	41.6%	16.6%	33.3%	26.9%	7.6%	19.2%

DISC: Cut off applied using automatic scoring software (C-DISC 4)

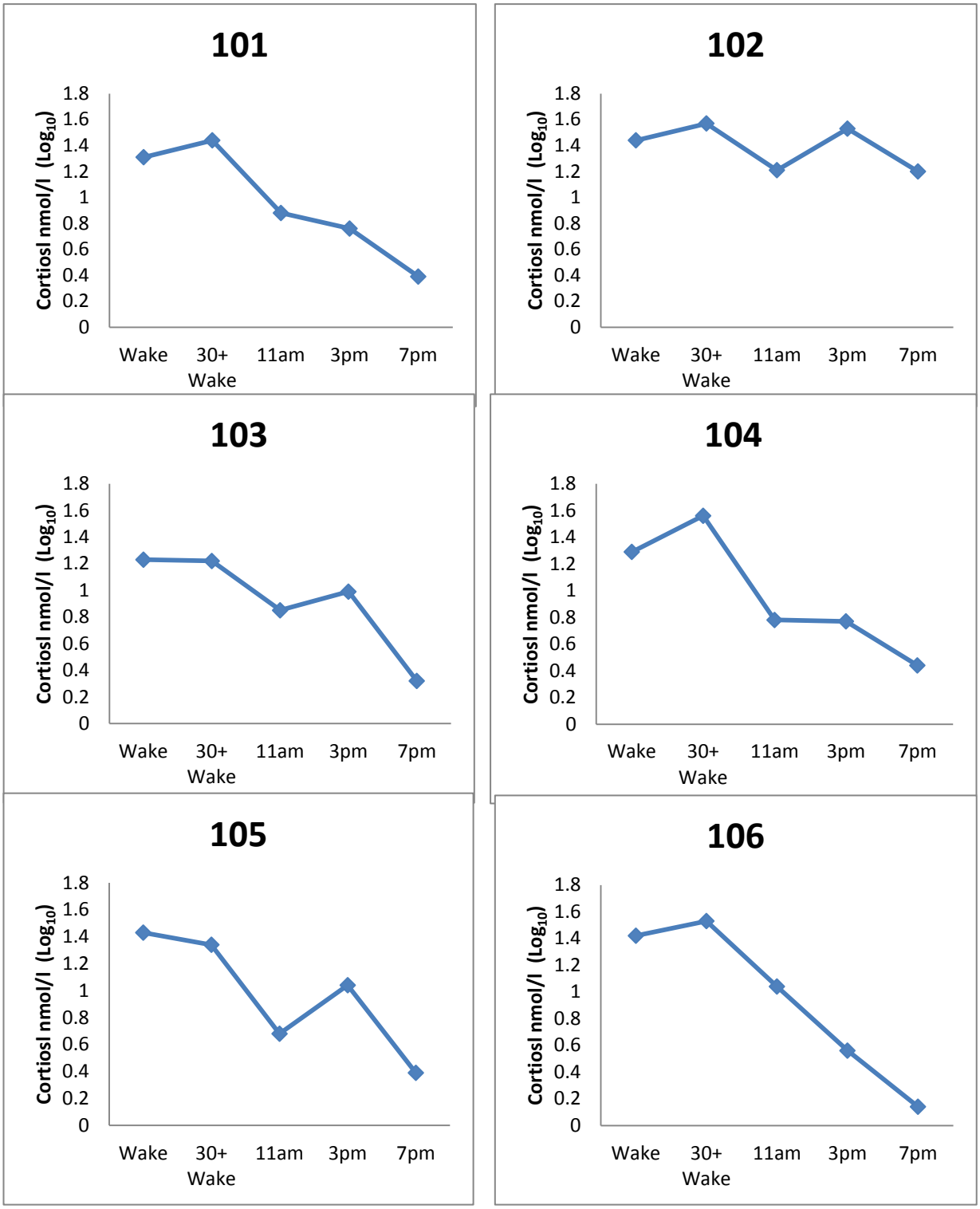
Diagnosis	Percentage meeting criteria		
	TD	ASD	Total
Separation Anxiety	28.5%	58.3%	42.3%
Social Anxiety	50%	66.6%	57.6%
Panic	14.2%	0%	7.6%
Specific Phobia	64.2%	75%	69.2%
OCD	21.4%	50%	34.6%
Generalised Anxiety	21.4%	58.3%	38.4%
Meeting 1 diagnosis	35.7%	8.3%	23.0%
Meeting 2	35.7%	25%	30.7%
Meeting 3	14.2%	16.6%	15.3%
Meeting 4	14.2%	50%	30.7%

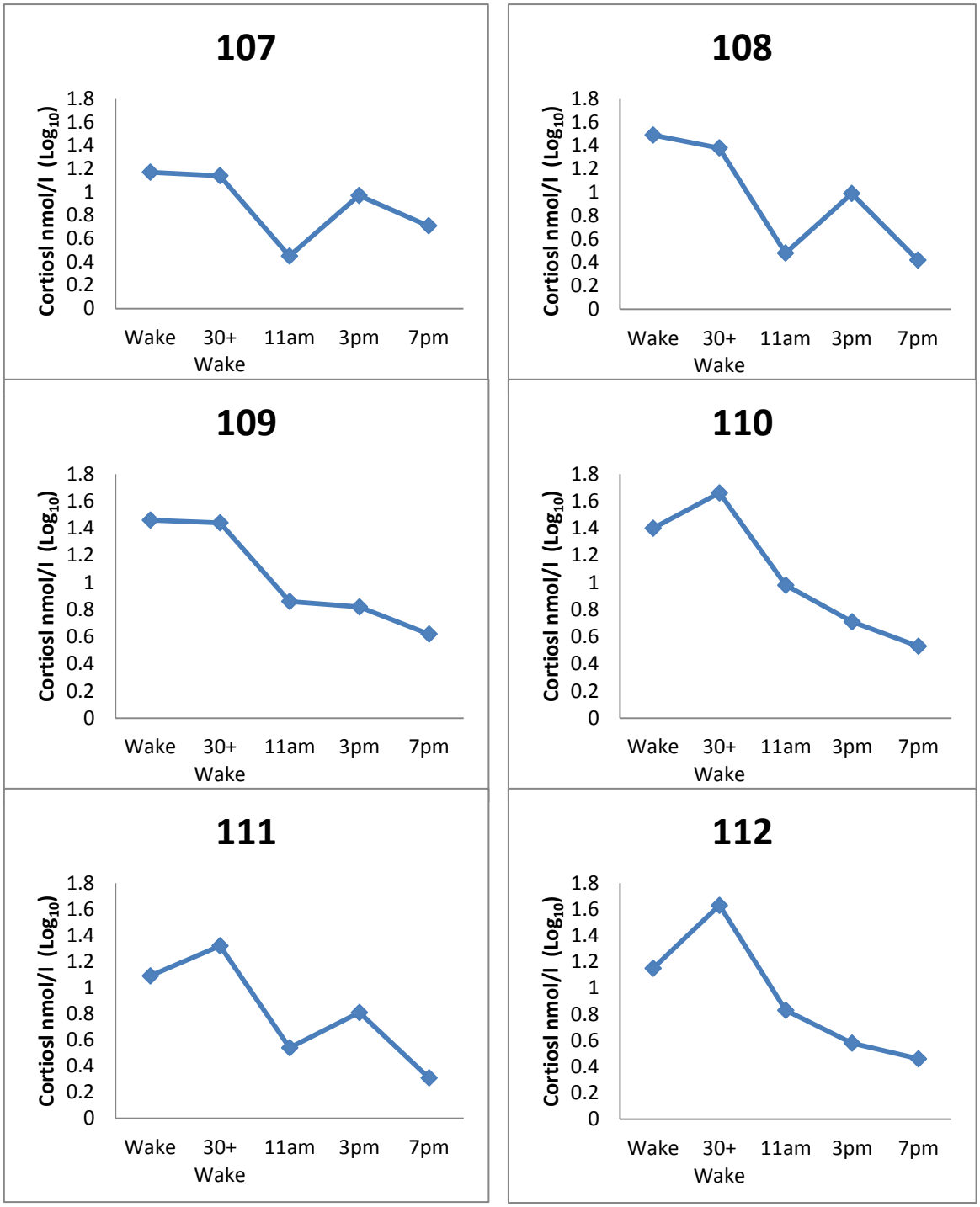
SAS-TR cut off:

Scores of 8 and more on the social anxiety, 10 or more on the generalized anxiety, and 17 or more on the total anxiety is considered to represent high anxious condition (Lyneham, Street, Abbott, & Rapee, 2008).

Scale	TD	ASD	Total
Generalised Anxiety	0%	83.3%	38.4%
Social Anxiety	7.1%	16.6%	11.5%
Total score	0%	41.6%	19.2%

Appendix Y: Pattern of each individual's cortisol levels, averaged for each time point across the two days

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109
110
111
112
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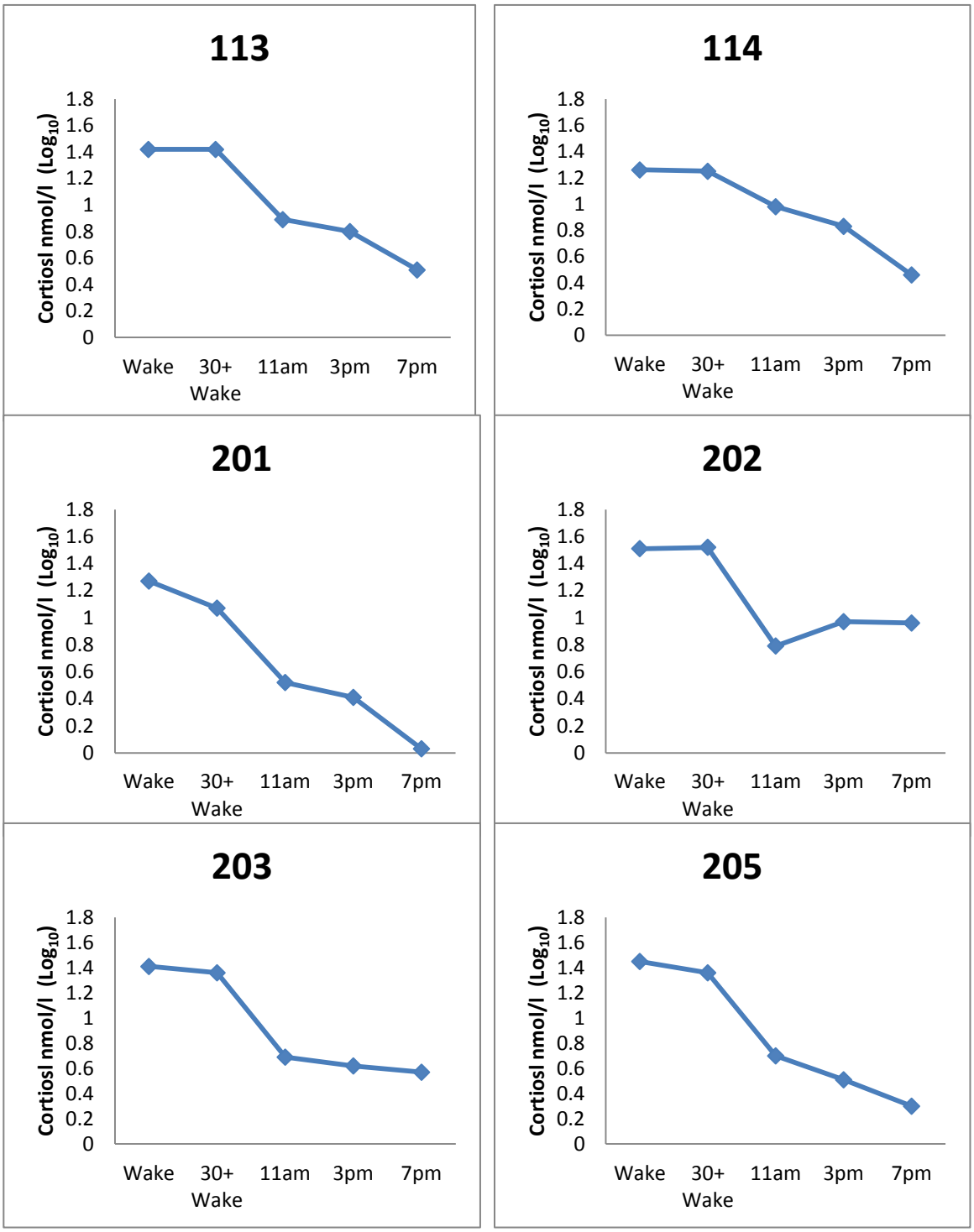
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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201
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203
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Cortiosl nmol/l (Log10)
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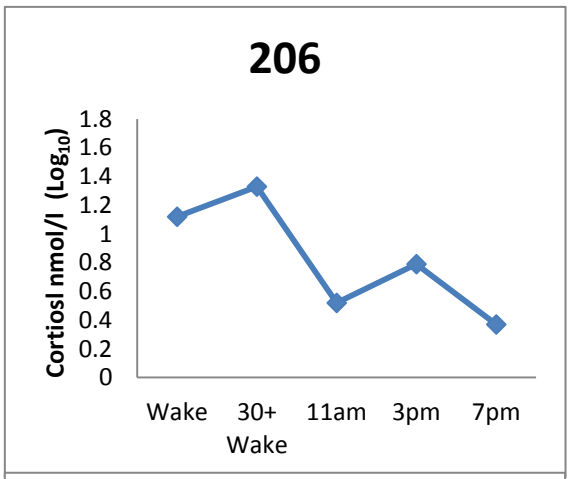
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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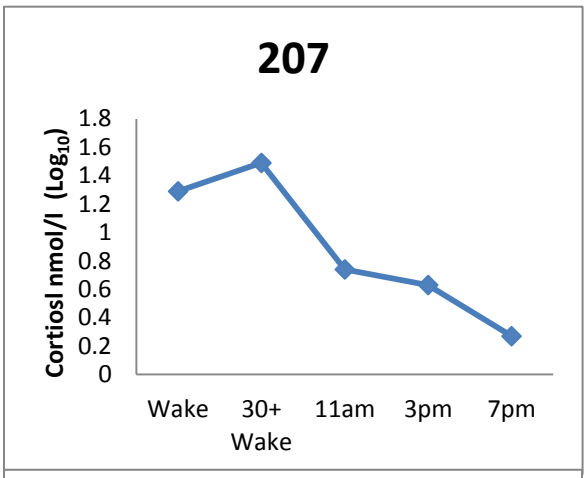
Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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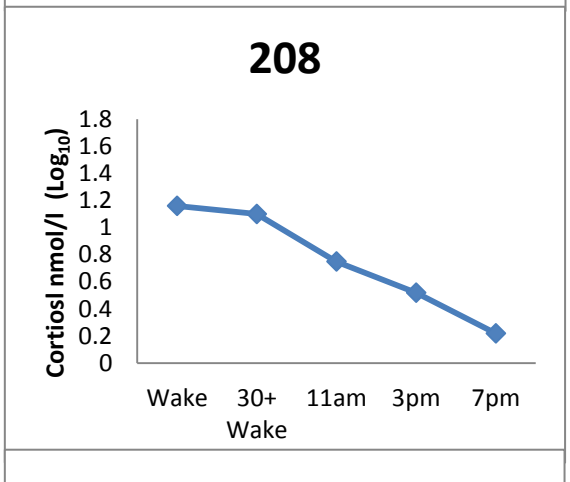
Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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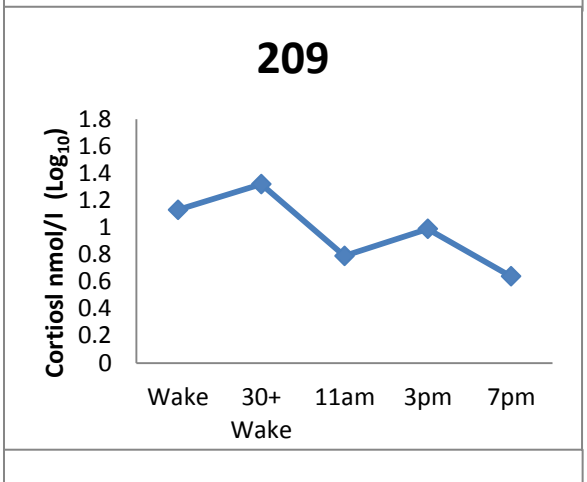
Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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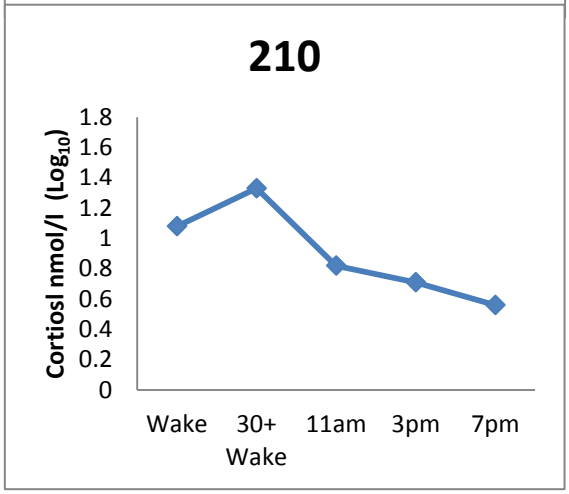
Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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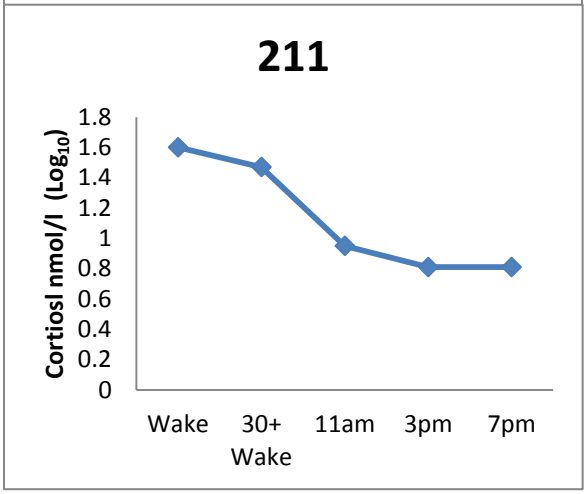
Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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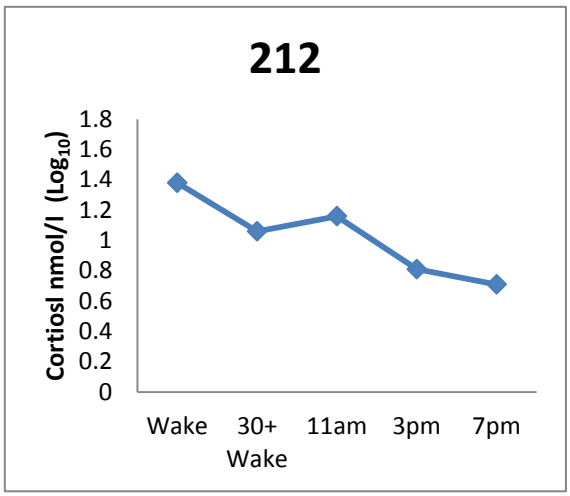
Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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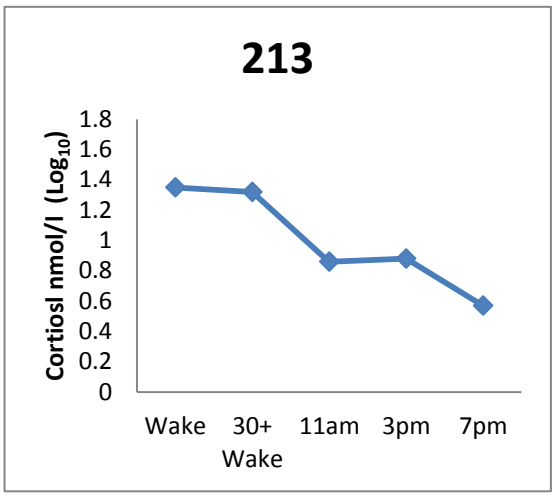
Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm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Cortiosl nmol/l (Log10)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Wake
30+ Wake
11am
3pm
7pm

Appendix Z: Cortisol data calculations and transformations

Each participant provided a sample for each of the 10 time points (5 per day for 2 days).

Each sample was analysed at the laboratory in Germany to give two values for each sample. This was then averaged to give a mean cortisol level for each sample.

As there was no significant difference in cortisol levels between the two days, an average for each time point was calculated from the mean of the time point for each day. This gave 5 cortisol values for each participant, 1 for each time point, averaged across the two days. To calculate the CAR, cortisol values at time point 1 (Wake) were subtracted from cortisol values at time point 2 (30 + wake), to give a value of cortisol change from Time 1 to Time 2. To calculate AUC, average values for each time point were added together (Time 1 + 2 + 3 + 4 + 5) to give a total value of cortisol released in the day. As there was no significant difference in cortisol values across the day, this too was calculated using the average of each time point across the two days. As AUC variable was not normally distributed, AUC values were then log transformed (base 10) when used in further analysis. Raw mean data for AUC is reported in tables.

When assessing correlations in the experience sampling data with cortisol levels and emotions, noise and other factors (social status, activity), log transformed values of cortisol were used in this analysis, as cortisol for each time point were not normally distributed.

When assessing interactions between time and cortisol between groups, raw mean data for each time point was calculated across the two days, then these 5 variables were log transformed (base 10) as they were not normally distributed. The log transformed variables were then used in the repeated measures ANOVA to assess for effect of time on cortisol, and interaction of ASD diagnosis.

Appendix AA: List of non-normal data scales

- SAS-TR:

- Both groups: Total anxiety score for both groups.

- ASD: General Anxiety sub scale

- TD: General Anxiety and Social Anxiety sub scale

- SDQ:

Teacher report SDQ:

- Both groups: Internalising Difficulties Scale and Emotional Problems scale

- TD: Behaviour difficulties, hyperactivity and peer difficulties scales, externalising difficulties scale

Parent report SDQ:

- TD: behaviour difficulties, peer relationship problems and pro-social scales

Self-report SDQ:

ASD: Behaviour difficulties scale

TD: Pro-social scale

- SCAS:

Parent report

TD: Panic and OCD scale

Self report

ASD: Panic scale for ASD

- DISC:

Both groups: total number of diagnoses met

- Experience sampling data:

Both groups: Anxiety, Happiness, Anger and Noise. Activity structure and social status.

- Cortisol

Data at each time point and AUC when calculated from raw data

Appendix AB: Experience sampling data correlations by group

Spearman's rho correlations between mood, noise, cortisol, activity and social status from experience sampling data, for the ASD group (top of table) and TD group (bottom of table) Significant associations are highlighted

Measure	1	2	3	4	5	6	7
ASD (n=12)							
1. Anxiety	-	**.66*****	**-.51*****	0.03	-0.13	0.11	-0.14
2. Anger		-	**-.55*****	0.07	-0.16	0.12	**-.19***
3. Positive Affect			-	-0.01	0.03	-0.10	0.15
4. Noise				-	**-.21***	**-.28*****	**.37*****
5. Cortisol					-	**.29*****	**-.20***
6. Activity						-	**-.48*****
7. Social Status							-
TD (n=14)							
1. Anxiety	-	**.55*****	**-.57*****	**.25*****	-0.00	**-.18***	**.32*****
2. Anger		-	**-.46*****	**.25*****	0.01	-0.09	0.15
3. Positive Affect			-	-0.04	-0.16	0.02	-0.09
4. Noise				-	**-.30*****	**-.26*****	**.39*****
5. Cortisol					-	**.18***	**-.20***
6. Activity						-	**-.31*****
7. Social Status							-

**p < .05, **p < .01, ***p < .008 (bonferroni adjustment)*

References

Alfano, C. A., Ginsburg, G. S., & Kingery, J. N. (2007). Sleep-Related Problems Among Children and Adolescents With Anxiety Disorders. *Journal of the American Academy of Child & Adolescent Psychiatry*, *46*(2), 224–232. https://doi.org/10.1097/01.chi.0000242233.06011.8e

Almela, M., van der Meij, L., Hidalgo, V., Villada, C., & Salvador, A. (2012). The cortisol awakening response and memory performance in older men and women. *Psychoneuroendocrinology*, *37*(12), 1929–1940. https://doi.org/10.1016/j.psyneuen.2012.04.009

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., text rev.). Washington, DC: Author

American Psychiatric Association, APA. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing

Ashburner, J., Bennett, L., Rodger, S., & Ziviani, J. (2013). Understanding the sensory experiences of young people with autism spectrum disorder: A preliminary investigation. *Australian Occupational Therapy Journal*, *60*(3), 171-180. doi: 10.1111/1440-1630.12025

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *The Lancet*, *368*(9531), 210-215. doi: 10.1016/S0140-6736(06)69041-7

Baron-Cohen, S., Hoekstra, R. A., Knickmeyer, R. & Wheelwright, S. (2006). The Autism-Spectrum Quotient (AQ) - Adolescent Version. *Journal of Autism and Developmental Disorders*, *36*, 343-350. doi: 10.1007/s10803-006-0073-6

Baron-Cohen, S., Lombardo, M., & Tager-Flusberg, H. (Eds.). (2013). *Understanding other minds: Perspectives from developmental social neuroscience*. Oxford: Oxford University Press

Bellini, S. (2004). Social skills deficits and anxiety in higher functioning adolescents with autism spectrum disorders. *Focus on Autism and Other Developmental Disabilities, 19 (3),* 78-86. doi: 10.1177/10883576040190020201

Ben-Sasson, A., Hen, L., Fluss, R., Cermak, S. A., Engel-Yeger, B., & Gal, E. (2009). A meta-analysis of sensory modulation symptoms in individuals with autism spectrum disorders. *Journal of autism and developmental disorders*, *39*(1), 1-11.doi: 10.1007/s10803-008-0593-3

Berggren, N., & Derakhshan, N. (2013) Attentional control deficits in trait anxiety: why you see them and why you don't. *Biological Psychology, 92* (3), 440-446. doi:10.1016/j.biopsycho.2012.03.007

Bitsika, V., Sharpley, C. F., Andronicos, N. M., & Agnew, L. L. (2015). Hypothalamus–pituitary–adrenal axis daily fluctuation, anxiety and age interact to predict cortisol concentrations in boys with an autism spectrum disorder. *Physiology & Behavior, 138*, 200–207. https://doi.org/10.1016/j.physbeh.2014.11.010

Boulter, C., Freeston, M., South, M., & Rodgers, J. (2014). Intolerance of uncertainty as a framework for understanding anxiety in children and adolescents with autism spectrum disorders. *Journal of Autism and Developmental Disorders, 44*(6), 1391- 1402. doi: 10.1007/s10803-013-2001-x

Brosnan, M., Turner-Cobb, J., Munro-Naan, Z., & Jessop, D. (2009). Absence of a normal Cortisol Awakening Response (CAR) in adolescent mates with Asperger Syndrome (AS). *Psychoneuroendocrinology*, *34*(7), 1095–1100. https://doi.org/10.1016/j.psyneuen.2009.02.011

Cath, D. C., Ran, N., Smit, J. H., Van Balkom, A. J., & Comijs, H. C. (2008). Symptom overlap between autism spectrum disorder, generalized social anxiety disorder and obsessive-compulsive disorder in adults: a preliminary case-controlled study. *Psychopathology*, *41*(2), 101-110. doi:10.1159/000111555

Carleton, R. N. (2012). The intolerance of uncertainty construct in the context of anxiety disorders: theoretical and practical perspectives. *Expert Review of Neurotherapeutics*, *12*(8), 937–947. https://doi.org/10.1586/ern.12.82

Carleton, R. N., Mulvogue, M. K., Thibodeau, M. A., McCabe, R. E., Antony, M. M., & Asmundson, G. J. G. (2012). Increasingly certain about uncertainty: Intolerance of uncertainty across anxiety and depression. *Journal of Anxiety Disorders*, *26*(3), 468–479. https://doi.org/10.1016/j.janxdis.2012.01.011

Chaste, P., & Leboyer, M. (2012). Autism risk factors: genes, environment, and gene-environment interactions. *Dialogues in Clinical Neuroscience*, *14*(3), 281–292.

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: A systematic review and meta-analysis. *Biological Psychology*, *80*(3), 265–278. https://doi.org/10.1016/j.biopsycho.2008.10.004

Chorpita, B. F., Yim, L., Moffitt, C., Umemoto, L. A., & Francis, S. E. (2000). Assessment of symptoms of DSM-IV anxiety and depression in children: a revised child anxiety and depression scale. *Behaviour Research and Therapy*, *38*(8), 835–855. https://doi.org/10.1016/S0005-7967(99)00130-8

Chorpita, B. F., Moffitt, C. E., & Gray, J. (2005). Psychometric properties of the Revised Child Anxiety and Depression Scale in a clinical sample. *Behaviour Research and Therapy*, *43*(3), 309–322. https://doi.org/10.1016/j.brat.2004.02.004

Clark, D. M., & Wells, A. (1995). A cognitive model of social phobia. In R. G. Heimberg (Ed.). *Social Phobia: Diagnosis, Assessment, and Treatment* (pp. 69–93). New York: Guilford Press

Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The awakening cortisol response: methodological issues and significance. *Stress*, *7*(1), 29-37. http://dx.doi.org/10.1080/10253890410001667205

Cochrane Collaboration. (2014). Review Manager (RevMan) (Version 5.3) [Computer program]. Copenhagen: The Nordic Cochrane Centre. Available from http://community.cochrane.org/tools/review-production-tools/revman-5

Comer, J. S., Roy, A. K., Furr, J. M., Gotimer, K., Beidas, R. S., Dugas, M. J., & Kendall, P. C. (2009). The Intolerance of Uncertainty Scale for Children: A Psychometric Evaluation. *Psychological Assessment*, *21*(3), 402–411. https://doi.org/10.1037/a0016719

Corbett, B. A., Mendoza, S., Abdullah, M., Wegelin, J. A., & Levine, S. (2006). Cortisol circadian rhythms and response to stress in children with autism. *Psychoneuroendocrinology*, *31*(1), 59–68. https://doi.org/10.1016/j.psyneuen.2005.05.011

Corbett, B. A., Mendoza, S., Wegelin, J. A., Carmean, V., & Levine, S. (2008). Variable cortisol circadian rhythms in children with autism and anticipatory stress. *Journal of Psychiatry & Neuroscience: JPN*, *33*(3), 227–234

Corbett, B. A., Schupp, C. W., Levine, S., & Mendoza, S. (2009). Comparing cortisol, stress, and sensory sensitivity in children with autism. *Autism Research*, *2*(1), 39–49. https://doi.org/10.1002/aur.64

Corbett, B. A., & Schupp, C. W. (2014). The cortisol awakening response (CAR) in male children with autism spectrum disorder. *Hormones and Behavior*, *65*(4), 345–350. https://doi.org/10.1016/j.yhbeh.2014.01.012

Craske, M. G. (1999). *Anxiety disorders: Psychological approaches to theory and treatment*. Boulder, CO, US: Westview Press

de Bruin E.I., Ferdinand R.F., Meesters S., de Nijs P.F.A. &, Verheij F. (2007).High rates of psychiatric co-morbidity in PDD-NOS. *Journal for Autism and Developmental Disorders, 37,*877–886. doi: 10.1007/s10803-006-0215-x

Derryberry, D., & Reed, M. A. (2002). Anxiety-related attentional biases and their regulation by attentional control. *Journal of Abnormal Psychology, 111,* 225– 236. doi:10.1037/0021-843X.111.2.225

De Los Reyes, A., Augenstein, T. M., Wang, M., Thomas, S. A., Drabick, D. A., Burgers, D. E., & Rabinowitz, J. (2015). The validity of the multi-informant approach to assessing child and adolescent mental health. doi: 10.1037/a0038498

De Los Reyes, A., & Kazdin, A. E. (2005). Informant Discrepancies in the Assessment of Childhood Psychopathology: A Critical Review, Theoretical Framework, and Recommendations for Further Study. *Psychological Bulletin*, *131*(4), 483–509.

Dugas, M. J., Gagnon, F., Ladouceur, R., & Freeston, M. H. (1998). Generalized anxiety disorder: a preliminary test of a conceptual model. *Behaviour Research and Therapy*, *36*(2), 215–226

Endler, N. S., & Kocovski, N. L. (2001). State and trait anxiety revisited. *Journal of anxiety disorders*, *15*(3), 231-245.

Essau, C. A., Conradt, J., & Petermann, F. (2000). Frequency, comorbidity, and psychosocial impairment of anxiety disorders in German adolescents. *Journal of anxiety disorders*, *14*(3), 263-279.http://dx.doi.org/10.1016/S0887-6185(99)00039-0

Essau, C. A., Sasagawa, S., Anastassiou-Hadjicharalambous, X., Guzmán, B. O., & Ollendick, T. H. (2011). Psychometric properties of the Spence Child Anxiety Scale with adolescents from five European countries. *Journal of Anxiety Disorders*, *25*(1), 19-27. http://dx.doi.org/10.1016/j.janxdis.2010.07.001

Evans, P. D., Fredhoi, C., Loveday, C., Hucklebridge, F., Aitchison, E., Forte, D., & Clow, A. (2011). The diurnal cortisol cycle and cognitive performance in the healthy old. *International Journal of Psychophysiology*, *79*(3), 371–377. https://doi.org/10.1016/j.ijpsycho.2010.12.006

Fleury, V. P., Hedges, S., Hume, K., Browder, D. M., Thompson, J. L., Fallin, K., … Vaughn, S. (2014). Addressing the Academic Needs of Adolescents With Autism Spectrum Disorder in Secondary Education. *Remedial and Special Education*, *35*(2), 68–79. https://doi.org/10.1177/0741932513518823

Fombonne, E., Quirke, S. & Hagen, A. (2011). Epidemiology of pervasive developmental disorders. In Amaral D.G., Dawson G. and Geschwind D.H. eds. (2011) *Autism spectrum disorders*. New York: Oxford University Press, pp. 90–111

Fox, N. A., Henderson, H. A., Marshall, P. J., Nichols, K. E., & Ghera, M. M. (2005). Behavioral inhibition: linking biology and behavior within a developmental framework. *Annual Review of Psychology*, *56*, 235–262. https://doi.org/10.1146/annurev.psych.55.090902.141532

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): facts and future directions. *International Journal of Psychophysiology*, *72* (1), 67-73. doi: 10.1016/j.ijpsycho.2008.03.014

Gabriels, R. L., Agnew, J. A., Pan, Z., Holt, K. D., Reynolds, A., & Laudenslager, M. L. (2013). Elevated repetitive behaviors are associated with lower diurnal salivary cortisol levels in autism spectrum disorder. *Biological Psychology*, *93*(2), 262–268. https://doi.org/10.1016/j.biopsycho.2013.02.017

Gartland, N., O'Connor, D. B., Lawton, R., & Bristow, M. (2014). Exploring day-to-day dynamics of daily stressor appraisals, physical symptoms and the cortisol awakening response. *Psychoneuroendocrinology*, *50*, 130–138. https://doi.org/10.1016/j.psyneuen.2014.08.006

Greaves-Lord, K., Ferdinand, R. F., Oldehinkel, A. J., Sondeijker, F. E. P. L., Ormel, J., & Verhulst, F. C. (2007). Higher cortisol awakening response in young adolescents with persistent anxiety problems. *Acta PsychiatricaScandinavica*, *116*(2), 137–144. https://doi.org/10.1111/j.1600-0447.2007.01001.x

Green, H., McGinnity, Á., Meltzer, H., Ford, T., & Goodman, R. (2005). Mental health of children and young people in Great Britain, 2004

Gröschl, M., Rauh, M., & Dörr, H.-G. (2003). Circadian Rhythm of Salivary Cortisol, 17α-Hydroxyprogesterone, and Progesterone in Healthy Children. *Clinical Chemistry*, *49*(10), 1688–1691. https://doi.org/10.1373/49.10.1688

Grupe, D. W., & Nitschke, J. B. (2013). Uncertainty and anticipation in anxiety: an integrated neurobiological and psychological perspective. *Nature Reviews. Neuroscience*, *14*(7), 488–501. https://doi.org/10.1038/nrn3524

Goodman, R. (1997). The Strengths and Difficulties Questionnaire: A Research Note. *Journal of Child Psychology and Psychiatry*, 38, 581-586

Goodman, R. (2001). Psychometric properties of the Strengths and Difficulties Questionnaire (SDQ). *Journal of the American Academy of Child and Adolescent Psychiatry, 40,* 1337-1345. http://dx.doi.org/10.1097/00004583-200111000-00015

Hadwin, J. A., Garner, M., & Perez-Olivas, G. (2006). The development of information processing biases in childhood anxiety: A review and exploration of its origins in parenting. *Clinical Psychology Review*, *26*(7), 876–894. https://doi.org/10.1016/j.cpr.2005.09.004

Hadwin, J. A., Visu-Petra, L., Muris, P., Derakshan, N., & Macleod, C. (2016). Introduction to the Special Issue: Understanding the role of attentional control in the development of anxiety in childhood, adolescence and across the lifespan. *Journal of Experimental Psychopathology*, *7*(3), 277–295

Happé, F. G. (1995). The role of age and verbal ability in the theory of mind task performance of subjects with autism. *Child Development*, *66*(3), 843–855.

Higgins, J. P. T., Thompson, S. G., Deeks, J. J., & Altman, D. G. (2003). Measuring inconsistency in meta-analyses. *BMJ : British Medical Journal*, *327*(7414), 557–560.

Hill, E., Berthoz, S., & Frith, U. (2004). Brief Report: Cognitive Processing of Own Emotions in Individuals with Autistic Spectrum Disorder and in Their Relatives. *Journal of Autism and Developmental Disorders*, *34*(2), 229–235. https://doi.org/10.1023/B:JADD.0000022613.41399.14

Hill, E. E., Zack, E., Battaglini, C., Viru, M., Viru, A., & Hackney, A. C. (2008). Exercise and circulating cortisol levels: the intensity threshold effect. *Journal of endocrinological investigation*, *31*(7), 587-591.doi:10.1007/BF03345606

Hucklebridge, F., Hussain, T., Evans, P., & Clow, A. (2005). The diurnal patterns of the adrenal steroids cortisol and dehydroepiandrosterone (DHEA) in relation to awakening. *Psychoneuroendocrinology*, *30*(1), 51–57. https://doi.org/10.1016/j.psyneuen.2004.04.007

Hurtig, T., Kuusikko, S., Mattila, M.-L., Haapsamo, H., Ebeling, H., Jussila, K., …Moilanen, I. (2009). Multi-informant reports of psychiatric symptoms among high-functioning adolescents with Asperger syndrome or autism. *Autism*, *13*(6), 583–598. https://doi.org/10.1177/1362361309335719

Judah, M. R., Grant, D. M, Mills, A. C., & Lechner, W. V. (2014). Factor structure and validation of the attentional control scale, Cognition and Emotion, 28:3, 433-451. doi:10.1080/02699931.2013.835254

Kaat, A. J., & Lecavalier, L. (2015). Reliability and Validity of Parent- and Child-Rated Anxiety Measures in Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *45*(10), 3219–3231. https://doi.org/10.1007/s10803-015-2481-y

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child, 2*, 217—250

Kerns, C. M., & Kendall, P. C. (2012). The presentation and classification of anxiety in autism spectrum disorder. *Clinical Psychology: Science and Practice*, *19*(4), 323-347.doi: 10.1111/cpsp.12009

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, *62*(6), 593–602. https://doi.org/10.1001/archpsyc.62.6.593

Kidd, S. A., Corbett, B. A., Granger, D. A., Boyce, W. T., Anders, T. F., & Tager, I. B. (2012). Daytime secretion of salivary cortisol and alpha-amylase in preschool-aged children with autism and typically developing children. *Journal of Autism and Developmental Disorders*, *42*(12), 2648–2658. https://doi.org/10.1007/s10803-012-1522-z

Kiess, W., Meidert, A., Dressendórfer, R. A., Schriever, K., Kessler, U., Kónig, A., … Strasburger, C. J. (1995). Salivary cortisol levels throughout childhood and adolescence: Relation with age, pubertal stage, and weight. *Pediatric Research*, *37*(4), 502–506. doi:10.1203/00006450-199504000-00020

Kossowsky, J., Pfaltz, M. C., Schneider, S., Taeymans, J., Locher, C., & Gaab, J. (2013). The separation anxiety hypothesis of panic disorder revisited: a meta-analysis. *American Journal of Psychiatry*, *170*(7), 768-781. http://dx.doi.org/10.1176/appi.ajp.2012.12070893

Kreslins, A., Robertson, A. E., & Melville, C. (2015). The effectiveness of psychosocial interventions for anxiety in children and adolescents with autism spectrum disorder: a systematic review and meta-analysis. *Child and Adolescent Psychiatry and Mental Health*, *9*, 22. https://doi.org/10.1186/s13034-015-0054-7

La Greca, A. M., & Lopez, N. (1998). Social Anxiety Among Adolescents: Linkages with Peer Relations and Friendships. *Journal of Abnormal Child Psychology*, *26*(2), 83–94. https://doi.org/10.1023/A:1022684520514

Leekam, S. R., Nieto, C., Libby, S. J., Wing, L., & Gould, J. (2007). Describing the sensory abnormalities of children and adults with autism. *Journal of autism and developmental disorders*, *37*(5), 894-910. doi: 10.1007/s10803-006-0218-7

Lord, C., Rutter, M., DiLavore, P. C., & Risi, S. (1999). Autism diagnostic observation schedule: Manual. Los Angeles, CA: Western Psychological Services.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders*, *24*(5), 659–685. doi:10.1007/BF02172145

Luxford, S., Hadwin, J. A., & Kovshoff, H. (2016). Evaluating the Effectiveness of a School-Based Cognitive Behavioural Therapy Intervention for Anxiety in Adolescents Diagnosed with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, 1–13. https://doi.org/10.1007/s10803-016-2857-7

Lyneham, H. J., Street, A. K., Abbott, M. J. & Rapee, R. M. (2008). Psychometric properties of the school anxiety scale—Teacher report (SAS-TR). *Journal of Anxiety Disorders, 22* (2), 292 - 300. doi: 10.1016/j.janxdis.2007.02.001

MacNeil, B. M., Lopes, V. A., & Minnes, P. M. (2009). Anxiety in children and adolescents with Autism Spectrum Disorders. *Research in Autism Spectrum Disorders*, *3*(1), 1–21. https://doi.org/10.1016/j.rasd.2008.06.001

Marinović-Ćurin, J., Marinović-Terzić, I., Bujas-Petković, Z., Zekan, L., Škrabić, V., Đogaš, Z., & Terzić, J. (2007). Slower cortisol response during ACTH stimulation test in autistic children. *European Child & Adolescent Psychiatry*, *17*(1), 39–43. https://doi.org/10.1007/s00787-007-0632-1

Merikangas, K. R., He, J., Burstein, M., Swanson, S. A., Avenevoli, S., Cui, L., … Swendsen, J. (2010). Lifetime Prevalence of Mental Disorders in U.S. Adolescents: Results from the National Comorbidity Survey Replication–Adolescent Supplement

(NCS-A). *Journal of the American Academy of Child & Adolescent Psychiatry*, *49*(10), 980–989. https://doi.org/10.1016/j.jaac.2010.05.017

Merikangas, K. R., Nakamura, E. F., & Kessler, R. C. (2009). Epidemiology of mental disorders in children and adolescents. *Dialogues in Clinical Neuroscience*, *11*(1), 7– 20.

Miller, L. D., Martinez, Y. J., Shumka, E., & Baker, H. (2014). Multiple informant agreement of child, parent, and teacher ratings of child anxiety within community samples. *The Canadian Journal of Psychiatry*, *59*(1), 34-39.

Mullen, E. (1997). *Mullen scales of early learning.* Los Angeles:Western Psychological Services.

Murray, L., Creswell, C., & Cooper, P. J. (2009). The development of anxiety disorders in childhood: an integrative review. *Psychological Medicine*, *39*(9), 1413–1423. https://doi.org/10.1017/S0033291709005157

Nauta, M. H., Scholing, A., Rapee, R. M., Abbott, M., Spence, S. H., & Waters, A. (2004). A parent-report measure of children's anxiety: psychometric properties and comparison with child-report in a clinic and normal sample. *Behaviour research and therapy*, *42*(7), 813-839.http://dx.doi.org/10.1016/S0005-7967(03)00200-6

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. *Archives of General Psychiatry*, *55*(1), 56–64.

Polk, D. E., Cohen, S., Doyle, W. J., Skoner, D. P., & Kirschbaum, C. (2005). State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology*, *30*(3), 261–272. https://doi.org/10.1016/j.psyneuen.2004.08.004

Powell, D. J., & Schlotz, W. (2012). Daily Life Stress and the Cortisol Awakening Response: Testing the Anticipation Hypothesis. *PLOS ONE*, *7*(12), e52067. https://doi.org/10.1371/journal.pone.0052067

Putnam, S. K., Lopata, C., Thomeer, M. L., Volker, M. A., & Rodgers, J. D. (2015). Salivary Cortisol Levels and Diurnal Patterns in Children with Autism Spectrum Disorder. *Journal of Developmental and Physical Disabilities*, *27*(4), 453–465. https://doi.org/10.1007/s10882-015-9428-2

Ranøyen, I., Stenseng, F., Klöckner, C. A., Wallander, J., & Jozefiak, T. (2015). Familial aggregation of anxiety and depression in the community: the role of adolescents' self-esteem and physical activity level (the HUNT Study). *BMC Public Health*, *15*, 78. https://doi.org/10.1186/s12889-015-1431-0

Rapee, R. M., & Heimberg, R. G. (1997). A cognitive-behavioral model of anxiety in social phobia. *Behaviour Research and Therapy*, *35*(8), 741–756. https://doi.org/10.1016/S0005-7967(97)00022-3

Rapee, R. M., Schniering, C. A., & Hudson, J. L. (2009). Anxiety Disorders During Childhood and Adolescence: Origins and Treatment. *Annual Review of Clinical Psychology*, *5*(1), 311–341. https://doi.org/10.1146/annurev.clinpsy.032408.153628

Rickard, N. S., Chin, T.-C., & Vella-Brodrick, D. A. (2015). Cortisol Awakening Response as an Index of Mental Health and Well-Being in Adolescents. *Journal of Happiness Studies*, 1–14. https://doi.org/10.1007/s10902-015-9706-9

Rieffe, C., Oosterveld, P., Miers, A. C., Terwogt, M. M., & Ly, V. (2008). Emotion awareness and internalising symptoms in children and adolescents: The Emotion Awareness Questionnaire revised. *Personality and Individual Differences*, *45*(8), 756-761. https://doi.org/10.1016/j.paid.2008.08.001

Rieffe, C., Oosterveld, P., Terwogt, M. M., Mootz, S., Van Leeuwen, E., & Stockmann, L. (2011). Emotion regulation and internalizing symptoms in children with autism spectrum disorders. *Autism*, *15*(6), 655-670. doi: 10.1177/1362361310366571

Rosmalen, J. G. M., Oldehinkel, A. J., Ormel, J., De Winter, A. F., Buitelaar, J. K., & Verhulst, F. C. (2005). Determinants of salivary cortisol levels in 10–12 year old children; a population-based study of individual differences. *Psychoneuroendocrinology*, *30*(5), 483-495.http://dx.doi.org/10.1016/j.psyneuen.2004.12.007

Rosmond, R., Holm, G., &Björntorp, P. (2000). Food-induced cortisol secretion in relation to anthropometric, metabolic and haemodynamic variables in men. *International Journal of Obesity & Related Metabolic Disorders*, *24*(4)

Rutter, M., Le Couteur, A., & Lord, C. (2003). Autism diagnostic interview-revised. *Los Angeles, CA: Western Psychological Services*, *29*, 30.

Salomone, E., Kutlu, B., Derbyshire, K., McCloy, C., Hastings, R. P., Howlin, P., & Charman, T. (2014). Emotional and behavioural problems in children and young people with autism spectrum disorder in specialist autism schools. *Research in Autism Spectrum Disorders*, *8*(6), 661-668. https://doi.org/10.1016/j.rasd.2014.03.004

Schulz, P., & Schlotz, W. (1999). Trierer Inventar zur Erfassung von chronischem Sre (TICS): Skalenkonstruktion, teststatistische Überprüfung und Validierung der Skala Arbeitsüberlastung. [The Trier Inventory for the Assessment of Chronic Stress (TICS).

Scale construction, statistical testing, and validation of the scale work overload.]. *Diagnostica*, *45*(1), 8–19. https://doi.org/10.1026//0012-1924.45.1.8

Schlotz, W., Hellhammer, J., Schulz, P., & Stone, A. A. (2004). Perceived work overload and chronic worrying predict weekend-weekday differences in the cortisol awakening response. *Psychosomatic Medicine*, *66*(2), 207–214

Schmidt-Reinwald, A., Pruessner, J. C., Hellhammer, D. H., Federenko, I., Rohleder, N., Schürmeyer, T. H., & Kirschbaum, C. (1999). The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences*, *64*(18), 1653–1660. https://doi.org/10.1016/S0024-3205(99)00103-4

Schwab-Stone, M. E., Shaffer, D., Dulcan, M. K., Jensen, P. S., Fisher, P., Bird, H. R., ...Rae, D. S. (1996). Criterion validity of the NIMH Diagnostic lnterview Schedule for Children, Version 2.3 (DISC- 2.3). *Journal of American Academic Child Adolescent Psychiatry, 35,* 878-888

Shaffer D., Fisher P., Lucas C., Dulcan M., & Schwab-Stone M. (2000). NIMH Diagnostic Interview Schedule for Children, Version IV (NIMH DISC-IV): description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child and Adolescent Psychiatry*, 39, 28-38. doi: 10.1097/00004583-200001000-00014

Sharpley, C. F., Bitsika, V., Andronicos, N. M., & Agnew, L. L. (2016). Further evidence of HPA-axis dysregulation and its correlation with depression in Autism Spectrum Disorders: Data from girls. *Physiology &Behavior*, *167*, 110–117. https://doi.org/10.1016/j.physbeh.2016.09.003

Shibuya, I., Nagamitsu, S., Okamura, H., Ozono, S., Chiba, H., Ohya, T., ...Matsuishi, T. (2014). High correlation between salivarycortisol awakening response and the psychometric profiles of healthychildren.*BioPsychoSocial Medicine, 8*, 9.doi:10.1186/1751-0759-8-9. Retrieved on 14.10.15 from http://www.bpsmedicine.com/content/8/1/9

Shirtcliff, E. A., & Essex, M. J. (2008). Concurrent and longitudinal associations of basal and diurnal cortisol with mental health symptoms in early adolescence. *Developmental Psychobiology*, *50*(7), 690–703. https://doi.org/10.1002/dev.20336

Silverman, W. K., & Ollendick, T. H. (2005). Evidence-Based Assessment of Anxiety and Its Disorders in Children and Adolescents. *Journal of Clinical Child & Adolescent Psychology*, *34*(3), 380–411. https://doi.org/10.1207/s15374424jccp3403_2

Simonoff, E., Pickles, A., Charman, T., Chandler, S. Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: prevalence,

comorbidity and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 47* (8), 921-29. doi:10.1097/CHI.0b013e318179964f

Smith, S. R. (2007). Making sense of multiple informants in child and adolescent psychopathology a guide for clinicians. *Journal of Psychoeducational Assessment, 25*(2), 139-149.doi: 10.1177/0734282906296233

Spence, S. H. (n.d.). Spence Children's Anxiety Scale Website; Information for Researchers and Practitioners. *Retrieved on 02.05.17 from* https://www.scaswebsite.com/index.php?p=1_9

Spence, S.H. (1998). A measure of anxiety symptoms among children. *Behaviour Research and Therapy, 36* (5), 545-566

Spence, S. H., Barrett, P. M., & Turner, C. M. (2003). Psychometric properties of the Spence Children's Anxiety Scale with young adolescents. *Journal of Anxiety Disorders, 17*(6), 605–625. https://doi.org/10.1016/S0887-6185(02)00236-0

Spielberger, C. D. (1966). *Anxiety and behavior, 1*.UK: Academic Press

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., ... & Miller, R. (2016). Assessment of the cortisol awakening response: expert consensus guidelines. *Psychoneuroendocrinology, 63*, 414-432. http://dx.doi.org/10.1016/j.psyneuen.2015.10.010

Steensel, F. J. A. van, Bögels, S. M., & Dirksen, C. D. (2012). Anxiety and Quality of Life: Clinically Anxious Children With and Without Autism Spectrum Disorders Compared. *Journal of Clinical Child & Adolescent Psychology, 41*(6), 731–738. https://doi.org/10.1080/15374416.2012.698725

Steensel, F. J. A. van, Bögels, S. M., & Perrin, S. (2011). Anxiety Disorders in Children and Adolescents with Autistic Spectrum Disorders: A Meta-Analysis. *Clinical Child and Family Psychology Review, 14*(3), 302. https://doi.org/10.1007/s10567-011-0097-0

Stiegler, L. N., & Davis, R. (2010). Understanding sound sensitivity in individuals with autism spectrum disorders. *Focus on Autism and Other Developmental Disabilities, 25*(2), 67-75. doi: 10.1177/1088357610364530

Taylor, J. L., & Corbett, B. A. (2014). A review of rhythm and responsiveness of cortisol in individuals with autism spectrum disorders. *Psychoneuroendocrinology, 49*, 207–228. https://doi.org/10.1016/j.psyneuen.2014.07.015

Tomarken, A. J., Han, G. T., & Corbett, B. A. (2015). Temporal patterns, heterogeneity, and stability of diurnal cortisol rhythms in children with autism spectrum disorder. *Psychoneuroendocrinology, 62*, 217–226. https://doi.org/10.1016/j.psyneuen.2015.08.016

Tordjman, S., Anderson, G. M., Kermarrec, S., Bonnot, O., Geoffray, M.-M., Brailly-Tabard, S., … Touitou, Y. (2014). Altered circadian patterns of salivary cortisol in low-functioning children and adolescents with autism. *Psychoneuroendocrinology, 50*, 227–245. https://doi.org/10.1016/j.psyneuen.2014.08.010

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research, 53*(4), 865–871. https://doi.org/10.1016/S0022-3999(02)00429-4

Van Ameringen, M., Mancini, C., & Farvolden, P. (2003). The impact of anxiety disorders on educational achievement. *Journal of Anxiety Disorders*, *17*(5), 561–571. https://doi.org/10.1016/S0887-6185(02)00228-1

Viau, R., Arsenault-Lapierre, G., Fecteau, S., Champagne, N., Walker, C.-D., & Lupien, S. (2010). Effect of service dogs on salivary cortisol secretion in autistic children. *Psychoneuroendocrinology*, *35*(8), 1187–1193. https://doi.org/10.1016/j.psyneuen.2010.02.004

Vreeburg, S.A., Zitman, F.G., van Pelt, J., Derijk, R.H., Verhagen,J.C.M., van Dyck, R., ...Penninx, B.W.J.H.(2010). Salivary cortisol levels in persons with and without different anxiety disorders. *Psychosomatic Medicine 72*,340-347. doi:10.1097/PSY.0b013e3181d2f0c8

Walker, S., O'Connor, D. B., Schaefer, A., Talbot, D., & Hendrickx, H. (2011). The cortisol awakening response: Associations with trait anxiety and stress reactivity. *Personality and Individual Differences*, *51*(2), 123-127. https://doi.org/10.1016/j.paid.2011.03.026

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence.*San Antonio:The Psychological Corporation

Wells, A. (1997). *Cognitive Therapy of Anxiety Disorders: A Practice Manual and Conceptual Guide.* UK:Wiley

Wessa, M., Rohleder, N., Kirschbaum, C., & Flor, H. (2006). Altered cortisol awakening response in posttraumatic stress disorder. *Psychoneuroendocrinology*, *31*(2), 209– 215. https://doi.org/10.1016/j.psyneuen.2005.06.010

Williams, D. (2010). Theory of own mind in autism: Evidence of a specific deficit in self-awareness? *Autism*, *14*(5), 474–494. https://doi.org/10.1177/1362361310366314

White, S. W., Oswald, D., Ollendick, T., & Scahill, L. (2009). Anxiety in children and adolescents with autism spectrum disorders. *Clinical Psychology Review*, *29*(3), 216–229. https://doi.org/10.1016/j.cpr.2009.01.003

White, S. W., Schry, A. R., & Maddox, B. B. (2012). Brief Report: The Assessment of Anxiety in High-Functioning Adolescents with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders*, *42*(6), 1138–1145. https://doi.org/10.1007/s10803-011-1353-3

Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W., & Wüst, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology*, *32*(4), 358–366. https://doi.org/10.1016/j.psyneuen.2007.01.008

Wood, J. J. & Gadow, K. D. (2010). Exploring the Nature and Function of Anxiety in Youth with Autism Spectrum Disorders. *Clinical Psychology: Science and Practice*, *17*, 281–292. doi: 10.1111/j.1468-2850.2010.01220.x

Woodward, L. J., & Fergusson, D. M. (2001). Life Course Outcomes of Young People With Anxiety Disorders in Adolescence. *Journal of the American Academy of Child & Adolescent Psychiatry*, *40*(9), 1086–1093. https://doi.org/10.1097/00004583-200109000-00018

World Health Organisation. (2006). *International Statistical Classification of Diseases and Related Health Problems*, (10th ed.). Geneva: World Health Organization

Wüst, S., Federenko, I., Hellhammer, D. H., & Kirschbaum, C. (2000a). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology*, *25*(7), 707–720. https://doi.org/10.1016/S0306-4530(00)00021-4

Wust, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000b). The cortisol awakening response-normal values and confounds. *Noise and health*, *2*(7), 79.

YouthinMind. (2016). SDQ info; Scoring the SDQ. *Retrieved from http://www.sdqinfo.com/py/sdqinfo/c0.py*

Zainal, H., Magiati, I., Tan, J. W.-L., Sung, M., Fung, D. S. S., & Howlin, P. (2014). A Preliminary Investigation of the Spence Children's Anxiety Parent Scale as a Screening Tool for Anxiety in Young People with Autism Spectrum Disorders. *Journal of*

Autism and Developmental Disorders, *44*(8), 1982–1994. https://doi.org/10.1007/s10803-014-2075-0

Zinke, K., Fries, E., Kliegel, M., Kirschbaum, C., & Dettenborn, L. (2010). Children with high-functioning autism show a normal cortisol awakening response (CAR). *Psychoneuroendocrinology, 35*(10), 1578–1582. https://doi.org/10.1016/j.psyneuen.2010.03.009