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Review

The role of the orexin system in the neurobiology of anxiety disorders: Potential for a novel treatment target

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ABSTRACT

Anxiety disorders constitute the most common group of psychiatric disorders with a lifetime prevalence of 14.5–33.7%. Despite efficacious pharmacological and psychological treatments, first line treatment is often not effective, and development of new therapies is needed. One area of interest is the orexin system, a neurotransmitter system centred in the lateral hypothalamus with widespread projections throughout the brain, including to several key areas involved in the modulation of fear and anxiety. In this article, we summarise findings from pre-clinical and clinical investigations of the potential role of the orexin system in the neurobiology of fear and anxiety. Pre-clinical studies in rodents generally indicate that orexin signalling promotes fear and anxiety-related behaviour, particularly in response to aversive stimuli. Orexin signalling in the amygdala, bed nucleus of the stria terminalis, paraventricular nucleus of thalamus, locus coeruleus and prefrontal cortex has been specifically implicated. Human studies are limited, with some evidence that orexin receptor antagonists are anxiolytic in experimental medicine models of anxiety, some indications from clinical populations of altered orexin signalling, and a molecular genetic study associating a non-synonymous variant in the orexin 1 receptor (HCRTR1) with panic disorder and agoraphobia. Given this emerging body of evidence, further human studies are required to fully assess the orexin system as a potential novel anxiolytic target.

1. Introduction

The anxiety disorders include several discrete mental disorders, characterised by persistent fear and anxiety with associated avoidance of potential threats and/or associated functional impairment (Penninx et al., 2021). They currently represent the most common group of mental disorders (Penninx et al., 2021; Bandelow and Michaelis, 2015). Epidemiological studies estimate a 12-month prevalence of 8.4–21.3% and a lifetime prevalence of 14.5–33.7% of at least one anxiety disorder (Bourdon et al., 1992; Kessler et al., 1994; Alonso and Lépine, 2007; Wittchen and Jacobi, 2005). Several anxiety disorders are recognised in the current diagnostic classification systems: the 2022 ICD-11 and the 2013 DSM-5 (World Health Organization (WHO), 2022; American Psychiatric Association, 2013). These include generalised anxiety disorder (GAD), panic disorder, agoraphobia, social anxiety disorder, specific phobia, separation anxiety disorder and selective mutism (World Health Organization (WHO), 2022; American Psychiatric Association, 2013). Although, obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) were considered as anxiety disorders in previous versions of the ICD and DSM, they are now separately classified and will not be addressed in this article (World Health Organization (WHO), 2022; American Psychiatric Association, 2013).

Broadly, treatment of anxiety disorders consists of a range of psychotherapies and/or pharmacological agents (Penninx et al., 2021; Baldwin et al., 2014; Bandelow et al., 2022). For psychological interventions, greatest evidence exists for cognitive behavioural therapy, with more limited evidence for psychodynamic and interpersonal psychotherapy (Bandelow et al., 2015). Several effective pharmacological agents are available including selective serotonin reuptake inhibitors (SSRIs), serotonin-noradrenaline reuptake inhibitors (SNRIs), the tricyclic antidepressant clomipramine, monoamine oxidase inhibitors, the second generation antipsychotic quetiapine, benzodiazepines and the gabapentinoid pregabalin (Baldwin et al., 2014; Bandelow et al., 2015, 2022). SSRIs are typically prescribed as a first line pharmacological treatment, due to their relatively favourable risk profile.

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List of abbreviations used in the text

ACTH	adrenocorticotrophic hormone
BDZ	benzodiazepine
BLA	basolateral amygdala
BNST	bed nucleus of the stria terminalis
CeA	central amygdala
CO2	carbon dioxide
CSF	cerebrospinal fluid
DORA	dual orexin receptor antagonist
DSM	Diagnostic and Statistical Manual of Mental Disorders
EPM	elevated plus maze
fMRI	functional magnetic resonance imaging
GABA-A	gamma-aminobutyric acid receptor A
GAD	generalised anxiety disorder
HCRTR1	hypocretin 1 receptor (orexin 1 receptor)
HCRTR2	hypocretin 2 receptor (orexin 2 receptor)
HPA	hypothalamic-pituitary-adrenal
IC	intracerebral
ICD	International Classification of Diseases
ICV	intracerebroventricular
IP	intraperitoneal
ITSSAD	InterneT based Stress test for Social Anxiety Disorder
LBD	light dark box
LC	locus coeruleus
MAP	mean arterial blood pressure
MDD	major depressive disorder
NPU	No-Predictable-Unpredictable threat paradigm
NT1	type 1 narcolepsy
NT2	type 2 narcolepsy
OCD	obsessive compulsive disorder
OFT	open field test
ORA	orexin receptor antagonist
OXR	orexin receptor
OX1R	orexin 1 receptor
OX2R	orexin 2 receptor
PFC	prefrontal cortex
PTSD	post-traumatic stress disorder
PTZ	pentylenetetrazol
PVN	paraventricular nucleus of hypothalamus
PVT	paraventricular nucleus of thalamus
RCT	randomised controlled trial
SAM	stress-alternatives model
SIT	social interaction test
SPT	social preference test
SNRI	serotonin-noradrenaline reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TSST	Trier social stress test

Benzodiazepines are also commonly offered as a short-term initial treatment for acute anxiety. Despite the range of effective treatments, anxiety disorders continue to be associated with considerable morbidity. Globally, across all physical and mental disorders, anxiety disorders are the 6th leading course of disability (as measured in years lived with disability) (Baxter et al., 2013). This reflects both the prevalence of anxiety disorders, and their significant under-recognition and under-treatment. A 2018 cross-sectional study across 21 countries identified a marked treatment gap in anxiety disorders, with less than 30% of patients receiving any treatment and less than 10% receiving adequate treatment (Alonso et al., 2018). However, even when recognised and appropriately treated, response to first line treatment is often modest. Failure of response to or remission with initial pharmacological treatment has been estimated at 40–50% (Roy-Byrne, 2015). Despite significant attempts to develop novel pharmacological treatments, successful translation has been relatively limited and there remains a clear need to develop improved treatments, with several active areas of research (Griebel and Holmes, 2013; Singewald et al., 2023).

An ongoing area of interest in the field of anxiety disorders is the orexin system, a neurotransmitter system identified at the end of the 1990s (de Lecea et al., 1998; Sakurai et al., 1998). Orexins are two neuropeptides (orexin-A and orexin-B, or hypocretin 1 and 2) synthesised by a small population of neurons in the lateral hypothalamus, with widespread projections throughout the brain (de Lecea et al., 1998; Sakurai et al., 1998; Fronczek et al., 2005; Peyron et al., 1998). Orexin signalling occurs through two different G-protein coupled receptors: orexin type 1 receptor (OX1R) and orexin type 2 receptor (OX2R) (Sakurai et al., 1998). The two receptors are differentially expressed throughout the mammalian brain and show differing affinities to the orexin neuropeptides: OX1R has a much greater affinity for orexin-A while OX2R has a similar affinity for orexin-A and orexin-B (Sakurai et al., 1998; Marcus et al., 2001) (Fig. 1). The orexin system was initially noted to regulate appetite and feeding behaviour (with the name orexin, derived from the Greek word *orexis* meaning appetite) (Sakurai et al., 1998). Subsequently, the roles of the orexin system in the regulation of motivated behaviour, anxiety-related behaviour, alerting, arousal and in the sleep-wake cycle have also been identified (Tsujino and Sakurai, 2013; Mahler et al., 2014; Geiger et al., 2014). The importance of the orexin system in the latter is particularly apparent in the sleep disorder narcolepsy type 1 (NT1), which is characterised by daytime somnolence, sleep paralysis and cataplexy and results from the selective loss of orexinergic neurons (Mahoney et al., 2019).

The orexin system was identified as a potential target for novel hypnotic agents, and a range of compounds with antagonistic action at orexin receptors have been identified (Winrow and Renger, 2014). Three antagonists to both OX1R and OX2R (dual orexin receptor antagonists/DORAs) have entered clinical practice since 2014: suvorexant, lemborexant and daridorexant, for the treatment of insomnia (Yang, 2014; Scott, 2020; Markham, 2022). Randomised controlled trials (RCTs) for DORAs *vs.* placebo treatment have demonstrated improvements across several sleep-related outcomes in insomnia, with a generally favourable adverse effect profile (Xue et al., 2022).

In summary, the orexin system is an important neurotransmitter system, involved in the regulation of several biological functions. The recent success in the development of orexin receptor antagonists in insomnia also indicates it can effectively be targeted by pharmacological agents in clinical populations (Ten-Blanco et al., 2023). Further research has also considered the potential role of the orexin system in several other psychiatric disorders, including major depressive disorder (MDD) (Nollet and Leman, 2013; Khairuddin et al., 2020; Fagan et al., 2023a), PTSD (Kaplan et al., 2022), substance misuse disorders (Giardino and de Lecea, 2014; James et al., 2017) and finally the anxiety disorders. In this review, we summarise the research literature investigating the role of the orexin system in the neurobiology of anxiety disorders. We consider first the available evidence from pre-clinical studies in animal models of anxiety, before discussing the limited number of human studies in patients with narcolepsy, healthy volunteers and clinical populations with anxiety disorders. Finally, we discuss the outstanding research questions in the published literature and suggest future directions for the translation of orexin-modulating treatment into the clinical treatment of anxiety disorders.

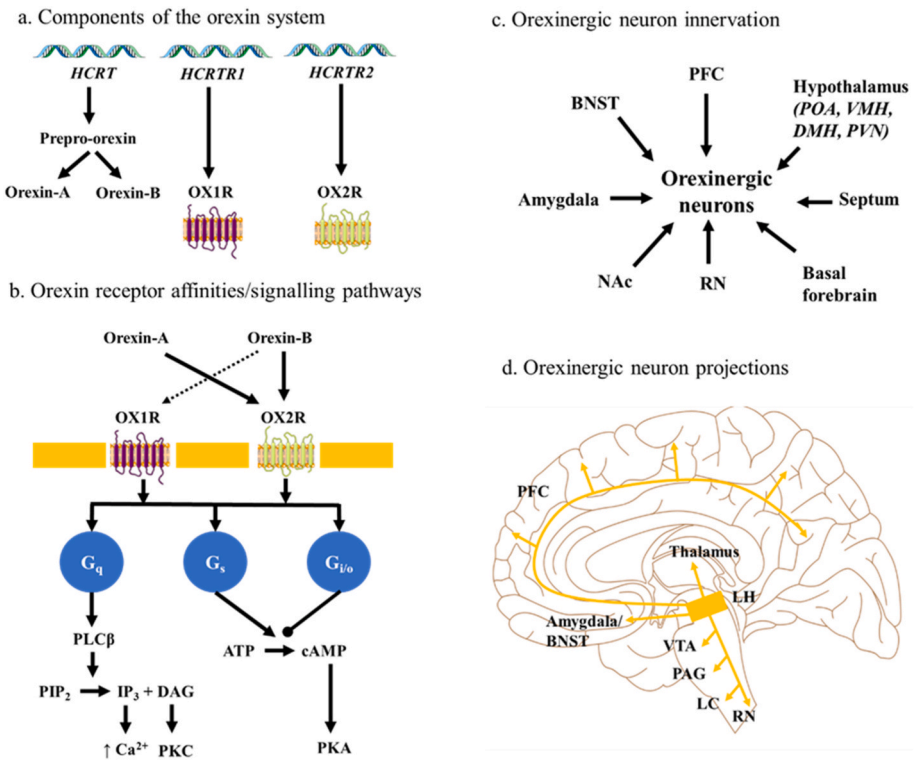

Fig. 1. The orexin neurotransmitter system. 1a: Summary of the relevant genes and encoded proteins products of the orexin system. 1b: Summary of relative affinities of the orexin neurotransmitters to the two orexin receptors OX1R and OX2R and intracellular signalling pathways. OX1R exhibits preferential affinity to orexin-A while OX2R exhibits similar affinity to both orexins (Sakurai et al., 1998). Both OX1R and OX2R are thought capable of activating G_q, G_s and $G_{i/o}$ proteins resulting in downstream intracellular signalling pathways (Kukkonen and Leonard, 2014; Dale et al., 2022). 1c: Diagram of afferent projections to orexinergic neurons in the lateral hypothalamus (Sakurai T et al., 2005). 1d: Diagram of neuroanatomy of the orexin system, demonstrating orexinergic cell bodies located in the lateral hypothalamus with wide widespread projections throughout the brain. Abbreviations used: ATP adenosine triphosphate, BNST bed nucleus of the stria terminalis, cAMP cyclic adenosine monophosphate, DAG diglyceride, DMH dorsomedial hypothalamus, IP_3 inositol trisphosphate, LC locus coeruleus, LH lateral hypothalamus, NAc nucleus accumbens, OX1R orexin 1 receptor, OX2R orexin 2 receptor, PAG periaqueductal grey, PFC prefrontal cortex, PIP_2 phosphatidylinositol 4,5-bisphosphate, PKA protein kinase A, PKC protein kinase C, PLCβ phospholipase beta, POA preoptic area, PVN paraventricular nucleus of the hypothalamus, RN raphe nuclei, VMA ventromedial hypothalamus, VTA ventral tegmental area (Gene and sagittal brain images from SciDraw (Kumar; Karamihalev), receptor image from Bioicons (Bioicons).).

2. Pre-clinical studies

2.1. Overview

The orexin system was first described in rats (de Lecea et al., 1998; Sakurai et al., 1998), and subsequently a large number of studies have been conducted, investigating the role of the orexin system in the neurobiology of fear and anxiety in animal models (see Table 1). Rodent models have been almost exclusively used in studies of fear and anxiety. However, earlier research into the role of the orexin system, particularly in sleep regulation and the pathophysiology of narcolepsy, was conducted in cat and dog models (Mahoney et al., 2019; Xi et al., 2001, 2002; Xi and Chase, 2006). A wide range of experimental techniques to model both fear and anxiety-like behaviours have been utilised with classical conditioned fear paradigms and the classical tests of the elevated plus maze (EPM), open field test (OFT), social interaction test (SIT) and light dark box test (LDB) being widely used (Calhoon and Tye, 2015). Animal models allow a wide range of invasive manipulations and monitoring of neural circuitry and have been considered essential in establishing the important neural circuitry underlying fear and anxiety (Calhoon and Tye, 2015; Robinson et al., 2019). Key regions identified in the fear and anxiety network include the amygdala, hypothalamus, ventral hippocampus, bed nucleus of the stria terminalis (BNST), paraventricular nucleus of thalamus (PVT), locus coeruleus (LC), periaqueductal grey and medial prefrontal cortex (PFC) (Calhoon and Tye, 2015; Robinson et al., 2019; Tovote et al., 2015).

To investigate the role of the orexin system in fear and anxiety, orexin modulating compounds have been used systemically or administered to localised brain regions. Animal models with selective knockdown of particular components of the orexin system have been developed and the behaviour of these animals assessed. Finally, the effect of the orexin system on the stress response and the hypothalamic-pituitary-adrenal (HPA) axis, has been investigated. Key findings from these differing experimental approaches will be discussed below.

2.2. Studies involving systemic modulation of orexin signalling

Suzuki and colleagues first demonstrated a role for the orexin system in anxiety through intracerebroventricular infusion of orexin-A into mice and rats (Suzuki et al., 2005). With no effect on spontaneous locomotion, orexin-A had an anxiogenic effect in the LDB and EPM in mice, although no significant effect was noted in rats in the EPM. This anxiogenic effect of orexin-A appears, at least in mice, to require GABAergic and α- and β-adrenoceptor adrenergic signalling as the anxiogenic effects of orexin-A in the EPM can be attenuated by antagonism of these neurotransmitters (Palotai et al., 2014). However, an anxiogenic effect of orexin-A was not found in a rat study (Magdaleno-Madrigal et al., 2019). In a separate study, Rodgers and colleagues, showed systemic pre-treatment with an OX1R antagonist (SB-334867) did not alter the behaviour of mice in the EPM (Rodgers et al., 2013). Systemic pre-treatment with OX1R antagonism likewise did not alter the behaviour of rats in the EPM although it did result in reduced avoidance

Table 1
Summary of discussed studies investigating the role of the orexin system in fear and anxiety-related behaviour in animal models.

Authors/year of publication	Animal model, sex and strain used.	Fear/anxiety model(s) used	Principal results	Overall evidence supporting role of orexin system in promoting fear and anxiety-related behaviour
1. STUDIES USING SYSTEMIC MODULATION OF OREXIN SIGNALLING				
Suzuki et al., 2005 (Suzuki et al., 2005)	Mouse (male C57BL), rat (male Wistar)	EPM, LBD	In mice, ICV administration of orexin-A had an anxiogenic effect in LDB and EPM (no effect on EPM in rats).	Supportive
Johnson et al., 2010 (Johnson et al., 2010)	Panic sensitive rat (see (Johnson and Shekhar, 2006) for details)	Panic attacks modelled with sodium lactate infusions. EPM and OFT used.	Orexin knock-out and systemic OXR1 antagonism reduced physiological and behavioural effects of sodium lactate infusions.	Supportive
Johnson et al., 2012 (Johnson et al., 2012a)	Rat (male Wistar)	Anxiety behaviour induced with BDZ inverse agonism (FG-7142) and caffeine. OFT and SIT used.	Caffeine and FG-7142 increase orexin neuron activation. OX1R antagonism blocks physiological effect and anxiogenic effect of FG-7142 in SIT.	Supportive
Steiner et al., 2012 (Steiner et al., 2012)	Rat (male F344 and Sprague-Dawley)	Fear conditioned startle. EPM also used.	The DORA almorexant reduced fear potentiated startle in response to an acoustic stimulus and a fear-conditioned stimulus. No effect of almorexant in EPM.	Partially supportive
Rodgers et al., 2013 (Rodgers et al., 2013)	Mouse (male BKW)	EPM	OXR1 antagonism had no anxiolytic effect in EPM.	Not supportive
Staples and Cornish. 2014 (Staples and Cornish, 2014)	Rat (male Wistar)	Cat odour avoidance test and EPM	OXR1 antagonism had an anxiolytic effect in cat odour avoidance model but no effect in EPM.	Partially supportive
Chen et al., 2014 (Chen et al., 2014)	Rat (male Sprague–Dawley)	Stress induced by single foot shock, OFT and EPM used.	Foot shocks increased orexin expression and neuron activity. OX1/2R blockade had an anxiolytic effect in OFT and in EPM (in subset of animals).	Supportive
Heydendael et al., 2014 (Heydendael et al., 2014)	Rat (male Sprague–Dawley)	Stress induced with resident-intruder model. SIT used.	Activation of orexin neurons decreased interaction in SIT and increased activity in PVT and LC.	Supportive
Palotai et al., 2014 (Palotai et al., 2014)	Mouse (female CFLP)	EPM	ICV administration of orexin-A had an anxiogenic effect in EPM. This was blocked by GABA-A and adrenergic receptor antagonism.	Supportive
Flores et al., 2014 (Flores et al., 2014)	Mouse (male C57BL/6J and 129S1/SvImJ)	Contextual fear conditioning, EPM, OFT.	OX1R antagonism reduced freezing in contextual and cued fear tests and accelerated extinction. OX2R antagonism reduced freezing in contextual fear tests with no effect on extinction.	Supportive
Johnson et al., 2015 (Johnson et al., 2015)	Rat (male Sprague–Dawley)	20% CO_2 inhalation panic model. SIT and OFT used.	Selective OX1R antagonism and dual OXR blockade reduced anxiogenic effect of CO_2 in SIT. Selective OX1R blockade reduced MAP rise associated with CO_2 inhalation.	Supportive
Vanderhaven et al., 2015 (Vanderhaven et al., 2015)	Rat (male Wistar)	Cat odour avoidance test	OX1R antagonism had an anxiolytic effect in cat odour avoidance test.	Supportive
Viviani et al., 2015 (Viviani et al., 2015)	Rat (male Sprague–Dawley)	Stressed with single foot shock, LDB and EPM.	Dual OXR blockage did not affect avoidance after foot shock in LDB but did have an anxiolytic effect in EPM.	Partially supportive
Bonaventure et al., 2017 (Bonaventure et al., 2017)	Panic sensitive rat (see (Staples and Cornish, 2014) for details)	20% CO_2 inhalation and sodium lactate induced panic models, SIT.	OX1R antagonism attenuated cardiovascular changes induced by CO_2 and reduced anxiogenic effects seen in SIT following both panic models.	Supportive
Blume et al., 2018 (Blume et al., 2018)	Rat (male female adolescent and adult Sprague-Dawley)	OFT	OX1R antagonism had an anxiolytic effect in adult male/females but an anxiogenic effect in adolescent males.	Supportive and opposing evidence
Staton et al., 2018 (Staton et al., 2018)	Mouse (male C57BL/6N)	SAM	ORX2 antagonism reduced escape behaviour from a hostile mouse. OX2R stimulation increased escape behaviour from a hostile mouse.	Supportive
Asadi et al., 2018 (Asadi et al., 2018)	PTZ-kindled rat (male Wistar)	EPM	ORX2 antagonism had no effect on anxiety behaviour.	Not supportive
Grafe et al., 2018	Rat (male Sprague–Dawley)	Stressed with 5 days of social defeat, SIT	Increased orexin activity is associated with resilience in social defeat paradigm. Orexin neuron inhibition has an anxiolytic effect in the SIT.	Supportive
Eacret et al., 2019 (Eacret et al., 2019)	Rat (male Sprague–Dawley)	Stressed with 5 days of social defeat, SIT	Orexin neuron activation enhanced time to social defeat and had an anxiogenic effect in the SIT.	Supportive
Magdaleno-Madrigal et al., 2019 (Magdaleno-Madrigal et al., 2019)	Rat (male Wistar)	EPM	Orexin-A had no effect on anxiety behaviour in EPM.	Not supportive
Salvadore et al., 2020 (Salvadore et al., 2020)	Rat (male Sprague–Dawley)	20% CO_2 inhalation induced panic model, SIT.	OX1R antagonism reduced anxiogenic effects seen in SIT following CO_2 inhalation.	Supportive
2. STUDIES USING LOCAL MODULATION OF OREXIN SIGNALLING				
Johnson et al., 2010 (Johnson et al., 2010)	Panic sensitive rat (see (Johnson and Shekhar, 2006) for details)	Panic attacks modelled with sodium lactate infusions. EPM and OFT used.	OX1R antagonism in the **BNST** had an anxiolytic effect after sodium lactate infusion.	Supportive

(*continued on next page*)

Table 1 (*continued*)

Authors/year of publication	Animal model, sex and strain used.	Fear/anxiety model(s) used	Principal results	Overall evidence supporting role of orexin system in promoting fear and anxiety-related behaviour
Li et al., 2010 (Li et al., 2010)	Rat (male Sprague–Dawley)	EPM	In the **PVT**, orexin-A and orexin-B infusions had an anxiogenic effect in EPM. This was inhibited by OX2R (but not OX1R) antagonism.	Supportive
Avolio et al., 2011 (Avolio et al., 2011)	Syrian golden hamster (male)	LDB, EPM	Orexin-A and orexin-B infusions into the **CeA** had an anxiogenic effect.	Supportive
Lungwitz et al., 2012 (Lungwitz et al., 2012)	Rat (male Sprague–Dawley)	EPM, SIT	In the **BNST**, orexin-A infusion has an anxiogenic effect in EPM and SIT.	Supportive
Soya et al., 2013 (Soya et al., 2013)	Mouse (male OX1R$^{-/-}$)	Fear conditioning paradigm.	In the **LC**, OX1R knockout resulted in reduced activity after conditioned fear (normalised by re-expression of OX1R in the LC).	Supportive
Sears et al., 2013 (Sears et al., 2013)	Rat (male Sprague–Dawley)	Fear conditioning paradigm.	In the **LC**, OX1R antagonism blocked threat learning while optogenetic activation of orexin neurons targeting LC enhanced it.	Supportive
Arendt et al., 2014 (Arendt et al., 2014)	Mouse (male C57BL/6)	Stress induced with 10 days of chronic social defeat. SPT, OFT and EPM used.	In the **BLA**, OX1R gene knockdown had no effect on anxiety behaviour. OX2R gene knockdown had an anxiogenic effect in the SPT and OFT.	Supportive
Avolio et al., 2014 (Avolio et al., 2014)	Syrian golden hamster (male)	LDB, EPM	In the **lateral amygdala**, orexin-A infusions had an anxiogenic effect while orexin-B had an anxiolytic effect in LDB and EPM.	Supportive and opposing evidence
Dong et al., 2015 (Dong et al., 2015)	Rat (male Sprague–Dawley)	Fear conditioning paradigm. SIT and OFT.	In the **PVT**, dual OXR antagonism did not alter response to cued or contextual fear but did have an anxiolytic effect in SIT and OFT.	Supportive
Flores et al., 2017 (Flores et al., 2017)	Mouse (male C57BL6/J)	Contextual fear conditioning	In the **BLA**, OX1R antagonism increased speed of fear extinction.	Supportive
Dustrude et al., 2018 (Dustrude et al., 2018)	Rat (male Sprague–Dawley)	Fear conditioning paradigm	In the **CeA**, OX1R (but not OX2R) antagonism reduces freezing in response to fearful stimuli.	Supportive
Giardino et al., 2019 (Giardino et al., 2018)	Mouse (male C57BL/6J)	OFT, EPM	In the **BNST**, two separate projections target the lateral hypothalamus with separate anxiogenic and anxiolytic effects.	Supportive and opposing evidence
Pan et al., 2020 (Pan et al., 2020)	Rat (male Wistar)	OFT, EPM	In the **CeA**, orexin-A (but not orexin-B) had an anxiolytic effect in OFT and EPM. This was inhibited by OX1R blockade.	Opposing evidence
Soares et al., 2021 (Soares et al., 2021)	Mouse (male C57BL/6N)	Mice expressed to erratically moving robotic beetle.	In the **prelimbic cortex**, OX1R and OX2R blockade increased tolerance and approach behaviour towards beetle. No effect on freezing behaviour.	Supportive
Wang et al., 2021 (Wang et al., 2021)	Mouse (male C57BL/6J)	SIT, OFT, EPM	Optogenetic activation of orexin projections to **lateral habenula** had an anxiolytic effect in EPM and OFT.	Opposing evidence
Yaegar et al., 2022 (Yaeger et al., 2022)	Mouse (male C57BL/6NHsd)	SAM	In the **BLA**, OX1R antagonism or gene knockdown increased escape behaviour from a hostile mouse.	Supportive
3. STUDIES USING OREXIN SYSTEM KNOCK OUT ANIMAL MODELS				
Soya et al., 2013 (Soya et al., 2013)	OX1R and OX2R knock out mouse (both male)	Fear conditioning paradigm.	OX1R knock out reduced freezing behaviour in response to contextual and cued fear tests. OX2R knock out reduced freezing behaviour in response to contextual fear only.	Supportive
Flores et al., 2014 (Flores et al., 2014)	OX1R knock out mouse (male C57BL/6J)	Contextual fear conditioning, EPM, OFT.	Reduced freezing in contextual and cued fear testing.	Supportive
Abbas et al., 2015 (Abbas et al., 2015)	OX1R knock out mouse (male C57Bl/6J)	LDB, EPM, SIT, startle response, OFT	Increased anxiety behaviour in EPM and SIT. Increased startle response. No different in OFT behaviour.	Opposing evidence
Flores et al., 2016 (Flores et al., 2016)	Orexin knock out mouse (male C57BL/6J)	EPM	No change in behaviour in EPM.	Not supportive
Khalil and Fendt. 2017 (Khalil and Fendt, 2017)	Orexin knock out mouse (male C57BL/6)	Fear conditioning paradigm, OFT, LDB, carnivore odour test.	No change in fear learning or extinction. Increased anxiety behaviour in OFT and LDB. Increased avoidance in carnivore odour test.	Opposing evidence
Faesel et al., 2021 (Faesel et al., 2021)	Orexin knock out mouse (male and female C57BL/6J)	Social fear conditioning paradigm, sociability test	Enhanced fear learning with delayed extinction and (in female mice) reduced sociability and social novelty preference.	Opposing evidence

from a threatening stimulus (predator odour) (Staples and Cornish, 2014). Of interest, a later study by Vanderhaven and colleagues demonstrated that OX1R antagonism was associated with reduced brain activity in two hypothalamic nuclei associated with the stress response: the paraventricular nucleus and the dorsal premammillary nucleus (Vanderhaven et al., 2015). The effect of OX1R antagonism may be related to both age and sex, as Blume and colleagues demonstrated an anxiogenic effect in adolescent male rats and an anxiolytic effect in adult male rats, with relatively limited effect in female rats (Blume et al., 2018).

Some studies have also used fear conditioning paradigms. Steiner and colleagues used a fear-potentiated startle paradigm in rats, where they were classically conditioned to associate a light with a foot shock, and the effect of the light to potentate the startle to an acoustic stimulus was then measured (Steiner et al., 2012). The DORA almorexant reduced the potentiation of the startle to the acoustic stimulus when combined with the light (Steiner et al., 2012). However, no effect was noted in the EPM (Steiner et al., 2012). In a mouse study, Flores and colleagues

showed that the consolidation of fear memory was inhibited and the extinction of fear memory enhanced by OX1R antagonism (Flores et al., 2014). OX2R antagonism reduced fear memory consolidation to a lesser extent, however, it did not affect the rate of extinction.

Several studies have utilised animal models that actively induce states of anxiety, for example through the induction of panic-like states via carbon dioxide (CO_2) inhalation, sodium lactate infusions, anxiogenic pharmacological agents or by exposing the animals to aversive stimuli such as pain or social defeat. In a panic-prone rat model (Johnson and Shekhar, 2006), several studies have demonstrated the panic-like response to CO_2 inhalation and sodium lactate infusion is attenuated by OX1R antagonism (Johnson et al., 2010, 2015; Bonaventure et al., 2017; Salvadore et al., 2020). Dual ORA antagonism had a lesser effect on the response to CO_2 inhalation, reducing social avoidance behaviour but not altering cardiovascular changes induced by CO_2 while OX2R antagonism had no significant effect (Johnson et al., 2015). OX2R antagonism also showed no change in anxiety-related behaviour in the EPM in pentylenetetrazol-kindled rats (Asadi et al., 2018). A similar attenuation of the panic-like response from FG-7142 (a partial inverse agonist of the benzodiazepine receptor) by OX1R antagonism was also noted in the same panic-prone rat model (Johnson et al., 2012a).

The response to other aversive stimuli was also influenced by orexin modulation. In rats, following a foot shock, the DORA TCS-1102 had an anxiolytic effect in the OFT, EPM and LDB and appeared to prevent the development of generalised avoidance behaviour (Chen et al., 2014; Viviani et al., 2015). Following repeated social defeat stress, specific activation of orexinergic neurons in rats increased social avoidance behaviour in the SIT, which could not be rescued by OX2R antagonism (Heydendael et al., 2014; Eacret et al., 2019). Interestingly, the behavioural response in rats exposed to repeated social defeat is associated with orexin gene expression (Grafe et al., 2018). Higher orexin expression is also associated with an faster latency to immobility in response to intruder animals, interpreted as a vulnerability to stress (Grafe et al., 2018). Finally, Staton and colleagues investigated the effect OX2R modulation in mice, using the Stress-Alternatives Model (SAM), a naturalistic model of anxiety and depression (Summers et al., 2020; Staton et al., 2018). In the SAM, the animal is placed in an enclosure with a larger, aggressive mouse for a 5-min period on 4 consecutive days. A small escape route is present. Mice are defined as showing either "Escape" or "Stay" behavioural phenotypes, dependent on whether they escaped or remained in the enclosure. OX2R agonism increased tendency to escape, in previously "Stay" mice, while OX2R antagonism reduced the tendency to escape in previously "Escape" mice (Staton et al., 2018). On day 5, in an SIT, OX2R agonism had an anxiolytic effect while OX2R antagonism had an anxiogenic effect.

2.3. Studies involving localised modulation of orexin signalling

Most key brain structures involved in the generation and regulation of fear and anxiety receive significant innervation from the orexinergic neurons in the hypothalamus. Further, these regions express one or both orexin receptors. These regions include the basolateral and central nuclei of the amygdala, BNST, PVT, LC, the lateral habenula and the medial PFC (Fronczek et al., 2005; Peyron et al., 1998), all of which have been investigated in animal models.

2.3.1. Orexin signalling in the amygdala

The amygdala is a key node in the fear and anxiety network and can be divided into two main clusters of nuclei: the basolateral amygdala (BLA) and central amygdala (CeA) (Davern and Head, 2011). Orexin signalling in both clusters has been shown to regulate fear and anxiety. Social defeat in mice increased expression of OX1R (and decreases expression of OX2R) in the BLA, with the selective knock down of OX2R in this region resulting in increased anxiety-related behaviour in the OFT and social preference test (SPT) (Arendt et al., 2014). Infusions of orexin-A into the lateral amygdala (part of the BLA) in hamsters had an anxiogenic effect in the EPM and LDB, while orexin-B infusions had an anxiolytic effect in these tests (Avolio et al., 2014). OX1R antagonism in the BLA in mice, also increased the speed of fear extinction in a fear conditioning paradigm (Flores et al., 2017). In the previously discussed SAM, OX1R antagonism or gene knock down in the BLA increased escape behaviour from the hostile mouse and reduced contextual and cued fear responses in a subset of vulnerable animals (Yaeger et al., 2022). BLA neurons with orexin receptors, also have established afferent connectivity with other key regions in the anxiety network including the medial PFC, PVT, ventral hippocampus, habenula and CeA (Kim and Han, 2016).

Regarding the CeA, infusions of orexin-A or orexin-B into this region in hamsters has an anxiogenic effect in the LDB and EPM (Avolio et al., 2011). In contrast, a rat study demonstrated an anxiolytic effect of orexin-A (but not orexin-B) infusions into the CeA that was inhibited by OX1R antagonism (Pan et al., 2020). Finally, localised OX1R antagonism in the CeA attenuated the response to a conditioned fear paradigm in rats (Dustrude et al., 2018).

An additional region of the amygdala implicated in the response to stressful and anxiogenic stimuli is the medial amygdala (Davern and Head, 2011). Neurons in this region show high levels of activation in response to the stress of forced swimming or restraint (Davern and Head, 2011), and this region additionally received moderate levels of orexinergic innervation and expresses both orexin receptors (OX1R > OX2R) (Peyron et al., 1998; Marcus et al., 2001). However, to date, no study has directly investigated the effect of localised modulation of orexinergic signalling in this region.

2.3.2. Orexin signalling in the BNST

The BNST in another key neurological region in the fear and anxiety network and is tightly connected with the amygdala (Robinson et al., 2019; Tovote et al., 2015). Orexin-A infusions in the BNST of rats has an anxiogenic effect in both the EPM and the SIT (Lungwitz et al., 2012) and localised OX1R antagonism in the BNST of panic prone rats attenuates the panic-like responses to sodium lactate infusions (Johnson et al., 2010). Finally, specific separate afferent projections from the BNST to the lateral hypothalamus have been identified in mice (Giardino et al., 2018). These separate pathways appear capable of stimulating both anxiogenic and anxiolytic behaviours in response to specific environmental stimuli (Giardino et al., 2018).

2.3.3. Orexin signalling in the PVT

The PVT is a thalamic nucleus which has been implicated in the neurocirculatory of fear and anxiety (Kirouac, 2021). Infusions of both orexin-A and orexin-B into the PVT of rats result in an anxiogenic response in the EPM (Li et al., 2010). Interestingly, following a single foot shock, the anxiogenic effect of orexin infusion could be attenuated by OX2R antagonism but not OX1R antagonism. In rats exposed to a fear conditioning paradigm, dual OXR antagonism did not alter the behaviour in response to cued or contextual fear but did have an anxiolytic effect in the OFT and SIT (Dong et al., 2015).

2.3.4. Orexin signalling in the LC

The LC is the main source of noradrenergic innervation of the brain and is involved in the regulation of arousal and in fear and anxiety-related behaviour in animal models (Morris et al., 2020). The importance of orexin signalling in the LC has been demonstrated where knockdown of both OX1R and OX2R has been conducted in mice (Soya et al., 2013). The OX1R appears particularly important in this region, with mice lacking it showing reduced freezing behaviour after fear conditioning, to both cued and contextual fearful stimuli. Re-expression of OX1R in the LC normalised cued fear responses in these mice. In rats undergoing an auditory threat conditioning paradigm, localised OX1R antagonism blocked normal threat learning while optogenetic activation of orexinergic projections to the LC enhanced it (Sears et al., 2013). Both studies identified an important projection from the LC to the lateral

amygdala nucleus (part of the BLA), the activation of which was required for threat learning (Soya et al., 2013; Sears et al., 2013).

2.3.5. Orexin signalling in the lateral habenula

The lateral habenula is a densely innervated structure in the basal forebrain involved in the regulation of motivation and aversive behaviour (Hu et al., 2020). This region receives orexinergic projections from the lateral hypothalamus, which in mice is activated by social stress (Wang et al., 2021). Interestingly, optogenetic activation of this projection in mice has an anxiolytic effect in both the EPM and OFT.

2.3.6. Orexin signalling in the medial PFC

The PFC - and particularly the medial PFC - appears to play a major role in the regulation of anxiety (Kenwood et al., 2022) and receives considerable orexinergic innervation (Peyron et al., 1998). In mice exposed to an erratically moving robotic insect model (the beetle mania test), OX1R and OX2R antagonism in the limbic cortex (equivalent to the primate PFC) promotes tolerance and approach towards to the robot (Soares et al., 2021). However, the amounts of freezing and jumping (fear-related behaviours) are unchanged.

2.3.7. Orexin signalling in additional brain regions

Several additional brain regions, including the paraventricular nucleus of hypothalamus (PVN), lateral septum and the dorsal raphe nucleus, are also implicated in the regulation of fear and anxiety and receive dense orexinergic innervation (Peyron et al., 1998; Wu and Zetter, 2022; Wirtshafter and Wilson, 2021; Paul and Lowry, 2013). While both orexin receptors are expressed in the dorsal raphe nucleus, the PVN and lateral septum primarily express OX2R only (Marcus et al., 2001). No animal study has directed investigated the effect of localised modulation of orexinergic signalling, on fear and anxiety-like behaviour, in the PVN or lateral septum. One animal study (in the Nile grass rat), investigated the effect of 5 days of systemic OX1R antagonism on serotonergic projections from the dorsal raphe nucleus (Adidharma et al., 2019). Reduced density of innervation was seen in the anterior cingulate cortex, BNST, the shell of the nucleus accumbens and the periaqueductal grey; regions implicated in fear and anxiety-related behaviour (Adidharma et al., 2019). However, no behavioural outcome was included in this study.

2.4. Studies involving orexin system knock out animals

Mouse strains lacking both orexin and the orexin receptor have been generated and analysed. Interestingly, mice lacking orexin appear to show no difference in behaviour in the EPM (Flores et al., 2016) but increased anxiety-related behaviour in the OFT, LDB and predator odour avoidance test (Khalil and Fendt, 2017). However, orexin deficiency has been associated with no change in normal fear learning or fear extinction (Khalil and Fendt, 2017). This contrasts with a separate study which demonstrated that orexin deficiency is associated with enhanced learning of a social fear conditioning stimulus and delayed extinction of this fear (Faesel et al., 2021). Global knock down of OX1R is associated with impaired fear learning but increased anxiety-like behaviour in the EPM and SIT, and no effect in the OFT (Soya et al., 2013; Abbas et al., 2015). The effect of knock down of OX2R has been less well studied, but in mice is associated with some degree of impaired fear learning and a reduced response to contextual fear stimuli (Soya et al., 2013).

2.5. Studies investigating the role of the orexin system on the stress response

The stress response is mediated by a combination of anatomical regions (principally the hypothalamus, anterior pituitary and adrenal glands or HPA axis) which coordinate behavioural and physiological changes to promote survival from aversive stimuli in the environment (Smith and Vale, 2006). Several lines of evidence support the role of the orexin system in regulating the HPA axis (Winsky-Sommerer et al., 2005; Spinazzi et al., 2006; Johnson et al., 2012b). Orexin receptors are expressed at each anatomical step in the HPA axis (Winsky-Sommerer et al., 2005). Orexin deficient mice showed attenuated responses to stress paradigms, including intruder animals and foot shocks (Kuwaki, 2011). Finally, direct stimulation of the orexin system in rats activates the HPA axis. ICV administration of orexin-A increased plasma adrenocorticotrophic hormone (ACTH) and corticosterone concentrations and increases activity in the PVN. (Kuru et al., 2000; Jászberényi et al., 2000). Similar responses are also noted with ICV administration of orexin-B, although no change in ACTH levels was noted and the effect on the PVN was less pronounced (Kuru et al., 2000).

3. Clinical studies

Evidence from human studies (and particularly patients with anxiety disorders) is more limited and circumspect. However, there are signs of promise that successful translation into human might be possible. Below, we discuss findings from observational studies in patients with narcolepsy, clinical studies where ORAs have been used in the treatment of insomnia, molecular genetic studies of the genes encoding key components of the orexin system, experimental medicine models of anxiety, and studies measuring serum or cerebrospinal fluid (CSF) levels in populations with anxiety.

3.1. Observational studies in patients with narcolepsy

The sleep disorder narcolepsy affects around 1 in 2000 of the population and is characterised by daytime somnolence and sleep paralysis (Mahoney et al., 2019). Narcolepsy is divided into the more severe type 1 (NT1) where patients also experience cataplexy and type 2 (NT2) narcolepsy (Mahoney et al., 2019). Animal and clinical studies have identified the selective loss of orexinergic neurons as the neurobiological basis of NT1, however the basis of NT2 (where CSF orexin levels are usually normal) is unclear (Mahoney et al., 2019). Observational studies of NT1 may therefore provide insight into the effect of orexin signalling on fear and anxiety.

A greater incidence of psychiatric comorbidities has been recognised in patients with narcolepsy, including MDD, eating disorders, attention deficient hyperactivity disorder and psychosis/schizophrenia (Fortuyn et al., 2011; Morse and Sanjeev, 2018; BaHammam et al., 2020). Multiple cross-sectional studies have also compared the prevalence of anxiety disorders in patients with narcolepsy to healthy controls (Ruoff et al., 2017; Ohayon, 2013; Fortuyn et al., 2010; Cohen A et al., 2018; Chen et al., 2020; Alasim H et al., 2020). Increased prevalence of at least one anxiety disorder was noted in every study, suggested a greater risk of developing an anxiety disorder. Although most studies were relatively small, the largest study which included over 9000 patients in the US identified an odds ratio of 2.5 for increased prevalence of any anxiety disorder in narcolepsy (Ruoff et al., 2017). However, it should be noted that most studies included patients with NT1 and NT2.

3.2. Insomnia trials in ORAs

Several RCTs have been conducted with ORAs in populations with insomnia (Xue et al., 2022). However, these RCTs have largely excluded patients with psychiatric co-morbidities and anxiety symptoms have not been measured as secondary outcomes. One small open-label trial of suvorexant has been conducted in psychiatric inpatients with insomnia (Nakamura and Nagamine, 2017). Most patients had an underlying diagnosis of schizophrenia, schizoaffective disorder, or a mood disorder. A range of outcomes were measured, including GAD-7 score, which showed a significant reduction following 4 weeks of treatment with suvorexant. However, most patients did not have a diagnosis of an anxiety disorder and patients were likely receiving additional psychiatric treatment which is likely to have affected anxiety symptoms.

3.3. Molecular genetic studies of the orexin system

The key components of the orexin system: orexin-A/orexin-B, the OX1R and the OX2R are encoded by the genes *HCRT*, *HCRTR1* and *HCRTR2* respectively. Initial studies into the genetics of narcolepsy identified one *HCRT* mutation, impairing peptide trafficking and processing, in a single case with early onset narcolepsy (Peyron et al., 2000). Polymorphisms in both *HCRTR1* and *HCRTR2* have been associated with panic disorder ± agoraphobia in clinical samples. In a Swedish sample of patients with panic disorder (85% with co-morbid agoraphobia) and healthy controls, the Iso allele of the *HCRTR2* Val308Iso polymorphism was significantly more common in patients with panic disorder (Annerbrink et al., 2011). No difference was seen for the *HCRTR1* Ile408Val (C/T) polymorphism (rs2271933) in this sample (Annerbrink et al., 2011).

However, a larger German sample of 613 patients and 1839 healthy controls did associate the *HCRTR1* Ile408Val (C/T) polymorphism (rs2271933) with panic disorder ± agoraphobia (Gottschalk et al., 2019). The rs2271933 T allele was associated with a significantly increased odd ratio of having panic disorder ± agoraphobia (either 1.51 or 1.82 depending on the comparison model used) (Gottschalk et al., 2019). Interestingly, the association was not significant when the subgroup of male patients was separately analysed. In smaller samples of patients, the T allele was also associated with a decreased treatment response to cognitive behavioural therapy. In patients, it was furthermore associated with increased avoidance behaviour and psychological and physiological measures of anxiety in a behavioural task where patients anticipated and underwent exposure to a dark confined space. Finally in healthy volunteers, the T allele was associated with increased activity in the LC during functional magnetic resonance imaging (fMRI) where a cognitive task was used to assess attention and alertness (Gottschalk et al., 2019).

These two molecular genetic studies are limited to a single polymorphism in the *HCRTR1* and *HCRTR2* genes, respectively, with inconsistent findings for the *HCRTR1* Ile408Val polymorphism across the two studies. The findings of the latter study on the *HCRTR1* Ile408Val polymorphism are stronger, however, considering the larger size of the sample and the additional supportive evidence from functional neuroimaging and behavioural outcomes. It has to be noted though, that the functional significance of both polymorphisms on receptor structure and function is currently not understood.

3.4. Experimental medicine models of anxiety

Further studies have investigated the role of the orexin system in experimental medicine models of anxiety in healthy human volunteers. These experimental approaches allow aspects of anxiety disorders to be mimicked in humans and can be used to screen novel treatments prior to expensive and time-consuming clinical trials (Grillon et al., 2019).

A widely used experimental medicine model of both GAD and panic disorder is the CO_2 inhalation model, where participants inhale air with elevated CO_2 concentrations (Bailey and Nutt, 2008; Bailey et al., 2011). A concentration of 7.5% CO_2 for 20 min mimics many of the psychological and physiological symptoms of GAD while a single inhalation of higher CO_2 concentrations (usually 35%) can induce panic symptoms. This model is sensitive to effective anxiolytic treatments in GAD, including benzodiazepines, SSRIs, SNRIs and pregabalin (Bailey et al., 2011). Two human studies have investigated the effect of a selective OX1R antagonist on the anxiogenic effects of CO_2 inhalation. The novel OX1R antagonist JNJ-61393215 and the benzodiazepine alprazolam (1 mg) were compared with placebo in healthy volunteers who underwent a single inhalation of 35% CO_2 (Salvadore et al., 2020). After 7 days of treatment, JNJ-61393215 resulted in a significant reduction in panic symptoms (as measured by the panic symptom list IV) although no difference in physiological measures (heart rate or blood pressure) were noted (Salvadore et al., 2020). A greater reduction in panic symptoms was noted with alprazolam, which also attenuated the increases in heart rate and blood pressure following CO_2 inhalation (Salvadore et al., 2020). In a similar study, the selective OX1R antagonist ACT-539313 was compared *vs.* placebo in healthy volunteers who underwent a 20-min inhalation of 7.5% CO_2 and a single inhalation of 35% CO_2 (Kaufmann et al., 2021). Subjective symptoms of anxiety as measured by the GAD-C and panic symptom inventory were not significantly different during either inhalation for ACT-539313 *vs.* placebo, although there was a trend for reduced anxiety symptoms in the ACT-539313 group (Kaufmann et al., 2021). No difference was noted for physiological measures (heart rate or blood pressure) between groups (Kaufmann et al., 2021).

An additional study assessed the effect of the DORA suvorexant in a model of anticipatory anxiety: the No-Predictable-Unpredictable (NPU) threat paradigm (Gorka et al., 2022). Healthy volunteers receive small electric shocks to the wrist which are either preceded by a visual countdown (predictable) or at random (unpredictable). The primary outcome was the startle eyeblink response. Unpredictable electric shocks were associated with an exaggerated magnitude of the startle response, but suvorexant significantly reduced this relative to placebo. However, no measure of subjective anxiety was assessed during the experimental sessions.

3.5. Orexin levels in clinical populations

The activity of the orexin system has been estimated in human studies where CSF or serum orexin concentrations are measured. Preclinical studies indicate that orexin-A (but not orexin-B) can rapidly cross the blood brain barrier (Kastin and Akerstrom, 1999) and one small clinical study found CSF and serum orexin-A concentrations were highly positively correlated ($r = +0.78$) (Strawn et al., 2010). In one study, a sample of 53 unmedicated adult patients who presented with suicidal behaviour were assessed for symptoms of MDD and panic anxiety (Johnson et al., 2010). Patients with panic anxiety only had significantly higher CSF orexin-A concentrations than patients with both panic anxiety and MDD and patients with neither panic anxiety or MDD (Johnson et al., 2010). However, these patients had panic symptoms rather than an anxiety disorder *per se*. In a second study, the serum concentrations of orexin-A and cortisol were compared between a group of unmedicated adolescent patients with an anxiety disorder ($n = 56$) and a group of healthy controls (Akça et al., 2020). The majority of the patient group had a disorder of GAD or social anxiety disorder. Significantly higher levels of orexin-A but not cortisol was noted in patients with anxiety disorders relative to controls.

4. Discussion

4.1. Summary of pre-clinical and clinical studies of the role of the orexin system in fear and anxiety

As described above, many rodent studies (chiefly mouse and rat) implicate the orexin system in the regulation of fear and anxiety behaviour. Although not comprehensively seen across all models of anxiety, systemic orexin signalling generally appears to promote fearful and anxiety-like behaviours. Orexin signalling appears particularly important in the response to aversive stimuli, such as CO_2 inhalation (Johnson et al., 2010, 2012a, 2015; Bonaventure et al., 2017; Salvadore et al., 2020), social defeat (Heydendael et al., 2014; Eacret et al., 2019) or to classical fear conditioning paradigms (Steiner et al., 2012; Flores et al., 2014; Viviani et al., 2015). Orexin signalling has also been implicated in the regulation of social behaviour (Dawson et al., 2023), supporting the potential the findings from social defeat paradigms and implicating the potential role of orexin signalling in social anxiety disorder. Orexinergic projections target multiple anatomical nodes in the fear and anxiety networks of the mammalian brain (Peyron et al., 1998) and play important modulatory roles in these regions (see Fig. 2 for

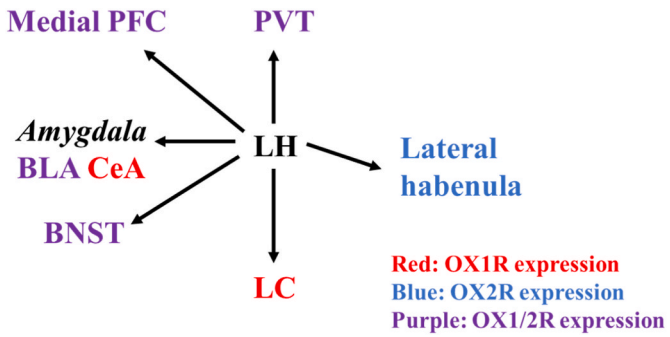

Fig. 2. Diagram showing orexinergic projections to brain regions implicated in fear and anxiety related behaviour in animal models. Expression of orexin receptor subtype is signified by text colour. Note that OX1R expression exceeds OX2R expression in BLA, BNST, medial PFC and PVT (Marcus et al., 2001). Abbreviations used: BLA basolateral amygdala, BNST bed nucleus of the stria terminalis, CeA central amygdala, LC locus coeruleus, LH lateral hypothalamus, OX1R orexin 1 receptor, OX2R orexin 2 receptor, PFC prefrontal cortex, PVT paraventricular nucleus of thalamus. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

summary). Enhancing orexin signalling in the amygdala (BLA and CeA) (Flores et al., 2014; Arendt et al., 2014; Avolio et al., 2011, 2014; Yaeger et al., 2022; Pan et al., 2020), the BNST (Johnson et al., 2010; Lungwitz et al., 2012; Giardino et al., 2018) the PVT (Li et al., 2010; Dong et al., 2015) the LC (Soya et al., 2013; Sears et al., 2013) and the PFC (Soares et al., 2021) promotes anxiety-like responses in a range of behavioural tests. An opposing effect of orexin signalling in the lateral habenula has been noted although this is based on a single study, and the role of the lateral habenula in anxiety disorders is less well established (Hu et al., 2020; Wang et al., 2021). Additional brain regions, involved in the regulation of fear and anxiety include the PVN, lateral septum and dorsal raphe nucleus. Although these regions all receive orexinergic innervation, further studies are needed to characterise the exact role of orexin signalling in these areas.

These findings are somewhat contradicted by the small number of studies with either orexin-deficient mice or selective knockdown of OX1R or OX2R. Orexin deficiency and OX1R loss appears to have an anxiogenic effect in several classical anxiety tests (Flores et al., 2016; Khalil and Fendt, 2017; Faesel et al., 2021; Abbas et al., 2015). Orexin deficiency has been shown to have contradictory effects on fear learning (Khalil and Fendt, 2017; Faesel et al., 2021) while OX1R loss, and to a lesser extent OX2R loss, have been associated with impaired fear learning (Flores et al., 2014; Soya et al., 2013). The contradictory results seen between mice with or without life-long orexin deficiencies may be related by compensatory up-regulation of other neurotransmitter systems in orexin-deficient mice. Orexinergic innervation and regulation of other monoamine systems and the cholinergic system is well established (Peyron et al., 1998; Sakurai et al., 2021) and mouse studies of orexin deficient or ablated mice have noted alterations in monoaminergic signalling (Mori et al., 2010; Tsujino et al., 2013). These animal findings in orexin-deficient mice are also consistent with the increased rates of anxiety disorders noted in orexin-deficient patients (i.e. patients with narcolepsy) (Ruoff et al., 2017; Ohayon, 2013; Fortuyn et al., 2010; Cohen A et al., 2018; Chen et al., 2020; Alasim H et al., 2020) and could also result from compensatory changes in other neurotransmitter systems.

The potentially different roles of the OX1R and the OX2R have been suggested by some studies. OX1R and OX2R expression in the mammalian brain is overlapping but distinct with an apparent greater representation of OX1R in many key brain regions in the fear and anxiety networks (Marcus et al., 2001; Tovote et al., 2015). Generally, the pre-clinical studies support the anxiogenic effect of orexin signalling being mediated through OX1R. Selective antagonism of OX1R alone is generally sufficient to reduce anxiety-related behaviours in response to a range of aversive stimuli including CO_2 inhalation, sodium lactate infusions, social defeat stress and anxiogenic medications (Staples and Cornish, 2014; Vanderhaven et al., 2015; Johnson et al., 2010, 2012a, 2015; Bonaventure et al., 2017). Where OX1R and OX2R antagonism have been directly compared, the anxiolytic effect of selective OX2R antagonism is limited or absent (Flores et al., 2014; Johnson et al., 2015). Finally in mice exposed to the Stress-Alternatives Model, selective OX2R antagonism appears to promote an anxiogenic response to social stress while selective OX2R agonism promotes escape from a larger aggressive animal (Staton et al., 2018). However, an anxiolytic response was seen with localised antagonism of OX2R in the PVT (Li et al., 2010) and PFC (Soares et al., 2021).

While animal models of anxiety disorders provide invaluable ways to monitor and manipulate neural circuitry, criticism of their applicability to human anxiety disorders have regularly been made. Most of the current studies employ long-used 'classical' tests of anxiety such as the EPM or OFT that have been criticised for a lack of validity in the disorder they purport to model (Haller et al., 2013; Blanchard et al., 2013). Many essential symptoms of anxiety disorders in our current diagnostic symptoms, are very difficult, if not impossible, to model in animal systems, for example the central symptom of worry in GAD or the persistent fear or embarrassment of scrutiny in social anxiety disorder. Translational studies in healthy human volunteers or clinical populations might be the only way of studying symptoms of this type.

To date, the small number of human studies conducted on the role of the orexin symptom and anxiety suggest a possible role for the orexin system in the pathogenesis of anxiety disorders, and orexin modulating agents as a potential treatment. The larger molecular genetic study found an association between the *HCRTR1* gene and panic disorder and agoraphobia (Gottschalk et al., 2019), which is generally consistent with pre-clinical studies of animal models of panic. Interestingly, the *HCRTR1* gene variant was also associated with increased LC activation in a fMRI study, again supported by the pre-clinical findings suggesting an anxiogenic role of orexin signalling in the LC (Soya et al., 2013). The findings of elevated orexin-A in CSF (Johnson et al., 2010) and serum of clinical samples (Akça et al., 2020) also implicate a potential contributory role of the orexin system. However, both samples were relatively small, and the CSF study only included patients with symptoms of anxiety rather than a specific diagnosis while the serum sample focused only on adolescents. A further question is to what extent CSF and particularly serum orexin levels accurately represent orexinergic activity in the human brain.

The finding that ORA treatment can reduce anxiety symptoms in psychiatric patients with insomnia in an open label trial (Nakamura and Nagamine, 2017) is also promising; however the trial was small, the patients largely lacked any diagnosis of an anxiety disorder and is vulnerable to all the potential confounders of a trial of this type. Finally, the reduction of anxiety in healthy volunteers in two experimental medicine models of GAD (CO_2 inhalation and an unpredictable threat paradigm) also support a potential role for an orexin receptor antagonist as a novel anxiolytic strategy (Salvadore et al., 2020; Kaufmann et al., 2021; Gorka et al., 2022). However, it should be noted that an anxiolytic effect was not seen for one of the selective OX1R antagonists tested in the CO_2 inhalation model (Kaufmann et al., 2021).

4.2. *Future directions for the translation into clinical practice*

Based on the current limited human studies, we suggest a number of potential research directions to further explore the orexin system and its effect on fear and anxiety in humans. Further research could be focussed on both healthy volunteers utilising experimental medicine models of anxiety and in clinical populations. Studies of both groups can include further neuroimaging studies, with techniques such as fMRI to investigate the activity of brain regions in the fear and anxiety networks with orexinergic innervations, including those already studied in animal

models such as the amygdala, PVT and medial PFC. In addition, the development of positron emission tomography probes targeting orexin receptors could provide a technique for specifically imaging orexinergic activity in the human brain (Bai et al., 2020).

A wide range of experimental medicine models of anxiety are available for the induction of symptoms of fear and anxiety in healthy volunteers (Grillon et al., 2019). Beyond the CO_2 inhalation model of either panic disorder (single breath of 35% of CO_2) or GAD (20-min inhalation of 7.5% CO_2), other models include the Trier social stress test (TSST), the InterneT based Stress test for Social Anxiety Disorder (ITS-SAD) or the display of anxiogenic stimuli using virtual reality testing (Kirschbaum et al., 1993; Biedermann et al., 2017; Huneke et al., 2022). Considering the effect of selective OX1R antagonism CO_2 inhalation has already been assessed in the CO_2 model, a further study investigating the effect of dual OXR antagonism or selective OX2R antagonism could be similarly conducted to further differentiate the roles of the two orexin receptors. We have recently proposed to conduct a study of this type assessing the effect of a single dose of the DORA daridorexant in healthy volunteers in the 7.5% CO_2 inhalation model (Fagan et al., 2023b). In clinical populations, further molecular genetic studies could be conducted to consider the role of *HCRT*, *HCRTR1* and *HCRTR2* gene variants in other anxiety disorders beyond panic disorder and agoraphobia. Further studies exploring serum and CSF concentrations of orexin-A and B in adult patients with a range of anxiety disorders are needed. Longitudinal studies could be performed to assess the effect of both pharmacological and psychological treatments and the remission of symptoms on the concentrations of orexin. Ideally, combined CSF and serum levels could be taken to further validate the use of serum concentrations as an accurate proxy marker for brain orexin activity. Finally, if the above studies show promise of an anxiolytic effect of orexin receptor modulation, RCTs of such agents could be performed in patients with anxiety disorders.

5. Conclusion

Anxiety disorders are common and a major cause of disability, worldwide. Although effective pharmacological and psychological treatments exist, many patients do not respond to initial treatment and there remains a pressing need to develop new treatments. The orexin neurotransmitter system has been implicated in the neurobiology of anxiety and fear in a wide range of rodent models and in a more limited number of human studies in healthy volunteers and clinical populations. In general, animal studies indicate that orexinergic activity promotes anxiety and fear-related behaviour, particularly in response to aversive or fearful stimuli. Of the two orexin receptors, OX1R appears particularly important to this function of the orexin system with the exact role of OX2R less clearly understood.

Translation of these findings into human studies has been relatively limited to date. Some evidence supports an anxiolytic effect of orexin receptor antagonists in healthy volunteers in experimental medicine models of anxiety. In addition, a large molecular genetic study has associated the gene variants of the *HCRTR1* gene with panic disorder and agoraphobia and two small clinical studies suggest an elevated level of orexin-A in CSF in patients with panic symptoms and in serum in adolescents with anxiety disorders. Further studies are needed to characterise the effect of orexin modulation in healthy human volunteers and to investigate the role of the orexin system in the neurobiology of patients with anxiety disorders, before clinical trials of orexin modulating treatments could be considered for anxiety disorders.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors intend to conduct a study investigating the anxiolytic effect of the dual orexin receptor antagonist daridorexant in healthy human volunteers. Complete funding to conduct this study, including provision of daridorexant, has been offered by Idorsia Pharmaceuticals Ltd. via their Investigator Sponsored Study (ISS) program.

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