Trends in neurosciences

Orexins and fear: implications for the treatment of anxiety disorders

África Flores, Rocío Saravia, Rafael Maldonado[#] and Fernando Berrendero[#]

Laboratory of Neuropharmacology, Department of Experimental and Health Sciences,
Universitat Pompeu Fabra, PRBB, C/ Doctor Aiguader 88, 08003 Barcelona, Spain

[#] Corresponding authors:

Rafael Maldonado, Phone: +34-93-3160824; E-mail: rafael.maldonado@upf.edu

Fernando Berrendero, Phone: +34-93-3160890; E-mail: fernando.berrendero@upf.edu

Keywords: orexin, fear, anxiety, amygdala, prefrontal cortex

Abstract

An understanding of the neurobiological mechanisms involved in the regulation of fear is essential for the development of new treatments for anxiety disorders such as phobias, panic and post-traumatic stress disorders. Orexins, also known as hypocretins, are neuropeptides located exclusively in hypothalamic neurons that have extensive projections throughout the central nervous system. Although this system was initially believed to be primarily involved in the regulation of feeding behavior, recent studies have shown that orexins also modulate neural circuits implicated in the expression and extinction of fear memories. Here, we discuss recent findings involving orexins in anxiety disorders and current clinical trials using orexin ligands that could be applied to identify new therapies for diseases characterized by pathological fear.

General overview of the orexin system

The orexin system consists of two neuropeptides, orexin A and B (also known as hypocretin 1 and 2), and their associated G-protein coupled orexin type 1 (OX1R) and 2 (OX2R) receptors [1,2]. Orexin A and orexin B are 33 and 28 amino acid peptides produced by the proteolysis of a common precursor, prepro-orexin. Radioligand binding assays have demonstrated that orexin B binds preferentially to OX2R whereas orexin A binds with equal affinity to OX1R and OX2R [1]. The signal transduction mechanisms triggered upon orexin receptor (OXR) activation mainly involved Gq proteins and the phospholipase C–protein kinase C (PLC-PKC) pathway, although a wide variety of signals seem to be originated from stimulation of these receptors ([3], for a detailed review). Orexin-expressing neurons represent a small population exclusively located in the lateral hypothalamus (LH), the perifornical area (PFA) and the dorsomedial hypothalamus (DMH). Although limited in number and localization, they have extensive projections throughout the brain [4]. OX1R and OX2R have a broad and partially overlapping distribution in many regions of the brain [5], although some areas have a selective expression of these receptors (Figure 1).

The widespread projections of the orexin system reflect the variety of physiological functions of orexin peptides, which include: the modulation of arousal and sleep/wake cycles, energy homeostasis, reward processing and stress ([6–8], for reviews) (Box 1). Moreover, a role for orexins in emotional behavior regulation has been recently proposed, consistent with the existence of orexin neuronal projections to several limbic areas [9] (Figure 1) (see Glossary). We review here recent studies involving the orexin system in the modulation of fear memory, and the potential usefulness of orexin ligands to treat trauma and anxiety-related disorders.

Functional circuitry of fear expression and extinction

Fear is an emotion that is crucial for the organization of defensive behaviors to threat, and therefore has an essential role in survival. Indeed, the formation of fear memories after stress exposure is an adaptive phenomenon that helps to avoid similar situations and to improve future coping strategies. Once acquired, however, the appropriate regulation of fear under varying environmental conditions is essential to maintain mental health [10]. Thus, deregulation of acquired fear, revealed by enhanced conditioning or resistance to fear extinction, is associated with anxiety disorders such as phobias, panic and posttraumatic stress disorders (PTSD). In rodents, the neurobiological bases of fear memory regulation can be studied by Pavlovian fear conditioning (Figure 2), an experimental scenario in which an initially neutral conditioned stimulus (CS), such as tone or context, is paired with a naturally aversive unconditioned stimulus (US), usually a footshock.

The use of complementary techniques including electrophysiology, immediate-early gene mapping, lesioning/inactivation approaches and optogenetics has revealed the critical brain areas involved in the regulation of fear. The amygdala (AMY), hippocampus (HPC), and the medial prefrontal cortex (mPFC) have emerged as the classical regions of the fear neural circuit [11,12]. However, other brain areas such as the bed nucleus of the stria terminalis [13], the locus coeruleus [14], the paraventricular nucleus of the thalamus [15] and the periaqueductal gray matter [16], among others, have also been implicated in the modulation of fear.

The basolateral amygdala (BLA), including lateral, basal and accessory basal nuclei, is significantly involved in both the formation of fear memory and its subsequent extinction (Figure 3A). Indeed, distinct neuronal populations of this brain structure are active during fear conditioning (“fear neurons”) and extinction (“extinction neurons”)

[17]. The BLA seems to be a crucial site where the associations between the CS (tone or context) and the US (shock) are created [18]. From the BLA, information is relayed to the central nucleus of the AMY which is responsible for the expression of fear responses through projections to downstream structures in the brainstem and the hypothalamus [19]. The formation of fear memories also requires the participation of the HPC and the prelimbic portion of the mPFC (dorsomedial PFC in humans). The HPC is responsible for assembling the different components of the contextual cues into a single representation of the context [20], and transmitting this information to the AMY. On the other hand, the prelimbic portion of the mPFC has excitatory projections to the BLA and constitutes a key pathway for cortical modulation of fear [21]. Recent research indicates that specific inhibition of parvalbumin interneurons in the prelimbic mPFC disinhibits prefrontal projection neurons leading to fear expression [22].

Extinction of aversive memories recruits similar brain regions to those involved in the acquisition of fear (Figure 3A). However, unlike fear memory formation, which recruits the prelimbic mPFC, fear extinction engages the infralimbic subdivision of the mPFC (ventromedial PFC in humans) [21]. In agreement, infralimbic mPFC neurons might project to “extinction neurons” within the BLA to reduce central AMY activity resulting in the suppression of fear. Alternatively, neurons in the infralimbic mPFC could also project to a group of inhibitory interneurons in the intercalated cell masses of the AMY to inhibit fear response in the central AMY. These interneurons are GABAergic, project to the central AMY and receive glutamatergic input from the infralimbic mPFC and the BLA [23,24]. The HPC, which has robust reciprocal connections with the AMY [25], appears to be also essential for fear extinction as revealed in studies using behavioral paradigms such as inhibitory avoidance and contextual fear conditioning [10].

Modulation of fear memory by orexins

Several recent reports have revealed a role of the orexin system in the regulation of emotional memories. This physiological function is consistent with the existence of bidirectional projections between orexin neurons and brain areas involved in the modulation of motivation and emotion (Figure 1). Specifically, orexin neuronal projections are particularly abundant in brain structures such as the mPFC, the bed nucleus of the stria terminalis, lateral septum, AMY, locus coeruleus, paraventricular hypothalamic and thalamic nuclei [26]. Reciprocally, orexin neurons receive input from several nuclei of the limbic system [27,28].

Studies in humans have shown that individuals with narcolepsy, a condition associated with a loss of orexin neurons [29], show reduced AMY activity and no increase in functional coupling between AMY and mPFC when exposed to a conditioned aversive stimulus [30]. This study suggests that human narcolepsy should be considered not only as a sleep-wake disorder but also as a condition that can impair emotional learning. In agreement, narcoleptic patients fail to exhibit startle potentiation during unpleasant stimuli [31] supporting the hypothesis of an AMY dysfunction in narcolepsy. The participation of the orexin system in the regulation of AMY function was also suggested by a recent microdialysis study in humans showing an increase of orexin A levels in this brain area during positive emotions, social interaction and anger, behaviors that induce cataplexy in narcoleptic patients [32].

The involvement of orexins in the modulation of fear has also been recently demonstrated in rodent models (Figure 2). Mice lacking the OX1R showed impaired freezing responses and reduced expression of *zif268* (an immediate-early gene which is considered a marker of neuronal activation) in the lateral AMY in both cued and contextual fear conditioning paradigms [33]. In agreement, the dual orexin antagonist

almorexant reduced fear-potentiated startle responses [34], and increased prepro-orexin mRNA levels were positively correlated with immobility time after footshock exposure in rats [35]. Notably, restoration of OX1R expression in noradrenergic neurons of the locus coeruleus by using an adeno-associated virus vector normalized cued fear behavior and increased lateral AMY activity after cued testing in OX1R knockout mice [33]. A similar involvement of OX1R in the locus coeruleus in AMY-dependent threat learning was revealed by pharmacological and optogenetic approaches [36]. In this study, pharmacological blockade of the OX1R in the locus coeruleus by microinjection of the OX1R antagonist SB334867 impaired threat memory formation induced by an auditory stimulus. Moreover, optical stimulation of orexin fibers in the locus coeruleus was sufficient to enhance this response [36]. These data are congruent with the previously established implication of the locus coeruleus noradrenergic neurons in emotional memory formation. Concordantly, the activity of these neurons increases after a fear conditioning test [14], and β -adrenergic blockade with propranolol was effective for treating patients with PTSD [37].

In contrast to the OX1R results discussed above, mice lacking OX2R showed reduced freezing behavior in a contextual fear test, but normal freezing in a cued fear paradigm [33]. Consistent with this, intracerebroventricular infusion of the OX2R antagonist TCS-OX2-29 did not modify freezing responses in a cue-induced fear conditioning paradigm [36]. These data suggest a different role for OX1R and OX2R in the formation of cued and contextual fear memories probably due to the different location of these receptors in brain structures such as the AMY and HPC [26].

Orexins also modulate the extinction of acquired aversive memory. For example, OX1R blockade with SB334867 facilitated the consolidation of fear extinction in both contextual and cued tests, while orexin A infusion impaired this response [38]. Direct

injection of SB334867 within the BLA mimicked the enhancement of fear extinction previously observed after systemic administration of this compound. The OX1R antagonist also rescued contextual and cued fear extinction in the 129S1/SvImJ inbred mouse strain [38], a well-established mouse model of impaired extinction [39]. These data suggest the potential usefulness of this pharmacological target in diseases characterized by inappropriate maintenance of aversive memories. On the contrary, OX2R blockade with the antagonist TCS-OX2-29 or orexin B infusion did not modify fear extinction [38]. Interestingly, a recent study in rodents has reported a link between the orexin system and generalized avoidance of situations with low similarity to the initial fear-associated context [40]. The presence of this maladaptive response after a traumatic experience predicts a higher probability for developing PTSD in humans [41], and is considered a symptom of anxiety disorders with similarities with PTSD. Chronic dual OXR blockade with almorexant reduced both the development and persistence of generalized avoidance behavior in rats [40], suggesting that orexins contribute to this effect.

Several studies measuring immediate-early gene expression conclude that hypoactivity of specific brain areas of the cortico-amygdala circuit, such as the infralimbic mPFC and the BLA, is related to impaired fear extinction [42–45]. On the contrary, complete extinction of conditioned fear is associated with increased immediate-early gene expression in these brain areas [42]. Notably, the extinction-facilitating effects of SB334867 were related to increased activity of both infralimbic mPFC and BLA (Figure 3B), suggesting that OX1R blockade facilitates the activation of pro-extinction circuits in specific cortical and AMY regions [38].

As previously mentioned, recent evidences indicate that the thalamic paraventricular nucleus, a subregion of the dorsal midline thalamus, is involved in conditioned fear

processing. This thalamic nucleus strongly innervates both prelimbic and infralimbic portions of the mPFC, as well as the basal and central nuclei of the AMY [46]. Temporary inactivation of midline thalamic nuclei by microinjecting the GABA agonist muscimol [47], and bilateral lesions of the thalamic paraventricular nucleus [48] after conditioning decreased freezing behavior produced by a tone conditioned fear. A recent study suggests that the thalamic paraventricular nucleus regulates fear responses modulating the lateral division of the central AMY [15], and seems crucial in the retrieval and maintenance of long-term fear memories [49]. The paraventricular thalamus receives dense projections from orexin neurons [50] (Figure 1) and seems to participate in certain negative emotional behaviors mediated by orexins. Indeed, microinjections of orexin A and B in this brain area promoted anxiogenic-like responses in the elevated plus maze [51], while optogenetic stimulation of orexin neurons induced OX1R internalization in the paraventricular thalamus and increased anxiety in the social interaction test [52]. Therefore, it is reasonable to hypothesize that orexins could modulate fear by acting also on the paraventricular nucleus of the thalamus considering its role in fear memory processing and its influence in certain anxiety-related actions of orexins.

In summary, orexins are critically involved in the neural mechanisms mediating emotional memory formation during fearful situations. Once acquired, orexin neurons are engaged to preserve fear during the normal physiological process of fear extinction through a mechanism that directly involves the activation of OX1R. Indeed, OX1R antagonism accelerates the extinction of aversive memories.

Implications of fear regulation by orexins for the treatment of anxiety disorders

As mentioned above, impaired fear processing is a key feature of a range of anxiety disorders. These pathological conditions, especially phobias, panic disorder and PTSD, are characterized by failure to extinguish fear memories or inadequate responsiveness to aversive situations. The current lifetime prevalence rate for anxiety disorders varies from 14% to 31% in developed countries [53,54], constituting together with mood disorders the most prevalent psychiatric pathologies. Traditional drug treatments for anxiety, such as benzodiazepines and selective serotonin reuptake inhibitors, offer transient symptom relief and are associated with negative side effects. In addition, a significant proportion of patients do not respond to treatment or relapse after treatment remission [55]. Combining cognitive-behavioral therapy with pharmacotherapy has not sufficiently filled this gap [56], leaving a strong need for the development of more effective and safer pharmacological treatments.

Orexin-related targets may represent promising opportunities for treating anxiety disorders considering the role for the orexin system in the modulation of the behavioral expression and extinction of fear responses. Hyperactivity of the orexin system has been suggested to be a critical factor in the maintenance of high arousal and anxiety, and to increase vulnerability to relapse to panic episodes in predisposed individuals [26]. In agreement, human subjects with panic anxiety have elevated levels of orexin A in the cerebrospinal fluid (CSF) compared with control subjects [57]. Interestingly, chronic treatment with sertraline, a well-known antipanic and antidepressant drug, reduces orexin concentrations in the CSF, whereas bupropion, an antidepressant with lower efficacy in treating panic disorder, fails to decrease orexin levels in the CSF [58]. These data suggest that orexin reduction might be a possible mechanism for the antipanic effects of some antidepressant drugs. In contrast, a clinical study showed a reduction of

orexin A levels in the CSF and plasma of combat veterans with chronic PTSD and these levels were negatively correlated with PTSD severity [59]. These unexpected results apparently contradict the association between a hyperactive orexin system and an enhanced vulnerability to develop maladaptive responses to fear events. However, possible depressive symptoms could be masking these findings, since depression has been associated with low orexin levels in the CSF even in the presence of comorbid anxiety [57].

Panic disorder, characterized by bursts of severe anxiety accompanied by multiple physical symptoms such as tachycardia and hyperventilation, has been robustly linked with orexins in animal models. Anatomical and pharmacological approaches in rodents have revealed that the DMH/PFA (containing an important proportion of orexin neurons), among other brain regions, is activated during panic attacks [60]. Moreover, orexin knockout mice display attenuated cardiovascular and behavioral defense responses to emotional stress after disinhibition of the DMH/PFA [61]. Based on these observations, a rat model of panic disorder involving chronic disinhibition of DMH/PFA and subsequent increase in orexin activity has been developed and validated over the last several years [62]. In this model, chronic reduction of GABA synthesis in the rat DMH/PFA produces anxiety-like states and enhanced vulnerability to panic-like responses following intravenous infusions of lactate [63]. Interestingly, systemic administration of the OX1R antagonist SB334867, as well as local silencing of the orexin precursor gene in the PFA region through siRNA injection, attenuated anxiety-like behavior and cardiorespiratory responses induced by the lactate challenge in panic-prone rats [57]. These findings suggest that OX1R antagonists may provide an alternative therapeutic approach for the treatment of panic disorder.

Orexin modulation of pH/CO₂ balance seems closely related to its role in panic disorder. Changes in CO₂ and acid concentration may trigger panic attacks after a challenge test by increasing autonomic symptoms and promoting behavioral arousal [64]. Notably, recent evidence has demonstrated that orexin neurons are highly sensitive to local changes in CO₂/H⁺ [65]. In a human study, orexin A plasma levels were dramatically increased in patients with chronic obstructive pulmonary disease, a condition associated with hypercapnia and a 10-fold increased risk of panic attacks [66]. Consistent with this, chronic exposure to cigarette smoke tripled hypothalamic orexin A expression in a rodent model of chronic obstructive pulmonary disease [67]. In addition, rat exposure to hypercapnic air increased c-Fos expression in orexin neurons, and promoted anxiety-like behavior, which was prevented by systemic injection of SB334867 [68]. These data provide new insights to support the potential interest of OX1R antagonists for treating panic disorders.

Even though most of the research performed so far supports a prominent role for the OX1R in anxiety, an association between a OX2R polymorphism (Val308Iso) and panic disorder has been reported in female patients [69]. Therefore, further research will be needed to fully understand the specific involvement of the different components of the orexin system in the pathophysiological substrate of panic disorders.

Current clinical trials with orexin receptor antagonists: potential design of novel trials for the treatment of anxiety disorders

The promising findings obtained with OXR antagonists in several animal models mimicking human pathological conditions have allowed for the design of clinical trials to evaluate the safety and effectiveness of these compounds in treating these disorders. At the present moment, the clinical trials with orexin antagonists have been

predominantly focused on insomnia treatment and all the compounds evaluated have been dual antagonists of both OX1R and OX2R (Table 1). The first dual orexin antagonist almorexant was evaluated in early clinical trials for insomnia treatment and its development was abandoned in 2011, probably due to tolerability concerns (see: <http://www1.actelion.com/en/our-company/news-and-events.page?newsId=1483135>).

In contrast, another dual antagonist, suvorexant, was recently approved by FDA for insomnia treatment in adults who have difficulty falling sleep and/or staying asleep (it is now available in the USA market as Belsomra®). Safety and efficacy of suvorexant have been demonstrated for treating insomnia for periods of 3 months [70] and 1 year in adult patients [71]. Suvorexant treatment improved sleep onset and maintenance in both clinical trials and was generally safe and well tolerated during the whole treatment period [70,71]. The most common adverse effect of suvorexant in both studies was day somnolence. In addition, recurrence of insomniac symptoms occurred when suvorexant was withdrawn after one year treatment [71]. In spite of these findings, a recent FDA report raises concerns about the safety of suvorexant for treating insomnia mainly when used at high doses (see: <http://www.fda.gov/downloads/.../UCM354215.pdf>). Indeed, the report suggests that the lowest dose developed of suvorexant (15 mg) may not be low enough for safe use, whereas phase II data suggest that a lower dose may be already effective for treating insomnia. These concerns were mainly raised with regards to the daytime somnolence that can occur suddenly as well as the inability to drive or operate heavy machinery in patients treated with suvorexant at doses over 20 mg. These adverse effects were dose-dependent and the FDA recommends additional efforts to find the lowest effective dose of suvorexant. Three other dual antagonists are also now under development for insomnia treatment. ACT-462206 (NCT01954589) [72] is under development in Phase I, and SB-649868 (NCT00426816) [73] and filorexant

(NCT01021852) are both under development in Phase II. The results of these clinical studies could provide additional valuable information to clarify the interest of this novel therapeutic approach for the treatment of insomnia.

Interestingly, novel clinical trials with dual orexin antagonists have also been designed to evaluate the possible effectiveness of this pharmacological approach in other pathologies. A recent clinical trial has demonstrated that filorexant was effective in preventing migraine when administered at night (NCT01513291) [74]. Similarly to the previous clinical trials, the most common side-effect in patients treated with filorexant was day somnolence. Other clinical trials are also currently under development to evaluate the possible efficacy of filorexant for the treatment of neuropathic pain (NCT01564459) and major depressive disorder (NCT01554176).

The involvement of the orexin system in the physiopathological control of fear and anxiety together with the preclinical efficacy reported for orexin antagonists in animal models of anxiety, panic disorder and PTSD (see previous sections) open now the possibility to design novel clinical trials to evaluate the effectiveness of orexin antagonists for anxiety treatment. It is important to underline that the doses required to treat anxiety are usually lower than those needed to treat insomnia, at least when using benzodiazepine ligands [75]. This could represent an important advantage in minimizing the dose-dependent side effects that appear when using orexin antagonists for insomnia treatment. Therefore, these possible clinical trials for anxiety treatment should be designed using an appropriate dose of the orexin antagonists and should predominantly include patients suffering anxiety linked to aversive memories, such as phobias, panic disorder and PTSD since the orexin system seems to be most directly related to these kind of anxiety disorders. In addition, the preclinical data support a predominant involvement of OX1R in anxiety disorders, which opens the possibility to

include more selective compounds in these clinical trials in order to minimize the incidence of adverse effects. Indeed, the blockade of OX2R seems to be sufficient to induce sleep, whereas OX1R antagonists are largely devoid of this side effect (Box 1) [76]. In agreement, new selective OX1R antagonists recently characterized such as [N-((3-[(3-ethoxy-6-methylpyridin-2-yl)carbonyl]-3-azabicyclo[4.1.0]hept-4-yl)methyl)-5-(trifluoromethyl)pyrimidin-2-amine] (compound 56) reduces anxiety-associated behavioral and physiological responses without hypnotic effects [77]. Therefore the use of OX1R antagonists in clinical trials for anxiety disorders could minimize the incidence of somnolence and impairment to drive or operate heavy machinery that constitute the most common side effects with previous dual orexin antagonists (see: <http://www.fda.gov/downloads/.../UCM354215.pdf>).

Concluding remarks

The orexin system is essential in maintaining wakefulness and arousal under non-stressful conditions, and it is responsible for mobilizing an adaptive response to stress challenges posed by anxiety associated behavior and autonomic responses. Emerging evidence indicates that dysregulation of the orexin system contributes to pathologies associated with generalized anxiety and/or impaired fear processing, such as phobias, panic disorder and PTSD. OX1R seems predominantly involved in these pathophysiological conditions. Preclinical data have revealed that OX1R blockade alters fear memory expression and extinction processes, and reduces panic behavioral and cardiorespiratory responses in panic-prone subjects. Consistent with these preclinical reports, human studies have shown an association between increased activity of the orexin system and acute anxiety states. Several clinical trials have validated the efficacy and safety of some dual OXR antagonists mainly in the treatment of insomnia.

Although the effectiveness of OXR antagonists for anxiety treatment has not been evaluated in humans yet (Box 2), their preclinical efficacy in animal models of anxiety suggests the possibility to design novel clinical trials to study these pathologies. These clinical trials could open novel therapeutic perspectives for this promising target in the central nervous system that has been demonstrated to be devoid of major side effects when an appropriate dose is used.

Acknowledgments

This work was supported by the Instituto de Salud Carlos III grants, #PI13/00042 and #RD12/0028/0023 (RTA-RETICS), by the Spanish Ministry of Science #SAF2011-29864 and #SAF2014-59648-P, National Plan on Drugs #2014I019, and the Catalan Government (SGR2014-1547). Important contributions from several authors could not be included due to space limitations. We thank Dr Miquel Angel Serra for invaluable assistance in the documentation of the clinical trials with orexin antagonists.

Glossary

Fear-potentiated startle: relative increase in the amplitude of an acoustic startle reflex when elicited in the presence of a neutral conditioned stimulus (CS) (e.g., a light) previously paired with an aversive stimulus. Clinically effective anxiolytic drugs decrease or block startle potentiation.

Generalized avoidance: maladaptive behavioral response consisting in over-interpretation of contextual cues, leading to generalized fear and to exaggerated avoidance of situations that resemble only slightly the context of the initial fearful event.

Hypercapnia: abnormally elevated levels of carbon dioxide (CO₂) in the blood. It is produced by accumulation due to deficient alveolar ventilation. If not restored, this condition leads to HCO₃⁻/CO₂ imbalance and subsequent acidosis (decreased blood pH).

Immediate-early gene (IEG): class of genes which respond transiently and rapidly to a wide variety of extracellular stimuli such as growth factors and neurotransmitters. These genes usually encode transcription factors that regulate the expression of downstream target genes. The IEGs most commonly used as tools for neuronal activity mapping are *c-fos*, *zif268* and *arc*.

Limbic system: set of functionally and anatomically interconnected brain structures, including, among others, the hypothalamus, the hippocampus, the amygdala, the nucleus accumbens and the cingulate gyrus. They regulate autonomic and endocrine function and set the level of arousal, particularly in response to emotional stimuli. They are also involved in motivation, reward and memory.

Panic disorder: anxiety disorders characterized by sudden and repeated attacks of fear that last for several minutes or longer, which are called panic attacks. During these

episodes, the fear response is out of proportion for the situation, which often is not threatening, and physical symptoms may appear, such as sweating and tachycardia.

Pavlovian fear conditioning: form of learning in which a neutral stimulus (designated the conditional stimulus or CS) is paired with an unconditional stimulus or US, which is biologically significant (in this case aversive, e.g. an electrical shock), conveying in the association of both stimuli. This will result in the expression of fear responses to the originally neutral stimulus or context.

Phobia: anxiety disorder characterized by persistent overwhelming and irrational fear of an object or situation that poses little real danger but provokes anxiety and avoidance. It is long lasting and causes intense physical and psychological reactions, which interfere in social or occupational activities.

PTSD: anxiety disorder that can develop after experiencing or witnessing a traumatic or terrifying event. It is diagnosed when a group of symptoms, such as disturbing recurring flashbacks, avoidance or numbing of memories of the event, and hyperarousal, persist for more than one month.

Box 1. Main physiological functions of the orexin system

Arousal/wakefulness: Orexin neurons project to regions implicated in the regulation of wakefulness such as the tuberomammillary nucleus and the locus coeruleus [4]. Indeed, orexins are strongly implicated in the pathogenesis of narcolepsy, a chronic neurological disorder characterized by sudden, brief episodes of sleep that interrupt normal waking. Genetic ablation of prepro-orexin or OX2R in rodents and dogs has been found to mimic human narcolepsy phenotype [78,79]. Thus, OX2R seems to be more important than OX1R in the regulation of sleep/wake cycle. Activation of orexin neurons increases the probability of transition from sleep to wakefulness [80], suggesting that

orexin ligands could be a potential treatment for sleep-related disorders. In this regard, suvorexant, a dual OXR antagonist, has been recently approved by FDA for the treatment of insomnia [70,71].

Energy homeostasis: Balance between food intake and energy expenditure can be maintained through orexin transmission [6]. Intracerebroventricular infusion of orexin A and B increases food intake [1] whereas OX1R antagonism reduces food consumption [81]. Orexin neurons in the LH seem to be sensitive to humoral and neural signals related to energy balance. Thus, ghrelin, an orexigenic peptide released by the stomach, activates orexin neurons whereas leptin, a hormone from adipose tissue reducing food intake, inhibits orexin cells [82]. The orexin system regulates food intake through the modulation of arousal, and directly acting on neurons that are involved in the control of feeding behavior.

Reward processing: There is strong evidence supporting a role for orexins in the reinforcing properties of different drugs of abuse [83]. The ventral tegmental area dopamine neurons might be a crucial site of action for orexins to mediate these effects [84]. Accordingly, direct injections of orexin A in the ventral tegmental area increase dopamine levels in the nucleus accumbens [85,86]. Although most of the research about the involvement of orexins in drug addiction has focused on elucidating the function of OX1R [83,87], recent studies point to a role for OX2R in reward regulation. Thus, antagonism of OX2R reduces heroin [88] and ethanol-self administration [89], as well as cue-induced reinstatement of nicotine-seeking behavior [90].

Stress: Orexinergic neurons mainly located in the PFA/DMH are activated by different stressors including immobilization, footshock, cold exposure and food deprivation [26,91]. Consistent with this, intracerebroventricular administration of orexin A induces anxiety-like effects in several behavioral models of anxiety [92]. The existence of

reciprocal interactions between orexin and CRF neurons [93] suggests that the orexin system is an important component of the pathways contributing to the physiological CRF-mediated behaviors that occur in response to stressful situations [94]. Dysregulation of stress responses could lead to the development of different anxiety disorders which might be influenced by the activity of the orexin system, as described in the present review.

Box 2. Outstanding questions

Anatomical targets of the orexin modulation of the fear neural circuitry. Preclinical studies have shown that orexins modulate fear memory by direct actions in the locus coeruleus [33,36] and the AMY [38]. Orexin transmission in the bed nucleus of the stria terminalis is crucial for panic expression. However, orexin projections are abundant also in other brain regions involved in fear processing and anxiety, such as the thalamic and hypothalamic paraventricular nuclei. Are these or other unexplored regions recruited by the orexin system during fear expression and extinction?

Molecular mechanisms involved in the modulation of fear memory processing by orexins. Some of the main downstream signaling pathways activated upon OXR stimulation, such as PKC and MAPK cascade, have been related to fear expression and/or extinction processes [95,96]. However, it is unknown at the present moment whether these or other molecular mechanisms are mediating the modulation of fear by the orexin system.

Heterogeneity of the anxiety clinical spectrum. Anxiety disorders include a wide range of heterogeneous clinical conditions showing different specific symptomatological profiles, probably reflecting multiple pathogenic mechanisms. Comorbidity in human subjects and limited existence of high predictive animal models

hinder the specific role of orexins in the pathophysiology of each anxiety disorder, an issue that should be considered in future research.

Effectiveness of OXR antagonism in humans. So far, the potential modulation of fear by OXR antagonists has not been evaluated in humans. Moreover, no clinical trials have tested the therapeutic potential of selective OX1R antagonists in human subjects. However, promising data in terms of safety and effectiveness from clinical trials evaluating dual OXR antagonists for the treatment of insomnia highlight the need for designing novel clinical trials to evaluate the efficacy of these compounds in anxiety disorders.

Genetic and epigenetic vulnerability to fear and anxiety. Panic disorder has been associated with the Val308Iso polymorphism in the OX2R gene [69]. In addition, epigenetic mechanisms regulate the formation and extinction of fear memories [97]. Might specific polymorphisms in genes of the orexin system or epigenetic profiles triggered upon OXR stimulation be critical for the development of anxiety disorders or impaired fear processing states?

References

- 1 Sakurai, T. *et al.* (1998) Orexins and Orexin Receptors: A Family of Hypothalamic Neuropeptides and G Protein-Coupled Receptors that Regulate Feeding Behavior. *Cell* 92, 573–585
- 2 De Lecea, L. *et al.* (1998) The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci.* 95, 322–327
- 3 Kukkonen, J.P. and Leonard, C.S. (2014) Orexin/hypocretin receptor signalling cascades. *Br. J. Pharmacol.* 171, 314–331
- 4 Peyron, C. *et al.* (1998) Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.* 18, 9996–10015
- 5 Marcus, J.N. *et al.* (2001) Differential expression of orexin receptors 1 and 2 in the rat brain. *J. Comp. Neurol.* 435, 6–25
- 6 Li, J. *et al.* (2014) The hypocretins/orexins: Integrators of multiple physiological functions. *Br. J. Pharmacol.* 171, 332–350
- 7 Mahler, S. V *et al.* (2014) Motivational activation: a unifying hypothesis of orexin/hypocretin function. *Nat. Neurosci.* 17, 1298–1303
- 8 Chen, Q. *et al.* (2015) The hypocretin/orexin system: an increasingly important role in neuropsychiatry. *Med. Res. Rev.* 35, 152–197
- 9 Sakurai, T. (2014) The role of orexin in motivated behaviours. *Nat. Rev. Neurosci.* 15, 719–731
- 10 Quirk, G.J. and Mueller, D. (2008) Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology* 33, 56–72
- 11 Maroun, M. (2013) Medial prefrontal cortex: multiple roles in fear and extinction. *Neuroscientist* 19, 370–83
- 12 Maren, S. *et al.* (2013) The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat. Rev. Neurosci.* 14, 417–428
- 13 Haufler, D. *et al.* (2013) Neuronal correlates of fear conditioning in the bed nucleus of the stria terminalis. *Learn. Mem.* 20, 633–641
- 14 Ishida, Y. *et al.* (2002) Conditioned-fear stress increases Fos expression in monoaminergic and GABAergic neurons of the locus coeruleus and dorsal raphe nuclei. *Synapse* 45, 46–51
- 15 Penzo, M.A. *et al.* (2015) The paraventricular thalamus controls a central amygdala fear circuit. *Nature* 519, 455–459

- 16 Johansen, J.P. *et al.* (2010) Neural substrates for expectation-modulated fear learning in the amygdala and periaqueductal gray. *Nat. Neurosci.* 13, 979–986
- 17 Herry, C. *et al.* (2008) Switching on and off fear by distinct neuronal circuits. *Nature* 454, 600–606
- 18 LeDoux, J.E. (2000) Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184
- 19 Orsini, C.A. and Maren, S. (2012) Neural and cellular mechanisms of fear and extinction memory formation. *Neurosci. Biobehav. Rev.* 36, 1773–1802
- 20 Rudy, J.W. (2009) Context representations, context functions, and the parahippocampal-hippocampal system. *Learn. Mem.* 16, 573–585
- 21 Sierra-Mercado, D. *et al.* (2011) Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology* 36, 529–538
- 22 Courtin, J. *et al.* (2014) Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. *Nature* 505, 92–96
- 23 Royer, S. *et al.* (1999) An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. *J. Neurosci.* 19, 10575–10583
- 24 Royer, S. and Paré, D. (2002) Bidirectional synaptic plasticity in intercalated amygdala neurons and the extinction of conditioned fear responses. *Neuroscience* 115, 455–462
- 25 Pitkänen, A. *et al.* (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Ann. N. Y. Acad. Sci.* 911, 369–391
- 26 Johnson, P.L. *et al.* (2012) Orexin, stress, and anxiety/panic states. *Prog. Brain Res.* 198, 133–161
- 27 Sakurai, T. *et al.* (2005) Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* 46, 297–308
- 28 Yoshida, K. *et al.* (2006) Afferents to the orexin neurons of the rat brain. *J. Comp. Neurol.* 494, 845–861
- 29 Peyron, C. *et al.* (2000) A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat. Med.* 6, 991–997
- 30 Ponz, A. *et al.* (2010) Reduced amygdala activity during aversive conditioning in human narcolepsy. *Ann. Neurol.* 67, 394–398

- 31 Khatami, R. *et al.* (2007) Amygdala dysfunction in narcolepsy-cataplexy. *J. Sleep Res.* 16, 226–229
- 32 Blouin, A.M. *et al.* (2013) Human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction. *Nat. Commun.* 4, 1547
- 33 Soya, S. *et al.* (2013) Orexin receptor-1 in the locus coeruleus plays an important role in cue-dependent fear memory consolidation. *J. Neurosci.* 33, 14549–14557
- 34 Steiner, M.A. *et al.* (2012) The brain orexin system and almorexant in fear-conditioned startle reactions in the rat. *Psychopharmacology (Berl)*. 223, 465–475
- 35 Chen, X. *et al.* (2014) Orexins (hypocretins) contribute to fear and avoidance in rats exposed to a single episode of footshocks. *Brain Struct. Funct.* 219, 2103–2118
- 36 Sears, R.M. *et al.* (2013) Orexin/hypocretin system modulates amygdala-dependent threat learning through the locus coeruleus. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20260–20265
- 37 Vaiva, G. *et al.* (2003) Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. *Biol. Psychiatry* 54, 947–949
- 38 Flores, A. *et al.* (2014) The Hypocretin/Orexin System Mediates the Extinction of Fear Memories. *Neuropsychopharmacology* 39, 2732–2741
- 39 Camp, M.C. *et al.* (2012) Genetic strain differences in learned fear inhibition associated with variation in neuroendocrine, autonomic, and amygdala dendritic phenotypes. *Neuropsychopharmacology* 37, 1534–1547
- 40 Viviani, D. *et al.* (2015) Orexin neuropeptides contribute to the development and persistence of generalized avoidance behavior in the rat. *Psychopharmacology (Berl)*. 232, 1383–1393
- 41 Van der Velden, P.G. *et al.* (2006) The independent predictive value of peritraumatic dissociation for postdisaster intrusions, avoidance reactions, and PTSD symptom severity: a 4-year prospective study. *J. Trauma. Stress* 19, 493–506
- 42 Herry, C. and Mons, N. (2004) Resistance to extinction is associated with impaired immediate early gene induction in medial prefrontal cortex and amygdala. *Eur. J. Neurosci.* 20, 781–790
- 43 Hefner, K. *et al.* (2008) Impaired fear extinction learning and cortico-amygdala circuit abnormalities in a common genetic mouse strain. *J. Neurosci.* 28, 8074–8085

- 44 Bilkei-Gorzo, A. *et al.* (2012) Dynorphins regulate fear memory: from mice to men. *J. Neurosci.* 32, 9335–9343
- 45 Holmes, A. and Singewald, N. (2013) Individual differences in recovery from traumatic fear. *Trends Neurosci.* 36, 23–31
- 46 Vertes, R.P. and Hoover, W.B. (2008) Projections of the paraventricular and paratenial nuclei of the dorsal midline thalamus in the rat. *J. Comp. Neurol.* 508, 212–237
- 47 Padilla-Coreano, N. *et al.* (2012) A time-dependent role of midline thalamic nuclei in the retrieval of fear memory. *Neuropharmacology* 62, 457–463
- 48 Li, Y. *et al.* (2014) Lesions of the posterior paraventricular nucleus of the thalamus attenuate fear expression. *Front. Behav. Neurosci.* 8, 94
- 49 Do-Monte, F.H. *et al.* (2015) A temporal shift in the circuits mediating retrieval of fear memory. *Nature* 519, 460–463
- 50 Kirouac, G.J. *et al.* (2005) Orexin (hypocretin) innervation of the paraventricular nucleus of the thalamus. *Brain Res.* 1059, 179–188
- 51 Li, Y. *et al.* (2010) Orexins in the paraventricular nucleus of the thalamus mediate anxiety-like responses in rats. *Psychopharmacology (Berl)*. 212, 251–265
- 52 Heydendaal, W. *et al.* (2014) Optogenetic examination identifies a context-specific role for orexins/hypocretins in anxiety-related behaviour. *Physiol. Behav.* 130, 182–190
- 53 Wittchen, H.U. *et al.* (2011) The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur. Neuropsychopharmacol.* 21, 655–679
- 54 Kessler, R.C. *et al.* (2012) Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int. J. Methods Psychiatr. Res.* 21, 169–184
- 55 Graham, B.M. and Milad, M.R. (2011) The study of fear extinction: implications for anxiety disorders. *Am. J. Psychiatry* 168, 1255–1265
- 56 Hofmann, S.G. *et al.* (2009) Is it Beneficial to Add Pharmacotherapy to Cognitive-Behavioral Therapy when Treating Anxiety Disorders? A Meta-Analytic Review. *Int. J. Cogn. Ther.* 2, 160–175
- 57 Johnson, P.L. *et al.* (2010) A key role for orexin in panic anxiety. *Nat. Med.* 16, 111–115
- 58 Salomon, R.M. *et al.* (2003) Diurnal variation of cerebrospinal fluid hypocretin-1 (Orexin-A) levels in control and depressed subjects. *Biol. Psychiatry* 54, 96–104

- 59 Strawn, J.R. *et al.* (2010) Low cerebrospinal fluid and plasma orexin-A (hypocretin-1) concentrations in combat-related posttraumatic stress disorder. *Psychoneuroendocrinology* 35, 1001–1007
- 60 Johnson, P.L. *et al.* (2008) Neural pathways underlying lactate-induced panic. *Neuropsychopharmacology* 33, 2093–2107
- 61 Kayaba, Y. *et al.* (2003) Attenuated defense response and low basal blood pressure in orexin knockout mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R581–593
- 62 Johnson, P.L. and Shekhar, A. (2012) An animal model of panic vulnerability with chronic disinhibition of the dorsomedial/perifornical hypothalamus. *Physiol. Behav.* 107, 686–698
- 63 Johnson, P.L. and Shekhar, A. (2006) Panic-prone state induced in rats with GABA dysfunction in the dorsomedial hypothalamus is mediated by NMDA receptors. *J. Neurosci.* 26, 7093–7104
- 64 Esquivel, G. *et al.* (2009) Review: Acids in the brain: a factor in panic? *J. Psychopharmacol.* 24, 639–647
- 65 Nattie, E. and Li, A. (2012) Respiration and autonomic regulation and orexin. *Prog. Brain Res.* 198, 25–46
- 66 Zhu, L.-Y. *et al.* (2011) Plasma orexin-a levels in COPD patients with hypercapnic respiratory failure. *Mediators Inflamm.* 2011, 754847
- 67 Liu, Z.-B. *et al.* (2010) Orexin-A and respiration in a rat model of smoke-induced chronic obstructive pulmonary disease. *Clin. Exp. Pharmacol. Physiol.* 37, 963–968
- 68 Johnson, P.L. *et al.* (2012) Activation of the orexin 1 receptor is a critical component of CO₂-mediated anxiety and hypertension but not bradycardia. *Neuropsychopharmacology* 37, 1911–1922
- 69 Annerbrink, K. *et al.* (2011) Panic disorder is associated with the Val308Iso polymorphism in the hypocretin receptor gene. *Psychiatr. Genet.* 21, 85–89
- 70 Herring, W.J. *et al.* (2014) Suvorexant in Patients with Insomnia: Results from Two 3-Month Randomized Controlled Clinical Trials. *Biol. Psychiatry* DOI: 10.1016/j.biopsych.2014.10.003
- 71 Michelson, D. *et al.* (2014) Safety and efficacy of suvorexant during 1-year treatment of insomnia with subsequent abrupt treatment discontinuation: a phase 3 randomised, double-blind, placebo-controlled trial. *Lancet. Neurol.* 13, 461–471

- 72 Hoch, M. *et al.* (2014) Entry-into-humans study with ACT-462206, a novel dual orexin receptor antagonist, comparing its pharmacodynamics with almorexant. *J. Clin. Pharmacol.* 54, 979–986
- 73 Bettica, P. *et al.* (2012) The orexin antagonist SB-649868 promotes and maintains sleep in men with primary insomnia. *Sleep* 35, 1097–1104
- 74 Chabi, A. *et al.* (2015) Randomized controlled trial of the orexin receptor antagonist filorexant for migraine prophylaxis. *Cephalalgia* 35, 379–388
- 75 Brunton, L.L. *et al.* (2011) *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, (12th edn) The McGraw-Hill Companies, Inc.
- 76 Hoyer, D. and Jacobson, L.H. (2013) Orexin in sleep, addiction and more: is the perfect insomnia drug at hand? *Neuropeptides* 47, 477–488
- 77 Bonaventure, P. *et al.* (2015) A selective orexin-1 receptor antagonist attenuates stress-induced hyperarousal without hypnotic effects. *J. Pharmacol. Exp. Ther.* 352, 590–601
- 78 Chemelli, R.M. *et al.* (1999) Narcolepsy in orexin Knockout MiceMolecular Genetics of Sleep Regulation. *Cell* 98, 437–451
- 79 Lin, L. *et al.* (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98, 365–376
- 80 Adamantidis, A.R. *et al.* (2007) Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 450, 420–424
- 81 Haynes, A.C. *et al.* (2000) A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul. Pept.* 96, 45–51
- 82 Yamanaka, A. *et al.* (2003) Hypothalamic Orexin Neurons Regulate Arousal According to Energy Balance in Mice. *Neuron* 38, 701–713
- 83 Plaza-Zabala, A. *et al.* (2012) The hypocretin/orexin system: Implications for drug reward and relapse. *Mol. Neurobiol.* 45, 424–439
- 84 Aston-Jones, G. *et al.* (2009) Role of lateral hypothalamic orexin neurons in reward processing and addiction. *Neuropharmacology* 56 Suppl 1, 112–121
- 85 Narita, M. *et al.* (2006) Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J. Neurosci.* 26, 398–405
- 86 España, R.A. *et al.* (2011) Hypocretin 1/orexin A in the ventral tegmental area enhances dopamine responses to cocaine and promotes cocaine self-administration. *Psychopharmacology (Berl)*. 214, 415–426

- 87 Khoo, S.Y.-S. and Brown, R.M. (2014) Orexin/hypocretin based pharmacotherapies for the treatment of addiction: DORA or SORA? *CNS Drugs* 28, 713–730
- 88 Schmeichel, B.E. *et al.* (2015) Hypocretin Receptor 2 Antagonism Dose-Dependently Reduces Escalated Heroin Self-Administration in Rats. *Neuropsychopharmacology* 40, 1123–1129
- 89 Brown, R.M. *et al.* (2013) Central orexin (hypocretin) 2 receptor antagonism reduces ethanol self-administration, but not cue-conditioned ethanol-seeking, in ethanol-preferring rats. *Int. J. Neuropsychopharmacol.* 16, 2067–2079
- 90 Uslaner, J.M. *et al.* (2014) Selective orexin 2 receptor antagonism blocks cue-induced reinstatement, but not nicotine self-administration or nicotine-induced reinstatement. *Behav. Brain Res.* 269, 61–65
- 91 Berridge, C.W. *et al.* (2010) Hypocretin/orexin in arousal and stress. *Brain Res.* 1314, 91–102
- 92 Suzuki, M. *et al.* (2005) Orexin-A (hypocretin-1) is possibly involved in generation of anxiety-like behavior. *Brain Res.* 1044, 116–121
- 93 Winsky-Sommerer, R. (2004) Interaction between the Corticotropin-Releasing Factor System and Hypocretins (Orexins): A Novel Circuit Mediating Stress Response. *J. Neurosci.* 24, 11439–11448
- 94 Giardino, W.J. and de Lecea, L. (2014) Hypocretin (orexin) neuromodulation of stress and reward pathways. *Curr. Opin. Neurobiol.* 29, 103–108
- 95 Tronson, N.C. *et al.* (2008) Regulatory mechanisms of fear extinction and depression-like behavior. *Neuropsychopharmacology* 33, 1570–1583
- 96 Cestari, V. *et al.* (2014) The MAP(K) of fear: From memory consolidation to memory extinction. *Brain Res. Bull.* 105, 8–16
- 97 Kwapis, J.L. and Wood, M.A. (2014) Epigenetic mechanisms in fear conditioning: implications for treating post-traumatic stress disorder. *Trends Neurosci.* 37, 706–720
- 98 Flores, A. *et al.* (2013) Cannabinoid-hypocretin cross-talk in the central nervous system: what we know so far. *Front. Neurosci.* 7, 256
- 99 Hoeber, P. *et al.* (2012) Orexin receptor antagonism, a new sleep-enabling paradigm: a proof-of-concept clinical trial. *Clin. Pharmacol. Ther.* 91, 975–985
- 100 Cox, C.D. *et al.* (2010) Discovery of the dual orexin receptor antagonist [(7R)-4-(5-chloro-1,3-benzoxazol-2-yl)-7-methyl-1,4-diazepan-1-yl][5-methyl-2-(2H-1,2,3-triazol-2-yl)phenyl]methanone (MK-4305) for the treatment of insomnia. *J. Med. Chem.* 53, 5320–5332

Legends of the figures

Figure 1. Schematic representation of the main orexin projections to brain areas related to motivated and emotional behaviors. OX1R and OX2R expression in these brain regions is also described. Effects of orexin peptides in emotional memories could be reflected by their innervations of the fear circuit composed of the hippocampus (HPC), medial prefrontal cortex (mPFC), and amygdala (AMY). Orexin terminals are also present in the bed of the stria terminalis (BNST), paraventricular thalamus (PVT), and septum which are critical for anxiety-related responses. The paraventricular nucleus of the hypothalamus (PVN) regulates stress responses and the hypothalamic-pituitary-adrenal axis hormone cascade. Projections to the ventral tegmental area (VTA) and nucleus accumbens (NAc) are related with the known role of orexins in the modulation of the rewarding properties of drugs of abuse. The locus coeruleus (LC) also has dense orexin innervations in concordance with its involvement in arousal and emotional memory. LH, lateral hypothalamus. Adapted with permission from [98]

Figure 2. Basis of fear conditioning and extinction in rodents. In this paradigm a particularly neutral conditioned stimulus (CS), usually a chamber in contextual fear conditioning (upper diagram) or a tone in cued fear conditioning (lower diagram), is presented together with an aversive unconditioned stimulus (US), typically an electrical footshock. As a consequence of this US-CS association, a new exposure to the CS in absence of the US evokes an evaluable conditioned response: the freezing behavior, a natural response in rodents experiencing fear. Repeated exposure to the CS in absence of the US progressively leads to fear extinction, i.e. the decrease of the freezing response weakening the US-CS association.

Figure 3. Circuitry of fear expression and extinction and its modulation by the orexin system. (A) Fear expression and extinction processes involve a neural circuit that includes both infralimbic (IL) and prelimbic (PL) prefrontal cortices, the hippocampus (HPC), and the amygdala. The critical components of the amygdala for fear conditioning are the lateral nucleus (LA), the basal nucleus (BA), intercalated (ITC) cells and the central nucleus (CeA). During fear expression (left diagram) thalamic and cortical inputs conveying information about the CS and the US arrive first at the LA, which excites the CeA through stimulation of a particular neuronal subpopulation in the BA known as “fear neurons”. Fear responses are elicited via successive projections to brainstem and hypothalamic sites. The BA receives also excitatory input from PL cortex, thereby promoting the expression of conditioned fear. In contrast, during fear extinction (right diagram) the IL cortex excites the GABAergic inhibitory neurons located in the ITC masses, either directly or through activation of the “extinction neurons” subpopulation in the BA. ITC neurons inhibit the CeA, thereby inhibiting conditioned fear and promoting extinction. In addition, the HPC modulates both PL and IL as well as amygdalar activity, integrating contextual information processing. (B) Extinction of conditioned fear is associated with increased activity of the cortico-amygdala circuit while impaired fear extinction is related to hypoactivity in relevant brain regions of this circuit [45]. Consistent with this, the number of neurons expressing the immediately-early gene c-Fos in the IL (upper panel) and the BLA (lower panel) in mice subjected to fear conditioning and undergoing extinction is higher than in animals exposed to the context without receiving footshock. OX1R blockade with the specific antagonist SB334867 (5 mg/kg, i.p.) during the extinction process enhances c-Fos expression in both the IL and the BLA, correlating with the extinction-facilitating effects of this OX1R antagonist. Data are expressed as mean $\pm$ SEM. ** $p < 0.01$

compared with the vehicle group; #p<0.05, ##p<0.01 compared with the control (no shock) group. Part B is adapted with permission from [38].

Table 1. Partial list of main clinical trials based on dual orexin receptor antagonists

Compound	Indication	Clinical Phase	Company	Reference
Almorexant (ACT-078573)	Insomnia; Primary Insomnia	1	Actelion	[99] Clinical trial ID: NCT00640848
Suvorexant (MK-4305)	Primary Insomnia	3	Merck Sharp & Dohme Corp.	[63] Clinical trial ID: NCT01097616, NCT01097629
	Insomnia	3	Merck Sharp & Dohme Corp.	[64,100] Clinical trial ID: NCT01021813
ACT-462206	Insomnia	1	Actelion	[65] Clinical trial ID: NCT01954589
SB-649868 or GW-649868	Sleep Initiation and Maintenance Disorders	2	GlaxoSmithKline	[66] Clinical trial ID: NCT00426816
Filorexant (MK-6096)	Primary Insomnia	2	Merck Sharp & Dohme Corp.	Clinical trial ID: NCT01021852
	Migraine; Headache	2	Merck Sharp & Dohme Corp.	[67] Clinical trial ID: NCT01513291
	Diabetic Neuropathy, Painful	2	Merck Sharp & Dohme Corp.	Clinical trial ID: NCT01564459
	Major Depressive Disorder, Recurrent	2	Merck Sharp & Dohme Corp.	Clinical trial ID: NCT01554176