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The Orexin/Hypocretin system and its role in the etiology of psychiatric deficits following Traumatic Brain Injury

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Abstract

Traumatic brain injury can cause long-term cognitive impairments and psychological symptoms such as anxiety, depression, as well as sleep disturbances. The orexin/hypocretin system, which controls feeding, sleep, mood, and reward functions, is affected as a result of TBI. This disruption can decrease the density of orexin neurons and dysregulate orexin receptor expression. Abnormal orexin levels and receptor expression may lead to psychiatric symptoms commonly experienced after TBI, such as sleep disorders including insomnia or excessive daytime sleepiness, and mood disorders such as major depressive disorder or anxiety. By understanding how TBI changes the orexin system, we may understand the connection between brain injury and the onset of sleep disorders, depression, and anxiety. Therapeutic strategies targeting the orexin system show promise for mitigating post-TBI psychiatric symptoms. Current treatments, such as orexin receptor antagonists, are being tested for their effectiveness in addressing sleep disorders after TBI. However, research is needed to progress our current understanding to develop targeted treatments that could improve outcomes for TBI patients. It may be advantageous to construct an algorithm that provides information on gender, along with biomarkers for the HPA axis (ACTH, cortisol, DHEA-S), inflammation and autoimmune disease (interleukins, ESR, CRP), blood glucose (diabetes), and genetic variant determination of ADH1B and ALDH2 (susceptibility to alcohol abuse) in order to determine how aggressive the post TBI orexin modulating therapy needs to be for that patient to achieve a favorable outcome. Such clinical algorithms, meta-analyses and more empirical studies are needed to progress our current understanding to develop patient-specific targeted treatments that could improve outcomes for TBI patients.

Keywords

Mood disorders, Major Depressive Disorder, Sleep-wake disorders, Anxiety, Excessive daytime sleepiness, Orexin, Hypocretin, Traumatic Brain Injury

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1 Introduction

1.1 Traumatic Brain Injury

A Traumatic Brain Injury (TBI) is one that is characterized as damage to the brain brought on by an external force (1). TBI is a significant problem in the United States, considering that it accounts for 2.5 million hospitalizations and deaths as estimated by the Centers for Disease Control and Prevention (2). A widely used TBI severity classification system is the Glasgow Coma Scale, a clinical tool for evaluating severity by an examination of the level of consciousness a patient exhibits (3). Moderate to severe TBI can cause various disruptions in the normal functioning of the brain, including alterations in the production of neuropeptides like orexin (4). These neuropeptides are pivotal to homeostatic functions such as feeding, sleep, and reward processing. The long-term effects of TBI on orexin/hypocretin (hereafter, orexin) function and its downstream effects on cognition are not clearly understood, despite studies showing that 24-65% of post-TBI patients have cognitive deficits after injury (5,6). Due to the prevalence of these deficits, discussing the orexin system as a potential target for addressing some of the observed symptoms is relevant. The aim of this review is to provide evidence for the role of the orexin system in post-TBI psychiatric deficits and avenues for future research and clinical therapeutics.

1.2 Orexin/Hypocretin

Orexin/Hypocretin are peptides produced by specific neurons found in the lateral hypothalamus. Orexins A (OXA) and B (OXB) are both produced from prepro-orexin. OXA and OXB are also known as hypocretin-1 and-2, respectively (13). Orexin receptor 1 (OXR1) and orexin receptor 2 (OXR2) are the receptors for these neuropeptides. Compared to OXR2, the OXR1 receptor has a 100–1000-fold greater affinity for orexin A, yet both orexin A and B have similar affinity for the OXR2 receptor (14). It is believed that the orexins function mainly as excitatory neurotransmitters (15,16). Orexin-containing neurons are concentrated within the hypothalamus but occur widely throughout the brain, including in the limbic system (notably the hippocampus and amygdala), the cerebral cortex, and brainstem (locus coeruleus and raphe nuclei) (17). They may have an excitatory effect on the neurotransmission of dopamine, histamine, serotonin, and acetylcholine, as well as a mediating function in the neurotransmission of glutamate and Gamma-AminoButyric Acid (GABA) (18, 19). Moreover, excitatory or inhibitory neurotransmitters can be released onto orexin neurons by afferent neurons to modify the activity of the former. It has been found that a number of brain regions, including the accumbens nucleus shell, bed nucleus of stria terminalis, infralimbic and prelimbic cortex, ventromedial hypothalamus, dorsomedial hypothalamus, and serotonergic neurons in the raphe nuclei, are known to directly connect to orexin neurons (20). Orexin neurons activate nuclei that are thought to promote wakefulness, such as basal forebrain cholinergic neurons (21,22), raphe serotonergic neurons (23,24), TMN histaminergic neurons (25), and pedunculopontine tegmentum /laterodorsal tegmental neurons (21,22). Dysregulation of the orexin neurotransmitter pathway has been linked to narcolepsy. Most narcoleptic patients have orexin levels that are

low or undetectable (26). Therefore, it is now known that a decrease in orexin levels may lead to sleep/arousal disorders. Given the established link between orexin and narcolepsy (26), cases of narcolepsy associated with the Pandemrix® vaccine have been linked to a loss of orexin (66). A study investigating this association vaccinated mice during prepubescence and then with a second injection of a booster during peri-adolescence. A 60% decrease in orexin was observed in comparison to the controls at the 21 day mark following the booster, thus suggesting that the Pandemrix® vaccine may modulate orexin expression during adolescence (66). A mechanism for the subsequent loss of orexin post Pandemrix® vaccination and the resulting narcolepsy was suggested to involve H1N1-specific T cells targeting orexin-producing neurons. Components of the vaccination or antigens of the virus were suggested to elicit an immune response that then activated autoreactive cytotoxic T cells; which in turn targeted orexin neurons (68). It was hypothesized that the loss of orexin after the vaccination occured due to an immune response triggered by similarities between the influenza A nucleoprotein in the Pandemrix® vaccine and an extracellular domain of OXR2. However, a subsequent study found no specific antibody or immune responses against orexin receptors in vaccinated individuals (67). The role of orexin is clear in many physiological phenomena, such as in the previously mentioned role in wakefulness. However, its role in psychiatric disorders is less known, especially when the orexin system is dysfunctional.

2 Discussion

2.1 Post Traumatic Brain Injury symptoms

~75% of post-TBI patients receive a psychiatric diagnosis within the first five years (7), making it a frequent occurrence. Psychiatric symptoms can be debilitating for a patient and may lead to worse health outcomes. It is therefore relevant to discuss psychiatric symptoms post-TBI in order to develop strategies for mitigating these negative effects.

Sleep disorders post-TBI, such as Excessive Daytime Sleepiness (EDS), occur in over 40% of patients after TBI (8). Psychiatric disorders such as Major Depressive Disorder (MDD) also have an increased prevalence. Throughout the first year after TBI, 33% of patients fit the criteria for MDD, and 76% of the patients in this subgroup also commonly exhibit comorbid anxiety (10). This may suggest a correlation and/or a carryover of damage that results from TBI to the development of psychiatric disorders. These post-TBI changes that may lead to patients developing MDD therefore also seem to correlate with comorbid anxiety. In one study, out of 559 patients with mild to severe TBI, 53% satisfied the MDD criteria during the first year after sustaining TBI (11). The median duration of depression was four months for individuals who had screened positive for MDD in the first three months, and with individuals who were depressed at the time of the injury excluded, 49% of new instances of MDD occurred. There was an association with anxiety as well, since following a traumatic brain injury, patients with MDD were more likely than those without it to have co-occurring anxiety disorders, the percent being 60% versus 7% respectively (11). This suggests that a potential correlation

between post-TBI changes and the development of MDD that co-occurs with anxiety disorders. Another study including 774 participants post-TBI, found that 56.1% of them were diagnosed with depression 3 months after the injury. Comorbid anxiety was also present in 90.8% of the individuals with depression (12). These studies collectively indicate that TBI may have an effect on the brain's neurobiological systems, which could lead to a higher prevalence of MDD and anxiety compared to those without TBI. A possible reason for this difference may be a disregulated orexin system, which may disrupt mood regulation and cognitive functions, potentially explaining the observed symptoms. Understanding this connection could lead to targeted treatments that could correct TBI associated orexin dysregulation, and that may improve outcomes for TBI patients.

2.2 Psychiatric disorders correlating with orexin dysfunction

As previously stated, orexin has a role in wakefulness. Therefore, disorders that are characterized by abnormally low levels of motivated arousal such as depression can be linked to the orexin system (29-32). Thus, it is important to determine how orexin deficiency may be implicated in the development of psychiatric disorders.

Orexin acts mainly as an excitatory neurotransmitter which regulates the activity of monoaminergic and cholinergic neurons (17-19). As such, a cholinergic–monoaminergic imbalance can be caused by a lack of orexin, and this primarily affects alertness and other bodily systems including mood control (27). Irregularities of the orexin system may cause mood disorders and psychological changes through several pathways since orexin is also engaged in stress reactions by stimulating the Hypothalamus-Pituitary-Adrenal (HPA) axis (27). The role of the orexins and their receptors in the development of mood disorders such as MDD is further reinforced because the substantia nigra and the dopaminergic ventral tegmental nucleus, which are important modulators of emotional activity, connect to neurofibers from orexin-containing neurons (28). Decreased orexin levels of those orexin projection sites were linked to depressive-like symptoms in one study, as it was shown that rats exhibiting symptoms of depression had less orexin in the ventral tegmental area (VTA) in comparison to controls (29). The hippocampus and amygdala, which are also orexin projection sites, were found to have abnormalities in orexin and/or orexin receptor expression which also correlated with depressive behavior (30). One study examined the relationship between orexin and its receptor mRNA distribution in conjunction with a forced swim test (FST) immobility. There was a negative relationship found in the hippocampus between FST immobility and orexin expression. OXA was expressed less in the hippocampus of animals that exhibited significantly more depressed behavior. Additionally, there was a positive correlation between depressive behavior and OXR1 in the amygdala (30). These results supported the hypothesis that orexin was associated with psychiatric deficits, in particular, with depression. Reduced levels of orexin were also observed in the post-mortem assessment of CSF hypocretin in depressed individuals who

committed suicide, which further linked depression to a loss of orexin (32). Analysis performed on CSF OXA indicated that those with MDD showed significantly decreased CSF-Orexin levels compared to other suicidal patients. Severe MDD accompanied by suicidality may be the end stage of a hypothalamic disorder that starts with hyperactivity of the HPA axis and progresses to hypoactivation of certain hypothalamic regions (32). Therefore, the orexin system could play a role in the symptoms of depressive disorders. A coincident upregulation in orexin receptor expression may contribute to the comorbidity of anxiety that occurs alongside MDD. The correlation of OXR1 to anxiety is suggestive because OXR1 antagonist SB334867 reduces anxiety by preventing sodium lactate-induced freezing, a sign of anxiety akin to panic, in the defensive burying test performed on rats prone to panic attacks. It also promoted social contact and field exploration (33), therefore, hyperactivity of OXR1 may potentially be attributed to anxiety symptoms.

Orexin is believed to be primarily a REM sleep inhibitor (69). The activity of orexin is lowest at the time of waking and highest immediately before the start of sleep (70). This coincides with it being a REM sleep inhibitor, since it gradually reduces activity throughout the night as REM sleep increases, which differentiates orexin from a neurotransmitter that promotes wakefulness. These observations are consistent with symptoms of narcolepsy type 1, a disorder with symptoms that implicate the REM/NREM ratio. Nocturnal sleep is often disrupted, not due to increased non-REM sleep but rather due to an elevation in REM sleep (69) that would be expected to occur with the loss of orexin neurons. Moreover, disordered sleep is commonly observed in cases of depression, and can be partly characterized by derepression of REM sleep, shortened REM latency, a prolonged first REM period, and increased REM density (71); this therefore may involve dysfunction of the orexin system.

Orexin is implicated in autoimmune diseases such as type-1 diabetes and multiple sclerosis (72, 73), which raises concerns for the severity of post-TBI psychiatric symptoms in individuals that present with these diseases. A study on streptozotocin-injected rats, in a model for type-1 diabetes, found that hypothalamic orexin mRNA levels were significantly reduced in diabetic rats compared to non-diabetic controls. Furthermore, adrenal OX1 receptor mRNA levels were elevated in diabetic rats, while OXR2 was reduced (72). This suggests a potential link between orexin signaling within the HPA axis and the pathophysiology of type-1 diabetes. As such, the further dysfunction of the orexin system as a consequence of TBI may lead to more significant psychological distress in individuals with type-1 diabetes considering the pre-existing association of disordered orexin signaling and this disease. A study using Experimental Autoimmune Encephalomyelitis (EAE), a rat model of multiple sclerosis, found a reduction in the number of orexin-producing neurons in specific regions of the hypothalamus; these findings suggested that EAE disrupted the balance between orexin production and utilization, with orexin being consumed at a faster rate than it was produced (73). Similarly to what was discussed with type-1 diabetes, post-TBI psychiatric disorders

could be exacerbated by the already occurring disruption of the orexin system potentially seen in multiple sclerosis.

Multiple studies have implicated both orexin hyperactivity (34-36), and the previously mentioned hypoactivity, in the development of depressive symptoms. As proposed by Khairuddin et al. (37), unbalanced levels of orexin in the brains of clinical and preclinical subjects, that are either higher or lower than normal, exhibit depressed behavior. Excessive or decreased expression of orexin, comparable to a hormonal imbalance, may play a role in depressive-like behavior, since neurotransmitters are examples of signaling molecules whose concentrations are modulated within a limited physiological range. However, there is not a presently known normal physiological range of orexin activity in patients with depression or in clinically healthy individuals (37).

The above mentioned studies strongly suggest that the orexin system modulates the pathophysiology of psychiatric disorders, such as depression. Therefore, it is not unreasonable to propose that the development of psychiatric disorders after TBI can be attributed – in part - to a dysfunction of the orexin system.

2.3 Orexin loss/dysfunction post-TBI

Patients with depression may experience symptoms of EDS, as well as cognitive impairment and energy loss. Depression and sleep - wake disorder symptoms have a close association that affects each other's development and prognosis (38). Considering this, a dysfunction in the Orexin system, characterized by either increased Orexin receptor 1 or 2 expression and decreased Orexin levels, might explain the common occurrence of sleep disorders, depression, and anxiety following TBI.

Orexin expression is subject to sexual dimorphism (74). It has been demonstrated that adult female rats exhibit elevated levels of prepro-orexin mRNA in the hypothalamus (76), and proestrus female rats have a greater number of orexin-producing neurons in the lateral hypothalamus than their male counterparts (77). Conversely, the expression of ORX-2 receptor mRNA in the adrenal gland and ORX-1 receptor mRNA in the pituitary gland is significantly higher in male rats than in females (78). Differences in orexin neuron activity between males and females have also been observed in post-TBI mice (75). In female mice, TBI led to an increase in the size of an action potential after hyperpolarization, which was not observed in males. On the other hand, male mice specifically exhibited a reduction in the action potential threshold following TBI (75). These observed differences between sexes both before and after TBI may lead to distinctions in the resulting psychiatric disorders that are associated with the orexin system, such as depression, which could manifest differently in males and females due to sex-specific variations in orexin signaling. For instance, orexin A levels in patients with depression were higher in the brains of deceased females compared to males, and there was increased OXR1 mRNA in the frontal cortex particularly in female rats that were exposed to chronic mild stress (79). These

findings may suggest different gender related psychiatric outcomes.

Based on unilateral left-sided hypothalamic lesions discovered in three patients with severe TBI, orexin insufficiency following TBI was thought to be driven by damage to the posterolateral hypothalamus. Nonetheless, mean OXA levels were not considerably less than those of severe TBI patients without hypothalamic lesions (4). This may suggest that orexin levels after TBI are influenced by factors other than direct hypothalamic damage. For instance, loss of orexin neurons after TBI could also be due in part to an inflammatory response to the injury since orexin neurons are susceptible to neuroinflammation (39). In CSF, bolus injections of lipopolysaccharide reduce orexin due to sterile inflammation (40-42), while activating microglia and triggering an inflammatory response (43,44). Microglia are activated as part of the TBI causative neuroinflammatory state (45-47), and hence, the effects of lipopolysaccharide-induced inflammation on orexin-producing hypothalamic cells in the absence of damage are somewhat comparable to what is seen in TBI. Thus, inflammation could mediate the subsequent loss of orexin neurons seen after TBI (39).

After TBI, patients are often administered anti-inflammatory agents to manage inflammation, and a decrease in orexin levels may further contribute to this need. In cases of intracerebral hemorrhage, OXA was found to improve neurofunctional outcomes and modulate inflammatory responses by upregulating anti-inflammatory cytokines while down regulating pro-inflammatory cytokines (80). Similarly, in a model symptom of rheumatoid arthritis, OXA demonstrated anti-inflammatory properties by reducing the secretion of pro-inflammatory cytokines IL-1β, IL-6, and IL-8, as well as inhibiting the NF-κB signaling pathway (81). It may be inferred that orexin is susceptible to inflammation (39) but has an anti-inflammatory effect. A decrease in orexin levels thus could contribute to the need for further reliance on anti-inflammatory treatment after TBI.

Several studies have found that a significant loss of orexin does occur following TBI (4, 39, 48, 49). Thirty out of 31 patients (97%) with severe traumatic brain injury and seven out of eight patients (88%) with a moderate traumatic brain injury in the acute stage (1-4 days) of the injury showed a significant reduction of OXA concentrations in the CSF. Of the thirty-nine patients with moderate to severe TBI, nine had undetectable CSF OXA levels (4). This indicates a significant loss of orexin in the first few days after TBI. Furthermore, patients with TBI showed a significant loss of orexin neurons, according to the autopsy results from four patients who died between seven and forty-two days after suffering a severe TBI. Compared to controls, TBI patients had 27% fewer orexin neurons (48). More recently, reduced levels of orexin A were found in the cytoplasm of hypothalamic neurons in 49 patients whose survival duration extended from the initial occurrence of TBI to 1.5 months. Nine instances (18.4%) showed a moderate reduction in ORXA immunoreactivity, while seven cases (14.3%) showed a severe reduction. In five (10.2%) of the cases, no neurons were found and there was no detectable ORXA immunoreactivity in the

hypothalamus (49). Both of these studies suggest that TBI can lead to a decrease in orexin levels that persist past the acute phase of TBI, since patients included in the sample had sustained TBI up to 42 days and 1.5 months before the data was collected.

OXA levels seem to recover 6 months after TBI, yet individuals with EDS had CSF orexin levels that were significantly lower at six months after traumatic brain injury than those without EDS (50). The drop in CSF OXA levels during the acute phase of a traumatic brain injury, followed by the levels returning to normal six months later, might be explained by damage to axons or orexin neurons caused by TBI, as according to autopsy reports, up to 66% of TBI patients had necrosis, hemorrhage, or structural abnormalities in the hypothalamus (51,52). Recovery of orexin levels following TBI would indicate that other cells that produce orexin had adapted, but the orexin synthesis of the surviving neurons may be insufficient in individuals with increased sleep propensity following TBI, leading to residually low levels (53). Therefore, post-traumatic EDS or hypersomnia may be related to insufficient adaptation by surviving orexin cells (50).

In animal models of TBI, there was also a decrease in hypocretin-producing neurons. Mice with moderate TBI 15 days after surgery had fewer numbers of orexin-producing neurons in the hypothalamus, as well as having fewer observed amounts when compared to orexin-positive cells present after 7 days (39). Another study showed similar results (54), as one month following traumatic brain injury, there was a significant decrease in OXA-positive cells in the lateral hypothalamus, further suggesting that TBI may cause a decrease in orexin neurons. An additional study did not find loss of orexin neurons, as there was a slight increase, rather, an activity impairment was observed. Measures of arousal were impaired and extracellular orexin release was disrupted three days after Controlled Cortical Impact (CCI) induced TBI (55). Despite there not being a decrease in orexin neurons, TBI still led to a disruption of the orexin system. Further studies demonstrated a disruption in the activity of orexin neurons, as it was found that there were noticeably fewer activated orexin neurons in the mice post-TBI (56).

The effect of TBI on orexin receptors, specifically OXR1, was observed in two studies. Firstly, it was found that in the penumbra after CCI-TBI, elevated OX1R immunoreactivity increased starting from six hours after TBI and peaked on the first day before progressively decreasing from day two (57). The distribution of OXIR was predominantly seen in the immediate penumbra of the injury, but in certain mice, as the injury progressed, it gradually extended to the periphery, including the thalamus and hippocampal regions (57). In the second study, there was a significant decrease in the number of OXR1-positive cells observed 14 days post-TBI at the injury site compared to controls (58). The results of the first study suggested an onset of increased OXR1 activity after TBI that later began to decrease. The second study provided results consistent with this decrease, suggesting that at 14 days post-TBI, OXR1 levels were reduced from baseline.

The variation observed in the studies may be due to the time the results were observed among other factors, however, they still collectively suggested that the orexin system may be altered post-TBI, regardless of whether due to neuronal loss or functional impairment. These findings are summarized in Table 1. This alteration may be a potential explanation to some of the symptoms experienced by patients after a traumatic brain injury. Considering a dysregulation of the Orexin system may lead to psychiatric disorders (37), the elevation of OXR1 immunoreactivity, and the loss of OXA in those post-TBI could be the explanation for the comorbidity of increased depression in conjunction with anxiety. The long-term effects of these disorders may be attributed to the orexin system remaining in a dysfunctional state, as seen in those with EDS post-TBI (50), or of adapting to the effects of the injury. Whether the orexin decrease coincides with OXR1 upregulation post-TBI is unclear and warrants further exploration

Table 1. Studies that have collected data on how the Orexin system changes h (hours), d (days), and m (months) after TBI.

	Study	Subject	Time Data was collected post-TBI	Orexin system change
Orexin peptide levels (OXA)	Baumann (4)	human	1d - 4d	**Decreased** OXA in CSF
	Baumann (48)	human	7d - 42d	**Decreased** OXA seen in hypothalamus
	Kousi (49)	human	1h - 1.5m	**Decreased** OXA seen in the cytoplasm of hypothalamic neurons
	Baumann (50)	human	1d - 4d, 6m	**Decreased** OXA levels in CSF 1d - 4d, Normal OXA levels at 6m for 81% of patients, low for 19% of patients.
	Thomasy (39)	mouse	7d, 15d	**Decrease** OXA seen in hypothalamus at both 7 and 15 days post-TBI (slightly lower at 15d)
	Skopin (54)	rat	29d	**Decreased** OXA seen in hypothalamus
	Willie (55)	mouse	3d	Small **increase** in OXA, extracellular Orexin release was disrupted in hypothalamus
OXR1	Mihara (57)	mouse	0h - 7d	**Elevated** OXR1, peaking at day 1, decreasing from day 2 (detected from 6 hours to 2d in surrounding penumbra of injury, left parietotemporal cortex, which extended to other brain regions in the non-injured hemisphere days 4 and 7).
	Ren (58)	rat	14d	**Decreased** OXR1 expression in damaged regions of the brain (left parietal cortex).

2.4 Clinical outlook on orexin's role in TBI

Sleep and mood are both commonly disrupted post-TBI as seen by the prevalence of sleep disorders such as EDS or insomnia and mood disorders such as MDD. This makes the orexin system a promising target for TBI-related therapeutic interventions.

There is further research needed to understand how the orexin system is affected after TBI before it can be targeted clinically. This is especially the case with orexin receptors due to

the current lack of information on their expression post-TBI. Orexin receptor antagonists could be used to reduce orexinergic activity if increased receptor expression or activation is indeed confirmed. One such antagonist is Suvorexant (MK-4305, Merck), which functions as a treatment for insomnia (59). Suvorexant acts differently from benzodiazepines and nonbenzodiazepines in that it does not affect GABA. It inactivates wakefulness rather than encouraging sleep (13). Therefore, the side effects associated with conventional insomnia treatment, such as those frequently linked to benzodiazepines and nonbenzodiazepines are no longer of concern. Hence, the chance of rebound insomnia or physical dependence while using suvorexant regularly is no longer a risk (59). This antagonist could be considered for TBI patients suffering from insomnia as a result of upregulated orexin receptor expression that persists for longer periods of time. More research and clinical trials are necessary to determine whether this medication can be used to treat symptoms similar to depression post-TBI, such as being utilized in the case of abnormalities in the orexin receptor expression leading to depressive behavior (30).

Furthermore, antagonists that selectively inhibit OX1R or OXR2 may also have specific therapeutic benefits. A selective antagonist of OXR2, Selotorexant; in phase III clinical trials; showed antidepressant effects in patients with MDD. The results of the investigation indicated that Seltorexant at a 20 mg dose was associated with a clinically significant decrease in depression symptoms particularly in those with symptoms of insomnia (60). Considering that insomnia is frequently associated with depression, OXR2 antagonists could represent a significant advancement in MDD therapy options in patients who have not responded well to existing antidepressant therapy, as they may be able to provide antidepressant benefits with a favorable safety profile. Selotorexant may benefit patients post-TBI who display symptoms of coexisting insomnia with MDD due to receptor expression abnormalities as a result of their injury. Another selective antagonist of OX1R, JNJ-61393215, has been demonstrated to have anxiolytic effects. 90 mg of JNJ-61393215 showed a significant decrease in panic induced by CO_2 as well as in anxiety symptoms in phase I clinical tests (61), and is now being tested as a treatment for depression. It has also been demonstrated that anxiety and immobility brought on by acute stress were reduced by blocking OXR1 and OXR2 in the hippocampus (62). OXR antagonists could therefore be considered in the case of comorbid MDD and anxiety, which is often observed after TBI (11,12).

Receptor antagonists have been evaluated specifically post-TBI as a treatment to normalize sleep homeostatic drive and to ameliorate posttraumatic epilepsy. A measure of sleep homeostatic drive is the EEG delta power (NΔ) and how it changes depending on how much time was spent awake or asleep. In one study, total 24-hour NΔ increased after TBI and was then subsequently lowered by DORA-22 – a Dual Orexin Receptor Antagonist - treatment. A month-long treatment with the sleep aid DORA-22 starting from the day of TBI suppressed chronic posttraumatic seizures and restored the normal homeostatic oscillation

of NΔ (63). Although antiseizure medications and sleep aids can be used independently to treat sleep disturbances and seizures, treatment with DORA-22 after TBI can mitigate both these complications. This implies targeting the orexin system as a method of addressing multiple disorders that arise post-injury.

Despite the significant loss of orexin that occurs following TBI (4, 48, 49), administration of orexin and orexin agonist development has received relatively little attention. In patients with hypoactive orexinergic networks, increasing the amount of orexin available in the extracellular space may be used to increase orexinergic levels. However, there are currently no studies that have been conducted on orexin metabolism in the brain (37). There has been an OXR2 agonist developed (YNT-185, University of Tsukuba, Japan) and it was able to ameliorate narcolepsy-cataplexy symptoms in mouse models (64), however, it has not been tested in clinical trials. Intranasal administration of OXA is another method of treating orexin deficiencies and shows promise in mitigating symptoms of narcolepsy in human patients. In a study, patients who were administered intranasal OXA experienced fewer wake-REM sleep transitions and shorter REM sleep durations. This provides evidence for OXA's role as a stabilizing factor for REM sleep and the ability for it to possibly be used as a treatment for narcolepsy with cataplexy (65). Therefore, this method can also treat other disorders, such as MDD, that may result from orexin deficiency post-TBI in conjunction with sleep disorders that arise due to the same deficiency, such as potentially EDS.

Orexin receptor antagonists, as well as potential orexin agonists could be considered for TBI patients with sleep disorders and depression. However, further research is needed to identify the dysfunction of orexin receptors after brain injury considering the current lack of preclinical and clinical studies. Orexin is especially intriguing as a target for the treatment of TBI, since it could potentially mitigate several disorders and complications that arise post-injury, namely MDD and sleep disorders such as EDS or insomnia.

OXR antagonists have been used in trials as a treatment for substance use disorders (82,83). Thus, targeting the orexin system may further improve outcomes for TBI patients due to the possibility of decreasing the motivation to abuse the substances that could otherwise worsen their psychological symptoms. Beyond being used as a sleep aid, suvorexant was investigated to determine its efficacy in reducing alcohol consumption and stress-triggered relapse of alcohol-seeking behavior in rats. It was found that suvorexant was indeed able to specifically reduce alcohol consumption in dependent rats and effectively block relapse of alcohol-seeking behavior (82). Furthermore, the OXR1 specific receptor antagonist, SB-334867 (SmithKline Beecham, UK), was tested as a treatment for drug addiction via suppressing seeking behavior. The findings indicated that SB-334867 was effective in decreasing the drive to obtain food rewards, suggesting that OXR1 plays a role in modulating motivational processes (83). Thus, the orexin system has potential as a therapeutic strategy for Substance Use Disorders (SUDs). Mitigation of these disorders are especially

pertinent in adults with TBI as there is a strong association between TBI and an increased risk of alcohol abuse or other SUDs. For instance, one study found a sixfold increase in alcohol abuse rates among TBI patients over a 10-year period (84), and other studies have reported several fold increases as well (85,86). Moreover, MDD and other mood disorders are also often accompanied by SUDs (87). Considering this, OXR antagonists could serve to further alleviate some of the psychological symptoms post-TBI which may be specifically exacerbated by, and/or simultaneously drive, substance use.

Orexin may affect carcinogenesis. There is a higher risk of certain cancers linked to insomnia (88), which may be mediated through immune dysregulation caused by inflammatory cytokines—IL-6—that are increased in cancer as well as in sleep disorders (89,90). This inflammation could be conducive to tumor development, and long-term exposure to high IL-6 levels may make it more difficult for the immune system to identify and destroy cancer cells due to the interference it may cause in the equilibrium of inflammatory responses (91). Therefore, by working to regulate sleep, orexin antagonists could potentially reduce the risk of the cancers found to be associated with insufficient sleep. Orexin's role in cancer development and mitigation may be more direct as well. OXR2 is expressed in a variety of cancer types (92), and OXR1 is found to be expressed in certain tumors, such as adrenocortical adenomas, as well (93). OXR-mediated signaling causes certain tumor cells to undergo apoptosis, but in other tumor cells, they enhance proliferative activity (94). Considering this, OXR antagonists have the potential to prevent the proliferative effect mediated by orexin and also encourage the death of tumor cells. For instance, Almorexant and Suvorexant caused apoptosis in AsPC-1 cells and also reduced tumor volume (95), and blocking OXR1 caused pancreatic cancer cells to undergo apoptosis (96). Further research is needed to explore the role of orexin in cancer development, but targeting the orexin system may contribute to tumor regression.

3 Conclusion

3.1 Hypothesized changes to the orexin system post-TBI

The frequent development of sleep and psychiatric disorders after TBI, along with their common comorbidities, highlights the importance of understanding their underlying causes. Changes to the orexin system have been observed both after TBI, as well as alongside sleep disorders such as narcolepsy, and alongside psychiatric disorders such as MDD or anxiety. Therefore, the purturbances to the orexin system following TBI elucidate a potential common mechanism underlying these psychiatric and sleep disturbances commonly observed in TBI patients. The exact timeline for changes to the Orexin system after TBI are not clearly established, especially for orexin receptors. However, analysis of the various studies that have observed the orexin system post-TBI can provide a relative timeline with regard to these changes (Figure 1).

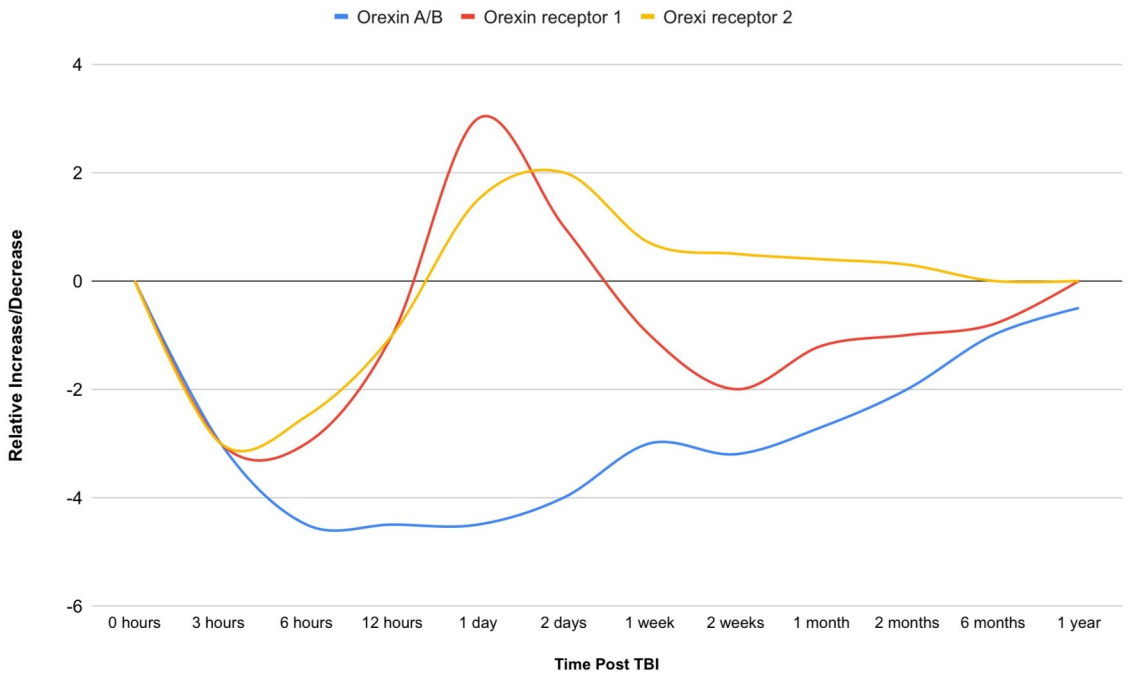

Figure 1. Hypothesized orexin system changes after moderate to severe TBI for patients exhibiting symptoms of sleep/mood disorders

There have been multiple studies observing orexin neuron counts after TBI (Table 1), and there appears to be a significant decrease in orexin during the acute phase of the injury, that being from the initial injury to ~ day four. Orexin neurons may remain decreased compared to normal levels even at a time point of 6 months, and could stay this way for longer due to potentially insufficient orexin synthesis of surviving neurons that had not adapted (53). This could be the case for those experiencing symptoms of EDS and perhaps MDD which may result from the same deficiency that would be characterized by the loss of orexin neurons. In this case, targeted ways to increase the levels of orexin neurons may improve these symptoms. For orexin receptors, there seems to be an initial upregulation in OXR1 after the first few hours following TBI (57), suggesting a compensatory response to the injury and the decreased orexin neurons. This later decreases below normal levels at a time point of 14 days (58), indicating that this increase in OXR1 occurs during the acute phase of the injury. Dual Orexin Receptor Antagonist (DORA) treatment starting immediately after the injury might stabilize the increased OXR1, and although there are no studies conducted on OXR2 expression post-TBI, there may be an increase in OXR2 that persists further past the acute phase which could then be targeted by this antagonist. This may be the case as a dual orexin receptor antagonist, DORA-22, was used to normalize sleep homeostatic drive (63),

and could suggest that a dysregulation of orexin receptors persists up to at least one month; since that is when the treatment was tested.

Considering the anxiolytic effect of orexin receptor antagonists (61,62), a targeted treatment of OXR antagonists during periods of increased OXR expression could also help alleviate the symptoms of anxiety that are often present in conjunction with MDD and sleep disturbances after TBI (11,12). Essentially, understanding the changes in the orexin system after TBI may provide insight into a possible mechanism behind the psychiatric and sleep disorders frequently seen in TBI patients, and targeting the orexin system at specific time points may be a viable treatment option for a number of post-TBI symptoms.

3.2 Perspectives

The role of the orexin system in the pathophysiology of psychiatric disorders post-TBI is still relatively unclear, as the exact progression of changes to the Orexin system is not known. Yet, understanding these changes can profoundly benefit both research and clinical care. A clear timeline of the increase or decrease of several components of the Orexin system such as OXR1 and OXR2 receptors may aid in the understanding of why certain post-TBI psychiatric disorders develop when they do, and could inform the development of targeted treatments. For instance, therapies could be tailored to stabilize Orexin neuron or Orexin receptor levels during periods where it is either abnormally increased or decreased to improve symptoms of sleep or mood disorders. As hypothesized, OXR1 and subsequently OXR2 levels may be increased post-TBI, and stabilizing the activity with a dual OXR antagonist may alleviate the symptoms of sleep disorders that could arise from that dysfunction; that has been seen to be the case (63). Ultimately, a detailed timeline of Orexin system changes could pave the way for more effective strategies in managing TBI and improving patient care trajectories.

As seen from previous studies performed on the orexin system after TBI, there are still significant gaps in data that impede understanding the temporal and spatial progression of changes to the orexin system. The data on the orexin neuron counts are relatively more robust, with decreases seen during the acute phase (1-4 days), two weeks to a month later, and even partially at a time point of 6 months. However, a consistent observation of orexin levels starting from the first or second day post-TBI to the fourth day, still need clarity. There are also no studies on OXR1 beyond a time point of 14 days, which could be crucial in identifying whether there is an upregulation or decrease in expression or activity of these receptors and the long-term effects of their dysregulation. Likewise, there are no studies on OXR2 as of now. There is a significant lack of understanding in how these receptors are expressed post-TBI. Moreover, the data for the study of OXR1 expression at 14 days only observed OXR1-positive cells at the site of injury. Therefore, it may also be relevant to observe the expression of these receptors as the injury progresses in brain regions associated with mood. Research into these gaps can help with understanding how the spatial and temporal targeting of the orexin system

post-TBI can be utilized for better outcomes for patients.

In addition, it may be advantageous to construct an algorithm that provides information on gender, along with biomarkers for the HPA axis (ACTH, cortisol, DHEA-S), inflammation and autoimmune disease (interleukins, ESR, CRP), blood glucose (diabetes), and genetic variant determination of ADH1B and ALDH2 (susceptibility to alcohol abuse) in order to determine how aggressive the post TBI orexin modulating therapy needs to be for a particular patient to achieve a favorable outcome. Such clinical algorithms, meta-analyses and more empirical studies are needed to progress our current understanding to develop patient-specific targeted treatments that could improve outcomes for TBI patients.

Abbreviations

TBI: Traumatic Brain Injury, Orexin: orexin/hypocretin, OXA: Orexin A, OXB: Orexin B, OXR1: GABA, Gamma-aminobutyric Acid, Orexin Receptor 1, OXR2: Orexin Receptor 2, TMN: tuberomammillary nucleus, EDS: Excessive Daytime Sleepiness, MDD: Major Depressive Disorder, VTA: Ventral Tegmental Area, FST: Forced Swim Test, CSF: Cerebrospinal Fluid, HPA: hypothalamic-pituitary-adrenal, CCI: Controlled Cortical Impact, EEG: Electroencephalography, NΔ: Electroencephalography Delta Power, DORA: Dual Orexinergic Receptor Antagonist, REM: Rapid Eye Movement, EAE: experimental autoimmune encephalomyelitis, IL: interleukin, ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein, HPA: Hypothalamus Pitutary Axis, DHEA-S: DeHydroEpiAndrosterone Sulfate, SUD: Substance Use Disorder

4 References

1. Menon, D. K.; Schwab, K.; Wright, D. W.; Maas, A. I. Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements for Research on Traumatic Brain Injury and Psychological Health. Position Statement: Definition of Traumatic Brain Injury. *Arch. Phys. Med. Rehabil.* **2010**, 91 (11), 1637–1640. https://doi.org/10.1016/j.apmr.2010.05.017

2. National Center for Injury Prevention and Control (U.S.). Division of Unintentional Injury Prevention. Report to Congress on Traumatic Brain Injury in the United States : Epidemiology and Rehabilitation. Centers for Disease Control and Prevention, March 27, **2015**. https://stacks.cdc.gov/view/cdc/29215

3. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Board on Health Care Services; Committee on the Review of the Department of Veterans Affairs Examinations for Traumatic Brain Injury. *Evaluation of the Disability Determination Process*

for Traumatic Brain Injury in Veterans; National Academies Press (US): Washington (DC), **2019**. https://nap.nationalacademies.org/catalog/25317/evaluation-of-the-disability-determination-process-for-traumatic-brain-injury-in-veterans

4. Baumann, C. R.; Stocker, R.; Imhof, H.-G.; Trentz, O.; Hersberger, M.; Mignot, E.; Bassetti, C. L. Hypocretin-1 (Orexin A) Deficiency in Acute Traumatic Brain Injury. *Neurology.* **2005**, 65 (1), 147–149. https://doi.org/10.1212/01.wnl.0000167605.02541.f2

5. Devi, Y.; Khan, S.; Rana, P.; Deepak; Dhandapani, M.; Ghai, S.; Gopichandran, L. Cognitive, Behavioral, and Functional Impairments among Traumatic Brain Injury Survivors: Impact on Caregiver Burden. *J. Neurosci. Rural Pract.* **2020**, 11 (4), 629–635. https://doi.org/10.1055/s-0040-1716777

6. Whiteneck, G. G.; Gerhart, K. A.; Cusick, C. P. Identifying Environmental Factors That Influence the Outcomes of People with Traumatic Brain Injury. *J. Head Trauma Rehabil.* **2004**, 19 (3), 191–204. https://doi.org/10.1097/00001199-200405000-00001

7. Alway, Y.; Gould, K. R.; Johnston, L.; McKenzie, D.; Ponsford, J. A. Prospective Examination of Axis I Psychiatric Disorders in the First 5 Years Following Moderate to Severe Traumatic Brain Injury. *Psychol. Med.* **2016**, 46 (6), 1331–1341. https://doi.org/10.1017/S0033291715002986

8. Crichton, T.; Singh, R.; Abosi-Appeadu, K.; Dennis, G. Excessive Daytime Sleepiness after Traumatic Brain Injury. *Brain Inj.* **2020**, 34 (11), 1525–1531. https://doi.org/10.1080/02699052.2020.1810316

9. Nishino, S.; Ripley, B.; Overeem, S.; Lammers, G. J.; Mignot, E. Hypocretin (Orexin) Deficiency in Human Narcolepsy. *Lancet.* **2000**, 355 (9197), 39–40. https://doi.org/10.1016/S0140-6736(99)05582-8

10. Jorge, R. E.; Robinson, R. G.; Moser, D.; Tateno, A.; Crespo-Facorro, B.; Arndt, S. M. Depression Following Traumatic Brain Injury. *Arch. Gen. Psychiatry.* **2004**, 61 (1), 42–50. https://doi.org/10.1001/archpsyc.61.1.42

11. Bombardier, C. H.; Fann, J. R.; Temkin, N. R.; Esselman, P. C.; Barber, J.; Dikmen, S. S. Rates of Major Depressive Disorder and Clinical Outcomes Following Traumatic Brain Injury. *JAMA.* **2010**, 303 (19), 1938–1945. https://doi.org/10.1001/jama.2010.599

12. Singh, R.; Mason, S.; Lecky, F.; Dawson, J. Prevalence of Depression after TBI in a

Prospective Cohort: The SHEFBIT Study. *Brain Inj.* **2018**, 32 (1), 84–90. https://doi.org/10.1080/02699052.2017.1376756

13. Mieda, M.; Sakurai, T. Overview of Orexin/Hypocretin System. *Prog. Brain Res.* **2012**, 198, 5–14. https://doi.org/10.1016/B978-0-444-59489-1.00002-1

14. Trivedi, P.; Yu, H.; MacNeil, D. J.; Van der Ploeg, L. H.; Guan, X. M. Distribution of Orexin Receptor mRNA in the Rat Brain. *FEBS Lett.* **1998**, 438, 71–75. https://doi.org/10.1016/s0014-5793(98)01266-6

15. de Lecea, L.; Kilduff, T. S.; Peyron, C.; Gao, X.; Foye, P. E.; Danielson, P. E.; et al. The Hypocretins: Hypothalamus-Specific Peptides with Neuroexcitatory Activity. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, 95 (1), 322–327. https://doi.org/10.1073/pnas.95.1.322

16. Sutcliffe, J. G.; de Lecea, L. The Hypocretins: Excitatory Neuromodulatory Peptides for Multiple Homeostatic Systems, Including Sleep and Feeding. *J. Neurosci. Res.* **2000**, 62 (2), 161–168. https://doi.org/10.1002/1097-4547(20001015)62:2

17. Date, Y.; Ueta, Y.; Yamashita, H.; Yamaguchi, H.; Matsukura, S.; Kangawa, K.; et al. Orexins, Orexigenic Hypothalamic Peptides, Interact with Autonomic, Neuroendocrine and Neuroregulatory Systems. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, 96 (2), 748–753. https://doi.org/10.1073/pnas.96.2.748

18. Ida, T.; Nakahara, K.; Kuroiwa, T.; Fukui, K.; Nakazato, M.; Murakami, T.; et al. Both Corticotropin Releasing Factor and Neuropeptide Y Are Involved in the Effect of Orexin (Hypocretin) on the Food Intake in Rats. *Neurosci. Lett.* **2000**, 293 (2), 119–122. https://doi.org/10.1016/s0304-3940(00)01498-1

19. van den Pol, A. N.; Gao, X. B.; Obrietan, K.; Kilduff, T. S.; Belousov, A. B. Presynaptic and Postsynaptic Actions and Modulation of Neuroendocrine Neurons by a New Hypothalamic Peptide, Hypocretin/Orexin. *J. Neurosci.* **1998**, 18 (19), 7962–7971. https://doi.org/10.1523/JNEUROSCI.18-19-07962.1998

20. Sakurai, T.; Nagata, R.; Yamanaka, A.; Kawamura, H.; Tsujino, N.; Muraki.; et al. Input of Orexin/Hypocretin Neurons Revealed by a Genetically Encoded Tracer in Mice. *Neuron.* **2005**, 46 (2), 297–308. https://doi.org/10.1016/j.neuron.2005.03.010

21. Eggermann, E.; Serafin, M.; Bayer, L.; Machard, D.; Saint-Mleux, B.; Jones, B. E.; Mühlethaler, M. Orexins/Hypocretins Excite Basal Forebrain Cholinergic Neurones.

Neuroscience. **2001**, 108 (2), 177–181. https://doi.org/10.1016/s0306-4522(01)00512-7

22. Eriksson, K. S.; Sergeeva, O.; Brown, R. E.; Haas, H. L. Orexin/Hypocretin Excites the Histaminergic Neurons of the Tuberomammillary Nucleus. *J. Neurosci.* **2001**, 21 (23), 9273–9279. https://doi.org/10.1523/JNEUROSCI.21-23-09273.2001

23. Brown, R. E.; Sergeeva, O.; Eriksson, K. S.; Haas, H. L. Orexin A Excites Serotonergic Neurons in the Dorsal Raphe Nucleus of the Rat. *Neuropharmacology.* **2001**, 40 (3), 457–459. https://doi.org/10.1016/s0028-3908(00)00178-7

24. Liu, R.-J.; van den Pol, A. N.; Aghajanian, G. K. Hypocretins (Orexins) Regulate Serotonin Neurons in the Dorsal Raphe Nucleus by Excitatory Direct and Inhibitory Indirect Actions. *J. Neurosci.* **2002**, 22 (21), 9453–9464. https://doi.org/10.1523/JNEUROSCI.22-21-09453.2002

25. Yamanaka, A.; Beuckmann, C. T.; Willie, J. T.; Hara, J.; Tsujino, N.; Mieda, M.; et al. Hypothalamic Orexin Neurons Regulate Arousal According to Energy Balance in Mice. *Neuron.* **2003**, 38 (5), 701–713. https://doi.org/10.1016/s0896-6273(03)00331-3

26. Melberg, A.; Ripley, B.; Lin, L.; Hetta, J.; Mignot, E.; Nishino, S. Hypocretin Deficiency in Familial Symptomatic Narcolepsy. *Ann. Neurol.* **2001**, 49 (1), 136–137. https://doi.org/10.1002/1531-8249(200101)49:1%3C136::AID-ANA24%3E3.0.CO;2-P

27. Kuru, M.; Ueta, Y.; Serino, R.; Nakazato, M.; Yamamoto, Y.; Shibuya, I. Centrally Administered Orexin/Hypocretin Activates HPA Axis in Rats. *Neuroreport.* **2000**, 11 (9), 1977–1980. https://doi.org/10.1097/00001756-200006260-00034

28. Wang, C.; Wang, Q.; Ji, B.; Pan, Y.; Xu, C.; Cheng, B.; et al. The Orexin/Receptor System: Molecular Mechanism and Therapeutic Potential for Neurological Diseases. *Front. Mol. Neurosci.* **2018**, 11, 220. https://doi.org/10.3389/fnmol.2018

29. Nocjar, C.; Zhang, J.; Feng, P.; Panksepp, J. The Social Defeat Animal Model of Depression Shows Diminished Levels of Orexin in Mesocortical Regions of the Dopamine System, and of Dynorphin and Orexin in the Hypothalamus. *Neuroscience.* **2012**, 218, 138–153. https://doi.org/10.1016/j.neuroscience.2012.05.033

30. Arendt, D. H.; Ronan, P. J.; Oliver, K. D.; Callahan, L. B.; Summers, T. R.; Summers, C. H. Depressive Behavior and Activation of the Orexin/Hypocretin System. *Behav. Neurosci.* **2013**, 127 (1), 86–94. https://doi.org/10.1037/a0031442

31. Lutter, M.; Sakata, I.; Osborne-Lawrence, S.; Rovinsky, S. A.; Anderson, J. G.; Jung, S.; et al. The Orexigenic Hormone Ghrelin Defends against Depressive Symptoms of Chronic Stress. *Nat. Neurosci.* **2008**, 11 (7), 752–753. https://doi.org/10.1038/nn.2139

32. Brundin, L.; Björkqvist, M.; Petersén, A.; Träskman-Bendz, L. Reduced Orexin Levels in the Cerebrospinal Fluid of Suicidal Patients with Major Depressive Disorder. *Eur. Neuropsychopharmacol.* **2007**, 17 (9), 573–579. https://doi.org/10.1016/j.euroneuro.2007.01.005

33. Johnson, P. L.; Truitt, W.; Fitz, S. D.; Minick, P. E.; Dietrich, A.; Sanghani, S.; Träskman-Bendz, L.; Goddard, A. W.; Brundin, L.; Shekhar, A. A Key Role for Orexin in Panic Anxiety. *Nat. Med.* **2010**, 16 (1), 111–115. https://doi.org/10.1038/nm.2075

34. Kim, T.-K.; Kim, J.-E.; Park, J.-Y.; Lee, J.-E.; Choi, J.; Kim, H.; et al. Antidepressant Effects of Exercise Are Produced via Suppression of Hypocretin/Orexin and Melanin-Concentrating Hormone in the Basolateral Amygdala. *Neurobiol. Dis.* **2015**, 79, 59–69. https://doi.org/10.1016/j.nbd.2015.04.004

35. Li, H.; Lu, J.; Li, S.; Huang, B.; Shi, G.; Mou, T.; Xu, Y. Increased Hypocretin (Orexin) Plasma Level in Depression, Bipolar Disorder Patients. *Front. Psychiatry.* **2021**, 12, 676336. https://doi.org/10.3389/fpsyt.2021.676336

36. Mikrouli, E.; Wörtwein, G.; Soylu, R.; Mathé, A. A.; Petersén, Å. Increased Numbers of Orexin/Hypocretin Neurons in a Genetic Rat Depression Model. *Neuropeptides.* **2011**, 45 (6), 401–406. https://doi.org/10.1016/j.npep.2011.07.010

37. Khairuddin, S.; Aquili, L.; Heng, B. C.; Hoo, T. L. C.; Wong, K. H.; Lim, L. W. Dysregulation of the Orexinergic System: A Potential Neuropeptide Target in Depression. *Neurosci. Biobehav. Rev.* **2020**, 118, 384–396. https://doi.org/10.1016/j.neubiorev.2020.07.040

38. Morse, A. M.; Garner, D. R. Traumatic Brain Injury, Sleep Disorders, and Psychiatric Disorders: An Underrecognized Relationship. *Med. Sci. Basel Switz.* **2018**, 6 (1), 15. https://doi.org/10.3390/medsci6010015

39. Thomasy, H. E.; Febinger, H. Y.; Ringgold, K. M.; Gemma, C.; Opp, M. R. Hypocretinergic and Cholinergic Contributions to Sleep-Wake Disturbances in a Mouse Model of Traumatic Brain Injury. *Neurobiol. Sleep Circadian Rhythms.* **2017**, 2, 71–84. https://doi.org/10.1016/j.nbscr.2016.03.001

40. Grossberg, A. J.; Zhu, X.; Leinninger, G. M.; Levasseur, P. R.; Braun, T. P.; Myers, M. G.;

Marks, D. L. Inflammation-Induced Lethargy Is Mediated by Suppression of Orexin Neuron Activity. *J. Neurosci.* **2011**, 31 (31), 11376–11386. https://doi.org/10.1523/JNEUROSCI.2311-11.2011

41. Qin, L.; Li, G.; Qian, X.; Liu, Y.; Wu, X.; Liu, B.; Hong, J.-S.; Block, M. L. Interactive Role of the Toll-like Receptor 4 and Reactive Oxygen Species in LPS-Induced Microglia Activation. *Glia.* **2005**, 52 (1), 78–84. https://doi.org/10.1002/glia.20225

42. Vasconcelos, A. R.; Yshii, L. M.; Viel, T. A.; Buck, H. S.; Mattson, M. P.; Scavone, C.; Kawamoto, E. M. Intermittent Fasting Attenuates Lipopolysaccharide-Induced Neuroinflammation and Memory Impairment. *J. Neuroinflammation.* **2014**, 11, 85. https://doi.org/10.1186/1742-2094-11-85

43. Camara, M. L.; Corrigan, F.; Jaehne, E. J.; Jawahar, M. C.; Anscomb, H.; Baune, B. T. Effects of Centrally Administered Etanercept on Behavior, Microglia, and Astrocytes in Mice Following a Peripheral Immune Challenge. *Neuropsychopharmacol.* **2015**, 40 (2), 502–512. https://doi.org/10.1038/npp.2014.199

44. Thomson, C. A.; McColl, A.; Cavanagh, J.; Graham, G. J. Peripheral Inflammation Is Associated with Remote Global Gene Expression Changes in the Brain. *J. Neuroinflammation* **2014**, 11, 73. https://doi.org/10.1186/1742-2094-11-73

45. Febinger, H. Y.; Thomasy, H. E.; Pavlova, M. N.; Ringgold, K. M.; Barf, P. R.; George, A. M.; et al. Time-Dependent Effects of CX3CR1 in a Mouse Model of Mild Traumatic Brain Injury. *J. Neuroinflammation.* **2015**, 12, 154. https://doi.org/10.1186/s12974-015-0386-5

46. Harish, G.; Mahadevan, A.; Pruthi, N.; Sreenivasamurthy, S. K.; Puttamallesh, V. N.; Keshava Prasad, T. S.; et al. Characterization of Traumatic Brain Injury in Human Brains Reveals Distinct Cellular and Molecular Changes in Contusion and Pericontusion. *J. Neurochem.* **2015**, 134 (1), 156–172. https://doi.org/10.1111/jnc.13082

47. Hernandez-Ontiveros, D. G.; Tajiri, N.; Acosta, S.; Giunta, B.; Tan, J.; Borlongan, C. V. Microglia Activation as a Biomarker for Traumatic Brain Injury. *Front. Neurol.* **2013**, 4, 30. https://doi.org/10.3389/fneur.2013.00030

48. Baumann, C. R.; Bassetti, C. L.; Valko, P. O.; Haybaeck, J.; Keller, M.; Clark, E.; Stocker, R.; Tolnay, M.; Scammell, T. E. Loss of Hypocretin (Orexin) Neurons with Traumatic Brain Injury. *Ann. Neurol.* **2009**, 66 (4), 555–559. https://doi.org/10.1002/ana.21836

49. Kousi, C.; Lampri, E.; Voulgaris, S.; Vougiouklakis, T.; Galani, V.; Mitselou, A. Expression of Orexin-A (Hypocretin-A) in the Hypothalamus after Traumatic Brain Injury: A Postmortem Evaluation. *Forensic Sci. Int.* **2021**, 327, 110961. https://doi.org/10.1016/j.forsciint.2021.110961

50. Baumann, C. R.; Werth, E.; Stocker, R.; Ludwig, S.; Bassetti, C. L. Sleep-Wake Disturbances 6 Months after Traumatic Brain Injury: A Prospective Study. *Brain J. Neurol.* **2007**, 130 (Pt 7), 1873–1883. https://doi.org/10.1093/brain/awm109

51. Crompton, M. R. Hypothalamic Lesions Following Closed Head Injury. *Brain J. Neurol.* **1971**, 94 (1), 165–172. https://doi.org/10.1093/brain/94.1.165

52. Benvenga, S.; Campenní, A.; Ruggeri, R. M.; Trimarchi, F. Clinical Review 113: Hypopituitarism Secondary to Head Trauma. *J. Clin. Endocrinol. Metab.* **2000**, 85 (4), 1353–1361. https://doi.org/10.1210/jcem.85.4.6506

53. Gerashchenko, D.; Murillo-Rodriguez, E.; Lin, L.; Xu, M.; Hallett, L.; Nishino, S.; et al. Relationship between CSF Hypocretin Levels and Hypocretin Neuronal Loss. *Exp. Neurol.* **2003**, 184 (2), 1010–1016. https://doi.org/10.1016/S0014-4886(03)00388-1

54. Skopin, M. D.; Kabadi, S. V.; Viechweg, S. S.; Mong, J. A.; Faden, A. I. Chronic Decrease in Wakefulness and Disruption of Sleep-Wake Behavior after Experimental Traumatic Brain Injury. *J. Neurotrauma.* **2015**, 32 (5), 289–296. https://doi.org/10.1089/neu.2014.3664

55. Willie, J. T.; Lim, M. M.; Bennett, R. E.; Azarion, A. A.; Schwetye, K. E.; Brody, D. L. Controlled Cortical Impact Traumatic Brain Injury Acutely Disrupts Wakefulness and Extracellular Orexin Dynamics as Determined by Intracerebral Microdialysis in Mice. *J. Neurotrauma.* **2012**, 29 (10), 1908–1921. https://doi.org/10.1089/neu.2012.2404

56. Lim, M. M.; Elkind, J.; Xiong, G.; Galante, R.; Zhu, J.; Zhang, L.; et al. Dietary Therapy Mitigates Persistent Wake Deficits Caused by Mild Traumatic Brain Injury. *Sci. Transl. Med.* **2013**, 5 (215), 215ra173. https://doi.org/10.1126/scitranslmed.3007092

57. Mihara, Y.; Dohi, K.; Yofu, S.; Nakamachi, T.; Ohtaki, H.; Shioda, S.; Aruga, T. Expression and Localization of the Orexin-1 Receptor (OX1R) after Traumatic Brain Injury in Mice. *J. Mol. Neurosci.* **2011**, 43 (2), 162–168. https://doi.org/10.1007/s12031-010-9438-6

58. Ren, B.; Kang, J.; Wang, Y.; Meng, X.; Huang, Y.; Bai, Y.; Feng, Z. Transcranial Direct Current Stimulation Promotes Angiogenesis and Improves Neurological Function via the OXA-TF-AKT/ERK Signaling Pathway in Traumatic Brain Injury. *Aging.* **2024**, 16 (7), 6566–6587.

https://doi.org/10.18632/aging.205724

59. Bennett, T.; Bray, D.; Neville, M. W. Suvorexant, a Dual Orexin Receptor Antagonist for the Management of Insomnia. P T Peer-Rev. *J. Formul. Manag.* **2014**, 39 (4), 264–266. https://pmc.ncbi.nlm.nih.gov/articles/PMC3989084/

60. Savitz, A.; Wajs, E.; Zhang, Y.; Xu, H.; Etropolski, M.; Thase, M. E.; Drevets, W. C. Efficacy and Safety of Seltorexant as Adjunctive Therapy in Major Depressive Disorder: A Phase 2b, Randomized, Placebo-Controlled, Adaptive Dose-Finding Study. *Int. J. Neuropsychopharmacol.* **2021**, 24 (12), 965–976. https://doi.org/10.1093/ijnp/pyab050

61. Salvadore, G.; Bonaventure, P.; Shekhar, A.; Johnson, P. L.; Lord, B.; Shireman, B. T.; et al. Translational Evaluation of Novel Selective Orexin-1 Receptor Antagonist JNJ-61393215 in an Experimental Model for Panic in Rodents and Humans. *Transl. Psychiatry.* **2020**, 10 (1), 308. https://doi.org/10.1038/s41398-020-00937-9

62. Bahramzadeh Zoeram, S.; Elahdadi Salmani, M.; Lashkarbolouki, T.; Goudarzi, I. Hippocampal Orexin Receptor Blocking Prevented the Stress Induced Social Learning and Memory Deficits. *Neurobiol. Learn. Mem.* **2019**, 157, 12–23. https://doi.org/10.1016/j.nlm.2018.11.009

63. Konduru, S. R.; Isaacson, J. R.; Lasky, D. J.; Zhou, Z.; Rao, R. K.; Vattem, S. S.; et al. Dual Orexin Antagonist Normalized Sleep Homeostatic Drive, Enhanced GABAergic Inhibition, and Suppressed Seizures after Traumatic Brain Injury. *Sleep.* **2022**, 45 (12), zsac238. https://doi.org/10.1093/sleep/zsac238

64. Irukayama-Tomobe, Y.; Ogawa, Y.; Tominaga, H.; Ishikawa, Y.; Hosokawa, N.; Ambai, S.; et al. Nonpeptide Orexin Type-2 Receptor Agonist Ameliorates Narcolepsy-Cataplexy Symptoms in Mouse Models. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, 114 (22), 5731–5736. https://doi.org/10.1073/pnas.1700499114

65. Weinhold, S. L.; Seeck-Hirschner, M.; Nowak, A.; Hallschmid, M.; Göder, R.; Baier, P. C. The Effect of Intranasal Orexin-A (Hypocretin-1) on Sleep, Wakefulness and Attention in Narcolepsy with Cataplexy. *Behav. Brain Res.* **2014**, 262, 8–13. https://doi.org/10.1016/j.bbr.2013.12.045

66. Pagh-Berendtsen, N.; Pavlovskyi, A.; Téllez, D.F.; Egebjerg, C.; Kolmos, M.G.; Justinussen J.; et al. Downregulation of hypocretin/orexin after H1N1 Pandemrix vaccination of adolescent mice, *Sleep.* **2024**, 47 (4), zsae014. https://doi.org/10.1093/sleep/zsae014

67. Melén, K.; Jalkanen, P.; Kukkonen, J.P.; Partinen, M.; Nohynek, H.; Vuorela, A.; et al. No evidence of autoimmunity to human OX1 or OX2 orexin receptors in Pandemrix-vaccinated narcoleptic children. *Journal of Translational Autoimmunity.* **2020**, 3, 100055. https://doi.org/10.1016/j.jtauto.2020.100055

68. Singh, A. K., Mahlios, J., & Mignot, E. Genetic association, seasonal infections and autoimmune basis of narcolepsy. *Journal of Autoimmunity.* ***2013,*** 43, 26–31. https://doi.org/10.1016/j.jaut.2013.02.003

69. Mogavero MP.; Godos J.; Grosso G.; Caraci F.; Ferri R.; et al. Rethinking the Role of Orexin in the Regulation of REM Sleep and Appetite. *Nutrients.* **2023**, 15(17), 3679. https://doi.org/10.3390/nu15173679

70. Narita, N.; Yamada, R.; Kakehi, M.; Kimura, H. Diurnal Fluctuations of Orexin-A and -B in Cynomolgus Monkey Cerebrospinal Fluid Determined by a Novel Analytical Method Using Antiadsorptive Additive Treatment Followed by Nanoflow Liquid Chromatography-High-Resolution Mass Spectrometry. *ACS Chem. Neurosci.* **2023**, 14, 609–618. https://doi.org/10.1021/acschemneuro.2c00370

71. Steiger, A.; Pawlowski, M. Depression and Sleep. *Int J Mol Sci.* **2019,** 20 (3), 607. https://doi.org/10.3390/ijms20030607

72. Jöhren, O.; Gremmels, J. A. F.; Qadri, F.; Dendorfer, A.; Dominiak, P. Adrenal expression of orexin receptor subtypes is differentially regulated in experimental streptozotocin induced type-1 diabetes. *Peptides.* **2006**, 27 (11), 2764–2769. https://doi.org/10.1016/j.peptides.2006.05.008

73. Perekrest, S. V.; Shteintcaig, A. D.; Kawakami, N.; Wekerle H.; Korneva, H. A. Morpho-Functional Characteristics of Hypothalamic Orexin Neurons During Experimental Autoimmune Encephalomyelitis. *Advances in Neuroimmune Biology.* **2014**, 5(3), 171–180. https://doi.org/10.3233/nib-140089

74. Zhang, J.; Jin, K.; Chen, B.; Cheng, S.; Jin, J.; Yang, X.; Lu, J.; Song, Q. Sex-dimorphic functions of orexin in neuropsychiatric disorders. *Heliyon.* **2024**, 10 (16), e36402–e36402. https://doi.org/10.1016/j.heliyon.2024.e36402

75. Somach, R. T.; Jean, I. D.; Farrugia, A. M.; Cohen, A. S. Mild Traumatic Brain Injury Affects Orexin/Hypocretin Physiology Differently in Male and Female Mice. *Journal of Neurotrauma.* **2023**, 40 (19-20), 2146–2163. https://doi.org/10.1089/neu.2023.0125

76. Jöhren, O.; Neidert, S. J.; Kummer, M.; Dominiak, P. Sexually dimorphic expression of prepro-orexin mRNA in the rat hypothalamus. *Peptides.* **2002**, 23 (6), 1177–1180. https://doi.org/10.1016/s0196-9781(02)00052-9

77. Gargiulo, A. T.; Hu, J.; Ravaglia, I. C.; Hawks, A.; Li, X.; Sweasy, K.; Grafe, L. Sex differences in cognitive flexibility are driven by the estrous cycle and stress-dependent. *Frontiers in Behavioral Neuroscience.* **2022**, 16. https://doi.org/10.3389/fnbeh.2022.958301

78. JöhrenO.; Neidert, S. J.; Kummer, M.; Dendorfer, A.; Dominiak, P. Prepro-Orexin and Orexin Receptor mRNAs Are Differentially Expressed in Peripheral Tissues of Male and Female Rats. *Endocrinology.* **2001**, 142 (8), 3324–3331. https://doi.org/10.1210/endo.142.8.8299

79. Lu, J.; Zhao, J.; Balesar, R.; Fronczek, R.; Zhu, Q.-B.; Wu, X.-Y.; Hu, S.-H.; Bao, A.-M.; Swaab, D. F. Sexually Dimorphic Changes of Hypocretin (Orexin) in Depression. *EbioMedicine.* **2017**, 18, 311–319. https://doi.org/10.1016/j.ebiom.2017.03.043

80. Li, T.; Xu, W.; Ouyang, J.; Lu, X.; Sherchan, P.; Lenahan, C.; Irio, G.; Zhang, J. H.; Zhao, J.; Zhang, Y.; Tang, J. Orexin A alleviates neuroinflammation via OXR2/CaMKKβ/AMPK signaling pathway after ICH in mice. *Journal of Neuroinflammation.* **2020**, 17 (1), 187. https://doi.org/10.1186/s12974-020-01841-1

81. Sun, M.; Wang, W.; Li, Q.; Yuan, T.; Weng, W. Orexin A may suppress inflammatory response in fibroblast-like synoviocytes. *Biomedicine & Pharmacotherapy.* **2018**, 107, 763–768. https://doi.org/10.1016/j.biopha.2018.07.159

82. Flores-Ramirez, F. J.; Illenberger, J. M.; Pascasio, G.; Matzeu, A.; Mason, B. J.; Rémi Martin-F. Alternative use of suvorexant (Belsomra®) for the prevention of alcohol drinking and seeking in rats with a history of alcohol dependence. *Frontiers in Behavioral Neuroscience.* **2022**, 16. https://doi.org/10.3389/fnbeh.2022.1085882

83. Wiskerke, J.; James, M. H.; Aston-Jones, G. The orexin-1 receptor antagonist SB-334867 reduces motivation, but not inhibitory control, in a rat stop signal task. *Brain Research.* **2020**, 1731, 146222. https://doi.org/10.1016/j.brainres.2019.04.017

84. Wu, C.-H.; Tsai, T.-H.; Su, Y.-F.; Zhang, Z.-H.; Liu, W.; Wu, M.-K.; Chang, C.-H.; Kuo, K.-L.; Lu, Y.-Y.; Lin, C.-L. Traumatic Brain Injury and Substance Related Disorder: A 10-Year Nationwide Cohort Study in Taiwan. *Neural Plasticity.* **2016**, 2016, 1–8. https://doi.org/10.1155/2016/8030676

85. McKinlay, A.; Grace, R.; Horwood, J.; Fergusson, D.; MacFarlane, M. Adolescent Psychiatric Symptoms Following Preschool Childhood Mild Traumatic Brain Injury. *Journal of Head Trauma Rehabilitation.* **2009**, 24 (3), 221–227. https://doi.org/10.1097/htr.0b013e3181a40590

86. Ilie, G.; Mann, R. E.; Hamilton, H.; Adlaf, E. M.; Boak, A.; Asbridge, M.; Rehm, J.; Cusimano, M. D. Substance Use and Related Harms Among Adolescents With and Without Traumatic Brain Injury. *Journal of Head Trauma Rehabilitation.* **2015**, 30 (5), 293–301. https://doi.org/10.1097/htr.0000000000000101

87. Calarco, C. A.; Lobo, M. K. Depression and substance use disorders: Clinical comorbidity and shared neurobiology. *International Review of Neurobiology.* **2021**, 157, 245–309. https://doi.org/10.1016/bs.irn.2020.09.004

88. Shi, T.; Min, M.; Sun, C.; Zhang, Y.; Liang, M.; Sun, Y. Does insomnia predict a high risk of cancer? A systematic review and meta-analysis of cohort studies. *Journal of Sleep Research.* **2020**, 29 (1), https://doi.org/10.1111/jsr.12876

89. Chang, S.-L.; Durocher, F.; Diorio, C. Sleep quality traits correlate with inflammatory markers in the breast tissue of women. *Cytokine.* **2022**, 160, 156028–156028. https://doi.org/10.1016/j.cyto.2022.156028

90. Zielinski, M. R.; Gibbons, A. J. Neuroinflammation, Sleep, and Circadian Rhythms. *Frontiers in Cellular and Infection Microbiology.* **2022**, 12. https://doi.org/10.3389/fcimb.2022.853096

91. Tong, H.; Li, J.; Feng, Y.; Li, S.; Qiu, H.; Hong, J. The Association Between Sleep Disturbance and Proinflammatory Markers in Patients With Cancer. *Cancer Nursing.* **2022.** https://doi.org/10.1097/ncc.0000000000001055

92. Kishida, M.; Kazunori, I.; Tomohisa, H.; Tada, N.; Nobutaka, K.; Shoda, J.; Kita, K.; Kawakami, K. Orexin 2 receptor as a potential target for immunotoxin and antibody-drug conjugate cancer therapy. *Oncology Letters.* **2011**, 3 (3), 525–529. https://doi.org/10.3892/ol.2011.528

93. Blanco, M.; García-Caballero,T.; Fraga, M.; Gallego, R.; Cuevas, J.; Forteza, J.; Beiras, A.; Diéguez, C. Cellular localization of orexin receptors in human adrenal gland, adrenocortical adenomas and pheochromocytomas. *Regulatory Peptides.* **2001**, 104 (1-3), 161–165.

https://doi.org/10.1016/S0167-0115(01)00359-7

94. Marcos, P.; Coveñas, R. Involvement of the Orexinergic System in Cancer: Antitumor Strategies and Future Perspectives. *Applied Sciences.* **2023**, 13 (13), 7596–7596. https://doi.org/10.3390/app13137596

95. Dayot, S.; Speisky, D.; Couvelard, A.; Bourgoin, P.; Gratio, V.; Cros, J.; et al. In vitro, in vivo and ex vivo demonstration of the antitumoral role of hypocretin-1/orexin-A and almorexant in pancreatic ductal adenocarcinoma. *Oncotarget.* **2018**, 9 (6), 6952–6967. https://doi.org/10.18632/oncotarget.24084

96. Suo, L.; Chang, X.; Zhao, Y. The Orexin-A-Regulated Akt/mTOR Pathway Promotes Cell Proliferation Through Inhibiting Apoptosis in Pancreatic Cancer Cells. *Frontiers in Endocrinology.* **2018**, 9. https://doi.org/10.3389/fendo.2018.00647