Peer review

Papamichail, Nina. 2025. “The Orexin/Hypocretin System and Its Role in the Etiology of Psychiatric Deficits Following Traumatic Brain Injury.” *Journal of High School Science* 9 (1): 183–208.

The manuscript is well researched and well presented. However, I found that substantial contant was left out; especially when addressing mechanisms. The manuscript also did not put forward or speculate new or different ways to think about this content. All the content presented is obtained from the public domain and hence does not significantly add to the corpus of knowledge in this field. The manuscript does not satisfy the Journal’s expectations for a review paper found here: https://jhss.scholasticahq.com/for-authors, types of manuscripts, review manuscripts. Hence, it is not acceptable for publication as written. However, if the author addresses my comments below, it can then be considered for publication. I encourage the author to address these comments and submit the revised manuscript for review.

1.sexual dimorphism of orexin needs discussion along with if there is a common (temporal and/or spatial) pattern of expression post TBI in females and in males and the strength of the association with psychological disorders in the sexes. See: https://doi.org/10.1016/j.heliyon.2024.e36402

2.decreased orexin associated with the Pandemrix vaccine needs discussion along with the mechanism(s). see: https://doi.org/10.1093/sleep/zsae014, https://doi.org/10.1016/j.jneuroim.2024.578383, https://doi.org/10.1126/scitranslmed.3007762 (retracted), there may be other references. Please perform a thorough search of the literature.

3.From point 2, autoimmune disorders and orexin expression may be associated. See for example, https://doi.org/10.1016/j.peptides.2006.05.008; this poses the question whether psychological symptoms pose TBI are more severe in individuals who suffer from autoimmune diseases such as type 1 diabetes or arthritis or MS… Needs discussion in the manuscript.

4.discussion on “…. orexin should be considered essentially as a neurotransmitter inhibiting REM sleep and, to a much lesser extent, a wake promoting agent……” is necessary in the manuscript with implications for psychological disorders that implicate the REM/NREM ratio.
See: https://doi.org/10.3390/nu15173679, and https://doi.org/10.3389/fnins.2018.00892

5.Orexins modulate carcinogenesis pathways. How might orexin agonist or antagonist drugs affect the risk of developing cancer ? Discuss in the manuscript.
See: https://doi.org/10.3390/app13137596, https://doi.org/10.3390/cells13151246

6.Discuss the following in the manuscript: After TBI, it may be safely assumed that patients are administered analgesics and/or anti-inflammatory agents. How much of this can be attributed to the decrease in orexin levels (given that orexin also modulates analgesic and inflammatory responses). Is there an inverse temporal correlation betweeen orexin and administered analgesic/antinflammatory drugs post TBI? Please discuss in the manuscript.

7.Orexin blockers reduce the motivation for Substance use (see: https://www.scripps.edu/news-and-events/press-room/2023/20230110-martin-fardon-alcohol.html, https://doi.org/10.1159/000514965. TBI increases the risk of alcohol abuse (see: https://doi.org/10.1089/neu.2008.0849) and substance abuse is linked to psychological disorders (numerous references). Discuss the possibility that orexin

blockers may improve post TBI psychological disorders (such as depression) at least partly by decreasing the motivation for substance abuse.
Please make sure you include all the references in your revised manuscript.

The following comments were given, and the following paragraphs address those comments:

1. Sexual dimorphism of orexin needs discussion along with if there is a common (temporal and/or spatial) pattern of expression post TBI in females and in males and the strength of the association with psychological disorders in the sexes.

It is worth noting that sexual dimorphism of orexin expression has been observed between male and female rats (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 notably higher in male rats than in females (78). Differences in Orexin neuron activity between males and females have also occurred 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 that different sexes could exhibit different psychiatric outcomes.

2. Decreased orexin associated with the Pandemrix vaccine needs discussion along with the mechanism(s):

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 gave mice an injection of the vaccination during prepubescence and then 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 implicating the Pandemrix vaccine during adolescence with orexin expression (66). A mechanism for the subsequent loss of orexin post Pandemrix vaccination and the resulting narcolepsy could be a result of H1N1-specific T cells targeting orexin-producing neurons: components of the vaccination or antigens of the virus may lead to an immune response that then activate autoreactive cytotoxic T cells which target orexin neurons (68). It has been hypothesized that the loss of orexin after the vaccination occurs due to an immune response triggered by similarities between the influenza A nucleoprotein in the Pandemrix vaccine and an extracellular domain of OXR2, however, it was found in one study that there were no specific antibody or immune responses against orexin receptors in vaccinated individuals (67).

3. From point 2, autoimmune disorders and orexin expression may be associated; this poses the question whether psychological symptoms pose TBI are more severe in individuals who suffer from autoimmune diseases such as type 1 diabetes or arthritis or MS… Needs discussion in the manuscript.

Orexin has been found to be 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 have these diseases. A study on streptozotocin-injected rats, a model for type-1 diabetes, revealed that hypothalamic orexin mRNA levels were significantly reduced in diabetic rats compared to non-diabetic controls. Alongside this, 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 the disease. Furthermore, a study using experimental autoimmune encephalomyelitis (EAE), a rat model of multiple sclerosis, revealed a reduction in the number of orexin-producing neurons in specific regions of the hypothalamus; these findings suggest that EAE disrupts the balance between orexin production and utilization, with orexin being consumed at a faster rate than it is 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.

4. Discussion on "…. orexin should be considered essentially as a neurotransmitter inhibiting REM sleep and, to a much lesser extent, a wake promoting agent……" is necessary in the manuscript with implications for psychological disorders that implicate the REM/NREM ratio.

Orexin is believed to be primarily a REM sleep inhibitor (69). The activity of Orexin is lowest at the time of waking and highest right at 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. This aligns with what can be observed in 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 with the loss of Orexin neurons. Moreover, disordered sleep is commonly observed in cases of depression, and can be partly characterized by disinhibition 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.

5. Orexins modulate carcinogenesis pathways. How might orexin agonist or antagonist drugs affect the risk of developing cancer ? Discuss in the manuscript.

Beyond the role they may play in treating sleep and mood disorders, Orexin's effect on the development of cancer is worth considering. 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; research has demonstrated that 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 has been seen to cause 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 offer a novel treatment approach.

6. Discuss the following in the manuscript: After TBI, it may be safely assumed that patients are administered analgesics and/or anti-inflammatory agents. How much of this can be attributed to the decrease in orexin levels (given that orexin also modulates analgesic and inflammatory responses).

After TBI, patients are often administered anti-inflammatory agents to manage inflammation, and a decrease in Orexin levels may further contribute to this need due to the effect they have on inflammation. 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 like IL-1β, IL-6, and IL-8, as well as inhibiting the nuclear factor-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.

7. Orexin blockers reduce the motivation for Substance use. TBI increases the risk of alcohol abuse and substance abuse is linked to psychological disorders. Discuss the possibility that orexin blockers may improve post TBI psychological disorders (such as depression) at least partly by decreasing the motivation for substance abuse.

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, SB-334867, 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 substance use.

Thank you for addressing my comments. I have taken the liberty to add the highlighted sections in the abstract and in the conclusion so that you have an actionable recommendation in your paper. Please change as necessary. You can also pen a different alternative. This is necessary so that your manuscript confirms to the expectations of a review paper as described here;

https://jhss.scholasticahq.com/for-authors, types of manuscripts, review papers.
Please resubmit when done. I look forward to the resubmission.

As far as I am aware I needed to re-submit with this section added:

"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."

The new document has this included.

Thank you for addressing my comments. Accept.