nature portfolio

Peer Review File

Orexin neurons inhibit sleep to promote arousal

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Editorial Note: This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at Nature Communications.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Orexin has clear sleep-suppressive effects, yet the mechanisms/circuit through which orexin acts to inhibit sleep have not been defined. It is known that orexin induces wake, that orexin delivered to the VLPO induces wake, and that orexinergic neurons release glutamate in addition to orexin. Both glutamate and orexin are both excitatory, so how the excitatory neuromodulator/transmitter could inhibit sleep has been a puzzle. The present work is an elegant and certainly a most robust study of orexin VLPO circuitry. The authors clearly establish that there are two populations of GABAergic neurons in VLPO (VLPOGABA and VLPOGABA/Gal). They show that orexin subtype 2 receptors (OX2R) are found only on the VLPOGABA neurons, which Orexin excites. In contrast, VLPOGABA/Gal neurons do not have OX2R and are inhibited by Orexin in the VLPO. This sets up an orexin excitation of VLPOGABA that then inhibits VLPOGABA/Gal. This is not a surprising circuit, given the background info leading up to this study, but the work is a fantastic example of how robust circuit mapping should be done.

Nothing imperative to change but the following points should be addressed:

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Additionally, orexin, should have unique effects in the VLPO, relative to noradrenaline (NA) and other neuromodulators, as orexin contributes to state (wake and sleep) stabilization. The authors compare the response with the VLPO response to NA, but a contrast would be more insightful. It would be helpful to know whether stimulation of orexinergic neurons results in a more prolonged wake by resulting in a longer activation of the VLPOGABA, relative to stimulation of locus coeruleus neurons. They should be able to comment on duration of wake after optogenetic stim. They may also have data from slice recordings and it should be determined wgether orexin’s effects at VLPOGABA neurons are more prolonged than with NA.

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Reviewer #2 (Remarks to the Author):

Orexin/hypocretin neurons play a major role in wakefulness generation and maintenance, and impaired orexin signaling (mainly by Ox cell loss) produces the sleep disorder narcolepsy. Current circuit models, supported by ample empirical data, suggest that orexins stabilize wakefulness by direct excitatory inputs onto other arousal-promoting systems. While intra-VLPO administration of orexins has been shown to increase wakefulness, suggesting a potential effect on sleep-promoting processes within this region, the precise cellular and circuit mechanisms are unknown.

In this study, the authors conducted an impressive array of experiments including optogenetic stimulation, optogenetic-assisted circuit mapping, in-vitro electrophysiology, and transcriptomics, demonstrating that orexin neurons promote arousal by indirect inhibition of sleep promoting (Galanin+) neurons in the ventrolateral preoptic nucleus of the hypothalamus. The studies were carefully designed and executed, the data are compelling and clearly presented, the manuscript is very well written, and the conclusions are fully supported by the data. The present work is thus novel, highly relevant, scientifically solid, and exciting.

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My sincere complements! I believe this is the first paper I have reviewed in my 50+ years of reviewing that I would say is as close to perfect as you could get. Your work will be a text-book case of how to investigate and describe a neural control circuit. Next challenge is to discover and incorporate the essential feedback loops to make it a regulatory circuit. The text is clearly written, the figures are beautiful, and the conclusions are straight-forward and solidly based on the data. Most significant is that you answer a conundrum in narcolepsy -- why without the arousal stimulus, you still have normal sleep amounts, but fragmented.

Your provocative suggestion at the end that disruptions of this circuit could be involved in parasomnias leads me to wonder what changes/deficits in the circuit could lead to the diversity of parasmonias. This would make an interesting hypothesis paper.

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Decision on Nature Communications NCOMMS-22-02307-T

Senior Editor & the three Reviewers

We thank all three Reviewers for their positive and supportive comments. During the manuscript revision process we have endeavored to address all of the critiques in full. All changes to the manuscript text have been highlighted in **red** to facilitate a rapid re-review.

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We agree with the reviewer, and as was acknowledged, we did address this issue in our original discussion. We know from our work and work by many others that there is considerable redundancy in the brain's arousal circuitry, with only a few 'nodes' having been established as truly necessary for arousal maintenance. We further agree with the reviewer that orexin neurons likely produce arousal - and are of course necessary for proper state stabilization - through multiple projections and that likely many of the post-synaptic 'targets' of orexin neurons contribute to arousal control, but that each 'target' is unlikely completely necessary for arousal maintenance. Our revised manuscript includes a more expansive discussion of the reviewer's excellent point(s) on page 14. Testing this hypothesis, which we fully agree is an important future direction, is however simply beyond the scope of our current project, which we continue to feel represents a substantial literature contribution unto itself.

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It would be helpful to know whether stimulation of orexinergic neurons results in a more prolonged wake by resulting in a longer activation of the VLPO^{GABA}, relative to stimulation of locus coeruleus neurons. They should be able to comment on duration of wake after optogenetic stim.

The reviewer makes a good point, but we would note that it is always difficult to interpret differences in results obtained by studies that use different stimulation paradigms. We found for example that optogenetic stimulation of orexin input within the VLPO rapidly and reliably rouses mice from both NREM and REM sleep, and that the mice did not immediately go back to sleep following the stimulation events. In fact, the opto-evoked arousals were of similar duration to spontaneous arousals. Carter and colleagues (PNAS 2012) as another excellent example, produced arousals in mice when stimulating orexin input to the LC, but unfortunately the authors did not report the length of the opto-evoked wake bouts, making a direct comparison difficult, if impossible.

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We fully agree with the reviewer's statement and hope to make continued contributions to a more complete understanding of the neurobiology of the Ox system.

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