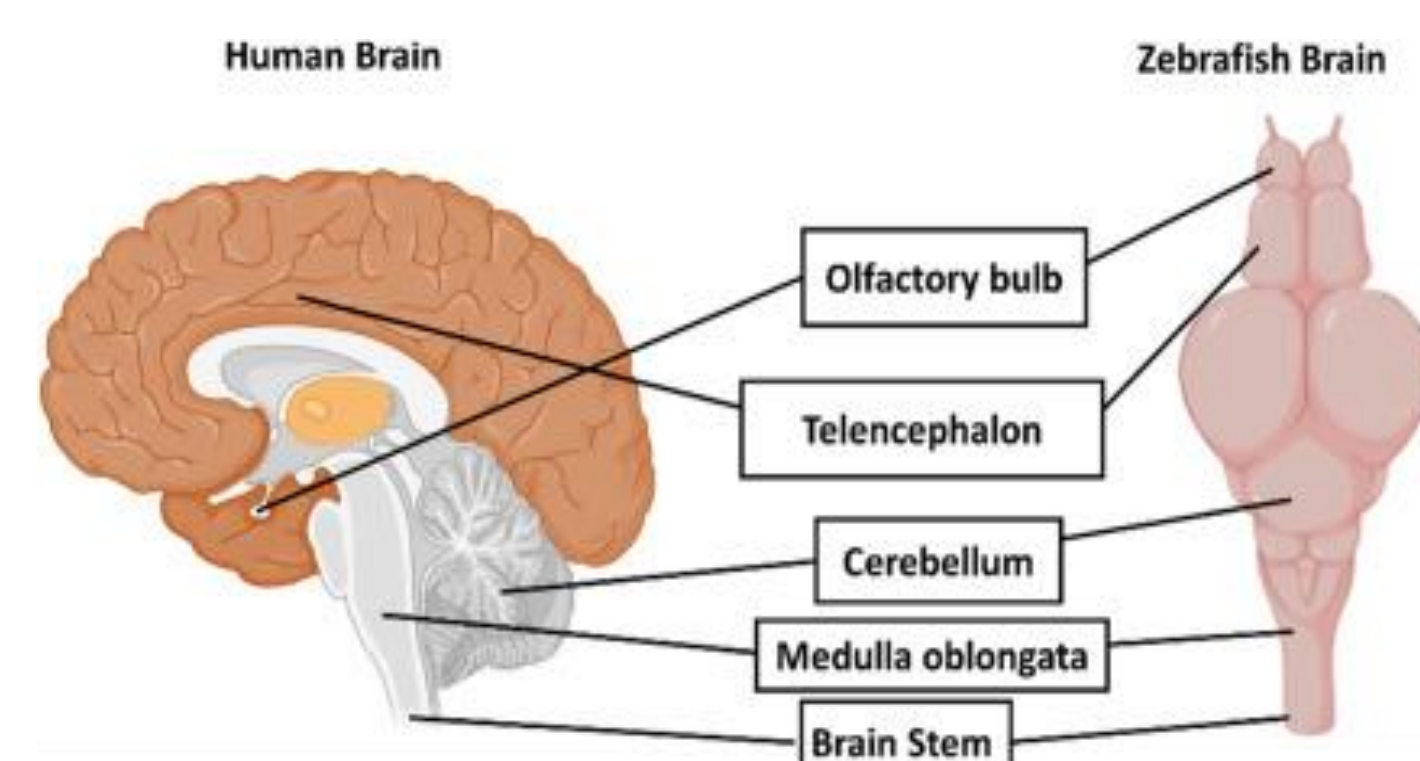




Thank you to Dr. Murugan, Sonia Karkare, and Max Cao!

Background

REM (rapid eye movement) sleep is a stage of the sleep cycle crucial for memory consolidation and learning and widely explored in mammals. REM is responsible for increasing heart rate and breathing and is generated by specialized brainstem nuclei and pontine cholinergic and glutamatergic networks (Patel et al., 2024). However, Zebrafish are unique in that they lack the mammalian brainstem structures for REM yet are still able to form a similar REM-like state known as propagating wave sleep (PWS). The PWS produces wave-like brain activity, rapid eye movements, and neural waves, giving the zebrafish comparable REM-like abilities despite a very different brain structure (Leung et al., 2019).



Due to the transparent, genetically accessible nature of larval zebrafish, they easily allow for testing and scanning of the whole brain and cellular imaging during sleep. Nonetheless, although previous studies have shown that zebrafish can exhibit REM, the specific brain regions and cell populations that initiate and sustain REM-like sleep remain unknown.

To test this, three key regions of the zebrafish brain are monitored.

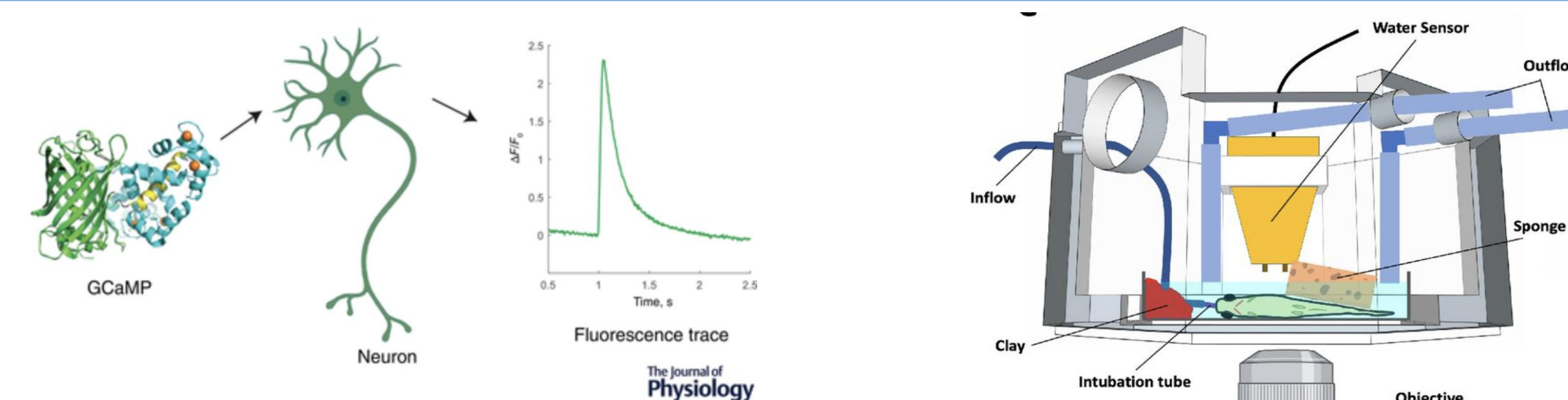
1. The **ventral prefrontal** which is a region that shows increase activity and acts as a hindbrain to produce REM-like waves across the brain.
2. The **ventral hypothalamus** which contains melanin-concentrating hormone neurons indicating a strong REM-like activity and activates at the onset of PWS.
3. The **dorsal pallidum** which displays desynchronized wave-like firing activity during PWS.

Question & Hypothesis

Question: Which brain regions and cell types in the zebrafish brain are responsible for initiating and generating REM-like propagating wave sleep (PWS)?

Hypothesis: We hypothesize that the ventral hypothalamus is sufficient to trigger the onset of PWS, while the ventral prefrontal region is required to maintain the propagating wave dynamics that are characteristic of PWS.

Methods



Calcium Imaging

1. Diazepam and melatonin are administered before imaging to induce and promote PWS sleep in subjects
2. Perform calcium imaging across the brain in zebrafish during wakefulness, slow-wave sleep, and episodes of PWS
3. Quantify % of active neurons in 3 ventral prefrontal, ventral hypothalamus, and dorsal pallidum
4. Compare brain-wide activity maps during wakefulness, slow-wave sleep, and REM-like sleep. Neurons that show a spike in activity before and during REM-like activity are the most likely REM-initiation centers.

Testing sufficiency of optogenetic activation of ventral hypothalamus (channelrhodopsin)

1. Split into two groups. One group will have Channel rhodopsin inhibited, while the other will be normal
2. During non-PWS sleep, deliver blue-light pulses every 5 seconds targeted to ventral hypothalamus while recording behavior/ brain activity
3. Score whether each pulse triggers a transition into PWS
4. Compare the fraction of light pulses that trigger PWS in channel-expressing fish versus inhibited fish

Results

REM-Related Neural Activity by Brain Region

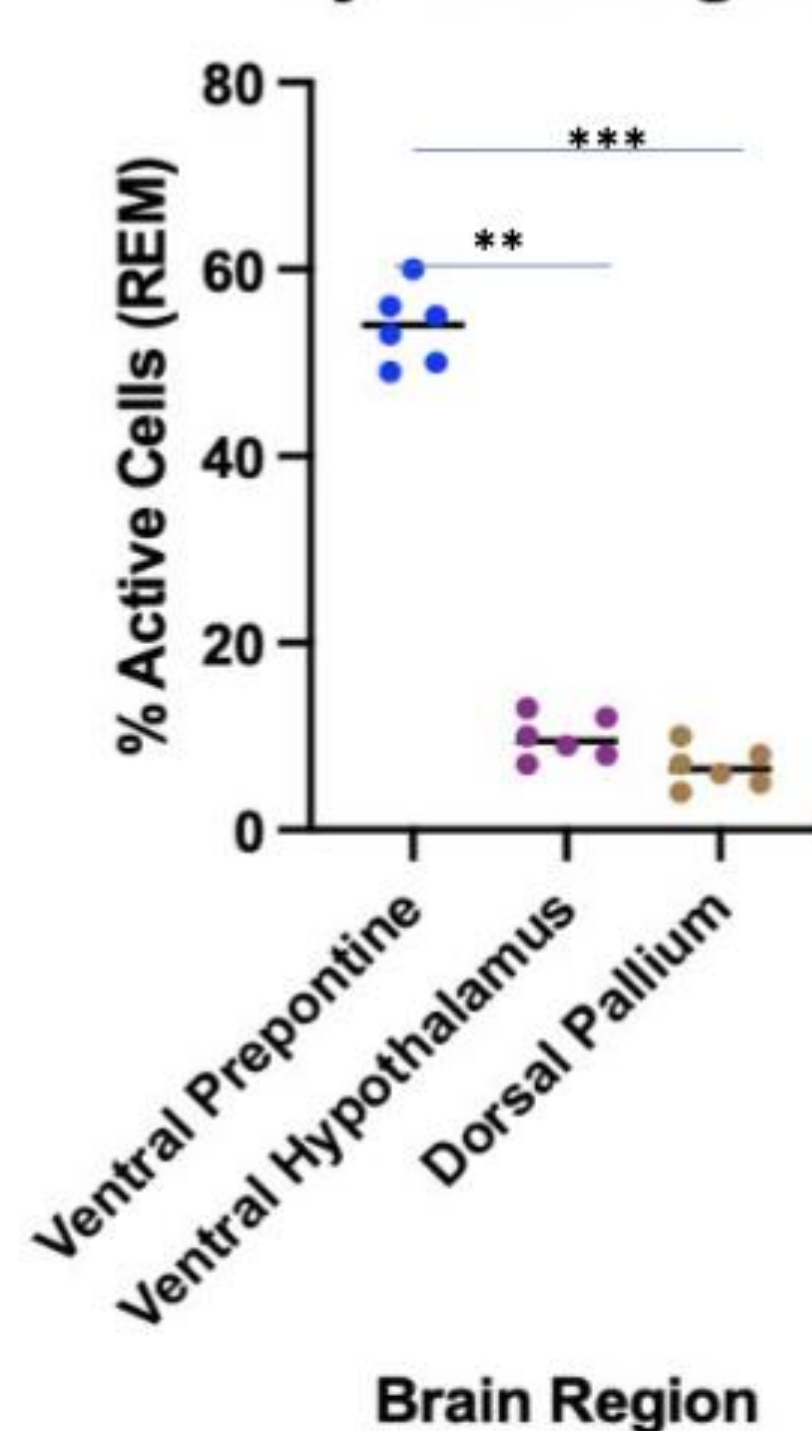


Figure 1: Neuronal activity during REM sleep across three brain regions. Ventral prefrontal shows the highest % of recorded cells active during REM, whereas ventral hypothalamus and dorsal pallidum show lower activation. one-way ANOVA, $F(2, 27) = 52.4, p < 0.0001$; ventral prefrontal > ventral hypothalamus > dorsal pallidum (Tukey post-hoc).

Pre-REM Neural Activity by Brain Region

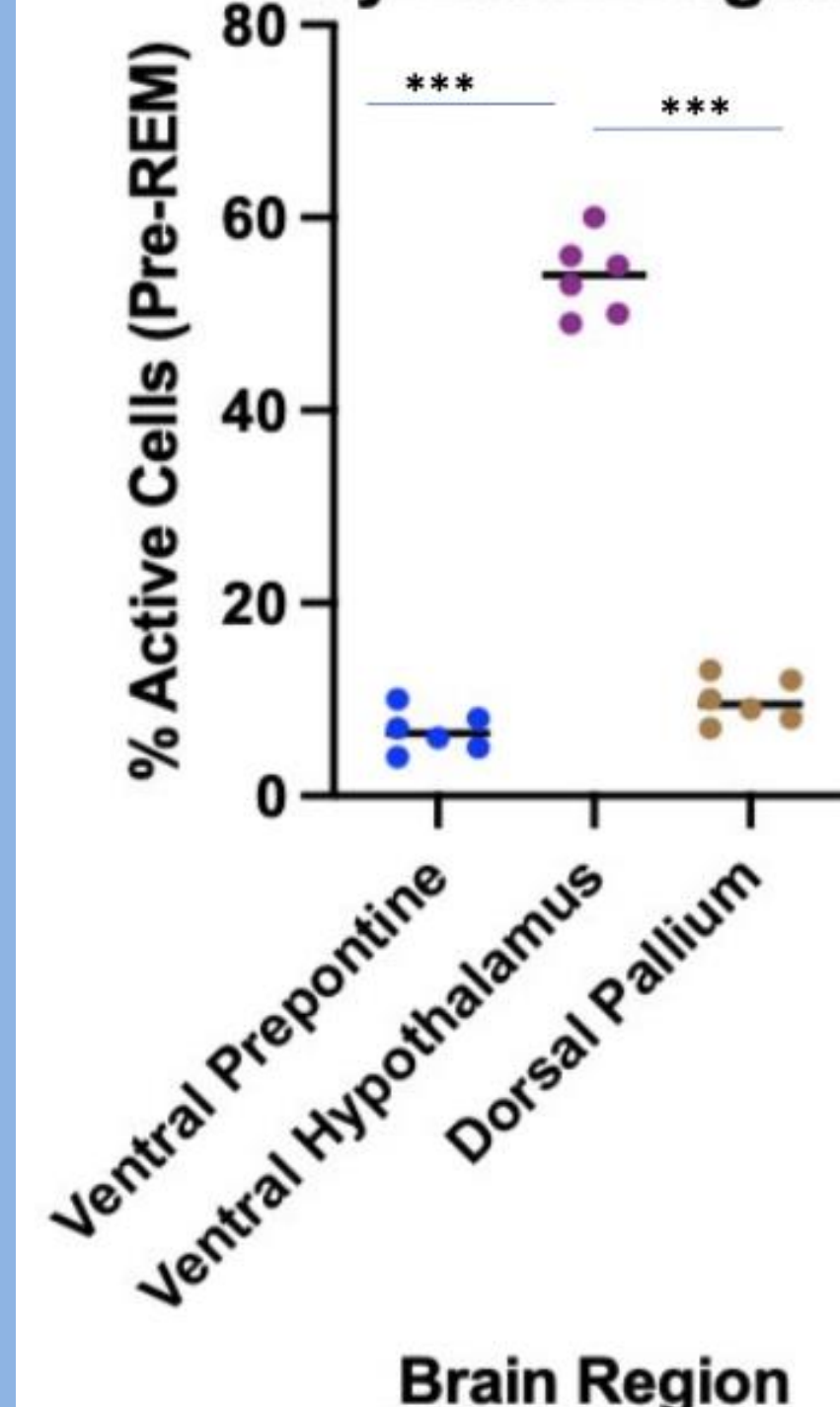


Figure 2: Neuronal activity immediately preceding REM sleep across three brain regions. The ventral hypothalamus shows increased activity just before REM onset, suggesting it may act as a pre-REM trigger, while the ventral prefrontal and dorsal pallidum show lower pre-REM activity. one-way ANOVA, $F(2, 27) = 52.4, p < 0.0001$; ventral prefrontal > ventral hypothalamus > dorsal pallidum (Tukey post-hoc).

Optogenetic Activation of Ventral Hypothalamus

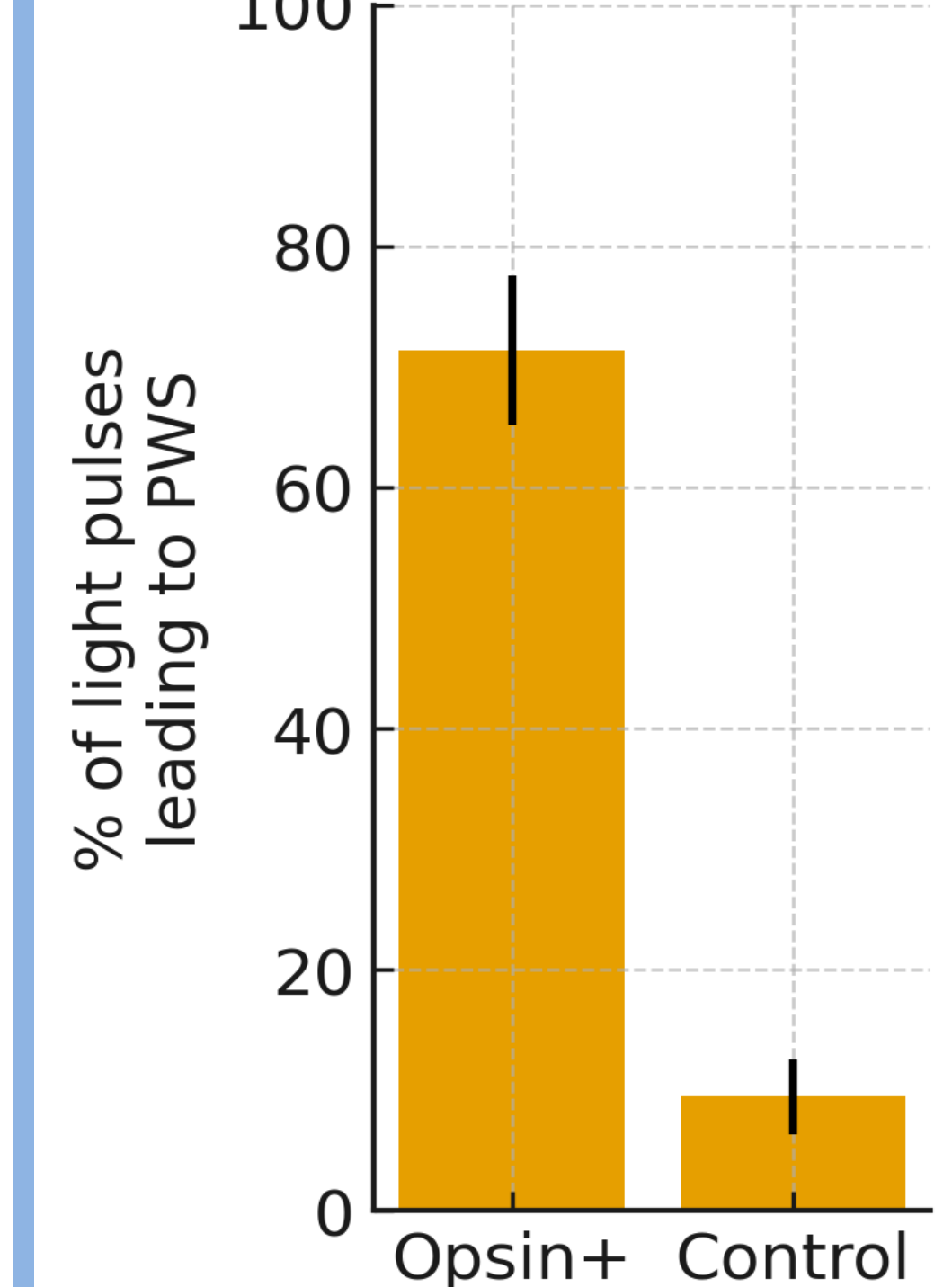


Figure 3: Brief optogenetic activation of ventral hypothalamus neurons during non-PWS sleep triggered PWS in $71.4 \pm 6.2\%$ of light pulses in opsin-expressing fish, compared with $9.5 \pm 3.1\%$ in control siblings. A χ^2 test showed a significant difference between groups ($\chi^2(1, N = 84) = 28.7, p < 0.0001$).

Future Directions

Manipulation of Pre-REM and REM circuits

- In fish expressing light-sensitive channels such as Channelrhodopsin or Halorhodopsin, brief pulses of light can probe whether ventral hypothalamus activation alters the probability of PWS initiation and how it affect subsequent prefrontal activity. These manipulations will help define how initiation and maintenance circuits interact.

Neural Circuit Tracing

- Use anterograde tracing to map downstream targets of ventral hypothalamus neurons and identify the brain regions involved in supporting or maintaining PWS. Complement this with retrograde tracing to reveal the upstream inputs that drive VPH activation prior to PWS onset. These approaches will outline broader circuit controlling sleep-state transitions.

Cross-State Dynamics

- Examine how PWS-initiating neuron interact with neural activity during other sleep states like NREM-like sleep to better understand transitions between states.
- Future work can also test whether the same hypothalamic neurons participate across multiple sleep stages or whether distinct subpopulations specialize in PWS initiation versus NREM-like regulation.

Conclusion

Our results support a model in which Ventral Prefrontal and Ventral Hypothalamus regions contribute to both the initiation and maintenance of REM-like sleep (PWS) states in zebrafish.

- Ventral hypothalamus neuronal activity increases right before PWS onset, indicating it is a likely initiator of REM-like sleep
- Ventral prefrontal region results in the highest activity during PWS onset, indicating it plays a role in sustaining REM-like activity
- Optogenetic activation of ventral hypothalamus neurons is sufficient to trigger transitions into PWS, further supporting its role as an initiator
- The dorsal pallidum region shows relatively low activation in both phases, therefore being unlikely to contribute (if any) to PWS and REM-like activity.

Together, these findings provides insight for how REM-like sleep may be organized in the zebrafish brain. Additionally, it supports that conserved mechanisms of sleep-state control could potentially exist across vertebrates despite absence of conserved anatomy. Further causal experiments will be required to test necessity and circuit-level pathways underlying PWS.

Limitations

- Artificially induced sleep patterns could be a potential confounder
- Calcium imaging tests and optogenetics test sufficiency but not necessity of regions

References

- <https://www.ncbi.nlm.nih.gov/books/NBK526132/>
- <https://pubmed.ncbi.nlm.nih.gov/31292557/>
- <https://www.biorxiv.org/content/10.1101/2021.03.19.436170v2.full>
- <https://physoc.onlinelibrary.wiley.com/doi/full/10.1113/JP28383>
- <https://www.nature.com/articles/s41586-019-1336-7>
- <https://pubmed.ncbi.nlm.nih.gov/26003945/>
- <https://blog.addgene.org/using-aav-for-neuronal-tracing>
- <https://www.nature.com/articles/s41467-019-11936-w?utm>
- https://www.researchgate.net/figure/Melatonin-and-diazepam-affect-locomotor-activity-in-zebrafish-via-specific-membrane-fg4_247044183