Octopaminergic Sleep Regulation in the Fruit Fly

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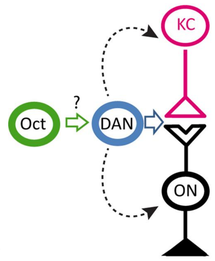
Purpose

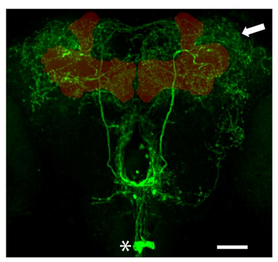
- To combine genetic manipulations and behavioral assessments on the fruit fly model to investigate sleep/wake promoting neurocircuits and pathways by which octopamine (OA), an invertebrate homolog of norepinephrine (NE), regulates sleep.

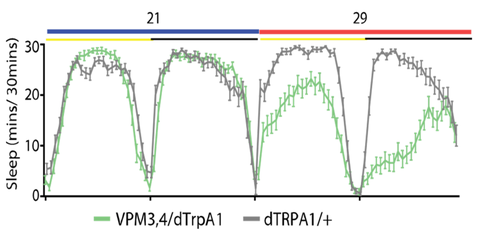
Background

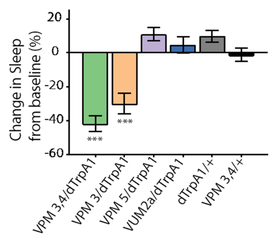
- The Fruit Fly (*Drosophila melanogaster*) serves as a great model organism for neural investigations due to their relatively small neural network, their short life cycle, and the molecular, genetic, and behavioral approaches available to understand underlying mechanisms of sleep regulation.
- The fruit fly shares many characteristics with human sleep, including well established diurnal sleep/wake cycles, homeostatic reaction to sleep deprivation, and an elevated arousal threshold (Nall & Sehgal, 2014).
- Many classes of OA neurons project to the mushroom body (MB), a region of the fly brain that shares similarities with the mammalian neocortex known to be critical for propagation of homeostatic sleep signals (Sitaraman et al., 2015).
- Subset of OA releasing neurons in the ventral paired median (OA-VPM 4-6) identified as a **wake promoting signal** in the fly brain.
  - Activation of OA = wakefulness promotion
  - Inhibition of OA = sleep promotion (Crocker & Sehgal, 2008)
- The details of communication in neural circuits involving OA and MB on sleep and arousal remain unclear.
- Anatomical analysis reveals putative interactions with the dopamine sleep circuit in the fly brain.

One of the mechanisms by which NE is hypothesized to promote wakefulness is by providing excitatory synaptic input to wake-promoting DA neurons (reviewed in Mitchell and Weinshenker, 2010).



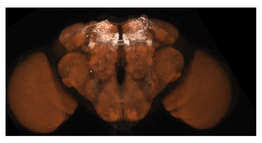




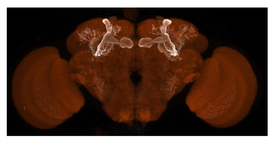


Gal4 Expression Patterns

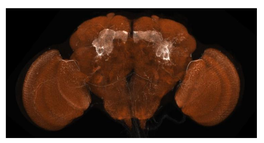
58E02-Gal4



35B12-GAL4

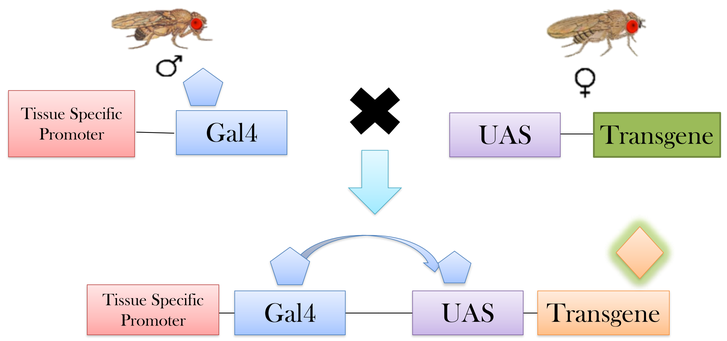


14H06-GAL4

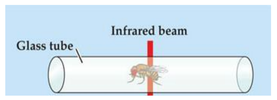


Methods

GAL4-UAS System: Two part-system used to express target genes, where UAS (Upstream Activating Sequences) serves as the binding site for GAL4, a promoter for the transcription of the desired gene. Thus, genes under control of UAS are only expressed in cells containing GAL4.



Sleep Experiments



- Set up genetic crosses
- Collected male progeny
- Loaded males into sleep tubes, which are placed in Drosophila Activity Monitors (DAMs) within an incubator
- Experiment lasts 5 days total:
  - Days 1 & 2: Entrainment
  - Day 3: Baseline
  - Day 4: Temperature Elevation
  - Day 5: Recovery

- Temperature change on Day 4 activates and/or inhibits targeted neurons that may be implicated in sleep behavior.
  - Experiment 1: dTrpA1 activation of OA receptor neurons.
  - Experiment 2: Activation of OA-releasing neurons (24E06) and simultaneous inhibition of downstream targets.
- Sleep was quantified as *D. melanogaster* inactivity tracked using Drosophila Activity Monitor System.
- Data was analyzed using MATLAB software.

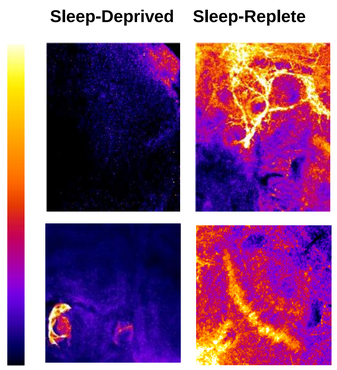
Sleep Deprivation

- CaLexA was crossed to 24E06-Gal4 and progeny was collected.
  - Ca^{2+} is an indicator of neuronal activity and allows a quantification of OA-VPM activity.
- Experimental flies were mechanically shaken for a 16 hour period to induce acute sleep deprivation.
- Control flies slept normally on a 12 hour light/dark cycle.
- Brains were dissected in 1xPBS, fixed in 2% PFA, and had undergone IHC steps adapted from Janelia FlyLight Protocols.

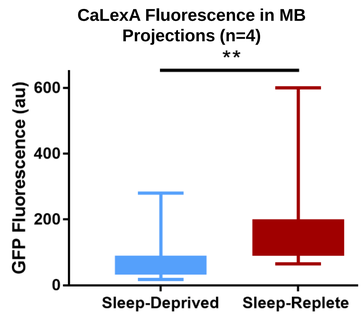
Results

Sleep Deprivation Alters OA-VPM Activity

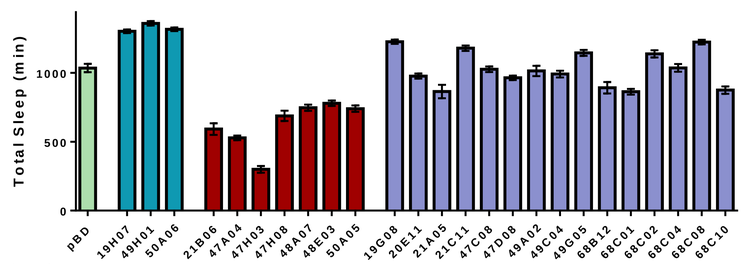
CaLexA in the Octopamine VPM Neurons Using the R24E06 Driver



Top Row: Mushroom Body Neurons
Bottom Row: Central Complex Neurons

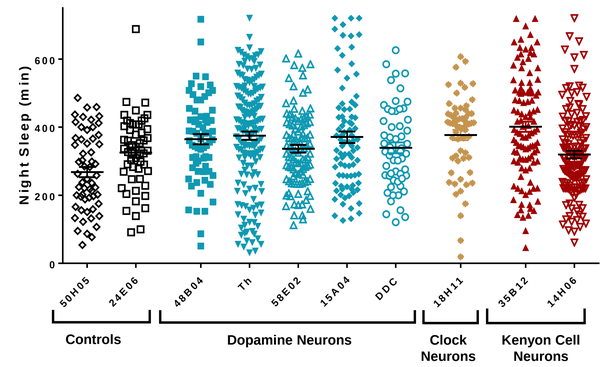


OA Receptor Neuron Activation Effects on Sleep



Control: pBD
OAMB Lines: 47A04, 47C08, 47D08, 47H03, 47H08, 48A07, 48E03, 49A02, 49C04, 49G05, 49H01, 50A05, 50A06
Octβ1R Lines: 19G08, 19H07, 20E11, 21C11, 21A05, 21B06
Octβ2R Lines: 68C04, 68C08, 68C10
Octβ3R Lines: 68C01, 68C02, 68B12

OA-VPM Activation and Downstream Inhibition Effects on Sleep



Summary and Implications

- Sleep-deprived flies displayed significantly reduced OA-VPM neuronal activity than sleep-replete flies.
  - This supports the finding that OA is wake-promoting because flies that are sleep-deprived are expected to rebound from sleep loss: OA levels are lowered → flies sleep more.
- Through a screen of multiple lines with downstream OA receptor neuron expression, we have identified some to be sleep-promoting and sleep-suppressing when activated.
  - Future direction: To activate OA-VPM neurons and inhibit the activity of these downstream OA receptor neuron targets.

References

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