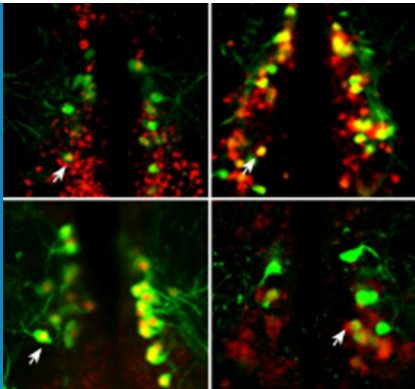


## TRANSCRIPTOMICS

# Resting easy with a sleep regulator

**Potassium ion channels in a subset of neurons in the brain of zebrafish may have a role in promoting sleep.**

WILLIAM J GIARDINO AND LUIS DE LECEA



**Related research article** Yelin-Bekerman L, Elbaz I, Diber A, Dahary D, Gibbs-Bar L, Alon S, Lerer-Goldshtein T, Appelbaum L. 2015. Hypocretin neuron-specific transcriptome profiling identifies the sleep modulator *Kcnh4a*. *eLife* 4:e08638. doi: [10.7554/eLife.08638](https://doi.org/10.7554/eLife.08638)

**Image** Mutations in the *Kcnh4a* gene reduce sleep in zebrafish.

the regulation of arousal (Yelin-Bekerman et al., 2015).

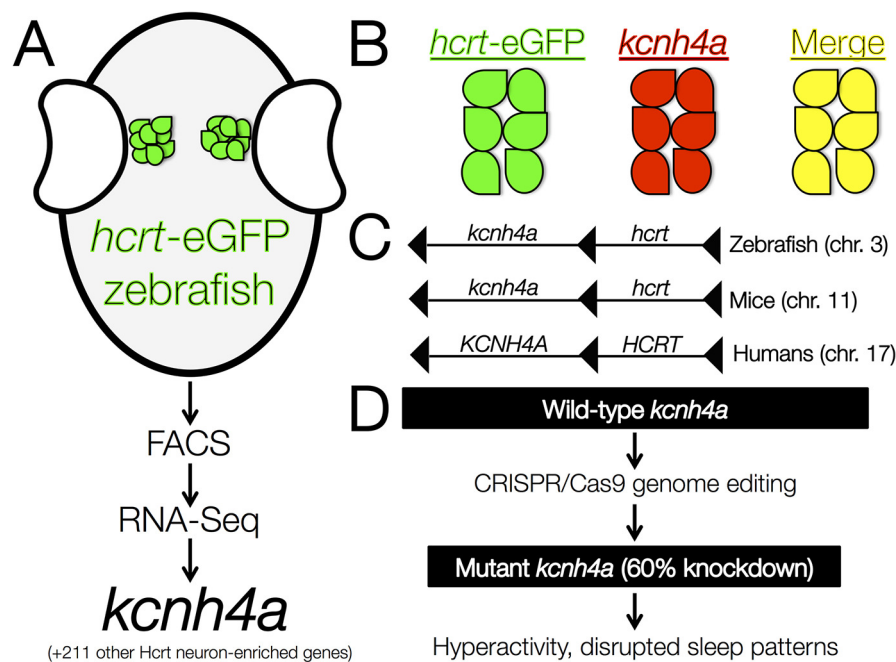
Yelin-Bekerman et al. – who are based at Bar-Ilan University, Toldot Genetics, the Weizmann Institute of Science and the Massachusetts Institute of Technology – studied zebrafish (*Danio rerio*), which display decreased movement patterns during the night that are characteristic of sleep. Moreover, they only have 16–20 Hcrt neurons, compared to more than 5000 cells in mammals. Using transgenic zebrafish that express enhanced green fluorescent protein only in their Hcrt neurons (*hcrt-eGFP*) (Faraco et al., 2006), Yelin-Bekerman et al. used a technique called fluorescence-assisted cell sorting to isolate the *hcrt-eGFP* cells and extract messenger RNA (Figure 1A). They then amplified the RNA material before carrying out whole-transcriptome sequencing to measure gene expression. Applying their most conservative criteria, Yelin-Bekerman et al. generated a list of 20 gene transcripts that are found at higher levels in Hcrt neurons than in other brain cells. These genes included *fam46a* and other markers of Hcrt neurons that have previously been reported in higher vertebrates (Dalal et al., 2013). With a more relaxed threshold, 212 transcripts met the criteria for being enriched in Hcrt neurons.

Using *in situ* hybridization, Yelin-Bekerman et al. then tested the degree to which the *hcrt-eGFP* and the 20 gene transcripts co-localize: they were able to confirm that several of the candidates are highly expressed within Hcrt neurons, and that others were actually expressed in nearby non-Hcrt neurons instead. Further analysis revealed that many of the genes contained conserved motifs for the binding of transcription

We are able to sleep as a result of a delicate balance between competing signals from complex circuits of neurons. In the hypothalamus, neurons that produce a neuropeptide called hypocretin (Hcrt; also known as orexin) control the transition between being asleep and being awake (Adamantidis et al., 2007; de Lecea, 2015). These neurons also coordinate waking up (arousal) in response to various stimuli (Giardino and de Lecea, 2014; Bonnavion et al., 2015). Thus, finding out how the activity of Hcrt neurons is regulated will be vital for understanding how these neurons maintain a healthy balance between the sleep and the awake states, and how this equilibrium is disturbed in individuals with sleep disorders. Now, in eLife, Lior Appelbaum and colleagues – including Laura Yelin-Bekerman as first author – report a new role for a potassium ion channel in

© Copyright Giardino and de Lecea. This article is distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use and redistribution provided that the original author and source are credited.

© Copyright Giardino and de Lecea. This article is distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use and redistribution provided that the original author and source are credited.



**Figure 1.** The *kcnh4a* gene is enriched in Hcrt neurons and promotes sleep in zebrafish. (A) Fluorescent-assisted cell sorting (FACS) of neurons from *hprt-eGFP* zebrafish followed by all-mRNA sequencing (RNA-seq) identified 212 genes that are significantly enriched in Hcrt neurons, including the voltage-gated potassium channel *kcnh4a*. (B) In situ hybridization for *kcnh4a* confirmed its expression in 100% of *hprt-eGFP* neurons. (C) Genomic analyses revealed that *kcnh4a* lies directly upstream of the *hprt* gene, a phenomenon that is conserved from zebrafish to humans. (D) CRISPR/Cas9 genome editing produced a mutant zebrafish with a 60% reduction in *kcnh4a* levels, resulting in hyperactivity and disrupted patterns of nighttime sleep.

factors, such as *pax4*, which is predicted to regulate as many as 44 genes in Hcrt neurons.

Of the transcripts that were verified as being enriched in Hcrt neurons, Yelin-Bekerman et al. focused on one called *kcnh4a*, which was the only one that was expressed in all *hprt-eGFP* neurons in both zebrafish larvae and adults (Figure 1B). It encodes a potassium channel and further genetic analyses revealed that *kcnh4a* is located directly upstream of the *hprt* gene in the zebrafish genome. In fact, these two genes are directly adjacent to each other throughout the animal kingdom, including in mice and humans (Figure 1C). The expression patterns and tight genetic linkage of the two genes suggests that well-preserved mechanisms govern their interactions at the protein level.

Pursuing this rationale, Yelin-Bekerman et al. examined whether the Kchn4a protein is important for behaviors that are regulated by Hcrt neurons (Figure 1D). The authors used CRISPR/Cas9 genome editing to produce mutant zebrafish that had reduced levels of *kcnh4a* expression. Compared to wild-type zebrafish, these mutants showed significantly increased

movement in both the light and dark periods of a 24-hour cycle. In further analyses in which sleep was defined as a period of inactivity that is longer than six seconds (Yokogawa et al., 2007), Yelin-Bekerman et al. reported decreased overall sleep time and shorter average bouts of sleep in *kcnh4a* mutants relative to control zebrafish, specifically during the dark periods.

An obvious next step is to determine whether Kchn4a is also co-expressed with the Hcrt neuropeptide in mammals, and whether it plays a similar role in the sleep-wake transition. In vivo imaging with genetically-encoded calcium or voltage sensors in *kcnh4a* mutants will likely be useful in understanding the role of this potassium channel in the intrinsic properties of Hcrt neurons. Also, the unique properties of the zebrafish model make it possible to image the activity of single neurons and entire networks during 24-hour periods, and to monitor changes induced by sleep or wake-promoting drugs. In fact, Kchn4a is an intriguing potential new drug target, and may be highly relevant for

therapeutic approaches to treat conditions like insomnia and narcolepsy.

**Competing interests:** The authors declare that no competing interests exist. **William J Giardino** is in the Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, United States

**Luis de Lecea** is in the Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, United States  
llecea@stanford.edu

**Published** 10 December 2015

## References

- Adamantidis AR**, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. 2007. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* **450**:420–424. doi: [10.1038/nature06310](https://doi.org/10.1038/nature06310)
- Bonnavion P**, Jackson AC, Carter ME, de Lecea L. 2015. Antagonistic interplay between hypocretin and leptin in the lateral hypothalamus regulates stress responses. *Nature Communications* **6**:6266 doi: [10.1038/ncomms7266](https://doi.org/10.1038/ncomms7266)
- Dalal J**, Roh JH, Maloney SE, Akuffo A, Shah S, Yuan H, Wamsley B, Jones WB, Strong CDG, Gray PA, Holtzman DM, Heintz N, Dougherty JD. 2013. Translational profiling of hypocretin neurons identifies candidate molecules for sleep regulation. *Genes & Development* **27**:565–578. doi: [10.1101/gad.207654.112](https://doi.org/10.1101/gad.207654.112)
- Faraco JH**, Appelbaum L, Marin W, Gaus SE, Mourrain P, Mignot E. 2006. Regulation of hypocretin (orexin) expression in embryonic zebrafish. *Journal of Biological Chemistry* **281**:29753–29761. doi: [10.1074/jbc.M605811200](https://doi.org/10.1074/jbc.M605811200)
- Giardino WJ**, de Lecea L. 2014. Hypocretin (orexin) neuromodulation of stress and reward pathways. *Current Opinion in Neurobiology* **29**:103–108. doi: [10.1016/j.conb.2014.07.006](https://doi.org/10.1016/j.conb.2014.07.006)
- de Lecea L**. 2015. Optogenetic control of hypocretin (orexin) neurons and arousal circuits. *Current Topics in Behavioral Neurosciences* **25**:367–378. doi: [10.1007/7854\\_2014\\_364](https://doi.org/10.1007/7854_2014_364)
- Yelin-Bekerman L**, Elbaz I, Diber A, Dahary D, Gibbs-Bar L, Alon S, Lerer-Goldshtein T, Appelbaum L. 2015. Hypocretin neuron-specific transcriptome profiling identifies the sleep modulator Kcnh4a. *eLife* **4**:e08638 doi: [10.7554/eLife.08638](https://doi.org/10.7554/eLife.08638)
- Yokogawa T**, Marin W, Faraco J, Pézéron G, Appelbaum L, Zhang J, Rosa Frédéric, Mourrain P, Mignot E, Stemple DL. 2007. Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. *PLoS Biology* **5**:e277 doi: [10.1371/journal.pbio.0050277.sv005](https://doi.org/10.1371/journal.pbio.0050277.sv005)