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Beyond the Symptom: The Biology of Fatigue
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Invertebrates to study fatigue

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Disclaimer and Disclosures

Disclaimer

This certifies that the views expressed in this presentation are those of the author and do not reflect the official policy of the NIH.

Disclosure

This certifies that I, David Raizen, have no financial relationship that is relevant to the subject matter of this presentation.

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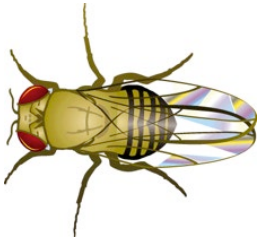
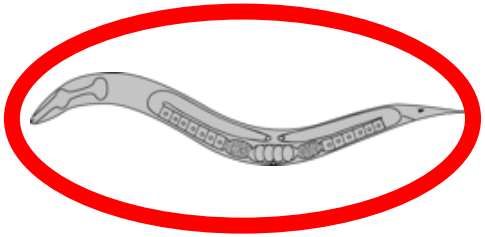
Studying fatigue in animals

The subjective patient symptom of fatigue corresponds to a phylogenetically conserved and objectively quantifiable program of sickness behavior. Sickness behavior includes reduced movement and feeding, social withdrawal, reduced motivated behavior, and sleep.

Fatigue mechanisms questions

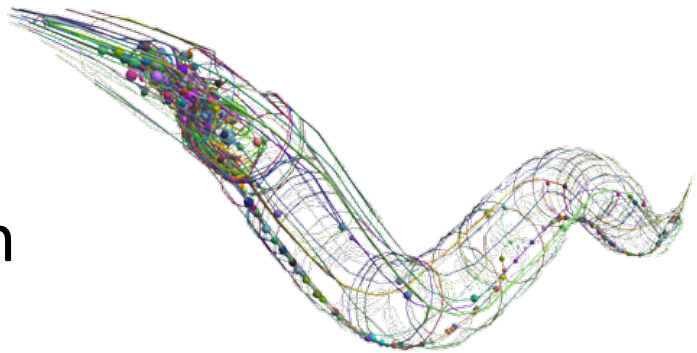
(1) Can we study fatigue in invertebrate animals?





(2) Are mechanisms of fatigue in sickness the same as those of fatigue in health, like after exercise or sleep deprivation?

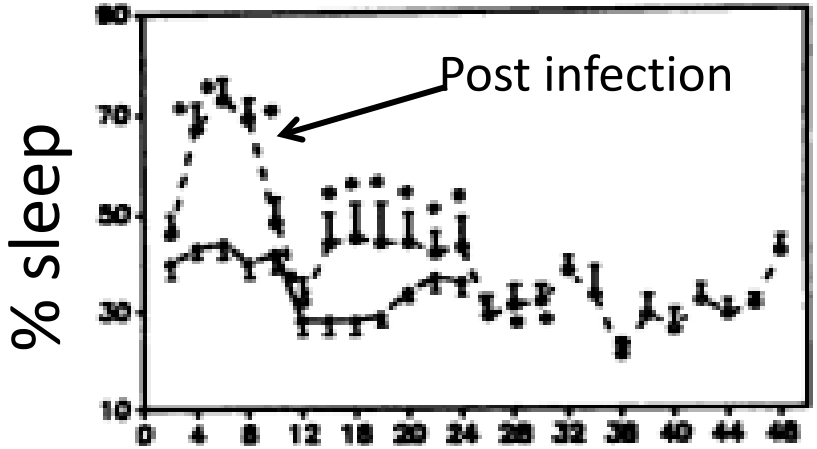
Caenorhabditis elegans: model organism to study mechanisms of human disease



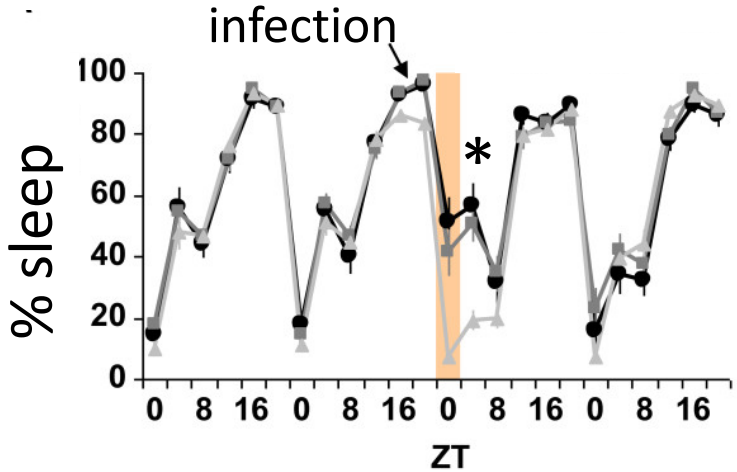
- 60-hour life cycle
- Well-described nervous system
  - 302 neurons, Connectome known
- Transparent
- Human disease genes and physiology highly conserved in *C. elegans*: RNAi, cell death, etc.
- Can do phenotype-driven discovery genetics
- Worms feed, sleep, and have social behaviors.
- Worm sickness induced by infection, high heat, UV light, others

Infection promotes sleep

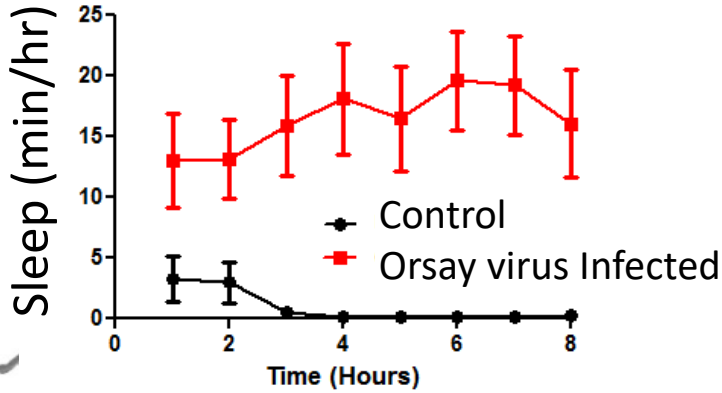
in mammals (Toth and Krueger, FASEB 1989)



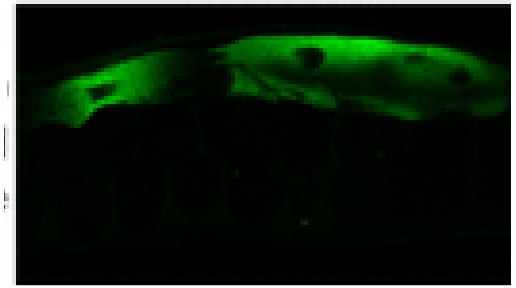
in fruit flies (Kuo et al, BMC Neurosci 2010)



in round worms (unpublished)

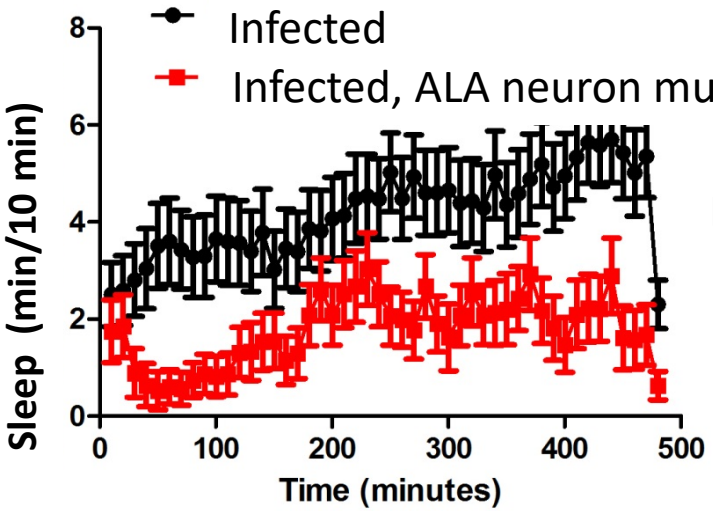


Orsay virus infects non-neural (intestinal) cells



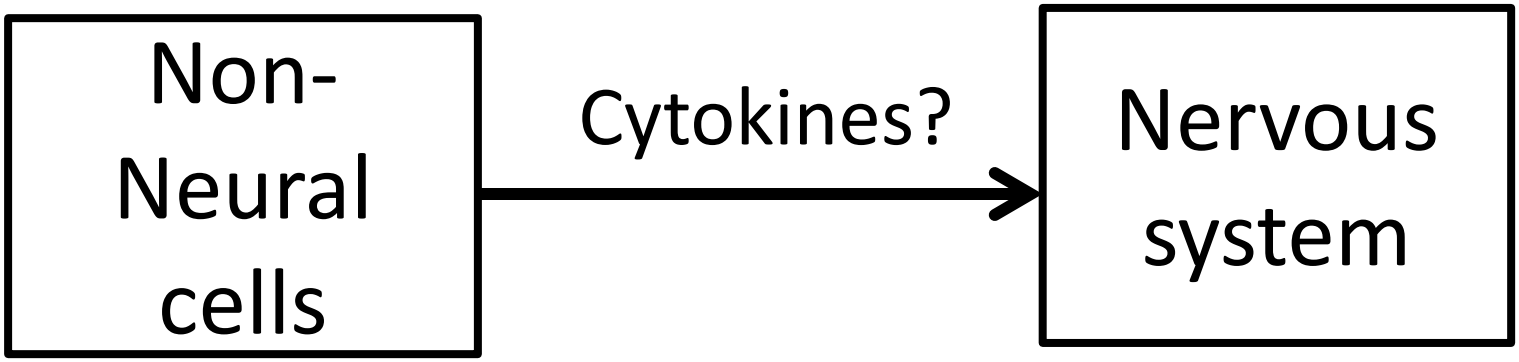
Felix, PLoS Biol '11

Sickness sleep is regulated by the CNS



N=24 per group

Non-neural cells → Cytokines? Enteric Neurons? Metabolites? → Central Nervous system

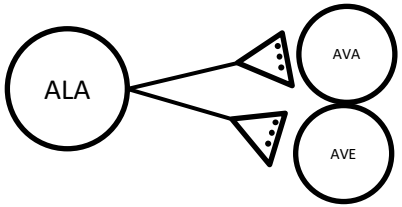


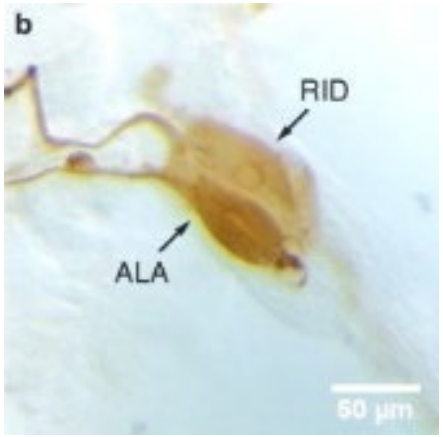
- Epidermal growth factor
  - Activates the ALA neuron
  - Promotes sleep in worms, flies, mammals
- Antimicrobial peptides (AMPs)
  - 17 distinct AMPs required for sickness induced sleep in worms. Each promotes sleep when over-expressed.
    - Sinner et al, Current Biology 2021.
  - *Nemuri* promotes sleep in flies.
    - Toda et al, Science 2019.

ALA contains FLP-13 neuropeptides

Dense core vesicles noted in ALA by EM

White J. *Philos Trans R Soc Lond* 1986

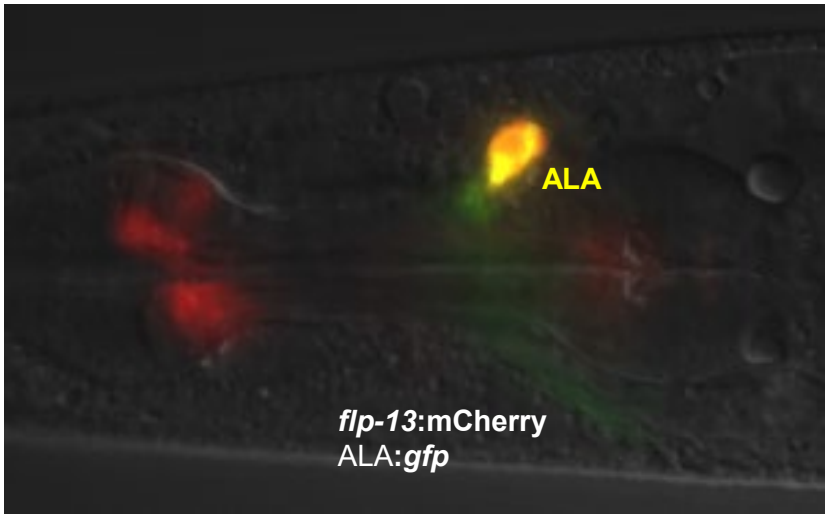




FLP-13 neuropeptides are in the *Ascaris* ALA neuron

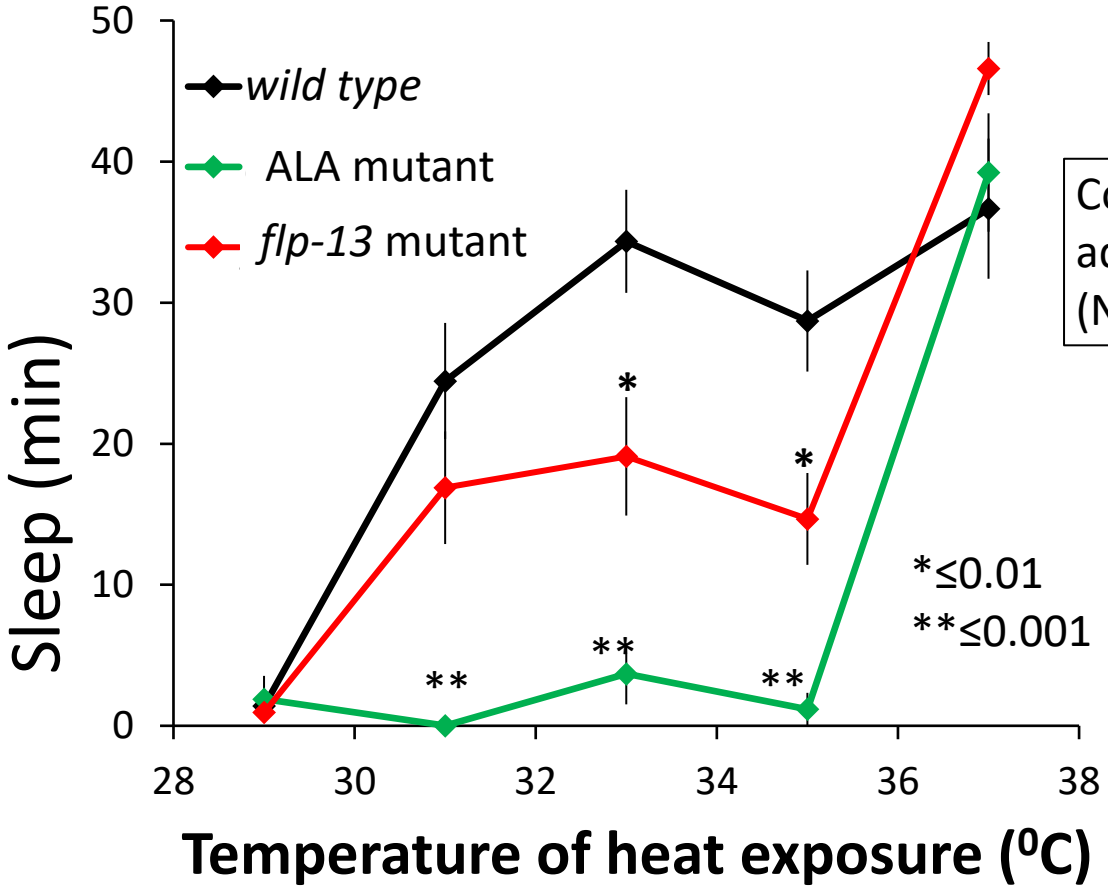
Jarecki et al. *ACS Chem Neuro* 2010

FLP-13 is also in the *C. elegans* ALA



Nelson et al, Current Biology 2014

FLP-13 neuropeptides are required for sickness sleep

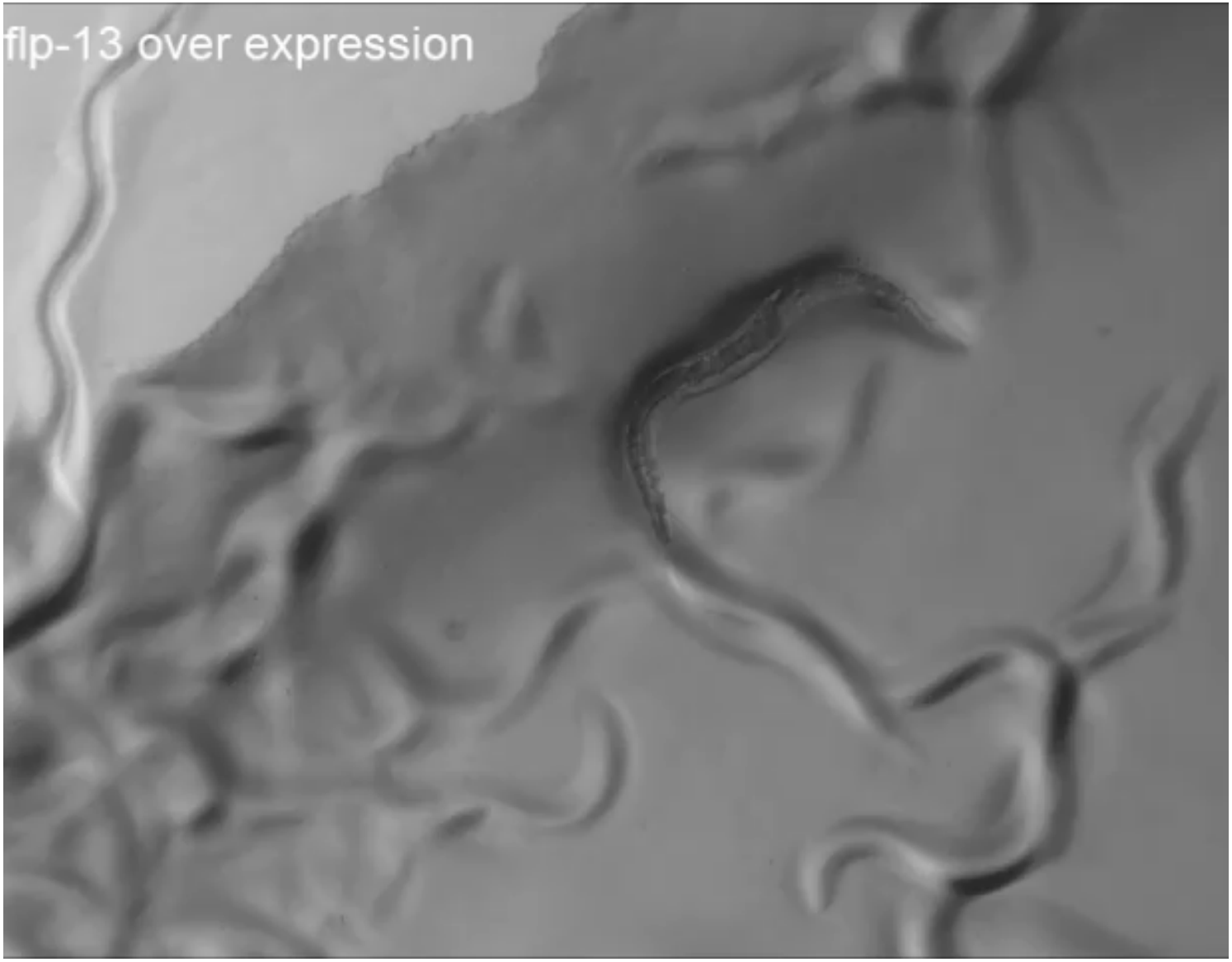


Contributing to sickness sleep are additional ALA neuropeptides (Nath, et al, Curr. Biol. '16)

FLP-13 neuropeptides have an amidated Arginine-Phenylalanine (RFa) at their C-terminus. RFamide neuropeptides are found in animals from humans to jellyfish. Their function is largely unknown

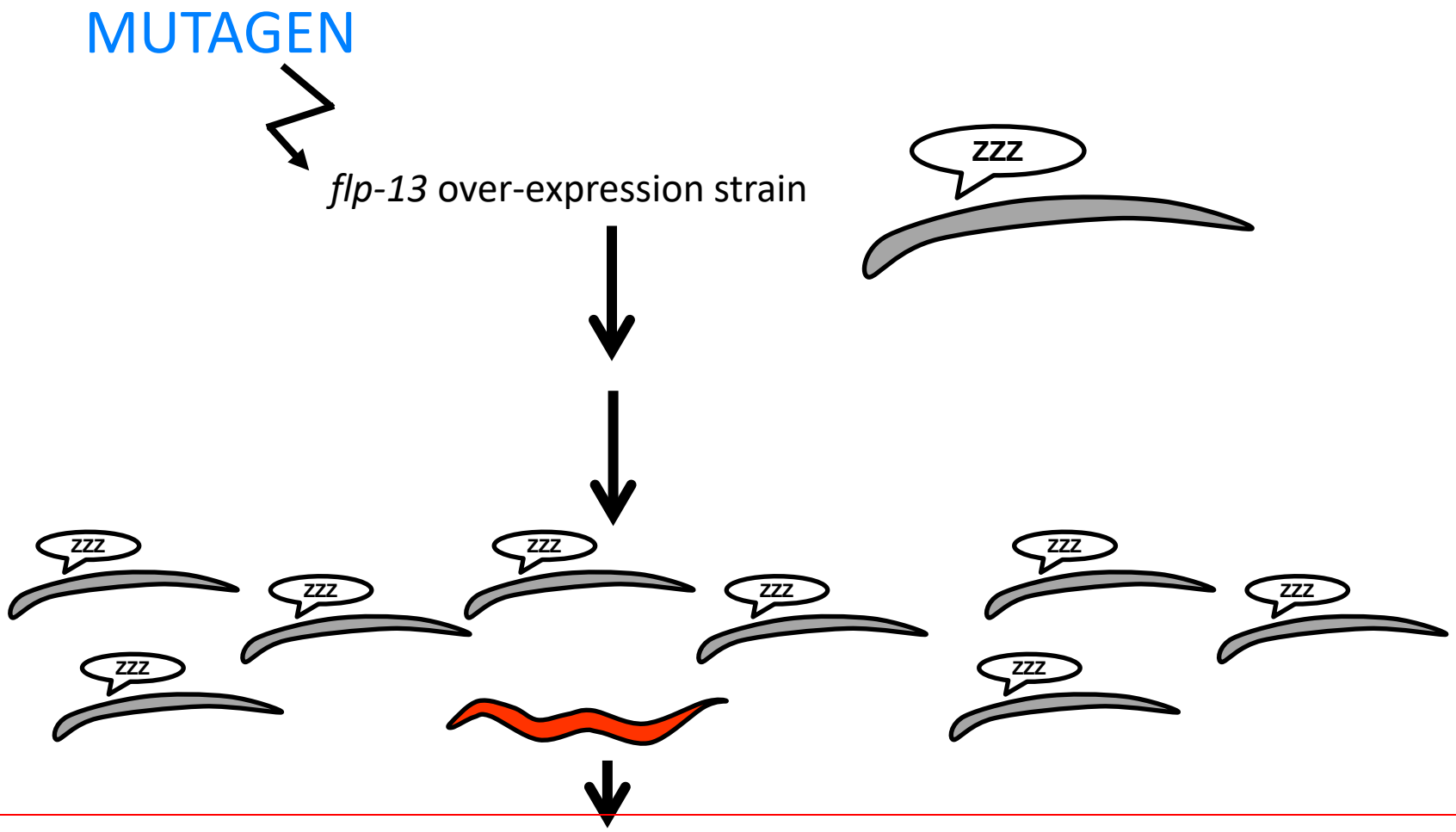
Nelson et al, Current Biology 2014; Hill et al, Current Biology 2014, Trojanowski et al, J. Neuroscience 2015

Over-expression of *flp-13* induces sleep



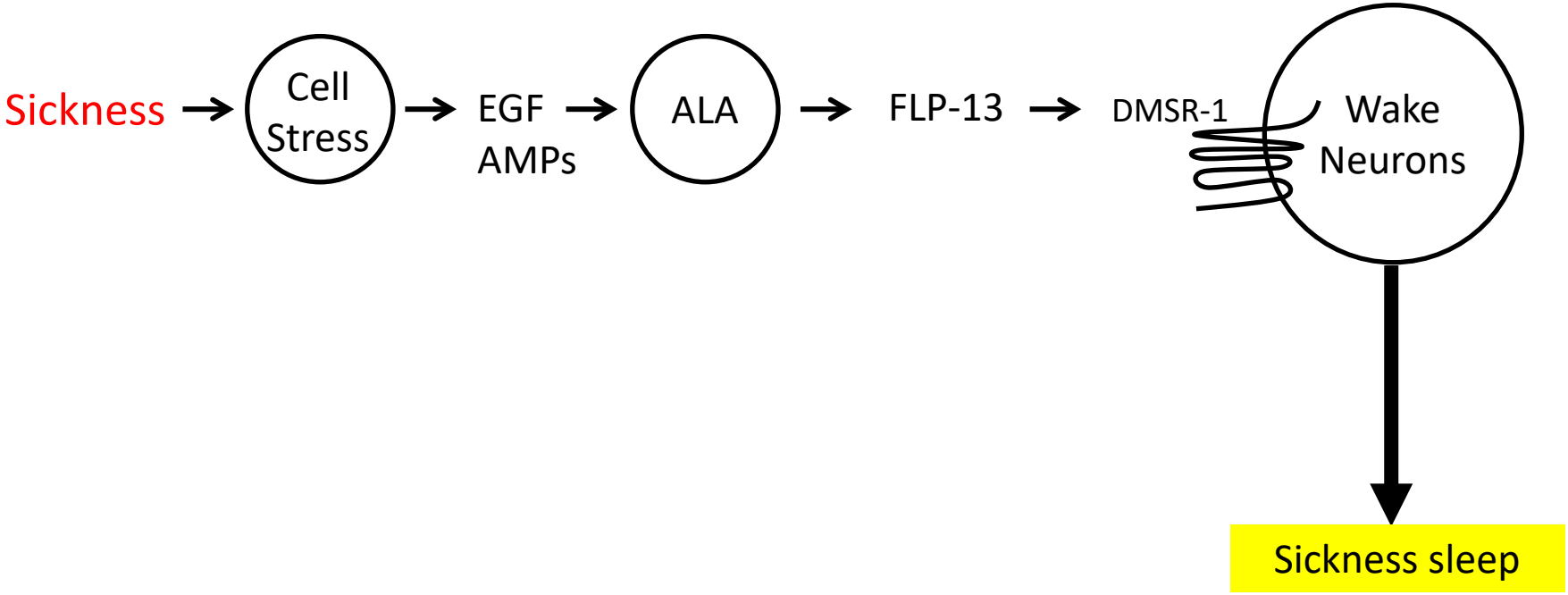
Nelson et al, Current Biology 2014

Finding genes required for somnogenic effects of FLP-13



Screened ~200,000 animals in a few weeks—found 12 mutants. 7 mutations in the same gene, which encodes a predicted RFamide neuropeptide receptor DMSR-1

Neural/molecular pathway for sleep during sickness



is the role of FLP-13 conserved?

FLP-13 orthologues

Brain, Behavior, and Immunity 2015

FMRFamide signaling promotes stress-induced sleep in *Drosophila*

Olivia Lenz [a,1], Jianmei Xiong [a,1,2], Matthew D. Nelson [b,c], David M. Raizen [a,b], Julie A. Williams

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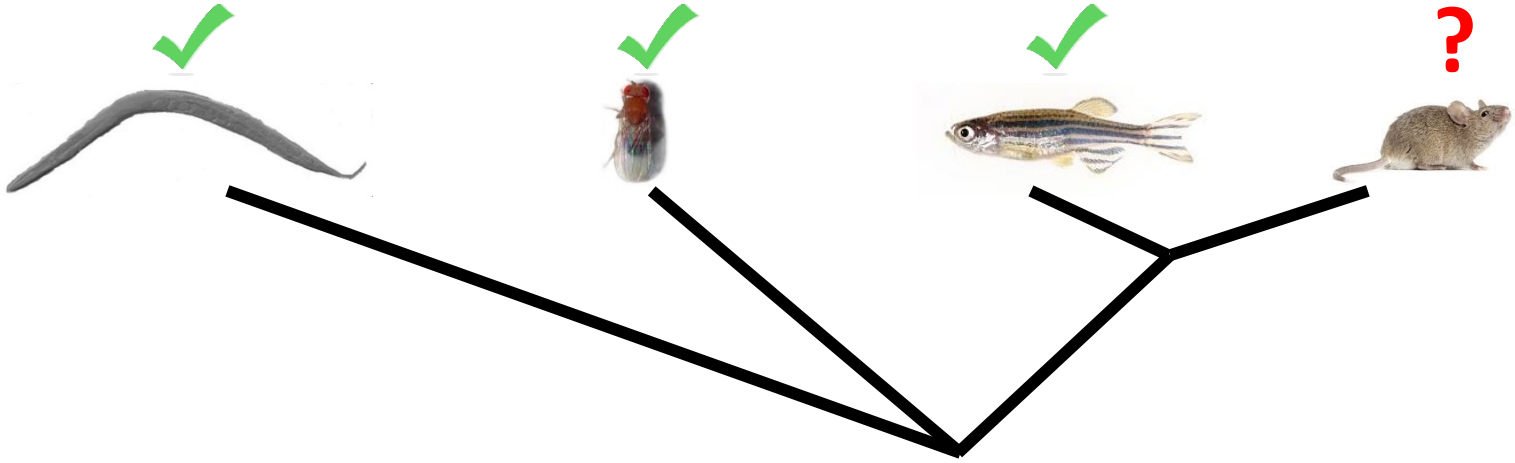
Genetic and neuronal regulation of sleep by neuropeptide VF

fe 2017

Daniel A Lee[1], Andrey Andreev[2], Thai V Truong[3], Audrey Chen[1], Andrew J Hill[1], Grigorios Oikonomou[1], Uyen Pham[1], Young K Hong[1], Steven Tran[1], Laura Glass[1], Viveca Sapin[1], Jae Engle[1], Scott E Fraser[2,3], David A Prober[1]*



(2) Are mechanisms of fatigue in sickness the same as those of fatigue in health?

?

- EGF signaling, ALA, and FLP-13/DMSR-1 required for worm sleep in sickness but not for sleep in health
- Antimicrobial peptides required for worm sleep in sickness but not for sleep in health.
- *Nemuri* not required for fly circadian (healthy) sleep

SUMMARY

- Sleep in sickness is observed throughout phylogeny. Invertebrates such as *C. elegans* and *Drosophila* offer experimental advantages, especially for discovery genetics.
- We can study mechanisms of sickness behavior using invertebrate model systems. These mechanisms inform our understanding of fatigue.
- RFamide neuropeptides (FLP-13/NPVF) play a phylogenetically conserved role in regulation of sleep in sickness.
- Mechanisms of sleep in sickness are at least partially distinct from those regulating sleep in health.

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Annesia Lamb (Post doc, UK)
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Robert Driver
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