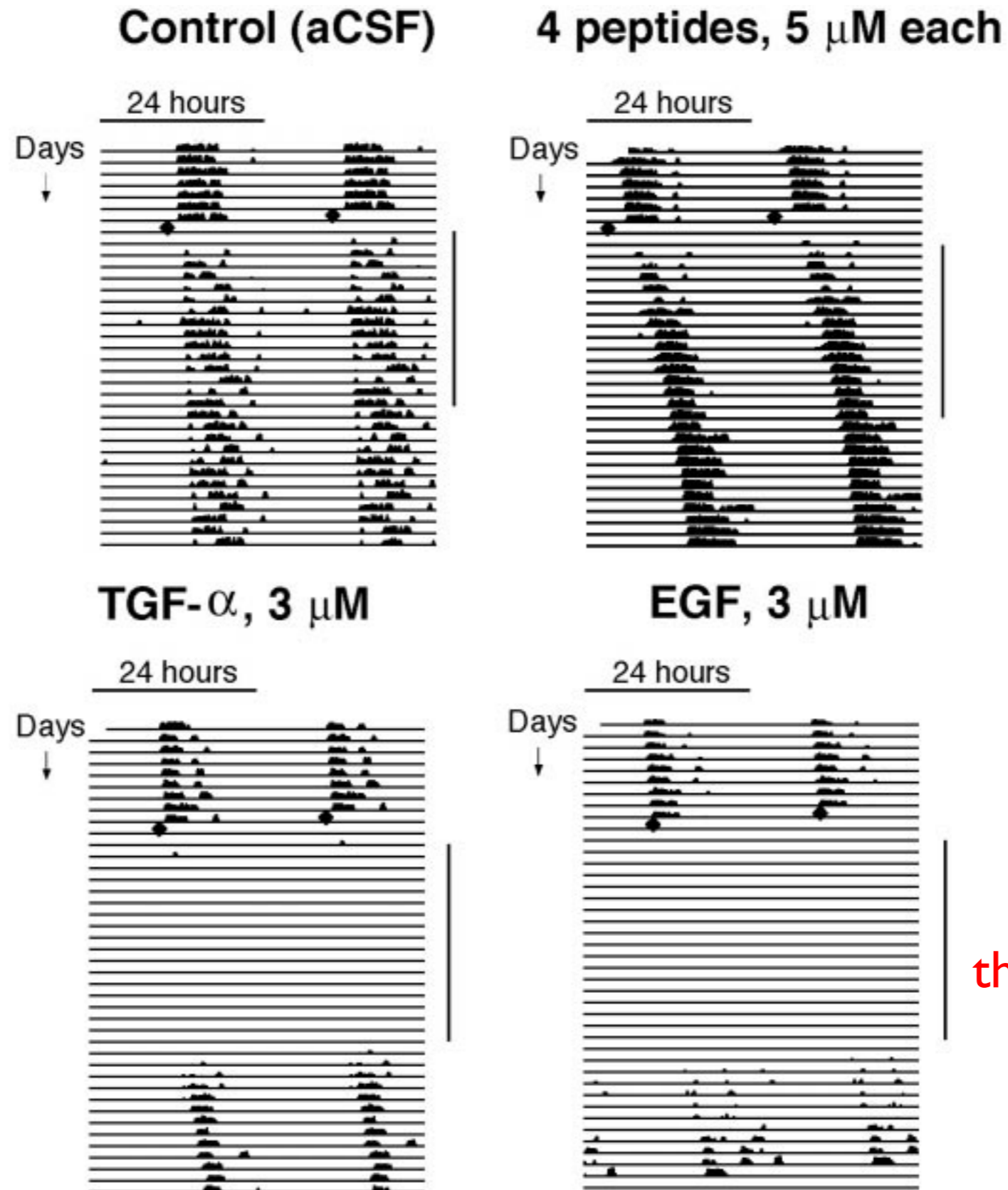


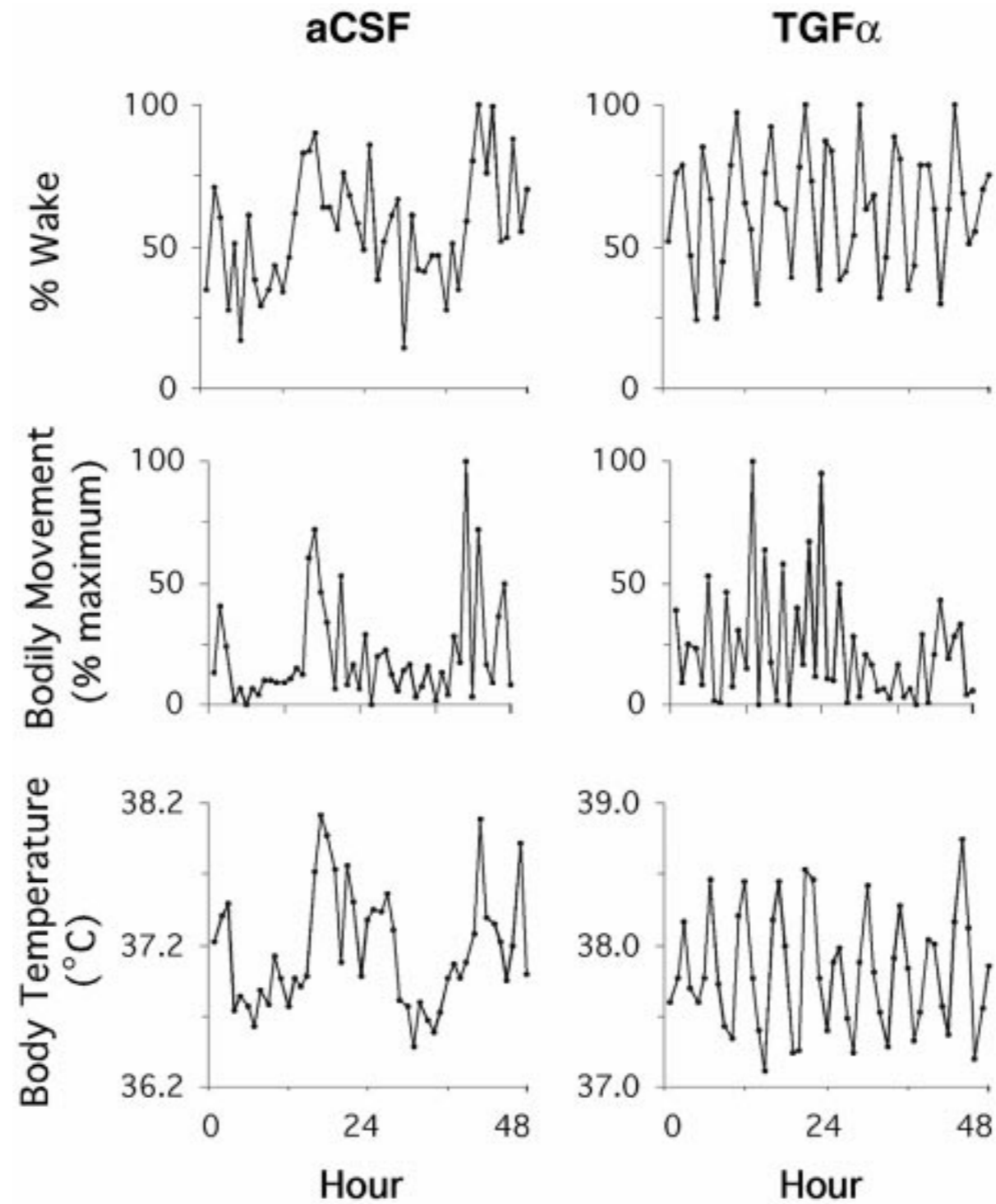
# Infusion of EGF blocks locomotor activity in hamsters



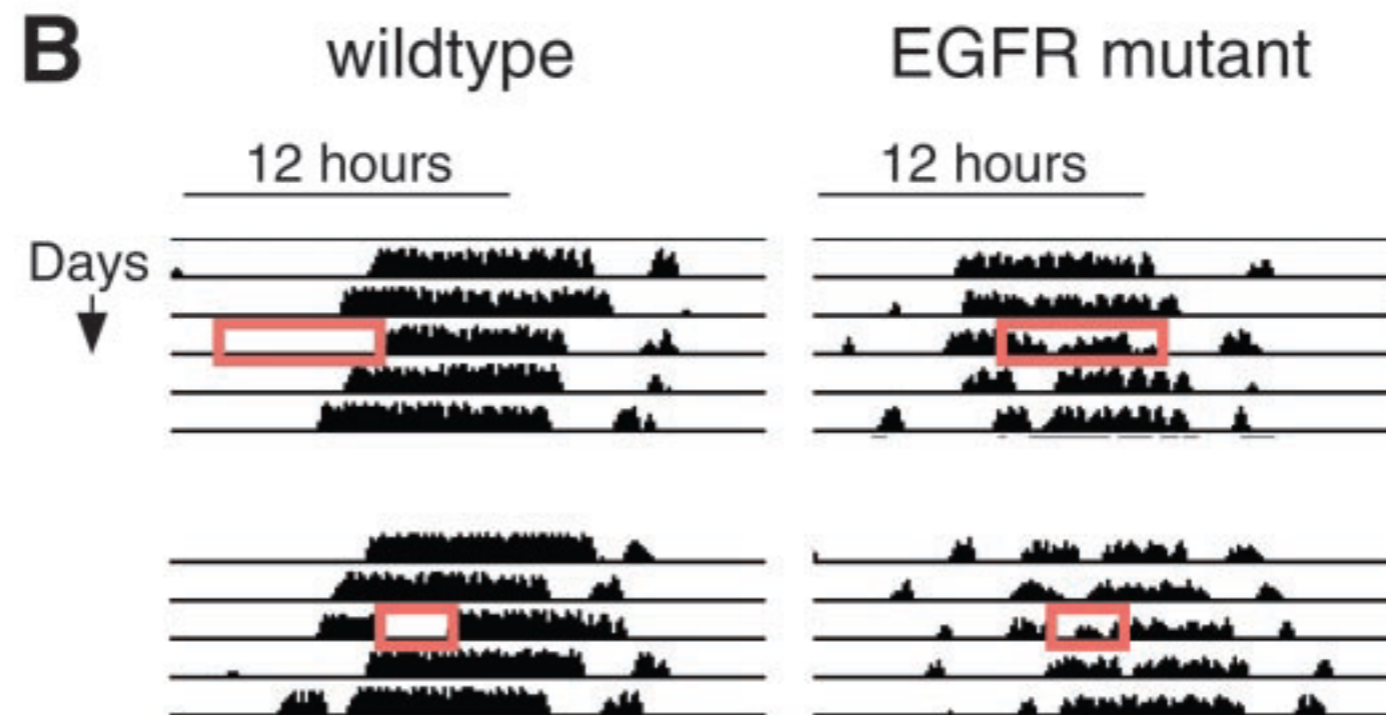
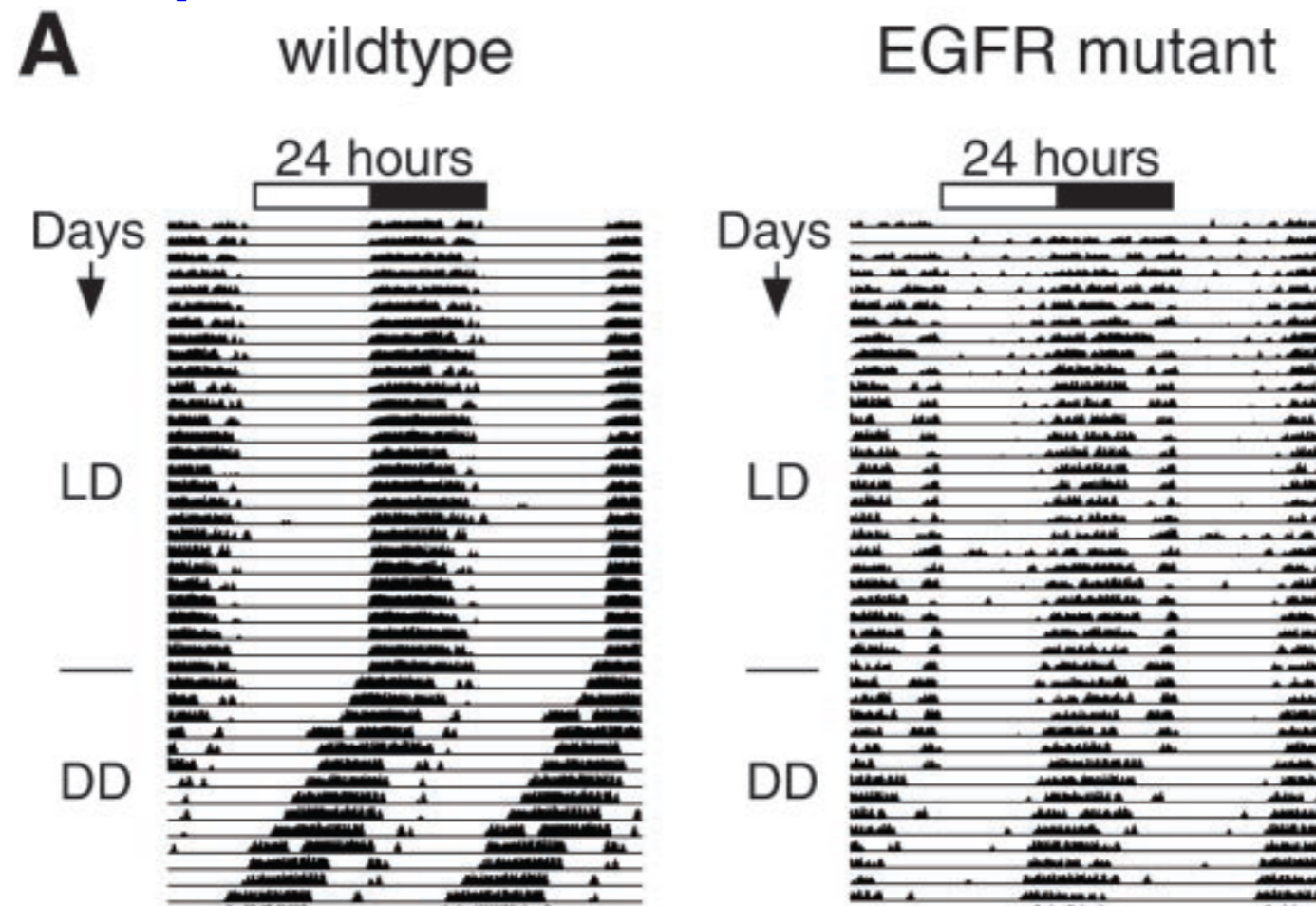
Period of infusion;  
therefore it's reversible

Running wheel assay

# TGF $\alpha$ , an EGF like factor, disrupts sleep-wake behavior



# Mouse EGF Receptor mutant is defective in light response



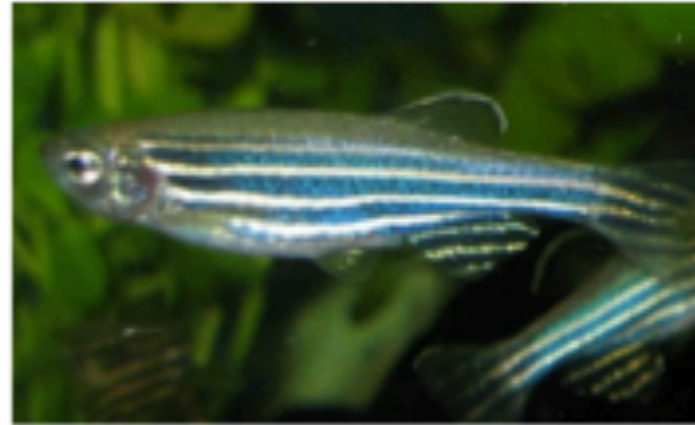
Together, these experiments suggest EGF is necessary and sufficient for activity/sleep cycle

# Sleep: A conserved physiology in animals

- Consolidated period of inactivity
- Increased arousal threshold
- Homeostatic drive to recover after sleep deprivation

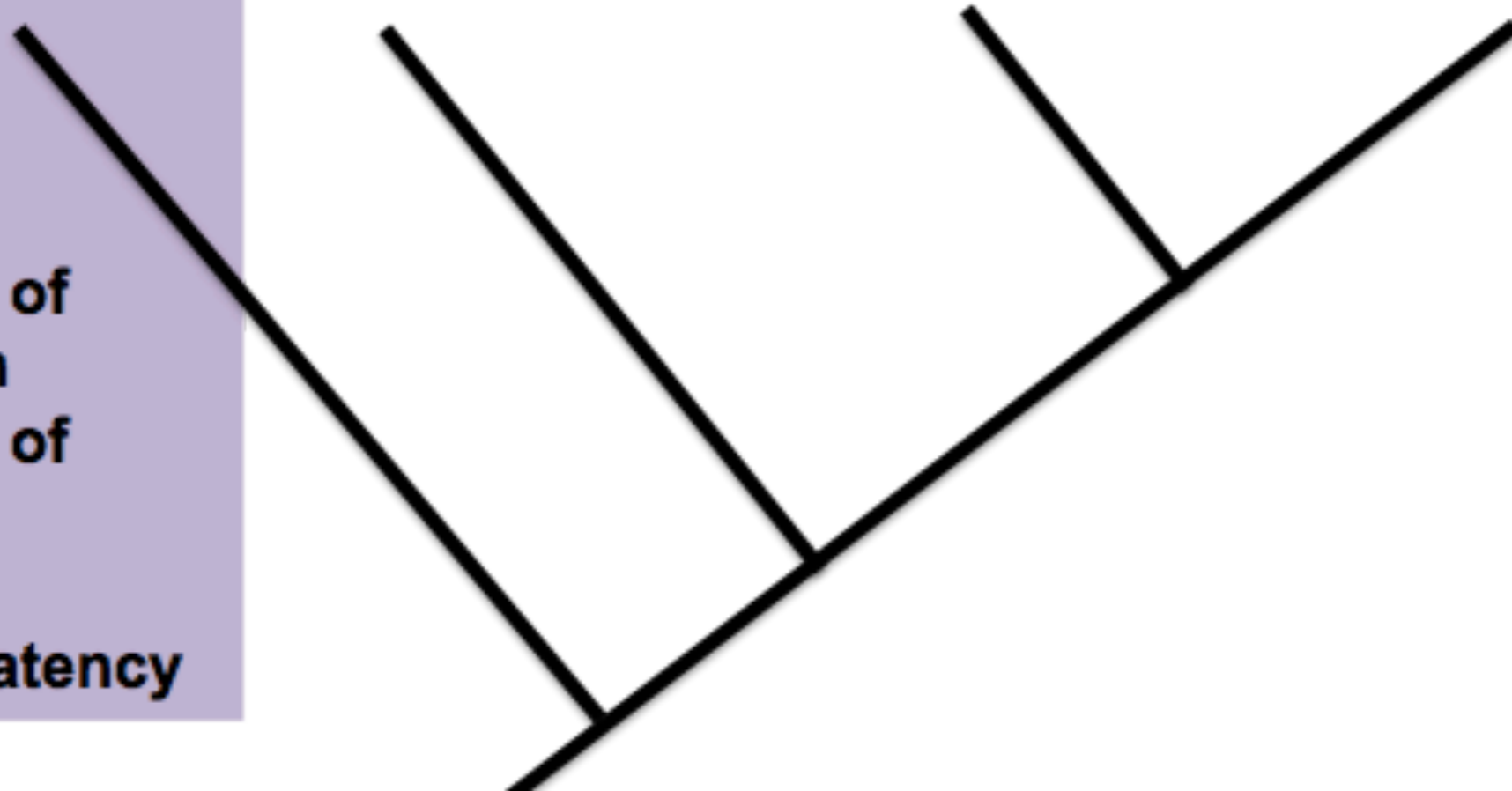
Quiescence/sleep-like state

Sleep



*C.elegans*

- Cessation of locomotion
- Cessation of feeding
- Increased response latency



# Sleep in worms

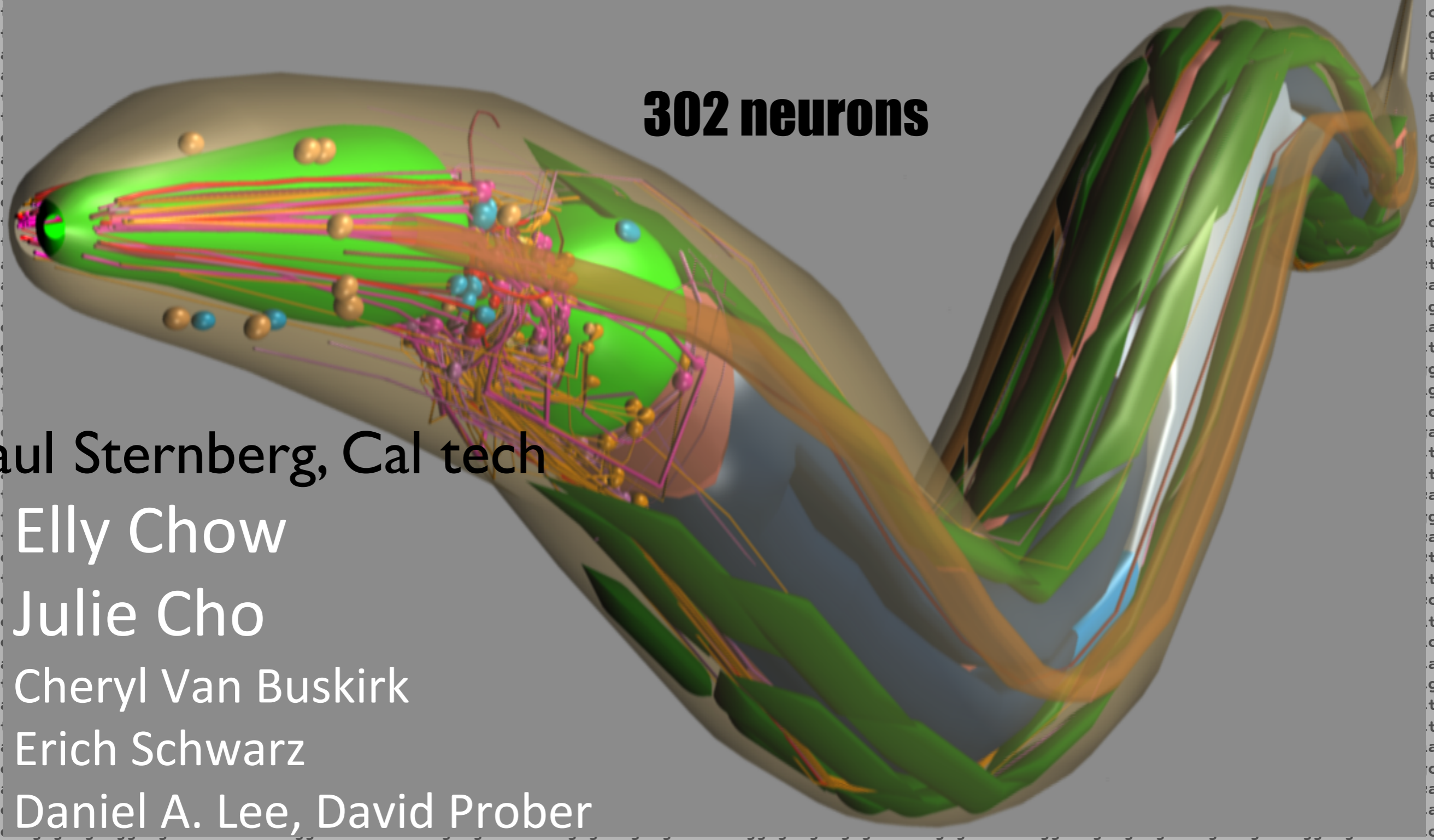
EGF and EGF-R

Target neuron - ALA

Neuropeptides and receptors

# C. elegans SLEEP: molecules & circuits

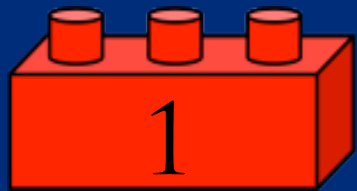
**302 neurons**



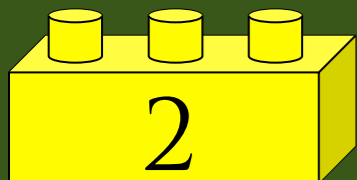
**Paul Sternberg, Cal tech**  
**Elly Chow**  
**Julie Cho**  
**Cheryl Van Buskirk**  
**Erich Schwarz**  
**Daniel A. Lee, David Prober**

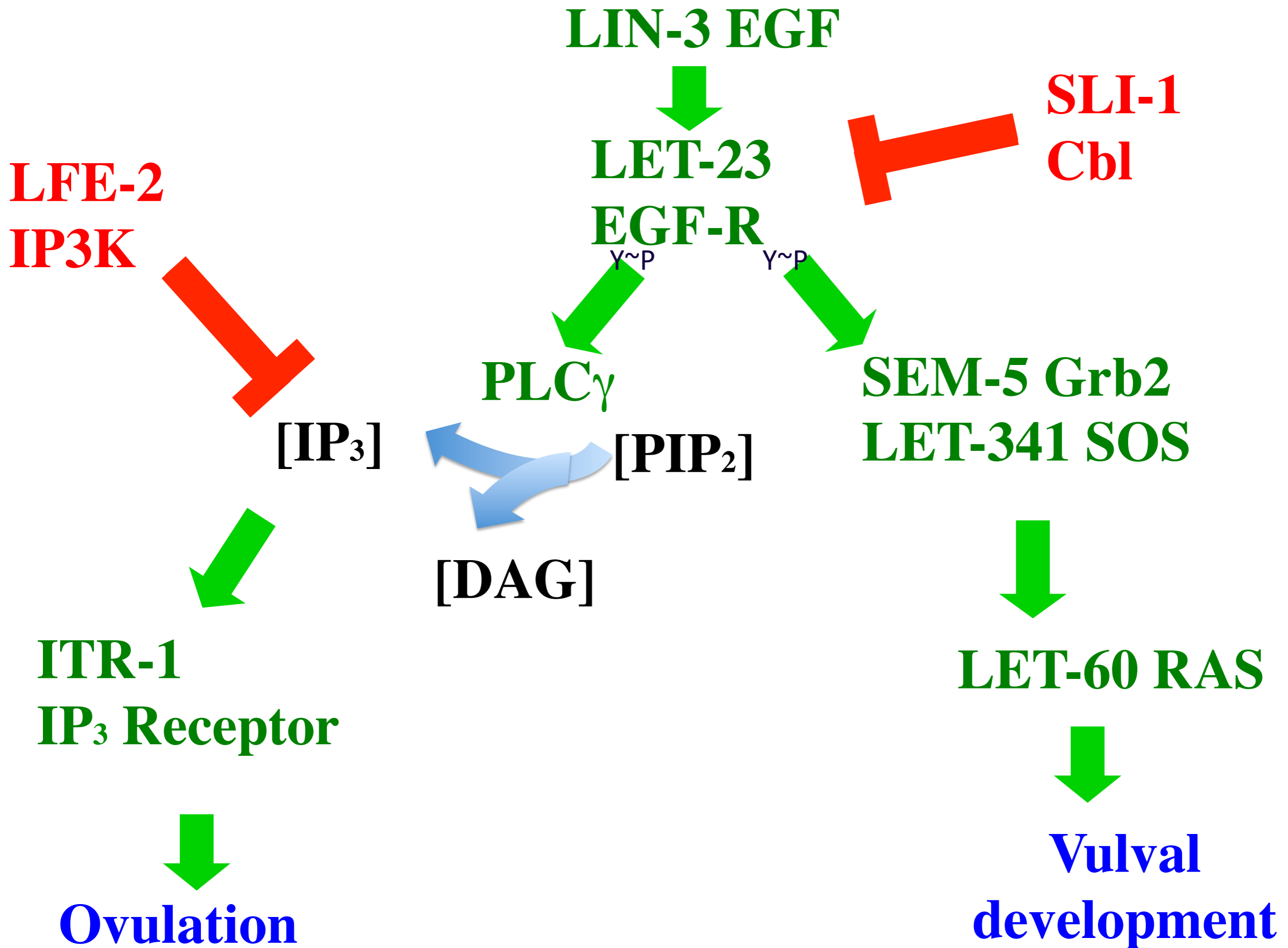
graphic by Chris Grove [WormBase-Caltech]

# Neuropeptide signaling network promotes EGF-dependent sleep



Sleep dampens sensory response by multilevel modulation of a sensory-motor circuit







# Wild-type adult worms are almost constantly moving



**Van Buskirk & Sternberg**  
**Nat Neuro 2007**

**Cheryl Van Buskirk**

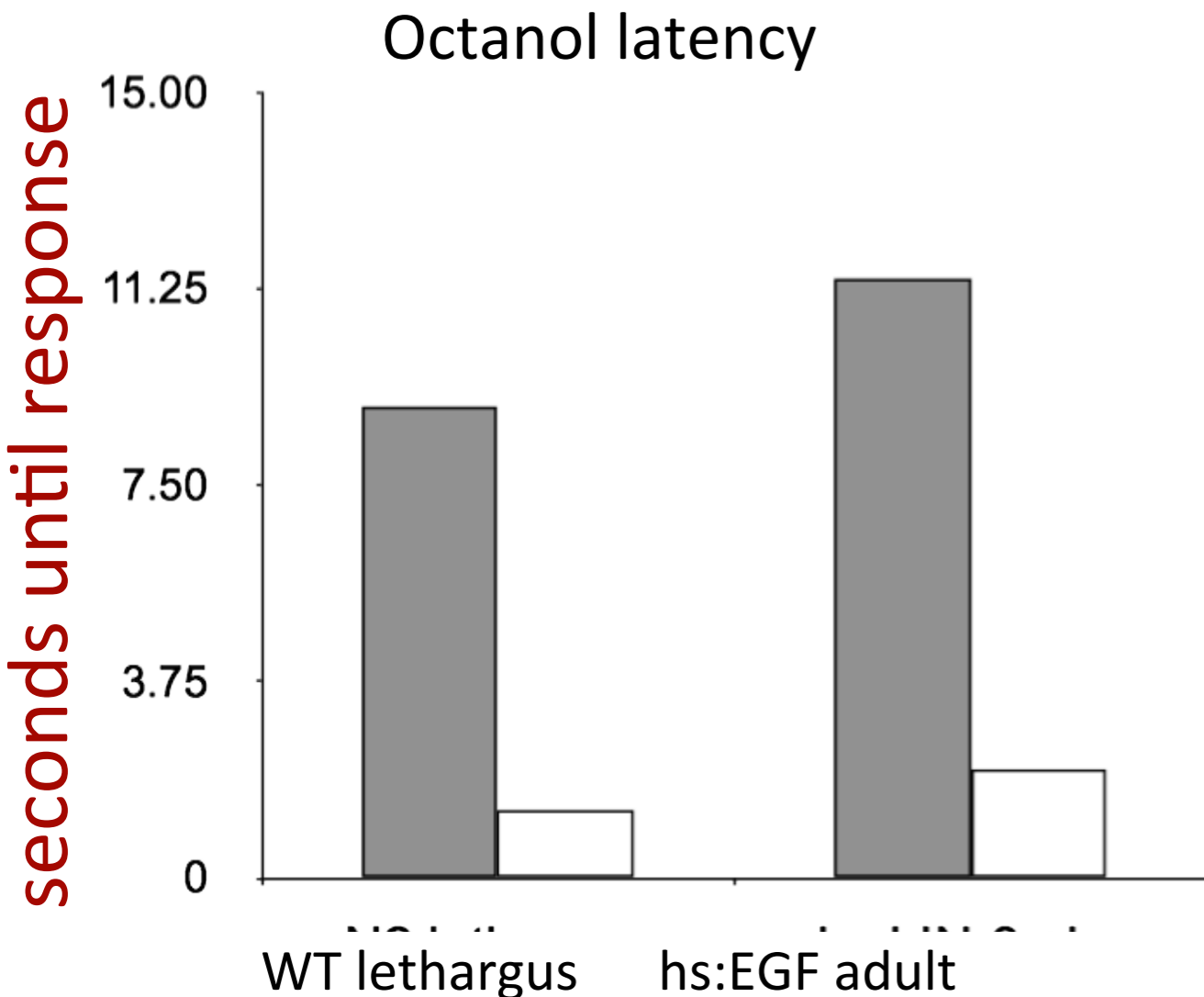
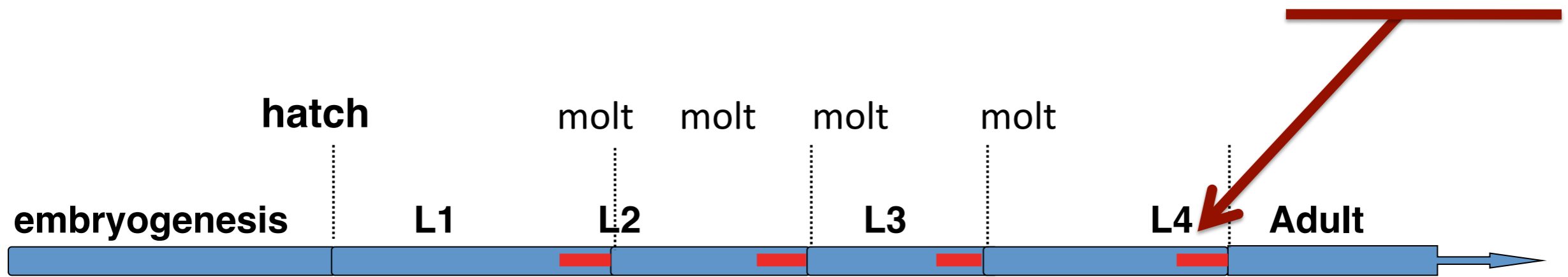
# EGF expression severely inhibits locomotion



(no movement unless perturbed)

**Cheryl Van Buskirk**

# hs:EGF induces sleep-like behavior outside of lethargus



cessation of feeding  
and locomotion

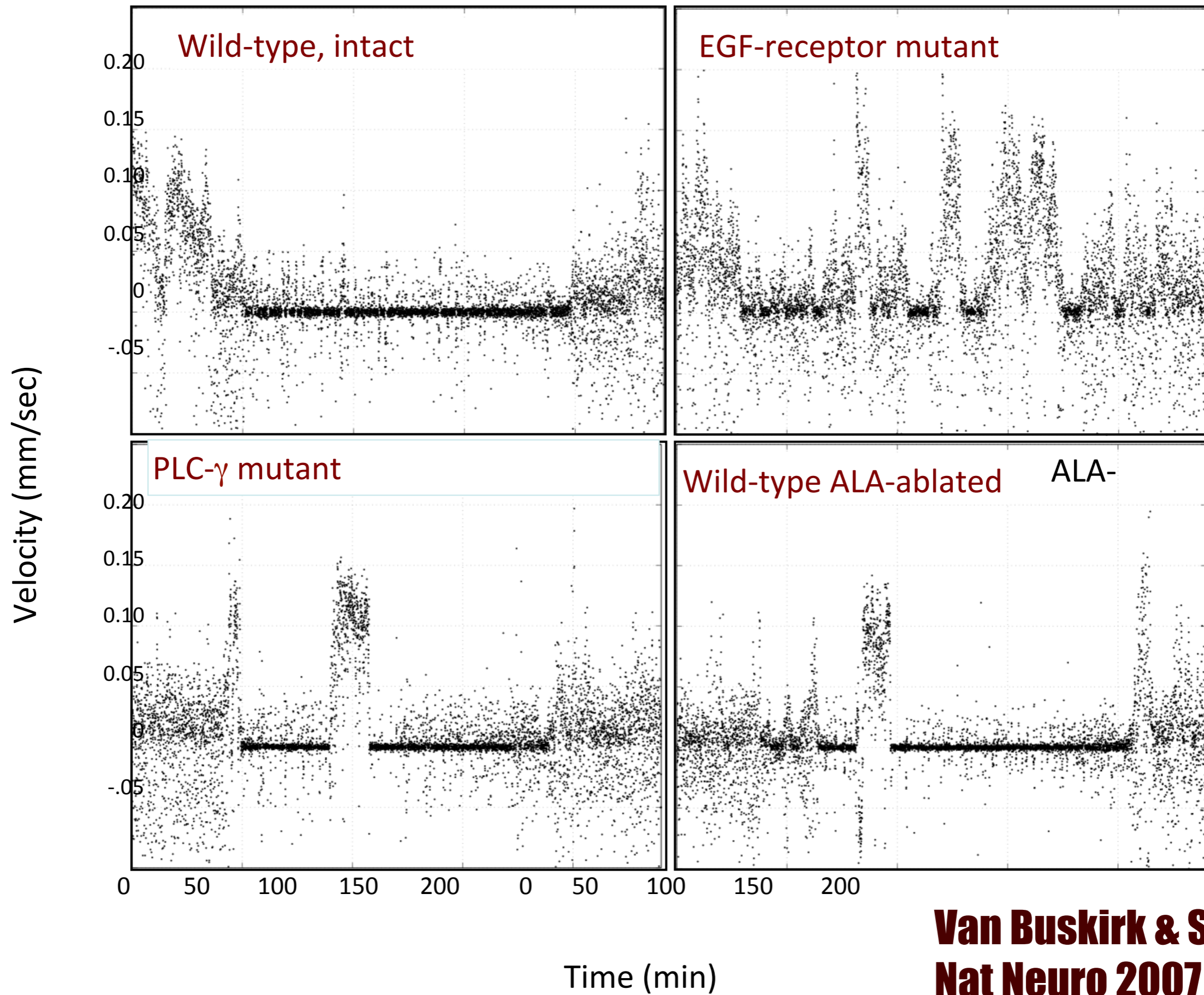
reduced sensory  
responsiveness

quick reversibility

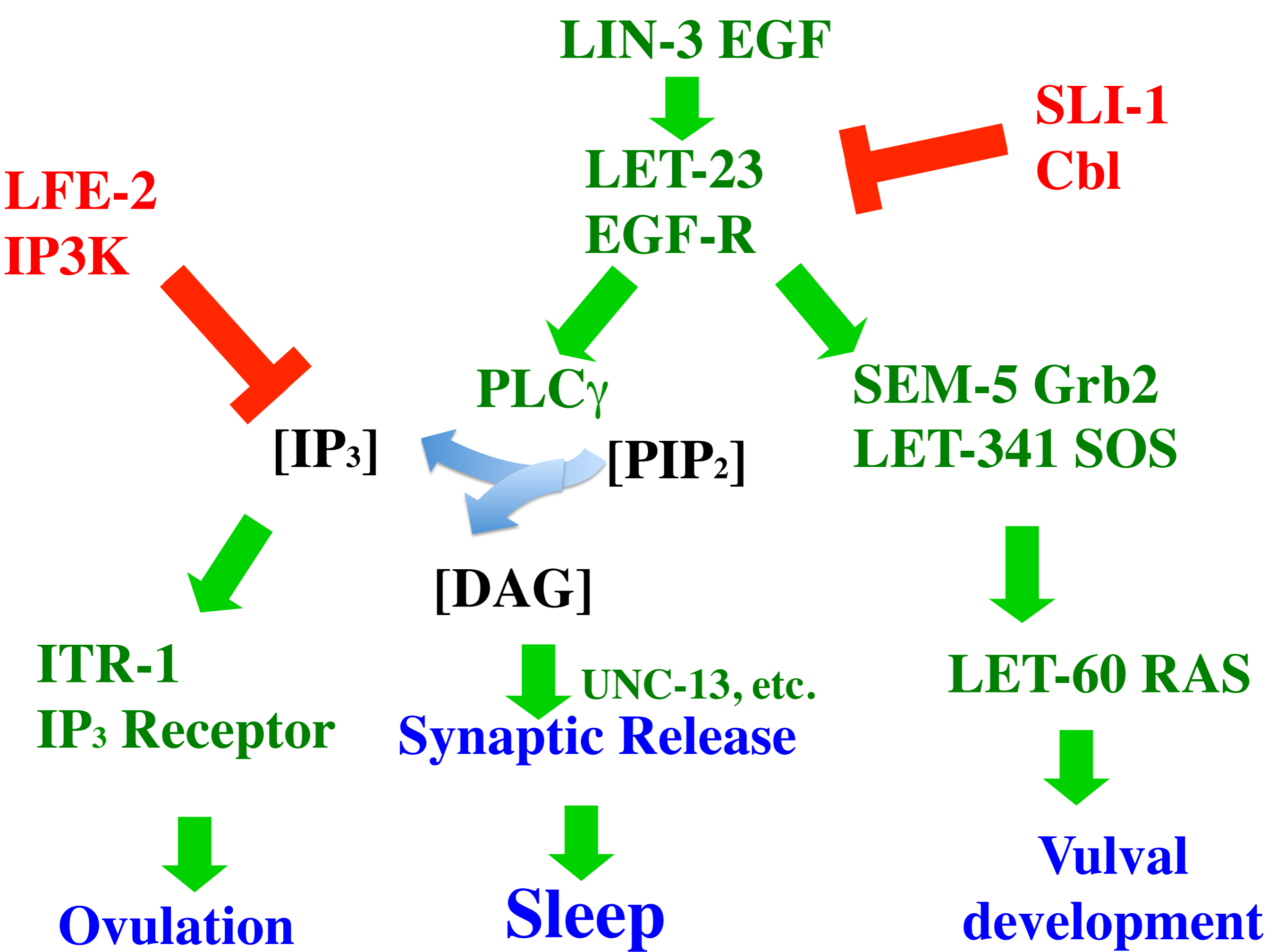
# EGF promotes quiescence during molting via ALA neuron



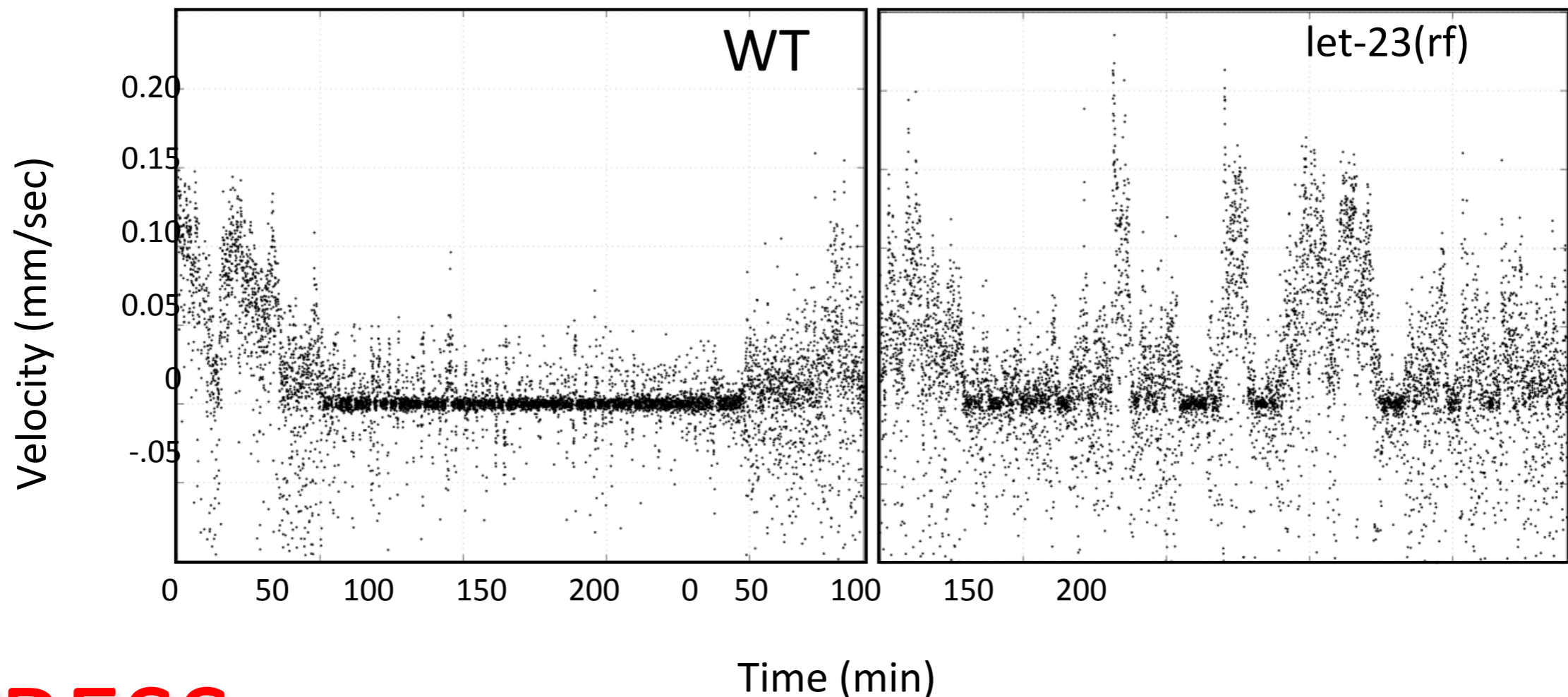
# EGF promotes quiescence during molting via ALA neuron



**Van Buskirk & Sternberg**  
**Nat Neuro 2007**



# EGF signaling mediates stress-induced sleep



**STRESS**



**EGF/EGFR**



**lethargus behavior**



**stress-induced sleep**

# The single ALA neuron is necessary for EGF-dependent sleep



ALA

- ~ALA-ablated animals are insomniac
- ~Expression of EGFR only in ALA is sufficient for EGF-induced sleep
- ~ALA synapses not necessary for function

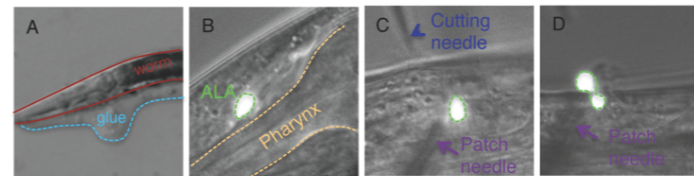
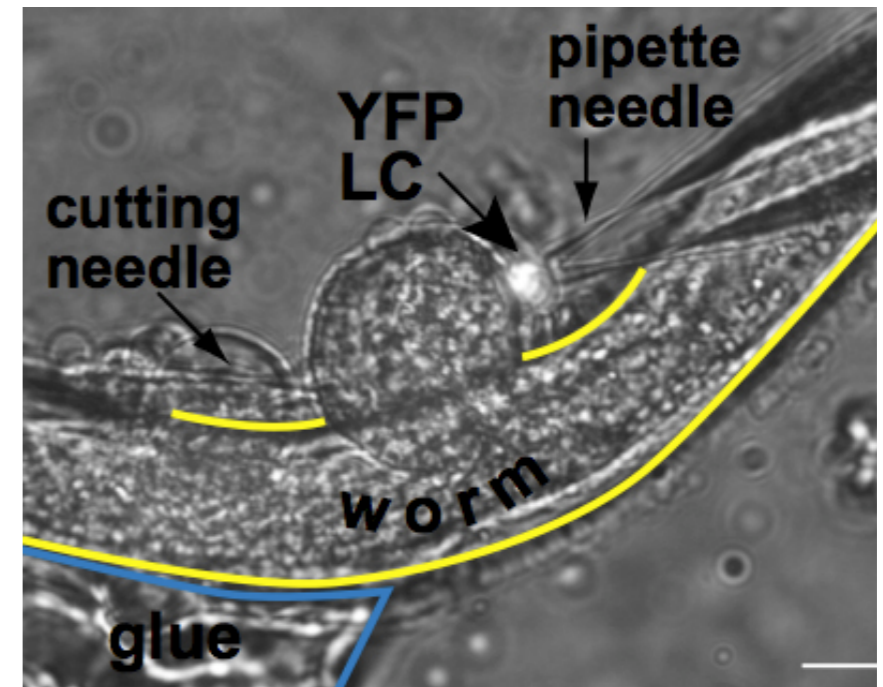
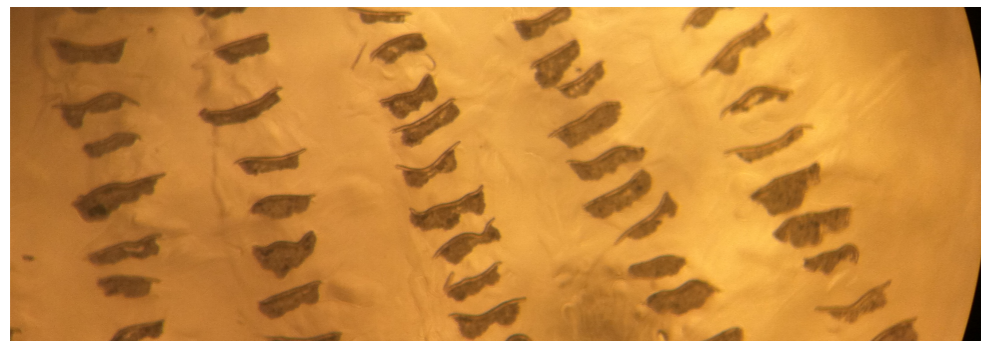


# Micro-dissection & Transcriptional profiling of single ALA neurons



4-5hr

L4 Lethargus



Single cell RT-PCR  
cDNA amplification  
9 cells (2 pools)



Igor Antoshechkin,  
Caltech Jacobs Genome Center

Erich Schwarz, Miriam Goodman, Mihoko Kato  
Schwarz, Kato & Sternberg [PNAS 2012]

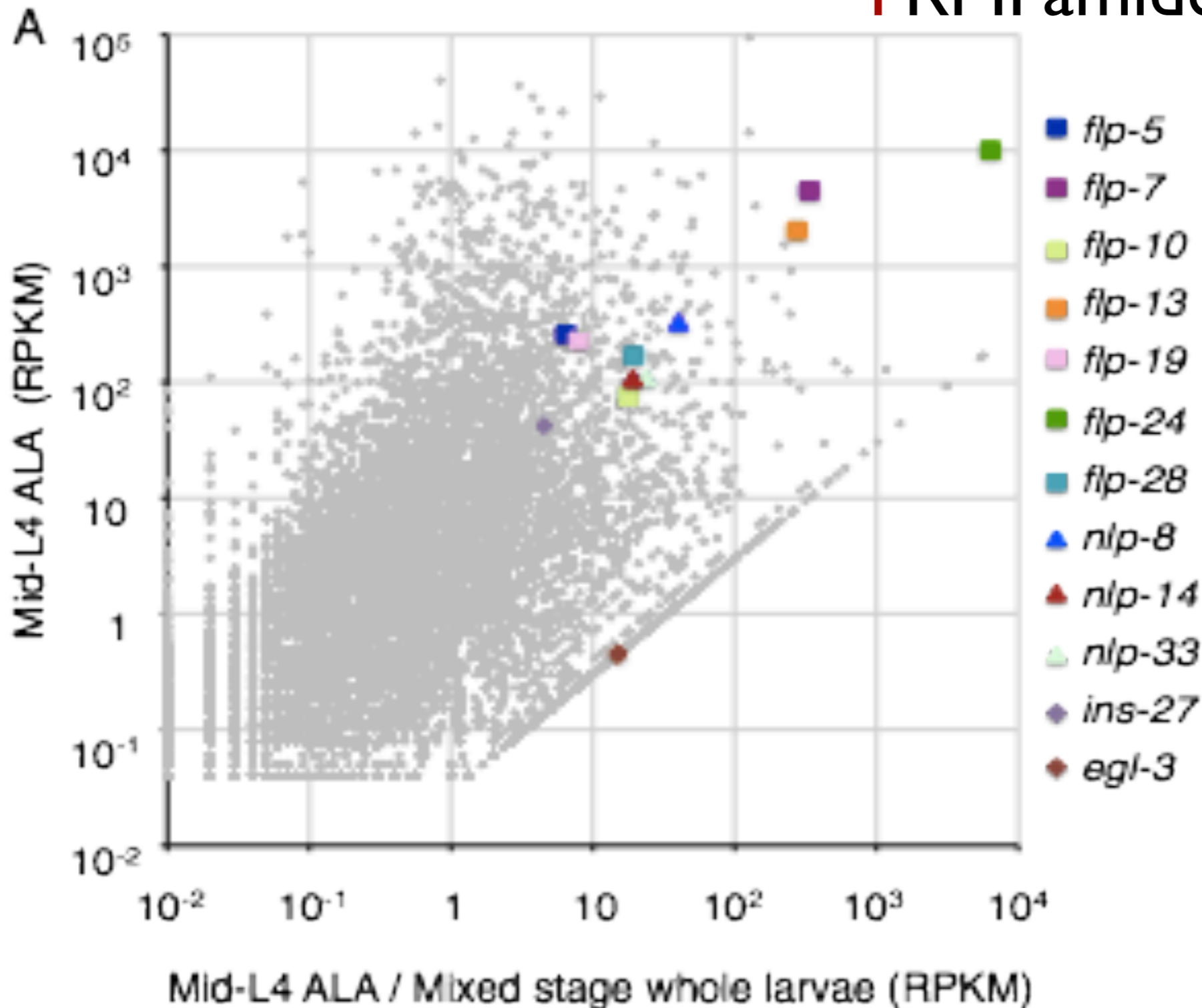
Elly Chow

8110 protein coding genes detected in ALA

# ALA enriched genes: 10 neuropeptide genes

FRMFamide-Like Peptide

Expression Level

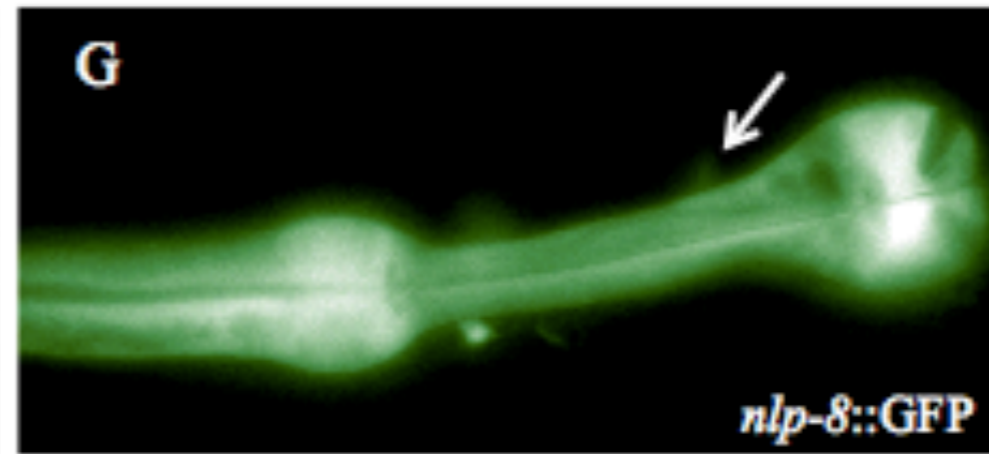
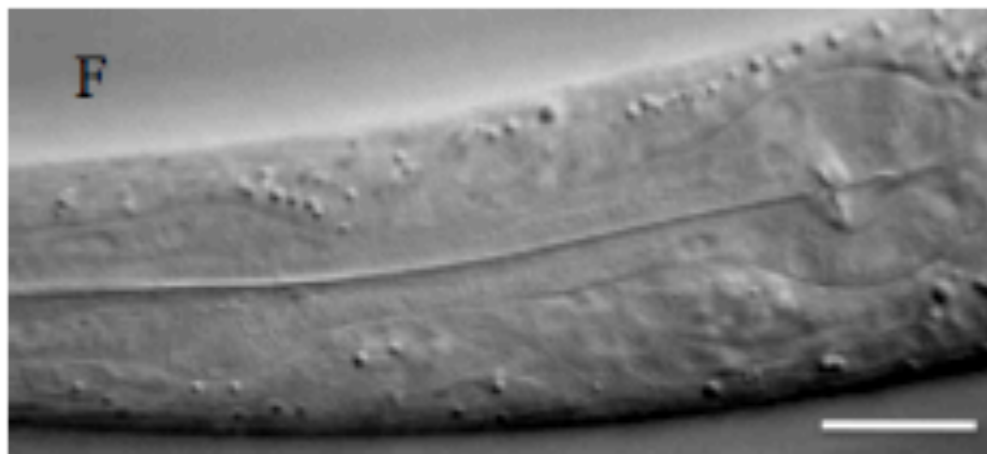
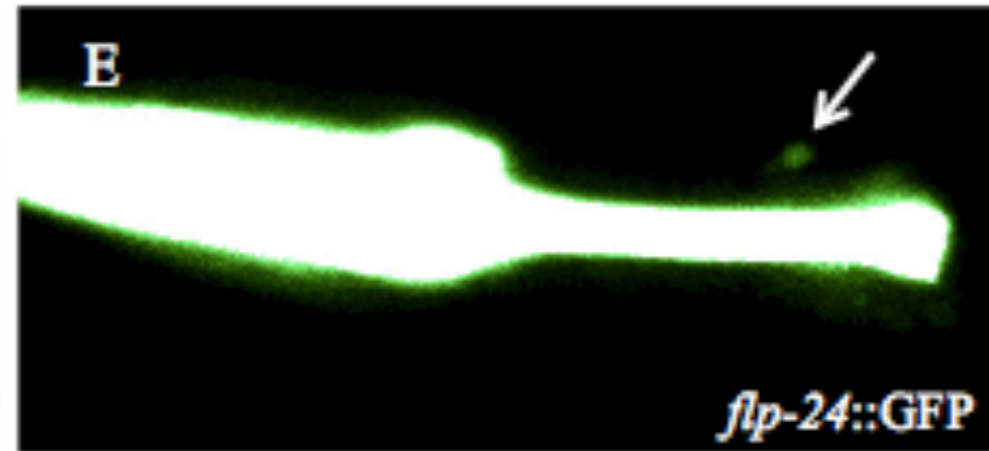
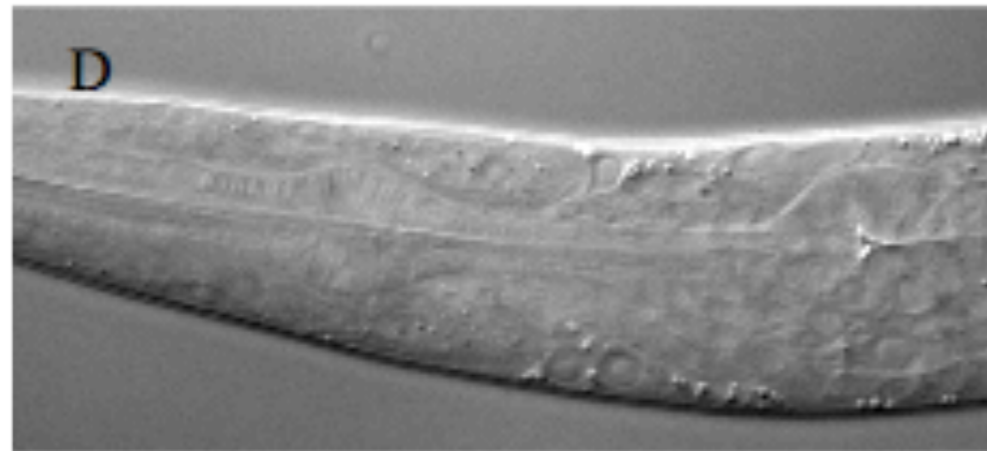
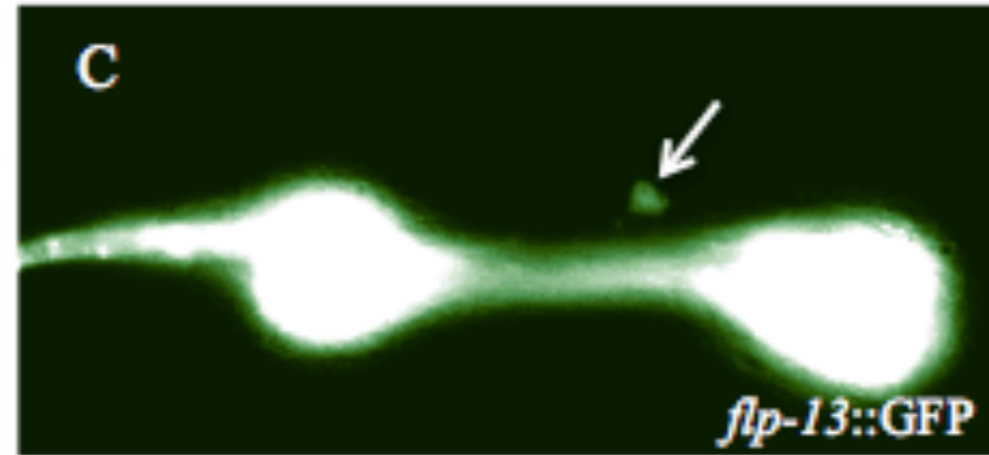


Erich Schwarz

# ALA-enriched neuropeptides (>12-fold over whole larvae)

<b>Genes</b>	<b>ALA</b>	<b>Whole larvae</b>	<b>ALA/W. larvae</b>
<i>flp-24</i>	10176.34	1.59	6400.21
<i>flp-7</i>	4518.55	13.51	334.46
<i>flp-13</i>	2063.11	7.52	274.35
<i>nlp-8</i>	332.58	8.25	40.31
<i>nlp-33</i>	109.35	4.58	23.88
<i>ins-9</i>	0.63	0	21
<i>flp-28</i>	169.94	8.76	19.4
<i>nlp-14</i>	106.87	5.56	19.22
<i>flp-10</i>	74.42	4.25	17.51
<i>nlp-42</i>	0.5	0	16.67
<i>ins-27</i>	0.45	0	15
<i>nlp-30</i>	5.38	0.41	13.12

# Validation of expression in ALA neuron using enhancers predicted from “ALA motif”



flp-24

**FLP-24** VPSAGDMMVRF

**FLP-7** SPMQRS SMVRF

TPMQRS SMVRF

SPMERS AMVRF

SPMDRS KMVRF

KRVPSAGDMMVRF GKR



VPSAGDMMVRF G



VPSAGDMMVRF<sub>amide</sub>

**FLP-13** AMDS PLIRF

AADGAP LIRF

APEAS PFIRF

ASPSAP LIRF

SPSAV PLIRF

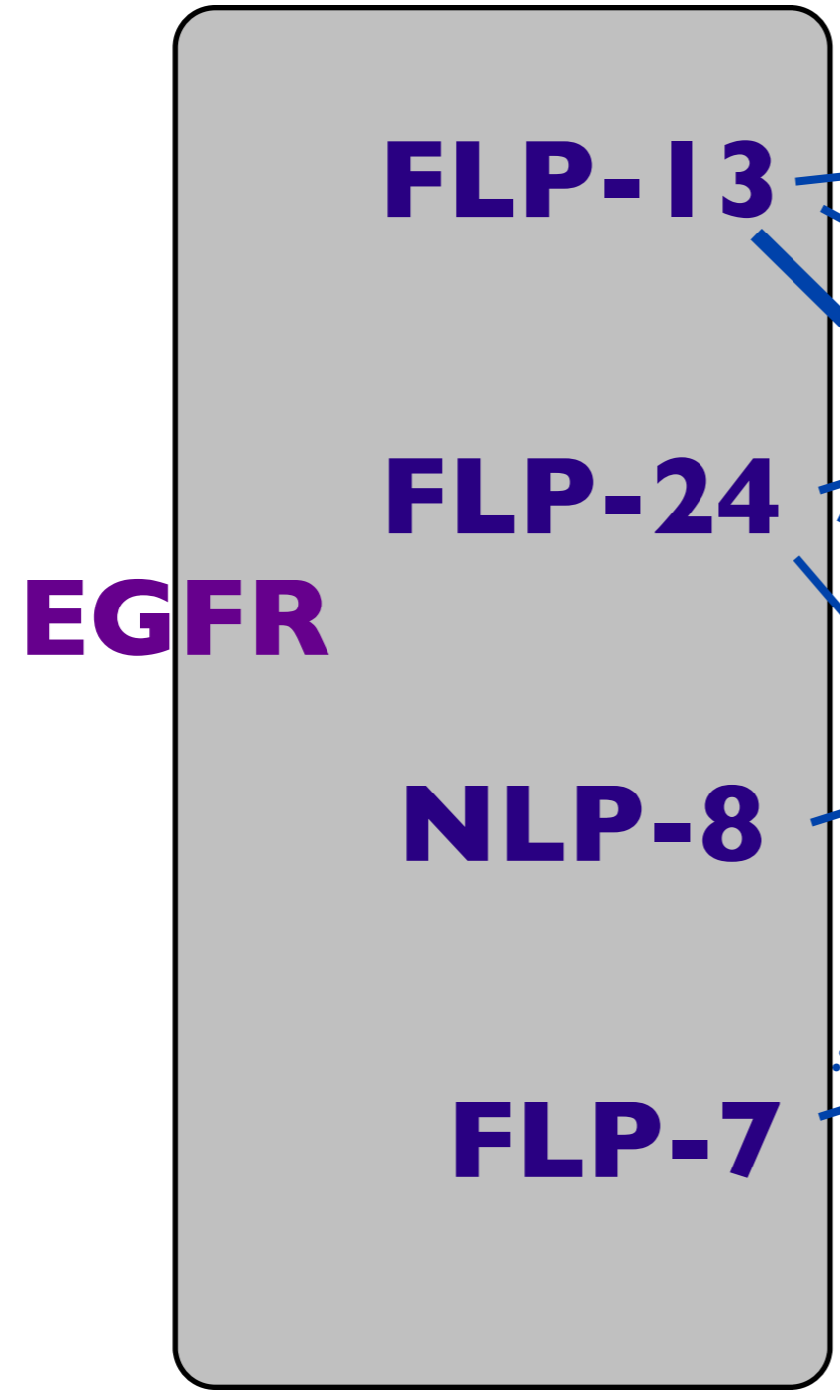
ASSAP LIRF

SAAAP LIRF

**STRESS**

**neuropeptides**

**receptors**



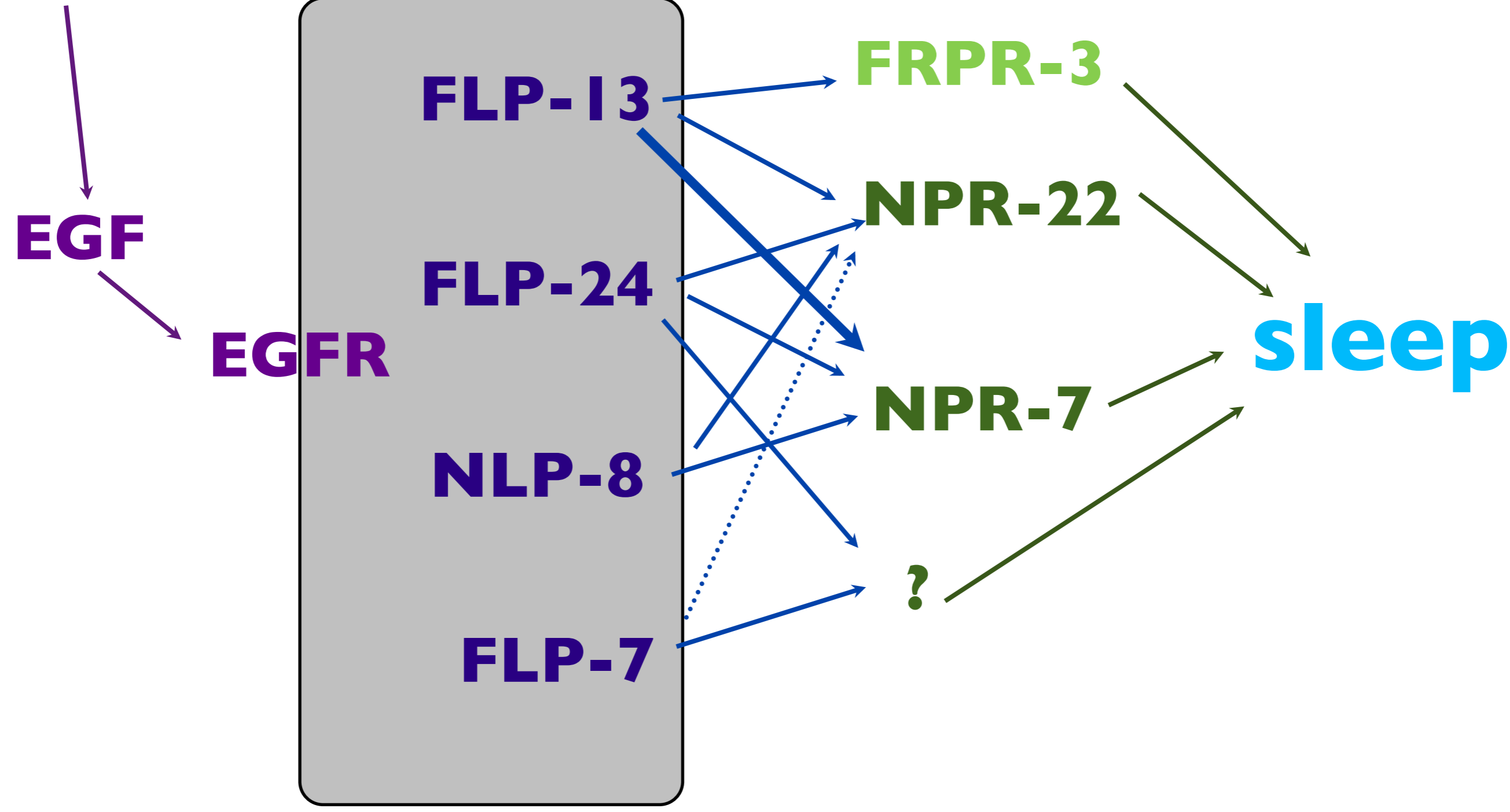
**FRPR-3**

**NPR-22**

**NPR-7**

**?**

**sleep**



**ALA neuron**

Elly Chow

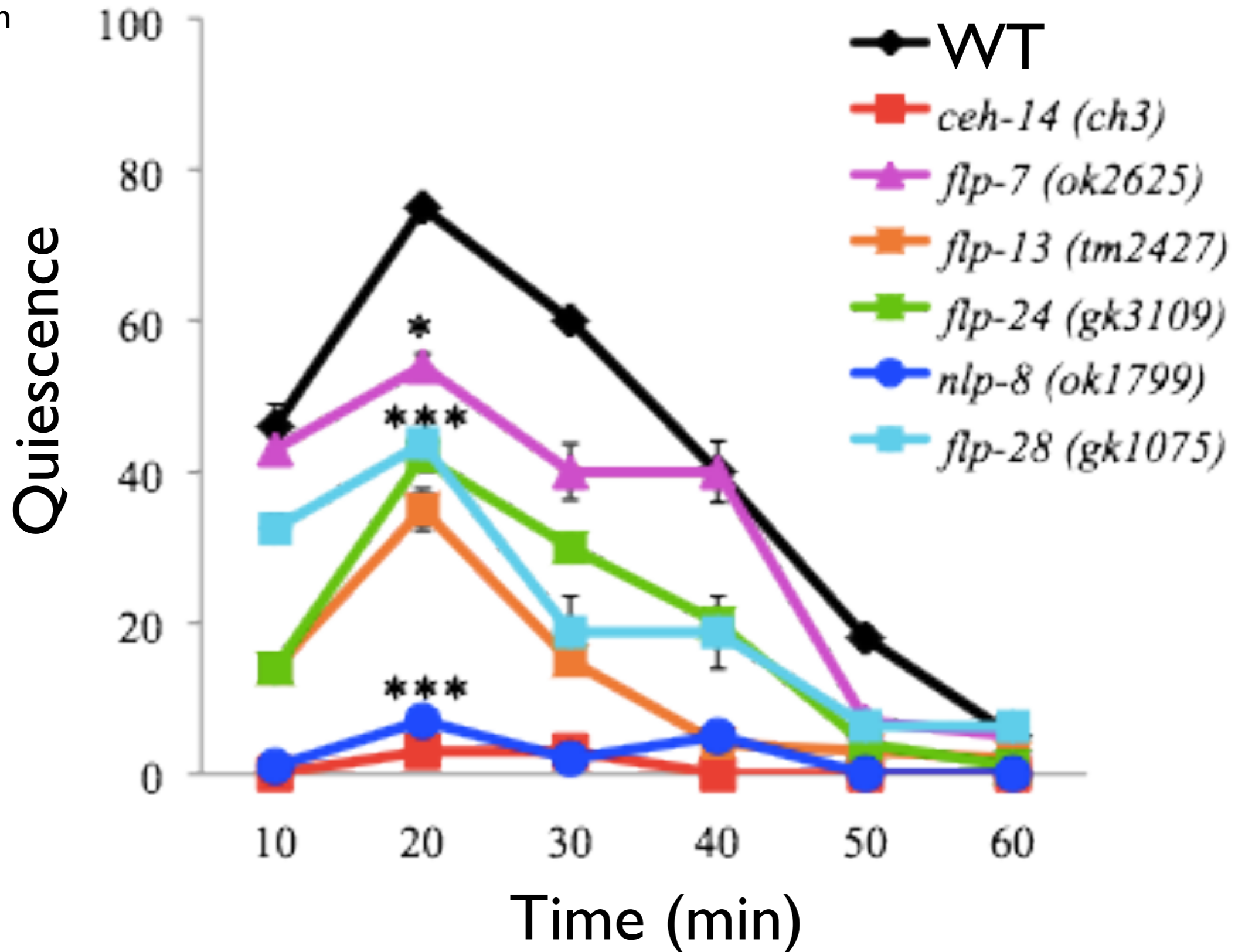
**neuropeptides** → **receptors** → **sleep**

~~**neuropeptides**~~ → **receptors** → **sleep**

Elly Chow

# 4 neuropeptides necessary for EGF response

35°C, 30 min  
recover RT, 10 min  
score





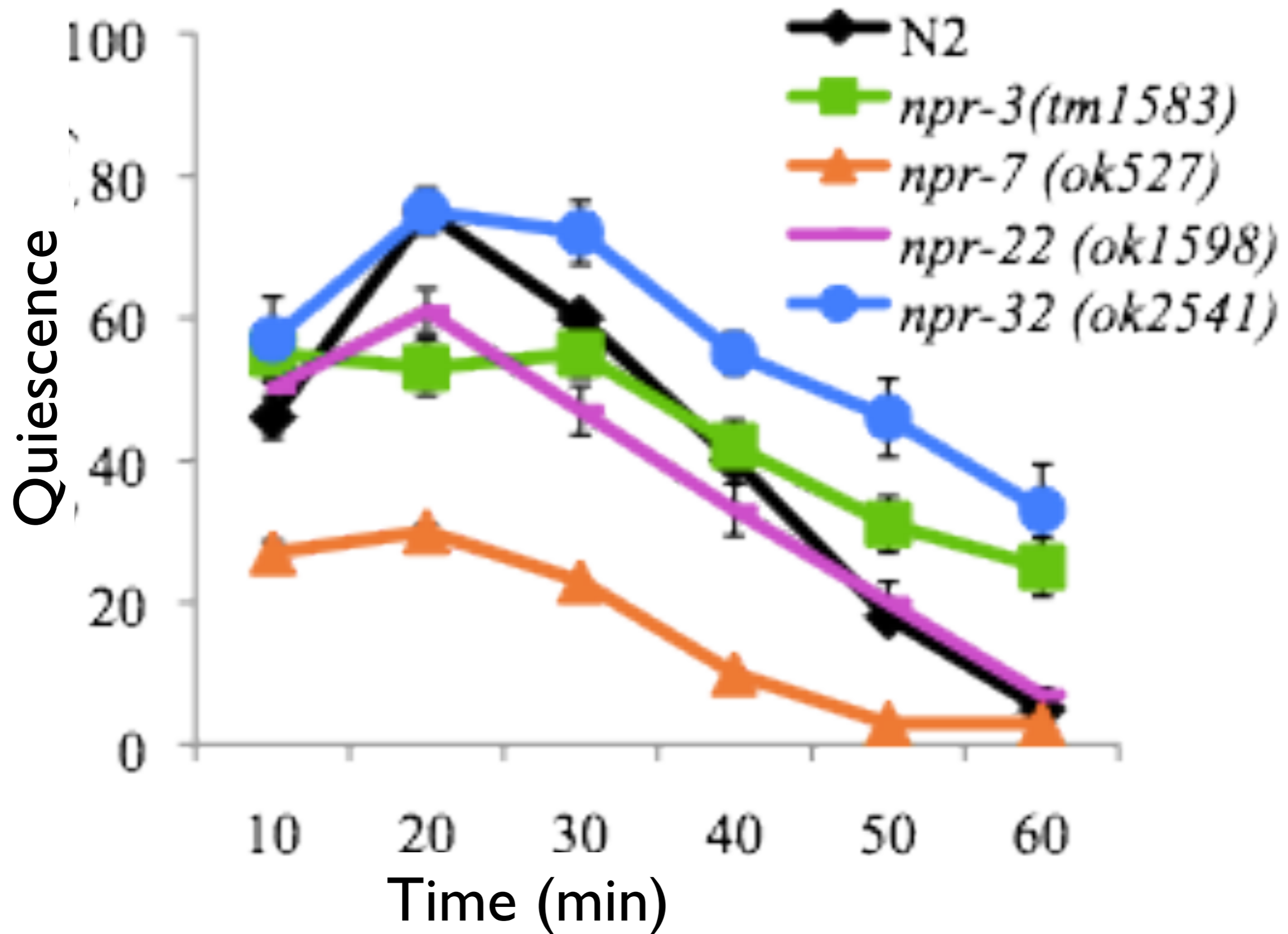
**neuropeptides** → **receptors** → **sleep**

~~**neuropeptides**~~ → **receptors** → **sleep**

**neuropeptides** → ~~**receptors**~~ → **sleep**

Elly Chow

# Multiple receptors are necessary



**neuropeptides** → **receptors** → **sleep**

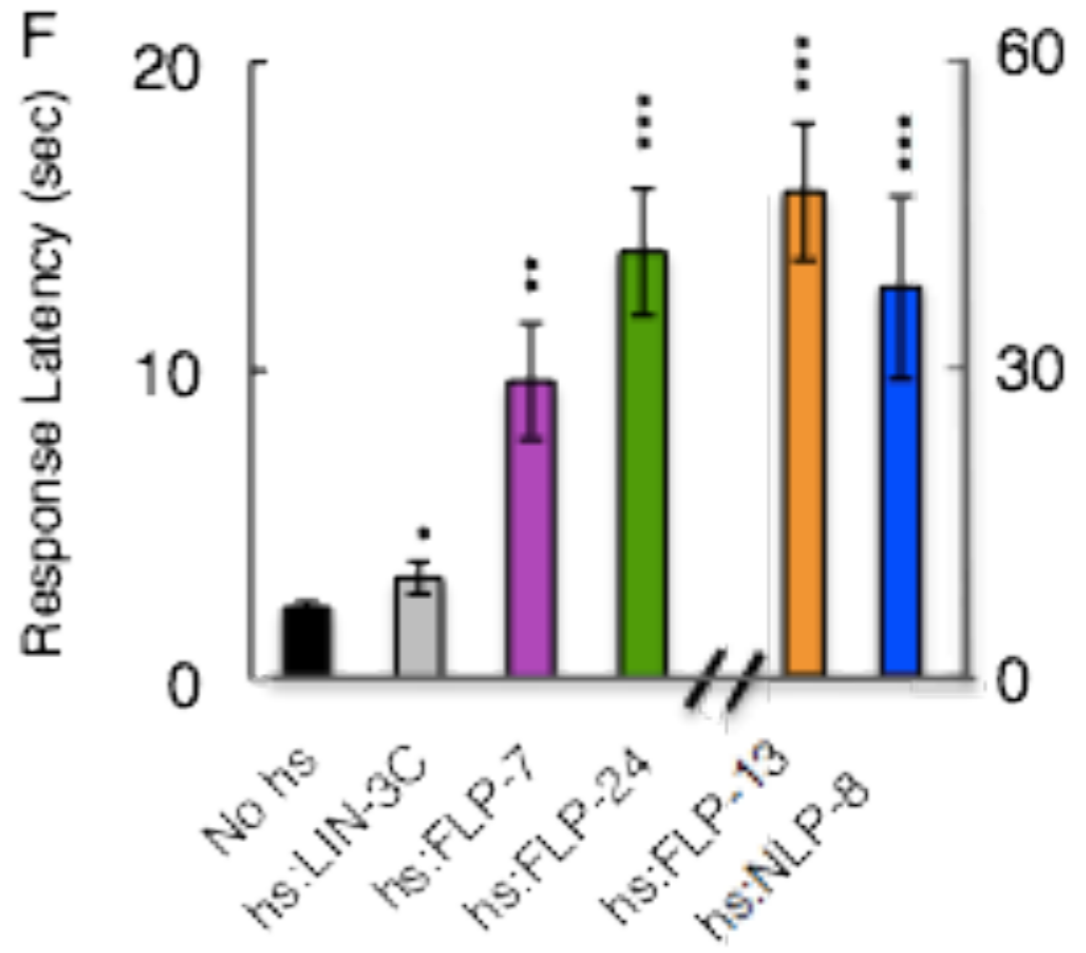
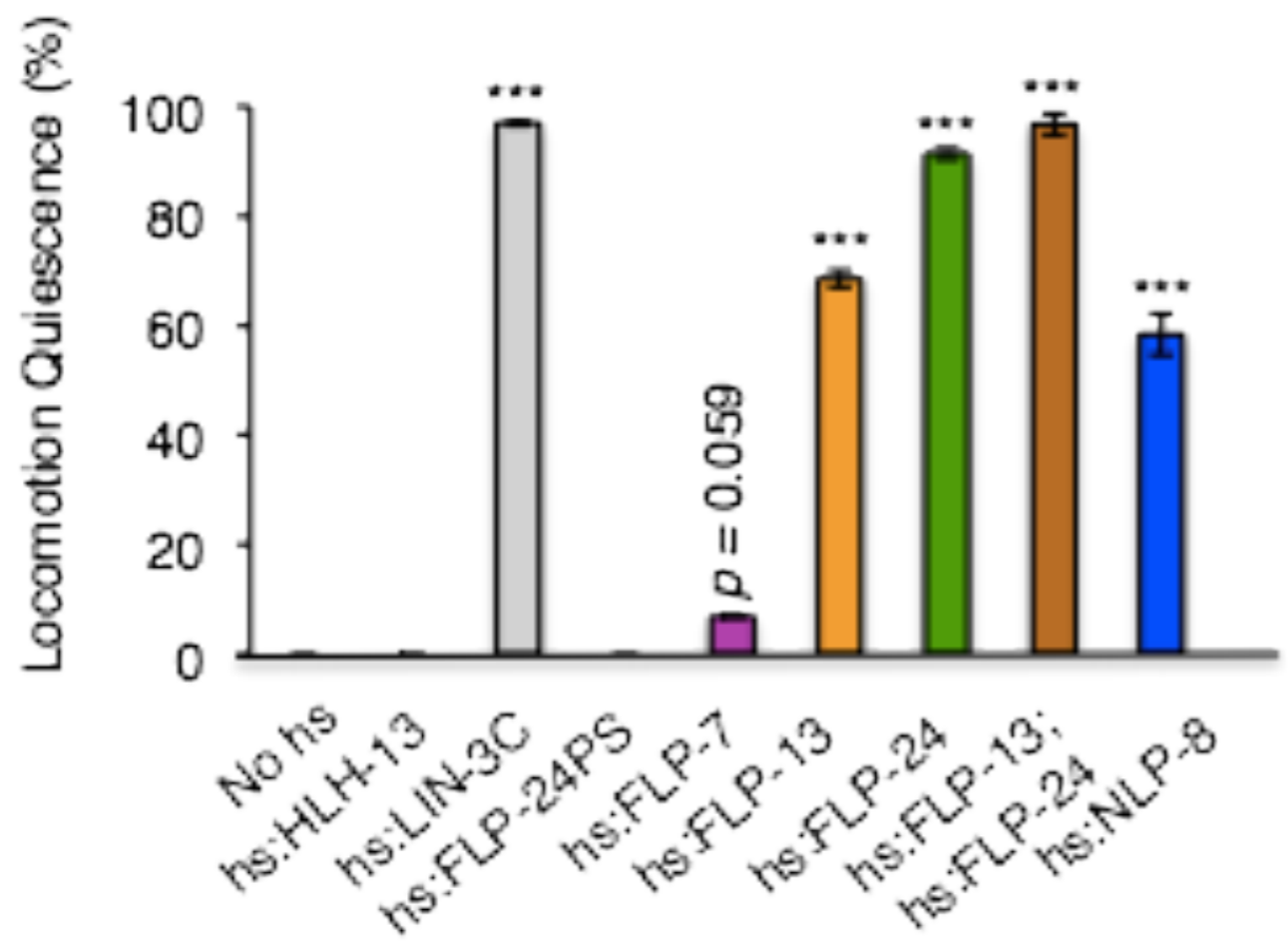
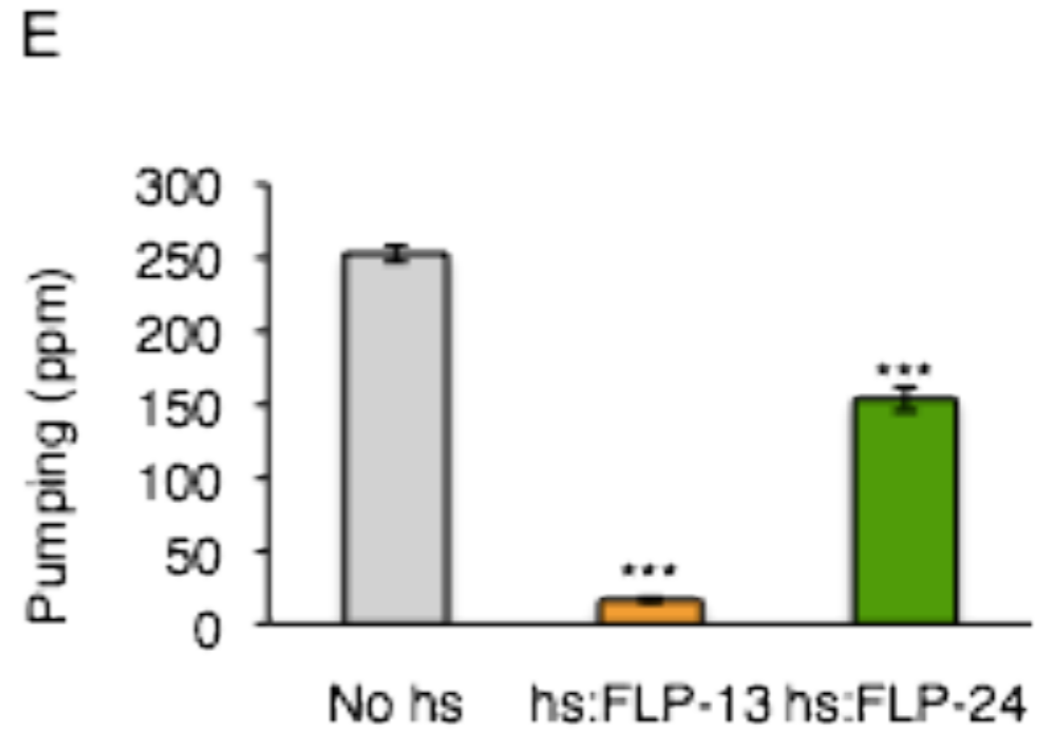
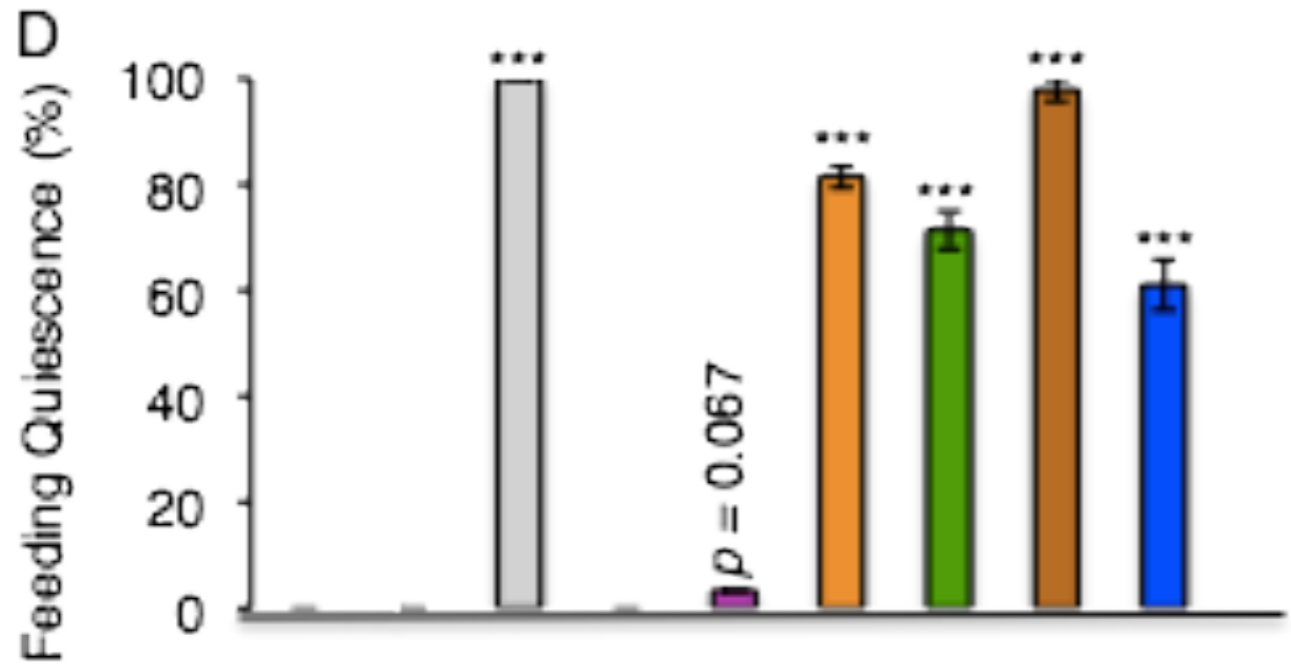
~~**neuropeptides**~~ → **receptors** → **sleep**

**neuropeptides** → ~~**receptors**~~ → **sleep**

**neuropeptides** → **receptors** → **sleep**

Elly Chow

# FLP peptides increase response latency



**neuropeptides** → **receptors** → **sleep**

~~**neuropeptides**~~ → **receptors** → **sleep**

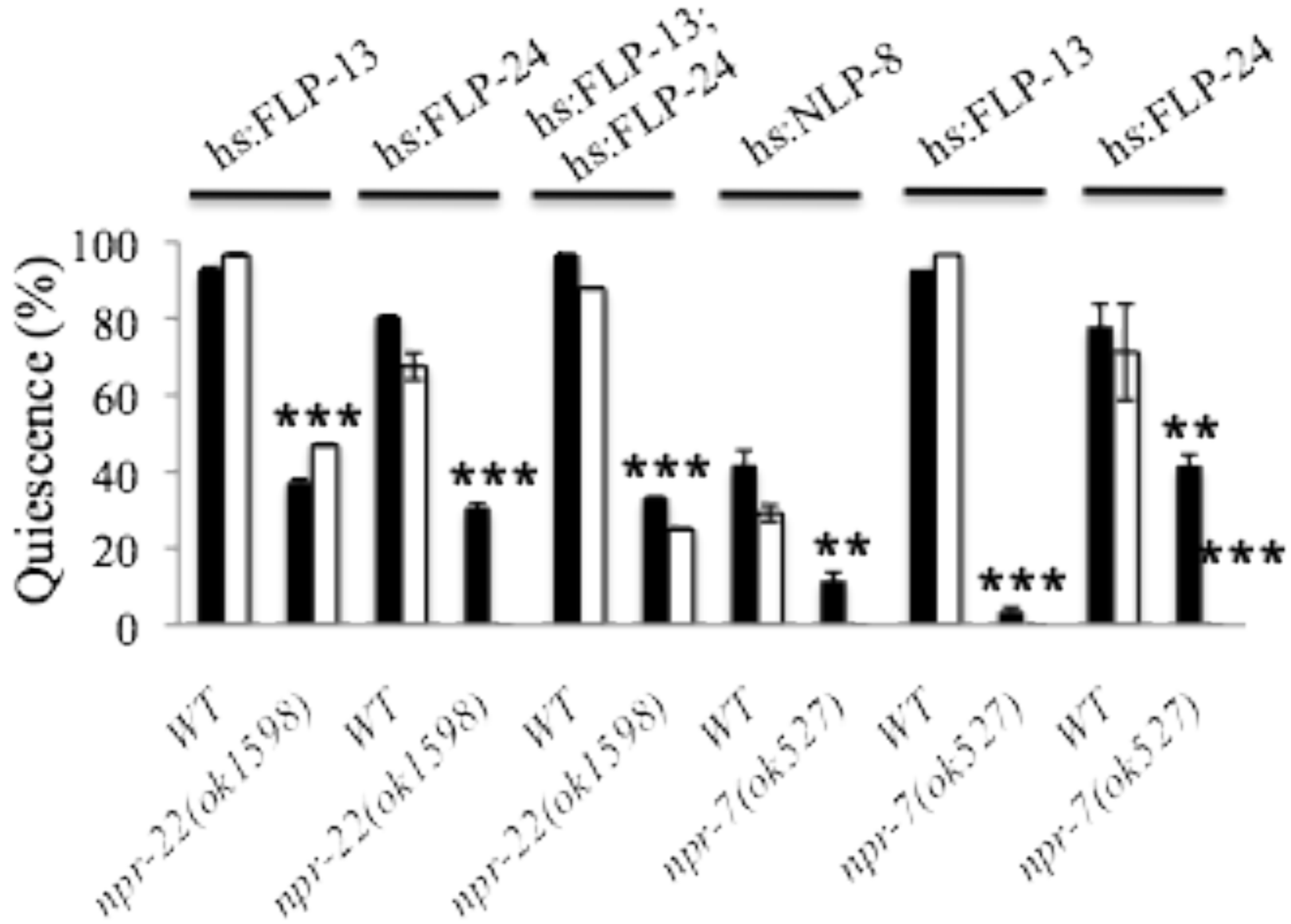
**neuropeptides** → ~~**receptors**~~ → **sleep**

**neuropeptides** → **receptors** → **sleep**

**neuropeptides** → ~~**receptors**~~ → **sleep**

Elly Chow

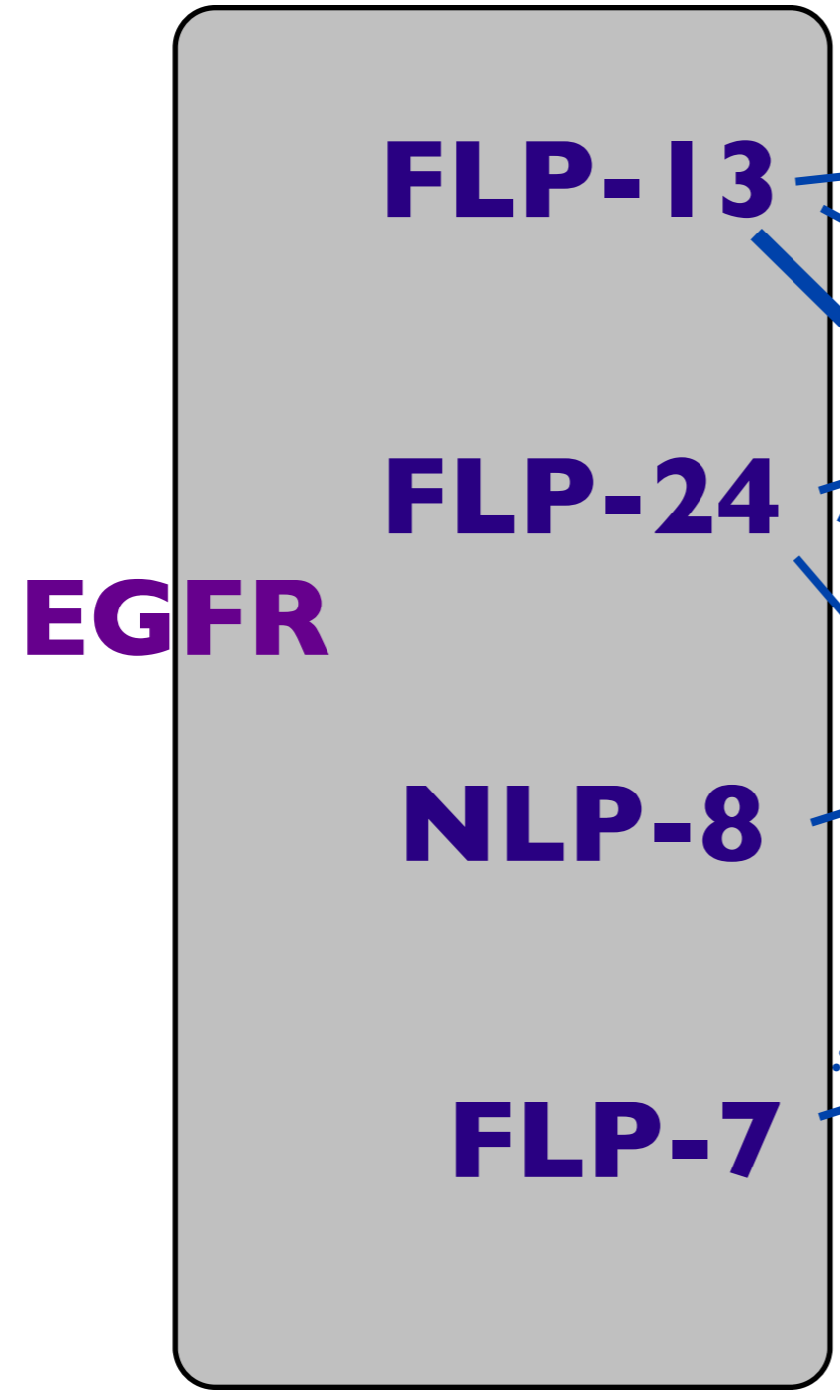
# receptor mutant block peptide overexpression



**STRESS**

**neuropeptides**

**receptors**



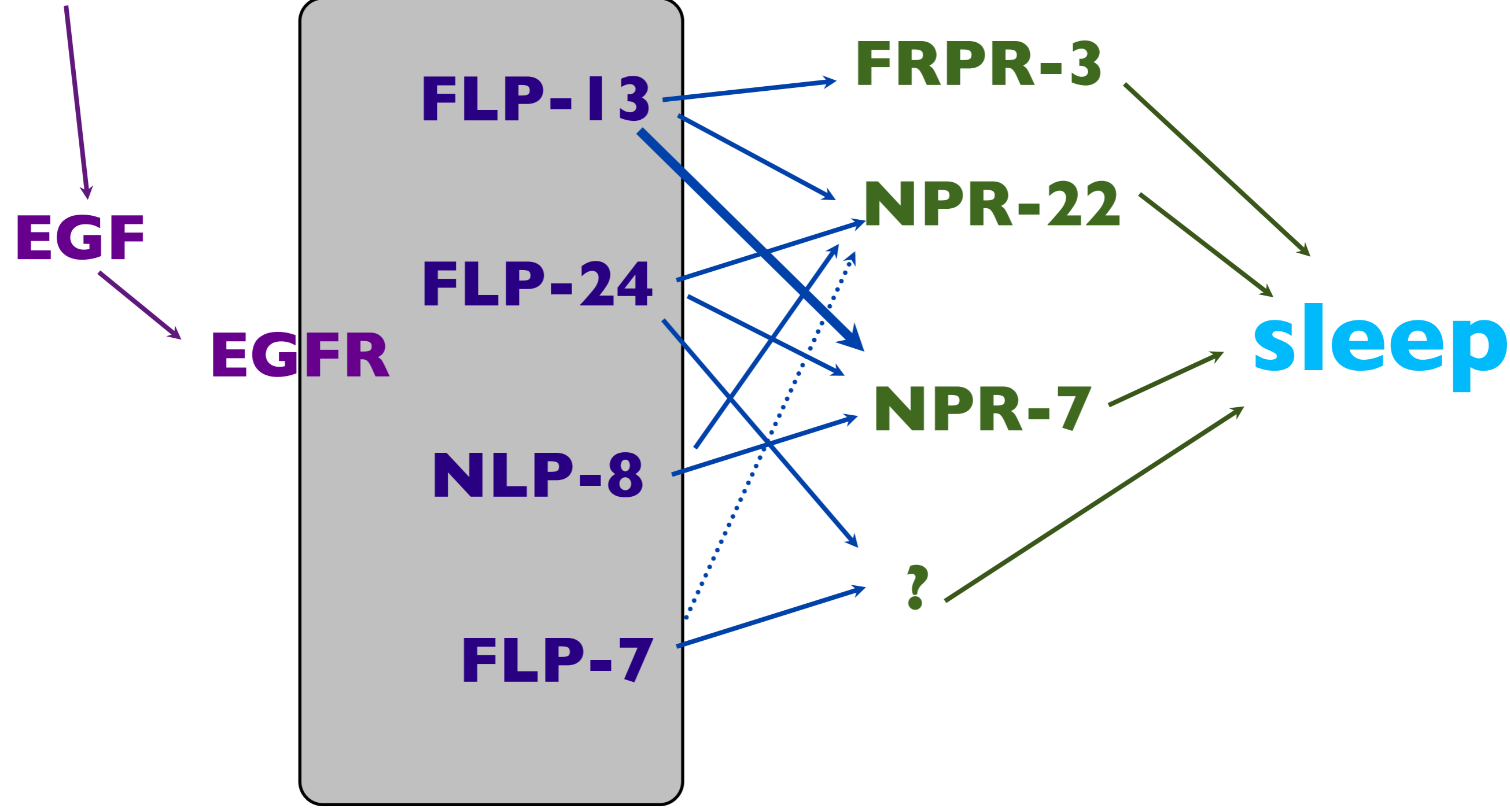
**FRPR-3**

**NPR-22**

**NPR-7**

**?**

**sleep**



Elly Chow