Nocieptor sensitivity and plasticity in *Drosophila melanogaster* is regulated by translation initiation factors

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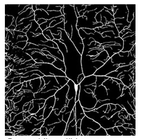
Key Points

- Plasticity of nociceptor neurons is associated with hypersensitivity during chronic pain states and this process can be controlled by regulation of protein synthesis
- Assembly of the eIF4F translation initiation complex is required for nociceptor function and injury-induced sensitization in a *Drosophila* model
- Cellular signaling pathways that regulate eIF4F assembly also regulate the ability of *Drosophila* larvae to undergo injury-induced sensitization following injury

Drosophila larvae are a model for studying nociceptor function

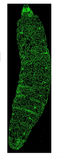


Drosophila mdIV neuron
Han et al 2007

The Class IV multidendritic (mdIV) neurons tile the larval epidermis and are the primary nociceptors of *Drosophila* larvae

Ion channels including TRPA1, Deg/ENaC channels, Piezo, and voltage-gated sodium channels have evolutionarily conserved roles in *Drosophila* nociception

Genetic tractability and well-characterized behavior make *Drosophila* larvae a powerful model system for studying the mechanisms that shape nociceptor sensitivity



Han et al 2007

Elevated Temperature (>39°C)
Harsh Mechanical Stimulation
Short-wavelength Light

→

Response latency inversely proportional to stimulus strength

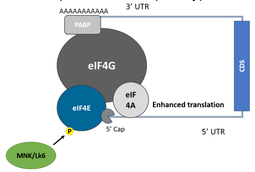
Drosophila larvae exhibit nocifensive escape locomotion (NEL) in response to potentially tissue damaging stimuli

Adapted from Robertson et al. 2013

Regulation of protein synthesis is a mechanism for regulating sensory neuron function

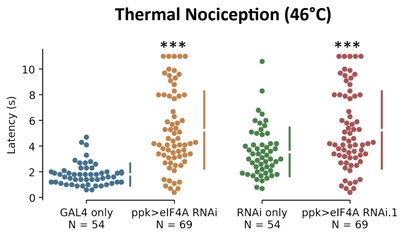
Regulation of protein synthesis is a well-understood mechanism for controlling many aspects of neural development and synaptic plasticity

Control of translation via regulated assembly of the eIF4F complex is thought to control some aspects of nociceptor hypersensitization following tissue damage in mice



How does translational regulation in *Drosophila* nociceptors control sensitivity to noxious stimuli?

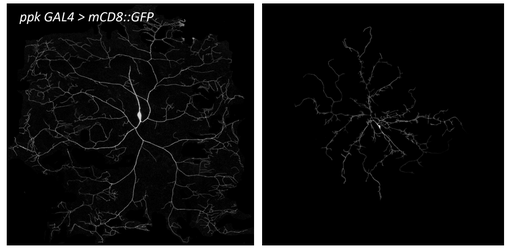
eIF4A proteins are required for normal sensitivity to noxious thermal stimuli



Compared to transgenic controls, larvae with nociceptor-specific *eIF4A* knockdown show reduced sensitivity to noxious thermal stimuli

*** p≤0.001, Brunner-Munzel Test

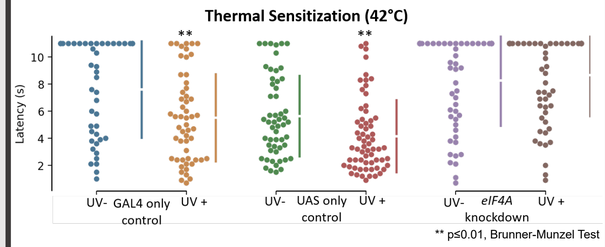
eIF4A proteins are required for normal nociceptor morphology



Compared to transgenic controls, larvae with nociceptor-specific *eIF4A* knockdown show strong defects in nociceptor dendrite morphology

eIF4A proteins are required for nociceptor sensitization following injury

Experimental paradigm: Control and knockdown larvae are exposed to UV-induced tissue damage. Eight hours later, they are tested for nociceptor sensitization (reduced response latency compared to sham-exposed control)

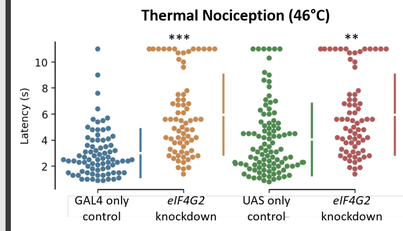


** p≤0.01, Brunner-Munzel Test

Compared to transgenic controls, larvae with nociceptor-specific *eIF4A* knockdown show defects in nociceptor sensitization

Conclusions: eIF4A function in the nociceptors is required for normal nociceptor sensitivity, normal nociceptor morphology, and for sensitization of nociceptors following injury

eIF4G2 proteins are required for normal sensitivity to noxious thermal stimuli

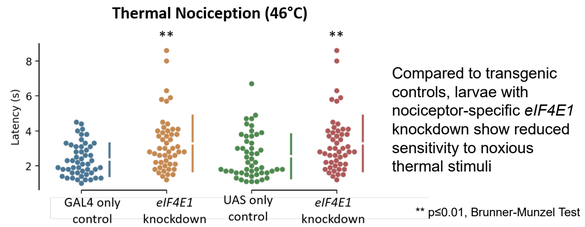


Compared to transgenic controls, larvae with nociceptor-specific *eIF4G2* knockdown show reduced sensitivity to noxious thermal stimuli

** p≤0.01, Brunner-Munzel Test

*** p≤0.001, Brunner-Munzel Test

eIF4E1 proteins are required for normal sensitivity to noxious thermal stimuli

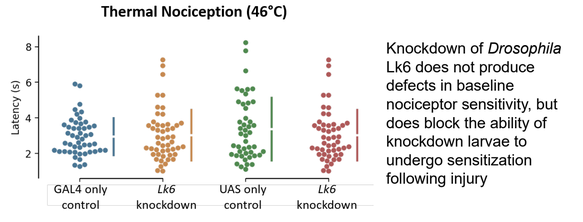


Compared to transgenic controls, larvae with nociceptor-specific *eIF4E1* knockdown show reduced sensitivity to noxious thermal stimuli

** p≤0.01, Brunner-Munzel Test

Conclusions: eIF4G and eIF4E subunits are also required in the nociceptors for normal nociceptor function (sensitization experiments are forthcoming)

MNK/Lk6 proteins are required for nociceptor sensitization following injury

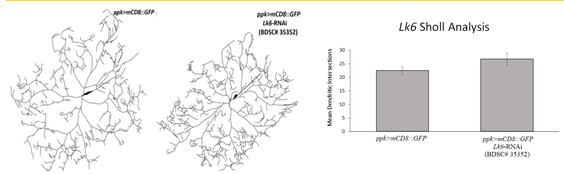


Knockdown of *Drosophila* Lk6 does not produce defects in baseline nociceptor sensitivity, but does block the ability of knockdown larvae to undergo sensitization following injury

* p≤0.05, Brunner-Munzel Test

** p≤0.01, Brunner-Munzel Test

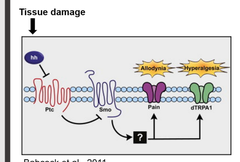
MNK/Lk6 proteins are not required for normal nociceptor morphology



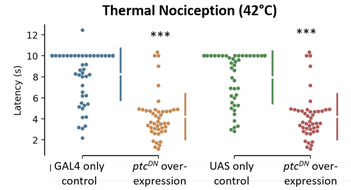
Compared to transgenic controls, larvae with nociceptor-specific *Lk6* knockdown do not show strong defects in nociceptor dendrite morphology

Activated Hedgehog signaling induces sensitization in the absence of injury

Previous studies have demonstrated that Hedgehog signaling pathways are required for nociceptor sensitization. We activated Hedgehog signaling in the nociceptors by overexpressing a dominant-negative form of the Patched regulatory protein.

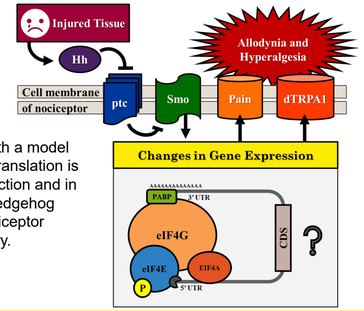


Babcock et al., 2011



*** p≤0.001, Brunner-Munzel Test

A model for translational regulation of nociception in *Drosophila*



Our data are consistent with a model in which eIF4F-regulated translation is required for nociceptor function and in which signaling through Hedgehog and MNK/Lk6 activate nociceptor sensitization following injury.

Conclusions and future directions

- Nociceptor-specific RNAi knockdown experiments demonstrate that Drosophila larval nociception is controlled by regulated protein synthesis
- Future experiments will determine how translation is regulated following tissue damage and during sensitized nociception states
- Ongoing experiments will identify the transcripts that are translationally regulated during nociceptor sensitization and the signaling pathways that activate MNK/Lk6 and eIF4F assembly

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