Chemical nociception in Wild-type and dYPEL3 Drosophila larva mutants

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Abstract

Drosophila larval nociception is widely used for studying various basic circuit mechanisms. Traditionally, the behavioral assay for Drosophila nociception relied on either optogenetic or physical stimulation. The behavioral response upon chemical stimulation has not been widely used due to high levels of variability. We found that larva require co-stimulation with blue light to exhibit consistent nociceptive behavior upon a noxious chemical stimulation using allyl isothiocyanate (AITC). Introducing constant blue light illumination during AITC treatment greatly increased the consistency of behavioral output. dYPEL3 mutations have been identified in a number of neurological disorders such as schizophrenia and autism. We used our refined AITC-induced larval nociceptive behavior and found a reduction in nociceptive behavior in dYPEL3 mutants. Currently, larval nociceptive behavior is quantified by manual scoring. We are working to develop automatic quantitation of nocicieptive behavior using machine-learning techniques from software such as DeepLabCut (DLC).

Materials and Methods

All fly larvae used completed development 5 days after egg laying. A solution of 25 mM allyl isothiocyanate (AITC) and 0.3625 M dimethyl sulfoxide (DMSO) was prepared 3 days prior to experiments. Experiments were conducted in 24-well plates with grape agar background. Solution was added to each plate and then fly larvae were placed in each well and observed for one minute. All experiments were recorded and manually analyzed.

Light Intensity Nociception

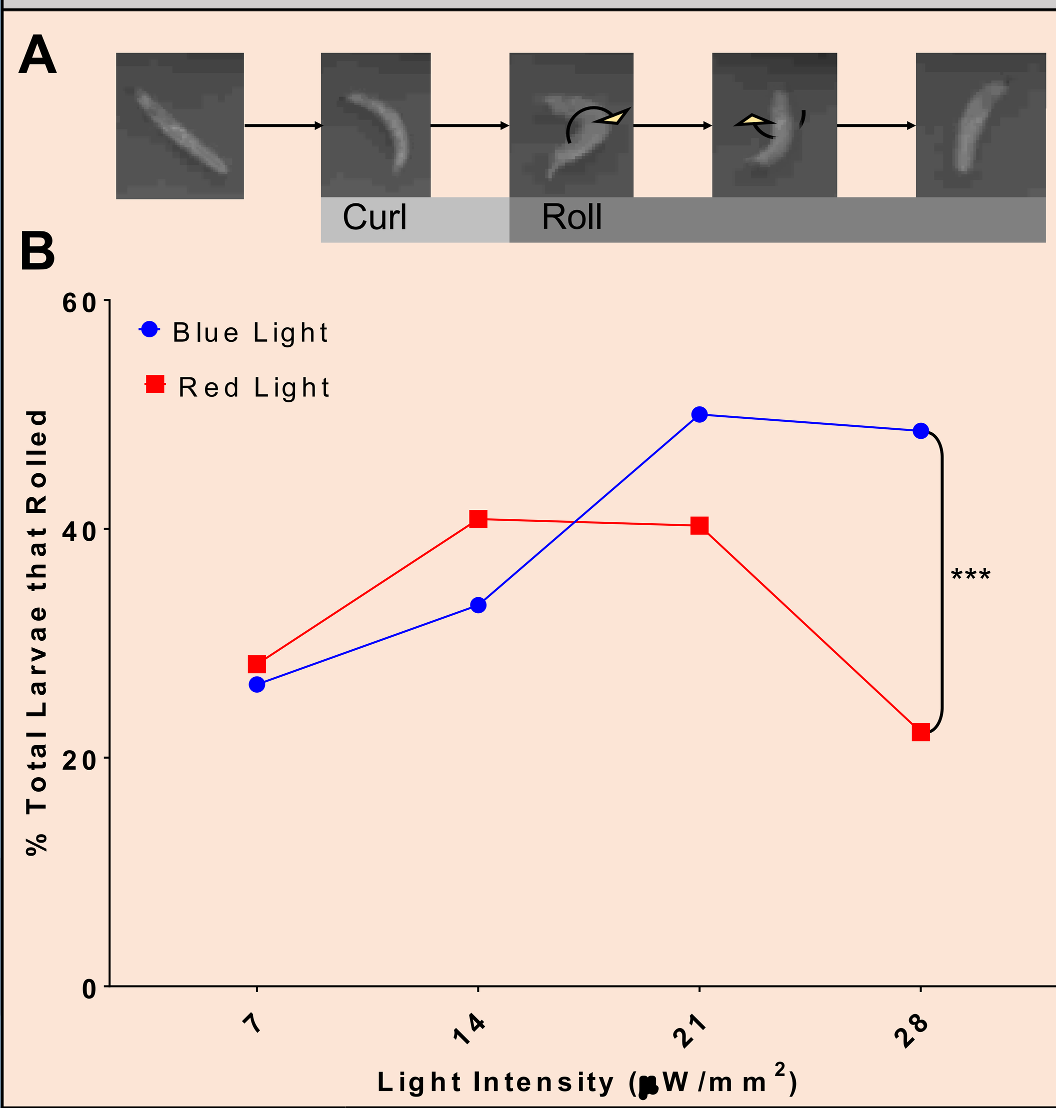
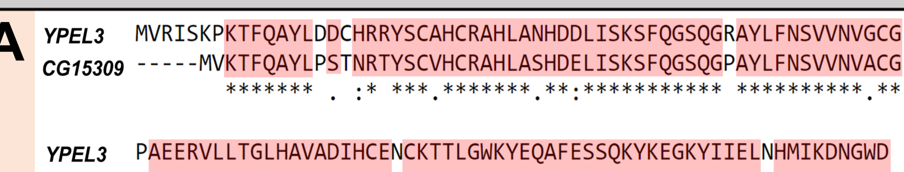


Figure 1. Activation of larval nociception at varying light intensity.
(**A**) A montage displaying third-instar larval nociceptive behavior, including curling and full-roll.
(**B**) Larval nociceptive behavior was tested at 4 varying red and blue light intensities. n=72 for 7 µW/mm², 14 µW/mm², and 21 µW/mm² blue, 21 µW/mm² and 28 µW/mm² red. n=71 for 7 µW/mm² and 14 µW/mm² red. n=70 for 28 µW/mm² blue

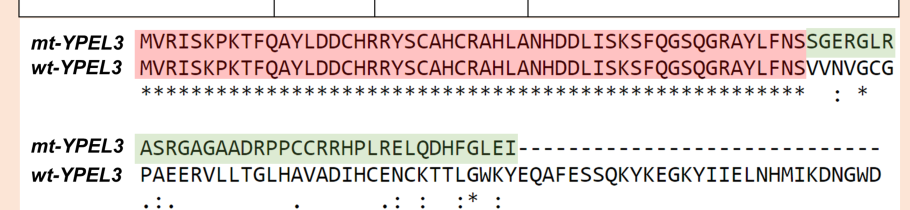
dYPEL3 Mutant Nociception

A



B

Transcript	Exon	Type	Protein
NM_031477.4 (YPEL3):c.273dup	exon2	insertion frameshift	p.Val92Serfs*38 (STOP codon 37 positions)



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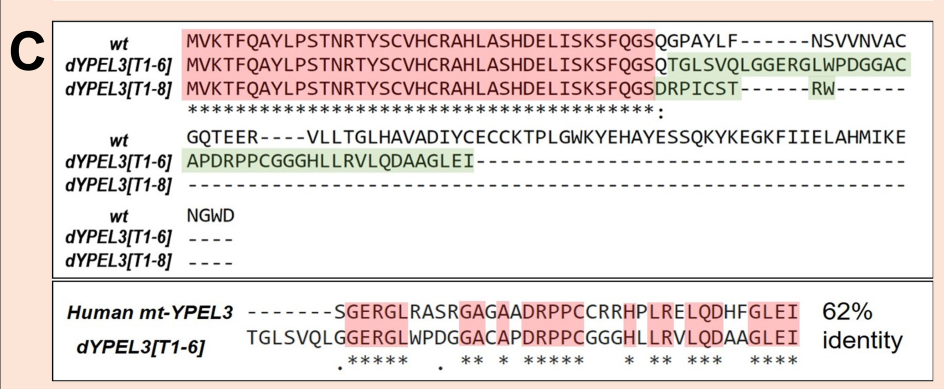

Figure 2. Amino acid sequences of dYPEL3 wild-type and mutant variants.
(**A**) The homology between human YPEL3 and a *Drosophila* CG15309.
(**B**) The frameshift mutation found in human *YPEL3* followed by the predicted molecular lesion.
(**C**) The isolated In-del mutations and predicted molecular lesion in dYPEL3, followed by the homology between the extended sequences in human mutant and *dYPEL3[T1-6]*

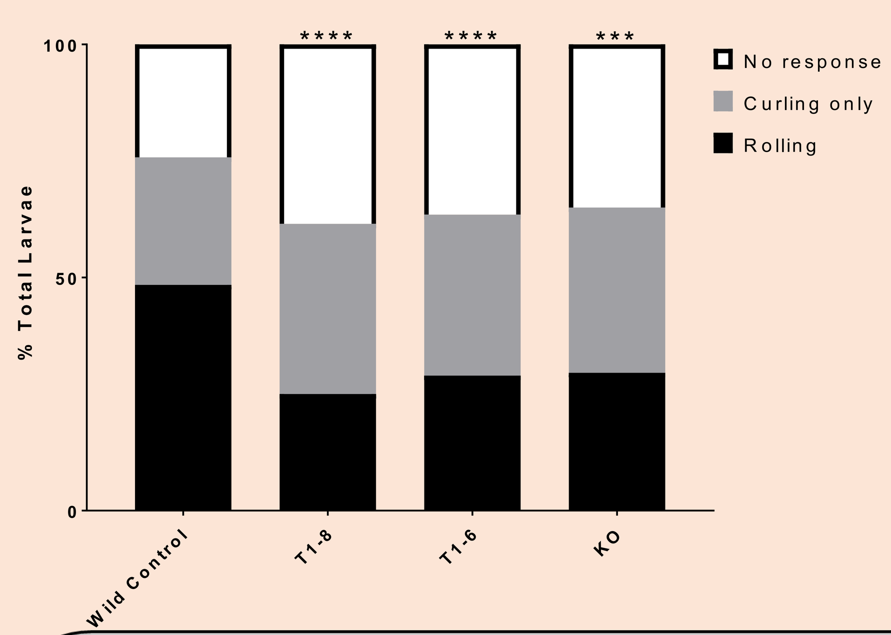

Figure 3. Nociceptive behavior in Drosophila larvae. Wild-type and three dYPEL3 mutant type larvae were tested for nociceptive behavior in AITC. All were conducted in 21 µW/mm² blue light. Significant decreases in behavior occurred in T1-8 ($p<0.0001$), T1-6 ($p<0.0001$), and knockout (KO, $p=0.0002$)variants. n=252 for wild, T1-8, and T1-6 variants. n=203 for KO variant.

Automatic Behavior Detection

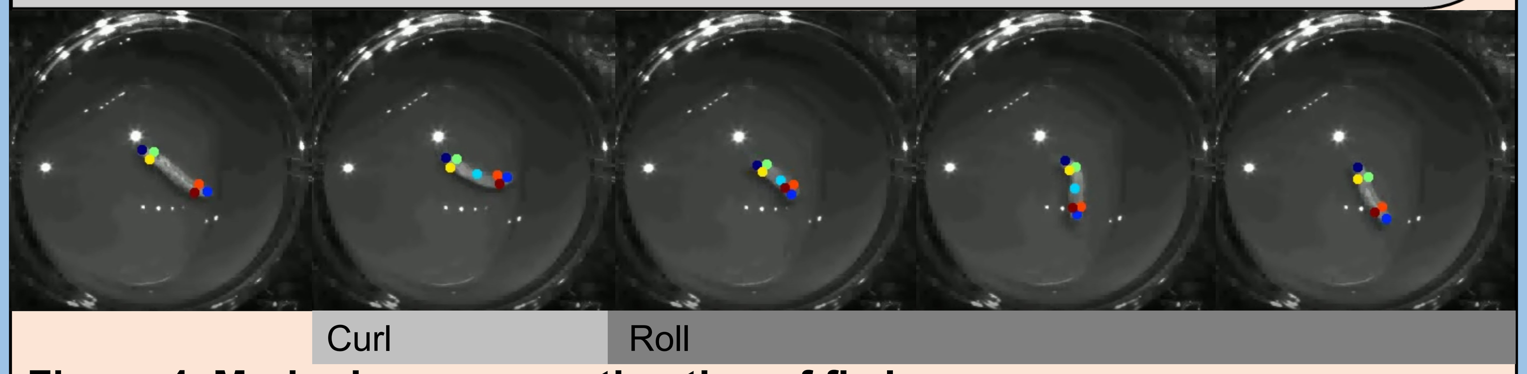

Figure 4. Markerless pose estimation of fly larva.
The software DeepLabCut (DLC), a machine-learning algorithm, was used to begin development of automatic nociceptive behavior detection in larvae. Tracked points above are the head, tail, relative left and right of both the head and tail, and underbelly of the larva. Outputted data is currently being studied to develop a program to automatically detect rolling and curling behavior.

Conclusions

While largely statistically insignificant, the general trend of the larval nociceptive behavior to noxious chemical stimuli increased as blue light intensity increased. Furthermore, blue light created a more consistent rolling behavior in the larvae at higher intensities (21 and 28 µW/mm²), suggesting multi-modal integration of light intensity for the nociceptive behavior when chemically stimulated. While under the 21 µW/mm² blue-light intensity, the rolling nociceptive behavior of the mutant larvae was significantly decreased, as well as any overall positive response to the noxious chemical stimulus. Also of note was the generally increased curling-only response of all mutant variants. From this we can conclude that mutations in the dYPEL3 gene significantly decrease nociceptive response in Drosophila larvae.

Future Directions

Going forward, we will study this multi-modal integration of noxious chemical stimuli and light stimuli. To do so, we will perform calcium imaging to map areas of interest in the fly nociceptive circuit to find areas of potential cross-over with light sensing circuits. Additionally, we will study nociceptive behavior of larvae with and without Bolwig organs, primitive eye-like structures used by the larvae to detect light, known to sense blue light. Further development of the machine-learning techniques to automatically detect and classify Drosophila larvae behavior will also continue.

References

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