Modeling Epilepsy in Larvae of Seizure-Prone Flies

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Background

• The *prickle* gene was first discovered in *Drosophila melanogaster* as a component of the planar cell polarity (PCP) pathway, which controls the orientation of cells and their organs and is within a layer of cells (Gubb 1999).
• There are three isoforms of *prickle*, two of them being *prickle* (pk) and *prickle* (spl) required for PCP in the larva and adult.

![Figure 1](image1.png)

Figure 1. *prickle* and *prickle* mutants display polarity defects in the wings, while *prickle* and *prickle* isoforms exhibit abnormally-bristled legs. However, the *prickle* flies express an intermediary phenotype of both. (from Lin & Gubb 2008)

• Recent studies have shown that mutations in *prickle* orthologs begin seizure phenotypes in flies, mice, and humans (Tao et al. 2011, Ehaideb et al. 2014).
• *prickle* mutants are prone to epileptic seizures, while *prickle* mutants are less prone to seizures than wild-type *Drosophila* (Tao et al. 2011, Ehaideb et al. 2014).
• Longevity studies have found that *prickle* flies have a reduced viability in comparison to *prickle* flies (Ehaideb et al. 2014), suggesting that the *prickle* isoform is important even if the *pk* mutants were not seizure prone.
• Although the *prickle* adults displayed varying seizure phenotypes, larvae of this genotype were never analyzed.

Methods

• Wild-type, *prickle*, and *prickle* third-instar *Drosophila* larvae were placed on 1% agarose pads.
• Natural, unmanipulated movements were recorded through a Leica MZ 16 F microscope, aided by a Q-Capture Q21350 camera.
• Acquired videos were analyzed through the DIAS (Digital Imaging Analysis System) software.
• DIAS analyzed parameters such as the larval speed, total path length, larval stamina (peristalsis), and the directional changes of larval paths.
• Persistence: higher number = higher stamina
• Directional Change: higher number = less change in direction

To determine if results were statistically significant, data were run through a Student’s t-test.

Objectives

• To test whether seizure-prone *prickle* mutants have a characteristic seizure phenotype in the larval stage. We predicted the following outcomes:
  1. *prickle* will move in a non-fluid, uncoordinated motion in comparison to controls.
  2. *prickle* will move slower in comparison to controls.
  3. To test whether both the *prickle* and *prickle* mutant larvae have locomotion defects compared to controls.

Results

• *prickle* larvae had less deviation in movements in comparison to the wild-type and *prickle* larvae

![Figure 2](image2.png)

Figure 2. While the paths of wild-type and *prickle* larvae are curved and inconsistent, *prickle* paths are generally consistent in one direction.

• *prickle* larvae have greater speed and path length in comparison to the wild-type and *prickle* larvae.

![Figure 3](image3.png)

Figure 3. Movement of larvae at increments of 0.1 frames. While wild-type and *prickle* tend to exhibit curved movements, *prickle* tends to move in a straight path.

<table>
<thead>
<tr>
<th>DIAS Data</th>
<th><em>prickle</em></th>
<th>wild-type</th>
<th><em>prickle</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed (um/sec)</td>
<td>18.69</td>
<td>14.96</td>
<td>12.92</td>
</tr>
<tr>
<td>Directional Change (higher = less)</td>
<td>42.16</td>
<td>36.61</td>
<td>30.47</td>
</tr>
<tr>
<td>Persistence</td>
<td>4.13</td>
<td>2.55</td>
<td>2.22</td>
</tr>
<tr>
<td>Total Path Length (um)</td>
<td>92.05</td>
<td>73.78</td>
<td>64.37</td>
</tr>
</tbody>
</table>

![Figure 4](image4.png)

Figure 4. Speed, directional change, persistence, and total path length of larvae were analyzed through the DIAS software. The Student’s t-test showed that the differences between the path lengths of the *prickle* vs. wild-type (*p* = 0.03), as well as the speeds of the *prickle* vs. wild-type (*p* = 0.03), were statistically significant.

Conclusions

• Surprisingly, the *prickle* mutant larvae did NOT exhibit uncoordinated movements, nor did they move more slowly than wild-type controls.
• In fact, the larvae moved significantly faster than wild-type controls, and showed an increased persistence with less directional change.
• Given that the *prickle* mutant adults have a reduced seizure threshold compared to controls (Ehaideb et al. 2014), these data suggest that *prickle* mutant larvae (although not seizure-prone) are able to more efficiently activate the neuronal circuitry required to engage the musculature responsible for crawling.

Implications

• These data are consistent with the hypothesis that there is a fine balance between being able to efficiently fire the neuronal circuitry required for a response to stimuli (such as escape from a predator) and the seizure phenotype that results from too low an excitability threshold. Intriguingly, the mutation that allows the *spile* larvae to more efficiently crawl away from danger, or towards food sources, predisposes the *prickle* adult to epileptic seizures.

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References


