The $p^{kspile}$ seizure-associated isoform of prickle is co-expressed in the same cells as the $p^{kpk}$ isoform in Drosophila larval brains

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Key Findings
We have demonstrated for the first time that prickle (pk) and spiny-leg (spl) proteins are expressed in similar locations of the Drosophila larval brain. This finding suggests that these proteins are localized in the same cell types, and supports the antagonistic relationship observed between these isoforms previously. Furthermore, cleaved caspase-3 levels were comparable between prickle (pk) and spiny-leg (spl) mutant and wild-type (Oregon-R) larvae, indicating that there is no significant neurodegeneration in the larval stages of this mutant, even though this genotype is prone to seizures.

Introduction
The prickle gene was originally discovered in flies as part of the planar cell polarity (PCP) pathway, controlling the orientation of cells and their structures within the plane of a layer of cells. $p^{kspile}$ mutant alleles cause an extreme polarity phenotype in the wing and notum, while $p^{kpk}$ mutant alleles cause an extreme phenotype in the abdomen and legs. Mutation of both isoforms creates intermediary phenotypes in the wings and legs, suggesting that these two isoforms are in opposition to one another (Gubb et al., 1999; Lin & Gubb, 2009).

Methods
1. Larvae of Oregon-R (OR), prickle (pk), and prickle (pk) mutant genotypes were inverted and fixed in 4% paraformaldehyde for 30 minutes.
2. Carasses were washed thoroughly with PBST and blocked with PBSTB to prevent non-specific binding.
3. Larvae were stained using an Elton (E10) antibody (designed to detect both the prickle and splg proteins), or cleaved caspase-3 antibody (to detect signs of apoptosis) overnight at 4°C, washed, incubated with a fluorescent secondary antibody, washed again, and mounted on slides for imaging.
4. A confocal microscope was used to capture images and the pictures were analyzed.

Conclusions
• Combining data from this poster with data generated by Gina Yuan, it is clear that expression patterns of the prickle isoforms were similar, suggesting the presence of both isoforms in comparable locations in the Drosophila larval brain, and indicating that these two isoforms function in the same cells.
• In the neurodegeneration experiment, there is minimal caspase activity in the larval stage of $p^{kspile}$ mutants, even though this genotype is prone to seizures. This may reflect the possibility that the third instar larvae were too young to show cytotoxic activity.

Study Implications
• We have shown for the first time that Prickle protein isoforms are expressed in similar locations in the Drosophila larval brain. This suggests that the two isoforms are acting in the same cells.
• Knowledge of the location and function of prickle, a gene that has been linked to epilepsy, can help elucidate the function of this gene, in hopes of eventually developing highly specific treatments for prickle-associated disorders

Future Directions
• We plan to determine which types of neurons express detectable levels of Prickle and whether there is a correlation between expression levels and neuronal function.
• We also plan to examine brains of aged flies to look for signs of neurodegeneration and damage due to cytotoxicity.

Objectives
1. We wanted to determine if there is differential expression of the prickle isoforms in larval brains. We predicted two potential outcomes: 1) The two Prickle protein isoforms are expressed in different places because in the PCP phenotype, mutations affect different parts of the body, or 2) since a moderate PCP phenotype results when both isoforms are knockouted, both isoforms could be expressed in the same cells.
2. Additionally, due to increased neuronal signalling events that occur as a result of epilepsy, we determined whether cytotoxicity and neuronal cell death were increased in the brains of knockouted larvae.

Results

1. OR larvae brains stained with E10 antibody showed very bright staining in the ventral nerve chord (VNC) and brain hemispheres.
2. Brains of the prickle mutant larvae also had staining in the VNC and brain, albeit slightly dimmer, as only the spl protein was produced.

References