Exploring Learning and Locomotion Deficits in a *Drosophila* Seizure Model

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Introduction/Background

The *prickle* gene encodes two adult protein isoforms of the Planar Cell Polarity (PCP) complex, and mutations in both isoforms have been associated with different neurological phenotypes. For instance, when the *prickle-spiny-legs* (*sple*) isoform is mutated, the fly exhibits seizures and locomotor defects that mimic those found in human patients with *PRICKLE* mutations (Ehaideb et al., 2016). Conversely, when the *prickle-prickle* isoform (*pk*) is mutated, preliminary data in the Manak lab revealed widespread neurodegeneration in the mutant brains, and *these* mutants showed a pronounced reduction in lifespan. Finally, when a mutation affects the entire gene (*pk-sple13*), intermediate phenotypes between *sple/sple* and *pk/pk* are observed.

While individuals with *PRICKLE* mutations can present with seizures, they also have an increased likelihood of presenting with an autism spectrum disorder (ASD) that includes intellectual disability (i.e., learning and memory deficits) (Paemka et al., 2013). Preliminary data in the Manak laboratory has shown that adult *sple/sple* mutants, in addition to presenting with seizures, show learning deficits. This study sought to determine whether locomotor or learning deficits were also manifest in earlier life stages (pre-adult) of the *sple/sple* mutants.

Research Objectives

Our goal was to investigate the effects of isoform-specific *prickle* mutations on the neurological dysfunction of *prickle* larvae. Given that seizure and locomotor defects - as well as learning deficits - were observed in adult *sple/sple* mutants, we sought to assess whether earlier stages of *sple/sple* development (larvae) presented with crawling and/or olfactory learning deficits using assays specific for larvae.

Methods

All larvae were outcrossed to a w^{1118} genetic background and were raised at 25°C on a 12h/12h light/dark cycle. Prior to analysis, stage L3 larvae were washed in filtered tap water for 2-5 minutes to clean off any food particles and/or additional bodily wastes. Afterward, the larvae were placed into a dry Petri dish and immediately used for analysis.

To assess locomotion, the Crawling Assay was performed. L3 larvae were individually placed onto an 85 mm 2.5% agarose plate. Larvae crawling behavior was recorded using a Canon High Definition Vixia HFM31 Camcorder to capture 30 seconds of active crawling. Larval crawling speed was manually tracked by marking the center of the larvae and tracking its position using the Manual Tracking plugin in FIJI. Crawling speeds were converted from pixels/frame and reported as mm/second.

To assess learning and memory, the Olfactory Learning Assay was performed. The two olfactants used were 3-Octanol (OCT) and 4-Methylcyclohexanol (MCH). Preliminary assays performed during the SSTP program showed that 1:100 OCT and 1:25 MCH (diluted in light paraffin oil) were sufficiently odorous to induce an association between smell and a gustatory reward (i.e., fructose). Additional tests were performed to ensure that larvae did not have any

preference for either scent when no training was performed. All experiments were performed at room temperature in low light conditions.

L3 larvae were trained on a 2M fructose, 2.5% agarose plate with 2 caps of either 1:100 OCT or 1:25 MCH for 5 minutes. All scent caps were covered in perforated aluminum foil to prevent larvae from crawling in the scent. Afterward, the larvae were transferred onto a fresh agarose plate without fructose with 2 caps of the opposite scent for 5 minutes. After repeating this training procedure twice, larvae were immediately tested to see if they formed an association between the reward (fructose) and the first scent. To do this, larvae were placed in the center of a fresh agarose plate (without fructose) with 1 cap of each scent equidistant from the center. After 5 minutes, the position of each larva on the test plate was recorded. If the larvae had crawled closer to the 1st scent, then this was evidence of a successful association between reward and odor. The scent associated with the reward alternated between OCT and MCH.

Results

The Crawling Assays revealed *pk/pk* mutant larvae, but not the *sple/sple* mutant larvae, show a significant increase in locomotor speed when compared to controls. This suggests that the *sple/sple* mutant larvae do not have an obvious locomotor defect.

The Olfactory Learning Assays showed that neither the *sple/sple* mutant nor the *pk/pk* mutant larvae exhibited learning deficits, suggesting that neither mutation in the *prickle* gene drastically impacted the learning ability of *Drosophila* larvae.

Conclusions/Implications

These data suggests that *sple/sple* mutants only show locomotor defects and learning deficits as adults. This is the first indication that the locomotor and learning defects found in *sple/sple* mutant *Drosophila* are unique to the adult life stage. The increased crawling speed of *pk/pk* mutants was another surprising observation, one we hope to explore further in future research.

References

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