Visualizing the expression of Tolls 7 and 8 in adult and embryonic *Gryllus bimaculatus* CNS tissue by HCR *in situ* hybridization

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Neuroplasticity refers to the ability of a nervous system to modify itself, sometimes in response to stress or injury. The ligand-receptor interaction between neurotrophins and their receptors contributes to neuroplasticity in mammals. Spätzle-Toll signaling has recently been found to play a neurotrophic-like role in *Drosophila melanogaster* (Mcllroy et al. 2013); however, the extent to which they serve as neurotrophins in other invertebrates has yet to be evaluated.

Gryllus bimaculatus (the Mediterranean field cricket) displays neuroplasticity throughout embryogenesis; in the mushroom bodies of the brain; and most unusually, in the adult auditory system in the prothoracic ganglion (PTG). The plasticity displayed by the cricket in these three areas position it as an ideal candidate for investigating the role of Spz-Toll signaling in invertebrate neuroplasticity.

This summer, I leveraged Hybridization Chain Reaction (HCR) *in situ* hybridization to characterize the expression of Tolls 7 and 8 in the adult and developing cricket Central Nervous System. This technique allowed our lab to: 1) corroborate previous findings for Toll-7 in the embryo, PTG, and brain (Fig. 1A-C); 2) visualize the ring-like organization of Toll-7s in the mushroom bodies (Fig. 1D); and 3) understand that Toll-8 is expressed along the perimeter of the embryo's abdomen, in bands in the limb buds, and at the base of the cerci (Fig. 2), but not internally in the adult PTG or brain.

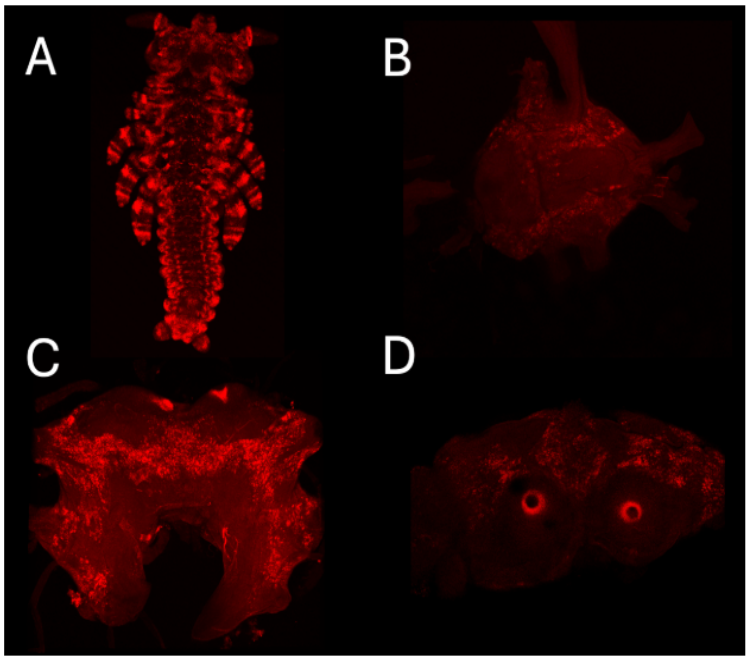

Figure 1. HCR *in situ* hybridization visualization of Toll-7 expression in *G. bimaculatus* embryonic and adult CNS tissue. Images were captured with a Leica DM6 CS confocal microscope on 10x magnification. **A)** Toll-7 is expressed in distinct bands along embryo limbs the sides of the abdomen. It appears to be faintly expressed in a ladder structure along the midline. **B)** Toll-7 appears in bands across the superior and inferior parts of the adult PTG. **C)** Toll-7 is expressed prominently in the mushroom bodies of the adult brain. It is strongly apparent in a horseshoe-like band that extends from one tritocerebrum to the other, passing through the central complex. **D)** Dorsal view of Toll-7 ring-like expression in the mushroom bodies of the adult brain.

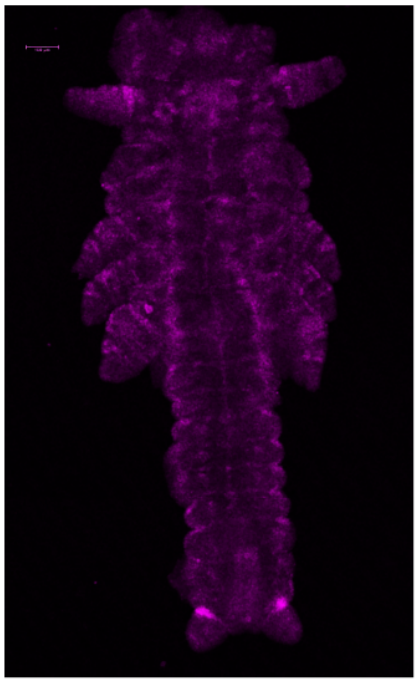

Figure 2. HCR in situ hybridization visualization of Toll-8 expression in G. bimaculatus embryonic CNS tissue. Images were captured with a Leica DM6 CS confocal microscope on 10x magnification. Toll-8 appears along the perimeter of the abdomen, in the circe, and across the midline of the cricket embryo. It is expressed in bands across the embryo limbs.

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References

McIlroy, G., Foldi, I., Aurikko, J., Wentzell, J. S., Lim, M. A., Fenton, J. C., Gay, N. J., & Hidalgo, A. (2013). Toll-6 and Toll-7 function as neurotrophin receptors in the Drosophila melanogaster CNS. Nature neuroscience, 16(9), 1248–1256. https://doi.org/10.1038/nn.3474