Elucidating expression patterns for Toll 2-5 protein that potentially underlies compensatory plasticity in *Gryllus bimaculatus*

Emily Jones, Class of 2024

Neuroplasticity refers to the ability of the nervous system to change and adapt through development and to new environments when needed especially after injury (von Bernhardi et al., 2017). During development, it is known that humans create new connections based on experiences which strengthen as one gets older, making it harder to alter those connections. When injured, however, the human central nervous system is often unable to recover, limiting its plasticity (Smith, 2013). Examples of unusual neuroplasticity and potential for neural regeneration are present in some invertebrates which raise questions about the mechanisms organisms have evolved that specifically permit this plasticity (Pfister et al., 2013).

The Mediterranean field cricket, *Gryllus bimaculatus,* sees an unusual amount of neural plasticity in the auditory system which has most of its circuitry located in the prothoracic ganglia (PTG). Within the PTG, dendrites will grow across a barrier that they normally respect after the loss of their ear (Horch et al., 2011). This results in the restoration of the auditory neuron connectivity allowing for the cricket to compensate for the loss of an ear. One potential explanation for this unusual plasticity involves the presence of Toll proteins. Toll receptors were originally known to function primarily as part of the insect immune response, but recently some Tolls have been found to influence the growth of dendrites and axons in another model organism, the fruit fly *Drosophila melanogaster* (Li et al., 2020).

To study how these proteins can contribute to the neuroplasticity seen in crickets, we need to characterize the sequences and expression patterns of the 11 Toll proteins found in crickets. This summer I chose to characterize Toll 2-5 since we have found *toll 2-5* mRNA to be significantly upregulated in PTG tissue 1-day after the loss of an ear. This leads us to question where the mRNA for *toll 2-5* is expressed in developing cricket embryos, and adult cricket tissue like the prothoracic ganglia and brains. I utilized a technique called *in situ* hybridization (ISH) to visulatize *toll 2-5* mRNA in cricket tissue. This involved creating a labeled probe that is complimentary to my mRNA region of interest which essentially binds to the region and recruits an enzyme to create a color reaction to stain the tissue where the mRNA is expressed. This can then be seen under a microscope and imaged.

Adult cricket brains and prothoracic ganglia were dissected and sectioned into 100 micron-thick slices before conducting the ISH. Whole-mount embryos at embryonic stages 7.0 to 11.0 (as described in Donoughe & Extavour, 2015) were used for the ISH. The ISH is about a 3-day process that involves incubating and washing the tissue in various solutions at different temperatures.

Preliminary results show that *toll 2-5* is expressed in the mushroom bodies of adult brains, and potentially expressed in adult PTG and developing embryos (Figure 1). Expression seen in the mushroom bodies makes sense due to their role in multimodal sensory integration, learning, and memory which requires high levels of plasticity (Figure 1A & B) (Eickhoff & Bicker, 2012). *toll 2-5* may be expressed in the midline of the PTG where the unusual dendritic growth occurs (Figure 1C & D). Potential striped expression in the limb buds of embryos indicates Toll 2-5 may play a role in leg development (Figure 1E & F). Because of high levels of nonspecific staining seen in the PTGs and embryos, our confidence that any *toll 2-5* staining is specific is decreased; however, I hope to conduct more ISH experiments to verify the results seen here. In the future, I would like to conduct a Toll 2-5 gene knockdown in adults to determine how the gene affects the structure of different tissue in the cricket.

Faculty Mentor: Hadley Horch
Funded by: Bowdoin Fellowship in the Life Sciences

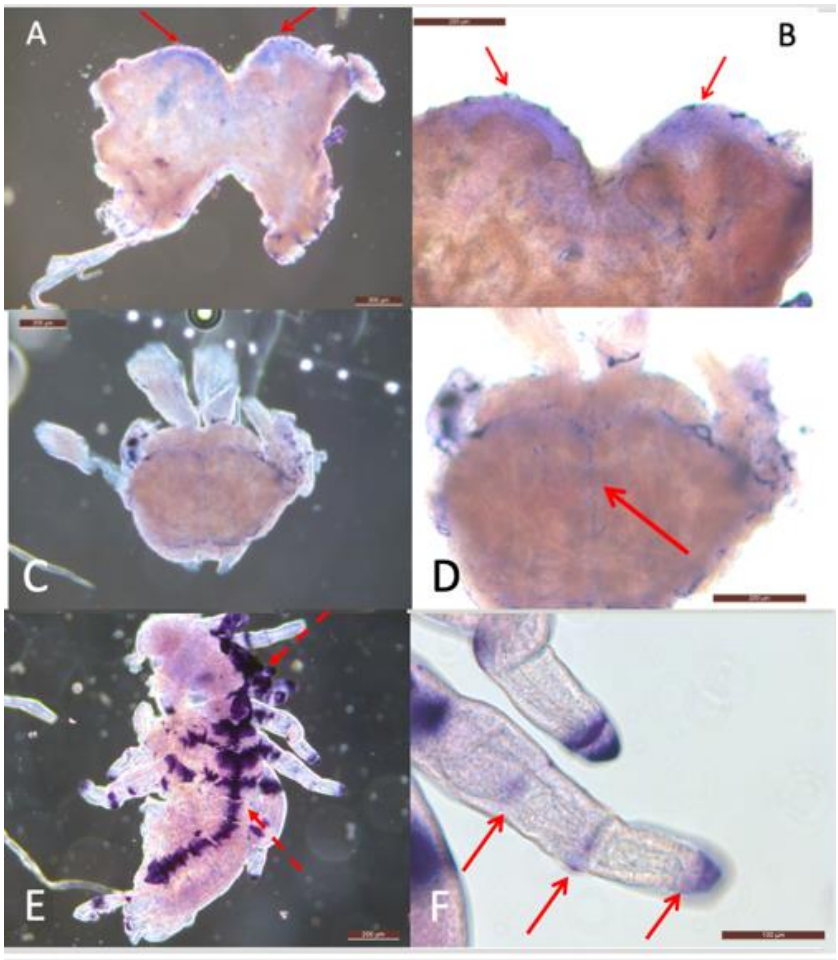

Figure 1: *toll 2-5* expression in adult brains and prothoracic ganglia and developing embryos in the cricket. A) Image of 100-micron slice of the brain and B) zoomed in image of the same brain. *toll 2-5* is expressed in the mushroom bodies of the brain which is noted with the red arrows. C) Image of 100-micron slice of the prothoracic ganglia (PTG) and D) zoomed in image of the same PTG. *toll 2-5* is potentially expressed in the midline of the prothoracic ganglia noted with the red arrow. E) Image of a developing embryo seen with nonspecific staining shown in the dotted red arrows. F) Zoomed in image of limb buds of the same embryo with potential *toll 2-5* expression in stripes indicated with the red arrows.

References

Bando T, Okumura M, Bando Y, Hagiwara M, Hamada Y, Ishimaru Y, Mito T, Kawaguchi E, Inoue T, Agata K, Noji S, Ohuchi H (2021) Toll signalling promotes blastema cell proliferation during cricket leg regeneration via insect macrophages. Development 149:dev199916.

Donoughe S, & Extavour CG (2016) Embryonic development of the cricket Gryllus bimaculatus. Developmental Biology, 411(1), 140–156.

Eickhoff R, & Bicker G (2012) Developmental expression of cell recognition molecules in the mushroom body and antennal lobe of the locust Locusta migratoria. The Journal of Comparative Neurology, 520(9), 2021–2040.

Horch HW, Sheldon E, Cutting CC, Williams CR, Riker DM, Peckler HR, and Sangal RB (2011) Bilateral Consequences of Chronic Unilateral Deafferentation in the Auditory System of the Cricket *Gryllus Bimaculatus* Developmental Neuroscience 33 (1): 21–37.

Li G, Forero M. G, Wentzell J. S, Durmus I, Wolf R, Anthoney N. C, Jiang R, Hasenauer J, Strausfeld N. J, Heisenberg M, Hidalgo A (2020), A Toll-receptor map underlies structural brain plasticity. Elife 9:e52743.

Pfister A, Johnson A, Ellers O, and Horch HW (2013) Quantification of Dendritic and Axonal Growth after Injury to the Auditory System of the Adult Cricket *Gryllus Bimaculatus* Frontiers in Physiology 3: 367.

Smith GS (2013) Aging and neuroplasticity. Dialogues in Clinical Neuroscience, *15*(1), 3–5.

von Bernhardi R, Bernhardi LE, & Eugenín J (2017) What is neural plasticity? In R. von Bernhardi, J. Eugenín, & K. J. Muller (Eds.), The Plastic Brain (pp. 1–15). Springer International Publishing.