

The Influence of Toll Receptors on the Developing Peripheral Nervous System of

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Abstract

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in the Spätzle-Toll signaling system are hypothesized to be neurotrophin-like, where Toll receptors and their Spätzle ligands influence the growth of peripheral neurons toward their targets in the central nervous system. A specific protein, Toll-7, is expressed in the developing limb buds of the Mediterranean field cricket, **Gryllus bimaculatus**. Previous literature suggests that knocking down the expression of Toll-7 will disrupt axonal routing and decrease the volume of the limb buds and pioneer neurons. Our results show an additional process extending away from the central nervous system, implying Toll-7 is important in ensuring proper embryonic peripheral nervous system development in **Gryllus bimaculatus**.

Embryo Dissection

Five days after egg injection, the viable embryos were dissected from the eggs in phosphate-buffered saline (PBS) solution. The dissected embryos were transferred to a tube containing 4% paraformaldehyde in PBS for 30 minutes. They were washed with PBSTx (1X PBS with 0.1% TritonX-1000 three times, for five minutes each.

Immunohistochemistry

Alexa Fluor 488-conjugated anti-mouse HRP (1.5mg/ml stock concentration) was diluted 1:400 with PBSTx-HS (10% horse serum in PBSTx). The embryos were rinsed with PBSTx three times, for five minutes each, then blocked in the PBSTx-HS solution for one hour. Embryos were incubated in anti-HRP antibodies on a shaker, overnight at 4°C. Embryos were then ~~washed with PBS~~ washed with PBSTx 3 x five minutes and mounted in 50% glycerol in HBS.

Visualization and Analysis

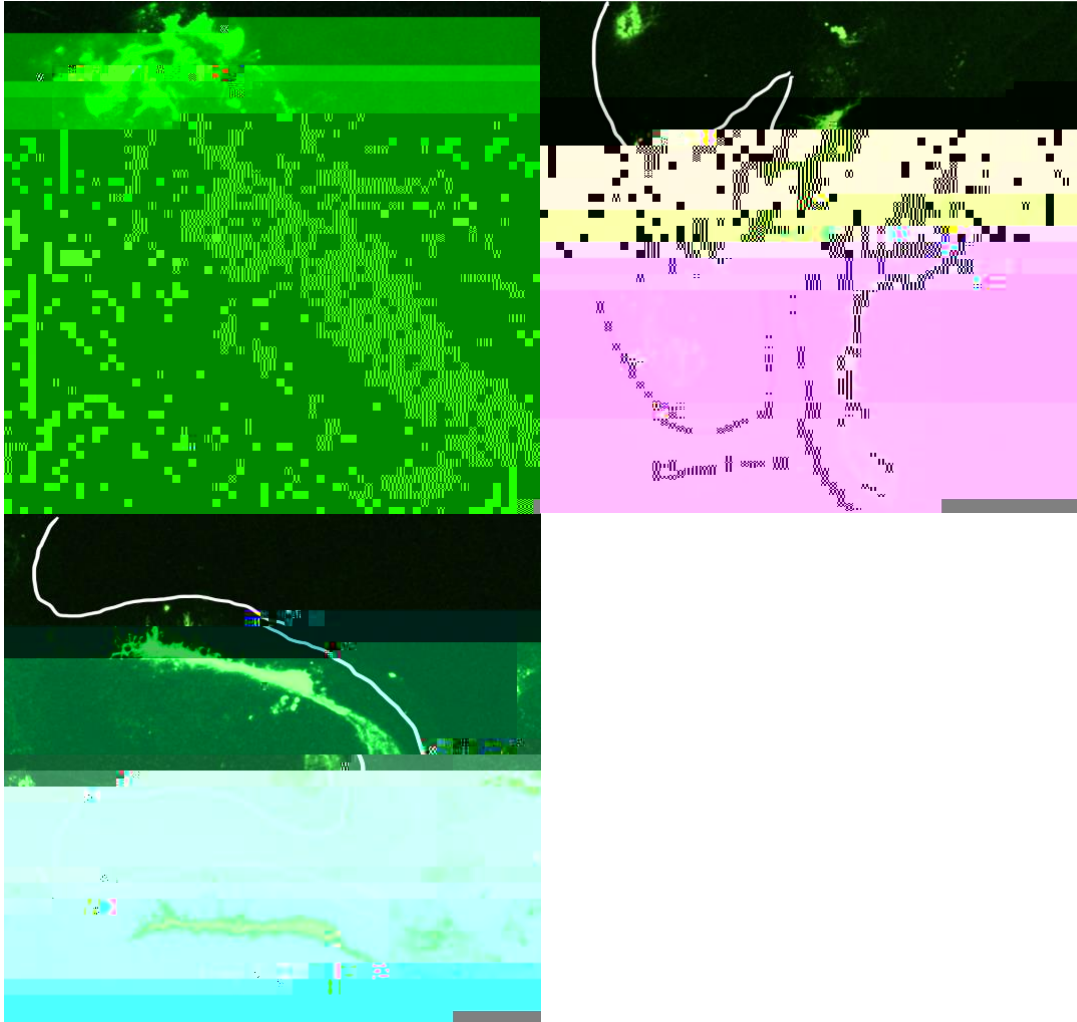


Figure 5. Confocal Images of Toll-7 dsRNA Injected Crickets

Top left image (10x) shows the ladder-like central nervous system structure, as well as the developing peripheral nervous system. Top right image (40x) shows the prothoracic limb bud, with a process extending towards the CNS as well as the distal tip of the limb bud. There is also a protrusion extending towards the anterior side of the limb bud, away from the central nervous system. Bottom left image (40x) shows the prothoracic limb bud, also with processes extending from the cell body to the central nervous system, distal tip, and anterior side of the limb bud.

Discussion

In the Toll-7 dsRNA injected crickets, there appears to be a process extending from the cell body towards the distal end of the limb bud that is not present in the GFP injected crickets. There are a few plausible explanations for this observed phenotypic abnormality. Toll-7 has been implicated with cell survival in **Drosophila melanogaster** where animals with mutant forms of Toll-7 experienced higher levels of programmed cell death than their wild-type counterparts (Li et al., 2020). Therefore, knocking down the expression of Toll-7 could cause increased death of guidepost cells or even pioneer neurons. This loss of axonal guidance may cause the processes to extend towards unintended targets.

Additionally, Bentley and Keshishian (1982) characterized limb bud development in grasshoppers, while Luo (2023) confirmed this progression in **Gryllus bimaculatus**. Specifically, around Stage 9.0 of development, tarsal neurons appear at the distal end of limb buds. Perhaps, these

