The Quantification of Toll candidates in control and deafferented tissue in the Cricket *Gryllus bimaculatus*

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Abstract

Neuronal plasticity is the capacity of a nervous system to alter itself in response to external forces including injury both functional or structural. The Lab's hypothesis is that Spz-Tolls signaling is present during the crickets response to injury. Specifically when the auditory system in the legs are damaged, dendrites sprout across their midline to form synapses with auditory cells on the opposite part of their body. Throughout the summer my role was to test the hypothesis by asking how expression of the Spz- Tolls changed after injury. I used the quantitative polymerase chain reaction to quantify expression levels.

Introduction

The expression of Spz-Tolls in response to injury is possibly linked to the phenomenon that happens in the Cricket *Gryllus bimaculatus. Spz-Tolls have shown to have similar functions to neurotrophin signaling.* Neurotrophins are a family of proteins that provide nerve growth function in mammals. *Although knowledge on neuronal plasticity is limited, exploration of the Spz-Tolls may provide new insights into how neurotrophins function in regard to nerve growth. This research specifically focused on the Tolls 6-2 and Toll 6-1. Along with how these tolls would be expressed on the third day after amputation which simulates the damage done in natural environments.* Quantitative polymerase chain reactions(qPCR) were eventually used to identify the levels of expressed Tolls in the cDNA collected from the crickets.

Methods

A period of isolation starts the timeline with crickets being individually separated into their respective habitats. Picked from the oldest bin, the crickets chosen are in their eighth larval instar in their life cycle. They remain there until adulthood and are removed after 3-5 days as adults. Once removed their front leg on one side was amputated either above (deafferented) or below ("foot chop") the ear (fig. 1). The footchop served as a control group that simulated damage to the leg that was not the area of focus. Deafferent amputations were done higher up on the leg and caused damage to the auditory system. Once amputated the crickets were placed back into their respective habitats and checked on daily for a 3 day period.

After the 3 day period the crickets were put on ice then dissected to obtain the prothoracic ganglion(PTG). Once removed the PTG is stored in 300ul of QAzol Lysis Reagent and the tissue was homogenized with a pestle. After a RNA purification followed by a DNase treatment the purified RNA was stored at -80 deg C. The RNA was synthesized into cDNA with a Invitrogen, SuperScript® IV First-Strand Synthesis System, stored at -20°C. Once synthesized the cDNA needed to be amplified through a PCR program on the thermocycler. After the PCR product was checked on an electrophoresis gel, the same cDNA used would then be sent into a quantitative polymerase chain reactions(qPCR)

Results

Over the course of the summer two large groups were tested (Table. 2). In these groups we predicted we would see increased expression in Toll 6-1 and 6-2 within 3 days of amputation. To identify if this reaction was happening in the PTG most of the summer was spent experimenting with a digital polymerase chain reaction (dPCR). Ideally the dPCR would have been able to not only amplify the cDNA processed from the PTG of the cricket but also give an approximate count of how much Toll-6 cDNA was already there. Unfortunately, due to continuous contamination and a lack of access to the dPCR machine, our final amplification had to be switched over. During the last week of research the switch to qPCR happened to properly amplify the cDNA. No contamination occurred in the qPCR and both groups of cDNA were tested successfully.

Interpretations/Future Directions

The goal was to determine the expression levels of Toll 6-1 and 6-2 after damage. The PTG was the main area of focus but in the future other areas of the nervous system along with other Tolls could be tested. In the past, 3 days after amputation has seen the highest levels of Toll expression but a range of 1-7 days could be revisited for a larger variety of results. This range helped with interpreting how long it takes for the dendrites to move across the midline. The use of dPCR could also be revisited since the device takes fewer steps and provides more accurate results of what exactly is being amplified in the cDNA. Using the qPCR for a longer time period in order to have results earlier in the summer could also be valuable. If I were to come back next summer, immediate use of the qPCR and more test groups would provide more results early on. Other Tolls could also be explored along with further support of the expression of Toll 6-2 or Toll 6-2.

Figures

- Deafferent or footchop 3-5 d following final molt.
  - **Animal's left** limb. Cut above elbow for deafferent or above tarsus for footchop.
    - Footchopped just above forked end/toe as shown in red. Deaff in blue:

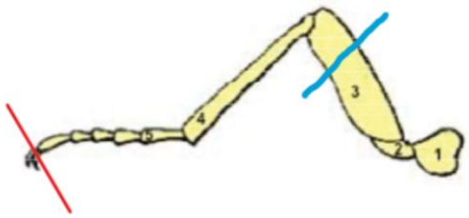

Figure 1.
Visual of deafferent versus footchop for amputation after a 3-5 day period of adulthood

Cricket	Isol.	Adult on	Days as adults	FC or DA on	Harvested	1d/3d/7d	RNA prep	RNA ng/ul	Notes
Leo	25/6/3	25/5/4	5	25/6/9 FC	25/6/12	3d		22.3	
Donnie	25/6/3	25/6/5	4	25/6/9 FC	25/6/12	3d		27.5	Might have tore bottom of ptg from pulling too hard on one bottom connector More water added than needed in cdna original component tube
Mikey	25/6/3	25/6/5	4	25/6/9 DA	25/6/12	3d		32.7	More water added than needed in cdna original component tube
Ralph	25/6/3	25/6/9	4	25/6/13 DA	25/6/16	3d		21.1	A little less amount of master mix for PT cdna
Romeo	25/6/3	25/6/9	4	25/6/13 FC	25/6/16	3d		34.7	Missing some body pieces
Allen	25/6/3	25/6/9	4	25/6/13 DA	25/6/16	3d		43.4	
uno	25/6/3	25/6/10		DA				27.4	Missing one back leg

Table 2.
Tracking chart of individual crickets timeline and RNA counts.

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