

FP6-016039

CILIA

Customized Intelligent Life-Inspired Arrays

Integrated Project

Information Society Technologies
Future & Emerging Technologies
Proactive Initiative BIO-I3

DELIVERABLE: D2.2.10 - Public

**PHYSIOLOGICAL AND MORPHOLOGICAL
CHARACTERIZATION OF THE NEMOBIUS
GINS**

Actual submission date:	March 18, 2009		
Start day of project:	September 1st, 2005	Duration:	48 months
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1. EXECUTIVE SUMMARY

The abdominal cerci of the wood cricket, *Nemobius sylvestris*, are covered by filiform hairs sensitive to air. When stimulated by the air-flow produced by the attack of a predator, this mechanosensitive system triggers jumping or running, in order to move away from the danger. The filiform hair axons project into the terminal abdominal ganglion (TAG) through bilateral cercal nerves to contact local interneurons and projecting ascending interneurons inside the TAG. In order to analyze how the information concerning the air-currents generated by an attacking predator is processed by the cricket's nervous system, we applied different electrophysiological methods for recording from the TAG and connectives. For the present work, we have mounted a setup for the intracellular recording. With advice from experts we adjusted the recording and dye injection parameters of our equipment. We started using well-studied model systems for this kind of recording, the tobacco hornworm *Manduca sexta* and *Gryllus bimaculatus*. However, when switching to *Nemobius sylvestris*, we found that the impalement of the TAG was quite difficult because of the mechanical resistance of the neurilemma to be pierced by the electrode. We succeed using protease and a hypotonic Ringer solution. After many unsuccessful attempts, we were able to obtain some intracellular recordings, which were very difficult to stabilize. Different techniques to stabilize the recordings during a longer time have being tested. Given the remaining time and the numerous difficulties, we stop this line of investigations and concentrate our efforts on extracellular recording of free crickets.



2. PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION OF THE *NEMOBIUS SYLVESTRIS* GIANT ASCENDING INTERNEURONS

2.1. INTRODUCTION

Insects such as cockroaches, locusts, crickets possess a pair of cerci bearing air-sensitive filiform hairs (mechanoreceptive sensilla) at the end of the abdomen. When stimulated by the air-flow produced by the attack of a predator, this mechanosensitive system triggers jumping or running, in order to move away from the danger. Cercal sensory inputs and motor units are usually connected by a reduced number of interneurons having axons of large diameters, allowing the information to be quickly transmitted to command units (Edwards and Palka, 1974; Palka and Olberg 1977; Camhi, 1980; Edwards and Williams, 1981; Ritzmann, 1984; Boyan and Ball, 1986, 1989, Insausti et al. 2008).

The abdominal cerci of the wood cricket, *Nemobius sylvestris*, are covered by a variety of hair-like sensilla sensitive to air-currents differing in their length, thickness and articulation with the exoskeleton. Their axons project into the TAG through bilateral cercal nerves. Some of them seem to continue into the connectives, ascending the ventral nervous chain, while others contact neural elements inside the TAG. These elements are local interneurons and projecting ascending interneurons, which also interact with local neurons. There are seven pairs of giant ascending interneurons (GINs) organized symmetrically in *N. sylvestris*. Their somata are located contralateral to their axons (diameters between 20 and 45 μm). The cercal projections overlap extensively with the dendritic fields of the giant interneurons. The axons of GINs ascend through the ventral nervous chain to reach higher centres (Insausti et al., 2008).

In order to analyze how the information concerning the air-currents generated by an attacking predator is processed by the cricket's nervous system, we applied different electrophysiological methods for recording from the TAG and connectives. As indicated in a previous report to CILIA, our laboratory has already successfully adapted an extracellular recording technique from the connectives for both, laboratory- and field-based biotests, using as stimulus the air flow produced by an approaching piston. The piston generates a controlled air current which simulate a predator attack. After having succeeded and standardized the extracellular recording of the projecting ascending interneurons, the next



step is the study of the relationship between the sensory input and the ascending output from the TAG.

The aims of our work were:

- The intracellular recording and iontophoretic marking of the giant interneurons (GINS) in the TAG of the cricket *Nemobius sylvestris*.
- The characterization of the response of individual GINS to air currents (piston).
- The integration of the results with those obtained by means of extracellular recording methods in the laboratory and in the field.



2.2. WORK DEVELOPMENT

2.2.1. Standardization of microelectrodes

With the advice of Sutter international (USA) we have calibrated our laser puller (Sutter P-2000) for making microelectrodes adequate to our preparation.

- Outcome: succeeded. We are able to make glass or quartz microelectrodes with the characteristics required for our preparation.

2.2.2. Installation of a set up for intracellular electrophysiology

We have mounted a setup for the intracellular recording of the neurons in the cricket's TAG, with the advice of Dr. Carolina Reisenman (member of John Hildebrand Lab Group - University of Arizona - Tucson, who came to Tours) and Dr. S. Anton (Physiologie de l'Insecte - Signalisation et Communication – Versailles; a week of training of F. Dupuy and T. Insausti). We standardized the recording and injection parameters for our equipment. For this, we started using a well-studied model system for this kind of recording, the tobacco hornworm *Manduca sexta*, where intracellular recordings from the adult olfactory lobe could be obtained. Afterwards, we started recording from the TAG of *Gryllus bimaculatus*, because this species is bigger than *Nemobius sylvestris* and its escape system is well known.

- Outcome: succeeded.

2.2.3. Preparation of the TAG of *N. sylvestris*

Different dissection techniques (ventral or dorsal approach) were tested to reduce damage to the TAG. The dorsal approach was the most appropriate. The surgical intervention is kept minimal. The legs are cut and the insect is immobilized using wax melting at low temperature (36°-37°C), which does not affect the insect. Also the cerci are immobilized, keeping a natural position and avoiding affecting sensory hairs (**Figure 1A**). A small window is cut in the dorsal cuticle of the abdomen, and the posterior digestive tube and reproductive organs are taken away. The trachea and fat body directly attached to the TAG are conserved intact (**Figure 1B**).

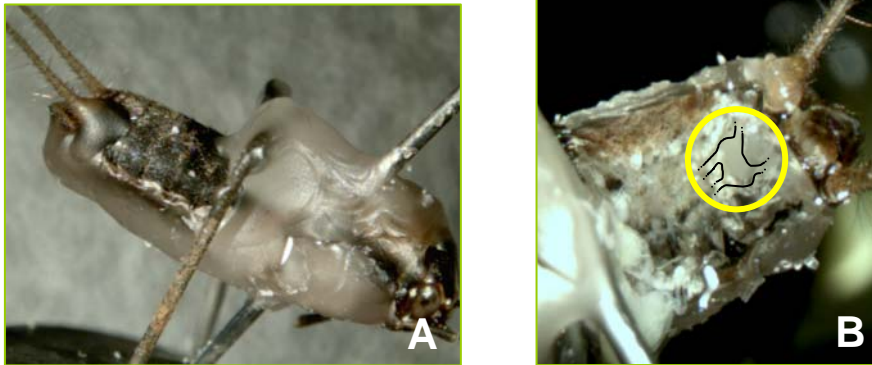


Figure 1: **A**, photograph of the preparation mounted on the recording platform. Note the minimal obstruction to airflow in the vicinity of the cerci. **B**, photograph showing the TAG dissection. With the purpose of indicating the place of the TAG, their outlines were drawn into the photograph (dark lines within yellow circle).

2.2.4. Piercing the TAG

The impalement of the TAG is quite difficult, due to the mechanical resistance of the neurilema to be pierced by the electrode. In this species, the neurilema is particularly hard. Thus, we tested different kinds of electrodes (borosilicate glass of different wall thickness and quartz capillaries) and dissected the neurilema, either chirurgically (microdissection) or enzymatically (protease). We tested different concentrations and application methods of protease, as well as digestion times.

After many unsuccessful attempts working with different compositions of isotonic Ringer solutions, we succeed using a Hypotonic Ringer solution with a composition suggested by Dr. John Miller (Department of Cell Biology and Neuroscience at Montana State University)

- Outcome: The protease application gave the best results. The concentration and time of application was standardized for our model.

2.2.5. Intracellular recording



Once the method and equipment were adjusted, we started to work in the determination of the recording places. Based on our previous anatomical studies, the electrodes were inserted in the GINs bodies (1), in the cercal neuropile, where the fibers of GINs branch profusely (2) and in the connective (3).

- Outcome: (1) We were not able to obtain recordings from GINs bodies.

(2) We have recorded spontaneous activity of local interneurons from the cercal neuropile. Four neurons were successfully marked by Lucifer Yellow injection (**Figure 2**).

(3) Neurons sensitive to air currents were recorded from the connectives right as they leave the ganglion. The records were unstable (short time, ca. 5 min). It was not possible to mark the cells.

We are able to obtain intracellular recordings, but for short times (max. 5 min). These short-lasting recordings allowed us to record only neuronal spontaneous activity. Recordings of responses to sensory stimulation and/or injection of dye was not possible.

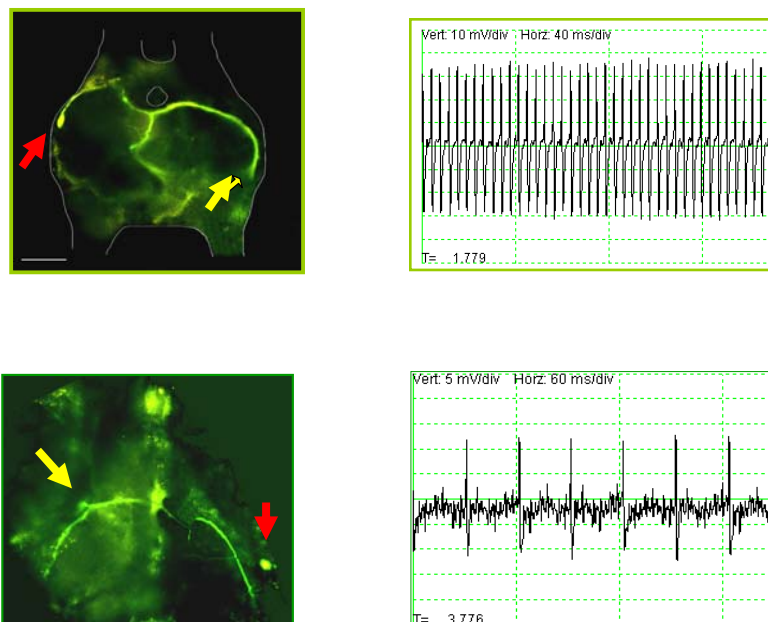


Figure 2: Samples of two intracellular recorded and marked TAG local interneurons. The yellow arrow indicates the recording site and the red one the cell body.



3. CONCLUSION

Around 200 crickets were prepared. We succeeded in impaling the TAG and obtaining intracellular recording in 40 preparations. The spontaneous activity of four local interneurons was recorded and the cells were marked. We have recorded other cells sensitive to air-currents, but we have not been able of injecting the dye due to instability of recordings.

Different techniques to stabilize the recordings during a longer time are being tested. At present, we are trying to impale GINs at the connectives and testing different ways and angles for microelectrode positioning.

The installation and adjustment of a set-up for intracellular electrophysiology is a task that needs a lot of experience in the topic and a lot of time. We are in the initial stage, for which we have requested the experts' advice with long experience in this kind of work. However, this is a long-term project; too long for the remaining time frame of the project CILIA.



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