

Technical Report

Replacement of Vertebrates by Locust in Student Laboratory Exercises

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Summary

The student laboratory exercises are a very important part in the curriculum of physiology, neurophysiology and biophysics. Knowledge from lectures and books should be supported with some practical experience based on work with live subjects. But this raises a very important question – is the use of animals for teaching purposes acceptable? This is a controversial question for a long time in physiology teaching and has numerous arguments for and against. With respect to efficiency and quality of teaching it is essential. However, is this enough to justify the use of animals?

Traditionally laboratory exercises are performed on frogs or rodents. Despite obvious advantages this has serious disadvantages, namely relatively high costs related to animal facility and care of the animals. Applying the 3R's principle, we replaced vertebrates by invertebrates and introduced laboratory exercises based on recording of action potentials from wing stretch receptors of the locust (*Robertson, 1992*). Locusts are cheap to buy (approx. 2 locust for 1 euro) and easy to care for. Preparation of locusts for experiment is very simple. However, in this laboratory exercise students can learn such basic concepts like generation of action potential, natural variability of recorded signals in a live system, adaptation and principles of coding in the nervous system. Apart from this, modification of the experimental setup enabled us to investigate physiological concepts like neural coding in more natural conditions.

Introduction

Knowledge about the mechanisms underlying brain activity is a very important part of biomedical education because it has strong practical impact: having this knowledge medical doctors and psychologists may better understand the basis of emerging health and social problems and find a proper solution.

It is difficult to imagine high quality learning without proper practical and laboratory exercises. And this is especially true for such complicated subjects

as physiology and particularly neurobiology. It is not enough for students to hear about information coding, transmission and integration in live organism, they should see how it really works.

The small laboratory animals (frogs, rats, mice) are usually used for practical training. However, here we are faced with ethical and financial problems. The ethical problem is that the use of animals is necessary for a proper education, but animals are used just for demonstration of well-described phenomenon. Could the use of animals be justified or should be they replaced? The financial problem is that the maintenance of animals according to EU regulations requires special facilities, expensive to install and to run.

In order to solve this dilemma we applied the of 3R's principle, proposed by Russell and Burch (*1956*), and replaced vertebrates by invertebrates

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by introducing laboratory exercises described by Robertson (1992). This laboratory exercise is based on the extra-cellular recording of action potentials from the wing-stretch receptors of locust (*Locusta migratoria*). The wing is lifted to different positions and action potentials are recorded. In locusts, the stretch receptor sensory organ consists of the cell body and dendritic arbor of a single neuron embedded within a strand of connective tissue that spans the base of the wing to an internal diaphragm. The main function of the locust wing receptor is to control frequency of wing movement, compensate the influence of external disturbances and coordinate action of different muscle groups (Frey, 2001). The locust wing-stretch receptor encodes the wing position by a series of action potentials – the higher wing is lifted, the higher the frequency of action potentials (Robertson, 1992). However, this encoding is not linear - the longer the wing stays in the same position, the lower is the frequency of action potentials (Robertson, 1992).

The locust wing moves up and down at approximately 23 Hz during flight (Robertson, 2004). The characteristics of the locust wing-stretch receptor presented above are obtained in a static mode, where the wing is kept in a constant position for one minute or more. But in natural conditions when the locust is flying the wings are periodically moving up and down. Therefore we decided to improve the experimental setup by investigation the characteristics of the stretch receptor during the dynamic state, which is more physiologically relevant.

This preparation is very attractive - from one side it is a very cheap object to buy (approx. 0.5 euro per locust), easy to keep and handle. On the other side it illustrates a number of fundamental neurobiological concepts like action potential, information coding, adaptation and intrinsic regulation of firing pattern. Apart from fundamental concepts, students get practical experience like experiment planning, work with experimental equipment, variability of biological signals, signals and noise, analysis and presentation of experimental data.

Here we present data obtained with a modified ex-

perimental setup, where stretch receptor action potentials are recorded while the wing is periodically moving up and down.

Materials and Methods

The locust is prepared for the experiment as described by Robertson (1992). Shortly, the legs are removed at the level of the coxae. Then the locust is decapitated and the gut removed. The preparation is glued to the stand and installed in the test apparatus. The grounded silver wire is inserted into the abdomen. The wing is fixed in an apparatus allowing measuring the angle of the wing position (Fig. 1). The wing was released from the clamp and attached to the wing lifter during wing movement experiments (Fig. 1). Wing was moving at 10 Hz frequency.

The signal from the wing-stretch receptor inside the locust trunk is recorded by a monopolar electrode guided by micromanipulator.

The extracellular recordings were performed with amplifier DAM 80 (WPI, Sarasota, USA), (gain 10000, high pass filter 300 Hz, low pass filter 3 kHz). Data was digitized by Digidata 1200 (Molecular Devices, Sunnyvale, USA) and by means of Clampex (Molecular Devices, Sunnyvale, USA) software stored on computer for analysis.

All experiments were performed at room temperature (20-22 °C).

The action potentials were automatically detected by *Pick Peaks* function from the Origin (OriginLab, Northampton, USA) software. Inverted interspike interval was used as an instant action potential frequency. During periodic wing movement the stretch receptor responded by bursts of action potentials. Only interspike intervals inside burst were used for analysis.

The differences between action potentials frequency in different experimental conditions were checked for significance by paired t-test. Frequencies are considered significantly different when $p < 0.05$.

Results

The locust wing-stretch receptor encodes the position of the wing by a series of action potentials – the

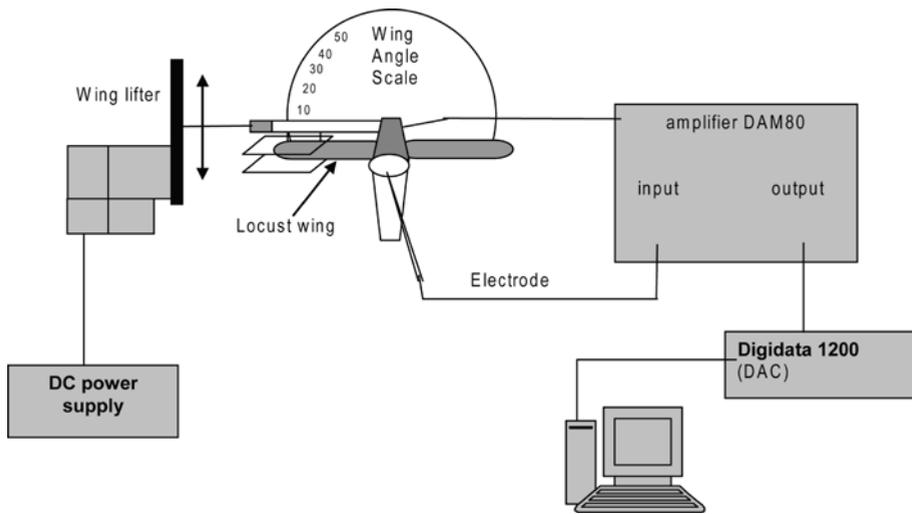


Figure 1. Experimental setup for recording activity from locust wing stretch receptor.

higher the wing, the higher the frequency of action potentials (Robertson, 1992; Gray *et al.*, 1994; Wolf *et al.*, 1988; Frye, 2001). However, this encoding is not linear.

When the wing is elevated and kept in constant position the frequency of action potentials decreases in time (Robertson, 1992; Gray *et al.*, 1994). This phenomenon is intrinsic to the wing-stretch receptor and called adaptation of action potential frequency. Adaptation is a common feature of neurons from various parts of the nervous system (Powers *et al.*, 1999; Yen *et al.*, 1999; Madison *et al.*, 1984). It is thought that adaptation contributes to integration, input filtering and saving of metabolic resources (Kohn *et al.*, 2002). Multiple mechanisms may contribute to the adaptation (Powers *et al.*, 1999).

First we repeated experiments described before (Robertson, 1992). As expected, the locust wing-stretch receptor responded by a series of action potentials of decreasing frequency when the wing was lifted to the 30 degree position (Fig.2, lower trace, triangles).

The adaptation of the wing-stretch receptor action potential frequency was observed in all animals tested ($n = 26$).

However, it is not known if adaptation of action potential frequency takes place during more physiological conditions, when the wing is moving up and down periodically. To test this we recorded action potentials from the wing-stretch receptor when the wing was periodically moved up and down at 10 Hz frequency (Fig.2, upper trace, circles $n = 6$).

The locust wing-stretch receptor responded by a series of bursts of action potentials when the wing was periodically moved up and down. As described before (Wolf *et al.*, 1988; Frye, 2001, Frye, 2005), one burst corresponded to one cycle of a wing movement. The action potential frequency (see methods) decreased during time tested (Fig.2, upper trace, circles) but less than when the wing was lifted and kept at the same position (Fig.2, lower trace, triangles). These qualitative observations were quantified by comparing action potential frequencies when wing was kept at the 30 degree position and periodically moved to the same position at 5, 10, 15, 20, 25, 30, 35 seconds after stimulation starts (Fig. 3). We can see that at all times tested (Fig. 3, $n=6$) frequency of locust wing stretch receptor action potentials is significantly higher when the wing is periodically moved (Fig. 3; white bars) as compared to the wing

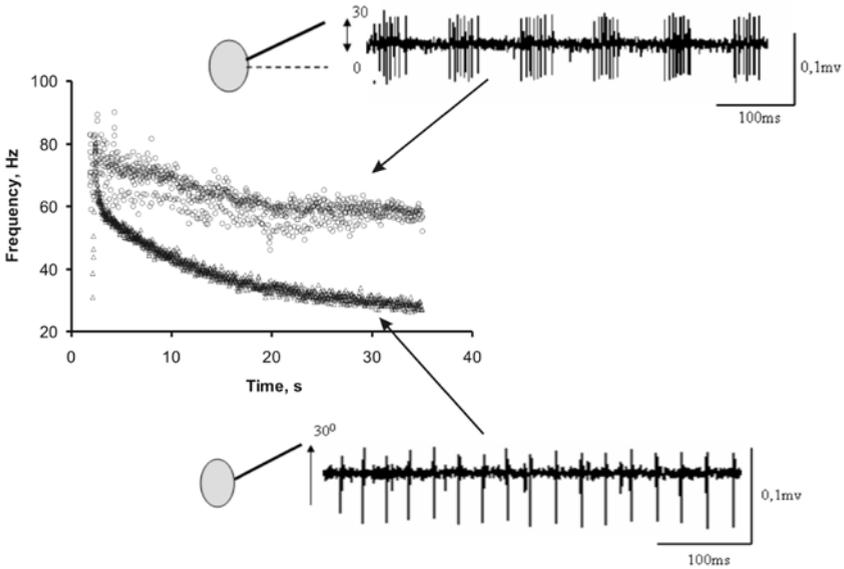


Figure 2. Frequency of stretch receptor action potentials when wing is lifted to the constant position at 30 degree (lower trace, triangles) and is periodically moved from 0 to 30 degree position (upper trace, circles). Both traces are from the same locust.

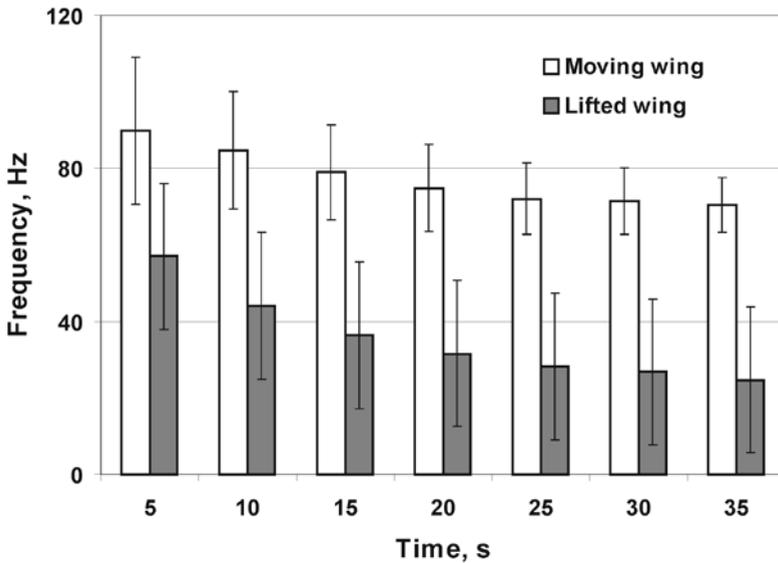


Figure 3. Averaged frequency of stretch receptor action potentials when wing is lifted to the constant position at 30 degree (white bars) and is periodically moved from 0 to 30 degree position (grey bars) at different time moments after stimulus starts.

kept at constant elevated position (Fig. 3; grey bars).

Discussion

The frequency adaptation of the locust wing stretch receptor action potentials in static mode, when the wing is lifted to a constant position, was known before (Robertson, 1992; Gray et al., 1994). In this investigation we show, that apart from static mode, adaptation occurs in dynamic mode, when the wing is periodically lifted up and down, as well. However, adaptation in dynamic mode is significantly weaker than in the static one.

The adaptation process in the stretch receptor starts from the moment the wing is lifted (Robertson, 1992; Gray et al., 1994) which corresponds to stimulus onset in other types of neurons (Powers et al., 1999; Gray et al., 1994). The recovery from adaptation is not instantaneous; it follows some time course (Kawai, 2002). When the wing is periodically moved up and down adaptation and recovery from adaptation occurs at the same time. We think that adaptation is weaker in the dynamic condition for two reasons: because wing spends less time in the elevated position (and therefore less time for the adaptation process) and because of recovery periods when the wing is in the down position.

To summarize, by introducing the locust experimental setup we solved two problems: replaced vertebrates in teaching by invertebrates (ethical problem) and maintained quality of teaching (financial problem). Actually, quality of teaching even increased due to the introduction of experiments with moving wings because this enables one to demonstrate more physiological principles using the same setup.

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