Adv Physiol Educ 33: 115–120, 2009;
doi:10.1152/advan.90200.2008.

Laboratory experience for teaching sensory physiology

Ana L. Albarracín,[1] Fernando D. Farfán,[2] and Carmelo J. Felice[2]

[1]*Cátedra de Neurociencia, Facultad de Medicina, Universidad Nacional de Tucumán, and* [2]*Laboratorio de Medios e Interfases, Departamento de Bioingeniería, Instituto Superior de Investigaciones Biológicas-Consejo Nacional de Investigaciones Científicas y Técnicas/Facultad de Ciencias Exactas y Tecnología-Universidad Nacional de Tucumán, Tucumán, Argentina*

Submitted 22 October 2008; accepted in final form 6 April 2009

Albarracín AL, Farfán FD, Felice CJ. Laboratory experience for teaching sensory physiology. *Adv Physiol Educ* 33: 115–120, 2009; doi:10.1152/advan.90200.2008.—The major challenge in laboratory teaching is the application of abstract concepts in simple and direct practical lessons. However, students rarely have the opportunity to participate in a laboratory that combines practical learning with a realistic research experience. In the Bioengineering Department, we started an experiential laboratory physiology to teach graduated students some aspects of sensorial physiology and exposes them to laboratory skills in instrumentation and physiological measurements. Students were able to analyze and quantify the effects of activation of mechanoreceptors in multifiber afferent discharges using equipment that was not overly sophisticated. In consequence, this practical laboratory helps students to make connections with physiological concepts acquired in theoretical classes and to introduce them to electrophysiological research.

vibrissal system; mechanoreceptors; multifiber recordings; signal processing

MULTIPLE BENEFITS during the learning process are obtained when students are offered practical laboratory experience. Bauer (2) affirmed that direct exposition to the research process develops both critical thinking and reflective judgment. This experience also helps to develop research skills and stimulates an interest in science (7). The objective of this article was to deepen theorical knowledge in physiology through the active participation of students in laboratory exercises. Laboratory experiences were also used to facilitate the understanding of electrical engineering applied to the biological sciences.

This laboratory exercise is part of a short course of Sistemas Fisiológicos I, an academic subject from the Master's Program in Bioengineering of the Universidad Nacional de Tucumán (Tucumán, Argentina). Electrophysiological concepts, biological membrane theory, biological transducers, real neural models, and vision systems are also included in this academic subject. The laboratory exercises include those on compound action potential, volume conduction, psychophysical analysis of vision, and the exercise described below.

The exercise described here is carried out every 2 years and helps students to develop practical skills in a research context. The course is taught jointly by instructors and researchers in neurophysiology and biomedical engineering.

An important issue in sensory physiology is the comprehension of how external stimuli are transduced in a code that the nervous system can interpret. This subject is rarely carried out in curricular programs, due to both its experimental design complexity and the mathematical skills required. These students already have the proper training in laboratory techniques and enough anatomic and physiological background. They are also trained in electronic and signal processing, which enable them to sample and analyze electrical neural activity.

The vibrissal system of the rat is used in this laboratory experience as a model to teach students some aspects of a sensory system. Thus, they are introduced to the study of sensory coding using a classical approach: vibrissa passive deflection. They observe and analyze the changes in the primary afferent discharge of a single vibrissal nerve produced during mechanical stimulation. In addition, they learn about background activity–the activity observed in all sensory systems in the absence of any stimulation.

Because many of the fundamentals underlying the laboratory experience go beyond introductory physiology texts, students were provided with some basic background on the sensorial vibrissal system and in electrophysiological recording. This provided them with a focused package of information with which to draw conclusions about their results. The following paragraphs summarize the points presented to the students in their laboratory guides.

Vibrissae are structures specialized in the reception and transmission of tactile information. Their arrangement, particularly in the rat, consists in five horizontal lines (*rows A–E*) and one vertical line (α, β, γ, δ) on both sides of the animal muzzle (11). It has been postulated that rats, due to their poor vision, use their vibrissae to explore the environment (12).

Each vibrissa sits on a structure called the follicle-sinus complex. There, mechanoreceptors, such as Merkel cells, lanceolate terminals, and free nerve endings, transduce tactile information to an electric signal (10). This information travels from the deep vibrissal nerves (branches of the infraorbital nerve) to the somatosensory primary cortex. Two branches of the facial nerve innervate follicular muscles, and the contractions of these muscles produce the forward vibrissal movement (5).

Classical studies in vibrissal sensory coding have involved head-fixed animals, controlled whisker deflection (passive deflection), and recordings of the evoked response. The authors demonstrated that the first-order afferent fibers encode a variety of hair deflection parameters, including amplitude, velocity, duration, frequency, and angular direction (9, 13).

On the other hand, behavioural studies in tactile discrimination have suggested that rats use their vibrissae to distinguish objects differing in physical characteristics of the surfaces, such as roughness and texture (3, 6). It has been recently demonstrated that the information about texture surfaces is present in the vibrissal nerve and contains the code that travels to the central nervous system (1).

Address for reprint requests and other correspondence: A. L. Albarracín, Cátedra de Neurociencia, Facultad de Medicina, Universidad Nacional de Tucumán, Tucumán 4000, Argentina (e-mail: ana@fm.unt.edu.ar).

METHODS

Students

No more than five students can participate in this laboratory exercise. The students were given a handout on the practical protocol and on the theoretical basis of the experiment (as mentioned above). Thus, they were given a background in electrophysiological aspects of extracellular multifiber recordings. A bipolar electrode was used in this experimental setup to record the discharge of many fibers activated simultaneously by mechanical stimulation. This activity, the compound action potential of the nerve, was observed in *experiment 2*. On the other hand, the same electrode was used to record spontaneous activity (i.e., the activity of a few fibers) in the absence of any stimulation. The students observed this in *experiment 1*.

Before the beginning of the experiments, students were familiarized with the software of the acquisition system so that they could change the acquisition parameters for the different experiments proposed. In addition, they learned how to retrieve the data obtained so that it could be analyzed after the laboratory work.

Materials

The following pieces of equipment are necessary:

- Head holder
- Heating pad
- Dissecting microscope
- Surgical instruments
- Mineral oil
- Physiological saline solution (0.9%)
- Bipolar electrode
- Micromanipulator
- Loudspeaker
- Acquisition system (Axon Instruments)
- Connection wires

The experimental setup for this laboratory exercise basically consist of two holders to maintain the animal and electrodes in an adequate position, a bipolar electrode for recording the afferent discharge, a micromanipulator to control vibrissae stimulation, and the acquisition system to process the signals.

During the surgery, the instructor should use a high-magnification microscope to carefully dissect the vibrissal nerve and correctly place the nerve on the recording electrodes.

Animal Preparation

Although the surgery to access the afferent nerve (infraorbital) should be performed by a tutor with surgical experience, students should be able to assist the instructor with some simple procedures.

These procedures take ~2 h and are carried out as follows.

Procedure 1. The animal, one Wistar adult rat, came from the vivarium of the Medicine College where it was maintained on a light-dark cycle with food and water ad libitum. The procedures for these practice were performed in accordance with the recommendations of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

The animal was anesthetized with urethane (150 mg/kg), and its temperature was maintained at 37°C by a servo-controlled heating pad.

Procedure 2. To expose the infraorbital nerve, the instructor removed the zygomatic arch and the surrounding musculature from one side of the rat muzzle. A branch from the infraorbital nerve trunk was dissected to select the innervation of only one vibrissa. Once identified, the vibrissal nerve was transected proximally and maintained in physiological saline solution.

Procedure 3. The students, with the instructor's help, should be able to connect the wires to the acquisition system and to the recording electrodes using the guide. The electrodes must be sustained by a holder so that they can easily be moved to the animal preparation and an amplifier has to be situated between them.

In turn, students have to place the vibrissal nerve on the recording electrodes using the microscope and a glass hook. Both the nerves and electrodes must be immersed in a mineral oil bath during the experiments.

Before students begin the experiments, it is important that they make sure that the nerve is functionally active. To confirm this, students should connect the recording electrodes to a loudspeaker and touch the corresponding vibrissa. If the nerve is functioning, some discharges will be heard when the vibrissa is touched.

As soon as the experimental setup is prepared (Fig. 1), students may begin the experiments proposed for the laboratory practice by following their descriptions in the guide.

Experiment 1: Spontaneous Activity

In this exercise, the afferent discharge should be recorded from a vibrissal nerve without any external stimulation. Students should recognize the spontaneous activity in the signal, detect the action potentials, and calculate the firing rate of this nerve.

Steps to realize the task. STEP 1. In *step 1*, students must fix the acquisition frequency at 50 kHz using the data-acquisition system (Digidata 1322A, Axon Instruments).

STEP 2. Students should record the afferent discharge for 5 s (Fig. 2, *top*).

STEP 3. Students must analyze the data. Students should analyze the results after the laboratory work using a computer program (AcqKnowledge Software, Biopac Systems) provided by the Biomedical Department. If events such as action potentials are detected, the students must count all of them to obtain the firing rate (in spikes/s) of the spontaneous activity.

In this experimental approach, action potentials appear as deflections of different amplitudes (Fig. 2, *bottom*). Thus, it is expected that students can observe the effects of volume conduction in a multifiber recording and can learn that the amplitude of the action potential decreases with the distance to the recording electrode.

Experiment 2: Afferent Discharge Evoked by Passive Stimulation

This experiment consists of recording the multifiber activity of the vibrissal nerve during passive stimulation. This stimulation produces vibrissal movement without muscular activation.

Steps to realize the task. STEP 1. Students should mechanically induce whisker deflection and detect which vibrissa corresponds to the nerve selected, i.e., the vibrissa that produces the greatest electric activity when stimulated. They should identify the discharge using the loudspeaker connected to the recording system and visualize the discharge using the data-acquisition system.

STEP 2. To study the effect of different stimulation intensities in the afferent nerve response, students should bend the whisker in three different directions applied on the same plane (Fig. 3*A*). In addition, three successive deflection levels (1, 2, and 3 mm) are applied to the vibrissa contact point (mean point between the distal and proximal ends of the vibrissa) with a mechanically held metal probe (Fig. 3, *B–D*). The different vibrissal movements are controlled with a micromanipulator, and each displacement is maintained for 5 s while the discharge is recorded (Fig. 4). In addition, spontaneous nerve activity should be recorded and considered as the control situation.

STEP 3. The afferent activity registered for the different situations should be analyzed with a mathematical tool: root mean square (RMS) values. This parameter allows characterization of the signal according to its energy content, and this is related to the signal amplitude in a certain interval of time. Windows of 100 ms for each situation have to be selected from the data acquired to calculate the RMS values and compared with them with the control signal.

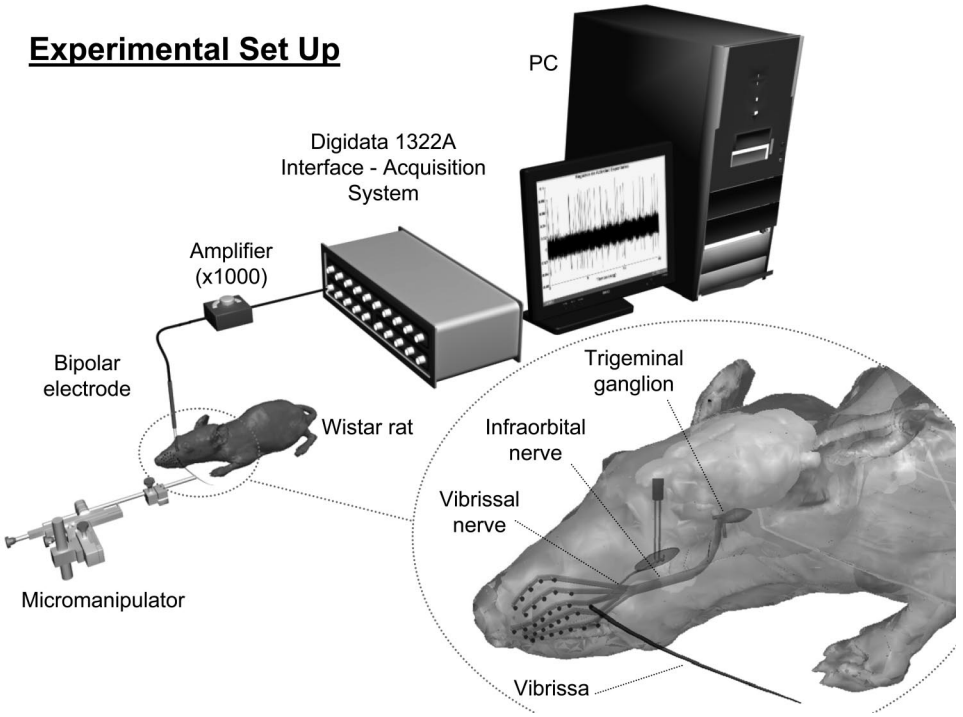

Fig. 1. Experimental setup. Shown is the preparation used for recording spontaneous and evoked activity. The micromanipulator is used to induce mechanical stimulation of the vibrissa. Afferent signals are amplified (×1,000) and acquired by a Digidata 1322A Interface (Axon Instruments). The acquisition parameters, such as sample rate, recording time, and data storage, are controlled by a personal computer (through Axoscope software). The enlarged area at the *right* shows a schematic diagram of the anatomy associated with the vibrissae afferent innervation and the recording electrode placement.

The RMS value is calculated as follows:

$$\mathrm{RMS} = \sqrt{\frac{1}{N}\sum_{k=1}^{N}[x(k)]^2} \quad k = 1, 2, \ldots, N$$

where N is the number of samples, $x(k)$ is kth sample of the signal, and RMS is the estimate of the energy.

This last part of the experiment (*step 3*) is done after the laboratory work. Students can use the computer programs MATHCAD or MATLAB for processing the data and obtaining the results.

RESULTS

Student Reports

As described previously, students have to process and analyze the data obtained from both experiments.

They have to count the spikes and calculate the firing rate offline during the first experiment. The recording files have to be saved as ASCII files. That way, the firings can be visualized and processed with several computer programs: AcqKnowledge (a program of Biopac Systems) or another processing program such as MATHCAD or MATLAB.

Students must compare and observe the distributions of RMS values obtained from different sorts of passive stimulation (direction and displacement) during the second experiment.

It is expected that due to the characteristics of the stimuli (invariant along time), the response will not change its amplitude over time (stationary signal). Therefore, RMS values should not vary significantly along time.

Students could represent the results using boxplot diagrams of RMS values. These diagrams allow students to observe the variability of RMS values. Boxplot diagrams are shown in Fig. 5 for each of the proposed experimental situations.

The procedure for the analysis of the afferent activity with RMS values requires the development of the following steps.

Step 1: ASCII file. If the processing is carried out with MATHCAD, the command for data reading is "READPRN," which returns an array containing the contents of the ASCII file. If the processing is done with MATLAB, the file can be read with the "DLMREAD" command, which reads an ASCII-delimited file of numeric data into the matrix.

Step 2: signal segmentation. Basically, this procedure consists of dividing the temporal series in segments of predefined times. In this process, a segmentation of 100 ms is required (without overlapping). The sampling frequency was 50 kHz; therefore, each segment had 5,000 samples.

Step 3: calculation of RMS values. One RMS value is calculated for each segment. If the students use MATHCAD, they must follow this command:

$$\mathrm{rms} = \frac{1}{n} \times \sum_{k=0+b}^{n+b} (x_k)^2$$

where n is the number of samples in each segment (5,000 samples); b has values of 0, 5,000, 10,000, 15,000, . . . , 50,000; and x_k is the kth sample of afferent activity recording.

If the processing is realized with MATLAB, the following sequence of commands must be used:

```
int=1: 5001;
N=length(int);
for i=1: 9
   rms(i)=(sum(x(int+(N*(i−1))).^2)/N)^0.5;
end
```

where **rms** is a vector with the RMS values for each segment.

Finally, students must present these results with the appropriate graphics.

DISCUSSION

In the laboratory experience described here, the students are expected to understand some aspects of a sensory system and

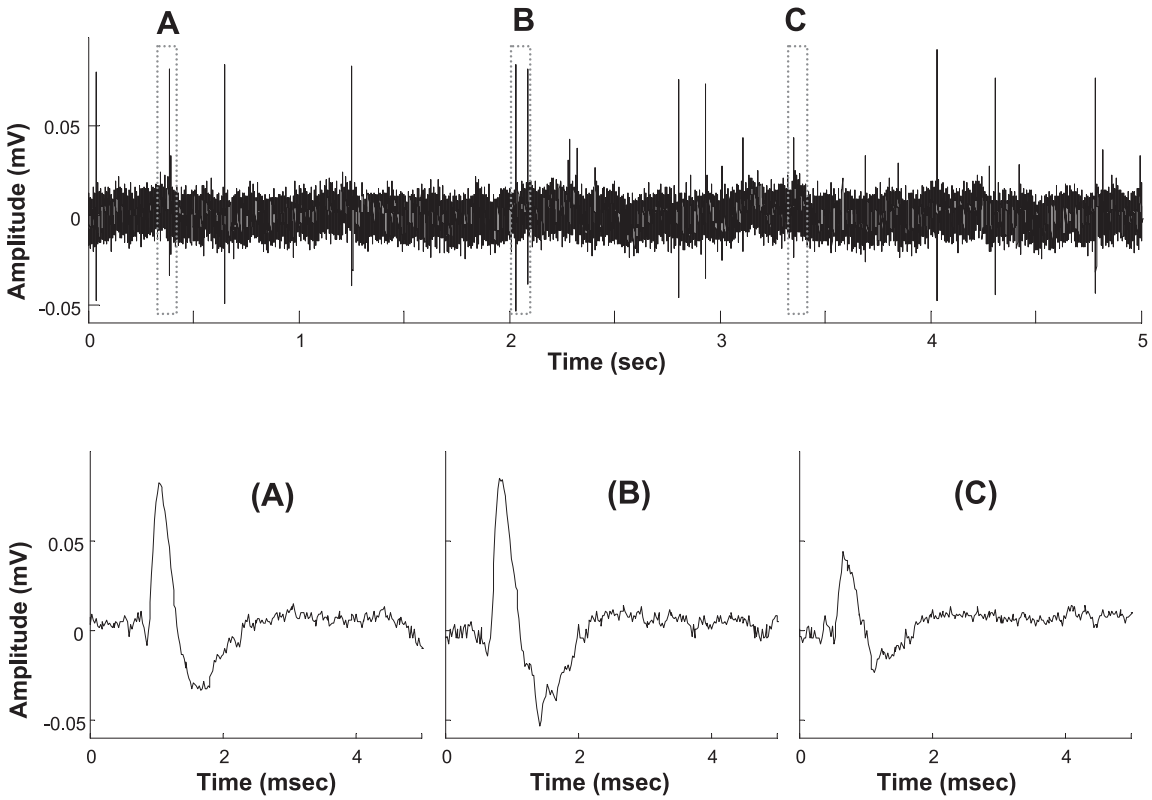

Fig. 2. Recordings of the afferent nerve discharge without stimulation. Three events (*events A*, *B*, and *C*; *bottom*) were extracted and amplified from the recording at the *top* (*boxes A*, *B*, and *C*).

to use mathematical and digital tools for processing biological signals.

The instructor talks about spontaneous activity and its random properties, such as Poisson's distribution of the interspike interval. These properties are related to stochastic resonance phenomenon, a possible way to improve the reliability of the transmission of information in the nervous system (4, 8).

The learning of signal processing methods is an important issue in biomedical engineering. Two main objectives were

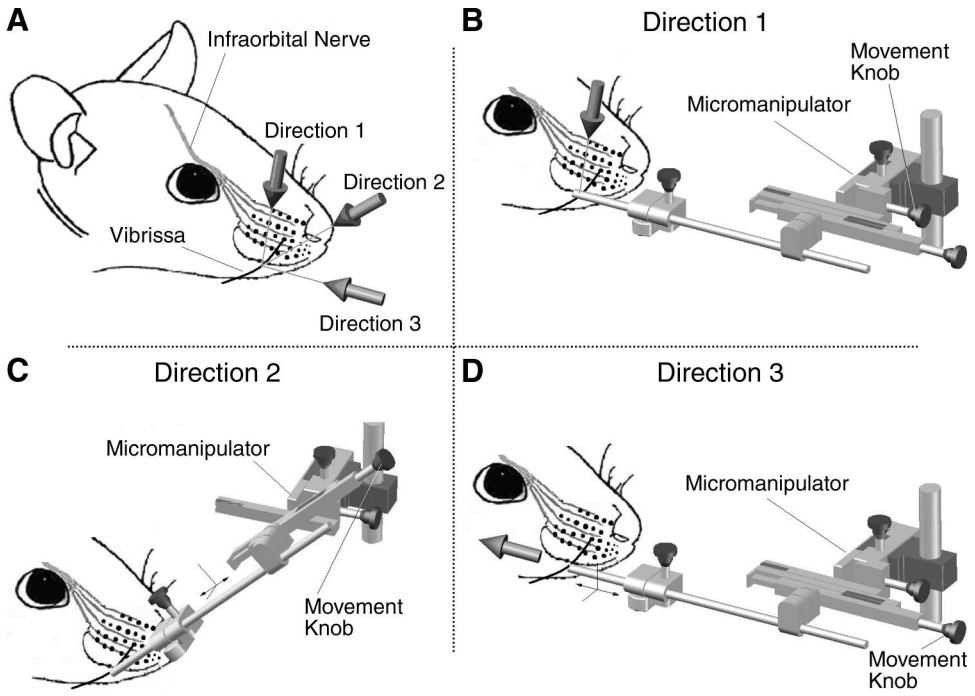

Fig. 3. Vibrissa mechanical stimulation. *A*: schematic showing vibrissa mechanical stimulation in three different directions applied on the same plane. *B–D*: vibrissa displacement directions were applied in the same vertical plane with a difference of 45° between each one.

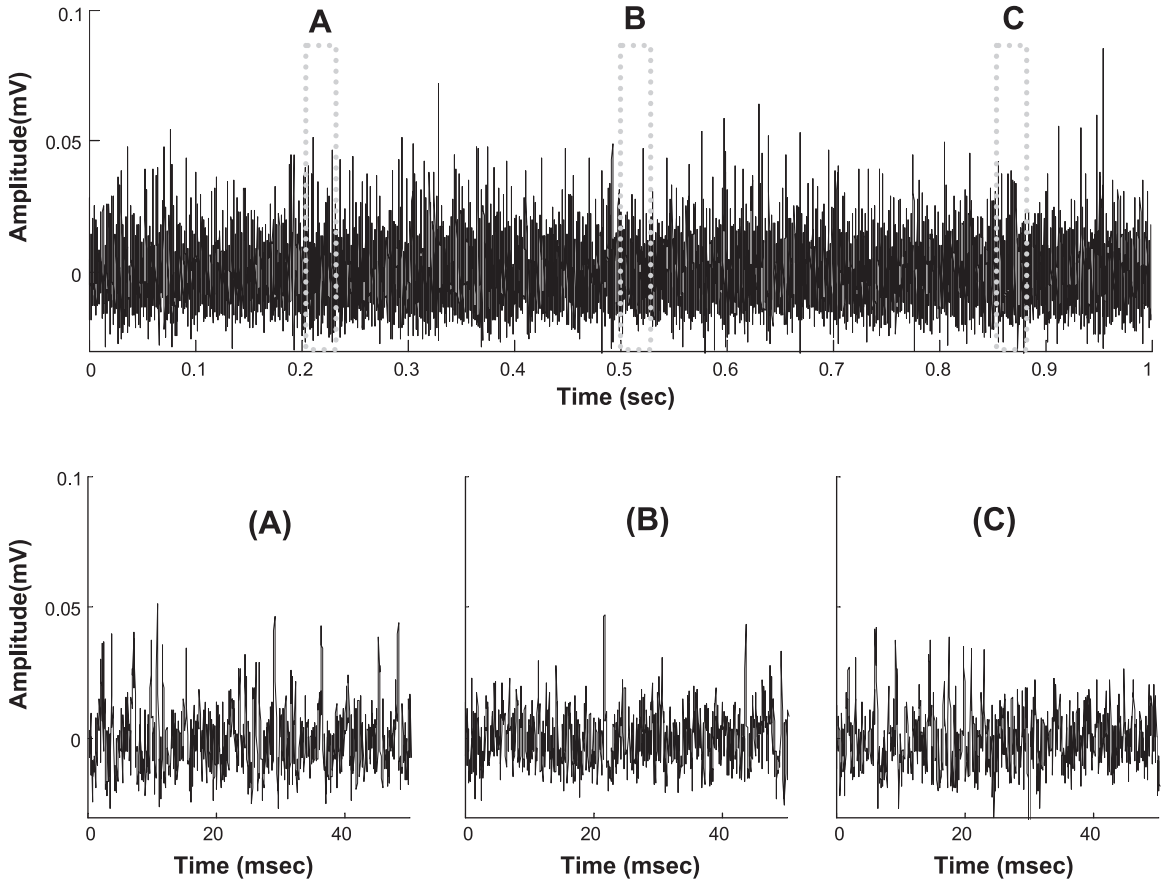

Fig. 4. Recordings of the afferent nerve discharge during mechanical stimulation. Three events (*events A*, *B*, and *C*; *bottom*) were extracted and amplified from the recording at the *top* (*boxes A*, *B*, and *C*).

proposed to the students in this practical work: *1*) to obtain the firing rate (in spikes/s) and *2*) to quantify the signal amplitude. It was necessary for the students to learn two processing methods. The determination of the firing rate was made using visual analysis, but the students could also develop some algorithms to analyze the signals automatically.

The analysis with RMS values is a useful method that characterize the signal in the time domain and is related to the number of mechanoreceptors electrically active during passive stimulation. The RMS value is a technique used to analyze stationary temporal series. Although the afferent activity recordings have a certain degree of nonstationarity, we (1) have shown that this technique can be used for the analysis of sensory signals.

Conclusions

The laboratory exercise presented in this article enables students to learn basic concepts in sensory physiology, such as receptor activation, background activity, and neural coding, by analyzing the afferent nerve discharge of the vibrissal system in the rat.

The vibrissal system is a model used by many researchers to study active sensation and sensory-motor integration in the central

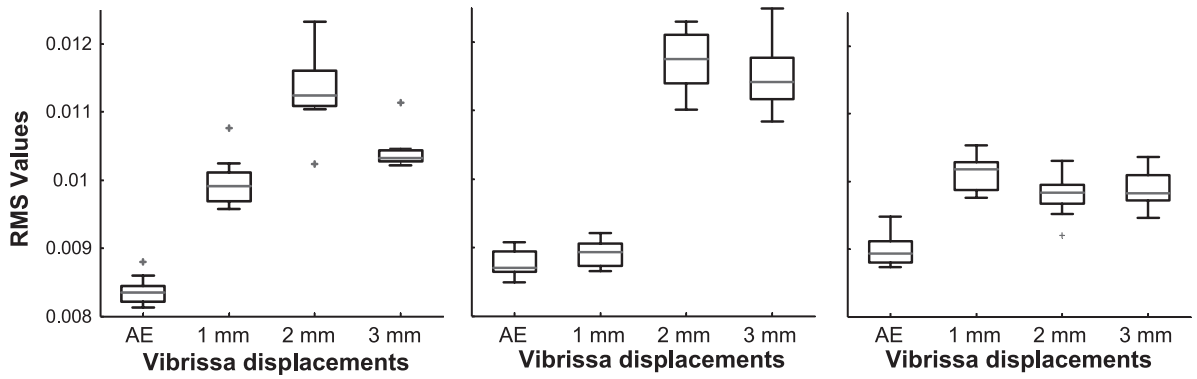

Fig. 5. Boxplot diagrams of root mean square (RMS) values obtained for the three directions and for the three levels of displacement proposed. SA, spontaneous activity (control).

nervous system. However, the simple stimulation protocol and the electrophysiological recordings used in this laboratory experience enable students to understand complex concepts and learn about experimental design in neurophysiology.

ACKNOWLEDGMENTS

The authors thank Patricia Black for help with the translation process.

GRANTS

This work was supported by grant from the Consejo de Investigaciones de la Universidad Nacional de Tucumán.

REFERENCES

1. **Albarracín AL, Farfán FD, Felice CJ, Décima EE.** Texture discrimination and multi-unit recording in the rat vibrissal nerve. *BMC Neurosci* 7: 42, 2006.
2. **Bauer KW.** *The Effect of Participation in Undergraduate Research on Critical Thinking and Reflective Judgement. AIR 2001 Annual Forum Paper* (online). http://eric.ed.gov/ERICDocs/data/ericdocs2sql/content_storage_01/0000019b/80/19/4e/af.pdf [9 April 2009].
3. **Carvell GE, Simons DJ.** Biometric analyses of vibrissal tactile discrimination in the rat. *J Neurosci* 10: 2638–2648, 1990.
4. **Chen Y, Yu L, Qin SM.** Detection of subthreshold pulses in neurons with channel noise. *Phys Rev E Stat Nonlin Soft Matter Phys* 78: 051909, 2008.
5. **Dörfl J.** The innervation of the mystacial region of the white mouse: a topographical study. *J Anat* 142: 173–184, 1985.
6. **Guic-Robles E, Valdivieso C, Guajardo G.** Rats can learn a roughness discrimination using only their vibrissal system. *Behav Brain Res* 31: 285–289, 1989.
7. **Halaby R.** Promoting undergraduate research in science. *Scientist* 15: 35, 2001.
8. **Kawaguchi M, Mino H, Durand DM.** Enhancement of information transmission with stochastic resonance in hippocampal CA1 neuron network. *Conf Proc IEEE Eng Med Biol Soc* 2008: 4956–4959, 2008.
9. **Lichtenstein SH, Carvell GE, Simons DJ.** Responses of rat trigeminal ganglion neurons to movements of vibrissae in different directions. *Somatosens Mot Res* 7: 47–65, 1990.
10. **Rice FL, Mance A, Munger BL.** A comparative light microscopic analysis of the sensory innervation of the mystracial pad. I. Innervation of vibrissal follicle-sinus complexes. *J Comp Rev* 252: 154–174, 1986.
11. **Van der Loos H, Dorfl J, Welker E.** Variation in pattern of mystacial vibrissae in mice. A quantitative study of ICR stock and several inbred strains. *J Hered* 75: 326–336, 1984.
12. **Vincent SB.** The function of the vibrissae in the behavior of the white rat. *Behav Monog* 1: 1–181, 1912.
13. **Zucker E, Welker WI.** Coding of somatic sensory input by vibrissal neurons in the rat's trigeminal ganglion. *Brain Res* 12: 138–156, 1969.