Electrophysiological study in the infraorbital nerve of the rat: Spontaneous and evoked activity

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Abstract. In this work we present some studies in the afferent nerve of the rat vibrissae. Studies on spontaneous activity (SA) in this sensorial system are of long data. Nevertheless, SA recordings in the nerve of a single vibrissa have not been made until present. In this work, we use an algorithm based on signal decomposition with Continuous Wavelet Transform (CWT) to analyse the discharges of two nerves. The action potentials of both nerves were detected and the firing rates were calculated. These results suggest that the firing rate of one vibrissa innervation is low considering that this nerve contains hundred of fibers. In addition, we present preliminary studies suggesting important effects of the hair shaft length in the afferent discharge during the vibrissae movements. The experiments consisted in recording the nerve activity after the vibrissae were sectioned at two different levels. The results showed important differences in the signal energy contents. It suggests that the hair shaft length would produce a differential activation of the mechanoreceptors located in the vibrissae follicle.

1. Introduction
Rodents, as well as many mammals, have vibrissae, whiskers or tactile hairs on both sides of the muzzle [1]. Vibrissae are structures specialised on reception and transmission of tactile information. Their arrangement, particularly in the rat, consists on five horizontal lines (rows A-E) and one vertical line (α,β,γ,δ) on both sides of the animal muzzle [2]. It has been postulated that rats, due to their poor vision, use this exploratory behaviour to explore their environment [3].

Each vibrissa sits on a structure called Follicle-Sinus Complex (F-SC). There, mechanoreceptors, such as Merkel cells, lanceolate terminals and free nerve endings transduce tactile information to an electric signal [4]. This information travels from the deep vibrissal nerves—branches of the infraorbital nerve— to the somatosensory primary cortex (S1).

Small muscles are associated with vibrissal follicles and have the form of a sling connecting two adjacent follicles of the same row [5]. Two branches of the facial nerve, the buccal branch and the upper division of the marginal mandibular branch, innervate follicular muscles and the contraction of these muscles produces the forward vibrissal movement [6].

Studies in tactile discrimination suggest that rats use their vibrissae to distinguish objects differing in the physical characteristics of the surfaces, such as roughness and texture [7][8][9]. The transduction mechanism related to this ability would be mediated by the mechanism of vibrissae movement, the follicular complex and the electrochemical mechanism of the mechanoreceptors [10].
It was demonstrated the presence of spontaneous activity (SA) in all sensorial systems. However, reports about the firing rates in the vibrissal system vary considerably. Zucker and Welker [11] reported spontaneous discharges for 6.5% of the trigeminal neurons in the rat. Gibson and Welker [12] suggested that the presence of SA could be due to the recording electrode, slight vibrissa movements or hair hysteresis. Lichtenstein et al. [13] reported that the ganglion appears practically silent but some cells fired at a low rate. In contrast, Dykes [14] reported high activity rates in cats and seals. Lichtenstein et al. [13] consider that, given the extraordinary sensitivity of mechanoreceptors, it is improbable that the trigeminal ganglion be completely quiet at any moment.

In this work, we present studies about the conduction in the infraorbital nerve (IO). We also analyse the spontaneous nerve discharges using an event detection algorithm based on Continuous Wavelet Transform (CWT) [15].

In addition, we recorded the spontaneous activity (SA) from the deep vibrissal nerve innervating the DELTA (δ) vibrissa and from the nerves innervating three vibrissae. We also analysed the action potentials (AP) and the firing rates for both cases.

Finally, we present preliminary studies suggesting important effects of the hair shaft length in the afferent discharge during the vibrissae movements or whisking. The results suggest that the hair shaft length would produce a differential activation of the mechanoreceptors located in the vibrissae follicle.

2. Materials and Methods

1.1. General response characteristics

Three Wistar adult rats (300 g – 350 g) were used in our experiments. They were anaesthetised with urethane (130 mg/Kg) and the temperature of the animal was maintained at 37° by a servo-controlled heating pad. Surgery consisted on exposing the infraorbital nerve. The nerve was dissected and transected proximally, and stimulation electrodes (nichrome wires) were placed on the nerve trunk (figure 1).

The nerve activity was recorded using bipolar electrodes; one was placed on the nerve and the other at the surrounding musculature. The stimulation inter-electrode distance was 2.5 mm and the cathode-recording electrode distance was 12 mm.

We computed the conduction velocity with 1 and 4 Hz stimulation pulses (30 µs duration; supra-maximals). On the other hand, we determined the refractory periods and studied the excitability of the infraorbital nerve. We used trains of rectangular pulses of 500 and 1000 Hz.

We acquired 40 recording windows (7msec each), and obtained the average signals from them.

1.2. Spontaneous activity

The spontaneous activity recordings were obtained: 1) from the nerves that supplies innervation to three vibrissae and 2) from the deep vibrissal nerve of the DELTA vibrissa. Both responses were compared.

For both preparations, the nerves were dissected and transected proximally. The recording electrodes were placed on their distal stumps to record the afferent discharge of the corresponding vibrissae (figure 2).

The SA recordings were acquired at 50 KHz; using a data acquisition system, Digidata 1322A, Axon Instruments. The parameters for acquisition were controlled with the software AxoScope.

Afferent activities were analysed with a spike detection algorithm proposed by Nenadic and Burdick [15].

Data processing and event detection -based on multiscale decomposition of the signal- were carried out using MATLAB.

All these procedures were done in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (National Research Council, NRC).
1.3. Study of the afferent discharges evoked by motor stimulation. Effect of the hair shaft length.

The next experiments were carried out to consider the possible effects of the whisker length in the mechanoreceptors activation and, consequently, in tactile information travelling throughout vibrissal sensory pathways.

The studies consisted on sectioning the whiskers into two different levels and recording the afferent discharge during the active whisking.

The vibrissae movements were produced using electrical stimulation of the facial motor branches innervating the mystical muscles. The motor branches were dissected and transected proximally, and stimulation electrodes were placed on their distal stumps to produce the contraction of the mystacial muscles. On the other hand, the nerves innervating 4 or 5 vibrissae were dissected and transected and a bipolar electrode was placed on them to record the afferent discharge of the corresponding vibrissae. The recording electrodes as well as the nerves were immersed in a mineral oil bath during all recording set (figure 2).

The hair shafts were transected at two levels: 1) at half of a way, eliminating the thinner part of the hair and 2) at the evenness of the skin, sectioning completely the hair. For the control recordings the normal vibrissal length was maintained.

Fifty recording windows were obtained (50 ms each one) and the average signal was used to calculate the RMS values.

Data processing was carried out using the MATLAB software.

All these procedures were done in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (National Research Council, NRC).

3. Results

Figure 3 shows the compound action potentials recorded from the IO nerve to determine the conduction velocity and refractory period.

The time delay between the beginning of the stimulus artefact and the peak of the action potential was 0.35 msec for 1 Hz stimulation pulses (figure 3A). Since the distance between the stimulating electrode (negative electrode) to the recording electrode was 12 mm, the velocity conduction was
34.30 m/sec. However, the time delay for 4-Hz stimulation pulses was 0.30 msec. and the conduction velocity was 40 m/sec (figure 3B).

Figure 3C shows the discharges induced by 500 Hz stimulation trains. The time delay of the first response was 0.321 msec. and the amplitude was 450 µV. On the other hand, the time delay of the second and the third responses increased to 0.346 msec, but the amplitude fell to 300 µV.

The discharges produced by 1000 Hz stimulation pulses are shown in figure 3D. The discharge amplitude produced by the first pulse was 470 µV. The second stimulus did not produce any response, but the following stimuli produced discharges of amplitudes: 160 µV, 100 µV and 150 µV. The time delay of the first response was 0.346 msec; the third, fourth and fifth responses were of 0.410 msec, 0.447 msec and 0.396 msec.

Figure 3. Responses in the infraorbital nerve induced by electrical stimulation. A. with 1 Hz pulses; B: with 4 Hz pulses. C. with trains of 500 Hz. D. with trains of 1000 Hz (D). Vertical calibration: 100 µV.

Figure 4A shows 1-sec duration recordings of the spontaneous activity (SA) in the nerves supplying innervation for three vibrissae. The temporal position of the action potentials detected is shown in B, C, D and E.

Figure 5A shows 15-sec duration recordings of the SA obtained from the deep vibrissal nerve of DELTA vibrissa.

Table 1 shows the firing rate calculated for both nerves. These results demonstrate an important difference between both firing rates, with is related to the amount of fibres included in each nerve.

The preliminary studies about the effect of the whisker length on tactile information travelling throughout vibrissal sensory pathway showed that qualitative and quantitative changes of the afferent discharge take place (figure 6). These differences are mainly observed on the amplitude of the initial discharge located at 15 msec from the beginning of the signal.

In order to quantify these observations, the amplitude of 50 recording window (sweeps) of each situation was determined. Later, the average value and standard deviation for each one was calculated.

Figure 6D shows that the amplitude of the initial discharge in A and B recordings does not present important differences; however, in 6C, the amplitude decreases a third of the value observed in A.
Figure 4. (A) Spontaneous activity recorded from the nerves innervating three vibrissae. (B) Temporal position of the AP detected (AP duration: 0.6 msec). (C), (D), (E) AP duration: 0.8, 1.0 y 1.2 msec, respectively.

Figure 5. (A) Spontaneous activity recorded from DELTA vibrissal nerve. (B) Temporal position of the AP detected (AP duration: 0.6 msec). (C), (D), (E) AP duration: 0.8, 1.0 y 1.2 msec, respectively.
Table 1. Firing rate calculated using AP detection.

| Duration | SA obtained for one vibrissa innervation | SA obtained for three vibrissae innervation |
|----------|------------------------------------------|------------------------------------------|
|          | 0.6 0.8 1.0 1.2 1.4                     | 0.6 0.8 1.0 1.2 1.4                      |
| Firing rate (firing / sec.) | 26.2 25.6 22.4 17.8 12.9 | 4 3.6 2.8 2.7 3 |

Figure 6. Effect of the hair shaft section in the afferent discharge. A. Control, B. Recordings after the vibrissae half section, C. Recordings after the vibrissae complete section, D. Average values and standard deviation of the initial discharge (at 15 msec).

4. Discussion

Previous works, using single unit recordings in the trigeminal ganglion, reported the presence of spontaneous activity in the afferent of the vibrissal system. Nevertheless, the reports on the firing rate vary considerably.

The present results confirm the presence of spontaneous activity in the vibrissal afferents at peripheral level. The average firing rate in a bundle of fibers innervating three vibrissal follicles was 21 firing/sec. On the other hand, the SA in the nerve of DELTA vibrissa showed an average firing rate of 3.2 firing/sec. These results suggest that the firing rate of one vibrissa innervation is low considering that this nerve contains hundred of fibers.

The vibrissae sit in a Follicle-Sinus Complex (F-SC), an anatomical structure that present a sinus and a great number of mechanoreceptors surrounding the follicle. These characteristics are the cause that different changes in the external conditions of the hair shaft activate the mechanoreceptors in different ways. On the other hand, the hair shaft has a conical form, with a diameter that decreases progressively towards its end.

The preliminary results presented in this work show that the whisker length could represent an important parameter for consideration in the mechanoreceptors activation.

The complete section of the hair shaft causes an important reduction of the mass that moves during whisking and, consequently, a reduction of the inertial moment of the hair. Therefore, the necessary
force moment to move the whiskers would be smaller in these conditions than that needed when these have their normal length. This speculation is consistent with the results of the present study and with current anatomical data. However, more studies would be necessary to confirm them.

5. Conclusions

The analysis of the spontaneous activity with the CWT allowed to detect the action potentials and to calculate the firing rate in afferent vibrissal nerves. The average firing rate was of 21 firing/sec for three vibrissae innervation and 3.2 for vibrissal nerve of one vibrissa.

The preliminary studies that analyse the change of the afferent discharge in relation with the vibrissae length has not been described previously and show that this aspect could represent an important parameter in tactile stimuli detection.

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