Temporal code in the vibrissal system – Part I: Vibrissa response to passive stimulation

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Abstract. In this paper we analyzed the afferent discharges of two nerves: one innervating three vibrissae (N1) and another one innervating the DELTA vibrissa (N2). Sustained mechanical stimulations were applied to the hair shaft of a whisker innervated by N1 in three different directions. The vibrissa selected for stimulation was the one that produced the higher electrical activity in the N1. The vibrissa was bent 1, 2 and 3 mm in each direction. The manual stimulation was applied to DELTA vibrissa and its electrical activity was registered. A custom-made photoresistive sensor registered the vibrissa displacements. We analyzed the multifiber discharge with a spike detection algorithm using the continuous wavelet transform (CWT). Directional sensibility in afferent responses was observed. RMS values had higher sensibility to deflection intensity in direction 1, while the responses in direction 2 and 3 were different. Median and dispersion of the inter-event times were decreased during different levels of the bent (events of 0.2 msec). The statistical analysis of the inter-event times showed significant differences among different stimulation levels. The inter-event times are decreased during passive movement with bent quantity induced to DELTA vibrissa. In this case the events duration was 0.6 ms.

1. Introduction

During exploration, rats actively sweep their vibrissae in a rhythmic forward and backward motion. It has been stated that rats, due to their poor vision, use this behavior to explore their environment [1] [2] [3]. The vibrissae or tactile hairs are located at the both sides of the muzzle in a specific arrangement of horizontal lines. Each vibrissa sits in a follicle innervated by a deep vibrissal nerve arising from the infraorbital branch of the trigeminal nerve [4]. Mechanoreceptors, such as Merkel cells, lanceolate terminals and free nerve endings, are activated during the vibrissae movements and this information travels along the trigeminal pathway.

Classical studies in vibrissal sensory coding involved head-fixed animals, controlled whisker deflection (passive deflection) and recordings of the evoked response [5] [6]. These studies classified 75% of the neurons as slowly adapting (SA) and 25% as rapidly adapting (RA) and demonstrated that cells respond differently to different whisker shaft directions and have different velocity sensitivities.

In this study we used passive stimulation to analyze the afferent discharges of two nerves. This stimulation produces the vibrissal movement without muscular activation.
The afferent activity of the nerve innervating three vibrissae (N1) was recorded during the whisker deflection in three directions and with three stimulation intensities each one.

On the other hand, the afferent discharges of the nerve innervating the DELTA vibrissa (N2) were recorded using manually stimulation.

The recordings were analyzed using an algorithm proposed by Nenadic y Burdick [7]. This detects events between 0.2 and 1.6 msec duration. We used the RMS (Root Mean Square) values to quantify the signal amplitude.

Our results show that the electric nerve activity (RMS values) increased with the deflection level for direction 1 but it did not happen in the other cases (direction 2 and 3). In addition, it was observed that the inter-events time decreased when the deflection increased, especially for events with 0.2-msec duration. On the other hand, the deflection applied to the DELTA vibrissa produced a decrease of the inter-events time when the deflection increased.

These results could be showing that a temporal code related with the vibrissal position is present at the peripheral nerves.

2. Materials and Methods

2.1. Procedures
Three Wistar adult rats (300 g – 350 g) were used in our experiments. They were anesthetized with urethane (130 mg/Kg) and the temperature of the animal was maintained at 37° by a servo-controlled heating pad. Surgery consisted of exposing the infraorbital nerve. The branches innervating three vibrissae (N1) and the nerve innervating the DELTA vibrissa (N2) were identified with the high magnification of a dissecting microscope. The dissected nerves were transected and a bipolar electrode was placed on it to record the afferent discharge of the corresponding vibrissa. The recording electrodes as well as the nerves were immersed in a mineral oil bath during all recording. The vibrissa DELTA displacements were registered using a custom-made photoresistive sensor (developed at the Neuroscience Laboratory, Facultad de Medicina, Universidad Nacional de Tucumán, Argentina) [8].

The experimental protocol used for multifiber recordings was described in previous papers [9] [10]. 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).

2.2. Recordings
The experiments consisted in recordings the multifiber activity of the N1 branch during sustained mechanical stimulation of a vibrissa (the stimulated vibrissa was the one that produced the higher electric activity in the branch N1). The stimulation consisted in bending the whisker in three different directions applied in the same plane (Figure 1). Three successive deflection levels (1, 2 and 3 mm) were applied to the vibrissa contact point (mean point between distal and proximal end of the vibrissa) with a mechanically held metal probe. The afferent activity was registered for the situations proposed. Spontaneous Nerve Activity (SA) was also recorded and considered as the control situation. The recordings time was 1 sec in all cases.

On the other hand, the afferent activity of the DELTA vibrissa was recorded during the passive stimulation. The whisker deflection was manually induced.

The sensory signals and the vibrissae displacements were digitalized using a data acquisition system, Digidata 1322A, Axon Instruments, at 50 KHz. The parameters of the acquisition were controlled using the software AxoScope.
2.3. Digital Processing
We calculated the RMS values to analyze the amplitude of the afferent nerve discharges in relation with the mechanoreceptors activation [9] [11]. Afferent activities were analyzed with a spike detection algorithm proposed by Nenadic and Burdick [7]. The purpose of this implementation was to study the temporal pattern related with vibrissae displacement levels.

The events detection algorithm was used to analyze the afferents discharges during passive movement.

2.4. Event Detection with Continuous Wavelet Transform (CWT)
The methodology consists of a combination of several techniques stemming from multiresolution wavelet decomposition, statistics, detection theory and estimation theory. Next, we state the five major steps of the algorithm up-front.

1- Multiscale decomposition of the signal using an appropriate wavelet basis. The mother wavelet used in this paper belongs to the family of biorthogonal wavelets: ‘bior1.5’. This wavelet was chosen because its biphasic shape is reminiscent of action potentials. The wavelet decomposition scales were chosen in order to detect events duration from 0.2 to 1.0 ms.

2- To separate the signal and noise at each scale. By applying the continuous wavelet transform we obtain a multiscale representation of the signal in terms of its wavelet coefficients. Then, the noise of each temporal series was eliminated by using simple threshold detection. For a near-optimal performance, it is sufficient to choose the threshold $T_j = \sigma_j \sqrt{2 \log_e(N)}$, where N is the number of samples of the analyzed time series, $\sigma_j$ is the variance of the noise coefficients $W(j,k)$ at scale $a_j$ and $T_j$ is the threshold of the time series. For a Gaussian random variable, it can be shown that the median of its absolute deviation effectively estimates the standard deviation:

$$\sigma_j = M \{ |X(j,0) - \overline{X}|, \ldots, |X(j,N-1) - \overline{X}| \}/0.6745$$

where $\overline{X}$ is the simple mean of $X_j$ and $M\{\}$ denotes the sample median.

3- On the basis of steps 2) and 3), it was made a Bayesian hypothesis in order to ensure the presence of spikes at different scales.

4- To combine the decisions at different scales.

5- To estimate the arrival times of individual spikes.

The detection algorithm description used in this paper is detailed in [7].
Data processing, RMS calculations and events detection with CWT were made with MATLAB®.

2.5. Statistics
Statistical analysis was done with Kruskal Wallis ANOVA. This test analyzes inter-event times detected in the afferent activity. The Kruskal Wallis test is a nonparametric alternative to the One-Way ANOVA. The test assesses the hypothesis that the different samples in the comparison were drawn from the same distribution or from distributions with the same median. Thus, the interpretation of the Kruskal-Wallis ANOVA is basically identical to that of the parametric One-Way ANOVA, except that it is based on ranks rather than on mean values.

3. Results
Figure 2 shows the afferent discharges of the N1 branch during the sustained mechanical stimulation of a vibrissa in direction 1. The SA recorded is also shown in figure 2.

Box plot diagrams showing the distribution of the RMS values for each direction are presented in figure 3.

The detection algorithm identified events of 0.2, 0.4, 0.6 and 0.8 ms. Figure 4 shows the inter-event times distribution detected during displacements in direction 1.

Figure 5 shows the afferent activity (A) and the displacement (B) of the DELTA vibrissa registered during the passive stimulation.

Events of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ms of durations, detected with event detection algorithm are shown in figure 5C.

Figure 6 shows the mean of the inter-event times for different displacement degrees.

The statistical results are shown in table 1. This table presents the comparison among different displacement situations as well.

![Figure 2. SA and afferent activity recordings obtained during vibrissa mechanical stimulation in the direction 1.](image-url)
Figure 3. Box-plots diagrams of the RMS values from afferent activity registered during vibrissa displacements in: (A) direction 1; (B) direction 2 and (C) direction 3.

Figure 4. Inter-event times distribution in direction 1. (A) Inter-event times distribution of the events of 0.2 ms duration. (B), (C) and (D) idem to (A) with events of 0.4, 0.6 and 0.8 ms duration.

Table 1. Results of the Krustal Wallis ANOVA applied to different displacement levels in direction 1. D1, D2 and D3 are the displacements of 1, 2 and 3 mm, respectively. The statistic used by the Krustal Wallis ANOVA is Chi².

|       | D1 vs D2 | D1 vs D3 | D2 vs D3 |
|-------|----------|----------|----------|
|       | Chi²     | p > Chi² | Chi²     | p > Chi² | Chi²     | p > Chi² |
| 0.2 ms| 7.45     | 0.0063   | 0.17     | 0.6764   | 4.8      | 0.0285   |
| 0.4 ms| 4.96     | 0.0259   | 0.25     | 0.6173   | 6.81     | 0.0091   |
| 0.6 ms| 1.62     | 0.2033   | 0.03     | 0.87     | 2.02     | 0.155    |
| 0.8 ms| 0.36     | 0.5513   | 0.00001  | 0.99     | 0.34     | 0.5604   |
Figure 5. (A) Afferent discharge of DELTA vibrissa during passive stimulation. (B) Vibrissa displacement recorded with a photoresistive sensor. (C) Events detected by algorithm based on TWC. The events detection procedure was carried out for events of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ms duration.

Figure 6. Mean of inter-event times vs vibrissa displacements. The inter-event times were averaged from ten movements of DELTA vibrissae applied with the hand (passive stimulation).
4. Discussion

The amplitude of the afferent discharge showed significant differences related with the level and direction of the deflection. Thus, during the sustained mechanical stimulation, the RMS values increased when the hair shaft was deflected in direction 1 and when the stimulation level increased.

Figure 3A shows that the signal amplitude increases when the deflection changes from 1 to 2 mm. But the RMS values fall when the higher deflection level (3mm) is applied. This effect could be explained from the lack of activation of the mechanoreceptors in same whisker positions.

The RMS values obtained for the direction 2 (Fig. 3B) did not show any significant difference between the deflection of 1 mm and the SA recordings. However, the values obtained for deflections of 2 and 3mm increased considerably. The displacements applied on direction 3 threw values slightly different to the control one (Fig. 3C). These results agree with studies suggesting the vibrissa directional sensibility [5][6].

The algorithm was adjusted to detect events from 0.2 to 0.8 ms duration. The median and the dispersion of the inter-event times decreased for deflection of 1 and 2 mm on direction 1 (Fig. 4A). This was observed for events of 0.2 ms duration but not for those of 0.4, 0.6 and 0.8 ms duration. The statistic analysis showed significant differences between the displacements 1 and 2 mm for events of 0.2 ms duration and between the displacements 2 and 3 mm for events of 0.4 ms duration (Table 1). The rest of the instances did not show significant results.

The analysis of the DELTA vibrissa discharge showed that the inter-event times decreased for all event detected (0.2, 0.4, 0.6, 0.8, 1.0 y 1.2 ms) (Fig. 5). The average of events using 10 displacements showed that events of 0.6 ms duration could be related with the deflection level (Fig. 6). However, the present results are insufficient for state a relation between inter-events time and the vibrissa position.

5. Conclusions

The afferent discharge was analyzed in two situations:
- Sustained and mechanical stimulation. The N1 afferent activity was analyzed. The stimulation was applied to the vibrissa that produced the higher electric activity.
- Manual stimulation. The stimulation was applied to the DELTA vibrissa.

Different responses were found according to different displacement directions. The RMS values showed high sensibility to the displacement for direction 1, while it was different for direction 2 and 3.

Median and dispersion of inter-event times decreased with the deflection levels (event of 0.2 ms duration).

The inter-event times decreased according to the displacement induced to DELTA vibrissa during the stimulation (event of 0.6 ms duration).

6. Acknowledgements

This work was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT), the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT), and Institutional funds from Instituto Superior de Investigaciones Biológicas (INSIBIO).

7. References

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