Temporal code in the vibrissal system – Part II: Roughness surface discrimination

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Abstract. Previous works have purposed hypotheses about the neural code of the tactile system in the rat. One of them is based on the physical characteristics of vibrissae, such as frequency of resonance; another is based on discharge patterns on the trigeminal ganglion. In this work, the purpose is to find a temporal code analyzing the afferent signals of two vibrissal nerves while vibrissae sweep surfaces of different roughness. Two levels of pressure were used between the vibrissa and the contact surface. We analyzed the afferent discharge of DELTA and GAMMA vibrissal nerves. The vibrissae movements were produced using electrical stimulation of the facial nerve. The afferent signals were analyzed using an event detection algorithm based on Continuous Wavelet Transform (CWT). The algorithm was able to detect events of different duration. The inter-event times detected were calculated for each situation and represented in box plot. This work allowed establishing the existence of a temporal code at peripheral level.

1. Introduction
Rodents as well as many mammals are characterized by the presence of vibrissae or whiskers located on both sides of the muzzle [1]. Studies in tactile discriminative behavior agree that rats are able to learn a rough-smooth discrimination task by actively palpating the discriminanda (sandpaper surfaces, grooved plastic cylinders, etc.) [2][3]. This capability to detect small differences in roughness was compared with that of humans using their fingertips.

A structure of the neural code was recently proposed for texture coding considering the mechanical characteristics of the vibrissae and their location in the whisker pad. The whiskers resonate at different vibration frequencies because of their different length [4][5][6].

Since the rats must make fast discriminations while they sweep the objects that surround them, the mechanoreceptors activation must produce a sufficiently robust and reliable code to capture complex and high frequency information. Jones et al. [7] demonstrated that the unique presentation of a complex stimulus produces patterns of specific and invariable firings in the trigeminal neurons.

Recent studies, using multifiber recordings in the vibrissal nerve, suggest that the afferent discharge at peripheral level would present codes related to the physical characteristics of the surfaces, such as roughness and texture [8].

In this work we analyzed the afferent discharges of two vibrissal nerves, one innervating the DELTA vibrissa and the other one innervating the GAMMA vibrissa.
The signals were recorded while the vibrissae were sweeping different rough surfaces (sandpaper #1000, #600, #380, #220 and #180). Control recordings were inserted between the surface recordings. The vibrissa movements were obtained using electrical stimulation of the facial motor branches.

In this work we used an algorithm - based on the wavelet decomposition of the signal - to find a temporal code that could be related to the roughness of the surfaces. The algorithm was previously purposed in other work [9] and evaluated by its authors using nervous signals simulated by computer. The algorithm was able to detect events of different durations. The inter-event times detected were calculated for each situation. The afferent discharge of the DELTA vibrissa was analyzed for two contact pressures. The same protocol was used for the GAMMA vibrissa analysis.

The statistical analysis of the inter-event times reveals significant differences between the situations purposed for both DELTA and GAMMA vibrissa.

It was observed that an increase in the contact pressure improves the discrimination between rough surfaces.

This work shows evidence about the existence of a temporal code that would be related to the discrimination of rough surfaces. The inter-event times detected would be a good parameter to characterize this code. It was also observed that when the contact pressure between the vibrissa and the surface was increasing, the performance of the discrimination improved.

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 as well as the two branches of the facial nerve (buccal and upper marginal mandibular) on the right side. 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. The deep vibrissal nerve innervating two vibrissal follicles (DELTA and GAMMA) were identified with the high magnification of a dissecting microscope. The dissected nerves were transected and a bipolar electrode was placed on each 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.

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. Recording of the vibrissa electrical activity

The afferent activity from dissected nerves was registered while the vibrissae were sweeping surfaces of different roughness.

Vibrissae movements were induced by electrical stimulation of motor branches of the facial nerve. Square-wave pulses (30 μs, 7V supramaximal, 5 Hz) simulated the vibrissal whisking at its natural frequency. Each whisking was recorded in a window (sweep) of 100 ms. The infraorbital nerve signals were digitalized using a data acquisition system, Digidata 1322A, Axon Instruments®, at 20 KHz.

Fifty windows were obtained for each surface, and another fifty for each control (vibrissa sweeping the air). The controls were inserted between the surface recordings.

Two different pressure levels were used. To obtain pressure 1, the tip of the whisker shaft was lightly placed on the surface (the platform surface was in a transverse position with respect to the whisker base). The pressure 2 level was obtained by moving the surface platform 1 mm closer (figure 1).
2.3. Rough surfaces

The swept surfaces used in this paper were sandpaper of different grain: Sandpaper #1000, #600, #360, #220 and #180. We measured the surfaces roughness using a Hommel Tester T1000 (Hommel Werke) and used the Ra parameter (arithmetical deviation of the assessed profile) as a roughness estimation (International Standards BS.1134 and ISO 468). Ra values obtained were: 2.97, 3.72, 4.14, 9.33 and 9.34 μm, respectively.

2.4. Digital processing and statistics

AE recordings were analyzed using an event detection algorithm based on multiscale decomposition of the signal (Continuous Wavelet Transform - CWT). The algorithm was proposed in [9]. The methodology consists of a combination of several techniques stemming from multiresolution wavelet decomposition, statistics, detection theory and estimation theory. The description of the event detection algorithm used in this paper was detailed in [7].

The events detected from afferent activity recordings were of 0.6 to 2 msec. Events detected during a sweeping are shown in figure 2. The method for obtaining the inter-event times is shown in figure 2G. In this last case, inter-event times were calculated within the contact region between the vibrissa and sandpaper (gray region, figure 2).

Statistical analysis was done with one-way repeated measure ANOVA on ranks (Friedman) and Dunn's method as a post hoc test (software SigmaStat®). Inter-event times were compared for recordings with the same level of pressure.

Data processing and event detection based on multiscale decomposition of the signal were performed with MATLAB®.
3. Results

The afferent activity from vibrissa nerves is shown in figures 3 and 4. Since our recordings are simultaneous with the vibrissae muscular activation, the stimulus artifact appears as the first signal followed by the deflection due to the muscle action current. Both deflections were removed before the data were processed and the start of the afferent discharge was estimated at 5 ms from the beginning of the recording.

Amplitude and shape differences of the afferent discharges from DELTA and GAMMA vibrissal nerves are observed. This is due to anatomical characteristics of the nerves.

Inter-event times detected were calculated from vibrissal nerves for each situation. Boxplot diagram showing the distribution of the inter-event times detected in DELTA vibrissal nerve with pressure level 2 are presented in figure 3. The detection algorithm identified events of 0.8, 1.2, 1.6 and 2.0 ms.

Figure 6 shows the boxplot diagrams of inter-event times detected from GAMMA vibrissal nerve with pressure level 2. Boxplot diagrams of the inter-event times belonging to pressure levels 1 were not shown. However, these were statistically analyzed.

Table I presents a summary of statistical results of the multiple comparisons applied to inter-event times detected from DELTA and GAMMA vibrissal nerves with pressure levels 1 and 2.
Figure 4. Afferent discharges from DELTA vibrissa nerve. All the records show a single vibrissa sweeping each surface at pressure level 1. (A) sweeping the air (control), (B) sweeping sandpaper #1000, (C) sweeping sandpaper #600, (D) sweeping sandpaper #360, (E) sweeping sandpaper, #220 and (F) sweeping sandpaper #180.

Figure 4. Afferent discharges from GAMMA vibrissa nerve. All the records show a single vibrissa sweeping each surface at pressure level 1. (A) Sweeping the air (control), (B) sweeping sandpaper #1000, (C) sweeping sandpaper #600, (D) sweeping sandpaper #360, (E) sweeping sandpaper, #220 and (F) sweeping sandpaper #180.
4. Discussions

In previous reports about neural coding in texture discrimination, it was noted that the afferent activity increased when increasing roughness. This phenomenon suggested that the RMS values, calculated from afferent nerve activity could be related to the physical properties of surfaces, specifically, to surface roughness [10].
The inter-event times detected at DELTA vibrissal nerve, for pressure level 1, showed significant differences among: Sa-2 vs Sa-3, and Sa-3 vs Sa-4 (analyzing events of 1.6 ms duration). Other instances were possible to be differentiated when increasing the contact pressure level. These were: Sa-1 vs Sa-2, Sa-1 vs Sa-3, Sa-1 vs Sa-4, Sa-1 vs Sa-5, Sa-3 vs Sa-4 and Sa-5 vs Sa-4 (table I). Similar results were found when evaluating the inter-event times of events of 2.0 ms duration.

The inter-event times obtained from GAMMA vibrissal nerve did not show any significant difference among sweep on different sandpapers (pressure level 1). However, when the contact pressure increased, it was possible to differentiate the following situations: Sa-1 vs Sa-5; Sa-2 vs Sa-5, Sa-3 vs Sa-4 y Sa-3 vs Sa-5 (analyzing events of 1.6 ms duration). Similar results were obtained after analyzing events of 2.0 ms duration.

Inter-event times dispersion decreased with increased contact pressure at all cases. This enabled for performing the discrimination among different sweeping situations. This effect was observed both in afferent discharge of DELTA vibrissal nerve and GAMMA vibrissal nerve.

5. Conclusions
In this work, it was possible to establish the existence of temporal code at the peripheral level in vibrissal systems. This temporal code allowed the discrimination of DELTA and GAMMA vibrissae whisking during different situations. An event detection algorithm (events of 0.6 to 2.0 ms durations)

### Table 1. Results of multiple comparisons applied to inter-event times detected from each sweep situation (Dunn method). The test computes statistic Q, the number of rank sums, and shows whether P < 0.05 or not, for the pair being compared. P is the probability that the null hypothesis may be rejected and, thus, it helps conclude that there are differences between treatments. Diff of ranks is the difference in the rank sum orders being compared. The rank sums are a measure of the difference between two treatments. Sa-1: #1000, Sa-2: #600, Sa-3: #360, Sa-4: #220 y Sa-5: #180.

| Event Dur. | DELTA Vibrissa | GAMMA Vibrissa |
|------------|----------------|----------------|
|            | Pressure 1     | Pressure 2     | Pressure 1     | Pressure 2     |
|            | Diff of Ranks  | Q   | P<0.05 | Diff of Ranks  | Q   | P<0.05 | Diff of Ranks  | Q   | P<0.05 |
| 1.6 msec   |                |     |        |                |     |        |                |     |        |
| Sa-1 vs Sa-2 | 9.291          | 0.939 | No    | 55.772         | 3.173 | Yes    | 13.405         | 0.87 | No    |
| Sa-1 vs Sa-3 | 17.094         | 1.438 | No    | 51.858         | 3.706 | Yes    | 1.872          | 0.117 | No   |
| Sa-1 vs Sa-4 | 18.936         | 1.718 | No    | 62.307         | 4.085 | Yes    | 22.049         | 1.31  | No   |
| Sa-1 vs Sa-5 | 2.885          | 0.275 | No    | 59.35          | 4.539 | Yes    | 27.354         | 1.434 | No   |
| Sa-2 vs Sa-3 | 77.803         | 3.753 | Yes   | 13.915         | 1.535 | No    | 11.533         | 0.779 | No   |
| Sa-2 vs Sa-4 | 9.645          | 1.031 | No    | 66.465         | 4.393 | Yes    | 35.455         | 2.267 | No   |
| Sa-2 vs Sa-5 | 6.405          | 0.735 | No    | 3.578          | 0.241 | No    | 40.759         | 2.26  | No   |
| Sa-3 vs Sa-4 | 71.842         | 4.161 | Yes   | 12.551         | 1.269 | No    | 23.922         | 1.47  | No   |
| Sa-3 vs Sa-5 | 14.208         | 1.301 | No    | 17.492         | 1.091 | No    | 29.226         | 1.572 | No   |
| Sa-4 vs Sa-5 | 16.05          | 1.609 | No    | 60.043         | 4.063 | Yes    | 5.305          | 0.275 | No   |
| 2.0 msec   |                |     |        |                |     |        |                |     |        |
| Sa-1 vs Sa-2 | 66.323         | 3.696 | Yes   | 71.457         | 4.669 | Yes    | 1.674          | 0.126 | No   |
| Sa-1 vs Sa-3 | 51.189         | 3.298 | Yes   | 1.55           | 0.126 | No    | 5.129          | 0.335 | No   |
| Sa-1 vs Sa-4 | 2.167          | 0.225 | No    | 4.733          | 0.402 | No    | 8.764          | 0.635 | No   |
| Sa-1 vs Sa-5 | 0.552          | 0.060 | No    | 7.15           | 0.478 | No    | 16.183         | 0.954 | No   |
| Sa-2 vs Sa-3 | 4.866          | 0.68  | No    | 79.907         | 4.315 | Yes    | 6.083          | 0.468 | No   |
| Sa-2 vs Sa-4 | 8.49           | 1.02  | No    | 66.19          | 3.969 | Yes    | 7.09           | 0.548 | No   |
| Sa-2 vs Sa-5 | 6.875          | 0.892 | No    | 58.607         | 4.375 | Yes    | 14.509         | 0.891 | No   |
| Sa-3 vs Sa-4 | 13.356         | 1.708 | No    | 6.283          | 0.913 | No    | 13.893         | 0.927 | No   |
| Sa-3 vs Sa-5 | 11.741         | 1.64  | No    | 8.7            | 0.757 | No    | 21.311         | 1.187 | No   |
| Sa-4 vs Sa-5 | 1.615          | 0.194 | No    | 2.417          | 0.222 | No    | 7.419          | 0.445 | No   |

The inter-event times detected at DELTA vibrissal nerve, for pressure level 1, showed significant differences among: Sa-2 vs Sa-3, and Sa-3 vs Sa-4 (analyzing events of 1.6 ms duration). Other instances were possible to be differentiated when increasing the contact pressure level. These were: Sa-1 vs Sa-2, Sa-1 vs Sa-3, Sa-1 vs Sa-4, Sa-1 vs Sa-5, Sa-3 vs Sa-4 and Sa-5 vs Sa-4 (table I). Similar results were found when evaluating the inter-event times of events of 2.0 ms duration.

The inter-event times obtained from GAMMA vibrissal nerve did not show any significant difference among sweep on different sandpapers (pressure level 1). However, when the contact pressure increased, it was possible to differentiate the following situations: Sa-1 vs Sa-5; Sa-2 vs Sa-5, Sa-3 vs Sa-4 y Sa-3 vs Sa-5 (analyzing events of 1.6 ms duration). Similar results were obtained after analyzing events of 2.0 ms duration.

Inter-event times dispersion decreased with increased contact pressure at all cases. This enabled for performing the discrimination among different sweeping situations. This effect was observed both in afferent discharge of DELTA vibrissal nerve and GAMMA vibrissal nerve.
was used for afferent discharges analysis. By means of analysing inter-event times distributions, it was possible to establish differences among vibrissae whisking situations (sweeping on different sandpapers). The discrimination among different situations was improved when contact the pressure level was increased.

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7. References
[1] Vincent S. B. (1912) The function of the vibrissae in the behavior of the white rat. Behav Monog 1: 1-181.
[2] Carvell, G.E., D.J. Simons, S.H. Lichtenstein, and P. Bryant (1991) Electromyographic activity of mystacial pad musculature during whisking behavior in the rat. Somatosens Motor Res 8: 159-164.
[3] Guic-Robles E., Valdivieso C., Guajardo G. (1989). Rats can learn a roughness discrimination using only their vibrissal system. Behav Brain Res 31: 285-289
[4] Mehta SB, Kleinfeld D (2004) Frisking the whiskers: Patterned sensory input in the rat vibrissae system. Neuron, 41: 181-184.
[5] Hartmann MJ, Johnson NJ, Towal RB, Assad C: Mechanical Characteristics of Rat Vibrissae: Resonant Frequencies and Damping in Isolated Whiskers and in the Awake Behaving Animal. J Neurosci 2003, 23(16):6510-6519.
[6] Mitchinson B, Gurney KN, Redgrave P, Melhuish C, Pipe AG, Pearson M, Gilhespy I, Prescott TJ: Empirically inspired simulated electromechanical model of the rat mystacial follicle-sinus complex. Proc R Soc Lond 2004, 271:2509-2516.
[7] Jones LM, Lee S, Trageser JC, Simons DJ, Séller A, 2004, Precise Temporal Responses in Whisker Trigeminal Neurons, J. Neurophysiol, 92: 665-668, 2004.
[8] Albarracin AL, Farfán FD, Felice CJ, Décima EE, 2006, Texture Discrimination and Multi-Unit Recording in the Rat Vibrissal Nerve, BMC Neuroscience 7:42.
[9] Nenadic Z and Burdick JW, 2005, Spike detection the continuous wavelet transform. IEEE Trans. Biomed. Eng. 52(1): 74-87.
[10] Farfán FD, Albarracin AL, Felice CJ, Décima EE, 2003, Analysis of the Rat Nerve Activity with an Autoregressive Parametric Model, 25th Annual International Conference of the IEEE Engineering in Medicine and Biology Society 2003:2063-2066.