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An Automated Method to Determine Angular Preferentiality using LFPs Recorded from Rat Barrel Cortex by Brain-Chip Interface under Mechanical Whisker Stimulation

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Abstract— **The sensory information processing in the rodents is mainly done by whisking, through which they explore the environment, perform object localization, texture and shape discrimination very precisely. During whisking, microcircuits in the corresponding barrel columns get activated to segregate and integrate the tactile information through the information processing pathway. To primarily understand the whisking mechanism angular preferentiality determination is very important. In this work we propose an automated method to determine different events present in the local field potentials (LFPs), calculate latencies and amplitudes related to those events and use them along with the stimulation angle information to determine the angular preferentiality. The method is extensively tested on LFPs recorded from S1 barrel cortex of anesthetized rats using EOSFET (Electrolyte-Oxide-Semiconductor Field Effect Transistor) based neuronal probes.**

Keywords—Barrel cortex; angular tuning; whisker stimulation; local field potentials; neuronal activity; EOSFET.

I. Introduction

RECENT developments in the neuronal probe technology allowed the neuroscientists to perform many complex experiments in deciphering cognition and perception which otherwise have been impossible even a decade ago. However, in sensory systems neuroscience and perception, the challenge to quantify brain activity and to explain this activity as the outcome of elementary neuronal response is still open. Through "whisking" rodents make precise discriminations of the environment, like object localization, based on shapes and textures of the objects [1]. In the rodent's S1 cortex there is a precise topological map of the mystacial pad, in which for each whisker there is a so called "barrel" that receives the tactile information [2]. Studies showed that intra- and transcolumnar microcircuits in a barrel segregate and integrate information during whisking [3], and have specific understanding of 'what' 'where' and 'when' aspects of the tactile information acquired by the whiskers. One of the salient features of primary vibrissal afferents is their sensitivity to the direction in which the vibrissae move. Directional sensitivity is also well conserved in brainstem, thalamic, and cortical neurons, indicating that this property plays a key role in the organization of the whisker system [4].

With the growth of the neuronal probe technology, scientists can use multisite recording devices now to record neuronal signals from the brain. These multisite neuronal probes generate a huge amount of data to be processed and analyzed. Scientists perform this kind of analysis manually spending lot of time. In this work, we present an automatic, simple to implement and computationally efficient method capable of detecting various events that characterize the LFPs recorded from different layers of the barrel cortex upon mechanical whisker stimulation. We then calculate latencies and amplitudes of these events and use them to determine the directional selectivity of stimulation. This program is a part of the SigMate software package which will be made available under the open source GNU-GPL [5].

II. Signal Acquisition

A. Animal Preparation

P30-P40 Wistar rats rats were anesthetized with an induction mixture of Tiletamine and Xylazine (2 mg and 1.4 mg/100 g weight, respectively). The rat's eye, hind-limbs' reflexes, respiration, and whiskers' spontaneous movements were monitored throughout the experiment to check the level of anesthesia and whenever necessary additional doses of Tiletamine (0.5 mg / 100 g weight) and Xylazine (0.5 g / 100 g weight) were provided.

Rats were positioned on a stereotaxic apparatus and fixed by teeth- and ear-bars. The body temperature was constantly monitored with a rectal probe and maintained at about 37°C using a homeotermic heating pad. Heart beat was monitored by standard ECG. Anterior-posterior opening in the skin was made in the center of the head, starting from the imaginary eye-line and ending at the neck. The connective tissue between skin and skull was removed by a bone scraper. The skull was drilled to open a window in the S1 (AP -1 ÷ -4, LM +4 ÷ +8) right cortex. In order to reduce brain edema, only a slit at coordinates AP -2.5, LM +6 was made [6].

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Throughout all surgical operations and recordings, the brain was bathed through a perfusion system by a standard Krebs solution (composition in mM: NaCl 120, KCl 1.99, $NaHCO_3$ 25.56, KH_2PO_4 136.09, $CaCl_2$ 2, $MgSO_4$ 1.2, glucose 11), constantly oxygenated and warmed to 37°C.

At the end of the surgery the contralateral whiskers were trimmed at about 10 mm from the rat snout.

B. *Microchip prototype and setup*

Figure 1(a) shows a chip prototype featuring a line of 4 round EOSFETs of 10 µm diameter, spaced 80 µm [7].

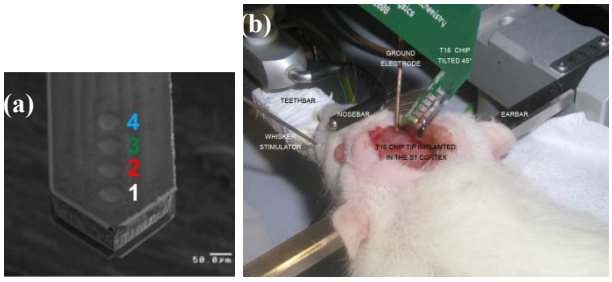

Fig. 1. (a). Scanning electron microscope image of the chip prototype with the 4 FETs numbered in different colors. Scale bar: 50 µm. (b). An experiment with the various components of the recording setup.

As seen in figure 1(b), the chip was correctly fixed to a micromanipulator so that it was inserted perpendicularly to S1 cortex and a silver electrode was used for grounding.

C. *Stimulation and signal recording*

Neuronal signals were evoked by stimulating single whiskers mechanically with a piezoelectric bender through a connected tube. The bender was driven by a waveform generator (Agilent 33250A 80 MHz, Agilent Tech.) providing square stimuli at 0.5 Hz. Each whisker, starting from the posterior group, was individually inserted into the metal tube and the corresponding response was checked in S1 cortex IV layer (at 640 µm depth). The most responsive whisker, i.e., the "principal whisker" was then chosen for the recording section, and signals were recorded by moving the chip up and down to have a complete depth profile of the cortex. At each recording depth LFPs were recorded by stimulating the whisker at different angles ranging from 0° to 315° at a step of 45°.

The chip was connected to the computer by custom-built amplifiers and the neuronal signals were recorded by custom-made signal acquisition software developed in LabView (http://www.ni.com/labview/).

D. *The Signals*

The LFPs recorded from a barrel column of the rat S1 cortex by stimulating the corresponding whisker can be differentiated by their specific characteristics based on the depth or layer they are recorded from. Figure 2 shows a depth profile in one of our experiments.

As illustrated in [8] and [9], usually in upper cortical layers (I, II) the signals are expected to have a small positive peak (E1), followed by a main negative peak (E2), a slow positive peak (E3) and a slow negative valley (E4) that gradually tends to reach the baseline at the end. In middle layers (III, IV, and V) the signals are expected to have the E2 (without E1) followed by the E3 and E4 tending to reach zero at the end. In deeper brain cortex (layer VI), the E2 becomes smaller and usually gets divided into two smaller negative peaks (E1 and E2), followed by the E3 and then the E4. These characteristics of the signals can be exploited in automated detection of the layers from the recorded signals.

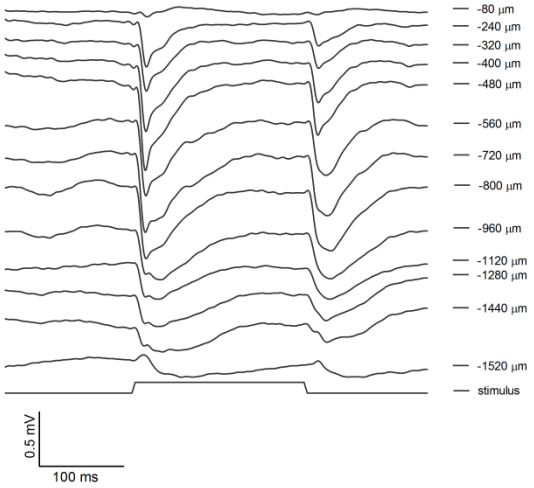

Fig. 2. LFP depth profile recorded from a barrel column by stimulating the corresponding whisker at 0° where the different features of the signals can be easily seen. Scale bar doesn't apply for the stimulus.

III. METHOD

This method for automatic angular preferentiality calculation in LFPs recorded from the rat barrel cortex is implemented using the MATLAB (www.mathworks.com) scripting with an easy to use Graphical User Interface (GUI). The Figure 3 shows the GUIs that encapsulate the code from the users. The GUIs provide an easy way for the non-programming background people to use the method in analyzing their data obtained from experiments.

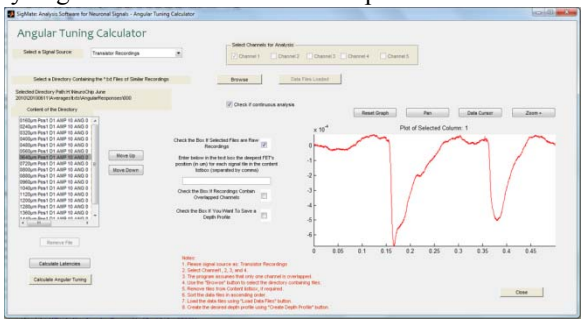

Fig. 3. GUI of the method.

A. *Preprocessing*

The method can accept two types of input files: (1.) a single file containing the complete cortical depth profile, and (2.) a depth profile consisting of individual signal files from each recording depths. Due to the fact that to date very few multisite neuronal probes are capable of recording signals from all cortical layers. Therefore, we have included a small and powerful utility in the method for calculating the depth profile from a set recording.

B. *Angular preferentiality determination*

The program requires the user to select sets of LFPs recorded with different angular stimuli one by one starting from 0°. For each depth profile induced by an angular stimulus, a function (algorithm in next section) is called. It can detect the events present in LFPs, determine various events' amplitude, and calculate their latencies. These information are saved in the user data area until LFPs for all angles are analyzed. Then, latencies and amplitudes are layerwise grouped basing on depth specific characteristics and a priori position information of the LFPs. The angular preferentiality is then calculated using latencies and amplitudes of E2. For latencies, it is the ascending ordered list of the minimum latencies of all angles and layers. For amplitudes, it is the ascending ordered list of the maximum amplitudes of all angles and layers.

C. *Latency and amplitude determination in LFPs*

This function detects various signal events (see Sec. II, *D*) present in the LFPs by calculating signal derivatives [10]. Major changes in derivatives are used to detect events. Once the events (E1-E4) are detected, latencies and amplitudes related to them are calculated. The latencies are calculated as the differences between time instances of the events and the stimulus-onset and the amplitudes are the recorded voltages at the time instances of the events. These information are saved for later usage in determining directional selectivity of the stimulation. The algorithm listed below is used in detecting the events, and calculating the latencies and amplitudes.

Function: *detectEvents( )*
Input: *Signal files for detecting the events*
Output: *Latencies and amplitudes of the detected events*
Method:
1. Lowpass filter the signal at 250 Hz;
2. Determine the stimulus-onset and end time;
3. Translate the signal by setting stimulus-onset to 0;
4. Detect the response-onset (RO);
5. Translate the signal by setting response-onset to 0;
6. Calculate signal derivative of the first 15 ms from RO;
7. Check the signal direction;
8. **if** *the signal is going upwards*
  tempEvent:=highest positive peak;
  if *E1 amplitude > 10 μV then*
    E1:=tempEvent;
  else *E1:=Absent;*
 elseif *the signal is going downwards*
  tempEvent:=highest negative peak;
  if *there is another negative peak in next ±5 ms then*
    secondTempEvent:=detect second negative peak;
    E1:= first occurring event;
  else *E1:=Absent;*
9. E2:=highest negative peak;
10. E3:=highest positive peak in next 100 ms from E2;
11. E4:=highest negative peak in next 200 ms from E3;
12. Calculate latencies and amplitudes of E1-E4;
13. Return latencies and amplitudes;

IV. Results and Discussion

The method was applied on 20 datasets and found to be working well except a few situations (5%) where an error of ± 300 μs was noticed in latency calculation. The error was seen in signals with slow stimulus artifacts (with frequency < 250 Hz). As the latencies are in terms of few tens of milliseconds, this error was considered negligible.

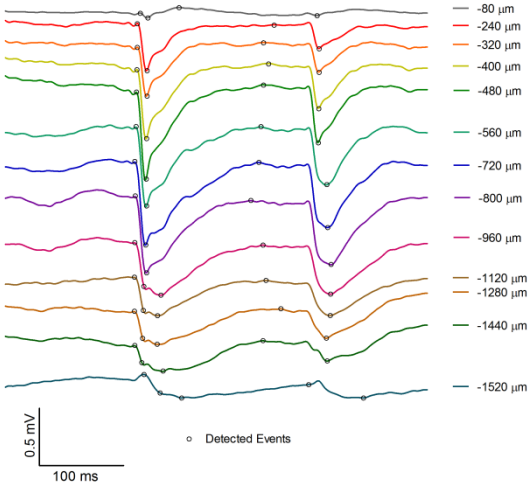

Fig. 4. Depth profile with detected events of representative LFPs from each layer by applying the above discussed methods for signals recorded from different layers of the rat barrel cortex with angular stimulus of 0°.

Figure 4 shows a depth profile with detected events by applying the above mentioned algorithm from a representative experiment. As one of the outputs, the program generates angular tuning curves for latencies and amplitudes of E2 as seen in figure 5 and 6. This type of curves is common in studying directional selectivity [11].

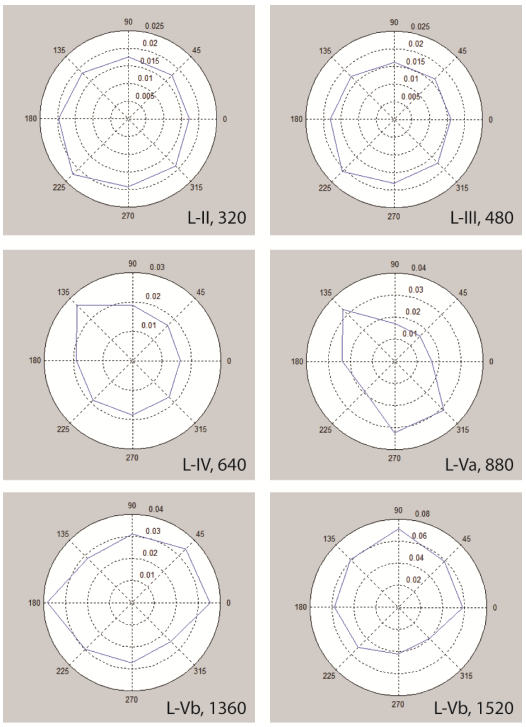

Fig. 5. Angular tuning curves for latencies of E2. The layer and recording depths are indicated at the right-bottom corner.

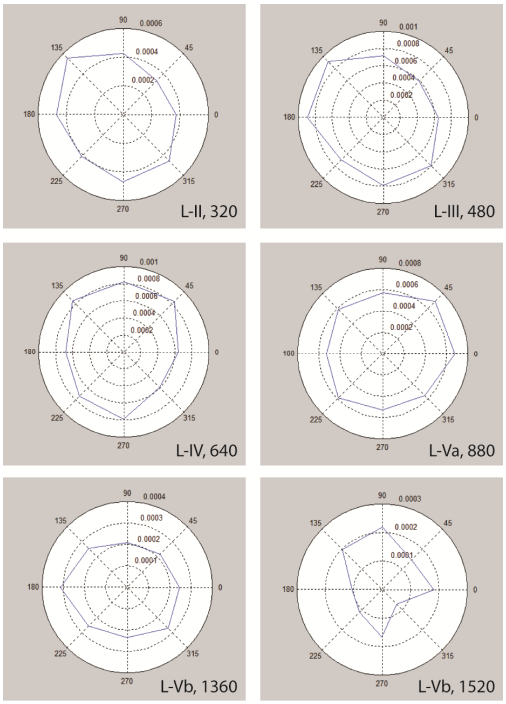

Fig. 6. Angular tuning curves for amplitudes of E2. The layer and recording depths are indicated at the right-bottom corner.

As a measure of accuracy in calculating latencies, we compared them with manually calculated latencies and found them to be similar as seen in figure 7.

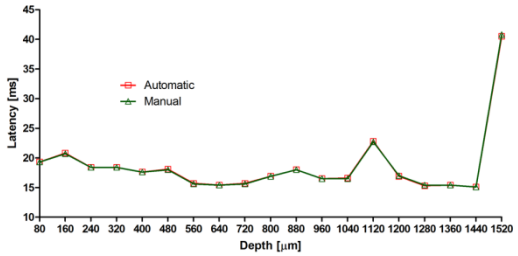

Fig. 7. Comparison of automated and manual latency calculation.

The program also generates graphs showing latencies, amplitudes at different depths and angles (figure 8) and directional selectivity (figure 9). The program provides directional selectivity using both latencies and amplitudes, and user will use the appropriate one for his/her analysis.

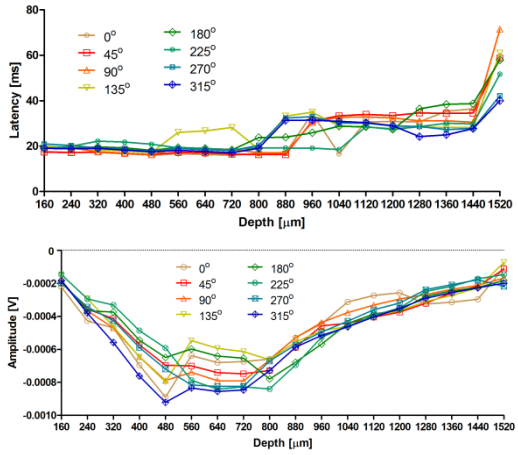

Fig. 8. Latencies (top) and amplitudes (bottom) calculated by the program at different depths of the cortex upon varied angle of stimuli.

Based on the evidences presented above, we can assert that the method presented above can accurately detect events, calculate latencies and amplitudes for the LFPs recorded using EOSFET based neuronal probes and provides directional selectivity based on them.

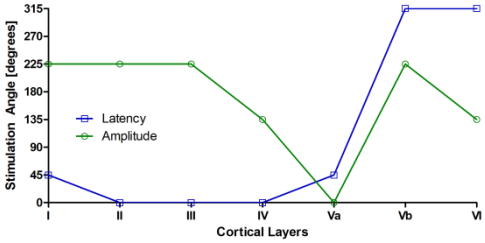

Fig. 9. Angular preferentiality determined using latencies and amplitudes of E2. It should be noted that using signals' the latency and the amplitude two different angular preferentiality profiles are obtained. This is due to the fact that the signals' amplitudes represent the cumulative current flow in a neuronal population, whereas the signals' latencies denote the time delay in the signal propagation among different cortical layers.

V. Conclusion

Whisking in the rodents is one of the most important ways in exploring the environments. To understand the whisking mechanism, its role in localize objects and discriminate among them based on shape and texture are under extensive study. To perform this kind of studies determining angular preferentiality is very important. Scientists perform this task manually which is time consuming and boring. As evidenced above, this work is an automated solution in performing this work. As evidenced, it can perform the calculation accurately. This method is a part of the SigMate software package and will be soon provided to the community [5].

Acknowledgment

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