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Sensory and cognitive neurophysiology in rats. Part 2: Validation and demonstration

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HIGHLIGHTS

- Multi-unit activity (MUA) can be measured using micro-ECoG.
- Pre-stimulus oscillatory phase modulates tactile evoked responses in the MUA.
- Sensory expectation modulates tactile evoked responses in the MUA.

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ABSTRACT

Background: We have developed a novel setup for rats that allows for controlled sensory input to an animal engaged in a task while recording both electrophysiological signals and behavioral output.
New method: Our setup is described in a companion paper.
Results: We validate our setup by replicating (1) the functionally nonspecific spread of neural activity following tactile stimulation, and (2) the effects of anesthesia on the tactile evoked responses.

We also demonstrate for the first time that the ECoG can be used to record evoked responses in a signal that reflects neural output (spiking activity), and illustrate the usefulness of our setup by demonstrating that these evoked responses are modulated by both the phase of pre-stimulus oscillations and by expectation.
Comparison with existing methods: Compared with high-density wire recordings, micro-ECoG offers a much more stable signal without readjustments, and a much better scalability. Compared with extracranial and regular ECoG recordings, micro-ECoG allows us to measure signals that reflect both neural input and neural output.
Conclusions: For sensory and cognitive research, our setup provides a unique combination of possibilities that cannot be achieved in other setups for rodents.

1. Introduction

This paper shows results that were obtained using a novel setup allowing for chronic electrophysiological recordings from a large part of the rat's brain surface, while at the same time providing controlled sensory input to the rat's snout. We both validate this novel setup by replicating some findings from sensory neurophysiology, and demonstrate its usefulness by novel sensory and cognitive neurophysiology results. The setup itself is described in a companion paper (*Sensory and Cognitive neurophysiology in rats, Part 1: combining controlled tactile stimulation and high-density micro-ECoG recordings in a freely moving animal*).

The importance of a number of our results depends on the ability of our electrophysiological recording setup to distinguish between neural input (postsynaptic potentials) and neural output (action potentials). Currently, electrophysiological measurements of neural output require wire or silicon probes in the neuropil, whereas neural input can also be measured using electro-encephalography (EEG) or electrocorticography (ECoG) outside of the neuropil, provided this input is sufficiently synchronized. In this paper, we show that a signal related to neural output (multi-unit activity; MUA) can also be measured using micro-ECoG electrodes (100 μm diameter).

We will present results that depend on the precise spatiotemporal control of sensory stimulation obtained both under anesthesia and during wakefulness. The results obtained during wakefulness

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crucially depended on the head-mounted stimulation device that was described in the companion paper.

In sum, we show results that depend on the three novel aspects of our setup: obtaining chronic recordings from a large part of the rat's brain surface, which contain signals that relate to neural input as well as neural output, and with the possibility to provide controlled sensory input to a freely moving animal. We also validate our setup by replicating some findings from sensory neurophysiology, and demonstrate its usefulness by showing novel sensory and cognitive neurophysiology results.

2. Materials and methods

With respect to the following topics, we refer to the companion paper: animals, surgery, anatomical co-registration, 3D brain atlas, sound-proofed experiments under anesthesia, and head-mounted tactile stimulator (HMS).

2.1. Animal experiments

2.1.1. Recordings under anesthesia

Some experiments were performed under anesthesia. The anesthetic agent was a mixture of ketamine and dexamethazone (resp., 0.75 and 0.2 ml/kg, injected IP).

2.1.2. Random stimulation experiments using the HMS

Using the HMS we stimulated the rat's snout at three locations (upper lip rostral and caudal, D5 follicle) and used one motor (the one to which no pin was connected) to produce an auditory stimulus. These four stimuli were presented in a fixed sequence with a random inter-trial interval and governed by the same parameters as in the sound-proofed experiments. In a single recording session, a total of 800 trials was collected, 200 per stimulus. We collected three datasets under anesthesia and three during wakefulness.

2.1.3. Experiment comparing the effects of periodic vs. non-periodic stimulation

This experiment was conducted both under anesthesia and during wakefulness. In both conditions, we stimulated in either a periodic or non-periodic fashion, resulting in a 2-by-2 experimental design. In the periodic condition, the stimuli repeated themselves with a constant inter-trial interval of 2.6 s, aimed at inducing a rhythmic modulation of expectation for the upcoming stimulus. In the non-periodic condition, the stimuli would be presented with random inter-trial intervals generated from an exponential distribution (see Sound-proofed Experiments under Anesthesia).

In every recording session, we collected 200 trials in which the rostral part of the rat's upper lip was stimulated. Both during wakefulness and under anesthesia, there were six sessions, three with periodic and three with non-periodic stimulation. The six sessions during wakefulness were conducted on three different days, with on each day one session consisting of periodic and one consisting of non-periodic stimulation only. The six sessions under anesthesia were conducted on a single day.

2.2. Electrophysiology and preprocessing

Electrophysiological data were recorded using a Digital Lynx system (Neuralynx Inc.) at a sampling rate of 8139 Hz. As a reference, we used an electrode over motor cortex (hind limb area).

Trials were cut from −0.25 to 0.55 s relative to the onset of the movement of the pin. Because the pin took 5 ms to move from its initial (just touching the fur) to its final position (maximal skin indentation), there is a maximum uncertainty of 5 ms with respect to the exact time at which the pin touched the skin. Field potentials (FPs) were obtained by low-pass filtering the raw signals at 150 Hz and down-sampling them at 1000 Hz. Multi-unit activity *envelope* (MUA*e*) signals were obtained from the raw signals in the following four steps: (1) average re-referencing, (2) high-pass filtering (8th order Butterworth filter, two-pass), (3) rectification, (4) low-pass filtering at 150 Hz, and (5) downsampling at 1000 Hz. All analyses were performed using a combination of Fieldtrip (Oostenveld et al., 2010) and custom Matlab functions.

In one of the sessions involving the HMS, we observed an artifact in the MUAe data in the first 20 ms, the period during which the motor was moving. Because of the periodic nature of the artifact (exactly two peaks separated by 10 ms), we could remove them by means of a filter based on the discrete Fourier transform (DFT). In effect, we removed the 100 and the 200 Hz Fourier components and transformed the data back to the time domain using the inverse DFT.

2.3. Data analysis

2.3.1. Determining the beginning of a stimulus evoked response

We determined the beginning of a stimulus evoked response on the basis of *t*-statistics comparing the post-stimulus activity at every time point with the time-averaged activity in the pre-stimulus interval between −100 and 0 ms. As a statistical threshold, we used the critical *t*-value corresponding to a two-sided *t*-test at $\alpha = 0.01$. The first time at which one channel exceeded this critical *t*-value was taken as the beginning of the stimulus evoked response.

2.3.2. Quantifying the relation between prestimulus phase and stimulus evoked amplitude

Several indices could be used to quantify the relation over trials between the pre-stimulus FP phase and the post-stimulus (stimulus evoked) MUAe amplitude. Here, we present a measure that is based on complex linear regression of the baseline-corrected MUAe signal (the dependent variable) onto the Fourier coefficients calculated over the pre-stimulus interval (the independent variable). We use this measure for convenience, and not because we believe it to be superior to other indices; it is a straightforward quantification of the strength of the relation in which we are interested. The regression equation an which the measure is based can be written as follows:

$$y = b \times x + b' \times x' + e,$$

in which y is the stimulus evoked MUAe signal, x is the Fourier coefficient (complex-valued) of the signal in the pres-stimulus interval, b is a complex-valued regression coefficient, e is the error term, and the prime ($'$) denotes the complex conjugate. Crucially, the Fourier coefficient captures the phase of the signal in the pre-stimulus interval. Note that the right-hand side of this equation produces a real-valued number. Our quantification now is the correlation between y and the linear combination ($b \times x + b' \times x'$), calculated using the least-squares estimate of b: the better you can predict the stimulus evoked MUAe from the from the Fourier coefficients over the pre-stimulus interval, the larger the higher this correlation.

The dependent variable y was the baseline-corrected MUAe signal, averaged over the interval [10, 20] ms (the interval in which the MUAe shows the strongest stimulus-induced increase). As a baseline, we used the pre-stimulus interval [−25, 0] ms. The independent variable x was the Fourier coefficient calculated over a pre-stimulus interval of length equal to four cycles at a given frequency.