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Research article

Optogenetic noise-photostimulation on the brain increases somatosensory spike firing responses

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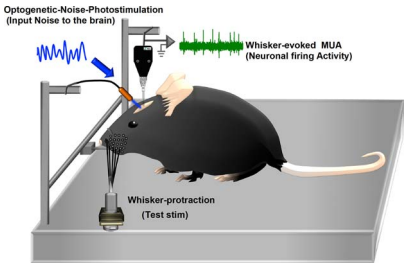
GRAPHICAL ABSTRACT



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ABSTRACT

We examined whether the optogenetic noise-photostimulation (ONP) of the barrel cortex (BC) of anesthetized Thy1-ChR2-YFP transgenic mice increases the neuronal multiunit-activity response evoked by whisker mechanical stimulation (whisker-evoked MUA). In all transgenic mice, we found that the signal-to-noise ratio (SNR) of such whisker-evoked MUA signals exhibited an inverted U-like shape as a function of the ONP level. Numerical simulations of a ChR2-expressing neuron model qualitatively support our experimental data. These results show that the application of an intermediate intensity of ONP in the brain can increase cortical somatosensory spike responses to whisker protraction. These findings suggest that ONP of the mice-BC could produce improvements in somatosensory perception to whisker stimulation.

1. Introduction

In the literature, there are numerous studies about the effects of noise of different nature on the electrophysiological and behavioral responses of the brain [1–3]. However, these studies had the difficulty of directly and selectively adjusting the noise level of neurons with continuous noise. In this context, the purpose of the present study was to examine possible changes in firing activity of neurons responsive to somatosensory stimulation during the application of optogenetic-noise-photostimulation (ONP). This type of stimulation selectively adjusts the noise level of neurons expressing the light-gated channel, channelrhodopsin-2 (ChR2), in transgenic mice Thy1-ChR2-YFP. The ONP employs an optic fiber that allows to directly control the level of noise in particular regions of the cerebral cortex and to examine causal aspects of

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the effects of noise on the *in vivo* Thy1-ChR2-YFP transgenic mouse brain. The acronym Thy1-ChR2-YFP means that the mice express the light-gated channel channelrhodopsin-2 (ChR2) under control of the Thy1 promoter fused to yellow fluorescent protein (YFP). The ChR2 is a cation channel that depolarizes neurons when illuminated with blue light [4]. Moreover, these transgenic mice express this ChR2 opsin in glutamatergic pyramidal cells in layers IV–V of the BC (see Fig. 1 from reference [5]). We adapted the optogenetics technique, using light with noisy variations in its intensity instead of square pulses of light. The novelty of our experimental approach is that we can selectively stimulate with noisy light the ChR2 neurons in layers IV–V of the barrel whisker cortex. We illuminated with noisy light the barrel cortical region in an area of about one mm^3, in which single neurons respond to sensory stimuli of the whiskers. This technique allowed us to examine whether the ONP on the BC of anesthetized Thy1-ChR2-YFP transgenic mice improves the neuronal multiunit-activity response to whisker mechanical stimulation (whisker-evoked MUA). We used the whisker-evoked MUA response as a first step to examine whether the ONP applied on the mice BC could produce improvements in somatosensory perception to whisker stimulation.

2. Methods

We employed six Thy1-ChR2-YFP transgenic mice (line 18) (mean weight 35 ± 3 g). Furthermore, we used other six wild-type littermate mice as a control (weight range 31 ± 3 g). We followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (85–23, revised in 1985) and the Norma-Oficial-Mexicana: NOM-062-ZOO-1999. The anesthesia consisted of an intraperitoneal injection of a mix of ketamine (90 mg/kg), xylazine (10 mg/kg) and acepromazine (2 mg/kg). During all the experiments we employed a radiant heating and a heating pad to maintain the temperature of the animals at about 37 °C. When the animals were anesthetized, they were fixed to a stereotaxic apparatus, and a craniotomy of 7 mm of diameter was performed to expose the barrel cerebral cortex. We removed the dura mater. Moreover, we employed dental acrylic to construct a small wall around the bone aperture. The space over the barrel cerebral cortex was filled with mineral oil.

2.1. Somatosensory stimulation: mechanical stimulation of the whole bundle of whiskers

The mechanical stimulation consisted of a protraction of the entire bundle of whiskers with a pulse of 5 ms and 2 Hz. We employed a mechanical stimulator-transducer Chubbuck to protract the entire bundle of the contralateral whiskers (Fig. 1A). The protraction strength was adjusted to produce multiunit activity (MUA) of neurons from the BC of about 30% of the maximal whisker MUA (100%) (Fig. 1D).

2.2. ONP of the BC

The ONP consisted of continuous noise blue light (470 nm) (Fig. 1C) administered with an optogenetics Thorlabs-DC4104 LED system (via an optic fiber of 200 micrometers, with a numerical aperture of 0.39) controlled with a WaveTek noise stimulator. We used an optical power meter PM100D to obtain the power spectrum of the optical noise administered. We found that such power spectrum is similar to Brownian noise (Fig. 1E). We applied ONP on the BC with a range of intensities from 0 to 0.67 mW.

2.3. Stimulation protocol

We followed three stimulation protocols: 1) the mechanical test stimulation of the whiskers alone; 2) the mechanical test stimulation of the whiskers during the ONP; 3) the ONP of the BC alone. The stimulation protocol 1, consisted of 32 trials of periodic whisker-stimulation during zero ONP (control). The stimulation protocol 2, consisted of 32 trials of periodic whisker-stimulation during the application of five intensities of continuous ONP. The five ONP levels were presented in a pseudo-randomized fashion. Furthermore, to avoid adaptation, rest intervals of 20 s were included between the noise levels. The stimulation protocol 3, consisted of the application of five intensities of continuous ONP alone.

2.4. MUA recording of neurons from the BC

We recorded the MUA of BC neurons with a 5-channel microelectrode system (MiniMatrix, SUA-Filter Version, Thomas Recording, GmbH, Giessen, Germany) referenced to an electrode placed on the head muscles. We employed quartz/platinum-tungsten fiber electrodes for the multi-unit neuronal activity (impedance 5–7 MΩ; fabricated with materials from Thomas Recording, GmbH, Giessen, Germany). The signals were amplified (with a gain of 100 or 1000) and high-pass filtered online (bandpass 0.5–5 kHz) with the same MiniMatrix system. We digitized the MUA with a Digidata System 1440A (Molecular Devices, Axon Instruments) with a sampling rate of 250 kHz. The signals were not filtered offline.

In the BC, we recorded multi-unit activity evoked by whisker protraction. Such response exhibited a typical burst of whisker-evoked MUA, as illustrated in Fig. 1B. Such neurons responding to whisker stimulation also responded to the noise light stimulation, as shown in Fig. 1C. The purpose of the whisker-evoked MUA was to characterize the effects of ONP on the output SNR of such MUA-response.

2.5. Rectified whisker-evoked MUA of neurons from the BC during ONP

The rectified whisker-evoked MUA during ONP was obtained by computing the absolute value of the whisker-evoked MUA raw signal plus noise (namely in Fig. 2B: “whisker evoked MUA + ONP). A rectified MUA provides a temporally precise index of global increases or decreases in neuronal firing (e.g., see [6]). This measurement is very often employed in the analysis of neuronal firing activity in the barrel somatosensory cortex [7,8], and it is not exclusive for the analysis of electromyographic signals. The rectified MUA signal plus ONP illustrated in Fig. 2B (namely, |whisker evoked MUA + ONP|) is a time series and not a point process. For the illustrative description of ‘time series’ and “point process” obtained from an MUA recording see Fig. 2 in reference [9]. In a physiological context, there is evidence suggesting that the rectified MUA in the BC mainly represents the compound spiking activity of multiple neurons around the recording electrode [7]. We employed the rectified MUA signal as it correlates well with the membrane potential of intracellular recordings in the BC and it is a good measurement of the global level of electrical activity in somatosensory neuronal ensembles in the cerebral cortex (e.g. see Fig. 1B and C in reference [7]).

2.6. SNR of the rectified whisker-evoked MUA

To compute the SNR, first, we calculated the absolute values of the rectified whisker MUA obtained for different levels of ONP, which we called |whisker evoked MUA + ONP|. Second, we calculated the values of the rectified MUA recordings during ONP conditions alone (namely, |MUA elicited by ONP but without whisker protraction|); i.e., without whisker stimulation. Third, the output SNR was calculated employing the formula illustrated in Fig. 2D.

We calculated this output SNR for every level of ONP. We followed the same procedure described in one of our previous articles (see Fig. 3 of reference [10]), in which we also applied periodic stimuli. In this section, we describe in detail such method adapted for the ONP.

We illustrate the procedure to calculate the output SNR for one animal (see Fig. 2). To simplify the explanation, Fig. 2A only shows three levels of input noise that were applied during the periodic test-