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Research report

Intrinsic optical imaging study on cortical responses to electrical stimulation in ventral posterior medial nucleus of thalamus

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ABSTRACT

Intracortical electrical micro-stimulation has been applied widely for the attempts on reconstruction of sensory functions. More recently, thalamic electrical stimulation has been proposed as a promising target for somatosensory stimulation. However, the cortical activations and mechanisms evoked by VPM stimulation remained unclear. In this report, the cortical neural responses to electrical stimulations were recorded by optical imaging of intrinsic signals. The impact of stimulation parameters was characterized to illustrate how the VPM stimulation alter cortical activities. Significant increases were found in cortical responses with increased stimulation amplitude or pulse width. However, frequency modulation exhibited significant inhibition with higher frequency stimulation. Our results suggest that optical imaging of intrinsic signals is sensitive and reliable to deep brain stimulations. These results may not only help to understand the modulation effects through thalamocortical pathway, but also show the possibility to use VPM stimulation to evoke frequency-tuned tactile sensations in rats.

1. Introduction

Electrical stimulation in the brain has long been used as a powerful tool in brain functional studies. Intracortical micro-electrical stimulation (ICMS) on primary sensory cortex has been shown to elicit discriminable tactile perceptions on rodents (Butovas and Schwarz, 2007; Houweling and Brecht, 2007) and primates (Romo et al., 1998; Romo et al., 2000). More recently, it has been reported that ICMS within the hand area of somatosensory cortex successfully evoked stable tactile sensations at appropriate locations in human (Flesher et al., 2016). Similar stimulation in rodent's whisker sensing area of somatosensory cortex (barrel field, S1BF) is believed to evoke virtual perceptions of whisker touching, resulting in whisking and turning behavior (Talwar et al., 2002). In rodent whisker sensing system, whisker-related peripheral nerve impulses firstly evoke action potentials in brain stem. The tactile information is then ascended to ventral posterior medial nucleus (VPM) of the thalamus before it reaches the somatosensory cortex barrel field (S1BF) (Chmielowska et al., 1989). Since the VPM involves in the whisker tactile processing, it may be a promising alternative stimulation site for sensation regeneration.

In previous behavioral studies, we have proposed that electrical stimulation in rat's VPM thalamus can be applied for turning control based on virtual "touch" sensation generated. Our results illustrated that while S1BF stimulation mainly enhanced whisking behavior, VPM stimulation evoked a more robust head and neck turning behavior (Xu et al., 2016). Particularly, the turning behavior of rats with VPM stimulation can be quantitatively adjusted by controlling the electrical stimulation parameters. Although there are close anatomical connections between VPM thalamus and S1BF, the two sites play different roles in whisker information processing. Functional studies have demonstrated that VPM neurons encode whisker information more stable than cortical neurons in S1BF (Brecht and Sakmann, 2002; Petersen et al., 2003). In S1BF, there are discrete structures termed as "barrels" in layer IV, which are arranged near identically to the layout of whiskers (Woolsey and Van der Loos, 1970). With clear anatomical maps, the barrel cortex processes neighboring whisker information segregated. Whereas in VPM, the clustered structures termed "barreloids" show broader receptive fields, where the majority of neurons response to neighboring whiskers as well as principal whisker (Veinante and Deschenes, 1999). Besides, the fact that neurons in one VPM barreloid (about 250 neurons) are fewer than those in corresponding S1BF barrel (about 2500 neurons) (Bruno and

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Sakmann, 2006) makes it reasonable to expect an amplification effect on VPM stimulation. Given all these anatomical and physiological evidences observed, we hypothesized that stimulation in VPM thalamus may activate larger portion of nuclei volume in cortex compared to direct stimulation in S1BF and thus evokes stronger sensations. However, there are few direct evidences how the cortex responds to electrical stimulation in VPM thalamus. In particular, the impacts of stimulation parameter changes needs to be quantified for optimization.

Previous functional MRI (fMRI) studies have shown similar sensory cortex response patterns evoked by nature whisker deflection and micro-electrical stimulation in VPM, which located mainly in the S1BF and upper lip region (Shih et al., 2014). A later micro-PET mapping work has also verified modulation effects evoked by VPM electrical stimulation (Zhu et al., 2016), that long-term consistent electrical stimulation would induced long-lasting cortical inhibition on primary sensory cortex. Both studies revealed the connection between VPM stimulation and cortex functional adjustment, nevertheless, the signals obtained in fMRI and PET examinations provide rather delayed information due to the relative long scanning time. To overcome this disadvantage, intrinsic signal optical imaging could be an alternative method that reflects oxygenated/deoxygenated hemoglobin concentration changes in local capillaries and provides up to 50 μm scale detailed signals captured within milliseconds (Vanzetta and Grinvald, 2008). Recent studies in mechanisms of neurovascular coupling have shown tight relationship between hemodynamic responses and neural activities (Boorman et al., 2010; Devor et al., 2003; Grinvald et al., 1986; Hillman, 2014), providing confident evidences of its reliability for functional brain researches in visual cortex (Grinvald et al., 1994; Jancke et al., 2004), auditory cortex (Bakin et al., 1996), olfactory bulb (Fletcher et al., 2009; Rubin and Katz, 1999) and sensory cortex (Blood et al., 1995; Brett-Green et al., 2001; Dunn et al., 2005; Martin et al., 2006). Moreover, this functional measurement is free of electrical stimulation artifacts, making the optical imaging of intrinsic signal suitable for functional researches with electrical stimulations.

In the current study, high speed intrinsic signal imaging was adopted on the exposed rat somatosensory cortex to understand the spatiotemporal neural activities elicited by electrical stimulation in VPM thalamus. The impacts of various stimulation parameters were characterized by analyzing cortical reflectance changes and corresponding time courses. The intrinsic signal imaging results were comparable to our previous behavioral observation, providing neural evidences to the feasibility of somatosensory stimulation in VPM.

2. Results

2.1. Intrinsic optical imaging responses of electrical stimulation

There are few intrinsic optical imaging studies on how the cortex responds to deep brain stimulation. We first examined if the electrical stimulation in VPM could induce an optical signal response in cortex. The imaging was focused on primary somatosensory cortex, mainly in the area of barrel field and its adjacent forelimb region (Fig. 1). In all imaged subjects, reflectance changes can be clearly observed after electrical stimulation onset, with an initial decrease in cortical reflectance followed by a large-spread increase (as shown in Fig. 2A). These signal changes in reflectance are believed to be analogous to the initial dip and positive components of the BOLD fMRI signals, respectively (Pouratian et al., 2002). In the current study, we mainly focused on the early component of darkening changes, a putative marker for localized metabolic activity (Malonek and Grinvald, 1996). Circular areas (about 100 μm in diameter) displaying the largest reflectance changes were selected as regions of interest (ROIs, arrowed dots in Fig. 2A) for further analysis. The reflectance changes of ROIs were plotted over time, and the time courses displayed typical intrinsic signal changes in response to electrical stimulation, starting with significant reductions and followed by long recover period (shown in Fig. 2B and C, *left*). Generally, after a 500-ms electrical stimulation, the reduction in tissue reflectance lasted about 2–3 s and peaked at around 2 s in time course. In contrast, time courses in blank trials showed no significant changes over time (Fig. 2B and C, black dashed lines), indicating the intrinsic signal changes observed is VPM stimulation-related. We then evaluated the cortical activation spreading range. Regions showing significant differences (student *t*-test, $p < 0.01$) before and after stimulation were marked as activated area (as shown in Fig. 2A, *far left*, red colored). The results showed wide spread activation in S1BF, about 1 mm in diameter. Furthermore, we also monitored the relationships between distances from the ROIs selected and corresponding peaking magnitudes. The calculated results showed no significant differences ($p > 0.05$) in peaking magnitudes between ROIs and distant sites within the activated area (as shown in Fig. 2B and C, *right*).

2.2. Effects of current amplitude and pulse width changes

We next estimated the effects of stimulation amplitudes and pulse widths. The amplitude changes were tested under fixed frequency of 20 Hz with pulse width of 2.5 ms. The images showed expanded activated area as current amplitudes increased (Fig. 3A). Two-way repeated measures ANOVA revealed significant main effect of amplitude ($F = 123.06$, $p < 0.0001$) modulations on the cortical responses. ROI analysis also displayed increased responses to higher stimulation amplitudes. Within the tested range of 10–90 μA, the peaking magnitudes of the ROI responses exhibited a near linear fit with stimulation amplitude while the peaking time remained stable (Fig. 3B and C).

As to pulse width changes, stimulation amplitude and frequency were fixed at 60 μA and 20 Hz, while the duration of each stimulation pulse was varied from 0.5 to 10 ms. In this test, the activated area displayed increase trend while pulse width lengthened (Fig. 4A). Two-way repeated measures ANOVA also revealed significant main effect of pulse width ($F = 62.55$, $p < 0.0001$) modulations. The ROI analysis showed slight differences from current amplitude changes. While the peaking time stayed consistent over all pulse widths tested, the peaking magnitudes exhibited power function fitting curve, with a quick increase at shorter pulse widths and then reached a plateau at higher pulse widths (Fig. 4C).

2.3. Effect of frequency variations

We then characterized the modulation impact of stimulation frequency on cortical responses. Two stimulus conditions were studied: frequency changes with fixed pulse width and frequency changes with fixed duty cycle ratio.

In the first condition, pulse width was fixed at 5 ms while the frequencies ranged from 10 to 100 Hz. Stimulation amplitude was fixed at 60 μA and total stimulation time was kept 500 ms for each trial. Different from amplitude and pulse width changes, higher frequency stimulation resulted in decreased activated area in S1BF (Fig. 5A). The cortical responses were consistent in peaking times (Fig. 5C), whereas displayed significant differences in peaking magnitude. The peaking magnitude curves revealed strong responses with lower stimulation frequencies at 10 Hz and 20 Hz, the responses dropped quickly at higher frequency of 50 Hz, and remained relatively stable at 100 Hz (Fig. 5D).