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Research Report

Timing-dependent effects of whisker trimming in thalamocortical slices including the mouse barrel cortex

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ABSTRACT

Whisker trimming produces depression of cortical responses in the barrel cortex. However, it is unclear how the developmental timing modifies the effects of whisker trimming. We investigated cortical responses in thalamocortical slices that included the mouse barrel cortex using flavoprotein fluorescence imaging. A topological relationship was observed between the thalamic stimulated sites and cortical areas showing fluorescence changes. By adjusting the position of the thalamic stimulated sites and the cortical windows in which amplitudes of the fluorescence changes were measured, we succeeded to reduce the variability of cortical responses between slices. We then investigated the effects of whisker trimming in the thalamocortical slices. Whisker trimming from 4 weeks to 8 weeks (at 4–8 weeks) of age significantly reduced cortical responses at 8 weeks. However, whisker trimming started before 4 weeks produced only slight depression or no significant effect on the thalamocortical responses. As sensory deprivation during a critical developmental period is known to prevent elimination of synapses, the presence of aberrant synapses may compensate the cortical depression induced by whisker trimming started before 4 weeks. To test this possibility, whisker trimming performed at 0–6 or 0–7 weeks of age was followed by regrowth of whiskers for 1–2 weeks. Clear and significant potentiation of cortical responses was observed in these mice at 8 weeks when compared with those of naive mice of the same age. Overall, these data suggest that whisker trimming, producing depression of thalamocortical responses, prevents elimination of aberrant synapses during a critical developmental period before 4 weeks in the mouse barrel cortex.

1. Introduction

Whiskers are important for rodents to obtain spatial information around the body, and whisker trimming has profound effects on the postnatal development of behaviors (Shishelova and Raevskii, 2010). The tactile inputs originating from a single whisker terminate on a single barrel in the primary somatosensory cortex (S1) through the ventral

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Abbreviations: S1, primary somatosensory; VPM, ventral posterior medial nucleus of the thalamus; POM, posteromedial nucleus of the thalamus; EPSP, excitatory postsynaptic potential; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; NMDA, N-methyl-D-aspartic acid; APV, D-2-amino-5-phosphonovalerate; CCD, charge coupled device

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posterior medial (VPM) nucleus of the thalamus (Woolsey and Van der Loos, 1970; Agmon et al., 1993). Inputs from whiskers are also mediated by the posteromedial (POM) nucleus of the thalamus (Lu and Lin, 1993). This system is widely used for investigating developmental plasticity (Brecht, 2007; Petersen, 2007). The basic neural circuits in the barrel cortex are formed during a critical period 1–2 weeks after birth, and sensory deprivation by whisker trimming modifies various aspects of these neural circuits during this period (Simons and Land, 1987; Fox, 1992; Schlaggar et al., 1993; Lendvai et al., 2000). Experience-dependent plasticity has also been found in adolescent or adult animals after this critical developmental period (Glazewski and Fox, 1996; Polley et al., 1999). In our previous study, whisker trimming produced depression of the cortical responses in coronal slices that included the barrel cortex obtained from adolescent rats (Kamatani et al., 2007). At the same time, it has been reported that aberrant cortical synapses are eliminated in an experience-dependent manner during a critical developmental period (Grutzendler et al., 2002; Jiang et al., 2009), suggesting that whisker trimming during a particular period could result in cortical potentiation. Therefore, the effects of whisker trimming on thalamocortical responses can be modulated by the timing of whisker trimming.

Slice preparation is suitable for detailed analysis of local neural circuits isolated from other part of the brain, and intact thalamocortical projections can be maintained in slices including the mouse barrel cortex and the VPM (Agmon and Connors, 1991). Furthermore, cortical activities of different layers can be visualized in a large area simultaneously in slice preparations. However, cortical responses are variable in each slice, and this variability prevents us to compare the experience-dependent effects recorded in different slices. In the present study, we overcame this problem by using flavoprotein fluorescence imaging. Endogenous green fluorescence signals derived from mitochondrial flavoproteins are activity-dependent (Chance et al., 1962), and this technique has been applied to functional brain imaging in the whole brain (Shibuki et al., 2003; Reinert et al., 2004; Husson et al., 2007) and in slices (Hishida et al., 2007; Kamatani et al., 2007; Llano et al., 2009; Theyel et al., 2010). The flavoprotein fluorescence is resistant to photobleaching, since denatured flavin is quickly replaced with newly synthesized molecules (Kubota et al., 2008). Mitochondrial flavoproteins are abundantly present in most neurons, and the variability of recorded data between different mice is sufficiently small. This method allows us to analyze experience-dependent plasticity by comparing response magnitudes recorded in different groups of anesthetized mice (Takahashi et al., 2006; Tohmi et al., 2006; Kitaura et al., 2010; Ohshima et al., 2010). In the present study, we reduced the variability of data recorded in thalamocortical slices by systematic positioning of the thalamic stimulated sites and the cortical windows in which the fractional fluorescence signal ($\Delta F/F_0$) was measured. Using this strategy, we succeeded to record significant effects of whisker trimming on thalamocortical slices obtained from different mice. Such effects of sensory deprivation were strongly affected by the timing of whisker trimming.

2. Results

2.1. *Properties of cortical fluorescence responses in thalamocortical slices*

Thalamic stimulated sites (sites 1–11) and the circular windows with a diameter of 500 μm (windows 1U–15U covering layers II–IV and 1L–15L covering layers V–VI) were determined based on the translucent image of fresh thalamocortical slices obtained from mice of 8 weeks old (Fig. 1A, B). Electrical stimulation with 20 pulses at 20 Hz produced stable responses (Fig. 1C, D), as reported previously in rat cortical slices (Hishida et al., 2007; Kamatani et al., 2007). The maximal $\Delta F/F_0$ was observed 1.5 s after the onset of the thalamic stimulation applied to site 4 (Fig. 1D), and the amplitudes around the stimulated site, layers V–VI at window 4L, and layers II–IV at window 4U were approximately 2%, 1%, and 0.5%, respectively. The relatively small standard error of the mean (SEM) in the data recorded from different slices (Fig. 1D) indicates that a statistical comparison of the cortical responses obtained from different groups of mice is practical.

We investigated the topographic relationship between the stimulated sites and cortical areas in the thalamocortical slices. Stimulation applied to sites 3–10 produced clear cortical responses, while stimulation to site 1, site 2, or site 11 produced almost no response or only small responses (Fig. 2A, B). Cortical responses were clearly observed in the barrel field (gray zone in Fig. 2B) when sites 3–6 were stimulated. The largest responses in the barrel field were observed after stimulation applied to site 4 (Fig. 2B). As a whole, a topographic relationship between the thalamic stimulated sites and the cortical areas showing activity was confirmed under the present experimental conditions (Fig. 2C). These results indicate that cortical responses in the barrel field appeared clearly after site 4 stimulation in windows 4–6. We used this combination of the thalamic stimulated site and cortical windows for later experiments.

To investigate the relationship between the flavoprotein fluorescence signals and neuronal activity, we recorded intracellular potentials from neurons distributed in layers II–V using a blind slice patch recording (Watanabe et al., 2007). Thirty two neurons were recorded from the active areas showing clear fluorescence responses in thalamocortical slices obtained from mice of 3 weeks old. At the stimulus intensity of 200 μA, these neurons did not show spike activity following the repetitive stimulation at 20 Hz (Fig. 3A–C). However, 11 neurons showed clear EPSPs with an amplitude larger than 5 mV. At the stimulus intensity around 1 mA, 16 neurons showed spike activity following the repetitive stimulation at 20 Hz, and 22 neurons showed clear EPSPs larger than 5 mV. No significant difference was found in neuronal responses between different layers, mainly because the response variability between neurons was too large (data not shown). Eleven neurons were recorded in the inactive areas showing no clear fluorescence responses. None of these cells showed spike activities or clear EPSPs larger than 5 mV in response to thalamic stimulation up to 1 mA. Therefore, the distribution of neurons activated by thalamic stimulation was well correlated to that of cortical fluorescence responses as a whole.