Neuroscience 281 (2014) 178–194

FREQUENCY-SPECIFIC RESPONSE FACILITATION OF SUPRA AND INFRAGRANULAR BARREL CORTICAL NEURONS DEPENDS ON NMDA RECEPTOR ACTIVATION IN RATS

N. BARROS-ZULAICA, C. CASTEJON AND A. NUÑEZ *

Departamento de Anatomía, Histología y Neurociencia, Facultad de Medicina, Universidad Autónoma de Madrid, 28029 Madrid, Spain

Abstract—Sensory experience has a profound effect on neocortical neurons. Passive stimulation of whiskers or sensory deprivation from whiskers can induce long-lasting changes in neuronal responses or modify the receptive field in adult animals. We recorded barrel cortical neurons in urethane-anesthetized rats in layers 2/3 or 5/6 to determine if repetitive stimulation would induce long-lasting response facilitation. Air-puff stimulation (20-ms duration, 40 pulses at 0.5–8 Hz) was applied to a single whisker. This repetitive stimulation increased tactile responses in layers 2/3 and 5/6 for 60 min. Moreover, the functional coupling (coherence) between the sensory stimulus and the neural response also increased after the repetitive stimulation in neurons showing response facilitation. The long-lasting response facilitation was due to activation of N-methyl-D-aspartate (NMDA) receptors because it was reduced by APV ((2*R*)-amino-5-phosphonovaleric acid, (2*R*)-amino-5-phosphonopentanoate) and MK801 application. Inactivation of layer 2/3 also blocked response facilitation in layer 5/6, suggesting that layer 2/3 may be fundamental in this synaptic plasticity processes. Moreover, i.p. injection of eserine augmented the number of layer 2/3 neurons expressing long-lasting response facilitation; this effect was blocked by atropine, suggesting that muscarinic receptor activation favors the induction of the response facilitation. Our data indicate that physiologically repetitive stimulation of a single whisker at the frequency at which rats move their whiskers during exploration of the environment induces long-lasting response facilitation improving sensory processing.

Key words: wavelet coherence, LTP, sensory plasticity, somatosensory system, thalamocortical network.

INTRODUCTION

The somatosensory barrel cortex is composed of local circuits heavily interconnected by vertical and horizontal projections (Feldmeyer, 2012; Feldmeyer et al., 2013). Sensory information from the whiskers passes via the brain stem and thalamus to layer 4 neurons in the barrel cortex. Sensory responses are relayed to layer 2/3 and then to layer 5 and layer 6, concomitant with feedback from layer 5 to layer 2/3 and layer 6 to layer 4. This vertical organization is linked horizontally by prominent projections within layer 2/3 and layer 5 (Douglas and Martin, 2004; Wester and Contreras, 2012). Distinct synaptic and intrinsic properties of these neurons may be involved in different sensory plasticity responses observed in the barrel cortex. Recently, it has been demonstrated that ''N-methyl-D-aspartate (NMDA) spikes'' and L-type voltage-gated Ca^{2+} channel activation increase the excitability of layer 5 neurons, thereby possibly mediating neuronal plasticity (Nuñez et al., 2012).

The barrel cortex of rodents is a remarkable structure that is capable of fine tactile discrimination based on whisker movements across objects or surfaces in repeated rhythmic sweeps at frequencies between 4 and 12 Hz (Carvell and Simons, 1990; Fanselow and Nicolelis, 1999), see for review (Moore, 2004). Sensory experience induces neuronal plasticity and has profound effects on synaptic responses in the neocortex. Long-term potentiation (LTP) of cortical synaptic potentials in response to repetitive stimulation is involved in sensory experience effects. For example, tetanic stimuli applied in layer 4 can induce LTP lasting several hours in layer 2/3 neurons (Glazewski et al., 1998). Repetitive whisker stimulation also induces a long-lasting increase in the amplitude of somatosensory-evoked potentials in layers 2/3 and 4 of the barrel cortex of neonatal rats or mice (Borgdorff et al., 2007; An et al., 2012), suggesting that it may participate in the activity-dependent wiring of the cortex during development. Moreover, multiwhisker stimulation at 2 or 8 Hz induces LTP in layers 2/3 and 4 of barrel cortical neurons of mature mice (Megevand et al., 2009), suggesting that sensory plasticity may contribute to information processing in adult animals.

Experiments on the possibility of inducing LTP in sensorially deprived barrel cortex provide further evidence on the role of LTP in cortical experience-dependent plasticity. In young adult rats with intact whiskers the incidence of LTP is relatively low,

*Corresponding author. Address: Departamento de Anatomía, Histología y Neurociencia, Facultad de Medicina, Universidad Autónoma de Madrid, c/ Arzobispo Morcillo 4, 28029 Madrid, Spain. Tel: +34-91-207-3755; fax: +34-91-397-5338.
E-mail address: angel.nunez@uam.es (A. Nuñez).
Abbreviations: Ach, acetylcholine; APV, (2*R*)-amino-5-phosphonovaleric acid, (2*R*)-amino-5-phosphonopentanoate; LTP, long-term potentiation; MK801, dizocilpine; NMDA, N-methyl-D-aspartate receptor; PSTH, peristimulus time histogram; RF, receptive field; SEM, standard errors of the mean; VPM, ventral posteromedial thalamic.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.057
0306-4522/

approximately 35% of neurons. However, this values rises to 70% following whisker deprivation (Hardingham et al., 2007), indicating also that LTP may contribute to sensory plasticity in adults.

In addition, several studies have shown that acetylcholine (Ach) regulates thalamocortical network synaptic plasticity in many important brain functions, such as arousal, attention, learning and memory (e.g. Celesia and Jasper, 1966; Sarter and Bruno, 2000; Oldford and Castro-Alamancos, 2003; Sarter et al., 2003; Hasselmo and Giocomo, 2006). Moreover, it has been demonstrated that Ach enhances synaptic plasticity in the hippocampus (e.g. Doralp and Leung, 2008; Fernandez de Sevilla et al., 2008; Navarrete et al., 2012) and neocortex (e.g. Metherate and Ashe, 1993; Kuo et al., 2009; Bueno-Junior et al., 2012; Nuñez et al., 2012) and may modulate tactile response facilitation. Here, we show that a brief period of repetitive whisker stimulation in anesthetized adult rats induces a frequency-specific long-lasting facilitation of tactile responses in layer 2/3 and layer 5/6 neurons. For this purpose we used single-unit recordings of rat barrel cortical neurons and analyzed tactile responses to whisker stimulation consisting of 20-ms air puffs at 1–8 Hz. Also, unit recordings were performed in the ventral posteromedial thalamic (VPM) nucleus to demonstrate that response facilitation was originated in the barrel cortex.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures were performed in accordance with the Ethics Committee of the Universidad Autonoma de Madrid, and with Council Directive 86/609/EEC of the European Community. Rats were group housed with a 12-h light/dark cycle and had free access to food and water. Every effort was made to minimize the number, and suffering, of the animals used.

Electrophysiological recordings

Experiments were performed on 122 urethane-anesthetized (1.6 g/kg i.p.) adult Sprague–Dawley rats weighing 200–250 g. Animals were placed in a Kopf stereotaxic device in which surgical procedures and recordings were performed. The body temperature was maintained at 37 °C; the end-tidal CO_2 and heart rate were monitorized. Local anesthetic (lidocaine 1%) was applied to all skin incisions and supplemental doses of anesthetic were given to maintain areflexia. An incision was made exposing the skull and a small hole was drilled in the bone over the barrel cortex. Single-unit recordings in the barrel cortex (*A* 1–3 mm, *L* 5–7 mm from bregma) were made 200–1500 μm below the surface with tungsten microelectrodes (2–5 MΩ) placed in both hemispheres. Units were recorded at different levels of the same track to ensure that we were moving along a single cortical column due to its response to specific whisker stimulation. After that, the stimulation train protocol was applied to that whisker (Fig. 1A). This recording protocol was repeated in different columns of the barrel cortex, applying the stimulation protocol to different whiskers. Also, unit recordings were performed in the VPM nucleus (*A* 3.2–3.8 mm, *L* 2.5 mm from bregma, *D* 6.5–7 mm from the surface) with tungsten microelectrodes. Unit firing was filtered (0.3–3 kHz), amplified via an AC preamplifier (DAM80; World Precision Instruments, Sarasota, USA), and fed into a personal computer (sample rate 10 kHz) with the temporal references of the stimuli for off-line analysis with Spike 2 software (Cambridge Electronic Design, Cambridge, UK). In some experiments (18 rats) the field potential was recorded through tungsten macroelectrodes (< 1 MΩ). The activity was filtered between 0.3 and 100 Hz, amplified and sampled at 500 Hz.

Sensory stimulation

Whisker deflections were performed by brief air puffs using a pneumatic pressure pump (Picospritzer) that delivers an air pulse through a 1-mm-inner diameter polyethylene tube (20-ms duration). To avoid complex responses due to deflections of multiple whiskers, all whiskers were trimmed to 5 mm in length, so that reproducible responses were evoked from a single, targeted, whisker. The pressure was set at 1–2 kg/cm^2, resulting in whisker deflections of ≈15°. When a single neuron was isolated, its cutaneous receptive field (RF) was carefully mapped with a small hand-held brush. RFs were monitored by listening to the audio conversion of the amplified activity signal. Thus, we could identify the whiskers belonging to the recorded neuron's RF. In this study two air tubes were used: one to stimulate the whisker that gave the highest spike response, called the principal whisker; the other to stimulate another whisker that gave a smaller response, called the peripheral whisker.

Pharmacological study

Drugs were injected (0.1 or 1 μl) through a cannula connected to a Hamilton syringe and targeted to layer 2/3 or 5/6. The complete experimental protocol began 5 min after the injection (see below; Fig. 1A).

The following drugs were used: (2*R*)-amino-5-phosphonovaleric acid, (2*R*)-amino-5-phosphono-pentanoate (APV; 50 μM), which is a selective NMDA receptor antagonist, and Muscimol (5-(aminomethyl)-isoxazol-3-ol) (8 mM), which is a selective agonist for $GABA_A$ receptors, were locally applied. Dizocilpine, also known as MK801 (0.5 mg/kg), which is a non-competitive antagonist of the NMDA receptor, eserine also known as physostigmine (0.1 mg/kg) and is an acetylcholinesterase inhibitor, and atropine sulfate (5 mg/kg), which is an antagonist of muscarinic receptors, were intraperitoneally (i.p.) injected.

Experimental protocol

The experimental protocol consisted of 30 pulses delivered to the principal or peripheral whiskers at 0.5 Hz (control period) followed by a train of 40 pulses at 0.5–8 Hz (stimulation train) delivered only to the