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SINGLE UNIT OSCILLATIONS IN RAT TRIGEMINAL NUCLEI AND THEIR CONTROL BY THE SENSORIMOTOR CORTEX

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Abstract—Oscillatory activity at both the single and multiunit levels has been reported in most central nervous system structures, and is postulated as a key factor in information processing and coding. Rats provide an excellent model for oscillation-based information processing, since tactile perception of the environment is achieved by rhythmic movements of their whiskers and information-related rhythmic activity has been identified in the thalamus and cortex. However, rhythmic activity related to information processing has never been reported in the sensory trigeminal complex (STC), the first brain stem relay station for whisker-related tactile information. In the present work, we demonstrate the existence of neural oscillations in the vibrissae-related neurons of the nuclei principalis (Pr5), oralis (Sp5o), interpolaris (Sp5i) and caudalis (Sp5c). Rhythmic activity was associated with the main task of each nucleus, prominent in nuclei responsible for tactile vibrissae information processing (up to 17% oscillating neurons in Pr5 and 26% in Sp5i) and less conspicuous in those concerned with pain (8% oscillating neurons in Sp5o and in Sp5c). The higher percentage of oscillating neurons and higher frequencies in Sp5i than in Pr5 suggests an active role for rhythmic activity in integrating multivibrissa inputs. Oscillations are generated within the brainstem; data obtained from decorticated animals suggest the existence of a differential cortical control of the rhythmic processes in STC nuclei. Corticofugal activity modifies oscillation frequency and synchronization strength of the rhythmic activity mainly during tactile stimulation of the vibrissae.

Key words : whisker, corticofugal, principalis, oralis, interpolaris, caudalis.

Rats provide an excellent model of oscillation-based perception and information processing and coding, since their tactile exploration of the environment requires both synergy and interactions among different oscillatory processes related to their vibrissae (30 large, thick hairs organized in five rows and nine columns on their snout; Carvell and Simons, 1990). The most visible of these oscillatory processes is whisking, a 4–12 Hz rhythmic movement of the vibrissae performed by the animal during active perception (Carvell and Simons, 1990; Nicolelis et al., 1995; Fanselow and Nicolelis, 1999). Besides these low frequency oscillations, whisking also causes vibration of the vibrissae. These oscillations and vibrations generate a complex signal that is coded by the receptors in the vibrissa follicle and transmitted to the CNS. An external object is perceived by interactions among these complex signals and new signals generated by the deflection of the vibrissae when they make contact with an external object (Hutson and Masterton, 1986; Carvell and Simons, 1990). Information about the texture of the objects touched is also obtained by additional vibrations caused by the displacement of the distal part of the whisker over their surface (Neimark et al., 2003; Andermann et al., 2004; Arabzadeh et al., 2005). This dynamic behaviour of the whiskers, either in unconstrained conditions or when they touch an external object, is transmitted to the cortex through the trigeminal somatosensory pathway (Woolsey and Van der Loos, 1970; Veinante and Deschenes, 1999).

The trigeminal pathway is the most highly-developed sensory system in rodents both at the cortical and subcortical levels, but despite a major role for oscillations and rhythmic interactions in information processing and coding, only a few studies have focused on rhythmic activity in this system: Nicolelis et al. (1995) observed 7–12 Hz synchronous activity at multiple levels of the whisker sensory pathway, brainstem, thalamus and cortex when the rats were in a state of attentive immobility preceding the onset of whisking; Ahissar et al. (1997) reported information coding-related oscillations of ~10 Hz in the barrel cortex of anaesthetized rats and guinea-pigs, while Hamada et al. (1999) detected cortical γ-band oscillations in the barrel region of awake rats. Surprisingly, despite the large size of the sensory trigeminal complex (STC), no research efforts have addressed the oscillatory activity of its nuclei (principalis-Pr5, oralis-Sp5o, interpolaris-Sp5i and caudalis-Sp5c).

In contrast to the limited number of studies devoted to oscillatory activity in the trigeminal sensory system, rhythmic activity has been recorded in all relay stations of the lemniscal pathway in both anaesthetized and awake animals. Rhythmic excitatory postsynaptic potentials (EPSPs) have been reported in the second relay station of the lemniscal somatosensory system, the ventroposterior nuclei of the thalamus, by Pinault and Deschenes (1992) and Nuñez et al. (1994), and 4–22 Hz oscillations have been

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Abbreviations: ACH, autocorrelation histogram; CCH, crosscorrelation histogram; CNS, central nervous system; CV, coefficient of variation; DCN, dorsal column nuclei, nuclei gracilis and cuneatus; EEG, electroencephalogram; EPSP, excitatory postsynaptic potential; gV, trigeminal ganglion; HF, high frequency neurons; ISI, inter-spike interval histogram; LF, low frequency neurons; Ph, phasic; POm, posteromedial nucleus of the thalamus; Pr5, nucleus principalis of the sensory trigeminal complex; PSTH, peristimulus histogram; RF, receptive field; SMCx, sensorimotor cortex; Sp5c, nucleus caudalis of the sensory trigeminal complex; Sp5i, nucleus interpolaris of the sensory trigeminal complex; Sp5o, nucleus oralis of the sensory trigeminal complex; STC, sensory trigeminal complex; T, tonic; Vg, trigeminal ganglion; VPM, ventral posteromedial nucleus of the thalamus.

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reported by Panetsos et al. (1997) in thalamic projecting neurons and putative interneurons of the dorsal column nuclei (DCN) upon stimulation of their receptive fields (RFs). These considerations, together with the report by Sandler et al. (1998) of 50% bursting neurons in slice preparations of the Pr5 of young gerbils, and evidence of oscillations (Puil et al., 1988) or even repetitive spike firing (Puil et al., 1989) of the membrane potential of trigeminal ganglion (gV) cells, led us to test for similar oscillatory activity in the first relay stations of the two main routes to the thalamus: from the nucleus principalis to the ventral posteromedial nucleus of the thalamus (Pr5 to VPM), and from the nucleus interpolaris to the medial region of the posterior thalamic nucleus (Sp5i to POm). Further evidence that oscillatory activity in the STC may play a role in information processing was provided by the detection of frequency-dependent information processing of somatosensory information at the levels of the brainstem (Sanchez-Jimenez et al., 2009), thalamus, and cortex via the trigeminal pathway (see Lak et al. (2008); Garabedian et al. (2003); Moore (2004) and references therein, and; Arabzadeh et al., 2003; Neimark et al., 2003; Andermann et al., 2004; Hipp et al., 2006). Furthermore, *in vivo* and *in vitro* experiments conducted on the homologous nuclei of the lemniscal somatosensory system have revealed that DCN cells have the capacity for rhythmic discharge patterns in the absence of cortical or peripheral inputs (Panetsos et al., 1997; Nuñez and Buño, 1999).

In the present paper, we report for the first time the presence of rhythmic activity in the whisker-related neurons of the STC, and relate this activity to incoming sensory stimuli. Our data reveal that such activity is generated inside the nucleus, yet not introduced from the trigeminal ganglion (Vg) nor imposed by the corticonuclear input. Finally, we examine relations between incoming stimuli and oscillatory processes according to the locations of these stimuli in the receptive field.

EXPERIMENTAL PROCEDURES

Data were obtained from 68 intact animals (58 anaesthetized with urethane (Sigma-Aldrich Chemie, Steinheim, Germany) 1.5 g/kg i.p., five with sodium pentobarbital 35 mg/kg i.p. (Sigma-Aldrich Chemie, Steinheim, Germany) and five with ketamine-xylazine 100 mg/kg and 20 mg/kg i.p. respectively (Sigma-Aldrich Chemie, Steinheim, Germany)) and 15 decorticated animals (10 anaesthetized with urethane and five with ketamine-xylazine). Additional recordings were made in seven animals with sectioned ganglion fibres and 10 animals in which several whiskers were stimulated, all anaesthetized with urethane (1.5 g/kg i.p.). Experiments were carried out according to national legislation (R.D.1201/2005) and EU Directives (86/609/EC) on this matter, minimizing the number of animals used and their suffering.

Surgical procedures and animal care

Rectal temperature was monitored and kept at 37°±0.1 °C using an abdominal heating pad under servo-control (Letica Scientific Instruments, Spain, model HB101/2). To obtain an electroencephalogram (EEG), a macroelectrode (1.0 mm diameter bluntly cut insulated nichrome wire (Advent, Eynsham, Oxford, England)) was lowered 1.5 mm from the cortical surface into the frontal lobe. The EEG was filtered between 0.3 and 30 Hz and was continuously monitored by the oscilloscope. Supplemental doses of anaesthetic were given when EEG slow waves decreased in amplitude.

Animals were placed in a stereotaxic device that allowed access to the vibrissae (Narishige Co., Ltd., Tokyo, Japan, model SN-3N). An incision was then made and the scalp removed to allow access to the brain at the appropriate coordinates (Paxinos and Watson, 1998). For Pr5, Sp5o and Sp5i, a hole was made in the skull 2.0–3.5 mm lateral to the midline and 8.0–12.5 mm posterior to bregma, the dura was removed and the recording microelectrodes introduced vertically into the brain. For Vg, we followed the same procedure 1.0–3.0 mm lateral and 0.0–4.5 mm posterior to bregma (Leiser and Moxon, 2006). To access Sp5c, the cisterna magna was opened, the dura removed and the recording microelectrodes introduced into the brain tissue at a 60° angle visually guided under a dissecting microscope. Drying of the exposed surface of the brain was prevented by covering it with vaseline.

Cortical lesions were produced by ablation of the sensorimotor cortex (SMCx) contralateral to the recording site. After fixing the animal to the stereotaxic apparatus, a hole was drilled in the skull 4.0–7.5 mm lateral to the midline and 0.5–4 mm posterior to bregma, the dura was removed and the SMCx was aspirated using a Pasteur micropipette connected to a vacuum pump (Vacumsol AS-60, ALSA Apparecchi Medicali SRL, Italy) taking care to spare the subcortical white matter (Rema and Ebner, 2003). The hole was then covered with vaseline and the animals were left for 3–4 h before starting the STC recordings.

To interrupt the efferent Vg fibers the cisterna magna was opened, the dura was removed and the nerve transected using a microsurgical blade under direct observation through a dissection microscope at the level of the dorsal column nuclei (14.0–15.0 posterior to bregma, 3.5–2.0 mm laterally and 8.4–9.0 mm depth; (Paxinos and Watson, 1998)). We considered the section acceptable when no responses were recorded when manually stimulating the vibrissae using a thin brush once the recording electrode was located into the suitable coordinates.

At the end of each experiment, the animals were injected with an overdose of sodium-pentobarbital (50 mg/kg) and perfused transcardially with saline followed by formalin (4% in saline). The brain was removed, stored in 20% sucrose saline and cut on a freezing microtome into 50-μm coronal sections. The sections were stained by either the Nissl method or CyO to locate the recording sites (Fig. 1A).

Stimulation and recordings

Extracellular recordings were obtained using 0.8–2.0 MΩ tungsten microelectrodes (World Precision Instruments, Sarasota, Fl, USA) placed in the zone of the barrelets of the STC nuclei ipsilateral to the stimulated whisker.

The recording microelectrodes were guided into the ipsilateral (with respect to the stimulated vibrissae) STC following the atlas coordinates. The location of the recording electrode was also inferred by the stereotyped somatotopy of the brainstem trigeminal nuclei (Ma, 1991). The vibrissae whose stimulation elicited changes in the discharge pattern of the neuron under observation were manually stimulated using a thin brush to determine their RFs. Spike amplitude and shape were continuously monitored on-line with an analogue oscilloscope; fine movement of the micromanipulator allowed a better approximation of the electrode tip to the recorded neuron, permitting a higher signal-to-noise ratio and isolation of single neurons. RFs were then mapped by a small hand-held brush while listening to their amplified neuronal discharges over a loud speaker. The whisker eliciting the greatest response (principal whisker) was considered the functional centre of the RF. Extracellular recordings were also filtered between 0.3 and 3 kHz, amplified (DAM80 bioamplifier, World Precision Instruments, Inc., Sarasota, FL, USA) and fed to a PC computer (sam-