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Research report

Involvement of trigeminal mesencephalic nucleus in kinetic encoding of whisker movements

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ABSTRACT

In previous experiments performed on anaesthetised rats, we demonstrated that whisking neurons responsive to spontaneous movement of the macrovibrissae are located within the trigeminal mesencephalic nucleus (Me5) and that retrograde tracers injected into the mystacial pad of the rat muzzle extensively labelled a number of Me5 neurons. In order to evaluate the electrophysiological characteristics of the Me5–whisker pad neural connection, the present study analysed the Me5 neurons responses to artificial whisking induced by electrical stimulation of the peripheral stump of the facial nerve. Furthermore, an anterograde tracer was injected into the Me5 to identify and localise the peripheral terminals of these neurons in the mystacial structures. The electrophysiological data demonstrated that artificial whisking induced Me5 evoked potentials as well as single and multiunit Me5 neurons responses consistent with a direct connection. Furthermore, the neuroanatomical findings showed that the peripheral terminals of the Me5 stained neurons established direct connections with the upper part of the macrovibrissae, at the conical body level, with fibres spiralling around the circumference of the vibrissae shaft. As for the functional role of this sensory innervation, we speculated that the Me5 neurons are possibly involved in encoding and relaying proprioceptive information related to vibrissae movements to other CNS structures.

1. Introduction

The sensory system of rodent vibrissae has been extensively analysed to understand how these animals may successfully explore the nearby environment, detect objects and orient their behaviour using the macrovibrissae system (Guic-Robles et al., 1989; Vincent, 1912). It is known that rats palpate objects using one or more macrovibrissae to recognise their size, shape and texture even in dark conditions (Arabzadeh et al., 2005; Carvell and Simons, 1990; Harvey et al., 2001; Metha et al., 2007; Prigg et al., 2002; von Heimendahl et al., 2007). Furthermore, they explore their surroundings either through active whisking (a rhythmical sweeping motion of their whiskers forwards and backwards; Berg and Kleinfeld, 2003), frequently associated with head movements (Hartmann, 2001; Mitchinson et al., 2007), or even by performing casual and free vibrissae movements in the air.

Somatosensory signals originating from the whisker pad and the vibrissae receptors (Ebara et al., 2002) enter the nervous system via the infraorbital nerve (ION; Vincent, 1913). Next, the central branch of the trigeminal ganglia (TG) neurons joins in the brainstem the trigeminal nuclei complex from which originate multiple efferent pathways targeting several brainstem structures, the cerebellum, as well as the thalamic nuclei that deduce higher-order information and project to the cerebral cortex (for review see Diamond et al., 2008; Kleinfeld et al., 2006).

As for the functional significance of the primary trigeminal afferents, a number of studies have shown that the neurons of the TG may also encode an object location by providing information about its spatial coordinates (Diamond et al., 2008; Krupa et al., 2001; Szwed et al., 2003, 2006). It has been shown that during active whisking, the TG touch and whisking/touch neurons modify their firing pattern. Indeed it has been proposed that their spiking frequency and/or timing may account for encoding spatial information, allowing the brain to reconstruct the spatial coordinates provided by macrovibrissae movements (Ahissar et al., 2000; Ahissar and Arieli, 2001; Ahissar and Zacksenhouse, 2001; Szwed et al., 2003, 2006; Yu et al., 2006; Solomon and Hartmann, 2006; Diamond et al., 2008; Knutsen and Ahissar, 2008).

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However, recent findings demonstrated that a retrograde tracer injected into the mystacial pad of the rat muzzle extensively labelled a significant number of trigeminal mesencephalic (Me5) neurons. The study in particular showed for the first time that besides the TG neurons, other sensory trigeminal primary neurons are involved in the innervation of the whisker pad (Mameli et al., 2010). Moreover, in the same study it has been found that the Me5 neurons were responsive to spontaneous movement of the macrovibrissae. In particular, during the so called "fan-like" movements of the vibrissae in the air, the spike activity of Me5 neurons tonically increased, whereas during rhythmic whisking their tonic activity turned to phasic firing; i.e. an activity characterised by bursts of spikes synchronised with the whisking frequency. Similar whisking neurons have been already described in the TG (Khatri et al., 2009; Leiser and Moxon, 2007; Lichtenstein et al., 1990; Pali et al., 2000; Szwed et al., 2003, 2006). On the basis of these results, it has been hypothesised that besides the TG neurons peripheral terminals, the vibrissae movements could also induce a mechanical distortion of the peripheral terminals of Me5 neurons which in turn elicited a volley of spikes into the nucleus. As for its functional role, we speculated that Me5 could be involved in relaying to other CNS structures proprioceptive information related to vibrissae displacements.

The present study was designed to electrophysiologically evaluate the neural connection between Me5 and the whisker pad by analysing the Me5 responses evoked by artificial whisking induced by electrical stimulation of the peripheral stump of the facial nerve (Brown and Waite, 1974; Semba and Egger, 1986). Furthermore, using neuroanatomical procedures, we tried to identify the peripheral terminals of the Me5 neurons within mystacial structures, with particular regard to the vibrissae follicle-sinus complex that it is known to retain several, variously distributed, receptors (Ebara et al., 2002).

2. Materials and methods

The experiments were performed on Wistar rats (Harlan, San Pietro al Natisone, Udine, Italy), weighing 250–350 g, which were submitted to electrophysiological (Groups I–II) and neuroanatomical procedures (Group III) in accordance with current institutional guidelines for the care and use of experimental animals (*EC Directive 86/609/EEC for animal experiments*). Furthermore, all experimental procedures were approved by the Italian Health Ministry and the local Veterinary Public Health Service.

Laboratory chow and water were available ad libitum, and the animals were housed under controlled conditions (room temperature: 23 ± 1 °C; lights on: 07:00–19:00).

2.1. Electrophysiological recordings

The animals were anaesthetised by an intraperitoneal injection of diazepam (30 mg/kg) and ketamine hydrochloride (45 mg/kg) and then mounted prone in a stereotaxic frame (David Kopf). A craniotomy was performed at the occipital bone level to expose the cerebellum and the obex. All the exposed surfaces were then protected with warm mineral oil and paraffin (37 °C) and the pressure's points were injected with xylocaine (0.3%) every 40 min. The ECG was continuously monitored throughout the experiment to assess the depth of the anaesthesia and the animal discomfort. The animals were then subdivided in two groups that were submitted to different experimental procedures.

2.1.1. Group I: Me5 neurons responses evoked by artificial whisking

Before general surgical procedures and craniotomy, a dissection nearby the whisker pad, that spared the vibrissae area, was carried out in 9 animals to expose the buccal branch of the facial nerve (approximately 1.0–1.5 cm) and prepare it for the electrical stimulation. It has been in fact accepted that this procedure induces an artificial whisking (Brown and Waite, 1974; Semba and Egger, 1986) that approximates the macrovibrissae synchronous protraction/retraction movement (whisking) that rats perform while exploring their surrounding environment. The peripheral branch of the facial nerve, previously exposed near to the muzzle, was mounted on a miniaturised pair of Ag electrodes each connected to an insulated Ag wire and then protected and covered with a solution of oil and paraffin (37 °C) that rapidly solidified. The surgical wounds were sutured, so that only the Ag wires were available for the electrical stimulation. A second dissection was then performed at the level of the mandibular angle to expose the proximal trunk of the facial nerve, identified by electrical stimulation, for its subsequent transection.

When the animal was set in the head holder, an automatic custom made stimulating device allowed eliciting a standard stretch of the jaw-closing muscles. The device consisted of a capacitor, powered by an AD 9 V battery, that when switched on delivered a standard pulse to a magnet. The magnet in turn pulled a tiny iron rod that, being sealed with dental acrylic cement to the mandibular symphysis, elicited a standard stretch of the jaw-closing muscles.

The spontaneous electrical activity of the Me5 neurons was recorded to identify the Me5 region where the neurons responsive to spontaneous movement of the vibrissae were located. The extracellular recordings were performed using tungsten-in-glass microelectrodes (impedance 700–1200 KΩ), carefully advanced into the Me5 using an electronic microdrive (David Kopf). Then, the facial nerve was tied and sectioned at the level of the mandibular angle and its proximal stump electrically stimulated to confirm that the lesion was complete. This procedure was carried out to avoid additional whisker pad stimulation that could in fact arise from antidromic stimulation of the facial motor neurons and/or by reflexes arising from activation of the trigeminal–facial sensory motor loop. It is known in fact that the facial motor-neurons, which induce contraction of the whisker pad muscles and vibrissae movements, could be activated by the principal and spinal trigeminal nuclei (Nguyen and Kleinfeld, 2005; Erzurumlu and Killackey, 1979; Hattox et al., 2002). These nuclei receive in fact vibrissae signals from TG neurons when stimulated by artificial whisking (Diamond et al., 2008; Krupa et al., 2001; Szwed et al., 2003, 2006).

The spontaneous electrical activity of Me5 neurons was extracellularly recorded as were their evoked responses to the electrical stimulation of the peripheral stump of the facial nerve. The stereotaxic coordinates used for the electrophysiological recordings into the Me5 were the following: −9.68 to −10.04 mm posterior to the bregma (Paxinos and Watson, 1997), which corresponded to the medial-caudal part of the nucleus (Fig. 3).

The peripheral stump of the facial nerve was stimulated using an insulated stimulator (Grass S11), which delivered square-wave pulses (0.05 ms wide, 0.5–1.0/s frequency) at threshold intensity (0.1–0.5 V amplitude) to induce vibrissae whisking.

The spontaneous activity of Me5 neurons, previously identified by their responses to masseter muscle stretch, was extracellularly recorded, relayed to conventional preamplifiers and then fed to computers for A/D conversion and subsequent analysis (Tecfen computerscope analysis ISC-16 software, and PowerLab 4/30 Chart 5, V 5.4.2 software). In particular the spontaneous electrical activity of single Me5 neurons, as well as that of a population of Me5 neurons (multi-unit electrical activity: MUA) was recorded in resting conditions (i.e. vibrissae motionless) and during masseter muscle stretch. For quantitative analyses this test was repeated four times. The averaged evoked potentials and the Me5 neurons responses to artificial whisking were successively recorded and analysed. Responses latencies, considered as the delay from the