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# ONGOING ACTIVITY IN TRIGEMINAL WIDE-DYNAMIC RANGE NEURONS IS DRIVEN FROM THE PERIPHERY

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**Abstract—Ongoing activity of spinal trigeminal neurons is observed under various conditions and suggested to be responsible for ongoing headache. It can be spontaneous, i.e. arising intrinsically from the neuron, or the product of descending influences from other central neurons, or maintained by ongoing afferent input. The aim of the present study was to examine if ongoing activity of neurons in different subnuclei of the spinal trigeminal nucleus is driven from peripheral afferent input.**

**Experiments were performed in Wistar rats anesthetized with isoflurane or Nembutal/urethane. Ongoing activity of single wide-dynamic range (WDR) neurons was recorded with carbon fiber glass microelectrodes in two subnuclei of the spinal trigeminal nucleus: oral (Sp5O) and caudal (Sp5C). Peripheral receptive fields were evaluated using von Frey filaments. Sp5O neurons received peripheral input from facial areas innervated by the mandibular branch of the trigeminal nerve. Units in Sp5C had receptive fields in the surgically exposed dura mater and in facial areas innervated by the ophthalmic and maxillary branch of the trigeminal nerve. Saline or the local anesthetic lidocaine was locally applied onto the exposed dura mater or microinjected into V3 (for Sp5O units) or V1/V2 (for Sp5C units) divisions of the trigeminal ganglion via the infraorbital channel.**

**Local application of lidocaine onto the exposed dura caused mechanical insensitivity of dural receptive fields but not significant decrease in ongoing activity. Microinjection of lidocaine but not saline into the trigeminal ganglion was followed by a substantial decrease in both the receptive field size and the activity of the recorded WDR units. Mechanical insensitivity of receptive fields after trigeminal ganglion blockade was accompanied by the disappearance of ongoing activity.**

**We conclude that the ongoing activity of WDR neurons in the spinal trigeminal nucleus, which may be indicative for processes of sensitization, is driven remotely by ongoing afferent input.** 

**Key words: trigeminal system, nociception, sensitization, headache, local anesthetics.**

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*Abbreviations:* Sp5C, caudal spinal trigeminal nucleus; Sp5O, oral spinal trigeminal nucleus; V1, V2, V3, divisions of the trigeminal ganglion: ophthalmic (V1), maxillary (V2), mandibular (V3); WDR, wide-dynamic range.

The origin of ongoing activity in central neurons that relay sensory information is of considerable clinical interest, since it is suggested to determine essentially the level of postinjury and chronic pain (Sorkin and Wallace, 1999; Suzuki and Dickenson, 2006). Particularly, the ongoing activity observed in the neurons of the spinal trigeminal tract is suggested to be responsible for ongoing headache (Burstein et al., 2000). Being recorded during *in vivo* electrophysiological experiments the ongoing activity in spinal trigeminal neurons is often referred to as spontaneous (Bolton et al., 2005; Bouhassira et al., 1995; Jakubowski et al., 2005; Kawamata et al., 2006; Knight et al., 2002; Park et al., 2006; Sessle et al., 1986; Suzuki and Dickenson, 2006). However, it is still not clear whether this ongoing firing is spontaneous, i.e. produced by the neuron itself, whether it is the result of descending central signals or if it is driven remotely by ongoing convergent afferent input (Collins, 1987; Collins et al., 1987; Herrero and Headley, 1995; Menetrey et al., 1984). This is largely because it is difficult to dissociate reliably spinal and medullary neurons from all of their peripheral inputs.

In the present study we examined the origin of ongoing activity in wide-dynamic range (WDR) neurons from the oral (Sp5O) and caudal (Sp5C) subnuclei of the spinal trigeminal nuclear complex in the rat. These structures receive afferent input from intra- and extracranial trigeminal nociceptive afferents (Davis and Dostrovsky, 1988; Schepelmann et al., 1999; Strassman et al., 1994), and are therefore of special interest for the investigation of mechanisms underlying craniofacial and orofacial pain (Bolton et al., 2005; Sessle, 1987).

In previous experiments, in which we recorded from neurons in the caudal spinal trigeminal nucleus (Fischer et al., 2005; Koulchitsky et al., 2004; Schepelmann et al., 1999), we noticed that ongoing activity was frequently observed in neurons that received afferent input from the experimentally exposed cranial dura mater or the skin areas damaged during the preparation, whereas ongoing activity was almost never seen in neurons with input from intact facial areas. This could possibly indicate a role for primary afferent activity in the maintenance of ongoing activity, assuming that the incised tissues and microlesions in the exposed dura cause activation of primary afferents. However, central components contributing to ongoing firing in these structures cannot be excluded. For example, the principal trigeminal nucleus possesses neurons that show bursting and tonic firing behavior (Sandler et al., 1998), and there is a projection, albeit sparse, from this nucleus to each of the caudal trigeminal subnuclei (Jacquin et al., 1990). Multielectrode recordings from neurons

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in the spinal trigeminal nucleus of awake animals have demonstrated synchronous oscillatory activity, and authors suggested that the activity is of central origin (Nicolelis et al., 1995).

The anatomy of the trigeminal system provides the unique possibility to remove reliably all trigeminal afferent input from central trigeminal structures, because local anesthetic can be injected directly into the trigeminal ganglion accessed via the infraorbital channel. This allows the contribution of peripheral input to the ongoing activity in neurons in the trigeminal nucleus to be examined.

In summary, the contribution of peripheral sensory input to the maintenance of ongoing activity in WDR neurons in the trigeminal nucleus is unclear. To clarify this issue we recorded from active trigeminal neurons in the subnuclei oralis and caudalis and examined changes in activity after microinjection of local anesthetic into the trigeminal ganglion.

## EXPERIMENTAL PROCEDURES

The study was performed in accordance with the ethical guidelines of the International Association for the Study of Pain and the German laws for animal protection (Tierschutzgesetz). The experimental procedure was reviewed by an ethics committee and approved by the local district government. Every effort was made to minimize the number of animals used and their suffering.

In total 45 Wistar rats with body weights ranging from 250 to 350 g were used. Activity was recorded from neurons located in Sp5C ($n=26$), and Sp5O ($n=19$). Observations from a group of 60 animals of similar weight obtained in previous experiments (Fischer et al., 2005; Koulchitsky et al., 2004; and unpublished observations from S. Koulchitsky) were included in this study for comparison of receptive fields and the level of ongoing activity.

### Anesthesia and general treatment of animals

For recordings from Sp5C, animals were anesthetized with isoflurane (Abbott, Wiesbaden, Germany). They were placed in a closed box with an initial concentration of 5% isoflurane in air for about 5 min before a mask was securely fixed to the animal's nose through which they breathed 3.5% isoflurane in oxygen-enriched air. In this state animals were tracheotomized to allow artificial ventilation (rodent ventilation system Hugo Sachs Elektronik KG, March, Germany) with a mixture of 2% isoflurane and oxygen-enriched room air using an evaporator system (TKM 0902; Föhr Medical Instruments, Frankfurt, Germany) during the remainder of the experiment. For recordings from Sp5O, animals were anesthetized with an initial i.p. injection of pentobarbital sodium (30 mg/kg, Nembutal; Sigma-Aldrich, Steinheim, Germany) mixed with ethyl carbamate (500 mg/kg urethane; Sigma-Aldrich). Supplemental doses of this mixture were administered as required. In all experiments the depth of anesthesia was held at a level at which noxious pinch stimuli did not evoke nociceptive reflexes or changes in blood pressure.

The right femoral artery was cannulated to monitor systemic blood pressure. The femoral vein was cannulated for slow continuous infusion of saline (0.5 ml/h) and administration of substances. The animals were additionally paralyzed with i.v. gallamine triethiodide (40 mg/kg; Relaxan, Sigma-Aldrich) supplemented by small doses (5–10 mg/kg) when required. Expiratory $CO_2$ was monitored ($CO_2$ analyzer from Carl Heyer GmbH, Germany) and maintained at 4.5–5% throughout the experiment by adjusting the ventilation frequency with a fixed stroke volume of 3.5 ml. Body temperature was maintained at 37–37.5 °C with a feedback-controlled blanket (TKM 0902; Föhr Medical Instruments). Vital parameters (blood pressure, heart rate, expiratory $CO_2$ level, and body temperature) were continuously recorded and monitored throughout the experiment.

### Head surgery

The animal's head was mounted in a stereotaxic frame and secured with ear bars. An incision was made along the midline of the scalp extended caudally to the cervical segments C4-5. The skin and periosteum were retracted and fixed with thread to expose the skull. To gain access to Sp5C neurons the neck muscles were divided along the midline and held apart with a clamp. The atlanto-occipital ligament including the underlying spinal dura was cut to expose the medulla oblongata. To expose the left side of the cranial dura mater including parts of the superior sagittalis sinus, a craniotomy of the parietal bone was made using a dental drill that was continuously cooled with saline. Another small opening was cut into the right parietal bone for the indifferent stimulating electrode (Fig. 1A). Special care was taken not to damage the exposed dura mater and to prevent bleeding from dural blood vessels. During further surgery and throughout the experiment, the dura was protected from drying with isotonic saline.

Sp5O neurons were accessed through a trepanation in the occipital bone made as described above. The cranial dura was not exposed in these experiments.

### Stimulation and electrophysiological recordings

Extracellular recordings were made using self-made carbon fiber glass microelectrodes (tip diameter 15–20 $\mu$m, impedance 0.5–5 M$\Omega$). To minimize vibration the entire preparation including the micromanipulators and stereotaxic frame with the animal was placed on an air pressure–supported table. For recordings from Sp5C neurons, the electrodes were inserted into the brain stem in a region 1.5–2.5 mm caudal to the obex using a microstepper. The position of recording sites was determined by measuring the distance caudal and lateral to the obex and by reading the depth of the microdrive. This measurement has been calibrated with electrolytic lesions and standardized in previous studies (Koulchitsky et al., 2004; Schepelmann et al., 1999). A pair of gold-plated electrodes with a convex contact surface (1 mm diameter) was placed onto the parietal dura on both sides of the sagittal sinus. Rectangular pulses of 0.5 ms duration with intensities of 7–11 V were delivered at a frequency of 0.2 Hz to the dura, while the recording electrode was slowly advanced through the trigeminal nucleus at steps of 2.5 $\mu$m until a single unit responding with constant latency to the electrical stimuli was found.

For recordings from Sp5O neurons the electrodes were inserted through the opening in the occipital bone and the underlying cerebellum to a depth of 7.9–8.1 mm from the skull surface in accordance with stereotaxic coordinates for this region (Paxinos and Watson, 1982). To detect single units in these experiments regular touch stimuli were applied to the facial skin with a fine glass rod.

Units were characterized according to their activation threshold and latency of response to electrical stimulation applied to the dura (for Sp5C neurons) or facial skin (for Sp5O neurons). The position and size of dural receptive fields were determined with graded von Frey filaments. Facial receptive fields were located by probing the skin and the muscles with von Frey filaments and a fine glass rod.

Signals were amplified, filtered and collected on-line using CED 1401 hardware with Spike2 software (Cambridge Electronic Design, Cambridge, UK). In some cases, two units with different spike sizes or forms were recorded simultaneously and discriminated off-line.

### Application of local anesthetic

In six animals from the Sp5C group, lidocaine (2% in saline) was applied topically onto the exposed dura mater. In 39 animals (20