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Thermoreceptive neurons in the dorsal portion of the trigeminal principal nucleus in rats

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HIGHLIGHTS

- Cold neurons in the trigeminal principal nucleus did not project to the thalamus.
- Responses of cold neurons had dynamic, suppressive, and static phases.
- Cold neurons had a large receptive field on the anterior tongue.

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ABSTRACT

The dorsal margin of the trigeminal principal nucleus (PV) contains neurons responsive to innocuous thermal stimulation of the tongue and maybe a thermal relay (Hayama and Hashimoto, 2011). The present electrophysiological study examined whether PV thermoreceptive neurons project to the thalamus and investigated response properties to cold (20 °C) or warm (40 °C) stimulation of the tongue. Twenty-three thermoreceptive neurons were identified in the dorsal portion of the PV. Twenty of the 23 neurons were examined but none projected to the thalamus. Impulse frequencies of 8 of the 11 thermoreceptive neurons examined rapidly increased with cold stimulation, then decreased and gradually increased to steady state level, and rapidly decreased with warm stimulation. Thermal receptive fields were examined for six PV thermoreceptive neurons; five had a large receptive field extending over the whole anterior tongue ipsilateral to the recording side. These findings suggest that the dorsal portion of the PV is not a thermal relay mediating thermal information from the tongue to the thalamus.

1. Introduction

The caudal subnucleus of the spinal trigeminal nucleus (SpVc), the primary thermal relay, receives thermal information conveyed by the trigeminal nerve from the trigeminal field and relays thermal information to the thalamic relay in many animal species [2–5,7,13–17,24]. Our recent electrophysiological study showed that the dorsal portion of the trigeminal principal nucleus (PV) contains neurons responsive to innocuous thermal stimulation of the tongue in rats [10]. Our previous neuroanatomical study demonstrated connections of the dorsal portion of the PV with the thalamic area and with the dorsal portion in the SpVc, which both receive thermal information from the tongue, and suggested that the dorsal portion of the PV is a possible new relay for thermal information from the tongue to the thalamus [10]. However, we did not examine whether thermoreceptive neurons in the PV project axons to the thalamus, which would demonstrate that the dorsal portion of the PV is a new relay.

The present electrophysiological study investigated the projection of PV thermoreceptive neurons to the thalamus and examined the response properties of such neurons. Our results were previously reported in abstract form [9].

2. Materials and methods

All surgical procedures were conducted following the Guidelines for Animal Treatment issued by our institution and by the Physiological Society of Japan. Ten albino Sprague-Dawley male rats weighing 200–350 g were anesthetized with intraperitoneal administration of urethane (1–1.5 g/kg) and maintained areflexia by additional intraperitoneal doses. The animals were mounted on a stereotaxic instrument. A portion of the parietal bone and/or a

Abbreviations: PV, Trigeminal principal nucleus; RF, Receptive field; SpVc, Caudal subnucleus of the spinal trigeminal nucleus.

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rostral portion of the interparietal bone were removed to allow electrical stimulation of the thalamic area, which receives thermal information from the tongue, and recording of neural activity in the dorsal margin of the PV, as described previously [10]. Rectal temperature was maintained at about 37 °C with a water heater. Electrocardiographical monitoring was continued throughout the experiment.

A glass micropipette (tip diameter 1–3 μm), filled with 0.5 M sodium acetate containing 2% pontamine sky blue or 2 M NaCl solution, was inserted into the dorsal portion of the PV through the occipital cortex rostrocaudally at 5–15° from the vertical to explore neuronal activities. An indifferent silver wire electrode was placed on the neck muscle. To examine projection of the PV thermoreceptive neurons to the thalamus, monopolar stimulating electrodes insulated down to the tip (tungsten, 0.2–0.5 MΩ) were inserted into the thalamus at 3.0–3.5 mm posterior to the bregma, 1.5–2.0 mm lateral to the midline, and to a depth of ca. 6.0 mm from the dorsal surface of the cerebral cortex [12]. The mouth of the subject animal was pulled open wide with a weight attached to the lower incisors with a piece of thread. The tongue was positioned ventrolaterally with its tip extending from the oral cavity.

Neuronal activities responsive to thermal stimulation of the tongue were explored by application of distilled water at 20 °C or 40 °C with a pipette to the tongue anterior to the intermolar eminence. Electrical pulses (duration 0.2 ms, intensity 300 μA) were then applied to the thalamic site receiving thermal information [12]. If electrical stimulation evoked impulses in PV thermoreceptive neurons, antidromic evocation of the impulse was examined with the collision test [8,11]. The response properties of the neurons were investigated by passing a continuous flow of distilled water at 20 °C and 40 °C over the anterior tongue from an overhead funnel via gravity flow at a rate of about 3 ml/s for more than 2 min. A large thermal stimulation consisting of a 20 °C change within the physiological range was used to ensure detection of even the slightest thermal responses [20]. The temperature of the surface of the anterior tongue was monitored with a thermistor (PTC-201; Unique Medical Co., Ltd., Komae, Tokyo, Japan). Single or multineuronal activity was passed to a preamplifier and displayed on a cathode ray oscilloscope, then to a data acquisition system (Unique Acquisition Ver.2.11.0.10; Unique Medical Co., Ltd.), digitized with a sampling frequency of 50 kHz, and stored in a hard disk for off-line analysis. Single neuronal activity was identified by examining the spike waveforms and impulse frequency histograms were prepared using a software program incorporated in the same data acquisition system.

Thermal receptive fields (RFs) on the anterior tongue were identified with a cold or warm metal probe (4 mm wide) connected with a glass tube containing water, which had been kept in a water bath at 20 °C or 40 °C. Responses to stimulation of other modalities, touch, mechanical nociceptive, and heat nociceptive, were also examined. Tactile stimulation was applied to the anterior tongue with a glass rod. Mechanical nociceptive stimulation was applied with pre-warmed non-serrated forceps. Heat nociceptive stimulation was applied using distilled water at 50 °C from a pipette soon after cessation of flow of distilled water at 40 °C. Stimulation sites in the thalamus and some recording sites of thermoreceptive neurons were marked by electrolytic lesions or electrophoretic deposition of dye from the recording electrodes. After the experiment, the animal was deeply anesthetized and perfused intracardially with 10% formalin solution in 0.1 M phosphate buffer (pH 7.4). The stimulation sites in the thalamus and recording sites of the thermoreceptive neurons were histologically identified on coronal brain sections (50 μm thick). The experiments were carried out at room temperature (25–28 °C).

3. Results

Twenty-three neurons responding to innocuous thermal stimulation of the tongue were identified in the dorsal portion of the PV at ca. 2.5 mm lateral to the midline, 0.8–2.5 mm posterior to the lambda, and at a depth of 6.2–7.2 mm from the dorsal surface of the cerebral cortex. Spontaneous activity of the neurons was suppressed by warm stimuli but potentiated by cold stimuli, indicating cold neurons. Twenty thermoreceptive neurons were examined to identify antidromic activation from the thalamus. Only 3 of these 20 neurons were activated with latencies of 0.9 ms, 1.0 ms, and 1.3 ms. Activated spikes did not collide with spontaneous spikes, thus excluding antidromic induction. Twenty-one neurons responding to tactile stimulation of the anterior tongue were also identified in the dorsal regions of the PV. Eight of these 21 neurons showed antidromic activation from the thalamus with a mean latency of 1.69 ± 0.34 ms (mean ± SD).

Impulse frequency histograms during cold and warm stimulation were prepared for 11 neurons. Impulse frequencies of 8 of the 11 neurons rapidly increased with cold stimulation, then significantly decreased and gradually increased to steady state level, and rapidly decreased with warm stimulation as shown in Fig. 1. Two of the remaining three neurons had both dynamic and static responses without an intervening suppression period. The other neuron showed only dynamic responses with cold stimulation and no spontaneous discharges. Mean impulse frequencies during the last 30 s of cold and warm water application were calculated as static frequencies of 15.7 ± 5.9 imp/s at 20 °C and 0.1 ± 0.3 imp/s at 40 °C (mean ± SD, n = 10), excluding the neuron with only dynamic

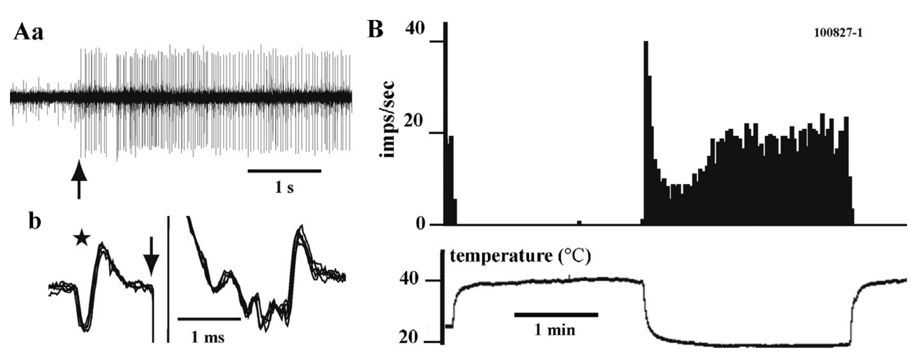

Fig. 1. Thermal responses of a single PV neuron (neuron no. 100827-1). (Aa) Thermal responses to cold water (20 °C). The arrow indicates onset of the stimulation. (Ab) Superimposed traces of field and/or action potentials activated with thalamic electrical stimulation (downward arrow) following action potentials of the thermoreceptive neuron (star mark). The thermoreceptive neuron was not considered to show antidromic activation because the shape of the action potential with latency of ca. 2.0 ms is similar but slightly different from that of the thermoreceptive neuron preceding thalamic stimulation. (B) Upper part, impulse frequencies during cold stimulation with bin width 2 s. The maximum discharge rate of the dynamic response was 41 imp/s. Lower part, temperature change of the tongue surface measured by a thermistor.