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# The locus coeruleus projects to the mesencephalic trigeminal nucleus in rats

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ABSTRACT

The ganglion-cells in the mesencephalic trigeminal nucleus (Me5) process proprioceptive signals from the masticatory muscles and the periodontal ligaments, and are considered to regulate the rhythm of biting and bite strength. The locus coeruleus (LC) is the major source of noradrenergic projections in the brain and plays an important role in stressful situations and aggressive behavior. The two nuclei are adjacently located to each other in the lateral part of the periaqueductal gray matter of the fourth ventricle. In the present study, a small number of neurons were labeled in the LC with a neuronal tracer biotinylated dextran amine. The labeled single axons were traced from the labeled LC neuronal somata to the ipsilateral Me5 region where they produced terminal-like swellings. Some of the swellings appeared to make contact with the ganglion-cells of the Me5. These results suggest that the LC regulates the bite strength by modifying the ganglion-cell activity in the Me5. Additionally, these findings shed light on the enigma of why the main part of the Me5 at the level of pons is located at the lateral end of the gray matter ventral to the fourth ventricle, instead of at the trigeminal ganglion.



## 1. Introduction

The mesencephalic trigeminal nucleus (Me5) is composed of pseudo-unipolar cells like other sensory ganglia and provides primary afferent fibers from the muscle spindles of jaw closers and the periodontal receptors (Alvarado-Mallart et al., 1975; Jacquin et al., 1983; Liem et al., 1991). It projects to the trigeminal motor nucleus and the premotor neurons and is thought to regulate biting rhythm (Shigenaga et al., 1988; Verdier et al., 2004). It is located at the ventrolateral end of the periaqueductal gray matter of the midbrain and at the lateral end of the gray matter of the floor of the fourth ventricle. As a result, at the level of pons, Me5 is located just lateral to the locus coeruleus (LC).

The LC is the major source of noradrenaline in the brain and it sends axons to most of the brain regions, including the cerebral cortex, basal forebrain, amygdala, and the hypothalamus (Foote et al., 1983). It is thus associated with emotion-related regions, such as the basal forebrain and the hypothalamus, and plays an important role in aggressive behavior, defense, and defeat (Moore and Bloom, 1979; Miczek et al., 2004). In addition to emotion-related projections, the LC also projects to a variety of regions throughout the trigeminal somatosensory pathway (Simpson et al., 1997).

The synapses on the neuronal somata in the Me5 are immunoreactive to noradrenaline, GABA, dopamine, serotonin, and glutamate (Copray et al., 1990). Therefore, the Me5 receives afferent projections from variety of brain regions that may regulate brain function. Among these, the noradrenergic fibers were suggested to originate from the LC (Copray et al., 1990); however, there has been no direct evidence for projection from the LC to the Me5. In the present study, a small amount of LC cells are labeled with an axonal tracer in order to identify whether the LC neurons directly project to the Me5 or not.

## 2. Materials and methods

### 2.1. Animals

Three adult male Sprague–Dawley rats weighing 250–300 g were housed individually and allowed free access for food and water. The animals were maintained at an ambient temperature (23 °C) and on a 12 h light–dark cycle (lights on at 08:00 h). All animals were cared in accordance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals* and the *Regulations for Animal Experiments in Gifu University*. This study was approved by the *Committee for Animal Research and Welfare of Gifu University*.

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### 2.2. Tracer injections

The animals were deeply anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and were then mounted on a stereotaxic frame (Narishige, Tokyo, Japan). A skin incision and a bone opening was made over the LC and a glass micropipette filled with biotinylated dextran amine (BDA; BDA-10,000, Molecular Probes, Eugene, OR) was inserted according to the stereotaxic atlas (Paxinos and Watson, 1998). The parameters of the tip of the glass micropipette were 0.7 mm posterior from the interaural line, 1 mm lateral from the midline, and 6.7 mm deep from the surface of the cortex. Under intermittent gas pressure using a Picospritzer II (General Valve, Fair Field, NJ), a small amount of BDA was injected into the LC.

Four days after the injection, the animals were anesthetized with a lethal dose of sodium pentobarbital (80 mg/kg, i.p.) and were then perfused through the ascending aorta with 300 ml of saline followed by 1 liter of a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer (PB), pH 7.4. The brain was removed from the skull and was immersed in a chilled solution of 30% sucrose in PB.

### 2.3. Histology

After 2 days, 50-μm thick coronal sections were cut frozen and collected in PB. All the sections were processed for BDA visualization. The sections were incubated with 1% hydrogen peroxide in PB for 2 h and then with 0.3% polyoxyethylene octylphenyl ether (Wako, Osaka, Japan) in PB for 3 h. The sections were rinsed and then processed with Elite ABC kit (Vector, Burlingame, CA) at 4 °C overnight. The sections were rinsed and further processed with a 0.1% diaminobenzidine tetrahydrochloride (DAB, Dojin, Kumamoto, Japan) solution with 2.5% ammonium nickel sulfate (Wako), 0.2% β-D-glucose (Sigma–Aldrich, St Louis, MO), 0.04% ammonium chloride (Wako), and 0.001% glucose oxidase (Sigma–Aldrich). The sections were mounted consecutively in gelatinized slide glasses, cleared with xylene, and coverslipped using DPX (Fluka, Buchs, Switzerland).

### 2.4. Observation and analysis

We used a bright-field microscope with a phase-contrast condenser (BX50, Olympus, Tokyo) and a differential interference contrast microscope (Axioskop2 MOT, Zeiss, Jena, Germany) to identify the positions and borders of the LC and the Me5. The injection site was photographed using the differential interference contrast microscope equipped with a CCD camera (AxioCam, Zeiss) and software (AxioVision Release 4.6.3, Zeiss). Labeled neurons were outlined under the phase-contrast field. The BDA-labeled axons were traced from the labeled LC cells using a microscope (DPTIPHOTO, Nikon, Tokyo, Japan) equipped with a camera lucida. The axons were reconstructed from the tracings over several sections. Photographs of these sections were taken under the phase-contrast microscope equipped with a CCD camera (CMOS300-USB2, Fortissimo, Tokyo, Japan). The BDA-labeled LC neurons were reconstituted from several photographs with different foci and photographs from different sections. These photographs were aligned using blood vessels and tissue artifacts.

## 3. Results

A total of 33 labeled neurons were identified within the LC. Fig. 1 shows a differential interference contrast photomicrograph of the injection site. The neurons of the LC were seen in the right side of Fig. 1B and the darkly stained labeled cells were localized in the LC. The ganglion-cells of the Me5 were apparent at the left of the figure. The labeled LC cells were multipolar (18%), triangular (48%), fusiform (24%), or oval (9%) in shape. The sizes of the perikarya of the labeled cells varied from 83 μm$^2$ to 466 μm$^2$ (mean: 252.4 μm$^2$; SD: 75.9 μm$^2$) (Fig. 2).

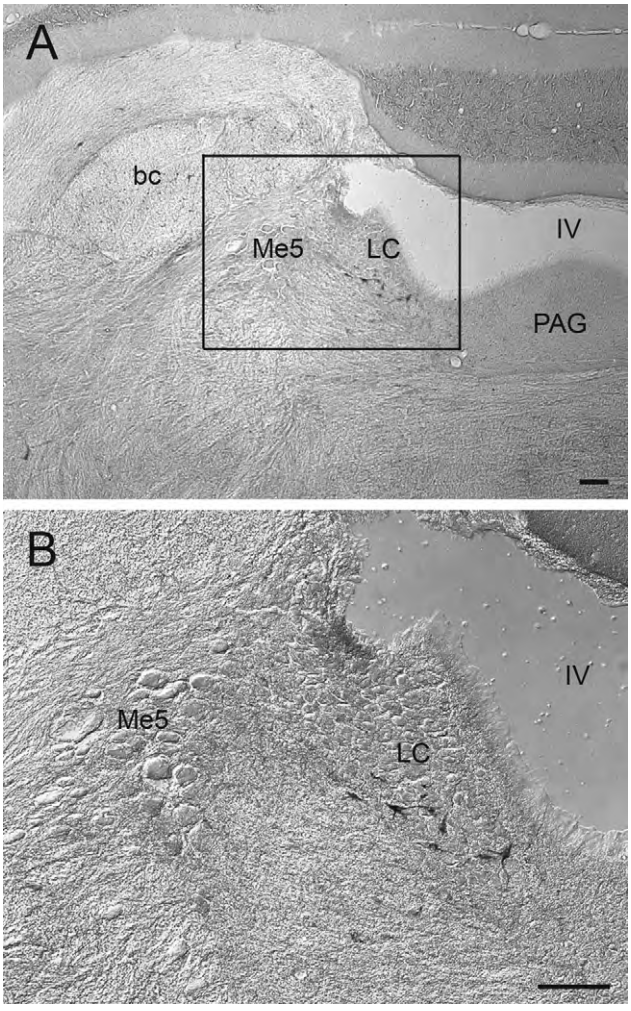


**Fig. 1.** Injection site showing darkly stained locus coeruleus (LC) neurons that are labeled with biotinylated dextran amine (BDA). (A) A photomicrograph using a low power objective lens. The box indicates the field shown in (B). (B) A photomicrograph using differential interference contrast microscope. Labeled neuronal somata are seen in the lower part of the LC near the border to the locus subcoeruleus. IV, fourth ventricle; bc, brachium conjunctivum; Me5, mesencephalic trigeminal nucleus; PAG, periaqueductal gray; scale bars, 100 μm.

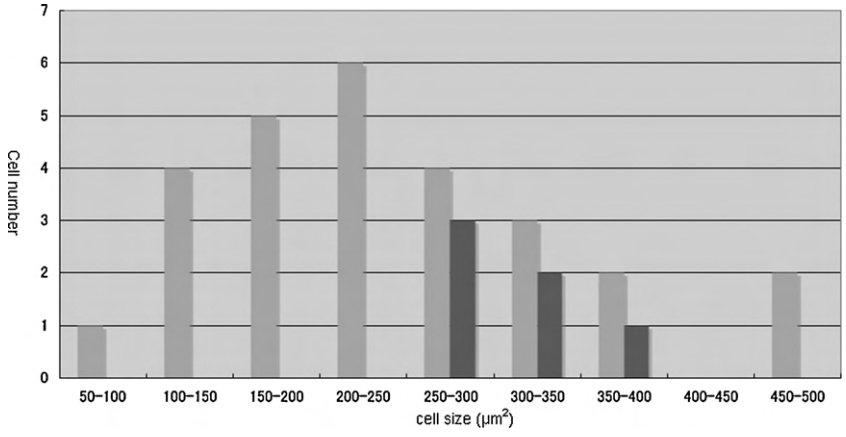


**Fig. 2.** Distribution of the size of labeled LC cells (gray columns) and that of Me5-projecting LC cells (black columns).