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Localization of masticatory motoneurons in the trigeminal motor nucleus of shrew and pig, with emphasis on the innervation ratio in the shrew

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ABSTRACT

Objectives: We investigated the topographical representation of the masticatory muscles, primarily in the trigeminal motor nucleus (TMN) of the house musk shrew (*Suncus murinus*) and miniature pig, in a series of mammalian comparative anatomical studies. Additionally, correlations between motoneurons and muscles were investigated in order to examine the functionality of each muscle in the trigeminal motor system.

Methods: Motoneurons were labeled using horseradish peroxidase (HRP) injection into each muscle innervated by the trigeminal nerve. In the shrew, the sizes and numbers of HRP-labeled neurons, muscle weight, and numbers of muscle fibers were measured in the innervated muscles, and correlation coefficients for the relationships among these parameters were calculated.

Results: The motoneuron cluster of each muscle was arranged in the TMN in a manner similar to that observed in other previously reported animals, but the distribution of the lateral pterygoid motoneurons varied between species. In addition, considerably higher overlap was observed in each pig jaw-closer motoneuron cluster compared with other animals. The approximate innervation ratios were as follows: masseter, 337; temporal, 322; anterior digastric, 137; medial pterygoid, 110; lateral pterygoid, 79; mylohyoid, 42; tensor veli palatini, 42; transverse mandibular, 16; and tensor tympani muscles, 5.

*Conclusions*The distribution pattern of the masticatory motoneurons was clearly observed in the shrew and pig TMN, and various correlations between the motoneuron and innervated muscles were determined.

1. Introduction

The myotopical arrangement of the masticatory motoneurons in the trigeminal motor nucleus (TMN) has been clarified in rats [1–3], guinea pigs [4,5], rabbits [6], cats [1,7], and monkeys [8], beginning when Mizuno et al. [1] introduced a practical retrograde horseradish peroxidase (HRP) method to this field of study. The localization of the tensor tympani muscle (TT) and tensor veli palatini muscle (TVP) has been identified in a number of animals, including rodents and primate species [9–20]. However, insectivores, which retain the primitive mammalian form [21], and ungulates have not yet been examined in this area of research. In the present study, the motoneuron distribution patterns of muscles innervated by the trigeminal nerve in the house musk shrew (*Suncus murinus*, Soricidae) and the miniature pig (an even-toed ungulate) were confirmed, using a retrograde HRP method for comparative anatomical study.

Several investigators have calculated the innervation ratios (IRs) of selected muscles in the lower limb [22–26], upper limb [24–26], and orbital muscles [27] of humans and some animals, by counting the number of muscle and nerve fibers according to the definition initially proposed by Eccles and Sherrington [22] (see [28]). In the present study, we investigated correlations, particularly IRs, between muscles and motoneurons in the trigeminal motor system by measuring the size and number of labeled motoneurons, and the number of muscle fibers.

2. Materials and methods

We used 58 house musk shrews (adult males, body weight 48–65 g), and 15 infant miniature pigs (both sexes, 32–44 days

Abbreviations: AD, anterior digastric muscle; DLD, dorsolateral division; DLDm, medial part of the dorsolateral division; DLDp, principal part of the dorsolateral division; IR, innervation ratio; lat, lateral direction; ll, lateral lemniscus; LP, lateral pterygoid muscle; M, masseter muscle; Mh, mylohyoid muscle; MP, medial pterygoid muscle; r, root of the trigeminal motor nerve; S, sensory nucleus of the trigeminal nerve; scp, superior cerebellar peduncle; T, temporal muscle; TM, transmandibular muscle; TMN, trigeminal motor muscle; TT, tensor tympani muscle; TVP, tensor veli palatini muscle; VMD, ventromedial division..

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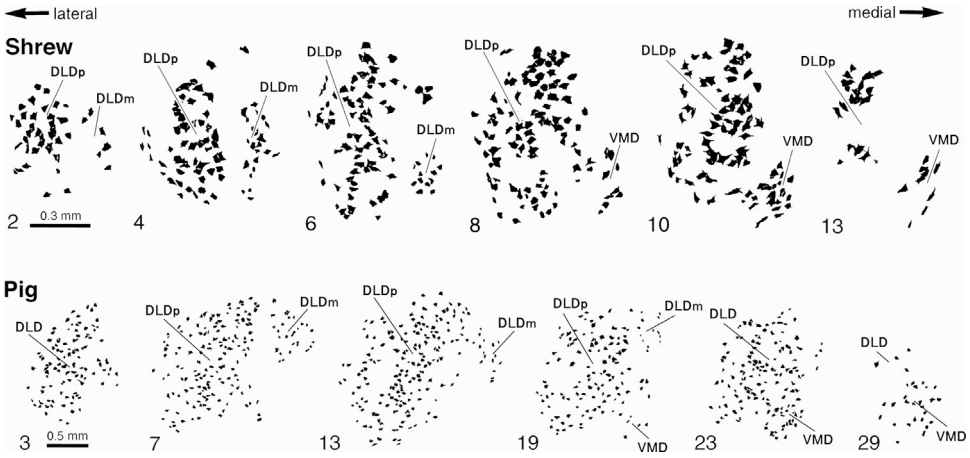

Fig. 1. Projection drawings of the trigeminal motor nucleus (TMN) of house musk shrew and infant miniature pig in serial cross-sections of 50- and 60-μm thickness, respectively, and numbered rostrocaudally. The nucleus is cytoarchitecturally divided into the dorsolateral (DLD) and ventromedial divisions (VMD). The DLD is further divided into the principal (DLDp) and medial parts (DLDm).

after birth, body weight 2.7–5.3 kg). The pigs were a hybrid between the Crown and Ohmini breeds, and were supplied by the Laboratory of Animal Reproduction, Department of Agricultural Sciences and Natural Resources, Kagoshima University Faculty of Agriculture (Kagoshima, Japan). The Animal Care and Use Committee of the Kagoshima University Dental School approved this study. A consecutive procedure from anesthesia to brain preparation was performed, as described in our previous report [29]. Ketamine (i.m., 50–60 mg every hour) was used to maintain anesthesia in the pig under basic narcosis using sodium barbiturate (i.p., 3.0–4.0 mg/kg body weight). The TMN cytoarchitectural structure with the standard Nissl stain in both the shrew and pig was observed and sketched through a camera lucida with a light microscope (Fig. 1). HRP saline solution (30%; 0.3–4.0 μl) in the shrew and HRP solution (5–30%; 3.5 μl–1 ml) in the pig were manually injected into each masticatory and tensor muscle.

2.1. Measurement of muscle weight and the number of muscle fibers

Intact individual muscles of the shrew were carefully removed at their origin and insertion under a dissection microscope. Moisture on the surface of the muscles was wiped away with absorptive paper before weighing the muscles using an electronic balance (Sartorius, H110). These muscles were assumed to consist only of muscle fibers, although muscle usually does include other tissues (Table 1, Item A). A maceration method was used to count the number of muscle fibers in order to avoid the high risk of count errors caused by length and direction of muscle fiber differences on observation of thin muscle sections [30]. Each isolated muscle was macerated with 5 N HCl saline solution at 60 °C for 0.5–3 h, rinsed with a buffered saline three times, and separated into individual muscle fibers on a Petri dish. A needle with a bent tip under a dissection microscope was used to pick up the muscle fibers individually for counting (Table 1, Item B).

In a previous study, some Soricidae family subjects had a small number of muscle spindles only in the temporal (T) and masseter (M) masticatory muscles [31], and the spindle had several intrafusal muscle fibers [32]. When these factors were taken into consideration, the α- and γ-motoneurons could not be clearly distinguished in each graph of Fig. 2. Consequently, it was considered that muscle fiber and motoneuron values were of the extrafusal muscle and α-motoneurons, respectively.

2.2. Measurement of the number and size of labeled neurons

The maximum number of HRP-labeled neurons in each injected muscle of the shrew was determined, and a camera lucida with a light microscope was used to sketch the neurons at a 1026× magnification (Table 1, Item C). The counted and sketched motoneurons clearly included a cell nucleus in the Nissl stained section. The long and short diameters of the sketched neurons were measured using a digitizer attached to a computer (Nikon Cosmoson 1S) (Table 1, Items D and E). The Schadé and Van Harreveld's formula [33] (volume $= 1.04 \times 1/6\pi ab\sqrt{ab}$; a: long diameter, b: short diameter) (Table 1, Item F) was used to calculate neuronal volume, and a Student's t-test was used to examine differences between the mean motoneuron volumes of each muscle. In addition, the total volume of motoneurons innervating each muscle was calculated (Table 1, Item G). Statistical software (Hulinks, KaleidaGraph, ver. 4.0) was used to create scatter diagrams with R to detect relationships between the expected muscle weight or volume of a single neuron, and the calculated values.

3. Results

The Nissl-stained TMN of the shrew appeared rostrocaudally in 13 cross-sections each of 50-μm thickness, and that of the infant pig in 30 cross-sections each of 60-μm thickness (Fig. 1). The TMN of these animals could be roughly divided into two cell cluster groups: the dorsolateral (DLD) and ventromedial divisions (VMD), as was reported in a previous study [1]. The DLD had a part of the medial prominence (DLDm) that was observed as a motoneuron pool, which was medially separated from the principal part (DLDp) of the DLD in a rostral level of the TMN (Fig. 1). In the shrew, it was sometimes difficult to distinguish the DLDm from the VMD in the Nissl stain because the DLDm was often seen as a rostral elongation of the VMD.

3.1. Topographical representation of the masticatory muscle in the trigeminal nucleus

Following HRP injections into the T, M, and medial pterygoid muscles (MP) of shrews and pigs, labeled motoneurons were observed in the dorsal, central or lateral, and ventral parts of the DLDp (Figs. 3a, b, a', b' and 4), and AD and Mh motoneurons were found in the dorsal and ventral parts of VMD, respectively (Figs. 3d, d', and 4). The T, M, and MP motoneurons were