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Original Article

Premotoneuronal inputs to early developing trigeminal motoneurons

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ABSTRACT

Objectives: We previously reported that masseter motoneurons (MMNs) and digastric motoneurons (DMNs) in postnatal day (P) 1–5 rats receive convergent inputs from the lateral (l-) and medial (m-) supratrigeminal regions (SupV), intertrigeminal region (IntV), and dorsal region of the principal sensory trigeminal nucleus (PrV). The l-SupV sends burst inputs predominantly to the MMNs. We compared the synaptic inputs to P9–12 rat MMNs and DMNs with those found in the previous study involving P1–5 rats.

Methods: We performed whole-cell recordings and laser photolysis of caged glutamate in the MMNs and DMNs of P9–12 rats.

Results: Similar to P1–5 rats, the photostimulation of multiple regions within the l-SupV, m-SupV, IntV, and dorsal PrV, induced postsynaptic currents (PSCs) in both P9–12 MMNs and DMNs. Photostimulation induced predominantly low-frequency PSCs in both P9–12 motoneurons, whereas l-SupV photostimulation predominantly induced burst PSCs in P1–5 rats. However, when the caged glutamate concentration was doubled, l-SupV photostimulation evoked burst PSCs in all P9–12 MMNs. Furthermore, l-SupV and m-SupV photostimulation evoked burst or low-frequency PSCs at significantly higher rates in the MMNs compared to in the DMNs.

Conclusions: These results suggested that, similar to P1–5 motoneurons, both P9–12 motoneurons received convergent inputs from the SupV, IntV, and PrV; however, the input–output gains of some of the premotor neurons decreased. These synaptic input changes may contribute to the proper development of chewing.

1. Introduction

Feeding behavior changes drastically during the postnatal period in mammals. Suckling is first performed, following which chewing develops. The muscles that close the jaw show little activity during suckling; however, these muscles require high levels of activity in animals chewing hard or tough food [1–3]. Thus, the motor commands sent to the masticatory muscles from the neural circuits that control suckling and chewing likely change as the teeth and orofacial musculoskeletal system develop. The last-order premotor neurons that transmit such motor commands to the trigeminal motoneurons play an important role in controlling masticatory muscle activity [4,5]. The supratrigeminal (SupV) and intertrigeminal (IntV) regions, the dorsal regions of the principal sensory trigeminal nucleus (PrV), and the reticular formation dorsal to the PrV (dRt) that surround the trigeminal motor nucleus (MoV) contain premotor neurons that innervate the motoneurons of the muscles that open and close the jaw [6,7]. Furthermore, the premotor neurons in these regions that target the MoV receive afferent inputs from a number of regions, such as the orofacial structures [8–10], central pattern generator for mastication [4,11], cerebral cortex [12], amygdala [13], and lateral hypothalamus [14]. Therefore, these premotor neurons might transmit the motor commands for suckling and/or chewing. We previously reported that, in postnatal day (P) 1–5 neonatal rats, both single masseter motoneurons (MMNs) and digastric motoneurons (DMNs) receive convergent glutamatergic inputs from the SupV, IntV, PrV, and dRt,

Abbreviations: ACSF, artificial cerebrospinal fluid; AP, action potential; DMN, digastric motoneurons; dRt, reticular formation dorsal to the PrV; IntV, intertrigeminal region; l, lateral; m, medial; MMN, masseter motoneurons; MoV, trigeminal motor nucleus; P, postnatal; PrV, principal sensory trigeminal nucleus; PSC, postsynaptic current; SupV, supratrigeminal region

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and that the lateral SupV sends burst inputs predominantly to the MMNs [15]. However, it is uncertain whether single motoneurons in juvenile (P9–12) rats receive convergent inputs from premotor neurons in the SupV, IntV, PrV, and dRt. Furthermore, it is unclear whether the premotor neurons in the lateral SupV of P9–12 rats send burst inputs predominantly to the MMNs. In this study, we investigated the synaptic inputs to MMNs and DMNs from the SupV, IntV, PrV, and dRt in P9–12 rats using the laser photolysis of caged glutamate. This allowed for the systematic stimulation of multiple confined regions, and simultaneous whole-cell recordings of motoneurons that were identified by a fluorescent dye.

2. Materials and methods

2.1. Slice preparation

The experiments were performed on coronal slices of the brainstem from P9–12 Wistar rats ($n = 59$). The slices were prepared as described in the Supplementary materials.

2.2. Retrograde labeling of jaw-closing and jaw-opening motoneurons

In order to discriminate between the motoneurons innervating the muscles that open or close the jaw, we used the fluorescence labeling technique [16,17] as described in the Supplementary materials.

2.3. Patch-clamp recordings

Whole-cell patch-clamp recordings were performed in the SupV neurons and the jaw-closing and jaw-opening motoneurons according to the procedures described in the Supplementary materials.

2.4. Photostimulation

We systematically stimulated various sites in the slices while simultaneously performing whole-cell recordings in the SupV neurons or motoneurons. Caged glutamate was subjected to laser photolysis by a 365-nm nitrogen-pulsed Micropoint laser system equipped with galvanometer-based steering lenses (Photonic Instruments, Inc., St. Charles, IL) [cf. 15]. The laser beam was positioned by steering the lenses with the MetaMorph software (Molecular Devices, LLC, Sunnyvale, CA). After using a 40X water immersion objective to establish the whole-cell recording configuration in an MMN, DMN, or SupV neuron, we changed the 40 × objective to an Olympus 4 × (0.28 NA) objective. Furthermore, 4-methoxy-7-nitroindolinyl-caged L-glutamate (MNI glutamate; Tocris Bioscience, Bristol, UK) was added to the recirculating artificial cerebrospinal fluid (ACSF) to a final concentration of 300 μM in 25 mL of ACSF. In some experiments, the final MNI glutamate concentration was increased to 600 μM. All the photostimulation experiments began at least 10 min after the addition of MNI glutamate. The laser beam was focused onto an area of approximately 10 μm in diameter on the brainstem slice using the 4 × objective. Single laser-beam pulses (2–6 ns) were delivered to the center of each site to trigger the focal photolysis of MNI glutamate. The photostimulation strength was always set at 70 (a.u.) in the MetaMorph software, which was the same setting used to stimulate the P1–5 rats in our previous experiment [15]. Each site was stimulated 4–8 times. The response pattern (burst PSC, low-frequency PSC, or no response) shown by over 60% of the responses across 4–8 trials was considered the representative response pattern of each sample at each stimulation site. Responses of more than 25 pA were considered as effectively evoking postsynaptic responses.

To determine the spatial resolution of the photostimulation, a single-pulsed laser beam was delivered at 5-s intervals to 100 different sites that surrounded the recording sites of the SupV neurons. The 100 sites were arranged in a 10 × 10 array with 40-μm spaces between adjacent rows and columns (Supplementary Fig. 1A).

As the brainstem is larger in P9–12 animals than in P1–5 animals, and the laser beam could only be positioned within an area of about 780 × 1200 μm^2 in our system, we could not stimulate all the areas in the SupV, IntV, and PrV of the P9–12 animals in single-motoneuron recording sessions. Thus, we stimulated 76 different sites in 36 slices at 2-s intervals. The photostimulation sites adjacent to the MoV, covering the SupV, IntV, and the medial border of the PrV, were arranged in an L-shape, with distances of 120 μm and 78 μm between the neighboring sites in the dorsal-ventral and medial-lateral orientations. The boundary of the MoV was identified using the 40 × objective. We assigned the areas of the m-SupV, l-SupV, IntV, and PrV based on histological observations made from Nissl-stained slices. In 9 different slices, photostimulation was delivered to 40 sites in the more lateral regions, including the medial PrV. These 40 sites were arranged in an I-shape with distances of 120 μm and 78 μm between the neighboring sites in the dorsal-ventral and medial-lateral orientations (Fig. 4A).

2.5. Histological procedure

The histological identification of the photostimulation sites was performed as described in the Supplementary materials.

2.6. Statistics

The data are presented as the mean ± SEM, except when specifically indicated. The data were compared within and between groups using the nonparametric Mann-Whitney *U*-test. Results with probability (p) values less than 0.05 were considered statistically significant. When the comparisons were performed among 4 groups, results with p values less than 0.0083 (0.05/6) were considered statistically significant. The statistical analyses were performed using the SPSS Statistics 17.0 (SPSS Japan, Tokyo, Japan) software.

3. Results

3.1. Spatial resolution of the photostimulation in P9–12 rats

In order to quantify the spatial resolution of the action potentials of putative premotor neurons by a single laser-beam pulse at P9–12 rats, we examined the effects of a single stimulation pulse on the action potentials (APs) of individual putative SupV premotor neurons with a method similar to that used to examine P1–5 rats [15]. Photostimulation applied close to the soma or proximal dendrites evoked APs (Supplementary Fig. 1A, 1D). Stimulation at some sites evoked bursts of APs at frequencies greater than 50 Hz (Supplementary Fig. 1Dc, e, 1Ec, e).

For the 19 SupV neuron recordings, sites in which stimulation evoked at least one AP were located within a 103 ± 43.4 μm (mean ± SD, median: 89.4 μm; range: 40–179 μm) radius from the tip of the patch pipette, which corresponded to the location of the soma (Supplementary Fig. 1De). This value was similar to the 126 ± 22.7-μm radius for P1–5 rats [15]. Thus, if the two effective photostimulation sites that evoked postsynaptic responses in a motoneuron were separated by more than twice the radius, the

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