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CONTRIBUTION OF THE LATERAL LEMNISCUS TO THE CONTROL OF SWALLOWING IN DECEREBRATE CATS

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Abstract—Lateral lemniscus, a relay nucleus of auditory sensation, is involved in the control of phonatory movements such as human speech and vocalization of animals. The present study was designed to test whether neurons in the lateral lemniscus contributed to the control of swallowing, one of non-phonic oro-pharyngolaryngeal movements. In acutely decerebrated cats (n = 15), swallowing was induced by electrical stimulation (20–80 μA at 10 Hz for 20 s with rectangular pulses of 0.2 ms duration) delivered to the superior laryngeal nerve (SLN). Repetitive electrical stimulation (30–50 μA at 50 Hz for 10–20 s) applied to the dorsal nucleus of the lateral lemniscus (LLD) increased the number and reduced the latency to the onset of the SLN-induced swallowing. On the other hand, stimulation of the ventral nucleus of the lateral lemniscus and the paralemniscal area, corresponding to the ventrolateral part of the parabrachial nucleus and the Kölliker–Fuse nucleus, often suppressed the SLN-induced swallowing. Microinjection of NMDA (0.1–0.15 μl, 5.0–10 mM) into the LLD through a stereotaxically placed glass micropipette facilitated the SLN-induced swallowing, i.e., the number was increased and the latency of swallowing was reduced. We also injected muscimol (a gamma amino-butyric acid (GABA)$_A$ receptor agonist), bicuculline (a GABA$_A$ receptor antagonist) and baclofen (a GABA$_B$ receptor agonist) into the LLD (0.1–0.15 μl and 5.0 mM for each substance). It was observed that an injection of muscimol suppressed the SLN-induced swallowing. However, an injection of bicuculline facilitated the swallowing. An injection of baclofen did not alter the swallowing. These results suggest the presence of functional topography in the lateral lemniscus and the paralemniscal area in relation to the control of swallowing. The facilitatory LLD-effects on swallowing are modulated by glutamatergic and GABAergic receptors on neurons in the LLD.

Key words: audiomotor control, brainstem, central pattern generator, GABAergic projection, oro-pharyngolaryngeal movement, respiratory movements.

INTRODUCTION

The brainstem plays important roles relevant to phonatory and non-phonatory oral–laryngeal–pharyngeal functions. Vocalization of animals and speech of human beings are representative phonatory behaviors. Non-verbal vocalization in humans, such as an infant's cry and laughter, represents innate vocal reactions whose acoustic structure is genetically programmed (Scheiner et al., 2004). Swallowing, which is one of the non-phonatory behaviors, is a coordinated motor sequence of muscles in the alimentary tract and consists of the orofacial, pharyngeal and esophageal phases. It is triggered by sensory signals from pharyngolaryngeal mucosa which are sent to the nucleus of the tractus solitarius (NTS) via internal branch of the superior laryngeal nerve (SLN). Because the stereotyped motor sequence of once-started swallowing is accomplished automatically without any further input, swallowing is controlled by the central pattern generator (CPG) in the medulla (Doty and Bosma, 1956; Weerasuriya et al., 1980; Miller, 1982; Harada et al., 2005; Barlow, 2009; Bianchi and Gestreau, 2009). Although swallowing and vocalization are different motor behaviors, both involve a precise coordination of a variety of orofacial, pharyngolaryngeal and respiratory muscles by the activation of common neuronal structures in the brainstem (Jean, 2001; McFarland and Tremblay, 2006; Simonyan and Horwitz, 2011).

Auditory sensation plays a critical role in the control of vocalization such as the Lombert reflex. Even in decerebrate cat preparations, auditory sensation

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Abbreviations: ANOVA, analysis of variance; CNF, cuneiform nucleus; CPG, central pattern generator; DC, data recorder; DIA, Diaphragm; DRG, dorsal respiratory group; DSG, dorsal swallowing group; EMG, electromyogram; GH, geniohyoideus muscle; H, horizontal; KFN, Kölliker–Fuse nucleus; L, lateral; LLD, dorsal nucleus of the lateral lemniscus; LLV, ventral nucleus of the lateral lemniscus; MRF, medullary reticular formation; NA, nucleus ambiguus; NMDA, *N*-methyl-D-aspartic acid; NTS, nucleus tractus solitarius; P, posterior; PBN, parabrachial nucleus; PCA, posterior cricoarytenoid muscle; PRF, pontine reticular formation; SCP, superior cerebellar peduncle; SD, standard deviation; SLN, superior laryngeal nerve; SNr, substantia nigra pars reticulata; SpO_2, saturation of percutaneous arterial oxygen; TA, thyroarytenoid muscle; VSG, ventral swallowing group.

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stimulation not only facilitated laryngeal reflex (Nonaka et al., 1997, 2006) but also enhanced vocalization which was evoked by electrical stimulation applied to the midbrain periaqueductal gray (Nonaka et al., 1997). Therefore, the auditory sensation signals have dual functions. One is to provide auditory sensation to the auditory cortices, and the other is to modulate oropharyngolaryngeal movements at the level of the brainstem. The latter function is supported by previous studies showing that neurons in the ventrolateral pons including the lateral lemniscus are involved in the generation of vocalization in various animal species (de Lanerolle, 1990; Kirzinger and Jürgens, 1991; Schuller et al., 1997; Sugiyama et al., 2010). However, no evidence has been shown whether an activation and inactivation of neurons in the lateral lemniscus modulated non-phonatory oropharyngolaryngeal movements such as swallowing. Because swallowing requires an activation of laryngeal reflex loops, we hypothesize that the auditory sensation pathway via the lateral lemniscus contributes to the control of swallowing.

The purpose of this study is to test the above hypothesis that neurons in the lateral lemniscus are involved in the control of swallowing. For this, we employed acute decerebrate cat preparations in which swallowing was readily initiated by stimulating the internal branch of the SLN. First, electrical stimulation was applied to the lateral lemniscus and paralemniscal area to determine optimal sites where the stimulation facilitated and suppressed the SLN-induced swallowing. Because lemniscal neurons receive glutamatergic (Wu, 1999) and gamma amino-butyric acid (GABA)ergic efferents (Yang and Pollak, 1994a,b; Chen et al., 1999), glutamatergic and GABAergic agents were microinjected into the region where electrical stimulation facilitated the SLN-induced swallowing. Particular attention has been paid to examine how the electrical and chemical stimulation modulated the stereotyped sequence of oro-pharyngolaryngeal muscle contractions during swallowing. Finally, role of auditory signals was discussed in the control of phonatory and non-phonatory oro-pharyngolaryngeal movements.

EXPERIMENTAL

Animal preparation

The present study was carried out in accordance with the Guidelines for Animal Experiments of the Asahikawa Medical University and National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. Every attempt was made to minimize animal suffering and to reduce the number of animals. The study is based on data obtained from 15 adult cats (nine male and six female, raised in an animal laboratory of the Asahikawa Medical University) that weighed from 1.8 to 3.3 kg.

Surgical procedures

The cats were anesthetized by halothane (Fluothane, Takeda Co., Osaka, Japan; 0.5–3.0%) and nitrous oxide gas (0.5–1.0 l/min) with oxygen (3.0–5.0 l/min). A cannula was placed in the femoral artery of each cat to monitor blood pressure. Another cannula was placed in the cephalic vein to administer epinephrine (Bosmin, Daiichi Co., Osaka, Japan). The left SLN was dissected, and a pair of silver wires (diameter of 100 μm) was attached to the proximal end. The silver wires were used to electrical stimulation of the SLN to evoke swallowing.

Bilateral carotid arteries were ligated. The cats were surgically decerebrated at the precollicular-postmammillary level. The dorsal portion of the occipital bone and tentorium was removed to allow access to the brainstem. The head and the dorsal processes of T_{1-3} were fixed in a stereotaxic apparatus. The body was supported by a rubber hammock at the abdominal level. After the completion of all surgical procedures, anesthesia was discontinued at least 1 h before data collection began. The body temperature was kept at 36–38 °C using radiant heating lamps. A cannula was placed in the nasal cavity to monitor expiratory endotidal CO_2 concentration using CO_2-monitoring system (OLG-2800, Nihon-Koden, Co., Tokyo, Japan). Saturation of percutaneous arterial oxygen (SpO_2) level was also measured by an oxygen sensor (disposable oxy-probe; type TL-252T, Nihon-Koden, Co.), which was tightly attached to the skin of the ear, and pulse-oximeter (OLV-2700, Nihon-Koden, Co.). The cats spontaneously breathed room air. Expiratory CO_2 was maintained between 3% and 5%, and SpO_2 level was maintained at more than 96% in the room air condition. The mean blood pressure was maintained higher than 100 mmHg by an intravenous infusion of epinephrine (0.1–0.3 mg/kg, infusion rate of 0.01 mg/min) if necessary.

Electromyogram (EMG) recordings and SLN-induced swallowing

EMG were recorded from the geniohyoideus (GH), the posterior cricoarytenoid (PCA; laryngeal abductor), thyroarytenoid (TA; the laryngeal adductor) muscles, and the diaphragm (DIA) using a pair of thin (50 μm) insulated stainless steel wires with 1 mm of the tips exposed. The EMG signals were amplified with low (100 Hz) and high (3 kHz) pass filters, monitored on an oscilloscope, and stored on a DAT data recorder (DC to 10 kHz, RD-135 T, TEAC Co., Japan) for off line analysis with an AD Instruments Powerlab system and softwares (Chart and Scope) running on a computer.

Swallowing was evoked by electrical stimulation delivered to the SLN. The stimulation consisted of rectangular pulses of 0.2-ms duration, an intensity of 20–80 μA, and a frequency of 10–20 Hz lasting for 20 s (mostly 20 s). Because SLN stimulation with either higher intensities or higher frequencies evoked coughing (Harada et al., 2005; Adachi et al., 2010), parameters of the SLN stimuli were carefully adjusted so that only swallowing could be elicited. Swallowing movements were identified by specific stereotyped contractions of GH, TA and PCA muscles and by visual observation of the characteristic laryngeal movements, such as