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Research Report

Organization of last-order premotor interneurons related to the protraction of tongue in the frog, *Rana esculenta*

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ABSTRACT

Moving visual stimuli elicit a sequence of coordinated activity of muscles including tongue protraction. Morphological and physiological studies fail to reveal any direct tectal projections to hypoglossal motoneurons suggesting that the last-order premotor interneurons (LOPI) are the direct recipients of neural activities generated in the optic tectum. The aim of this study is to analyze the topographical organization of the last-order premotor interneurons related to protractor muscles of the tongue. In *Rana esculenta*, biotinylated dextran amine (BDA) was injected by iontophoresis into the subnucleus of the hypoglossal nerve containing the motoneurons of protractor muscles of the tongue. For visualizing BDA, sections were treated with avidin–biotin complex and a nickel-enhanced DAB chromogen reaction. The position of labeled neurons was reconstructed with a Neurolucida equipment. Morphologically heterogeneous populations of neurons were detected bilaterally, the majority of them were distributed ipsilateral to the site of injection and extended 1200 μm in rostral and 500 μm in caudal directions. Labeled neurons were found in the rhombencephalic reticular formation, the vestibular nuclei, the nucleus prepositus hypoglossi, the nucleus of solitary tract, the spinal nucleus of trigeminal nerve and the dorsal column nuclei. Our results indicate that the majority of last-order premotor interneurons related to protractor muscles of the tongue are located in the reticular formation of the brainstem. Since this area also receives a significant input from the vestibular system and from proprioceptive fibers, the last-order premotor interneurons presented here may be the target of convergence of sensory modalities involved in prey-catching behavior.

1. Introduction

Prey-catching behavior of the frog consists of a sequence of coordinated activity of different muscles elicited by a moving visual stimulus (Ewert, 1984). Large part of the input is received by the contralateral optic tectum and the tectal output is conveyed by a set of tectal efferent neurons to the brainstem and spinal cord via the descending tectobulbar and tectospinal pathways. An important efferent component of the neuronal circuitry underlying prey-catching behavior is the hypoglossal nucleus responsible for the activation of various muscles controlling protraction and retraction of the tongue. Relatively little information is available about neuronal structures involved in the transmission of tectal output to hypoglossal motoneurons. It is known that electrical stimulations of different parts of the optic tectum evoke prey-catching behavior

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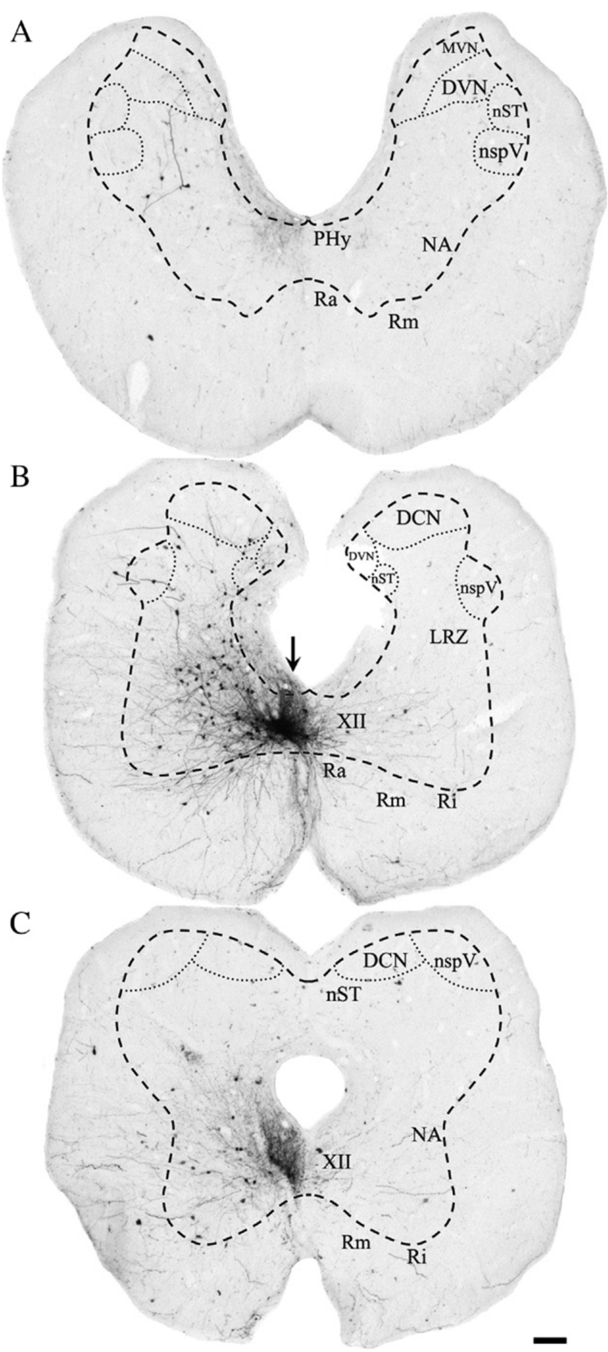

Fig. 1 – Photomicrographs showing the localization of last-order premotor interneurons following BDA injection (arrow in B) into the dorsomedial subnucleus of the hypoglossal nerve containing the motoneurons of protractor muscles of the tongue. Cross sections of the brainstem rostrally (A) and caudally (C) to the level of site of injection. Abbreviations: DCN, dorsal column nuclei; DVN, descending vestibular nucleus; MVN, medial vestibular nucleus; NA, nucleus ambiguus; nspV, spinal nucleus of trigeminal nerve; nST, nucleus of solitary tract; PHy, nucleus of prepositus hypoglossi; Ra, nucleus raphes; Ri, nucleus reticularis inferior; Rm, nucleus reticularis medius; XII, motor nucleus of hypoglossal nerve. Scale bar = 100 μm.

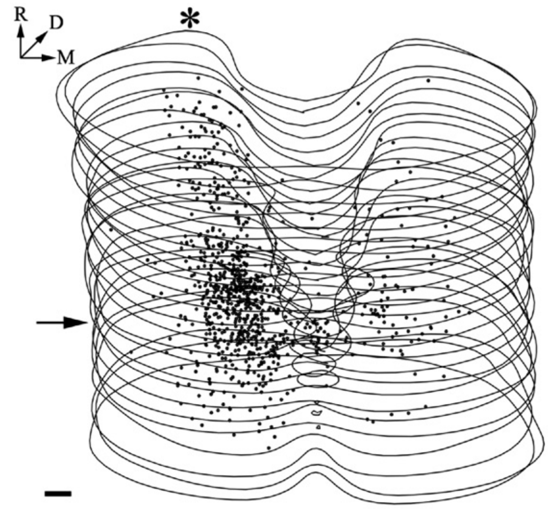

Fig. 2 – Neurolucida reconstruction of transverse sections of the brainstem following BDA injection into the dorsomedial subnucleus of hypoglossal nerve containing the motoneurons of the protractor muscles of the tongue. Dots represent the retrogradely labeled last-order premotor interneurons. The asterisk indicates the side of BDA injection, the arrow show the level of injection. D, dorsal; M, medial; R, rostral. Scale bar = 100 μm.

(Ewert, 1967, 1984), and that tectal neurons can be antidromically activated by electrical stimulation of the tectobulbar pathway. Nevertheless, the morphological substrate of the transmission of tectal activity to the hypoglossal nucleus has not yet been detected. The findings that direct fiber terminals of tectal origin cannot been shown on hypoglossal motoneurons (Rubinson, 1968; Lázár, 1969) suggest the presence of intercalated last-order premotor interneurons. No morphological data are available about premotor interneurons of the hypoglossal nucleus of lower vertebrates. Since the motoneurons of the hypoglossal nucleus display a musculotropic organization (Stuesse et al., 1983; Sokoloff, 1991; Matesz et al., 1999; Birinyi et al., 2004), we can assume matching organization in the spatial distribution of their LOPI as well. The aim of this study is to analyze the organization and morphology of the last-order premotor interneurons related to the protractor muscles of the tongue by using a retrograde tracer, the biotinylated dextran amine (BDA) (Rajakumar et al., 1993; Puskár and Antal, 1997; Birinyi et al., 2003).

2. Results

BDA labeling usually resulted in a well-circumscribed round injection site with a diameter of 200 μm in the dorsomedial part of the brainstem near the obex (Fig. 1B). In this area, the caudal part of the dorsomedial subnucleus of the hypoglossal nerve is situated which contains the motoneurons of the protractor muscles (genioglossus and geniohyoid) and a few motoneurons of the inner tongue muscles (Matesz et al., 1999; Birinyi et al., 2004). A group of BDA-labeled neurons were detected external to the site of the injection over a length of