Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 373 (2005) 134–137
Neuroscience Letters
www.elsevier.com/locate/neulet

Distribution of facial motor neurons in the pond turtle *Pseudemys scripta elegans*

Dantong Zhu, Joyce Keifer*

Neuroscience Group, Division of Basic Biomedical Sciences, University of South Dakota School of Medicine, Vermillion, SD 57069, USA

Received 6 July 2004; received in revised form 13 September 2004; accepted 1 October 2004

Abstract

A tract tracing study was performed to examine the localization of the facial nucleus in the brain stem of the pond turtle, *Pseudemys scripta elegans*. Neurobiotin and the fluorescent tracers alexa fluor 488 and 594 were used to retrogradely label neurons of the abducens or facial nerves. The results showed that the facial nucleus has two subnuclei, a medial group and a lateral group. Measurements of cell size revealed no significant differences between these populations. Double labeling studies showed that the medial cell group of the facial nucleus lies between the principal and accessory abducens nuclei in the pons, whereas the lateral group lies adjacent to the accessory abducens nucleus. The facial nucleus of pond turtles largely overlaps the rostrocaudal extent of the accessory abducens nucleus, but extends well beyond it into the medulla. These data elucidate the position and distribution of the facial nucleus in the brain stem of pond turtles and contribute to the body of comparative neuroanatomical literature on the distribution of the cranial nerve nuclei of reptiles.

Keywords: Facial nucleus; Abducens nucleus; Turtles; Reptiles

There are few studies describing the location and distribution of the cranial nerve nuclei of reptiles and the facial nucleus is frequently misidentified in neuroanatomical studies of these species. The facial nerve of reptiles is described as having the same nerve components as mammals: general and special visceral efferent fibers, and general somatic and special visceral afferent fibers [2–4]. However, there is little information on the distribution of the central neurons that send their axons into the facial nerve. During our studies of a neural correlate of eyeblink classical conditioning using an in vitro brain stem preparation from pond turtles we have worked extensively with the anatomy and function of the principal and accessory abducens motor nuclei which were identified previously by tract tracing [6,9,12]. The abducens nerve innervates the lateral rectus, retractor bulbi and pyramidalis muscles that control movements of the eye, eyelid and nictitating membrane. Immunocytochemical studies demonstrate these motor neurons to be cholinergic [10] and they are immunopositive for glutamate receptors of the NMDA and AMPA receptor subtypes [7,8]. However, the exact location of the accessory abducens motor nucleus in relation to neurons of the facial nucleus was uncertain. Therefore, it was important to establish the location of facial nucleus neurons as they are not likely to be involved in eyeblink reflexes as turtles lack an orbicularis oculi muscle that evokes contraction of the eyelid in mammals. The aim of present study was to determine the location and distribution of central neurons that extend their axons into the facial nerve of the pond turtle *Pseudemys scripta elegans*. Previous reports concerning facial nucleus neurons in turtles are sparse [5], although several studies have been performed on lizards and snakes [3,4,11]. These data elucidate the position and distribution of the facial nucleus in the brain stem of pond turtles and contribute to the body of comparative neuroanatomical literature on the distribution of the cranial nerve nuclei of reptiles.

Freshwater pond turtles *Pseudemys (Trachemys) scripta elegans* ($n = 10$) of approximately 12 cm carapace length and either sex obtained from commercial suppliers (Kons, Germantown, WI, USA) were anesthetized by hypothermia and

* Corresponding author. Tel.: +1 605 677 5134; fax: +1 605 677 6381.
E-mail address: jkeifer@usd.edu (J. Keifer).

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.10.022

decapitated. Protocols involving the use of animals complied with the guidelines of the National Institutes of Health and the Institutional Animal Care and Use Committee. The brain was transected at the level of the trochlear nerve to produce an in vitro brain stem preparation consisting of the pons and medulla. The tissue was continuously bathed in physiological saline (2–4 ml/min) containing (in mM): 100 NaCl, 6 KCl, 40 $NaHCO_3$, 2.6 $CaCl_2$, 1.6 $MgCl_2$ and 20 glucose, which was oxygenated with 95% O_2/5%CO_2 and maintained at room temperature (22–24 °C) at pH 7.6 [1,7]. A micropipette having an approximately 15 μm tip diameter was filled with a stock solution of the tracer neurobiotin (NB; 4%) dissolved in 0.5 M KCl and 0.5 M Tris buffer, or one of the fluorescent tracers alexa fluor 488 (AF488, green fluorescence; 0.5%; Molecular Probes, Eugene, OR, USA) or alexa fluor 594 (AF594, red fluorescence; 0.5%) mixed in distilled water. With the aid of a surgical microscope, the micropipette was inserted into the cut end of the facial or abducens nerve. A precise volume of 0.1–0.6 μl of tracer substance was pressure injected by direct visual monitoring of the meniscus in the pipette. Following injection, the brain remained in oxygenated physiological saline for 3–4 h to allow for transport of tracer substances. At the end of that period, the brain was immersion fixed in cold 3% paraformaldehyde for at least 24 h and processed for histology. Prior to sectioning, tissue was cryoprotected in 25% sucrose and sectioned at 60 μm on a sliding microtome. Cases using NB were processed with the avidin–biotin–peroxidase staining procedure [12]. Free floating sections were placed in 0.3% hydrogen peroxide for 30 min to inhibit endogenous peroxidase activity, washed twice in 0.1 M phosphate buffered saline (PBS) and incubated with avidin–biotin–peroxidase complex (Vector Labs, Burlingame, CA, USA) and 0.5% Triton X-100 in PBS for 2.5 h at room temperature while continuously shaking. Sections were washed twice in PBS followed by one wash in Tris buffer. Tissue sections were reacted with DAB for 10 min using nickel intensification. Cases using fluorescent tracers were sectioned without further processing. Sections were mounted on slides and dried overnight, dehydrated in alcohol, cleared in xylene and coverslipped. Images of fluorescent label from brain stem tissue sections were obtained by using epifluorescence on a Zeiss Axioskop microscope equipped with an Axiocam digital color camera. The double label cases were imaged by using a dual excitation/emission cube (peak excitation near 485 and 580). Specimens containing NB label were examined under brightfield microscopy. Images were processed for contrast and brightness by using Adobe Photoshop software.

The facial motor nucleus of turtles was retrogradely labeled following NB injections into the facial nerve in four cases (Fig. 1). Two groups of neurons were observed in the pons. First, a cluster of spindle-shaped neurons having dendrites that extend from dorsomedial to ventrolateral were observed and will be referred to as the medial group (arrow; Fig. 1A and D). Second, a more lateral and ventrally placed cluster of neurons with dendrites extending from medial to lateral was observed and will be referred to as the lateral group (arrowhead; Fig. 1A and D). The size of the somata of these two neuronal populations measured in the long axis did not differ significantly from one another (mean ± S.D., 26 ± 6,

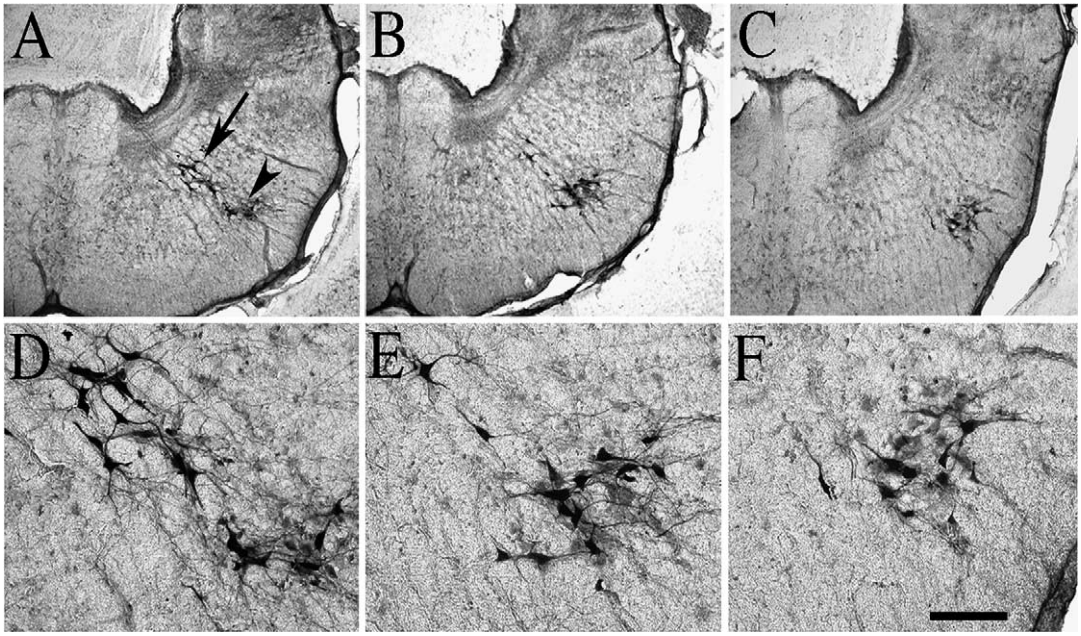

Fig. 1. Injections of NB into the facial nerve resulted in retrograde label of the facial nucleus. (A) Section taken through the pons shows two groups of retrogradely labeled neurons, a medial group (arrow) and a lateral group (arrowhead). (B) Section through the medulla shows that the medial group is no longer present. (C) Section taken farther caudally in the medulla shows the lateral group of facial neurons and that they have shifted slightly more laterally. (D–F) Higher magnification of the images in (A–C). Scale bar: 750 μm (A–C); 150 μm (D–F).