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Research article

Different discharge properties of facial nucleus motoneurons following neurotmesis in a rat model

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HIGHLIGHTS

- There are two types of facial nucleus motoneurons.
- Nerve injury can affect the discharge properties of motoneurons.
- Change of firing pattern was distinct in different motoneurons.

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ABSTRACT

Facial nucleus motoneurons innervating the facial expressive muscles are involved in a wide range of motor activities, however, the types of movement related neurons and their electrophysiological transformation after peripheral facial nerve injury haven't been revealed. This study was designed to elucidate the types of facial nucleus motoneurons and their alterations of discharge parameters following peripheral facial nerve injury in vivo. Here we set up a rat model by implanting electrode arrays into the brainstem and recorded the electrophysiological signals of facial nucleus neurons in the intact rats for 5 days, then transected the trunk of facial nerve (TF), and continued the record for 4 weeks. At the 4th week post-surgery, the morphological changes of TFs were analyzed. In this paper, we described two types of putative facial nucleus motoneurons based on their electrophysiological properties and their firing frequency adaptation. Type I motoneurons ($n = 57.6\%$) were characterized by a sustained spike adaptation, Type II motoneurons ($n = 26.2\%$) were identified by a phasic fast spike firing. Facial palsy and synkinesia, caused by neurotmesis of TF, were accompanied by firing rates reduction and firing pattern alteration of motoneurons. Our findings suggest the presence of two types of facial nucleus motorneurons, and their response patterns after neurotmesis support the notion that the discharge pattern of motorneurons may play an important role in the facial nerve function.

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1. Introduction

Peripheral facial palsy, resulted from trauma, tumor resection and surgical manipulation of peripheral facial nerves, and may accompanied by synkinesia, is frequently encountered in clinical practices, for which the neurotmesis has long been regarded as one of the key reasons for peripheral facial palsy. In recent years, the mechanisms of denervation and reinnervation of facial nerves after impairment have been widely studied, which demonstrated that the lack of functional recovery occurred at a number of locations along the facial nucleus-facial nerve trunk-facial nerve fascicles-facial muscles axis [1,2]. However, the roles of the facial nucleus motoneurons in regular facial movements, facial palsy and synkinesis remain inadequately studied.

The facial nucleus motoneurons are not only involved in many orofacial and auricular behaviors, but also disclosed as an integral part of the motor programme that maintains upper airway patency during breathing [3], and that involves in mastication and swallowing [4]. Previously, reports have proposed the presence of two types of motoneuron population in the facial nucleus based on their electrophysiological and morphological features in vitro [5,6]. In addition, the excitatory changes that occur in the facial nucleus after facial nerve compression have been reported [7]. Neverthe-

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less, there has been no direct investigation of the types of facial nucleus motoneurons in vivo and, moreover, studies investigating alteration of facial nucleus motoneuron activity after peripheral facial nerve neurotmesis in vivo are still lacking.

In this study, we set up a rat model by implanting electrode arrays into the brainstem to record the discharge properties of facial nucleus motoneurons before and after facial nerve neurotomy in vivo. A number of studies have demonstrated the degeneration and conduction block in injured nerve [8,9], so we simultaneously evaluated the histological changes of the TFs. This model is a powerful tool for examining the types of facial nucleus motoneurons and analyzing the electrophysiologic responses of different types of motoneurons to dyskinesia or synkinesia.

Overall, the purposes of the present work were to study the types of facial nucleus motoneurons and their changes of discharge properties after facial nerve neurotomy in vivo, further elucidating the role of facial nucleus motoneurons in nerve degeneration.

2. Materials and methods

2.1. Animals

Female Wistar rats (280–310 g, n = 8), which were provided by Animal Center of Shandong University, Jinan, China, were used in this study. All of these animals were kept on a standard laboratory diet, given tap water and housed in a room with a 12 h light/dark cycle. The care and use of the animals were approved by Animal Care and Use Committee of Shandong University and were performed by authorized investigators in accordance with Chinese law.

2.2. Surgical procedures

The rat was anesthetized by intraperitoneal injecting 10% chloral hydrate (400 mg/kg). The rat's head was fixed in a stereotaxic apparatus (Kopf Instruments, USA). The surgical area was trimmed of fur and disinfected with 10% povidone iodine. A 2-cm-long incision was made to expose the skull surface, connective tissue was removed and the skull surface was cleaned. The craniotomies, slightly larger than the electrode arrays, were made on the right side at the following coordinates: AP = −11.16 mm, ML = 2.3 mm, Depth = 10 mm, which was according to coordinates outlined by Paxinos and Watson (1998). The 4 × 4 electrode arrays made with insulator coated nichrome wires (diameters of 38 μm) were slowly implanted into the brainstem. Electrodes were fixed by dental cement, leaving only the connectors exposed. After inflammatory stage (3 days post- electrode implantation), continuous signals tracing was carried out for 5 days. After tracing signals for 5 days, rats were intraperitoneally anesthetized again. A 1–2-cm-long incision was made on opisthotic area of the right side to expose the TF leaving skull from stylomastoid foramen. Under an operating microscope (Leica, Germany), the TF was transected using dissection scissors. The trace record was lasted for 28 days post-surgery. Eventually, the electrode placement was confirmed histologically by performing electrolytic lesions. Then rats were deeply anesthetized, perfused by 4% paraformaldehyde and obtained the brainstems. The brainstems were dehydrated and then embedded in Tissue-Tek O.C.T compound (Sakura Finetek, Torrance, CA). 30 μm transverse sections were cut with a cryostat, followed by cresyl violet staining. Images were taken by confocal microscope with a 10× objective lens.

2.3. Histological observation of nerve degeneration

At the 4th week following neurotmesis operation, the rats were deeply anesthetized and obtained the TFs from both the injured and non-injured sides, then the segments were embedded in epoxy resin. Transverse semi-thin sections (2 μm) were cut with an ultramicrotome (Leica, RM2265) followed by 1% toluidine blue staining. The prepared sections were examined under a light microscope (Leica, DM400). Ultra-thin sections (70 nm thick) were observed under transmission electron microscope (JEM-1200EX, JEOL, Japan).

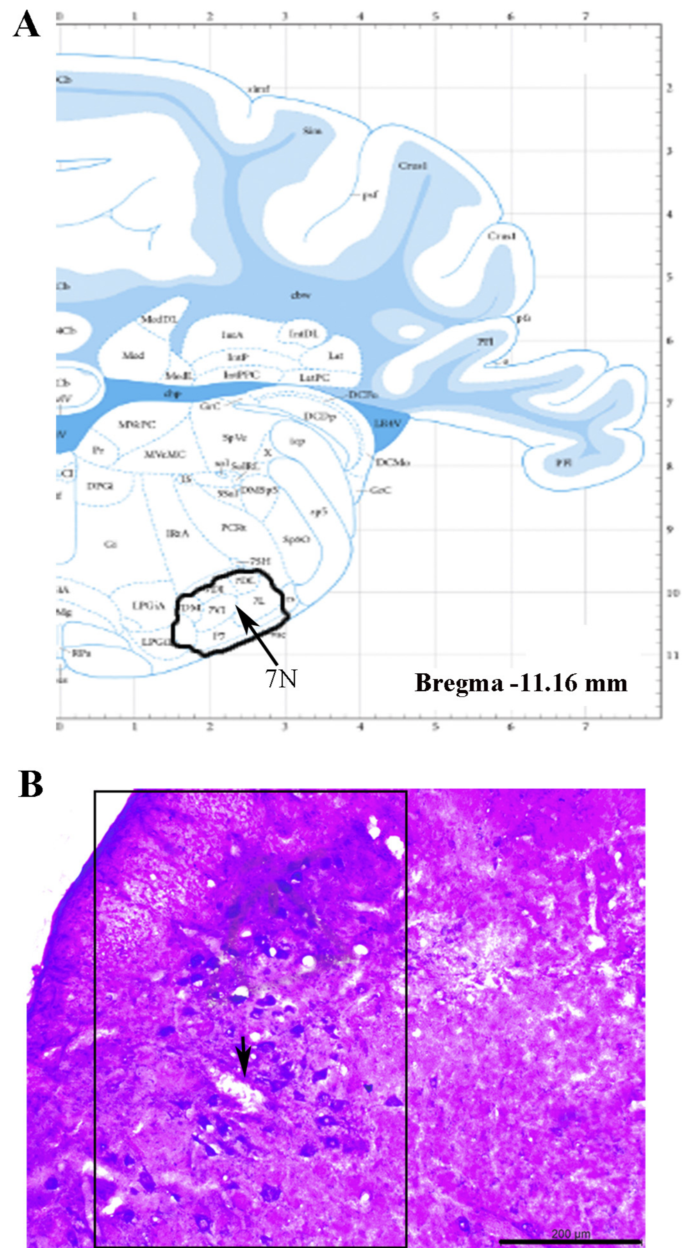

Fig. 1. Recording electrodes were positioned in 7N. (A) Diagram showed the location of 7N. (B) Location of 7N was framed by rectangle; locations of electrode tips, marked by electrolytic lesions, were labeled by black arrows. Scale bars: 200 μm.

2.4. Data collection

Signal tracing of intact rats was continued for 5 days. After nerve transecting, records were lasted for 28 days to trace the different neurons, finally we analyzed the data recorded at 28th days. The data were recorded both at quiescent and motorial condition. For the best performed session, the quiescent condition was confined by state without macroscopic tentacles or nictation movements.

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