Respiration-related rhythmic activity in the rostral medulla of newborn rats

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ABSTRACT

There are at least two respiration-related rhythm generators in the medulla: the pre-Bötzinger complex, which produces inspiratory (Insp) neuron bursts, and the para-facial respiratory group (pFRG), which produces predominantly pre-inspiratory (Pre-I) neuron bursts. The pFRG Pre-I neuron activity has not been correlated with motor neuron activity in slice or block preparations of rostral medulla. In this study, we attempted to detect pFRG Pre-I activity as motor output in the rostral medulla. We recorded respiratory activity of the facial nerve in the brainstem-spinal cord preparation of 0-2-day-old rats. Facial nerve activity consisted of pre-inspiratory, Insp, and post-inspiratory activity. Pre- and post-inspiratory activity corresponded well with membrane potential trajectories of Pre-I neurons in the rostral ventrolateral medulla. In response to perfusion of 1 µM DAMGO (a µ-opiate agonist), fourth cervical ventral root (C4) Insp activity was depressed, and facial nerve activity continued to synchronize with Pre-I neuron bursts. After transverse sectioning between the levels of the pre-Bötzinger complex and the pFRG, C4 Insp activity recovered within 15 min, but facial nerve activity was inhibited. When DAMGO was applied, C4 Insp activity was inhibited, and rhythmic facial nerve activity recovered. Subsequent elevation of K+ concentration reinduced C4 activity, but facial nerve activity was inhibited. Whole-cell recordings in the rostral block revealed the presence of putative Pre-I neurons, the activity of which was synchronized with facial nerve activity. These results show that the rostral medulla, not including the pre-Bötzinger complex, produces Pre-I-like rhythmic activity that can be monitored as facial nerve motor output in newborn rat in vitro preparations.

Keywords: para-facial, pre-inspiratory, respiratory rhythm, pre-Bötzinger, in vitro
INTRODUCTION

At least two respiration-related rhythm generators have been suggested to exist in the medulla and to produce intrinsic periodic burst activity under specific conditions. One is an inspiratory (Insp) neuronal network (i.e., Insp rhythm generator), which is localized predominantly in the pre-Bötzinger complex of the ventrolateral medulla (Rekling and Feldman 1998; Smith et al. 1991). Another is a pre-inspiratory (Pre-I) neuronal network (i.e., Pre-I rhythm generator), which generates activity prior to Insp bursts and typically also during the post-inspiratory period (for this reason, these neurons have also been referred to as biphasic expiratory neurons; Smith et al. 1990). The Pre-I neuronal network includes the para-facial respiratory group (pFRG) in the more rostral ventrolateral medulla (Ballanyi et al. 1999; Mellen et al. 2003; Onimaru and Homma 2003a). These rhythm generators can be distinguished by differences in burst phase and in their localization in the ventral medulla, although they may overlap to a certain extent in the rostro-caudal column (Arata et al. 1990; Smith et al. 1990). Mellen et al. (2003) suggested that these generators interact to form a coupled oscillator. Janczewski and Feldman (2006) showed that these two rhythm generators are normally coupled but can function independently in juvenile rats in vivo. Pre-Bötzinger complex Insp activity has been correlated with Insp motoneuron discharge, including phrenic and hypoglossal discharge in isolated en bloc medulla-spinal cord preparations, and with hypoglossal motoneuron discharge in medullary slice preparations (Smith et al. 1991). However, pFRG Pre-I neuron activity has not been correlated with motor neuron activity in slice or block preparations of rostral medulla that do not include the pre-Bötzinger complex. In this study, we showed that pFRG Pre-I neuron activity correlates with facial nerve activity in the newborn rat brainstem-spinal cord preparation.
We then examined the effects of transverse section between the levels of the pre-Bötzinger complex and the pFRG on Pre-I-like facial nerve activity and spinal Insp activity. Results suggest that these two generators can be independently active under the conditions required for burst generation. Some of these findings have been presented in abstract form (Onimaru 2005; Onimaru and Homma 2003b).

METHODS

Brainstem-spinal cords from 0-2-day-old rats (n = 62) were isolated under deep ether anesthesia as described previously (Onimaru and Homma 2003a; Suzue 1984). Experimental protocols were approved by the Animal Research Committee of Showa University, which operates in accordance with Law No. 105 of the Japanese Government for the care and use of laboratory animals. To monitor facial nerve activity, the right half of the pons was retained (Fig. 1). Insp activity corresponding to phrenic nerve activity was monitored from the fourth cervical ventral root (C4). Preparations were superfused continuously at 2.5-3.0 ml/min in a 2-ml chamber with the following standard solution (mM): NaCl, 124; KCl, 5.0; KH2PO4, 1.2; CaCl2, 2.4; MgCl2, 1.3; NaHCO3, 26; glucose, 30; equilibrated with 95% O2 and 5% CO2 at 25-26°C, pH 7.4 (Suzue 1984). DAMGO ([D-Ala2, N-Me-Phe4, Gly5-ol]-enkephalin; Sigma-Aldrich Co., St. Louis, MO) as a µ-opiate agonist and naloxone hydrochloride dihydrate (Sigma-Aldrich Co.) as an opiate antagonist were dissolved in the standard solution and were applied by superfusion for 10-15 min. The medulla was incised with a stainless steel blade (0.5-mm in width) attached to a manipulator while nerve activity was monitored. The blade was moved gently into the tissue to make the incision, and this manipulation was repeated several times until complete transverse section was achieved.
For membrane potential recordings from bursting neurons in the rostral block, brainstem-spinal cord preparations were cut transversely with a vibrating-blade tissue slicer (laboratory-made) in the dissection chamber, and the rostral block was transferred to the recording chamber. Nerve activities were recorded via glass capillary suction electrodes through a high-pass filter with a 0.3-s time constant. To clearly identify facial nerve activity, the signals in some experiments were integrated with a 0.1-s time constant after high-pass filtering with a 0.1-s time constant and rectification. Respiratory rate (bursts/min) was calculated from the mean C4 burst activity for 3-5 min. Values are shown as mean ± SD. Significance of differences (P < 0.05) was determined with Student’s t-test for paired samples.

Membrane potentials of Pre-I neurons in the rostral ventrolateral medulla just caudal (within 200 µm) to the caudal end of the facial nucleus and overlapping the caudal part of the pFRG (Onimaru and Homma 2003a) were recorded by a blind whole-cell patch-clamp method (Onimaru and Homma 1992). The electrodes, which had an inner tip diameter of 1.2-2.0 µm and a resistance of 4-8 MΩ, were filled with the following pipette solution (mM): K-gluconate, 130; EGTA, 10; HEPES, 10; Na2-ATP, 2; CaCl2, 1; and MgCl2, 1; with pH 7.2-7.3 adjusted with KOH. For histologic analysis of the location of recorded cells, the patch electrode tips were filled with 0.5% Lucifer yellow (LY, lithium salt; Sigma-Aldrich Co.) . Membrane potentials were recorded with a single-electrode voltage-clamp amplifier (CEZ-3100; Nihon Kohden, Tokyo, Japan) after compensation of series resistance (20-50 MΩ) and capacitance.

For histologic verification of the transection level and location of recorded neurons, preparations were fixed for more than 48 h at 4°C in Lillie solution (10% formalin in phosphate buffer, pH 7.0). Transverse 100-µm sections were then cut with a
laboratory-made vibrating-blade tissue slicer and stained with neutral red. LY-labelled neurons were reconstructed with the aid of a camera lucida attached to a fluorescence microscope (BH-2; Olympus, Tokyo, Japan).

RESULTS

Facial nerve and Pre-I neuron activity

The typical activity pattern of the facial nerve in the brainstem-spinal cord preparation is shown in Fig. 1. The onset of facial nerve activity preceded that of Insp (C4) activity by 555 ± 287 ms (n = 10), and the amplitude increased immediately before C4 onset in most cycles. The peak coincided with the C4 peak. Significant activity of variable amplitude also occurred during the post-inspiratory phase. Thus, facial nerve output consisted of pre-inspiratory, Insp, and post-inspiratory phase activity.

To compare facial nerve activity with the membrane potential trajectory of Pre-I neurons, we simultaneously recorded Pre-I neuron and facial and C4 nerve activity (n = 5) (Fig. 2, see Fig. 8B for location). In the half pons-attached preparations used in this study, Pre-I neuron bursts were frequently not accompanied by C4 Insp activity, indicating a quantal relation (Mellen et al., 2003). Small amplitude activity corresponding to Pre-I neuron bursts was observed in facial nerve recordings whether or not the Pre-I burst was accompanied by a C4 Insp burst (Fig. 2A). Application of 1 µM DAMGO (15 min) increased the burst rate of Pre-I neurons from 6.9 ± 2.4 bursts/min (control) to 8.9 ± 1.6 bursts/min (n = 5, P < 0.05), whereas the C4 rate decreased from 3.8 ± 0.5 bursts/min (control) to 2.8 ± 1.3 bursts/min (not significant). Small amplitude facial nerve activity, corresponding to Pre-I neuron bursts, was clearly identified after DAMGO application (Fig. 2B, C). The ratio of C4 burst rate/Pre-I neuron burst rate was
Effects of transverse section and DAMGO

We next examined the effects of transverse section at the medullary level between the pre-Bötzinger complex and the pFRG (at the approximate level of the Xth cranial nerve roots or the most rostral roots of the XIIth cranial nerve, see Fig. 8A) on facial and C4 nerve activities. Immediately after transection, rhythmic burst activity from these nerves disappeared, and transient tonic discharges appeared (Fig. 3B). C4 Insp activity recovered gradually, showing initially smaller amplitude and faster burst rate than were shown under control conditions (Fig. 3A). After 10-15 min, C4 amplitude and burst rate returned almost to control levels. The mean C4 burst rate was $4.6 \pm 1.0$ bursts/min (n = 22) under control conditions, $15.2 \pm 4.9$ bursts/min 3-5 min after transection ($P < 0.001$), and $5.0 \pm 1.3$ bursts/min 15 min after transection (not significant compared to control). In contrast, no clear rhythmic facial nerve activity was detected. C4 activity patterns were stable for more than 1 h. Subsequently, when 1 µM DAMGO was applied, C4 Insp activity was inhibited, and rhythmic facial nerve activity was consistently induced (Fig. 3B). This rhythmic facial nerve activity continued for more than 1 h after DAMGO was washed out. The burst rate of DAMGO-induced facial nerve rhythmic activity (after 10-15 min) was $9.3 \pm 2.2$ bursts/min, whereas the rate in controls was $7.3 \pm 2.0$ bursts/min (n = 7, not significant, $P = 0.053$). Application of 1 µM naloxone depressed the rhythmic facial nerve activity and restored C4 Insp activity (Fig. 3C). Further addition of 4-5 mM KCl (final concentration 10-11 mM K+) after application of 1 µM DAMGO induced rhythmic C4 activity ($4.4 \pm 1.1$ bursts/min), and depressed the rhythmic facial nerve activity (n = 5, Fig. 4).

When DAMGO was applied prior to transection, rhythmic facial nerve activity
continued (n = 2 of 6) or became disorganized and recovered spontaneously within 8 min (n = 4 of 6) (Fig. 5A). The burst rate was initially high (22.3 ± 7.5 bursts/min within 10 min) and decreased to a stable level (9.3 ± 1.7 burst/min) after 20-25 min.

C4 Insp activity recovered after transverse sectioning in the absence of DAMGO. Therefore, the caudal block was believed to include the pre-Bötzinger complex. The level of transection in these experiments was determined to be 200-400 µm (331 ± 108 µm, n = 21) caudal to the caudal end of the facial nucleus (Fig. 5B, see also Fig. 8C). Thus, the transection level was estimated to be 100-300 µm rostral to the rostral end of the pre-Bötzinger complex (Smith et al. 1991).

To determine whether induction of rhythmic facial nerve activity after transverse sectioning required the presence of DAMGO, we examined the effect of separating the caudal block from the rostral block after confirming the presence of rhythmic C4 activity after transverse sectioning (Fig. 6A). When the rostral and caudal blocks were separated (Fig. 6B), C4 Insp activity was greatly reduced (0.6 ± 0.9 bursts/min, n = 14). Recovery of stable, rhythmic facial nerve activity required 20 min or more in most cases (Fig. 6D). Facial nerve burst rate was 5.8 ± 1.2 bursts/min in controls and 6.4 ± 1.4 bursts/min (n = 6, not significant) 40 min after complete separation in the absence of DAMGO. DAMGO application rapidly restored rhythmic facial nerve activity (Fig. 6C).

**Intracellular recordings of putative Pre-I neurons in the rostral block after DAMGO application**

Facial nerve activity in the rostral block after transverse sectioning is presumed to be derived from Pre-I neurons that have lost their biphasic burst pattern due to absence of synaptic inhibition from Insp neurons. Accordingly, we recorded membrane
potentials from putative Pre-I neurons that showed burst activity synchronized with facial nerve activity in rostral block preparations. To obtain stable rhythmic facial nerve activity, 1 µM DAMGO was applied to the block preparation for 10–15 min and then washed out. In all preparations, rhythmic facial nerve activity continued for more than 1 h, and bursting neurons were identified in whole-cell recordings by the caudal cut-face approach. A representative example is shown in Fig. 7. After application of 1 µM naloxone (Fig. 7A), the burst activity of neurons as well as rhythmic facial nerve activity disappeared, accompanied by the appearance of slight depolarization of neurons (-51.8 ± 3.8 mV in controls and -49.3 ± 4.0 mV after naloxone application, n = 5, not significant). Further addition of 5 mM KCl (final concentration 11.2 mM K+) after application of 1 µM DAMGO (without naloxone) disturbed the burst generation of neurons as well as rhythmic facial nerve activity (Fig. 7B). The neurons showed tonic firing of action potentials with significant depolarization (-42.5 ± 4.3 mV in 11.2 mM K+ and -49.3 ± 2.7 mV in controls, n = 6, P < 0.01). The locations of recorded neurons stained with LY are shown in Fig. 8.

DISCUSSION

Facial nerve activity consists of pre-inspiratory, Insp, and post-inspiratory phases in the brainstem-spinal cord preparation of newborn rats. Pre-inspiratory and post-inspiratory activity of the facial nerve corresponded well with Pre-I neuron activity in the rostral medulla. Moreover, facial nerve activity without C4 Insp bursts, which occurred frequently after DAMGO treatment, also showed good correspondence with Pre-I neuron bursts. The results of this study show that facial nerve activity, with the exception of the Insp phase, is a good indicator of Pre-I neuron activity. Although
activity of the first lumbar root (L1) is also reported to be a good indicator of Pre-I neuron activity (Janczewski et al. 2002), it is noteworthy that the facial nerve as well as the major region of active Pre-I neurons are located rostral to the medullary Insp center. Indeed, we showed that rhythmic Pre-I neuron-like activity can be detected from the facial nerve root after separation of the rostral medulla from the caudal medulla, where Insp activity is produced. Our results suggest that the rostral block includes the Pre-I rhythm generator as well as a sufficient number of pre-motor neurons involved in activating facial motor neurons and that the caudal block includes the Insp rhythm generator as well as a sufficient number of Insp pre-motor neurons.

The observation that facial nerve activity is comprised of discharges preceding inspiration as well as inspiratory discharges has been reported in cats in vivo (Hwang et al. 1988). The burst pattern of facial nerve activity in newborn rats in the present study resembled that in cats. Facial nerve activity preceding inspiration in cats is likely active stage 2 expiration (Bianchi et al. 1995) (see below). Activity of the facial nerve, whose branches innervate muscles of the 'alae nasi', decreases nasal airway resistance during inspiration (Hwang et al. 1988; Strohl 1985). The pre- and post-inspiratory activities may be also involved in maintaining the patency of the nasal airway during the peri-inspiratory phase.

Our findings support the notion that respiratory rhythm generation comprises two distinct rhythm generators (Janczewski and Feldman 2006; Mellen et al. 2003). Janczewski and Feldman (2006) further suggested that role of Pre-I neurons is to drive expiratory rhythm generator. However, previous studies (Mellen et al. 2003; Onimaru et al. 1997; Takeda et al. 2001) and the present study also clearly indicate that Pre-I neurons generate the basic respiratory rhythm to which Insp burst activity is entrained in
the intact brainstem-spinal cord preparation. There are unresolved issues as to whether
Pre-I neuron activity is simply reflected in motoneuron discharge as active expiration.
In the brainstem-spinal cord preparation of newborn rats, we recorded activity of a
subtype of expiratory neuron that receives inhibitory synaptic inputs from Pre-I and
inspiratory neurons (Arata et al. 1998). Bursts of this type of expiratory neuron
commence after post-inspiratory activity and cease during the pre-inspiratory phase.
This type of expiratory neuron is clearly distinguishable from Pre-I neurons and is
thought to correspond to late expiratory (or E-augmenting) neurons in adult mammals
(Bianchi et al. 1995; Ezure 1990). In addition, the brainstem-spinal cord preparation can
produce complex expiration-related motor activity recorded from various cranial and
spinal rootlets (Iizuka 1999, 2001). Central mechanisms of origin of such
expiration-related activity remain to be elucidated.

After transection of the medulla into rostral and caudal blocks, each block
produced rhythmic bursts that could be monitored according to facial and C4 nerve
activities under specific conditions. Facial nerve activity disappeared after transection
and reappeared after exposure to DAMGO. In the absence of DAMGO, recovery of
stable rhythmic facial nerve activity took more than 20 min after complete separation of
the rostral block from the caudal block in most preparations. The specific mechanism of
induction of rhythmic facial nerve activity by DAMGO after transection is not clear.
Pre-I neurons, which are presumed to be the source of this rhythmic activity, are
insensitive to µ-opiate agonists (Takeda et al. 2001). Therefore, modulation of
excitatory or inhibitory inputs to Pre-I neurons may be involved in DAMGO-induced
rhythmic bursting. Two contrasting possibilities can be considered. One is that the
transection produces excessive excitatory effects on burst generation of Pre-I neurons

and that DAMGO inhibits this excitation. Another is that DAMGO facilitates Pre-I bursting by depressing the pontine inhibitory system that sends inhibitory inputs to Pre-I neurons (Tanabe et al. 2005). The rhythmic facial nerve activity showed an initially greater burst rate that gradually decreased after transection in DAMGO pre-treated preparations, suggesting that the transection caused excitatory effects on the rhythmic activity. Naloxone depressed the rhythm generation of putative Pre-I neurons in the rostral block without inducing any significant membrane hyperpolarization. Moreover, elevation of K⁺ concentration disturbed rhythm generation of the putative Pre-I neurons and induced membrane depolarization. These results suggest that the former possibility is plausible, but more complex mechanisms may be involved with respect to the effects of DAMGO. Effects on the pontine inhibitory system may also contribute to rhythm induction or modulation by DAMGO.

In contrast to facial nerve activity, C4 Insp activity appeared 2-5 min after transection, with a high burst rate that gradually decreased almost to the control level within 10-15 min. DAMGO may inhibit Insp neuron bursting directly (Gray et al. 1999; Mellen et al. 2003; Takeda et al. 2001) and may also inhibit it indirectly via reduction of excitatory effects induced by transection. Our results suggest that the conditions under which rhythmic facial nerve activity in the rostral block (i.e., Pre-I rhythm generator) and C4 Insp activity in the caudal block (i.e., Insp rhythm generator) can be produced may differ significantly, consistent with previous studies; burst generation of Pre-I neurons (but not of Insp neurons) was disturbed by elevated K⁺ concentration (Funk et al. 1993; Mellen et al. 2003). Janczewski and Feldman (2006) showed that complete transection of the brainstem at the caudal end of the facial nucleus abolished rhythmic abdominal muscle activity, whereas rhythmic inspiration continued. The transection
level was more caudal in the present study than in their study. In preliminary studies and in the present study, we did not identify conditions under which both generators are active with independent stable rhythm after transection. For example, 0.2 μM DAMGO induced effects similar to those induced by 1 μM DAMGO, but the time course was slower (unpublished observation). Therefore, it would be interesting to examine whether facial nerve activity and inspiratory activity continue independently after transection of the medulla at an appropriate level in rats in vivo.

In conclusion, our findings in the newborn rat in vitro preparation suggest that the rostral block of the medulla, not including the pre-Bötzinger complex, can produce rhythmic burst activity, likely derived from Pre-I neurons, and that Pre-I rhythm and Insp rhythm generators can be independently active under the conditions required for burst generation.

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Figure legends

Fig. 1. Facial nerve and C4 activity in a newborn rat brainstem-spinal cord preparation. A. Simultaneous recording of facial nerve (VII) and C4 activity. B. Faster sweep representation of a burst phase with integrated facial nerve activity (Int. VII). C. Integrated facial nerve activity averaged 30 times with the use of C4 activity as a trigger. Facial nerve activity consists of pre-inspiratory (Pre-I), inspiratory (Insp), and post-inspiratory (Post-I) activity. Inset, ventral view of the preparation.

Fig. 2. Simultaneous recordings of a Pre-I neuron and facial nerve and C4 activity. A. Control. Note that not all Pre-I neuron bursts are accompanied by C4 inspiratory activity. Small amplitude activity in the integrated facial nerve recording (dotted lines on the trace of Int. VII), corresponding to the Pre-I neuron burst, was observed. B. Application of 1 µM DAMGO facilitated the burst rate of Pre-I neurons, but the C4 rate decreased. Small amplitude activity in the facial nerve recording, corresponding to the Pre-I neuron burst, was clearly identified after DAMGO application. C. Integrated facial nerve activity (Int. VII) averaged 20 times with the use of the Pre-I neuron burst as a trigger, in cycles where C4 inspiratory bursts were not induced after DAMGO application.

Fig. 3. Effects of transverse section at the medullary level between the pre-Bötzing complex and the para-facial region on facial (VII) and C4 nerve activities. A. Control. B. Activity after bilateral transection. Immediately after transection (arrow), rhythmic activity from these nerves disappeared, and transient tonic discharges appeared. C4 inspiratory activity recovered gradually. After 10-15 min, the amplitude and burst rate of
C4 activity returned to almost the control levels. In contrast, no clear rhythmic activity was detected in the facial nerve recording. When 1 µM DAMGO was applied, rhythmic activity was induced in the facial nerve, and the C4 inspiratory activity was inhibited. Application of 1 µM naloxone depressed the rhythmic facial nerve activity and restored C4 inspiratory activity. Inset, approximate level of transection.

Fig. 4. Effects of elevated K+ concentration on facial (VII) and C4 nerve activities. A. Activities before and after transection (black bar below C4 trace). B. At 15 min after transection, application of 1 µM DAMGO depressed C4 bursting and induced rhythmic facial nerve activity. C. Subsequent addition of 5 mM KCl (11.2 mM K+ final concentration) depressed facial nerve activity and re-induced C4 bursting.

Fig. 5. Effects of DAMGO pretreatment on transverse sectioning. A. DAMGO was applied before sectioning. After sectioning (black bar below C4 trace), facial nerve activity (VII) continued to be rhythmically active, although the frequency was very high immediately after sectioning. C4 activity almost completely disappeared. B. Transection level represented in a sagittal slice compared with schematic drawing (traced and modified from Smith et al. 1991). BC, Bötzinger complex; NA, nucleus ambiguus; LRN, lateral reticular nucleus; nVII, facial nucleus; PBC, pre-Bötzinger complex; RFN, retrofacial nucleus; SO, superior olive.

Fig. 6. Effects of detaching the caudal block from the rostral block after transverse sectioning. A. Activity of facial (VII) and C4 nerves before and after transection at ‘a’. Note that the C4 burst reappears. B. The rostral block was moved to allow the cut
surface of the caudal block to be exposed to the flow of superfusate. C4 inspiratory activity was greatly inhibited immediately after this manipulation. C. DAMGO application restored rhythmic facial nerve activity. D. Facial (VII) and C4 nerve activities before transection in another preparation. D’. Activity 45 min after complete separation of the rostral block from the caudal block.

Fig. 7. Activity of putative Pre-I neurons in the rostral block synchronized with rhythmic facial nerve activity. Block preparations were treated with 1 µM DAMGO for 10-15 min and then washed out. MP, membrane potential recordings. VII, facial nerve recordings. A, B. Control burst activity. A’. Effect of addition of 1 µM naloxone (15 min). B’. Effect of addition of 5 mM KCl (11.2 mM K⁺ final concentration).

Fig. 8. Location of recorded neurons. A. Ventral aspect of preparation and approximate area of para-facial respiratory group (pFRG). B. Location of recorded neurons stained with Lucifer yellow, plotted in two sections denoted by dotted lines in A. Open circles, burst-generating neurons recorded in the rostral block preparations (e.g., in Fig. 7). Solid circles, Pre-I neurons recorded in intact brainstem-spinal cord preparations (e.g., in Fig. 2). C. A representative example of the rostral face of the caudal block after transverse sectioning at the level denoted by the solid line in A. The rostral part of nucleus ambiguus and the most rostral part of the inferior olivary nucleus are visible. AMB, nucleus ambiguus; CST, corticospinal tract; IO, inferior olivary nucleus; nVII, facial nucleus; RFN, retrofacial nucleus; STN, spinal trigeminal nucleus; XII, hypoglossal nerve roots.
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