原著論文

Postnatal changes in the repetitive firing properties of rat jaw-closing motoneurons

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Abstract: Feeding behavior drastically changes from suckling to chewing during the postnatal period. Thus, it is possible that the firing properties of jaw-closing motoneurons (JCMNs), one of the final common pathways in the oro-motor system, are also altered during the postnatal period. We examined the repetitive firing properties of JCMNs in developing rats from an early postnatal stage to a post-weaning stage using intracellular recording techniques. Firing frequency adaptation was observed within the first four spikes in a spike train. The neurons were classified into two groups according to the firing patterns subsequent to the first four spikes: neurons with a constant or slightly decrementing firing pattern (type I) and neurons with an incrementing firing pattern (type II). The percentage of type I neurons increased with age. In more than 50% of the neurons in each age group except for the group at 10-15 days of age, an extra spike was produced from the post-spike afterdepolarization (ADP) of the 1st spike in a spike train, leading to a high firing rate for the first inter-spike interval (1st ISI). The incidence of an extra spike also increased with age. The firing rate for the steady-state was high in JCMNs, even at 4-9 days of age, and approximately 50% of the neurons at this age fired at higher than 50 Hz. These findings demonstrate that JCMNs can fire at a high rate, even in the early postnatal period, and the firing rate for the 1st ISI further increases with age. These changes may contribute to the transition from suckling to chewing.

I. Introduction

Feeding behavior in mammals drastically changes from suckling to chewing during the early postnatal period. The activity of the jaw-closing muscle should be low during the suckling phase, whereas the activity increases when hard or tough food is chewed after early postnatal development¹⁻⁴⁾. Thus, motor commands to jaw-closing muscles change with development of the orofacial musculoskeletal system during the early postnatal period. It is possible that properties of the jaw-closing motoneurons (JCMNs), one of the final common pathways in the oro-motor system, are also altered with changes in the motor commands. We previously reported that the action potential half-duration, and the amplitude and half-duration of the medium-duration afterhyperpolarization (mAHP) decreased during postnatal development; in contrast, the incidence of post-spike afterdepolarization (ADP) progressively increased with the age of the JCMN⁵⁾. Furthermore, we reported that glycinergic and/or GABAergic inputs from the supratrigeminal nucleus and the reticular formation dorsal to the facial nucleus change from excitatory to inhibitory during postnatal development^{6, 7)}. However, the postnatal changes in firing properties of JCMNs are still unclear.

In this study, we used intracellular recordings to investigate the firing properties of JCMNs that innervate the jaw-closing muscle in brainstem slice preparations containing the trigeminal motor nucleus of developing rats from an early postnatal stage to the post-weaning stage. We demonstrate that JCMNs can be classified into two groups according to firing patterns after the first four spikes, including neurons with a constant or slightly decrementing firing pattern (type I) and neurons with an incrementing firing pattern (type II). In addition, the percentage of type I neurons and the incidence of an extra spike increase with age. These changes may contribute to the transition from suckling to chewing.

II. Materials and Methods

All experiments were approved by the International Animal Research Committee of Showa University in accordance with Japanese Government Law #105 concerning the care and use of laboratory animals. All efforts were made to minimize the number of animals that were used.

1. Slice preparation

Experiments were performed with brainstem slices obtained from P3-27 Wistar rats ($n = 52$). The day of birth was defined as P0. Neonatal (P3-P15) and juvenile (P16-P27) rats were deeply anesthetized by ether or combined injections of ketamine HCl (150 mg/kg i.m.) and chlorpromazine HCl (12.5 mg/kg i.m.), respectively. Following decapitation, the brain was removed rapidly and placed in cold oxygenated sucrose-based modified artificial cerebrospinal fluid (ACSF) because it has been shown that use of this ACSF during the slicing procedure is essential to maintain viable motoneurons in adult rats⁸⁾. The modified ACSF contained (in mM) 260 sucrose, 3 KCl, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃ and 10 glucose. The slices were incubated at 34°C in a holding chamber containing modified ACSF for 15 min, transferred to a holding chamber containing normal ACSF (N-ACSF) at 34°C for 30 min and were maintained at room temperature. N-ACSF contained (in mM) 130 NaCl, 3 KCl, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃ and 10 glucose.

2. Recordings

To identify the recorded cell as a JCMN, we used a fluorescence labeling technique⁵⁾. One to two days before the slices were prepared, animals were anesthetized with ether or combined injection of ketamine HCl and chlorpromazine HCl, and 2-5 μ l of 10% 3,000 or 10,000 MW dextran-tetramethylrhodamine-lysine (DRL, Molecular Probes, Eugene, OR, USA) dissolved in distilled water was injected bilaterally in the masseter muscles with a microsyringe (1010RN, Kloeckner, Las Vegas, NV, USA).

After a 2-10 h incubation in the holding chamber, the slices were transferred to an interface-type chamber. The recording chamber was continuously perfused with N-ACSF at a rate of 1-1.5 ml/min, and an atmosphere consisting of humidified 95% O₂-5% CO₂ flowed over the slice. All experiments were performed at $32 \pm 1^\circ\text{C}$. Intracellular recordings were obtained with glass microelectrodes (1.2 mm O.D., 20-40 M.) (Sutter Instruments, Novato, CA, USA) filled with 1% biocytin in 1 M KCl and 0.05 M Tris buffer (pH 7.6). The trigeminal motor nucleus was visible in the slice through the stereomicroscope (MZ FL III, Leica Microsystems GmbH, Wetzlar, Germany). The neurons retrogradely labeled by DRL were visualized with an epifluorescence unit attached to the microscope and localized in the dorsolateral portion of the trigeminal motor nucleus. Next, the electrode was

Table 1 Membrane and firing properties of jaw-closing motoneurons

	Age, days					
	4-9	10-15	16-21	22-27	4-15	16-27
Resting membrane potential (mV)	-68.3 ± 1.4 (17)	-70.0 ± 1.4 (24)	-62.7 ± 1.8 (7)	-68.8 ± 1.50 (4)	-69.3 ± 1.0 (41)	-65.2 ± 1.8 (11)
Spike amplitude (mV)	87.4 ± 1.4 (17)	83.9 ± 0.9 (24)	83.6 ± 1.7 (7)	87.2 ± 1.50 (4)	85.3 ± 0.8 (41)	83.5 ± 1.2 (11)
Number of neurons						
type I	9 (52.9%)	10 (41.7%)	6 (85.7%)	3 (75.0%)	19 (46.3%)	9 (81.8%)
type II	6 (35.3%)	13 (54.2%)	1 (14.3%)	1 (25.0%)	19 (46.3%)	2 (18.2%)
others	2 (11.8%)	1 (4.2%)	0 (0.0%)	0 (0.0%)	3 (7.3%)	0 (0.0%)
1st ISI f-I slope (Hz/nA)	36.8 ± 6.2 (17)	38.6 ± 3.2 (24)	35.4 ± 3.8 (7)	44.9 ± 7.3 (4)	38.5 ± 3.1 (41)	38.9 ± 3.7 (11)
Steady-state f-I slope(Hz/nA)	22.3 ± 2.0 (17)	22.7 ± 2.0 (24)	19.1 ± 2.5 (7)	30.2 ± 3.7 (4)	24.2 ± 1.3 (41)	23.1 ± 2.6 (11)
Incidence of extra spike	9/13 (69.2%)	7/16 (43.7%)	5/6 (83.3%)	4/4 (100%)	16/29 (55.2%)	9/10 (90.0%)
Incidence of secondary range	6/13 (46.2%)	4/16 (25.0%)	4/6 (66.7%)	2/4 (50%)	10/29 (34.5%)	6/10 (60.0%)

inserted into the cluster of DRL-labeled neurons in the slice. Following recording, we re-confirmed whether the tip of the electrode was located within the cluster of labeled neurons. It has been shown that motoneurons innervating the jaw-closing muscles, such as the masseter or temporalis muscles, are localized in the dorsolateral portion of the trigeminal motor nucleus of the rat, and neurons innervating the jaw-opening muscles, such as the anterior digastric or mylohyoid muscles, are located in the ventromedial portion^{9,10}. Furthermore, there are minimal interneurons in the trigeminal motor nucleus¹¹. Thus, we regarded the neurons located within a cluster of DRL-labeled neurons as JCMNs. In some neurons, we further confirmed if the recorded neurons were localized in a cluster of DRL-labeled neurons using histological observations (cf. Yamaoka et al.⁵). Membrane potentials were recorded using an Axoclamp 2B amplifier (Molecular Devices, Sunnyvale, CA, USA) in bridge mode (see Inoue et al.¹²). The membrane potential was filtered at 30 kHz with built-in single-pole low-pass filters, digitized at 40 kHz and stored on a computer hard disk using software (Clampex, Molecular Devices) through an A-D converter. Data were analyzed with Clampfit (Molecular Devices) and Excel (Microsoft Japan, Tokyo) software. Fifty-two JCMNs were used to analyze the electrophysiological properties, including 4 postnatal age groups: 4–9 days ($n =$

17), 10–15 days ($n = 24$), 16–21 days ($n = 7$) and 22–27 days ($n = 4$). To examine the properties of repetitive firing, trains of spikes were elicited in JCMNs by intracellular injections of long (1 s) depolarizing current pulses. The instantaneous firing frequency was defined as the reciprocal of the inter-spike interval (ISI), and the steady-state firing frequency was calculated as the average of the firing rate over the last half (500 ms) of the injected current pulse.

Data are presented as the mean ± SE. Comparisons of data between groups were completed using Student's *t*-test, Fisher's exact test or the χ^2 test. Probabilities < 0.05 were considered significant.

III. Results

1. Repetitive firing patterns

The present study was based on recordings from 52 JCMNs that had stable resting potentials more negative than -55 mV (-68.4 ± 0.9 mV) and displayed spikes larger than 70 mV (85.0 ± 0.7 mV). There were no significant differences in the resting potentials and spike amplitudes among the four age groups (Table 1).

Trains of spikes could be elicited in developing JCMNs ($n = 52$) by 1 s depolarizing current pulses (Fig. 1A, D).

Fig. 1 Repetitive firing characteristics of type I and II JCMNs.

A: Repetitive firing of an 8-day-old type I neuron induced by 1-s constant current pulses at 1.0 nA (*upper*) and 2.0 nA (*lower*).
 B: Frequency-time plot (*f-t* plot) for the neuron in A for 3 different current intensities. Note that there is a prominent early adaptation observed at current intensities of 1.0 and 2.0 nA.
 C: Frequency-current (*f-I*) relationship for 1st ISI, 3rd ISI and steady-state firing for the neuron in A.
 D: Repetitive firing of a 6-day-old type II neuron at 2.0 nA (*upper*) and 3.6 nA (*lower*) current intensities.
 E: Frequency-time plot (*f-t* plot) for the neuron in D. Note that the firing rate for the 1st ISI (*arrow*) was lowest in the spike train at a 2.0 nA current intensity; however, prominent early adaptation appeared at 3.6 nA.
 F: *f-I* relationship for the neuron in D. Note that *f-I* plots for the 1st ISI and steady-state intersect at approximately 2.7 nA.

The instantaneous firing rate was plotted against the time of occurrence of each spike (*f-t* plot) to analyze the firing properties (Fig. 1B, E). At moderate to high current intensities, the firing rates for the 1st ISIs were highest in the spike trains of all neurons tested, and they rapidly decreased by the fourth ISI at the latest (early adaptation). The subsequent firing patterns were different among the neurons and were classified into 2 groups. Type I neurons showed relatively constant or slowly decreasing firing during the current pulse (Fig. 1B, *n* = 28, 55% of the analyzed cells), and type II neurons showed a rapid acceleration of firing rate for 100–200 ms and a

subsequent constant or slowly increasing firing rate (Fig. 1E, *n* = 21, 41%). In 25% (7/28) of type I neurons, the firing rate continued to decrease after early adaptation at high current intensities (Fig. 1B 2.0 nA). In 47% (10/21) of type II neurons, the firing rate for the 1st ISI was lowest in the spike train at low current intensities (Fig. 1D *upper* and 1E *arrow*). Even in these 10 type II neurons, the firing rate for the 1st ISI became highest among the rate for the subsequent ISIs during higher current pulses (Fig. 1D *lower* and E 3.6 nA). The increase in the firing rate for the 1st ISI corresponded to the steeper slope of the relationships between the firing frequency and injected

Table 2 Relationships between the repetitive firing properties and incidences of extra spike and secondary range

	type I	type II	others	Total
Incidence of extra spike	12/23 (52.1%)	11/14 (78.5%)	2/2 (100%)	25/39 (64%)
Incidence of secondary range	11/23 (47.8%)	5/14 (35.7%)	0/2 (0%)	16/39 (41%)

current (f - I relationships) for the 1st ISI, compared with the slopes for the 3rd ISI and steady-state (Fig. 1F). The ratio of the 1st ISI slope to the steady-state slope was 1.96 ± 0.14 ($n = 10$). In the remaining 3 neurons, which were neither type I nor II, two neurons had a type II firing pattern during a smaller injected current and a type I firing pattern during a larger injected current. One neuron had a complex firing pattern.

At the age of 4–9 and 10–15 days the numbers of type I and type II neurons were similar; however, the majority of JCMNs ($\geq 75\%$) were type I neurons at 16–21 and 22–27 days (Table 1). When the data were pooled from 4- to 15-day-old animals and from 16- to 27-day old animals, the percentage of type I neurons in 16- to 27-day old animals (81.8%) was significantly greater than the percentage of type I neurons (46.3%) in younger animals (Fisher's exact test, $P < 0.05$). Neither the f - I slope for the 1st ISI nor did steady-state differ among the age groups (Table 1).

JCMNs could also fire at a high rate in the steady-state. Although we did not inject currents into all neurons large enough to determine the maximum steady-state firing rates, the steady-state firing rate exceeded 50 Hz at current intensities of 1.5–4.0 nA in 54% (28/52) of the neurons tested. Even at 4–9 days, approximately 50% (8/17) of the neurons fired at higher than 50 Hz, and the firing rate exceeded 67 Hz in 3 of the 8 neurons.

2. Production of an extra spike

When we injected large currents up to 4.0 nA into 39 JCMNs, an extra spike was generated from the ADP of the 1st spike in 64% (25/39) of the neurons (Table 1, 2). Figure 2Cc shows an example of the extra spike obtained from a 4-day-old JCMN. This neuron showed only a small depolarizing hump following a single action potential elicited by an injection of a short current pulse (Fig. 2A); however, a distinct ADP was induced by a long current pulse at low intensity (Fig. 2Ca arrow). At higher current intensities, the peak potential of the 1st ADP in the spike train became more depolarized (Fig.

2Cb) and exceeded the spike threshold, leading to generation of an extra spike from the 1st ADP (Fig. 2Cc arrowhead). In this neuron, the f - I slope for the 1st ISI before occurrence of the extra spike did not differ from the slope in the presence of the extra spike. An extra spike was also obtained from older JCMNs (Fig. 2B, D and F). When the data were pooled from 4- to 15-day-old animals and from 16- to 27-day old animals, the incidence of an extra spike was significantly higher in 16- to 27-day old animals than in 4- to 15-day-old animals (Fisher's exact test, $P < 0.05$) (Table 1). Furthermore, when an extra spike was generated, the firing rate for the 1st ISI jumped to significantly higher value in 16–27-day-old-rats than in 4–15-day-old-rats (188.5 ± 18.1 Hz, $n = 9$ vs. 108.8 ± 8.76 Hz, $n = 16$; $P < 0.005$; points indicated by 'c' in Fig. 2E and F). The incidence of the extra spike was not different between type I and type II neurons (χ^2 test, $P > 0.10$, Table 2). In the remaining 14 neurons that did not display an extra spike, 6 neurons had a distinct ADP following the action potential elicited by a short current pulse; however, the peak potential of the 1st ADP of these 6 neurons did not exceed the spike threshold, even when firing rate for the 1st ISI increased to 70–120 Hz (data not shown).

Sixteen neurons (41%) of the 39 neurons have bilinear f - I relationships for the 1st ISI, exhibiting a distinct primary and a steeper secondary range of firing (Fig. 2F) previously described in adult brainstem motoneurons^{13–16}. In contrast to earlier reports, we found that a steeper secondary range of the 1st ISI was observed in neurons from all age groups (Table 1). The incidence of the secondary range was not significantly different between age groups when the data were pooled from 4- to 15-day-old animals and from 16- to 27-day old animals (χ^2 test, $P > 0.10$). The occurrence of the secondary range was related to neither type I/type II classification (χ^2 test, $P > 0.25$, Table 2) nor the incidence of the extra spike (χ^2 test, $P > 0.05$).

Fig. 2 Generation of an extra spike from the 1st ADP in the spike train.

A, B: Action potentials from 4- (*A*) and 19-day-old (*B*) type I neurons. Note that a distinct ADP was not observed in 4-day-old neurons.

C: Repetitive firing was evoked by current injection at 0.7 (*a*), 1.2 (*b*) and 1.6 (*c*) nA for the neuron in *A*. Note that the extra spike generated from the 1st ADP (arrowhead).

D: repetitive firing was evoked by current injection at 0.5 (*a*), 1.3 (*b*) and 2.3 (*c*) nA for the neuron in *B*.

E: *f-I* relationship for the neuron in *A*. The points on the graph indicated by 'a-c' were obtained from the corresponding traces in *C*.

F: *f-I* relationship for the neuron in *B*. The points on the graph indicated by 'a-c' were obtained from the corresponding traces in *D*. The dotted lines in *Ca-c* and *Da-c* indicate the spike threshold of the 1st spike in *Ca* and *Da*, respectively.

IV. Discussion

Repetitive firing patterns of JCMNs were characterized by the higher firing rate for the 1st ISI than the rate for succeeding ISIs (early adaptation), as observed in trigeminal¹⁷⁾, genioglossal¹⁵⁾, and spinal¹⁸⁾ motoneurons. Regarding firing patterns following early adaptation, type II neurons showed an increasing firing pattern and were found in 4–15-day-old rats. In hypoglossal motoneurons, the majority of the cells in 8–15-day-old rats show an accelerating firing pattern, the f - I slope for the steady-state is steeper than the slope for the 1st ISI, and the firing rate for steady-state is higher than the rate for the 1st ISI, even at large current intensities¹⁶⁾. In contrast, when current pulses at high intensities were injected into type II JCMNs, the firing rate for the 1st ISI became highest in the spike train, and the f - I slope for the 1st ISI was steeper than the slope for the steady-state (Fig. 1F). Furthermore, the number of type II neurons was not different from the number of type I neurons in 10–15-day-old rats. Therefore, the firing properties of type II JCMNs were different from the hypoglossal motoneurons regarding the accelerating firing pattern.

It has been reported that maximal steady-state firing frequency of motoneurons is low in neonatal animals compared with adults^{15, 16, 18)}. The maximal rate is 33 Hz with a range of 19–44 Hz in neonatal rat spinal motoneurons¹⁸⁾, and the rate exceeds 50 Hz in only one of 51 neonatal (0–15-day-old) rat hypoglossal motoneurons¹⁶⁾. In contrast to these motoneurons, approximately 50% JCMNs at 4–9 days fired at higher frequencies than 50 Hz. The amplitude and half duration of the mAHP in JCMNs at 4–9 days are slightly smaller compared with hypoglossal motoneurons⁵⁾. Thus, a high steady-state firing rate may be due to the smaller mAHP because AHP is an important factor in the control of neuronal discharge¹⁹⁾. Furthermore, trigeminal motoneurons have been reported to display persistent sodium currents during the membrane depolarization¹⁷⁾. These persistent inward currents might have increased the steady-state firing frequency in neonatal JCMNs.

As mentioned above, the maximal firing rate in the steady-state was high in JCMNs from all age groups. Although generation of a large force by the jaw-closing muscles is not necessary during suckling, it is likely that JCMNs were preparing for producing relatively robust force early in postnatal life. In fact, when anything is placed in the mouth,

human infants show the bite reflex (a sudden closure of the mandible with activation of the masseter muscle), even at birth²⁰⁾. Extensive occlusal wear of the molar teeth is found in guinea pig fetuses, even ten days prior to birth²¹⁾. These results suggest that neonates before weaning appear to be able to generate a relatively strong jaw-closing force. The generation of the jaw-closing force in neonates may contribute to the proper development of the oro-motor system.

During postnatal development, the percentage of type I JCMNs increased by up to 82% at 16–27 days. This increase has been shown in adult hypoglossal motoneurons that mainly exhibit a decrementing firing pattern similar to type I¹⁶⁾. The type I firing property leads to an initial high firing rate of JCMNs in response to excitatory inputs, even when the inputs are not strong. Furthermore, the incidence of an extra spike that also produces an initial high firing rate increased in the JCMNs with age. The initial high firing rate of JCMNs would enable the fast rise of the jaw-closing force necessary to chew food because insertion of a single extra action potential in a low frequency spike train of a spinal motoneuron can cause the marked, long-lasting tension enhancement produced by the muscle fibers innervated by the motoneuron²²⁾. These developmental changes in firing properties of JCMNs, as well as the high maximal firing rate observed throughout the postnatal development, may contribute to the transition from suckling to chewing.

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