Neuroscience Letters 525 (2012) 163–167

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Four subtypes of motor neurons exhibiting mutually exclusive firing patterns in the spinal ventral horn

Junming Zhu[a,1], Fang Feng[a,1], Kunming Ni[b], Yuxin Zheng[a], Jianmin Zhang[a,*]

[a] Department of Neurosurgery, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China
[b] Department of Neurobiology, Key Laboratory of Medical Neurobiology of Ministry of Health of China, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

HIGHLIGHTS

- This research contributes to classification and study of motor neurons (MNs).
- MNs may regularly fire in patterns with unique characteristics.
- Four distinct MN subtypes are identified based on distinct firing patterns.
- Three typical forms of afterhyperpolarization were illustrated in these neurons.
- These differences of firing pattern may be associated to their functions.

ARTICLE INFO

Article history:
Received 24 May 2012
Received in revised form 15 July 2012
Accepted 31 July 2012

Keywords:
Interspike interval
Delayed onset
Transient
Spike frequency adaptation

ABSTRACT

Motor neurons (MNs) communications are thought to occur primarily through spike bursts and regularly firing action potential trains. Reports of both burst and nonburst firing MNs suggest that these neurons may regularly fire in a variety of controlled output patterns with unique characteristics. Based on the cellular response to somatic current injection in these neurons, four distinct MN subtypes are identified from the spinal ventral horn. Approximately 42% of MNs exhibited regular firing, with minimal current injection (rheobase) exhibited a short latency, and with stronger current intensities exhibited significant spike frequency adaptation (SFA). Another 30% of MNs exhibited delayed onset at rheobase with a weakly-adapting firing pattern as stimulation increased. The remaining 18% and 10% of MNs exhibited transient firing patterns or exhibited irregular firing patterns when strongly depolarized, respectively. Our results provide a basis for improvement in the classification and study of MNs.

1. Introduction

Motor neurons (MNs) in the spinal ventral horn play an essential role in directly regulating muscle contraction, and these neurons are thus implicated in a number of pathological conditions. Sherrington referred as the "final common pathway" to emphasize the independent and direct contribution to control of diverse locomotors [12]. MNs are unique sources of ascending fibres targeting multiple muscle units, receiving abundant descending input from central pattern generators (CPGs) [22], as well as afferent information from interneurons and sensory systems [1,6]. Due to the diversity of input, it is not surprising that spinal MNs have a heterogeneous organization that is reflected in a variety of classification schemes.

Numerous previous studies reported that muscle units are associated with many different types of MNs. Eccles described slow twitch and fast twitch muscles which were innervated by phasic and tonic MNs, respectively [7]. Later it was found that the fast MN units coordinating with fast twitch muscles generate occasional high-frequency action potential bursts (30–60 pulses/s). Conversely, slow MN units coordinating with slow twitch muscles are characterized by a relatively steady, low frequency activity (10–20 pulses/s) [9]. The distribution of interspike intervals (ISI) in MNs varies among cells, with some neurons exhibiting bimodal distributions, suggesting that there were at least two firing patterns. High frequency bursts generally comprise the fast ISI peak, while regular, and low frequency firing corresponds to a much longer ISI peak distribution.

MNs in the oculomotor and abducens nuclei have demonstrated notable variations in sustained, transient, and delayed firing

Abbreviations: ADP, afterdepolarization; AHP, afterhyperpolarization; ACSF, artificial cerebrospinal fluid; CPGs, central pattern generators; ISI, interspike interval; MN, motor neuron; SFA, spike frequency adaptation.

* Corresponding author. Tel.: +86 571 13805722695; fax: +86 571 87784755.
E-mail address: zjm135@vip.sina.com (J. Zhang).

[1] These authors contributed to this work equally.

0304-3940/$ – see front matter.
http://dx.doi.org/10.1016/j.neulet.2012.07.062

Table 1
Electrphysiological characteristics of MNs recorded in spinal cord slices.

Type	*n*	Resting V_m (mV)	AP amplitude (mV)	AP half-width (ms)	R_{in} (MΩ)
Delay onset	13	61.09 ± 2.2	79.9 ± 2.79	0.62 ± 0.18	63.4 ± 4.57
Regular	18	64.06 ± 2.56	83.8 ± 3.31	0.51 ± 0.18	50.8 ± 4.88
Irregular	8	66.7 ± 1.99	86.0 ± 2.72	0.44 ± 0.15	34.6 ± 3.67
Transient	4	66.5 ± 1.51	83.0 ± 2.81	0.36 ± 0.08	28.5 ± 3.59

Data show means ± S.E.M. Resting V_m, resting membrane potential; AP, action potential; R_{in}, input resistance;

patterns [8,19,20]. However, systematic research of firing patterns associated with different subtypes of MNs in spinal cord lacks consistency. To better understand the cellular neurobiology of MNs from spinal ventral horn, the present study used whole-cell recordings to study the firing patterns and the electrobiological properties of MNs within the anterolateral ventral horn. Our data demonstrate that four subtypes of MNs with distinct firing patterns are identified, providing a basis for improvement in the classification and study of MNs.

2. Materials and methods

2.1. Animal subjects and samples selection

A total of 12 male Sprague-Dawley rats aged 2–3 weeks in relatively healthy condition for whole-cell recording configuration were used in this study, producing a total of 43 sampled neurons recorded in 25 slices.

2.2. Slice preparation

Sprague-Dawley rat specimens were anesthetized with halothane and treated subsequently with pentobarbital (130 mg/kg i.p.). After anesthetization, animals were perfused with modified ice-cold (4 °C), oxygenated (95% O_2/5% CO_2) sucrose-substituted artificial cerebrospinal fluid (sucrose-ACSF) containing (in mM): 212.5sucrose, 3.5KCl, 26$NaHCO_3$, 1.3$MgSO_4$, 2.0$MgCl_2$, 1.2$CaCl_2$, 1.2KH_2PO_4, and 10glucose. Spinal cord tissues were removed from each rat specimen and blocked in order to prepare horizontal spinal cord slices (450–500 μm) containing transverse sections of the spinal cord using a Leica VT1000S. Slices were initially incubated for 30 min in a submerged holding chamber at 36–40 °C oxygenated with ACSF, where sucrose was replaced with NaCl, which contain (in mM): 125NaCl, 3.5KCl, 26$NaHCO_3$, 1.3$MgSO_4$, 2.4$CaCl_2$, 1.2KH_2PO_4, and 10 glucose. Subsequent to this 30 min period, tissues were allowed to return to room temperature (22–24 °C).

2.3. Patch-clamp recording

Whole-cell recordings were produced using MNs collected from the spinal ventral horn region. MNs were recorded from the whole cell configuration with a sharp electrode (3–5 MΩ) filled with either a solution of (in mM): 140KCl, 10Hepes and 2$MgCl_2$. Series resistance (<20 MΩ) was about 90% compensated via carefully adjusting bridge balance to obtain a clamp speed of <50 μs. The holding potential for action potential recordings was −60 mV. MNs were identified by antidromic activation originating from the ventral root of the L4–L6 [3].

2.4. Data analysis

Instantaneous firing frequency (*f*) was calculated as the reciprocal of the ISI. Rheobase was determined as the minimum current needed to elicit at least one action potential. Input resistance (R_{in}) was calculated from responses to hyperpolarizing pulses in the linear region of the current–voltage relationship. SFA was quantified by plotting the ISI versus the latency to the later of the two spikes. The slope of this plot was assessed by fitting using linear regression, and the slope used was presented as a measure of SFA. T Statistical analysis was performed on Systat19 (SPSS Inc.) and included one-way ANOVAs with post hoc Tukey tests, Kruskal–Wallis tests; statistical significance was defined as $P < 0.05$.

3. Results

3.1. Overview of distinguishing features

Four main subtypes of MNs were characterized by their firing patterns during depolarizing current injection. Regular MNs typically showed sustained spiking throughout the current step, and with mild current intensities, trains of spikes exhibited spike frequency adaptation (SFA) (Figs. 1Aa and 2Ac). Delayed onset MNs typically exhibited a marked delay to the first action potential at rheobase, and with mild current intensities exhibited a weakly-adapting firing pattern (Fig. 1Ab and 2Ac). Irregular cells did not fire repetitively in response to mild stimulation, and fired spikes distributed irregularly throughout current application (Fig. 1Ac and 3Ab). Transient spike cells typically exhibited a brief (<100 ms) train of high-frequency (≥50 Hz) spikes produced by strong depolarizing current injection (Figs. 1Ad and 3Ba). Passive membrane properties are summarized in Table 1.

3.2. Action potential shape

MNs with sustained spikes were, in turn, divided into Type 1, Type 2, and Type 3 groups based on the specific features of the action potential shape evoked by a brief depolarizing current pulse. Type 1 shows biphasic AHP, consisting of an early fAHP and a delayed sAHP. Meanwhile, Type 2 MNs consistently demonstrated an intermediate afterdepolarization (iADP) existing between observed fAHPs and sAHPs, and illustrated as deep sAHP (Fig. 1Bb). Regular and transient MNs primarily showed Type 1 biphasic AHP consisting of an early fAHP and a delayed sAHP (Fig. 1Ba and Bd). Delayed onset MNs showed Type 2 biphasic AHP, and the irregular MNs primarily showed Type 3 monophasic AHP (Fig. 1Bc).

Based on the analysis of the AHP following the first spike elicited by rheobasic stimulation in each cell type, the AHPs in all delayed onset MNs were best described with fAHP and sAHP ($t_1 = 2.42 \pm 1.03$ ms; $t_2 = 95.8 \pm 6.7$ ms). Similarly, regular MNs ($t_1 = 3.87 \pm 1.20$ ms; $t_2 = 81.4 \pm 4.64$ ms) and transient MNs ($t_1 = 3.05 \pm 0.99$ ms; $t_2 = 30.03 \pm 3.19$ ms). The AHPs in irregular MNs were best described with monophasic AHP ($t = 41.3 \pm 3.87$ ms). These differences in AHP kinetics among MN subtypes were still present when neurons were analyzed at equivalent V_m values.

3.3. Characterization of MN subtypes

3.3.1. Regular MNs

Regular MNs fired at low frequency in response to weak stimulation, and steadily increased over a wide range of stimulus