Development of Tonic Firing Behavior in Rat Soleus Muscle

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Eken T, Elder GC, Lømo T. Development of tonic firing behavior in rat soleus muscle. J Neurophysiol 99: 1899–1905, 2008. First published February 6, 2008; doi:10.1152/jn.00834.2007. Tonic firing behavior in soleus muscle of unrestrained rats aged 7 to ≥100 days was studied by chronic single-motor-unit and gross-electromyographic (EMG) recordings. Median motor-unit firing frequency at 10 days was 19–26 Hz and did not change appreciably after this time, whereas interval-to-interval firing variability was reduced with age. Two units with median frequencies 40 and 59 Hz were encountered in one 7-day-old rat. Integrated rectified gross EMG developed from being phasic only to predominantly tonic during the second and third postnatal week. From the end of the third week, rather short tonic periods with irregular amplitude were replaced by longer lasting constant-amplitude periods. Quantitatively, median duration of gross-EMG activity episodes more than doubled, while 90th-percentile values for episode duration increased 19-fold, from 7.4 s at 7 days to 140 s in adults. The main part of this increase took place after 22 days. Previous work in adult rats has indicated that descending monoaminergic innervation is essential for maintained tonic motoneuron activity, which probably is caused by depolarizing plateau potentials. Such innervation of the lumbar spinal cord matures gradually to an adult termination pattern in the lumbar spinal cord as late as 2–4 wk after birth. The present results, describing a concurrent considerable development of tonic firing behavior, support and extend these findings.

INTRODUCTION

Motoneurons (MN)s to soleus (SOL) muscles in unrestrained adult rats typically generate large numbers of impulses per day (300,000–500,000) at a relatively low, stable frequency of around 20 Hz (Eken 1998; Hennig and Lømo 1985). Often the discharges occur as long-lasting trains of impulses (up to 9 min), consistent with a postural function of SOL muscles (Hennig and Lømo 1985). This firing behavior gives rise to characteristic long-lasting tonic segments in the whole-muscle gross-EMG signal (Eken 1998).

Previous evidence regarding postnatal development of SOL firing behavior is conflicting and incomplete. Navarrete and Vrbová (1983) reported that tonic postural activity develops gradually between 12 and 21 days of age. They also reported that the mean MN firing frequency stays at ~10 Hz, even in the adult, in contrast to ~20 Hz reported by us for adult unrestrained rats (Eken 1998; Hennig and Lømo 1985) and ~28 Hz for 14- to 30-day-old tape-restrained rats reported by Buffelli et al. (2002). Westerga and Gramsbergen (1994) have also reported the appearance of tonic EMG activity with age. In their work, maximum MN firing frequency during nonlocomotor behavior increased from ~10 to ~50 Hz between 10 and 16 days; other firing-frequency characteristics were not reported.

Postnatal appearance of tonic activity in SOL muscles (Navarrete and Vrbová 1983) may account for much of the postnatal transformation of type II to type I fibers. SOL muscles in 8-day-old rats contain ~50% fast type II muscle fibers (Thompson et al. 1984), most of which are transformed into slow, fatigue resistant type I fibers during subsequent months (Kugelberg 1976). Results obtained by stimulating denervated muscles directly with different stimulus patterns show that tonic, low-frequency impulse activity determines the slow phenotype of adult SOL muscles (Ausoni et al. 1990; Eken and Gundersen 1988; Gorza et al. 1988; Westgaard and Lømo 1988) in agreement with earlier cross-reinnervation experiments (Close 1969).

In adult SOL MNs, long-lasting trains of impulses are turned on or off by excitatory or inhibitory inputs (Eken and Kiehn 1989) and disappear after chemical destruction of monoaminergic axons descending in the spinal cord (Kiehn et al. 1996). Monoamine-activated plateau potentials in MNs of reduced preparations respond similarly (Kiehn and Eken 1998), suggesting that monoamine-dependent plateau potentials underlie the tonic impulse trains of adult SOL MNs observed in intact animals during normal behavior. Serotonergic and noradrenergic pathways descending from the brain stem acquire their adult termination pattern in the lumbar spinal cord as late as 3–4 wk after birth (Bregman 1987; Rajaoefreta et al. 1989, 1992). Similarly, 5-HT2A receptors, presumed to be particularly important for serotonin-dependent depolarization, appear on rat SOL and extensor digitorum longus (EDL) MNs during the second postnatal week (Vult von Steyern and Lømo 2005) with distinct differences in distribution on individual MNs consistent with the hypothesis that tonic firing of SOL MNs depends on monoaminergic innervation.

The primary aim of the present work is to characterize in behaving animals the postnatal changes in SOL MN firing activity and gross EMG. Furthermore, due to the perceived importance of monoamine-dependent plateau potentials for long-lasting tonic muscle activity, we seek to relate postnatal development of tonic firing to the development of descending monoaminergic innervation to the lumbar spinal cord as documented in the existing literature.

METHODS

All experiments were performed in Mall-Wistar rats. Gross SOL EMGs were obtained from 15 rats ranging from 7 days old to adult (≥100 days old estimated from growth curve). Single-motor-unit activity was studied in six 7- to 22-day-old rats. The experiments were approved by the Norwegian Animal Research Authority in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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with Norway’s Animal Protection Act and overseen by the veterinarian responsible for the animal house at the department.

Surgery was performed under general anesthesia. Equithesin was used when possible as described in Eken (1998). However, in animals <2 wk of age, the use of Equithesin was associated with a considerable mortality. Thus in the youngest animals, ether was used as a general anesthetic as in previous experiments in rats of similar age (Buffelli et al. 2002; Navarrete and Vrbová 1983). When ether was used, one person performed the surgery, and one was responsible for keeping the animal adequately anesthetized. Regardless of anesthetic agent, an adequate level of anesthesia was ensured by regularly pinching the skin of the feet to test for the lack of withdrawal reflexes. Body temperature was maintained by radiant heat. Lidocaine local anesthetic (Xylocain, Astra) was applied to the wound before closing. After completion of surgery, the animal was placed in a cardboard box with tissue paper and kept warm by radiant heat until awake. The animals were offered drinking water with sugar before being put back with their mother. No animal was rejected by mother or sibs.

Single-motor-unit or gross-EMG electrodes were implanted in the SOL muscle using a technique modified from Eken (1998). Only one kind of electrode was implanted in each animal due to the small size of the muscle in young rats. All electrodes were made from 50- or 25-μm-diam Teflon-coated platinum/iridium wires (7760 and 7750, A-M Systems) that were each soldered to a separate multifilament stainless steel wire (AS 632, Cooner Wire). The soldering points were insulated with silicone elastomer (Silastic Medical-Grade Elastomer, MDX4-4210, Dow Corning). For gross-EMG recordings, two 25- or 50-μm wires with bare ends were implanted, one in the proximal end and another in the distal end of the muscle. For single-unit experiments, two to four 25-μm wires were twisted tightly together in a bundle, which was cut obliquely to obtain an electrode for differential recording and gently inserted into the muscle through a slit in the fascia. A ground electrode made from 50-μm wire was sutured to a fascia in the lower leg at some distance to the SOL. In the youngest animals, all wires were taken subcutaneously to the neck, out through a slit in the skin, and fixed by means of a suture. The wires were soldered to a socket contact (E363/0, Plastics One), which was attached to an electrode mount (MS363, Plastics One) to provide electrical connection during recording. In older animals, wires were taken subcutaneously to the head where the electrode mount was fixed by dental cement (Simplex Rapid, Austenal Dental). Electrode positions were verified in all experiments after the animal was killed (anesthetic overdose).

Recordings of gross EMG were performed median 1 day postoperatively (range: 1–3 days, except for 1 adult rat, which was recorded from 6–9 days after surgery). Single-unit EMG was obtained 1 day postoperatively except for one 7-day-old rat assessed on the day of surgery and one animal at 1 and 3 days. Recordings from the youngest animals were performed with several sibs in the cage, allowing them to crawl freely around and cuddle into a heap. Gross-EMG and single-motor-unit signals were obtained and stored on analog FM tape, as well as on video tape in some cases, as described in Eken (1998).

To quantify tonic gross-EMG activity, the duration of all activity episodes was computer measured as described previously (Kiehn et al. 1996): the integrated rectified gross-EMG signal was digitized on a computer (Macintosh IIX, Apple Computer) equipped with an analog/digital input/output card and a DMA card (NB-MIO-16H-9 and NB-DMA-8-G, National Instruments) and processed by a “virtual instrument” developed with LabVIEW (National Instruments). First, mean and standard deviation (SD) voltages from the longest-duration baseline segment were determined. The start of a gross-EMG activity episode was defined as the time when the gross-EMG signal passed upward through a threshold set at mean + 7 SD. The episode ended when the signal passed downward through mean + 1 SD. These values were chosen to provide reliable detection of activity episodes of varying amplitude while at the same time preventing erroneous measurements caused by oscillation of the analog signal between levels above and below thresholds. The algorithm proved highly robust, as start and endpoints were consistent if different baseline segments were chosen or thresholds were moved by 1 SD in either direction. Furthermore, results were comparable to manual episode definition (cf. Fig. 2 in Kiehn et al. 1996).

Interspike intervals from single-unit recordings were analyzed as in Eken (1998). Briefly, the unprocessed EMG signal was high-pass filtered and fed into a spike discriminator (Slope/Height Window Discriminator, Frederick Haer). The output detection pulses were displayed on a storage oscilloscope underneath the high-pass filtered single-unit EMG signal to ascertain that every spike in a train was detected. At the same time, the detection pulses triggered an oscilloscope with signal delay and storage circuitry (MS6 with SDS 6, Medelec), which displayed all the unprocessed motor-unit potentials superimposed at high sweep speed to verify that they were of similar shape (cf. Fig. 4). The detection pulses were also fed into a computer with custom-built software (see preceding text) that measured the interspike intervals. Firing frequencies >100 Hz were excluded from the final analyses because those values mainly represent noise due to interference from other units (cf. maximal phasic firing frequencies reported by Westerga and Gramsbergen 1994). Additionally, firing frequencies <2 Hz were excluded from analyses of interspike interval variability. Because the focus of the present study was on tonic motor behavior, only recordings containing ≥90 interspike intervals were included in the material.

Statistical analyses were performed with StatView 5.0 for Macintosh (SAS Institute). Values from adult rats for comparison were taken from material pertaining to a previous publication (Eken 1998). Trends during development were assessed with linear regression; probability of zero slope of the regression line is reported. In cases where the precise age of adult rats was lacking, age was set to 100 days. Group differences between young (present material) and adult (Eken 1998) rats were assessed with the Mann-Whitney U test.

RESULTS

Gross EMG

Examples of recordings of integrated rectified gross-EMG activity are shown in Fig. 1. In the youngest animals, only phasic activity could be seen (Fig. 1, 7 days). This pattern was consistent with behavioral observations; the animals mostly huddled with their sibs and occasionally padded a bit with their legs to change position. As the animals grew older, they started to rise up, move around more freely in the cage, and display initially irregular tonic episodes in gross-EMG records (Fig. 1, 11 days). These episodes were then replaced by more regular-looking gross-EMG profiles that first tended to slope downward (Fig. 1, 19 days) and later appeared more horizontal (Fig. 1, 24 days). Eventually, the tonic segments became more prolonged (Fig. 1, 34–71 days), reflecting longer periods of sustained muscle activity. Adult data resembled previously published results (Eken 1998).

A quantitative assessment was made of the durations of activity episodes during development (Fig. 2). Characteristics for each age were computed from pooled data with 478–5,073 episodes recorded during 3.2–56 h (total 15 animals; 21,239 episodes; 154 h). Median episode duration increased by a factor of 2.3 with age (1.6 s at 7 and 8 days; 3.6 s in 24-day-old, 57-day-old, and adult animals). However, the most striking finding was the increase in 90th-percentile values (7.4 s at 7 days; 140 s in adults), i.e., the duration of the longest activity periods increased by a factor of 19 in adult animals compared...
with young ones. The increase in duration of the longest activity periods made mean episode duration increase from 3.4 s at 7 days to 62 s in adults in spite of the rather moderate increase in median duration. Total activity duration, measured as the sum of all individual activity episode durations as fraction of total recording time, increased by a factor of 1.7 from 39% at 7–8 days (pooled data from 2 rats) to 67% in adults (3 rats).

Single-motor-unit EMG

Data were obtained from 19 units in six rats. Median recording duration per unit was 58 s (range: 8–2,698 s, total 4,368 s), and median number of intervals recorded per unit was 879 (range: 94–7,885, total 24,460). Characteristics of individual recordings are shown in Table 1 and Fig. 3. Two units with median frequencies 40 and 59 Hz were encountered in one 7-day-old rat; a firing episode of the latter unit is shown in Fig. 4. Apart from this, there was a striking similarity in firing frequency between age groups: median motor-unit firing frequency was 24 Hz at 10 days, 16 Hz at 15 days, 18 Hz at 20 days, and 23 Hz at 22 days. We have previously published a median firing frequency of 22 Hz in 10 adult SOL motor units (6 Møll-Wistar rats from the same supplier, estimated /H11350

Development of regularity of firing was also assessed using the present material and previously unpublished results from recordings of spontaneous motor behavior in adult rats (Eken 1998). The coefficient of variation (CV) of interspike intervals (ISIs) in the individual motor units showed no significant trend during development (P = 0.73), and no difference between the young and adult age groups (P = 0.94). Removing the units with particularly high firing frequencies from analysis (2 at 7 days, 1 in an adult, cf. Fig. 3) gave comparable results (P = 0.81 for trend; P = 0.96 for age group comparison).

Development of regularity of firing was also assessed using the present material and previously unpublished results from recordings of spontaneous motor behavior in adult rats (Eken 1998). The coefficient of variation (CV) of interspike intervals (ISIs) in the individual motor units showed no significant trend during development (P = 0.90), and there was no significant difference between the young and adult age groups (P = 0.71). However, the CV does not take into account that ISIs are

FIG. 1. Integrated rectified gross electromyogram (EMG) during spontaneous behavior at different ages. All recordings are from different animals and last 30 min. Note phasic appearance initially (7–9 days), irregular tonic activity (11 days), downward-sloping tonic episodes (19 days), and finally flattening and prolongation of tonic activity (24–71 days).

FIG. 2. Quantitative assessment of gross-EMG episode durations during development. ●, median duration (50th percentiles), vertical bars are drawn between 10th and 90th percentiles. Horizontal lines mark 10th, 25th, 75th, and 90th percentiles. Pooled data from 3 rats at 11 days and for adults; 2 rats at 9 days; 1 rat at other ages (total: 15 animals).
TABLE 1. Characteristics of the 19 included soleus motor units

<table>
<thead>
<tr>
<th>Unit ID</th>
<th>Age, days</th>
<th>No. of Intervals</th>
<th>Recording Duration, s</th>
<th>Firing Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>149.AL1</td>
<td>7</td>
<td>329</td>
<td>8</td>
<td>59 (53–67)</td>
</tr>
<tr>
<td>149.AT4</td>
<td>7</td>
<td>1,987</td>
<td>61</td>
<td>40 (37–45)</td>
</tr>
<tr>
<td>155.BL1</td>
<td>10</td>
<td>94</td>
<td>10</td>
<td>26 (19–34)</td>
</tr>
<tr>
<td>155.BL2</td>
<td>10</td>
<td>147</td>
<td>10</td>
<td>19 (14–22)</td>
</tr>
<tr>
<td>155.BL3</td>
<td>10</td>
<td>4,054</td>
<td>266</td>
<td>24 (18–32)</td>
</tr>
<tr>
<td>155.BL5</td>
<td>10</td>
<td>2,569</td>
<td>177</td>
<td>20 (16–24)</td>
</tr>
<tr>
<td>155.BT1</td>
<td>10</td>
<td>1,242</td>
<td>60</td>
<td>25 (19–31)</td>
</tr>
<tr>
<td>152.AT1</td>
<td>15</td>
<td>188</td>
<td>29</td>
<td>9 (7–11)</td>
</tr>
<tr>
<td>152.AT2</td>
<td>15</td>
<td>879</td>
<td>58</td>
<td>18 (16–19)</td>
</tr>
<tr>
<td>152.BT1</td>
<td>15</td>
<td>431</td>
<td>47</td>
<td>16 (12–20)</td>
</tr>
<tr>
<td>137.AL1</td>
<td>20</td>
<td>1,712</td>
<td>73</td>
<td>33 (26–42)</td>
</tr>
<tr>
<td>138.AT1</td>
<td>20</td>
<td>458</td>
<td>29</td>
<td>29 (21–34)</td>
</tr>
<tr>
<td>138.AT2</td>
<td>20</td>
<td>165</td>
<td>20</td>
<td>18 (12–23)</td>
</tr>
<tr>
<td>146.AT1</td>
<td>20</td>
<td>7,885</td>
<td>2,698</td>
<td>16 (11–20)</td>
</tr>
<tr>
<td>146.AT1</td>
<td>20</td>
<td>3,510</td>
<td>369</td>
<td>17 (14–19)</td>
</tr>
<tr>
<td>146.BL1</td>
<td>22</td>
<td>1,331</td>
<td>229</td>
<td>22 (14–29)</td>
</tr>
<tr>
<td>146.BL2</td>
<td>22</td>
<td>381</td>
<td>23</td>
<td>24 (19–29)</td>
</tr>
<tr>
<td>146.BL3</td>
<td>22</td>
<td>1,961</td>
<td>193</td>
<td>25 (20–29)</td>
</tr>
<tr>
<td>146.BT1</td>
<td>22</td>
<td>137</td>
<td>8</td>
<td>21 (18–24)</td>
</tr>
</tbody>
</table>

Number of intervals, recording duration, and firing-frequency characteristics (median and quartiles in parentheses) for each of the 19 included units. Unit ID consists of rat number and unit code in the individual rat separated by a point. Data from 6 rats; note that rat 146 was recorded from both at 20 and 22 days.

temporally related. Thus interval-to-interval variability was assessed by computing the difference between successive ISIs (ΔISIs) and using the inter-quartile range (IQR) of the resulting distribution as a measure of motor-unit firing variability. Results are shown in Fig. 5. There was a highly significant reduction in ΔISI IQR during development (trend: P = 0.003; young age group vs. adults P = 0.0008). Excluding the units with particularly high firing frequencies from this analysis (Fig. 5, ○) yielded even more significant results (trend: P = 0.0004; young age group vs. adults P = 0.0002).

DISCUSSION

The present work shows that tonic impulse activity in rat SOL MNs develops postnatally, starting 1–2 wk after birth and increasing into adult life. The duration of the longest episodes of tonic activity increased 19-fold from 7–8 days of age, and most of this increase occurred after 3 wk. In contrast, MN firing frequency during tonic activity remained relatively unchanged at ~20 Hz from 10 days of age. Interval-to-interval firing variability was reduced with age.

Single-motor-unit EMG

Recently Cangiano and coworkers have observed mean firing frequencies of rat SOL MNs that ranged from 10.3 to 15.9 Hz between embryonic day 21 and postnatal day 5 and from 22.0 to 29.2 Hz between postnatal days 8 and 30 (Buffelli et al. 2002; A. Cangiano, personal communication). The latter values are essentially the same as those obtained by us between days 10 and 22 (Table 1). Previously, Navarrete and Vrbová (1983) had found no appreciable changes in MN firing frequencies from 7 days to adult. In their work, however, mean firing frequency in MNs did not exceed ~10 Hz even in adult animals. The only other report on postnatal development of SOL MN firing is by Westerga and Gramsbergen (1994), who described an increase in maximal firing frequencies for nonlocomotor behavior from ~10 Hz at 10 days toward 50 Hz at 16 days. Measures of central tendency and variability of firing frequencies were not published.

Such discrepancies in observed firing patterns might be explained by differences in electrode design and recording conditions. Navarrete and Vrbová (1983) used 75-μm-thick Teflon-coated stainless-steel wires where ~100 μm of insulation was removed, whereas Westerga and Gramsbergen (1994) used obliquely cut 50-μm enamel-insulated Ni/Cr wires spaced 500 μm apart. Buffelli et al. (2002) used two 25-μm-thick Teflon-insulated platinum wires glued together and cut obliquely to obtain slightly separated tips, comparable to the single-motor-unit EMG electrodes utilized in the present study. The latter design ensures that recording surfaces are smaller and closer together (see METHODS), and therefore more selective (Andreasen and Rosenfalke 1978). Higher selectivity enables one to distinguish a single motor unit during higher total muscle activity, and thus to higher firing frequencies, because MN firing frequency is closely related to whole-muscle EMG activity (Hoffer et al. 1987). This may explain, at least in part, why we (Eken 1998; Eken and Kiehn 1989; Gorassini et al. 2000; Hennig and Lømo 1985) and Buffelli et al. (2002) find higher spontaneous firing frequencies in rat SOL motor units than Navarrete and Vrbová (1983).

Two motor units recorded in the youngest rat were clearly different from the rest. Contrary to what one would have expected from previous studies (Navarrete and Vrbová 1983; Westerga and Gramsbergen 1994), their firing frequencies were particularly high. However, they resembled a previously described fast unit in the adult SOL and may have been fast units containing Type IIA muscle fibers (Eken 1998).

Gross EMG

An increase in amount of tonic activity from 7 days to adult has been described previously. According to Buffelli et al. (2002), activity in SOL motor units progressed from short bursts in newborn rats to short locomotor bursts and tonical activity during standing after 2 wk. Westerga and Gramsbergen
(1994) described a gradual increase of activity level from 11 days of age but found no further changes after 14 days. According to Navarrete and Vrbová (1983), progression to the adult pattern occurred entirely between 12 days and 3 wk of postnatal development. These findings contrast with our own, which describe progressive increase in tonic activity until well after 2 mo of age, most of the increase occurring after 3 wk. A reason for the discrepancy between our results and previous work may be the duration of recording periods. The level of activity in the SOL of an unrestrained rat varies markedly from one 15- to 30-min period to the next (Fig. 1 in the present study) (see also Elder and Toner 1998). Previous reports are based on recordings lasting only for 10–15 min (Westerga and Gramsbergen 1994) or 1–2 h (Navarrete and Vrbová 1983).

The results from Buffelli et al. (2002) were obtained during 30- to 60-min-long recordings with animal containment obtained with tapes and pins. Our gross-EMG recordings in freely moving animals which lasted for 3.2–56 h may thus be more representative.

**Motoneuronal plateau potentials**

In intact adult rats, stable tonic activity of SOL motor units probably arises from monoamine-dependent depolarizing plateau potentials in the MNs (see Kiehn and Eken 1998). Long-lasting tonic segments in recordings of integrated rectified gross EMG from adult rats (cf. Fig. 1) have been attributed to this phenomenon (Eken 1998; Kiehn et al. 1996). Expression of plateau potentials in SOL MNs seems to require the presence of serotonin or noradrenaline normally released by monoaminergic axons descending from brain stem nuclei: Selective depletion of spinal monoamines in adult rats by intrathecal administration of neurotoxins (Kiehn et al. 1996) caused the tonic segments to become fewer, shorter, and downward sloping, resembling recordings from normal rats in their third postnatal week (cf. Fig. 1 in the present study). Quantitative analyses with the same algorithm as in the present study showed that monoamine denervation decreased mean activity duration to 34% and total activity duration to 46% of controls. The effect on the longest duration episodes was most pronounced; thus the 90th percentile values decreased to less than one-third at or above 20 days after drug administration. Interestingly, motor-unit firing frequencies remained unchanged. This is a mirror image of the developmental changes of tonic activity in SOL MNs during the second through fourth weeks after birth described in the present study.

The reduction of interval-to-interval firing variability seen in our material is also compatible with maturation of plateau-potential generating properties in SOL MNs. Plateau potentials are well suited for the maintenance of long-lasting stable MN firing due to uncoupling of spike
generation from the variability in synaptic input and from the effects of nonlinear summation of excitatory synaptic potentials (Kiehn and Eken 1998). Moreover, during the plateau, the input conductance increases two to three times compared with resting membrane potential (Hounsgaard and Kiehn 1989), making synaptic inputs less effective and further stabilizing firing frequency (Heckman et al. 2005; Kiehn and Eken 1998).

The relation of the changes in firing characteristics to the maturation of monoamine innervation of the lumbar spinal cord is remarkable: different types of 5-HT receptors may mediate the effects of serotonin on MNs, but 5-HT_2A receptors, and especially 5-HT_2A receptors, appear particularly important in facilitating neuronal activity (Wang and Dun 1990; White and Fung 1989). 5-HT_2A receptor immunoreactivity appears on rat SOL and EDL MNs during the second postnatal week, and 67% of retrogradely labeled SOL MNs display such reactivity at 2 wk (Vult von Steyren and Lømo 2005). In mice, the anatomical and physiological profile of L-type calcium channels believed to be responsible for motoneuronal plateau properties approximate adult distribution no earlier than postnatal day 18 (Jiang et al. 1999). Glucose utilization in the raphe nuclei that send serotonergic axons to the spinal cord increases markedly after 17 days and continues to increase between days 21 and 35, suggesting increasing activity in descending axons (Nehlig et al. 1988). Serotonin and noradrenaline immunoreactivity in the spinal cord reach adult pattern and density at 21 and 30 days, respectively (Bregman 1987; Rajaofetra et al. 1989, 1992). Together, these findings corroborate the notion that postnatal development of tonic firing in SOL depends on concurrent appearance of monoamine-dependent plateau potentials in MNs.

**Functional consequences**

Previous research indicates that changes in impulse activity, such as those described here, will strongly affect the development and expression of muscle fiber properties. In developing rats, primary generation SOL muscle fibers apparently give rise to the early type I fibers, while secondary generation fibers develop into the early type IIA fibers (Schiaffino and Reggiani 1996). Rat SOL muscles 1 wk after birth contain 50% type IIA fibers, and nearly all of them subsequently become transformed into type I fibers (Thompson et al. 1984). Hindlimb suspension, which reduces the amount of tonic motor activity (Blewett and Elder 1993), inhibits the transformation of fast type IIA to slow type I fibers in young developing rat SOL (≥18 days) (Elder and McComas 1987). Similarly, in adult rat SOL, removal of tonic motor activity by denervation induces expression of type IIA myosin heavy chain in a set of muscle fibers that probably belongs to the population of early type IIA fibers (Corza et al. 1988). Direct stimulation of denervated adult SOL with a slow tonic 15- to 20-Hz pattern prevents this transformation by maintaining essentially normal slow contractile properties (Ausoni et al. 1990; Corza et al. 1988; Westgaard and Lømo 1988). Intracellular signaling pathways involving MAPK/ERK and calcineurin that contribute to a slow phenotype by turning off slow muscle gene programs have been shown to be selectively activated by tonic low-frequency impulse activity (Murgia et al. 2000; Serrano et al. 2001). Larger amounts of activity also make fast muscles in the cat slower and more fatigue resistant (Eerbeek et al. 1984; Kernell et al. 1987a,b).

In conclusion, the postnatal appearance and development of tonic motor-unit activity described here has several functional implications: the tonic impulse activity generates sustained muscle contraction as needed for postural support, and it causes muscle fibers to become energy efficient and fatigue resistant by activating appropriate intracellular signaling pathways. Descending monoaminergic innervation facilitates this development by allowing generation of plateau potentials in SOL MNs that can maintain stable long-lasting tonic activity without the need for sustained synaptic drive.

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**Disclosure**

No conflicts of interest.

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