Effect of precocious locomotor activity on the development of motoneurones and motor units of slow and fast muscles in rat

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Abstract

We have investigated the effect of precociously increasing locomotor activity during early postnatal development by daily treatment with the monoaminergic precursor L-DOPA on the survival of motoneurones supplying the slow soleus (SOL) muscle and the fast, tibialis anterior (TA) and extensor digitorum longus (EDL) muscles as well as the contractile and histochemical properties of these muscles. L-DOPA treatment resulted in a significant loss of motoneurones to the slow SOL muscle, but not to the fast TA and EDL muscles. Moreover, motoneurones to fast muscles also die as when exposed to increased activity in early life, if their axons are repeatedly injured. The loss of normal soleus motoneurones was accompanied by an increase in force of the remaining motor units and sprouting of the surviving axons suggesting a remodelling of motor unit organisation. The time to peak contraction of both SOL and EDL muscles from L-DOPA treated rats was prolonged at 8 weeks of age. At 4 weeks the soleus muscles of the L-DOPA treated animal developed more tension than the saline treated one. This difference between the two groups did not persist and by 8 weeks of age the muscle weight and tetanic tension from either group were not significantly different from control animals.

The present study shows that early transient, precocious locomotor activity induced by L-DOPA is damaging to normal soleus but not to normal EDL/TA motoneurones.

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Keywords: Neuromuscular activity; L-DOPA; Muscle; Motoneurone; Motor unit; Development

1. Introduction

Motoneurone activity patterns play an important role in the development and plasticity of the motor unit. In adults, the physiological and biochemical properties of muscle fibres belonging to the same motor unit are similar, and are adapted to the functional demands imposed upon them by the motoneurone that supplies them. During development, a dynamic matching takes place between the motoneurones and their associated muscle fibres that result in generation of motor unit anatomical and functional diversity (for review see Ref. [20]).

In neonatal rodents, motor units are large and have overlapping territories as each muscle fibre is innervated by several axons from different motoneurones. The establishment of motor unit architecture, where only one axonal branch innervates a single muscle fibre is achieved by elimination of polyneuronal innervation and is an activity-dependent process [1,21,24].

In the rat, the differentiation of the activity patterns of motoneurones supplying slow and fast hindlimb muscles takes place during the first 3 postnatal weeks associated with the development of postural and locomotor functions [1,19,32]. Neonatal rats are unable to produce coordinated locomotor activity and the emergence of quadrupedal locomotion during the second postnatal week depends on the functional and neurochemical maturation of descending pathways associated with postural functions [30]. There is considerable evidence based on studies in adult animals that descending catecholaminergic and serotonergic pathways are involved in controlling the spinal interneuronal networks which generate locomotion ([2,5,6], for review see Ref. [25]).
2. Materials and methods

Similarly, studies using isolated in vitro spinal cord preparations in neonatal spinal cord have revealed that coordinated patterns of flexor and extensor muscle activity can be elicited by exogenous administration of neurotransmitter agonists including glutamate, dopamine, and serotonin [3,9,11,30]. These studies suggest that the spinal circuitry that generates locomotion is already established at birth. We have previously shown that exogenous administration of the monoaminergic precursor L-DOPA to neonatal rats triggers sustained episodes of stereotyped overground locomotion characterized by rhythmic reciprocal bursts of electromyographic (EMG) activity in flexor and extensor muscles resembling to some extent the rhythmic locomotor pattern seen in mature animals [18]. Not only motor unit size and muscle development are influenced by activity, but the survival of developing motoneurones is also critically dependent on activity. Reducing activity to axotomized neonatal motoneurones protects motoneurones destined to die from their fate and allows their survival [16], while increasing their activity precociously causes them to die [26]. In view of the important role of activity on the survival of motoneurones as well as development and differentiation of slow and fast muscles we have studied here the effect of precociously increasing locomotor activity during early postnatal development using L-DOPA. Unlike other methods of increasing the activity of motoneurones such as electrical stimulation, L-DOPA, by activating the central pattern generators involved in locomotion, induces the orderly recruitment of motoneurones to hindlimb flexor and extensor muscles thus inducing a more natural increase in the synaptic drive to the motoneurones [8,18]. Following such daily transient increases in locomotor activity during early postnatal development we have investigated the survival of motoneurones supplying the slow soleus muscle (SOL) and the fast, tibialis anterior (TA) and extensor digitorum longus (EDL) muscles and the contractile and histochemical properties of their respective muscles.

2.1. L-DOPA induced locomotion

In one group of animals where the effect of premature locomotor activity on the development of normal uninjured motoneurones and muscles was studied, activity was induced daily from birth until the animals were 12 days old by intraperitoneal (i.p.) injection of L-DOPA (100 mg/kg body weight). In another group of animals the effect of activity on injured motoneurones was investigated after unilateral sciatic nerve injury at 5 and 10 days of age. Unlike nerve injury at birth, where the majority of motoneurones die, the injury carried out at this age (5 days after unilateral sciatic nerve injury at 5 and 10 days of age). Unlike nerve injury at birth, where the majority of motoneurones die, the injury carried out at this age (5 and later) leads to only a moderate loss of motoneurones [15] and therefore the effect of excess activity on injured motoneurones could be studied. In this group, the treatment started on postnatal day 5 (P5) and lasted until postnatal day 17 (P17). After the age of 9–10 days, the decarboxylase inhibitor, Carbidopa (10 mg/kg body weight) was added to the L-DOPA solution to enhance the action of L-DOPA. Control littersmates were treated daily by i.p. injections with sterile saline for 12 days. They were kept away from their mother for the same period of time as the L-DOPA treated pups.

2.2. EMG recording

In 11 Wistar rat pups aged 7–9 days a pair of bipolar EMG recording electrodes was implanted into the SOL and either TA or EDL of one hindlimb as previously reported [18]. The EMG signals were amplified using a Neurolog NL 104 differential pre-amplifier (Digitimer, UK), filtered (bandpass 50Hz–50kHz) and recorded on a Racal 4DS FM tape recorder (Racal, UK). For quantitative analysis, the EMG recordings were played back from the tape recorder into a computer via an analogue to digital interface (CED 1401; Cambridge Electronic Design, UK). Spontaneous EMG activity was recorded for 30 min from the unrestrained animal. Following recording of spontaneous EMG activity, the animals were injected i.p. with a single dose (150 mg/kg) of L-DOPA (Sigma, UK) dissolved in 0.1 ml of saline and the EMG activity was monitored for about 90–120 min after injection. The aggregate EMG activity from each muscle was determined by counting all the muscle action potentials above the noise level (about 50 μV) using a spike trigger (Neurolog NL 200; Digitimer) and displayed on a computer using a software package (MRATE, Cambridge Electronic Design). In each recording session, aggregate EMG activity was measured and the results were expressed as spikes per minute [18].

2.3. Muscle tension recordings

At 4 and 8–10 weeks of age the animals were anaesthetised with chloral hydrate (i.p. 4.5%; 1 ml/100 g body weight) and the distal tendons of the SOL, EDL, and TA muscles of both legs were dissected free of their surrounding tissue, and attached to strain gauges (Dynamometer UFI, Devices). The sciatic nerve was exposed and cut. The nerve to soleus and the deep peroneal nerve were dissected and prepared for stimulation. Isometric contractions were elicited by electrical stimulation of the motor nerve, via bipolar silver electrodes using a pulse width of 0.02 ms. The length of the muscle was adjusted at the beginning of the contraction experiment so that it developed maximal twitch tension. During a single twitch, the time to peak (TTP), i.e. time taken by the muscle to produce peak tension and the half relaxation time (1/2RT), i.e. time taken for the peak tension to drop to half its value were measured. To estimate the number of motor units in each muscle the stimulus strength was gradually increased to obtain stepwise increments of twitch tension, as individual motor axons were recruited. The number of stepwise increments was counted to give an estimate of the number of motor units present in the muscle. The mean motor unit force was obtained by dividing the maximum tetanic tension of each muscle by the number of increments of twitch tension in response to stimulation of the motor nerve. To determine the maximum tetanic tension, the motor nerve was stimulated repetitively at frequencies ranging from 10 to 100 Hz, for 600 ms and the maximum tetanic tension was determined at the optimal frequency of repetitive stimulation. Statistical differences in the muscle contractile properties were tested by a Mann–Whitney U-test. This non-parametric test was used because of relatively small sample size and due to the fact that data was not normally distributed.

2.4. Histology and histochemistry

After the tension experiments had been completed, the investigated muscles were dissected out from the rats, weighed and frozen in cooled isopentane. The corresponding muscles from either L-DOPA or saline treated animals were mounted next to each other, so that the pair of muscles underwent the same processing, i.e. sectioning (10 μm) and staining. The muscles were stained for succinate dehydrogenase (SDH), which reflects the oxidative capacity of the muscle fibres or processed for immunocytochemistry using a specific antibody against slow myosin. To visualize the endplates and the axons a modified, combined cholinesterase-silver stain [17,21] was used.

2.5. Retrograde labelling of motoneurones

The number of motoneurones in animals treated either by L-DOPA or saline was assessed using the retrograde tracer horseradish peroxidase (HRP) method as previously described in more detail [8,20]. Briefly, under chloral hydrate anaesthesia and using sterile precautions 15% solution of HRP (type VI; Sigma)
was injected into either the SOL muscle (2 μl/100 g body weight) or the TA/EDL muscles (8 μl/100 g body weight) using a Hamilton micro syringe. Twenty-four hours later the rats were perfused transcardially with 2.5% glutaraldehyde in Millonig’s phosphate buffer (pH 7.3) and the spinal cord was dissected out. Free-floating sections (50 μm) were cut from spinal segments L2–L6 and processed for HRP histochemistry using a modified Hanker–Yates method [7]. Following HRP histochemical processing the spinal cord sections were lightly counterstained with a Nissl stain (gallocyanin). The number of HRP-labelled motoneurons in each ventral horn was counted under a light microscope. In order to avoid counting the same cell twice in consecutive section, only those labelled motoneurons in which the nucleus and nucleolus were clearly visible at high magnification were included in the counts. Our previous findings [13,26] demonstrated that this method provided accurate and reproducible counts of motoneurons to particular hindlimb muscles, which were consistent with results obtained by simultaneously counting Nissl stained motoneurones. The cross-sectional area of the labelled motoneurone perikarya was measured using a digitizing tablet linked to a computer. These methods have been described in detail previously [13].

3. Results

3.1. L-DOPA induced EMG activity in slow and fast muscles

In order to induce precocious motor activity over the first 2 postnatal weeks, daily injections of the monoaminergic precursor L-DOPA (100 mg/kg i.p.) were used. We have previously shown that this treatment induces episodes of stereotypic locomotor activity [18]. The patterns of EMG activity in flexor and extensor hindlimb muscles elicited by L-DOPA treatment resembled that seen during locomotion in more mature animals and involved the recruitment of a large fraction of the motoneurone pool to the muscles. The effect of L-DOPA on motor activity changed as the rats matured. In newborns, L-DOPA predominantly induced alternating movements of the forelimbs while the hindlimbs remained extended. When the pups were 2–3 days old administration of L-DOPA induced crawling-like movements in which the hindlimbs moved in tandem with the forelimbs while the abdomen was still on the ground. After 7–9 days L-DOPA induced quadrupedal locomotion with the hindlimbs providing sufficient postural support to maintain the pelvis raised above ground. Following L-DOPA treatment there was a dramatic increase in the aggregate EMG activity lasting for about 90 min. The time course and extent of this increase in the fast TA/EDL and the slow soleus muscles is illustrated in Fig. 1. During the first 10 days of treatment the repeated daily injection of L-DOPA produced a similar effect during each session but at later stages the effects of L-DOPA diminished and it became necessary to administer additionally the decarboxylase inhibitor Carbipoda to prolong the action of L-DOPA.

3.2. Effect of precocious activity on muscle properties

3.2.1. Changes seen immediately after completion of L-DOPA treatment

As it is well known that neuromuscular activity has profound effects on muscle properties we studied the effect of increased activity induced by L-DOPA on the development of some histochemical and contractile properties of slow and fast muscles. One of the enzymes known to increase during postnatal development [4] and also in response to increased activity [22] is succinate dehydrogenase. SDH can therefore be taken as an indicator of increased muscle activity. The increased EMG activity recorded from the muscles should be mirrored by an increase in SDH activity shortly after treatment with L-DOPA. Thus, SDH was visualized in frozen cross-sections of SOL and EDL muscles immediately after completion of the period of treatment with L-DOPA, i.e. at 12 days of age. There was no apparent difference between the intensity of staining in the SOL muscles of the saline (n = 5) and L-DOPA (n = 5) treated animals. The staining of the EDL muscle from L-DOPA treated animals was more intense than that of the control saline treated animals (see Fig. 2). This change was not permanent and 16 days after the last treatment with L-DOPA, there was no difference between the EDL muscles from L-DOPA and saline treated animals (data not shown). Thus the L-DOPA induced increase in motor activity during early postnatal development had only a temporary influence on muscle oxidative enzyme activity.

The muscles were also examined for the presence of fibres reacting with antibodies for slow myosin heavy chain. At 12 days of age there was no qualitative difference in the number of
Fig. 2. Examples of transverse sections from a 12 days old normal (A) and L-DOPA treated (B) EDL muscle reacted for succinate dehydrogenase (SDH) are shown. Scale bar: 50 μm.

Fig. 3. Changes of body weight of saline (solid line) and L-DOPA (interrupted line) treated rats are plotted against age.

fibres staining for this protein for either the SOL or EDL muscles from animals treated with saline or L-DOPA.

3.2.2. Changes at later stages after treatment

Whether the early activity influenced the time course of the development of contractile properties of slow and fast muscles was examined. Treatment with L-DOPA influenced the increase in body weight that takes place during normal development. During the first 2 postnatal weeks L-DOPA treated rats were smaller and weighed less than the saline treated littermates (Fig. 3). However, at 4 and 8 weeks of age, the difference in body weight was not significant (Table 1). We therefore examined the muscle contractile properties of L-DOPA and saline treated rats at 4 and 8 weeks of age.

3.2.2.1. Soleus. The weight and contractile properties of the SOL muscles from L-DOPA and saline treated animals were compared. Table 1 summarizes the weights of the animals, mus-

Table 1
The effect of L-DOPA treatment on the weight and force output of slow (SOL) and fast (EDL) muscles

<table>
<thead>
<tr>
<th></th>
<th>SOL muscle</th>
<th>EDL muscle</th>
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<tbody>
<tr>
<td></td>
<td>Muscle weight (g)</td>
<td>Twitch tension (g)</td>
</tr>
<tr>
<td>4 weeks old</td>
<td></td>
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<tr>
<td>Saline</td>
<td>Mean 86.1</td>
<td>0.043</td>
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<tr>
<td></td>
<td>S.E.M. 12.04</td>
<td>0.005</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>Mean 94.2</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>S.E.M. 8.9</td>
<td>0.0035</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>8 weeks old</td>
<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>Mean 295.6</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>S.E.M. 12.42</td>
<td>0.006</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>Mean 286</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>S.E.M. 8.84</td>
<td>0.007</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

* p < 0.05 Mann–Whitney U-test.
Table 2
The effect of L-DOPA treatment on the speed of contraction and relaxation of slow (SOL) and fast (EDL) muscles

<table>
<thead>
<tr>
<th></th>
<th>SOL muscle</th>
<th>EDL muscle</th>
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<tbody>
<tr>
<td></td>
<td>Time to peak tension (ms)</td>
<td>Time to 1/2 relaxation (ms)</td>
</tr>
<tr>
<td></td>
<td>Time to peak tension (ms)</td>
<td>Time to 1/2 relaxation (ms)</td>
</tr>
<tr>
<td>4 weeks old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>Mean 63.2 ± 6.59</td>
<td>Mean 30.9 ± 1.94</td>
</tr>
<tr>
<td></td>
<td>S.E.M. 7.59</td>
<td>S.E.M. 7.94</td>
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<tr>
<td></td>
<td>n 7</td>
<td>n 7</td>
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<tr>
<td>L-DOPA</td>
<td>Mean 74.3 ± 3.8</td>
<td>Mean 34.4 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>S.E.M. 9.16</td>
<td>S.E.M. 4.1</td>
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<tr>
<td></td>
<td>n 8</td>
<td>n 8</td>
</tr>
<tr>
<td>8 weeks old</td>
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<td></td>
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<tr>
<td>Saline</td>
<td>Mean 72.7 ± 3.0</td>
<td>Mean 36.1 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>S.E.M. 5.63</td>
<td>S.E.M. 2.71</td>
</tr>
<tr>
<td></td>
<td>n 7</td>
<td>n 7</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>Mean 114.8 ± 7.92</td>
<td>Mean 44.5 ± 3.03</td>
</tr>
<tr>
<td></td>
<td>S.E.M. 10.75</td>
<td>S.E.M. 2.89</td>
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<tr>
<td></td>
<td>n 7</td>
<td>n 7</td>
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</table>

* p < 0.05.
** p < 0.01 Mann–Whitney U-test.

The SOL muscle is a slow twitch muscle and the time course of contraction and relaxation becomes slower with age [12]. Whether this age related slowing is affected by L-DOPA treatment was examined. Table 2 shows that at 4 weeks of age the speed of contraction and relaxation of SOL muscles from animals treated with L-DOPA during the early stages of postnatal development was similar to that of saline treated rats, but was significantly slower at 8 weeks of age. The weights and tetanic tensions from either group were not different.

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motoneurones we counted the numbers of functional motor units in the SOL muscles of control and treated rats using physiological methods. Motor unit numbers in SOL were determined at 4 and 8 weeks of age in saline and L-DOPA treated rats. In 4 weeks old animals we found $31 \pm 1.1$ (mean ± S.E.M.; $n=6$) motor units in the control SOL and $23.9 \pm 0.7$ ($n=8$) in the L-DOPA treated rats. At 8 weeks of age there were $30.8 \pm 4$ ($n=5$) motor units in the control SOL and $26.7 \pm 0.47$ ($n=7$) in the L-DOPA treated rats. Fig. 5 summarizes these results and shows that at both time intervals the number of motor units in SOL of L-DOPA treated rats was significantly smaller. Thus, consistent with the counts of retrogradely labelled motoneurones, the numbers of motor units are significantly smaller in the L-DOPA treated rats at both ages studied.

The remaining soleus motor units in the L-DOPA treated animals developed more force than in the saline treated controls. This is probably due to an expansion of the motor territory induced by sprouting. Indeed, we found evidence of sprouting of the surviving axons using the silver-cholinesterase method [17]. In control animals aged 8–10 weeks there were no detectable signs of sprouting (data not shown), but in the animals that had been treated with L-DOPA shortly after birth sprouting was readily seen (Fig. 6).

The sizes of motoneurones were also compared in the control and L-DOPA treated rats. There was no difference in the cell body sizes between the saline treated (1069.47 ± 64.87 $\mu m^2$; $n=8$) and L-DOPA treated SOL motoneurones (1063.86 ± 65.1 $\mu m^2$; $n=8$).

### 3.3.2. Effect of L-DOPA on injured motoneurones

The differential effect of L-DOPA treatment on SOL motoneurones were consistent with our earlier findings that SOL motoneurones mature later than those to TA and EDL [26] and therefore are more vulnerable to increased activity. We therefore attempted to delay the maturation of motoneurones to TA and EDL by crushing the sciatic nerve at 5 and 10 days of age. This injury is known to produce a small but significant loss of motoneurones [14].

In 12 rats the sciatic nerve was crushed at 5 days that was followed by a similar injury 5 days later. Six of these rats had daily

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**Fig. 4.** The mean number (±S.E.M.) of motoneurones innervating the soleus (A) and TA/EDL (B) muscles from saline (grey columns) and L-DOPA (dark columns) treated 8 weeks old animals (**$p<0.001$ Mann–Whitney U-test).**

**Fig. 5.** The mean number (±S.E.M.) of soleus motor units from saline (grey columns) and L-DOPA (dark columns) treated 4 and 8 weeks old animals. The difference between the number of motor units from saline and L-DOPA treated animals is significant at both ages. The difference between the number of motor units from 4 and 8 weeks old saline treated animals is not significant, and in L-DOPA treated animals there is a slight but significant difference between the two age groups. (*$p<0.01$ Mann–Whitney U-test; **$p<0.001$ Mann–Whitney U-test).**

**Fig. 6.** Sprouting in soleus muscles of L-DOPA treated animals. Silver cholinesterase stained longitudinal sections of soleus muscles from 8 weeks old rats that had been treated with L-DOPA during the neonatal period are shown. (A) and (B) show examples of ultraterminal; (C) and (D) show examples of preterminal sprouting. Scale bar: 50 $\mu m$. 

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Fig. 7. The mean number (±S.E.M.) of motoneurones to TA and EDL muscles expressed as a percentage of control from 8 weeks old animals that had their sciatic nerve injured at 5 and 10 days are shown. Grey column shows results from saline and dark column from L-DOPA treated animals for 2 weeks after nerve injury (*p < 0.01 Mann–Whitney U-test).

injections of saline and another group of six animals had injections of L-DOPA between 12 and 17 days of age, i.e. 2–7 days after the last injury, a time during which the injured motoneurones were expected to be most vulnerable. The number of retrogradely labelled motoneurones was established 2 months later and compared to that in the contralateral ventral horn. Fig. 7 shows that the increased activity elicited by L-DOPA treatment during the period when the motoneurones were recovering from injury induced an additional loss of motoneurones. After a double crush to the sciatic nerve 19% of motoneurones were lost and this loss increased to 36% when the animals were treated with L-DOPA. Thus increasing the activity of injured motoneurones enhanced their death.

These results together with the previous findings show that activation of either immature or injured young motoneurones induces them to die. This deleterious effect of activity on these cells may be associated with their increased excitability, possibly because of their smaller size. Previous results show that the area of the motoneurone cell body that had their axons injured at 5 days was reduced [10]. Here we measured the areas of cell bodies of control motoneurones and compared those of motoneurones that had been injured and treated with either saline or L-DOPA. Table 3 shows that after a double-nerve crush the areas of motoneurone cell bodies were significantly reduced, but treatment with L-DOPA had no additional effect to this reduction.

4. Discussion

The present study shows that precocious locomotor activity induced by L-DOPA in normal rats is damaging to SOL but not to EDL/TA motoneurones from normal animals. Nevertheless, in L-DOPA treated animals, motoneurones to EDL/TA also die if their axons are repeatedly injured during the perinatal period. The surviving soleus motor units develop more force suggesting an expansion of their territory due to sprouting of the surviving axons. Additional adaptive changes to L-DOPA induced increased activity, were observed. The time of contraction of both SOL and EDL muscle from L-DOPA treated rats was prolonged at 8 weeks of age.

Motoneurone activity is a major factor influencing the development of skeletal muscles and its contractile properties (see Ref. [20]). During normal development the changes of contractile properties of the SOL muscles are brought about by the increased impulse activity that develops with age [19,32], and by the increase in load due to the changes of the animals body weight that extensor muscles such as SOL have to support [12]. In the present study we demonstrate that treatment with L-DOPA leads to a several fold increase in neuromuscular activity in both slow and fast muscles. Furthermore when activity is repeatedly elicited in this way over 12 days, it leads to long lasting changes in both motoneurones and muscles. We are uncertain as to whether the L-DOPA treatment, in addition eliciting increased motor activity, may also have direct effects on either or both motoneurones or muscles. However, in view of the well documented effects of activity on neuromuscular functions (see Ref. [23]) we believe our findings can best be interpreted in terms of activity-mediated effects on both motoneurones and muscle.

Although the SOL muscles of animals that had been subjected to increased activity during early postnatal life have fewer motor units, they produced more force than those of control animals. These results led us to investigate the possibility that remaining

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Mean motoneurone area (µm²)</th>
<th>Oop/con (%)</th>
<th>K–S test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Con</td>
<td>Op</td>
<td></td>
</tr>
<tr>
<td>Saline (n = 6)</td>
<td>1078.44 ± 12.80</td>
<td>811.80 ± 11.12</td>
<td>75.46 ± 2.83</td>
</tr>
<tr>
<td>L-DOPA (n = 6)</td>
<td>1074.90 ± 12.37</td>
<td>910.29 ± 12.45</td>
<td>83.78 ± 6.29</td>
</tr>
</tbody>
</table>

Statistical comparison was made using the Kolmogorov–Smirnov test.
motor units compensated for motoneurone loss by expansion of motor unit size due to sprouting. We have indeed found physiological evidence of increased mean motor unit size as well as histological evidence of sprouting. However, the transient increase in tetanic force of the whole muscle may be due to an increase in either the number or diameter of SOL muscle fibres.

In addition, the early treatment with L-DOPA also affects the contractile properties of the SOL muscle, which becomes slower contracting, and this effect is apparent long after cessation of the L-DOPA treatment. It is possible that the reason for the slowing of the SOL muscle phenotype is due to the fact that the muscle has fewer motor units and that the remaining motor units therefore have to work harder to compensate for this deficiency. Such an explanation is consistent with findings on partially denervated SOL and EDL/TA muscles, which show that when motor unit numbers are reduced both slow and fast muscles become slower contracting [27–29]. This slowing effect is greater when partial denervation was carried out in young animals.

The long term effects of precocious L-DOPA treatment on the contractile properties of fast twitch muscles also led to a slowing of the speed of contraction, confirming results of a recent study [6]. Interestingly, in a study where immature fast muscles were electrically stimulated for several days by a pattern of activity resembling that normally typical of a slow muscle, no slowing of the time course of contraction was observed, even though SDH histochemical analysis revealed that the stimulated muscle fibres appeared to have increase SDH enzyme activity [31]. This difference could be due either to the fact that electrical stimulation does not elicit locomotion and thus, the stimulated muscles are not subjected to the stretch and load that occurs during locomotor movements. Moreover, L-DOPA induces graded recruitment of motor units [18] in contrast to the synchronous nature of the activity induced by electrical stimulation. Our results therefore indicate that a more natural activity pattern such as the locomotor-like activity induced by L-DOPA, in addition to increasing impulse activity also induces stretching, shortening, and loading of the different muscle groups associated with the hindlimb movements.

Our earlier results showed that the number of motoneurones to SOL, but not to TA and EDL muscles was reduced by daily treatment of the animals with L-DOPA [26]. Since retrograde labelling as carried out in our previous study does not distinguish between alpha and gamma motoneurones we counted the number of motoneurones using physiological methods. We found that the reduction in the number of motoneurones was also reflected in the smaller number of motor units (reflecting the alpha motoneurones) using physiological methods. We found that the reduction in the number of motoneurones was also reflected in the smaller number of motor units in SOL muscles subjected to daily L-DOPA elicited activity. The present results clearly show that alpha motoneurones to SOL are induced to degenerate by the precocious motor activity. Thus, unlike SOL muscle fibres, which seem to survive and develop more force, SOL motoneurones are adversely affected by excessive, motor activity during early postnatal development. This is consistent with our hypothesis that immature motoneurones are vulnerable to increased excitatory inputs. In particular, when motoneurone maturation is prevented by axotomy, motoneurones destined to die after this procedure can be rescued by reducing excitatory inputs by an NMDA receptor blocker, MK 801 [16]. The lack of effect of increased precocious activity on motoneurones to TA and EDL muscles confirms our earlier findings that these motoneurones pools mature earlier in the postnatal rat than motoneurones to the slow SOL [26]. This is not surprising, since postural hindlimb functions in which SOL plays an important role develop later than rhythmic movements in which fast muscles participate [19]. However, our present findings show that if the development of motoneurones to fast muscles is slowed by axotomy, then these vulnerable motoneurones are unable to cope with the excess activity induced by L-DOPA. This provides further evidence that motoneurones have to reach a certain degree of maturity before they are able to survive the developmentally regulated increases in afferent activity.

References