Selective depletion of spinal monoamines changes the rat soleus EMG from a tonic to a more phasic pattern

Ole Kiehn, Jesper Erdal, Torsten Eken* and Torben Bruhn

*Division of Neurophysiology, Department of Medical Physiology, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark and *Institute of Neurophysiology, University of Oslo, PO Box 1104, N-0317 Oslo, Norway*

1. To assess the role of descending monoaminergic pathways for motor activity long-lasting EMG recordings were performed from the adult soleus muscle before and after selective depletion of spinal monoamines.

2. Rats were chronically implanted with an intrathecal catheter placed in the lumbar subarachnoid space and gross-EMG recording electrodes in the soleus muscle. EMG recordings were performed in control conditions and at different times after intrathecal administration of either 40–55 μg 5,6-dihydroxytryptamine (5,6-DHT) and 40–55 μg 6-hydroxydopamine (6-OHDA) or 80 μg 5,7-dihydroxytryptamine (5,7-DHT) alone. The depletions were evaluated biochemically in brains and spinal cords after recordings.

3. In agreement with previous studies the intrathecal administration of neurotoxins caused a reduction of the noradrenaline (NA) and serotonin (5-HT) content of the lumbar spinal cord to about 2–3% of control, with little or no changes in the monoamine content of the cortex.

4. In non-treated chronically catheterized rats the integrated rectified gross EMG displayed long-lasting EMG episodes composed of phasic high-amplitude events and tonic segments of varying duration and amplitude.

5. After intrathecal administration of neurotoxins the number of long-lasting gross-EMG episodes, the mean episode duration, and the total EMG activity per 24 h, were reduced. These changes were accompanied by a simultaneous increase both in the number of short-lasting EMG episodes and the total number of EMG episodes per 24 h period. The changes were apparent 5–6 days after drug administration and fully developed after 2–3 weeks.

6. No changes in general movement ability were observed, except that the denervated animals had a tendency to a less erect posture.

7. These results indicate that descending monoaminergic pathways are important for the maintained motor output in tonic hindlimb muscles.

Monoamines are strong modulators of spinal motor output. It has long been known that serotonin (5-HT), noradrenaline (NA), and dopamine (DA) precursors given intravenously can enhance reflexes (Carlsson, Magnusson & Rosengren, 1963; Andén, Jukes & Lundberg, 1964; Lundberg, 1965) and locomotor activity (Grillner, 1981). More recently it has also been shown that 5-HT and NA cause a long-lasting enhancement of spinal motoneurone excitability mediated by a slow depolarization or by induction of plateau properties (VanderMaelen & Aghajanian, 1980; Hounsgaard, Hultborn, Jespersen & Kiehn, 1988; Conway, Hultborn, Kiehn & Mintz, 1988; for reviews see VanderMaelen, 1985; Sigvardt, 1989; Kiehn, 1991*a*, *b*). The excitability increase amplifies synaptic input and supports or maintains motoneuronal spike activity, and the role of monoamines in motor function might therefore be, at least partly, to enable tonic motor output (Holstege & Kuypers, 1987; Kiehn, 1991*a*, *b*; Jacobs & Fornal, 1993).

Monoaminergic fibres descend to the spinal cord from nuclei in the brainstem (Björklund & Skagerberg, 1982; Holstege & Kuypers, 1987). The aim of the present study was to evaluate the modulatory role of descending monoaminergic systems on the activity pattern in the soleus muscle in intact rats. We have, therefore, performed long-lasting EMG recordings from soleus muscles in rats before and after administration of either 5,6-dihydroxytryptamine and 6-hydroxydopamine or 5,7-dihydroxytryptamine to the lumbar subarachnoid space through an intrathecal catheter. Given in this way these neurotoxins can deplete spinal monoamines selectively within 2–3 weeks, without

affecting the monoamine content in higher brain structures (Howe & Yaksh, 1982; Berge, Fasmer, Tveiten & Hole, 1985; Tremblay, Bédard, Maheux & Di Paolo, 1985).

Normal rat soleus motor units are tonically active for prolonged periods of time (Hennig & Lømo, 1985; Eken & Kiehn, 1989), and their activity is reflected in a characteristic gross-EMG pattern with long-lasting EMG episodes composed of high-amplitude phasic events and tonic segments (Eken & Lømo, 1993). Here we show that in monoamine-depleted animals the EMG pattern becomes more irregular and phasic, and we suggest that descending monoaminergic systems might be important for maintaining tonic EMG activity in intact animals, e.g. during postural activity.

Preliminary results from these experiments have been presented in an abstract (Kiehn, Erdal, Eken & Bruhn, 1992).

METHODS

Experiments were performed on male Wistar rats weighing 220–500 g ($n = 17$). For surgery rats were anaesthetized with Equithesin (9·7 mg pentobarbitone, 76 mg ethyl alcohol, 42·5 mg chloral hydrate, 428 mg propyleneglycol, 21 mg $MgSO_4$ per ml sterilized H_2O) given I.P. (0·5 ml (100 g body wt)$^{-1}$ initially, and supplemented every 1–2 h with 0·2 ml (100 g)$^{-1}$). An adequate level of anaesthesia during implantation was ensured by regularly testing for the lack of cutaneous withdrawal reflexes.

Implantation of intrathecal catheters

Rats were chronically implanted with intrathecal catheters placed in the spinal subarachnoid space. Briefly, thin polyethylene tubing (PE-10, Clay Adams, NJ, USA) filled with saline was inserted through the atlanto-occipital membrane and the tip advanced 8–8·5 cm caudally to the lumbar region. The intrathecal tubing was glued to the skull and the neck muscles with cyanoacrylate, and the neck muscles were sutured in several layers. Finally, a large-diameter tube which fitted the tip of a Hamilton syringe was connected to the intrathecal catheter. After recovery from anaesthesia rats were carefully observed for possible motor deficits. Animals showing any sign of discomfort were immediately given an overdose of pentobarbitone I.P. Further details of the implantation procedure can be found elsewhere (Yaksh & Rudy, 1976).

Implantation of EMG recording electrodes

Gross-EMG recording electrodes were implanted in the soleus muscle 3–6 days after catheterization. Electrodes were made from two individual 6 mm long 50 μm diameter platinum–iridium wires (7760, A-M Systems, Inc., Everett, WA, USA), both bieng soldered to a 30 cm long multi-filament stainless-steel wire (AS 632, Cooner Wire Company, Chatsworth, CA, USA). The terminal 1·5 mm of insulation was removed from each electrode, and the distal 2 mm segment was bent back 180 deg to make a hook. The gross-EMG electrodes were separately introduced in the proximal and distal part of the soleus muscle, and a ground electrode, consisting of a 30 cm long multi-filament stainless-steel wire with approximately 5 mm insulation removed, was placed in the lower leg at some distance from the muscle. The wires were secured with 6-0 sutures and taken subcutaneously to an electrode mount which was subsequently fixed to the head of the animal by stainless-steel bone screws and dental cement (see Eken & Kiehn, 1989, for details). A wire loop under the dorsal skin of the animal prevented pull on the electrodes during movements. Following recovery from anaesthesia the rats were transferred to separate cages and kept in a 12 h light–dark cycle.

Technique for recording

Recordings were usually started 1–3 days after electrode implantation, i.e. 4–20 days after catheterization. The rat was transferred to a show case and connected via a flexible shielded multistranded wire to a swivel which allowed the animal to move freely. The bipolar EMG signals were fed into an amplifier via a preamplifier and bandpass filtered (100–10 000 Hz). From the amplifier the signal was either rectified and integrated with a time constant of 200 ms and taken to the analog input on our computer (see below) or recorded on the voice channel of a videotape-recorder (frequency response, 50–15 000 Hz) along with a synchronized picture of the rat's movements (see Eken & Kiehn, 1989). The 12 h light–dark cycle was maintained during recording. Periods of up to 24 h of integrated EMG activity were sampled (sampling rate, 10–20 Hz) directly on the computer in control animals and after injection of monoaminergic neurotoxins. Electrode position was verified visually after the animals were killed.

Drug application and biochemical quantification of monoamines

Previous studies have shown that intrathecal administration of 20–40 μg 5,6-dihydroxytryptamine (5,6-DHT) and 6-dihydroxy-dopamine (6-OHDA) evokes a selective depletion of 5-HT and NA, respectively, in the lumbar spinal cord with little or no changes in the brain content of the same transmitters (Howe & Yaksh, 1982; Berge *et al.* 1985). Two to three weeks after intrathecal administration the concentrations of 5-HT and NA in the lumbar cord were approximately 90–95% of controls (Berge *et al.* 1985). Intrathecal administration of high doses (80–200 μg) of 5,7-dihydroxytryptamine (5,7-DHT) cause a simultaneous reduction in the lumbar spinal cord content of both NA and 5-HT (Berge *et al.* 1985; Tremblay *et al.* 1985). In the present study we required selective depletion of all major spinal monoamines, and have therefore used intrathecal injections of 10 μl solutions of either 40–55 μg 5,6-DHT in combination with 40–55 μg 6-OHDA or 80 μg 5,7-DHT alone. Weights were calculated as the base of the drugs. The neurotoxins were dissolved immediately prior to use in saline containing 0·2 mg ml^{-1} ascorbic acid, and were injected over a period of 60–90 s, followed by 10 μl 0·9% NaCl. All drugs were purchased from Sigma. The neurotoxins were usually given 1–2 days after the control EMG recordings. After drug application the rats showed normal behaviour and were feeding and sleeping regularly. Control animals were given 20 μl 0·9% NaCl through the intrathecal tubing.

To determine the degree of denervation brains and spinal cords were taken out for biochemical quantification of monoamines after recording. Animals were anaesthetized and decapitated and tissue was immediately removed. Tissue pieces from the frontal cortex, lower brainstem, and cervical and lumbar enlargements of the spinal cord were placed in cooled 0·1 M perchloric acid and homogenized on ice in a Teflon and glass homogenizer (500 r.p.m. for 40–60 s). The homogenates were subsequently filtered (molecular mass cut-off, 20 kDa) and samples of filtrate were kept protected from light at −80 °C until analysis. Separation and detection of monoamines was carried out by isocratic electrochemical high-pressure liquid chromatography. Using a cooled (8 °C) autoinjector

system, separation was carried out by injecting 20 ml of each sample onto a Phase II ODS column (length, 0·10 m; diameter, 3·2 mm; silica based; particle size, 3 μm; Bioanalytic Systems, West Lafayette, IN, USA). The mobile phase was perfused at 0·5 ml min^{-1}, and contained 100 mM chloroacetic acid, 2·5 mM octanesulphonic acid, 1·0 mM ethylenediaminetetraacetic acid, 6·0% methanol and 3·0% (by volume) tetrahydrofuran. The pH was adjusted to 3·0 using NaOH. Detection was achieved by applying +750 mV to a glassy carbon electrode, with a Ag–AgCl electrode serving as reference. Quantification was performed from peak areas by means of the external standard procedure.

Data analysis

A Macintosh IIci (Apple) equipped with an analog–digital input–output card and a DMA card (NB-MIO-16H-9 and NB-DMA-8-G, National Instruments, Austin, TX, USA) was used as the laboratory computer. The rectified and integrated EMG signal was sampled directly on the computer. The analog files were processed off-line for episode detection. First, the longest-duration baseline segment was analysed and the mean (μ) and standard deviation (S.D.) voltages computed. The detection algorithm defined an episode to start when the signal passed upwards through a level corresponding to $\mu + 7$ S.D., and to end when the signal passed downwards through $\mu + 1$ S.D. The algorithm gave results comparable to manual episode definition. It provided reliable detection of low-amplitude episodes, and initial analysis showed that start and end points were consistent if different baseline segments were chosen or the thresholds were moved by 1S.D. in either direction. Episode detection for a stretch of activity is shown below the rectified EMG activity in Fig. 2 (horizontal bars).

Mean firing frequencies of motor units were measured from tonic segments (15 s to several minutes) during the EMG episodes (see Fig. 2).

The level of statistical significance was set at 0·05.

RESULTS

Effect of intrathecally administered neurotoxins on brain monoamines

The time course of monoamine depletion was not investigated systematically due to the fact that we performed several long-lasting EMG recordings from each rat. However, from previous studies it is known that the lumbar monoamines start to decrease 24 h after intrathecal administration of the neurotoxins and that the decrease reaches a plateau after approximately 9–10 days (Howe & Yaksh, 1982; Berge *et al.* 1985). Furthermore, the regeneration of monoaminergic fibres is relatively sparse within the first 3 months of chemical depletion (Wiklund, Björklund & Nobin, 1978). For these reasons data were pooled from recorded rats killed 11–63 days after drug administration (31 ± 6 days, mean ± S.D.; $n = 7$).

Administration of neurotoxins caused a substantial reduction in the monoamine content of the lumbar spinal cord as compared with control (Fig. 1). The two major monoamines, 5-HT and NA, were reduced to about 2–3% of controls ($n = 4$), while the lumbar dopamine (DA) content was only slightly (and not significantly; ANOVA) reduced. As in previous studies we found a significant reduction ($P < 0·03$) in the DA and NA content of the cervical spinal cord and the brainstem (Berge *et al.* 1985). In contrast, no significant changes were seen in the 5-HT content of the cervical spinal cord and the brainstem. The cortical monoamine values were not significantly different from control.

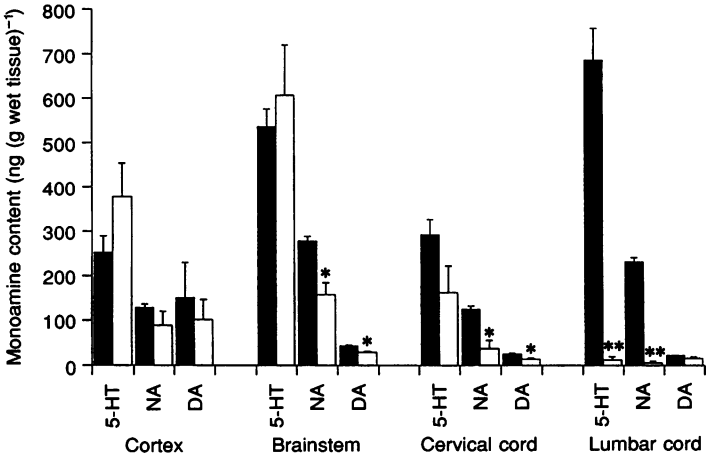

Figure 1. Brain and spinal cord monoamine content following intrathecal injections of neurotoxins

Animals were injected with either 40–55 μg 5,6-DHT in combination with 40–55 μg 6-OHDA ($n = 6$) or 80 μg 5,7-DHT alone ($n = 1$). Monoamines measured: 5-HT, noradrenaline (NA) and dopamine (DA). Controls (■) were taken from age-matched unimplanted animals ($n = 4$). All values represent means + S.E.M. ** $P \leq 0·0001$ and * $P \leq 0·03$, ANOVA. Data from denervated animals (□) were compiled from rats killed 11–63 days after the initial drug application (see text).

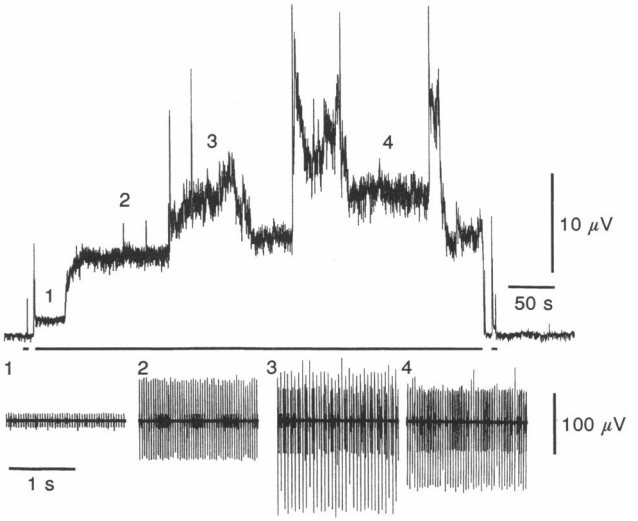

Figure 2. Soleus EMG episode in a non-treated catheterized rat

Upper trace illustrates integrated rectified gross-EMG activity, while lower traces show raw gross-EMG signals (note single motor units) taken from the numbered parts of the upper trace. Tonic segments in the integrated rectified EMG recordings reflect stable long-lasting firing in a few motor units. The recordings were taken before intrathecal drug administration, 20 days after the rat was catheterized. Horizontal bars indicate automatically detected EMG episodes (see Methods).

We conclude that our treatment, in agreement with previous studies, caused a substantial reduction in the monoamine content of the lumbar spinal cord, with little or no changes in the monoamine content of higher (cortical) brain structures.

EMG pattern in catheterized rats

Since the control condition in our experimental protocol was chronically (non-treated) catheterized rats it was important to compare the soleus EMG pattern in these animals with the EMG pattern observed in normal rats (Eken & Lømo, 1993).

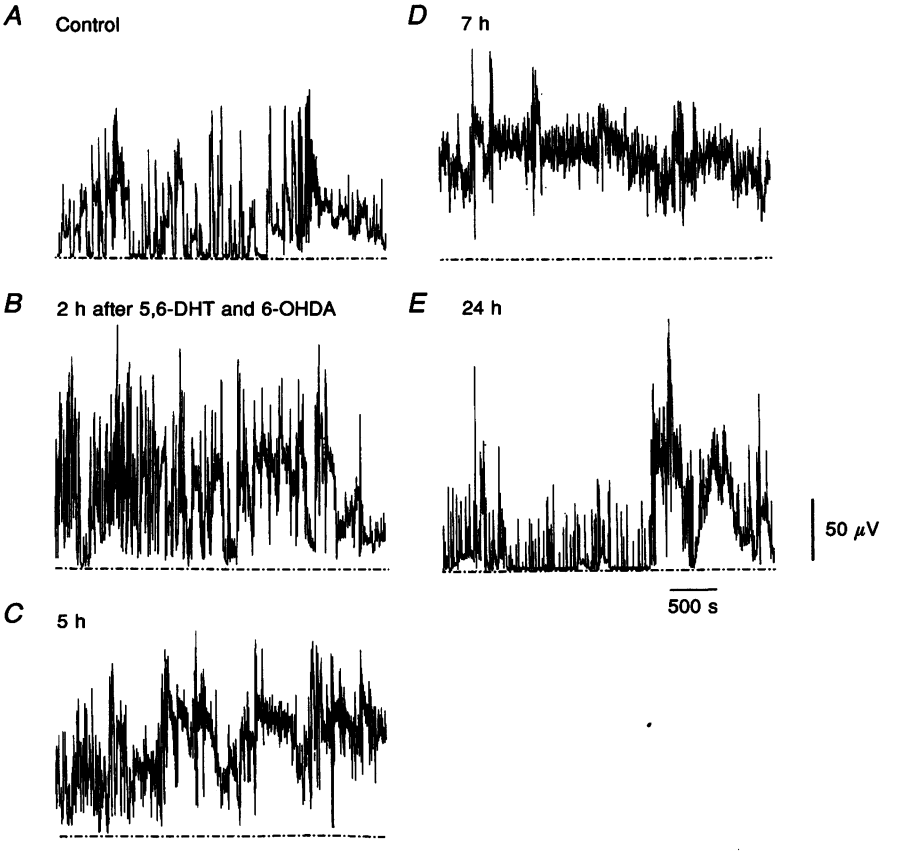

Figure 3. Initial changes in soleus EMG activity following intrathecal administration of 5,6-DHT in combination with 6-OHDA

A, integrated rectified gross-EMG activity in control (non-treated catheterized rat; 9 days after catheterization). *B–E*, integrated rectified EMG activity 2–24 h after intrathecal administration of 5,6-DHT (40 μg) in combination with 6-OHDA (40 μg). Note the initial increase in gross-EMG activity (*B–D*), which disappeared within 24 h (*E*).

As in normal rats we found that the integrated rectified gross-EMG activity in non-treated chronically catheterized rats revealed long-lasting EMG episodes, composed of phasic high-amplitude events and tonic segments of varying duration and amplitude. An example is illustrated in Fig. 2. The recording was taken from a non-treated rat catheterized 20 days previously. The tonic segments reflect recruitment and derecruitment of motor units of different amplitude firing for long periods of time with stable frequencies between 10 and 25 Hz as illustrated by the single-unit recordings in the lower traces in Fig. 2. This typical EMG pattern was present in all chronically implanted rats before drug administration independent of the time after implantation ($n = 10$; range, 4–20 days; mean, 10·6 days following catheterization; see also Figs 3*A*, 4*A* and 7). These findings support the general notion that, if properly performed, intrathecal catheterization itself has no observable effects on behaviour (for references see LoPachin, Rudy & Yaksh, 1981).

Changes in EMG pattern following chemical denervation

Qualitative EMG changes

In contrast to the above findings the EMG pattern changed after administration of neurotoxins. Initially after giving the drugs there was an increase in the EMG activity which persisted for less than 24 h (Fig. 3*A*–*E*). These initial changes were followed by slowly developing changes of the characteristic EMG pattern seen in normal and non-treated catheterized rats (Figs 2, 3*A* and 4*A*) to a denervation pattern (Fig. 4*B* and *C*) composed of longer periods of no activity and EMG episodes of shorter duration. The tonic EMG segments were fewer than in control and/or tended to decay slowly in amplitude. The denervation pattern was apparent 3–4 days after drug administration, progressed further during the following days (Fig. 4*B*) and was substantial 2–3 weeks after the start of denervation (Fig. 4*C*; all recordings in Fig. 4 were performed at the same time of the day).

From about 10–14 days to 20–30 days there was little development in the appearance of the gross-EMG pattern and in one rat the denervation pattern was seen 57 days after drug administration. The data were similar both with 5,6-DHT in combination with 6-OHDA and with 5,7-DHT alone.

In conclusion, it appears that the characteristic tonic soleus EMG pattern seen in normal and catheterized non-treated rats is replaced by a more phasic pattern after intrathecal administration of monoaminergic neurotoxins.

Changes in mean episode duration and total activity duration

In an attempt to quantify the development and degree of these EMG changes we analysed long-lasting recordings of integrated rectified EMG quantitatively (Fig. 5).

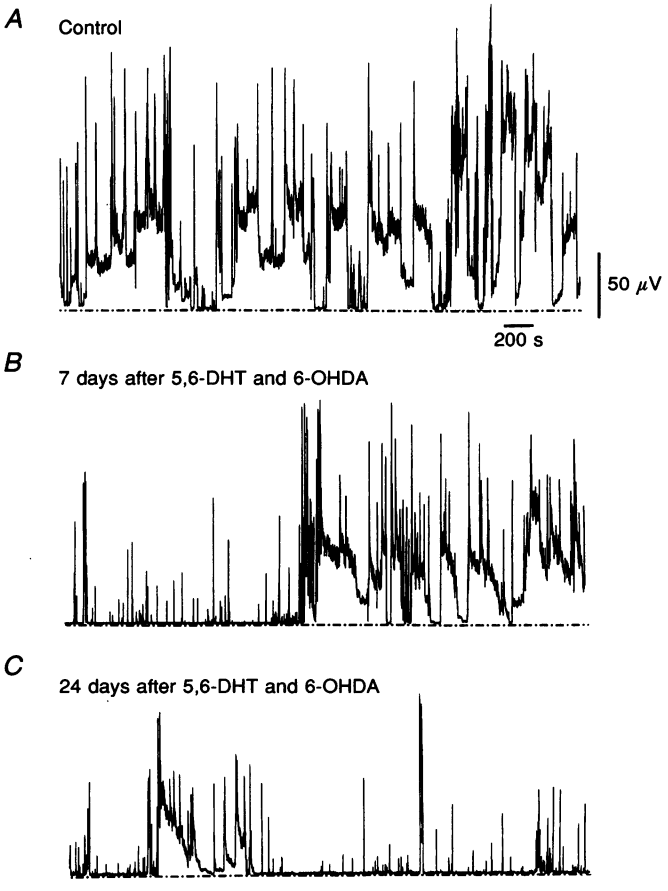

Figure 4. Long-lasting changes in soleus EMG pattern following intrathecal administration of neurotoxins

A, integrated rectified gross-EMG activity in control (non-treated catheterized rat; 13 days after catheterization). *B* and *C*, integrated rectified EMG activity 7 and 24 days after intrathecal administration of 5,6-DHT (40 μg) in combination with 6-OHDA (40 μg). The characteristic gross-EMG pattern with long-lasting EMG episodes composed of tonic segments and high-amplitude phasic events in catheterized rats and normal rats (*A*) was replaced by a denervation pattern with shorter EMG episodes and less pronounced tonic segments. All records were taken in the light period at 08.00 h.

The mean episode duration (Fig. 5*A*) was calculated from duration measurements of individual EMG episodes throughout the recording session in non-catheterized control rats, in catheterized non-treated rats, and at different times after intrathecal administration of monoaminergic neurotoxins. Figure 5*B* shows the total activity duration, i.e. the summed duration of all EMG episodes, as a fraction of total recording time. Data points in the graphs represent continuous recordings ranging from 6 to 24 h (except △ which represents a 2 h recording). Note that the mean episode duration and total activity in non-treated chronically catheterized rats was comparable to values obtained in normal unimplanted rats (crosses). Multiple linear regression analysis of data for all animals that were followed both before and after treatment (Fig. 5*A* and *B*, filled symbols) confirmed a clear time-dependent decrease in both mean episode duration ($-1{\cdot}4$ s $(24\text{ h})^{-1}$; $P = 0{\cdot}0006$) and total activity duration ($-1{\cdot}8\%$ $(24\text{ h})^{-1}$; $P < 0{\cdot}0001$). The statistical model assumes equal slopes, but allowed different intercepts for individual animals. At 20 days or more after drug administration the mean episode duration had decreased to 34% that of catheterized non-treated rats (15 s compared with 44 s), while the total activity duration was 46% of controls (37% compared with 79% of total recording time).

The changes in episode duration can be appreciated from Fig. 6, which illustrates the distribution of episode durations in catheterized non-treated controls (Fig. 6*A*; $n = 5$), 10–19 days after denervation (Fig. 6*B*; $n = 3$) and 20 days or more after drug administration (Fig. 6*C*; $n = 3$). In catheterized non-treated controls (as in unimplanted controls; authors' unpublished results) the majority of EMG episodes are rather short, but there is a considerable number of long-lasting episodes, which contribute significantly to the impression of muscle activity over time (Fig. 6*A*; cf. Figs 2, 3*A*, 4*A* and 7*A*). After denervation the distribution of episode durations changed significantly (Fig. 6*A–C*; $P < 0{\cdot}0001$ for a Kolmogorov–Smirnov test between any combination of groups; same significance level when only episodes $\leqslant 1000$ s were included). The number of episodes above 40 s decreased dramatically, while the number of shorter episodes increased (Fig. 6*B* and *C*). Thus, the 90th percentile for episode duration was reduced from 78 s in the controls to 25 s at 20 days or more after drug administration. These changes are highlighted in Fig. 6*D* and *E*, where the control histogram has been subtracted from the 10–19 and $\geqslant 20$ day histograms. In particular it is noted that the very long-lasting episodes disappeared in the denervated rats. Similar findings were obtained when histograms were compiled from data in individual rats.

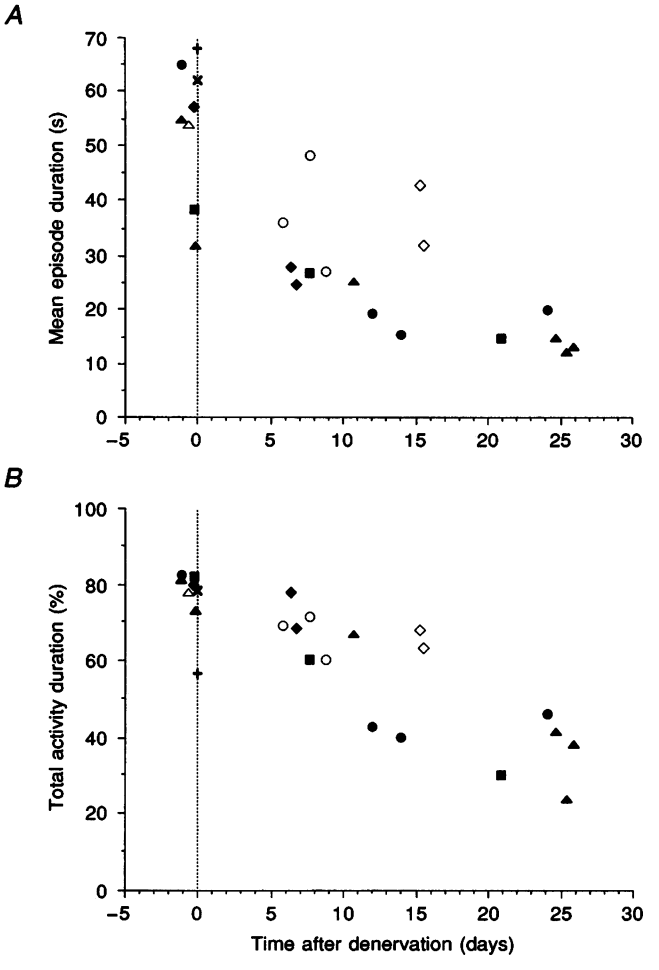

Figure 5. Development of neurotoxin-induced changes in mean EMG episode duration and total EMG activity duration over time

A, episode duration. Each data point represents 6–24 h continuous recordings (total 358 h). Open symbols indicate long-lasting recordings from rats either in control (non-treated catheterized) or after denervation. Filled symbols indicate long-lasting recordings obtained both in control (non-treated catheterized) and at different times after denervation. Crosses represent data from unimplanted controls. *B*, total EMG activity duration. Data from the same rats as in *A*.

The reduction in total activity duration occurred in spite of a 35% increase in the number of episodes per 24 h period at 20 days or more after denervation. Thus, we conclude that the denervation reduces the total activity duration through a reduction in soleus EMG episode duration alone.

In contrast to these dramatic changes in episode duration and total activity duration the firing frequency of individual motor units measured during the tonic EMG stretches (15 s to several minutes) appeared unchanged after drug treatment. Thus, the mean firing frequency was 20·95 ± 8·39 impulses s^{-1} (± S.D.) in catheterized non-treated rats (data from seven animals; 16867 spike intervals), 18·57 ± 7·65 impulses s^{-1} in animals 1–9 days after denervation (data from four animals; 53200 spike

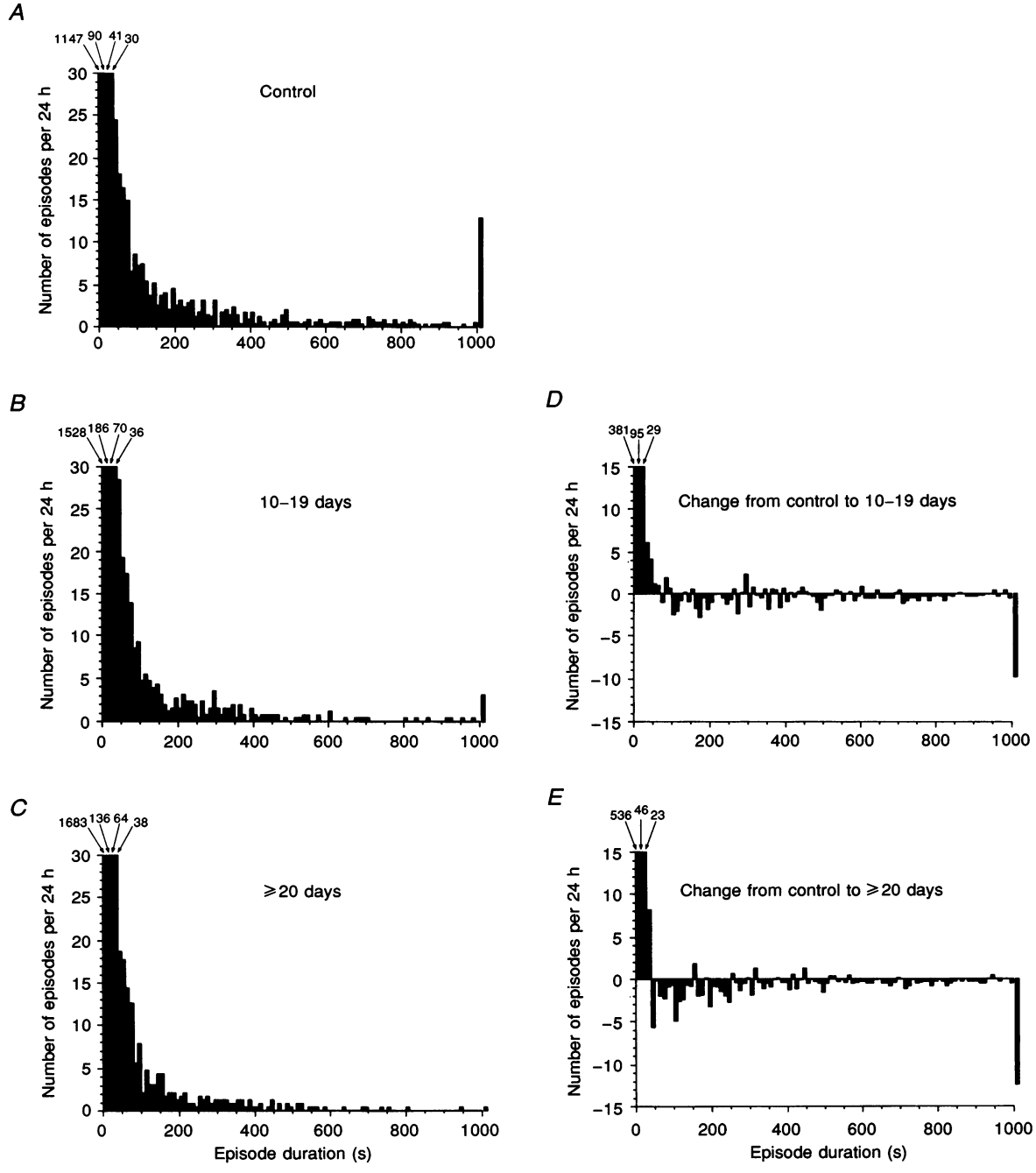

Figure 6. Distribution of episode durations before and after intrathecal drug administration

A–C, episode duration histograms in controls (*A*; non-treated catheterized), 10–19 days after denervation (*B*) and 20 days or more after drug administration (*C*). Data were normalized to number of episodes in a 24 h period. The bin width is 10 s and arrows with numbers indicate the actual values of the first bins. The last bin represents episodes with a duration of more than 1000 s. *D* and *E*, graphs where the control histogram has been subtracted from the 10–19 days and ≥20 days histograms.

intervals) and 19·41 ± 7·76 impulses s^{-1} in animals more than 10 days after denervation (data from five animals; 14 533 spike intervals). These mean firing frequencies were not significantly different (tested by ANOVA) from each other.

The changes in the EMG pattern can also be appreciated in a composed figure illustrating integrated rectified EMG recordings over a 24 h period (Fig. 7). The decrease in mean EMG episode duration and total EMG activity in denervated rats compared with control rats is most easily seen in the light period, in which rats normally show least exploring activity. In the dark period, there was also more vivid EMG activity after denervation and the changes are not so easily appreciated.

Behavioural observations

In spite of the considerable changes in soleus EMG pattern the denervated rats were not impaired in terms of moving around in the cage. It is, therefore, not likely that the pronounced changes in episode duration and total EMG activity can be attributed to a reduction in the general movement ability of the rats (see also Fig. 7*B*). In particular the locomotor behaviour (Steeves, Schmidt, Skovgaard & Jordan, 1980) and exploratory behaviour appeared unaffected. In some denervated rats there was a tendency to maintain a stance position where the back was less curved than in non-denervated rats and where the hindquarters tended to 'hang'. This would indicate that the general postural activity was reduced.

DISCUSSION

These experiments provide an attempt to assess the functional role of the descending monoaminergic fibres in modulating the tonic motor output in an intact animal. We have used the rat soleus muscle as a model system because previous studies have shown that the muscle is very active in postural tasks (Hnik, Vejsada & Kasicki, 1982;

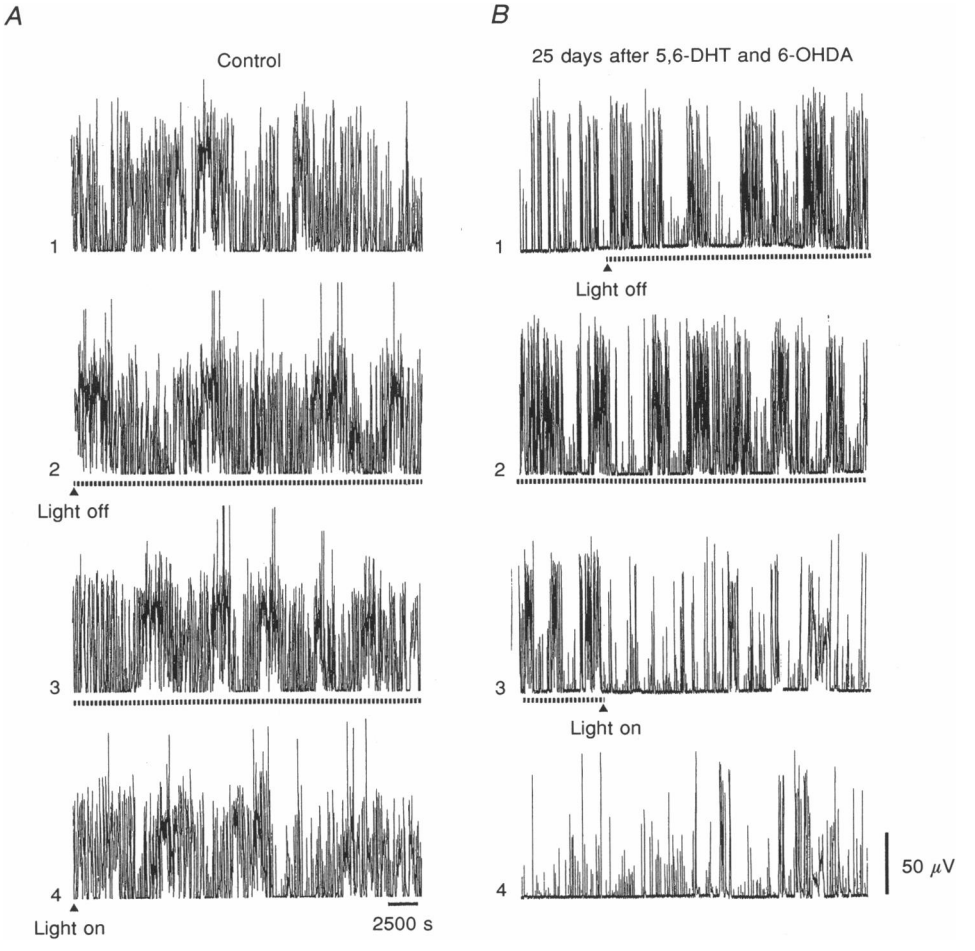

Figure 7. Twenty-four hour recordings in control and after intrathecal drug administration

A, control (non-treated rat 4 days after catheterization). *B*, same rat 25 days after drug administration (80 μg 5,7-DHT). Dashed lines below the EMG plots indicate the 12 h dark period. The difference between the control EMG pattern and the denervation pattern is especially pronounced in the light period. In *A* and *B*, the numbers 1–4 indicate the sequence of consecutive 8 h recordings over a 24 h period.

Navarrete & Vrbova, 1984; Roy, Hutchison, Pierotti, Hodgson & Edgerton, 1991). Furthermore, recent studies have demonstrated that the integrated rectified soleus gross EMG has a characteristic appearance composed of long-lasting EMG episodes, with phasic high-amplitude events and tonic segments of varying duration and amplitude (Eken & Lømo, 1993; T. Eken, unpublished observations). The tonic segments reflect recruitment and derecruitment of motor units of various sizes, which fire for prolonged periods of time with stable frequencies. In this study we have shown that after selective chemical depletion of descending monoaminergic fibres there is a change from the EMG pattern observed in normal rats to a phasic denervation pattern with reduced EMG episode duration causing reduced total EMG activity duration. The tonic EMG segments were fewer than in the controls and tended to decay slowly in amplitude. We suggest that descending monoaminergic pathways might be important for the maintained tonic motor output in the hindlimb muscles. Before discussing this topic, we will briefly comment on the intrathecal denervation method.

The intrathecal depletion technique

In the present study we have used intrathecal administration of either 5,6-DHT in combination with 6-OHDA or 5,7-DHT alone to obtain a selective depletion of all major spinal monoamines. In agreement with previous studies we find a substantial reduction of the 5-HT and NA content in the lumbar spinal cord (Howe & Yaksh, 1982; Berge *et al.* 1985). The NA content was also significantly reduced in the cervical spinal cord and the lower brainstem, while the changes in 5-HT content were not significant in these structures. Similar observations have been made previously (Berge *et al.* 1985) and apparently the noradrenergic fibres are more sensitive to retrograde degeneration than the serotonergic fibres. In addition to these changes there was a significant reduction in the DA content of the cervical spinal cord and the lower brainstem, while the change in the DA content of the lumbar spinal cord was not significant. The degree of dopaminergic denervation following intrathecal administration of neurotoxins has not been investigated previously and at present we have no explanation for the lack of full DA depletion in the lumbar cord. Most important is, however, the fact that the content of all three monoamines was not significantly changed in cortical structures. We therefore conclude that the intrathecal route of administration caused a selective loss of spinal monoamines. In this study we have not investigated the time course of the denervation. It is known, however, from studies using intraventricular and intrathecal administration, that the monoamine content starts to decrease shortly (hours) after drug administration, that the decrease levels off 2–3 weeks later, and that there is little fibre regeneration (especially in structures distant from the brainstem) during the first 2–3 months after a substantial denervation (Nobin, Baumgarten, Björklund, Lachenmayer & Stenevi, 1973; Wiklund *et al.* 1978; Berge *et al.* 1985).

When discussing the degree, time course, and regional selectivity of the denervation, it is also necessary to address the general cytotoxicity of the neurotoxins and possible non-specific damage caused by the catheter. Whereas 5,6-DHT in lower doses is regarded as a specific serotonergic neurotoxin, high doses (>75 μg) can lead to non-selective damage of myelinated fibres (Jonsson, 1983). 6-OHDA and 5,7-DHT have no non-specific cytotoxicity in the doses used in the present study (Jonsson, 1983). Similarly, no clear pathological changes, especially demyelinization, have been attributed to the intrathecal catheterization (Yaksh & Rudy, 1976; Hee, Klinken & Ballegaard, 1992). The EMG characteristics in catheterized non-treated rats were also similar to those found in unimplanted rats (cf. Fig. 5).

In conclusion, we propose that the physiological changes observed in the present study can probably be ascribed to a selective denervation of monoaminergic (at least the serotonergic and noradrenergic) descending pathways.

Functional interpretations of the EMG changes

Motoneurones in the ventral horn are strongly innervated by serotonergic and noradrenergic and to a lesser extent by dopaminergic terminals (Björklund & Skagerberg, 1982; Holstege & Kuypers, 1987). From experiments in reduced preparations it is known that monoamines can enhance or initiate tonic spinal motor behaviour. Thus, 5-HT and NA evoke long-lasting enhancement of motoneurone excitability by a subthreshold depolarization or induction of plateau properties. These mechanisms will, either by themselves or in combination with other synaptic inputs, provoke long-lasting motoneuronal spike activity (VanderMaelen, 1985; Hounsgaard *et al.* 1988; Conway *et al.* 1988; Sigvardt, 1989; Kiehn, 1991*a*,*b*). The decrease in mean soleus EMG episode duration and in total EMG activity duration in drug-injected rats with the simultaneous disappearance of the tonic EMG segments, which represent tonic firing in individual motor units (Fig. 2; Eken & Lømo, 1993), indicates that, as in reduced preparations, the descending monoaminergic fibres enable or facilitate spinal tonic motor output in intact animals. The parallel increase in the number of EMG episodes per 24 h period is caused by an increased number of short episodes and suggests that the changes are not due to a general inhibition of animal behaviour.

Monoamines can elicit spinal locomotor activity in reduced preparations (Smith, Feldman & Schmidt, 1988; Sigvardt, 1989; Cazalets, Grillner, Menard, Cremieux & Clarac, 1990), or enhance on-going rhythmic activity (Harris-Warrick, 1988), and it is likely that the observed EMG changes, in addition to changes in tonic motor output, reflect changes in locomotor behaviour. The slow time constant of the integrator (200 ms) did not allow us to

resolve single steps in the EMG episodes, but the locomotor behaviour appeared unaffected. The latter observation is in agreement with previous studies, which have shown that a substantial reduction of spinal monoamines in cats did not block the locomotor capability of the cord (Steeves *et al.* 1980). It is possible, however, that more refined EMG recordings will disclose denervation-induced changes in timing and phasing of the soleus locomotor pattern.

As a coarse indication of the firing characteristics of motor units we measured the mean firing frequencies of motor units during tonic segments of activity in non-treated catheterized rats and drug-treated rats. The mean firing frequency (which is the mean firing frequency of many units) was not significantly different before and after drug treatment, and the frequencies were comparable with previously reported values from untreated adult rats (Hennig & Lømo, 1985; Eken & Lømo, 1993). It is, therefore, unlikely that the temporal EMG changes are caused by an increase or decrease in the motor unit firing frequency. The most obvious explanation is that individual motor units fire for a shorter period of time as a consequence of the decreased monoaminergic drive onto motoneurones. Owing to the extreme difficulties in identifying individual motor units in consecutive recordings with several days in between, we have not addressed this issue further.

Interestingly, the changes in gross-EMG pattern developed with a similar time course (Figs 4 and 5) as has been noted for the decrease in spinal monoamines following intrathecal administration of neurotoxins. While the denervation pattern probably reflects decreased descending drive from monoaminergic pathways, the initial *increase* in gross-EMG activity (Fig. 3) might be due to neurotoxins acting as 'false transmitters' on postsynaptic receptors, competing for uptake sites on the monoaminergic terminals or causing an initial overflow of transmitter from the terminals (Jonsson, 1983). The latter mechanism has also been suggested as a cause of the rigid posture which occurs immediately after a systemic injection of *p*-chloroamphetamine, which lowers brain levels of 5-HT (Growdon, 1978).

It should be noted that monoamines are co-localized with peptides (e.g. substance P, TRH and neuropeptide Y) and that these transmitters are depleted by neurotoxins (Gilbert *et al.* 1982). Some of the observed effects might thus be attributed to loss of spinal neuropeptides. In general the peptides work in concert with the monoamines (Hökfelt, 1987) and the main conclusion that the descending monoaminergic pathways are important for maintained tonic motor output remains the same. The background for such a regulatory function is found in the firing behaviour of the monoaminergic brainstem cells. These cells discharge steadily and their activity will provide tonic influence to modulate spinal motor activity (Jacobs & Fornal, 1993).

Finally, it is well known that receptor supersensitivity develops after monoaminergic denervation (Nygren, Fuxe, Jonsson & Olson, 1974; Nygren & Olson, 1976; Howe & Yaksh, 1982; Berge, Fasmer, Flatmark & Hole, 1983) and it has been assumed that this mechanism, together with regeneration (Nygren *et al.* 1974; Nygren & Olson, 1976), contributes to the functional recovery which, with a variable time course, occurs after denervation. We cannot exclude that supersensitivity also plays a role in the present study. However, given the tonic activity in monoaminergic brainstem cells (Jacobs & Fornal, 1993) it is expected that denervation supersensitivity should *decrease* the effect of the denervation *per se*, by *increasing* the effectiveness of the remaining synapses; that is, an increase in EMG activity. It therefore appears that in the time window of this study the EMG changes caused by the denervation itself (loss of transmitter substances) are stronger than theoretically opposing changes caused by supersensitivity.

While our findings indicate that the descending pathways facilitate soleus EMG activity in intact animals, it is at present impossible to know the locus (or more likely loci) for this effect. Motoneurones, with their known ability to generate plateau potentials and slow depolarizations in the presence of monoamines, are probable candidates. These membrane properties obviate the need for continuous repetitive excitatory inputs to maintain continuous motor output (Hounsgaard *et al.* 1988; Conway *et al.* 1988; Eken & Kiehn, 1989; for reviews see Kiehn, 1991*a*, *b*), and an inhibition of such properties by denervation will decrease motor output. The activity of pre-motoneurones as well as presynatic modulation of sensory afferents and descending command signals will probably also change after denervation. The relative contribution of these different elements to the soleus motor output is unknown at present. Despite these uncertainties our study strongly indicates that the monoaminergic descending systems facilitate tonic motor output in the intact animal.

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Author's present address

Torben Bruhn: Institute of Neuropathology, University of Copenhagen, Frederik V's vej 11, DK-2100 Copenhagen, Denmark.

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