Facilitation of postural limb reflexes in spinal rabbits by serotonergic agonist administration, epidural electrical stimulation, and postural training

V. F. Lyalka, L.-J. Hsu, A. Karayannidou, P. V. Zelenin, G. N. Orlovsky and T. G. Deliagina
Department of Neuroscience, Karolinska Institute, SE-17177, Stockholm, Sweden.

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Corresponding author: Dr. T. G. Deliagina, Department of Neuroscience, Karolinska Institute, SE-17177, Stockholm, Sweden.
Phone: +(46)8-5248 6915. FAX: +(46)8-34 95 44. E-mail: Tatiana.Deliagina@ki.se.

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ABSTRACT

In quadrupeds, spinalization in the thoracic region severely impairs postural control in the hindquarters. The goal of this study was to improve postural functions in chronic spinal rabbits by regular application of different factors – intrathecal injection of 5-HT₂ agonist, DOI; epidural electrical spinal cord stimulation, EES; and specific postural training, SPT. The factors were used either alone (SPT group of animals), or in combination (DOI+SPT, EES+SPT, DOI+EES+SPT groups), or not used (control group).

It was found that in none of these groups normal postural corrective movements in response to lateral tilts of the supporting platform reappeared within the month of treatment. In control group, reduced irregular EMG responses, either correctly or incorrectly phased in relation to tilts, were observed. By contrast, in DOI+SPT and EES+SPT groups, a gradual threefold increase in the proportion of correctly phased EMG responses (as compared to control) was observed. The increase was smaller in DOI+EES+SPT and SPT groups. Dissimilarly to these long-term effects, short-term effects of DOI and EES were weak or absent. In addition, gradual development of oscillatory EMG activity in the responses to tilts, characteristic for the control group, was retarded in DOI+SPT, EES+SPT, DOI+EES+SPT and SPT groups. Thus, regular application of the three tested factors and their combinations caused progressive, long-lasting plastic changes in the isolated spinal networks, resulting in the facilitation of spinal postural reflexes and in the retardation of the development of oscillatory EMG activity. The facilitated reflexes, however, were insufficient for normal postural functions.
INTRODUCTION

When standing, quadrupedal animals maintain the dorsal-side-up body orientation and equilibrium due to the activity of the postural system (Deliagina et al. 2006, 2008; Horak and Macpherson 1996; Macpherson et al. 1997a; Massion and Dufosse 1988; Massion 1998; Orlovsky et al. 1999). Extensive spinal cord injuries (SCI) result in a dramatic impairment of the postural system. After a complete transection of the spinal cord in the thoracic region, chronic spinal cats exhibited poor responses to perturbations of posture and were, as a rule, not able to maintain the dorsal-side-up orientation of the caudal part of their body (Macpherson and Fung 1999). The postural functions practically did not recover (Barbeau et al. 2002; Macpherson et al. 1997b; Rossignol et al. 1999, 2002).

One reason for postural dysfunction in spinal animals is cessation of phasic supraspinal motor commands for postural corrections. These commands are generated on the basis of sensory information about postural perturbations, and are transmitted to the spinal cord by different descending pathways, as demonstrated for the corticospinal tract (Beloozerova et al. 2003a, 2005) and rubrospinal tract (Zelenin et al. 2010). Another reason for postural dysfunction is the cessation of tonic supraspinal excitatory drive to the spinal networks, resulting in a dramatic reduction of spinal postural reflexes. The importance of this drive was demonstrated in acute experiments on spinal rabbits (Musienko et al. 2010). It was shown that postural limb reflexes disappeared after acute spinalization, but their EMG pattern could be restored by means of electrical and/or pharmacological stimulation of the spinal cord below the lesion. This stimulation presumably substitutes the missing tonic supraspinal drive and activates the spinal postural networks underlying generation of postural limb reflexes. However, the efficacy of these reflexes is low and they cannot evoke corrective movements. Since spinalization results in a loss of all supraspinal commands addressed to the spinal networks, the
only way to improve postural functions in spinal subjects is to increase the efficacy of reflexes generated by the spinal postural networks.

Numerous evidences indicate that spinal networks, deprived of supraspinal influences, undergo considerable spontaneous changes (for review, see Frigon and Rossignol 2006). Spontaneous changes in the spinal postural networks are reflected in the modifications of spinal postural reflexes, observed at different post-SCI stages (Lyalka et al. 2008, 2009a). They are also reflected in the fact that the drugs, which improve locomotor or postural functions in spinal animals, are effective only during a definite post-lesion “time window” (Rossignol et al. 1998, 2001). For example, serotonergic drugs, which enhance postural limb reflexes at the earlier post-SCI stage, are inefficient at the later stages (Lyalka et al. 2008; Musienko et al. 2010). This could be caused by transient up- or down-regulation of corresponding receptors (Kim et al. 1999; Lee et al. 2007; Otoshi et al. 2009). Other reasons could be secondary processes like cell degeneration, sprouting, etc., initiated by partial or complete loss of descending fibres in the lumbar spinal cord after SCI (Holmes et al. 2005; Saruhashi et al. 1996; Hou et al. 2008, 2009).

It is known that regular application of some factors including training, electrical and pharmacological stimulation of the spinal cord, or their combination results in restoration of locomotor functions in spinal animals (Antri et al. 2003, 2005; Courtine et al. 2009; Edgerton et al. 2008; Fong et al. 2005, 2009; Gerasimenko et al. 2008; Rossignol et al. 2001). These findings suggest that such treatments prevent spontaneous changes in the isolated spinal network leading to disintegration of locomotor CPG, and induce plastic changes resulting in the restoration of locomotor function.

In the present study, we tested the hypothesis that repetitive, long-term application of some activating factors can cause plastic changes in the isolated spinal postural networks leading to restoration of postural limb reflexes and, possibly, to recovery of postural functions. Three approaches were used. First, we performed regular (every second day) pharmacological stimulation of the spinal cord by means of the 5-HT$_2$ agonist DOI [(±)-1-(2,5-Dimethoxy-4-}
iodophenyl)-2-aminopropane hydrochloride], which was applied intrathecally below the level of spinalization. A positive role of serotonergic drugs for the recovery of motor functions after SCI is well established. In acute spinal rabbits, 5-HTP induced locomotor rhythms (Viala and Buser 1971). In chronic rabbits with extensive incomplete SCI, a single intrathecal application of quipazine (5-HT1,2,3 agonist) facilitated postural reflexes, but the effect was limited to the first ten days post-lesion (Lyalka et al. 2008). Recently we have shown that epidural electrical stimulation, if it was combined with application of quipazine, resulted in much stronger facilitation of postural limb reflexes in acute spinal rabbits (Musienko et al. 2010). It was also shown that quipazine increased the efficacy of locomotor training in SCI-rats (Antri et al. 2002; de Leon and Acosta 2006), mice (Fong et al. 2005) and cats (Rossignol et al. 2001). It enhanced the effect of epidural stimulation (Gerasimenko et al. 2007), which restores the locomotor function in SCI-animals. In the present study we used DOI as an activating factor. This drug has been reported to increase H-reflex amplitude in chronic spinal rats (Lee et al. 2007). In acute spinal cats, DOI caused restoration of extensor excitability (Miller et al. 1996), which is an important condition for the generation of postural corrections.

Second, we used regular epidural electrical stimulation (EES) of the spinal cord below the level of spinalization. This technique was successfully used for activation of spinal locomotor mechanisms in rats, cats, and humans (for review, see Gerasimenko et al. 2008), as well as for facilitation of postural reflexes in acute spinal rabbits (Musienko et al. 2010). For testing the effects of activating factors on postural functions of spinal animals, we recorded postural reactions in the hindquarters evoked by lateral tilts of the supporting platform (this technique was described earlier, see Beloozerova et al. 2003b; Lyalka et al. 2005). To reveal their short-term and long-term effects, such postural tests were performed before and after application of the factor(s). With this procedure, however, in each session of postural testing, the animal was subjected to numerous tilts, which could be considered as specific postural training (SPT). Thus, the effects of DOI and EES could not be assessed separately, but
in combination with SPT (in the DOI+SPT and EES+SPT groups of animals). The effects of postural training alone were assessed in the SPT group of animals.

It is known that some rudiments of postural control, e.g. weight-bearing standing episodes, are present in spinal subjects (Giuliani and Smith 1985; Grillner 1973; Kellog et al. 1946) and can be improved by training (de Leon et al. 1998; Edgerton et al. 2001, 2004; Pratt et al. 1994). However, these animals demonstrated the lack of lateral stability and were not able to generate postural corrections in response to disturbances of body orientation. The SPT used in the present study was specifically aimed at improving the lateral stability in spinal animals.

We also tested postural effects produced by a combination of three factors (in the DOI+EES+CPT group of animals). No activating factors were used in the control group of spinal animals.

A brief account of part of this study has been published in abstract form (Lyalka et al. 2009b).

METHODS

Experiments were carried out on 16 adult male New Zealand rabbits (weighing 2.5-3.5 kg). All experiments were conducted with the approval of the local ethical committee (Norra Djurförsöksetiska Nämnden) in Stockholm.

Surgical procedures

Each animal was subjected to two operations under Hypnorm-midazolam anesthesia, using aseptic procedures. During the first surgery, bipolar EMG electrodes (0.2 mm flexible stainless steel Teflon-insulated wires) were implanted bilaterally into m. gastrocnemius lateralis (Gast, ankle extensor), m. vastus lateralis (Vast, knee extensor) and m. biceps femoris (Bic,
knee flexor). In addition, in a few rabbits, electrodes were implanted into *m. tibialis anterior* (Tib, ankle flexor) and *m. semitendinosus* (ST, knee flexor and hip extensor). The wires were led sub-cutaneously toward the head and then through a small incision in the skin on the dorsal aspect of the neck. The wound was sutured so that the wires were fastened to the skin. A small connector was soldered to each wire at a distance of 2-3 cm from the skin.

In 3-4 days, when the animal had recovered completely from the first surgery, its postural responses to tilts were tested (see below), and afterwards the second surgery was performed. There were four types of surgery; all of them included spinalization at T11. In animals from control and SPT groups, only spinalization was performed. In other groups, spinalization was combined with implantation of either intrathecal cannula (DOI+SPT group), or stimulating electrodes (EES+SPT group), or both (DOI+EES+SPT group).

During the second surgery in animals of all groups, an incision was made along the dorsal midline in the lower thoracic region, and laminectomy at the T11-T12 level was done. For implanting the cannula (in animals from DOI+SPT and DOI+EES+SPT groups), a small hole in the dura was made on the midline dorsum of the cord in the T12 segment. The cannula (silicone tube, OD = 0.7 mm, about 200 mm in length, filled with Ringer solution) was inserted under the dura in the caudal part of the T12 segment, and protracted caudally for ∼100 mm to reach the L5-L6 level, as shown schematically in Fig. 1A. The necessary length of the descending arm of the cannula was measured externally by counting spinous processes. The tube was glued to the T10 spinous process by dental cement; its rostral end was led subcutaneously toward the head and then through a small incision in the skin on the dorsal aspect of the neck. The wound was sutured so that the tube was fastened to the skin. An opening of the tube was closed with a small plug.

For implantation of electrodes for epidural electrical stimulation (EES) of the spinal cord (in animals from the EES+SPT and DOI+EES+SPT groups), an incision was made along the dorsal midline in the lumbar region, and laminectomy at the L5-L7 level was done. Two EES
electrodes were made of the thin flexible stainless steel wires (d=200 µm) Teflon-insulated except for the tips. They were positioned on the midline of the dorsal aspect of the spinal cord, one in the L6 segment and the other in the L7 segment. The electrodes were sutured to the dura. The electrode wires were glued to the L4 spinous process by dental cement. The rostral ends of these wires were led sub-cutaneously toward the head and then through a small incision in the skin on the dorsal aspect of the neck. The wound was sutured so that the wires were fastened to the skin. In some of the rabbits, both cannula and EES electrodes were implanted using the techniques described above.

In all groups of animals, the spinal cord injury, i.e., a complete transection of the spinal cord, was performed at T11 level. For this purpose, the dura in the rostral part of the T11 segment was opened, a few drops of xylocaine (2%) were placed on the spinal cord, and then a few injections (0.1-0.2 ml each) were made directly into the spinal cord at the level of transection. The spinal cord was completely severed progressively under the dissecting microscope by means of spring scissors, microsurgery forceps and a small scalpel. Afterward, the incisions were closed in anatomical layers. Animals from the DOI+SPT, EES+SPT, and DOI+EES+SPT groups were subjected to spinalization after implantation of the cannula and (or) the EES electrodes.

**Animal care**

Each rabbit was kept in an individual cage (80 x 70 x 65 cm). The cage was cleaned every day, and its bottom was covered with fresh absorbing tissue. The rabbits had access to water, and were fed dry rabbit food, hay, and carrots.

The rabbits were monitored closely after surgery, particularly during the first 24-48 h. An analgesic, buprenorphine hydrochloride (Temgesic, 0.01 mg/kg s.c.) was given every 12 h for 48 h. To reduce inflammatory reaction caused by surgery, Rimadyl (4 mg/kg s.c) was
injected pre-operatively and two days after surgery. In addition to their own water intake, the first two days after the second surgery the rabbits were administrated, twice daily, 25 ml of Ringer solution. During 3 days after the first surgery and 5 days after the second surgery, antibiotic (Baytril, 5 mg/kg i.m.) was daily administered prophylactically.

Only two rabbits with spinal cord lesions were kept at any particular time. They were attended to a few times daily, to express the bladder manually, and to inspect and clean the hindquarters.

Experimental design

Postural tests on a tilting platform have been described earlier (Beloozerova et al. 2003b; Lyalka et al. 2005, 2008). No special training of the rabbits was required prior to testing. For testing, an animal was positioned on the two platforms (P1 and P2 in Fig. 1A), so that P1 supported the forelimbs, and P2 supported the hindlimbs. The sagittal plane of the animal was aligned to the axis of the platform rotation (Fig. 1B). The surface of the platforms was covered with sandpaper to prevent sliding of the animal during tilts.

The platform supporting the hindquarters could be tilted periodically in the frontal (transverse) plane of the animal (angle α, Fig. 1, A and C), while P1 was kept horizontal. A trapezoidal trajectory of tilting was used (Fig. 1E) with transitions between stationary (extreme) positions lasting for 0.5-0.7 s, and with each position being maintained for 3-4 s. Tilts were symmetrical in relation to the horizontal position, with the peak-to-peak value of 40° in intact rabbits. A smaller tilt value (30° peak-to-peak) was used in spinal animals.

Earlier it was shown that lateral displacements of the trunk in relation to the tilting platform well characterize the efficacy of stabilization of the dorsal-side-up trunk position (Beloozerova et al. 2003b). In the present study we used the same method, and measured lateral displacements of the caudal part of the trunk in relation to the platform P2 (postural corrections
in the hindquarters). This was done by means of a mechanical sensor positioned at the half-height of the body (S in Fig.1, A-D). The sensor consisted of a variable resistor, the axis of which was rotated by means of a long lever; the latter was always touching (under a light pressure) the lateral aspect of the body. Recording of postural corrections was performed along with recording of EMGs from selected hindlimb muscles.

**Activating factors**

DOI INJECTION. The 5-HT_2_ agonist DOI [(±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride] from Sigma-RBI was used. It was dissolved in sterile saline and injected (concentration 1 mM) as a bolus of 100 μl into the subarachnoid space of the spinal cord through the inlet of the cannula, as described in the previous paper (Lyalka et al. 2008). A subsequent bolus injection of saline (100 μl) was made to flush the drug outside the cannula; the dead space of the cannula was 80 μl.

The position of cannula was verified *post mortem*. In all cases, the tip was positioned in the L5-L6 segments. In the previous study it was shown (by injecting 100 μl of fast green solution through the cannula) that all parts of the lumbar enlargement of the spinal cord were stained, suggesting that the injected drug affected a considerable part of the hindlimbs-related spinal networks (Lyalka et al. 2008).

**EPIDURAL ELECTRICAL STIMULATION (EES).** To set the optimal strength of current pulses during EES, in each experiment we initially determined the threshold current (that elicited motor responses in the hindlimbs), and then used a current 3-5 times stronger, which usually was within the range of 100-500 μA. In the previous study on acute spinal rabbits it was shown that an optimal frequency of EES for re-activation of spinal postural reflexes was 3 pps (Musienko et al. 2010). In the present study, three frequencies of stimulation (1 pps, 3 pps, and
5 pps) were tested in each experiment in the EES+SPT group of animals, and only one frequency (3 pps) in the DOI+EES+SPT group.

**Specific postural training (SPT).** All animals (except for the control group) were subjected to SPT. For this purpose, each second day the spinal rabbit was positioned on the tilting platform and subjected to periodical lateral tilts (120-440 tilt cycles; amplitude, ±15-20°; period, 8 s).

**Experimental protocol**

Experimental sessions started from day 3 after the second surgery. Animals from all groups were subjected to postural tests on the tilting platform. The sequence and number of tests in the session differed between the groups of animals.

In the control group (N=3), an experimental session consisted of only one postural test (15 tilt cycles). The session was performed each second day.

In the DOI+SPT group (N=3), the session consisted of the following series of tests: Control test (15 tilt cycles) → DOI injection → 7 postural tests (15 tilt cycles each) with an interval of 10 min. The session included about 120 tilt cycles and was performed every second day.

It is known that the effects of intrathecal DOI injection are observed for ~90 min post-injection (Lee et al. 2007). To trace the time course of the effects of DOI, the first postural test was done 5 min post-injection, and the following tests were done with intervals of 10 min. The session included about 120 tilt cycles and was performed every second day.

In the EES+SPT group (N=3), the session consisted of the following series of tests: Control test (15 tilt cycles) → 6 stimulation tests (with three frequencies of stimulation through each of the two electrodes), with an interval of 5 min. Each stimulation test consisted of: 10 tilt
cycles before EES \(\rightarrow\) 10 tilt cycles during EES \(\rightarrow\) 10 tilt cycles after EES. The session included about 200 tilt cycles and was performed every second day.

In the DOI+EES+SPT group (N=3), the session consisted of the following series of tests: Control test (15 tilt cycles) \(\rightarrow\) DOI injection \(\rightarrow\) 14 stimulation tests performed with an interval of 5 min (tests with stimulation through each of the two electrodes were alternated). The session included about 440 tilt cycles and was performed every second day.

In the SPT group (N=4), the session consisted of the following series of tests: Control test (15 tilt cycles) \(\rightarrow\) Training consisting of 7 postural tests (15 tilt cycles each) performed with intervals of 10 min. The session included about 120 tilt cycles and was performed every second day.

By comparing the data obtained in the control test with the data obtained after application of a given factor (DOI or/and EES), short-term effect(s) of the factor(s) were determined. By comparing the data obtained in the control tests at different post-lesion time points with the data obtained in the control group at the corresponding time points, long-term effect(s) of the factor(s) were characterized.

**Recordings and data analysis**

The signals from the EMG electrodes and from the position sensors were amplified, digitized with a sampling frequency of 5 kHz (EMGs) and 1 kHz (sensors), and recorded on a computer disk using the data acquisition and analysis software (Power-1401/Spike-2, Cambridge Electronic Design, Cambridge, UK). The EMG signals were rectified and smoothed (time constant, 50 ms). All quantitative data in this study are presented as the mean ± SE. Student’s t-test was used to characterize the statistical significance when comparing different means; the significance level was set at \(p = 0.05\).
Histological procedures

At the termination of the experimental series, rabbits were deeply anesthetized with pentobarbital sodium and perfused with isotonic saline followed by a 10% formalin solution. Frozen sections of 30 µm thickness were cut in the region of spinal cord damage. The tissue was stained for Nissl substance with cresyl violet. The completeness of the spinal transections in all animals was verified by observation of a series of magnified digital images of the sections.

RESULTS

Impairment of postural control caused by spinalization

POSTURAL PERFORMANCE BEFORE SPINALIZATION. All animals were subjected to postural tests before spinalization. Their postural reactions did not differ from those described in our previous papers (Beloozerova et al. 2003b; Lyalka et al. 2005, 2009a). Intact rabbits maintained balance when the platform under their hindlimbs (P2 in Fig. 1A) was periodically tilted, as shown schematically in Fig. 1C. The stereotypic postural responses included extension of the hindlimb on the side moving downward and flexion of the hindlimb on the opposite side. These flexion and extension limb movements displaced the trunk in the transverse plane, in the direction opposite to the platform tilt. The corrective trunk movements reduced the deviation of the body from the dorsal-side-up position.

Figure 2A illustrates kinematics and EMG pattern in rabbit 154 before spinalization. One can see that lateral displacements of the trunk in relation to the platform (S) were in anti-phase to platform tilts (α). The peak-to peak value of S in rabbits before spinalization ranged from 3.0 to 4.5 cm (4.0±0.5 cm) and was similar to that observed in our previous studies (Beloozerova et al. 2003b, Lyalka et al. 2009a). Tilt-related limb movements were caused by a specific pattern
of muscle activity (Fig. 2A). When the platform under a limb was moving downward, the limb was extending due to activation of its extensor muscles (Gast and Vast). When the platform under a limb was moving upward, the limb was flexing due to reduction in the activity of its extensors, as well as due to activation of some flexors (e.g. Bic, not illustrated) (see also Beloozerova et al. 2003b, Lyalka et al. 2009a). Such EMG pattern of postural corrections (shown schematically in Fig. 1E) was observed in all intact animals.

**POSTURAL PERFORMANCE AFTER SPINALIZATION.** Spinalization caused a dramatic impairment of postural control in the hindquarters. Observations of spinal animals in the cage and on the floor have shown that the rabbits were not able to maintain the dorsal-side-up orientation of their hindquarters. They did not use their hindlimbs for locomotion and moved around using their forelimbs only.

Figure 2B shows a postural test in one of the animals of control group (rabbit 154) performed on day 6 after spinalization (the postural test in this rabbit before spinalization is shown in Fig. 2A). When the platform under the hindlimbs was tilted, the hindquarters passively followed the platform movement and swayed toward the side tilting downward (as shown schematically in Fig. 1D). In contrast to anti-phase relationships between the platform tilts (α) and the lateral displacements of the trunk (S) in this rabbit before spinalization (Fig. 2A), the S trajectory after spinalization was in phase to the platform tilts, thus demonstrating the lack of postural corrections. In the postural test shown in Fig. 2B, the tilt peak-to-peak value was reduced from 40° (see Fig. 2A) to 30°, since with larger tilts the rabbit could fall sideways. As one can see in Fig. 2B, the EMG activity was either absent (as in Gast-L and Gast-R) or considerably diminished (as in Vast-L and Vast-R). When present, this small EMG activity was not modulated by tilts.

In all spinal rabbits of the control group (N=3), the ability to maintain the dorsal-side-up position of the hindquarters and to maintain equilibrium did not recover during the whole period of observations (30-45 days post-lesion), though some recovery of the EMG activity was
observed. This is illustrated in Fig. 2C, which shows a postural test (in the same rabbit as in A and B) on day 27 after lesion. Tilts of the platform evoked EMG responses in some hindlimb muscles. Vast-L was active in a correct phase (with left tilt) in all tilt cycles, but its responses varied considerably in amplitude and shape (onsets of these “correct” responses are indicated by black arrows). Gast-L was also activated by tilts in all cycles, but its responses overlapped the two phases (right and left tilts). We ascribed such EMG responses to the phase in which they started and had more than 50% of their duration. According to these criteria, responses of Gast-L in three tilt cycles, i.e., activation during right tilt, were considered “incorrect” (indicated by white arrows). Gast-R did not exhibit any stable responses, and Vast-R responded only in one of the tilt cycles, and this response was in a correct phase.

In addition, a new phenomenon – the repetitive EMG bursts – was observed in spinal animals, especially at the later post-lesion stages. This oscillatory EMG activity was often caused by tilts and superimposed on the ordinary EMG responses (Fig. 2C). Repetitive EMG bursts in different groups of rabbits is considered in the section Oscillatory EMG activity.

Thus, a well-coordinated EMG pattern observed in intact animals (Fig. 2A), after spinalization was transformed into poorly coordinated activity of individual muscles. Each muscle could spontaneously switch between three types of activity: correct response, incorrect response, and no response to tilt. Because of disintegration of the EMG pattern and a decrease in the response magnitude, corrective trunk movements were absent (see trace S in Fig. 2C). This was characteristic for all animals of the control group.

To characterize EMG responses at different post-lesion stages, we used a method described earlier (Lyalka et al. 2008, 2009a). In each postural test, we classified all EMG responses in individual muscles into three categories (Fig. 1F): a) correct responses (extensor EMG is timed to the ipsilateral tilt, and flexor EMG is timed to the contralateral tilt); b) incorrect responses (opposite phase relations); c) absence of responses. Then we calculated the relative number of tilt cycles with responses in each category for individual muscles. An
example of such representation of data is shown in Fig. 3A for the rabbit 154 (for the 2nd ten
day post-lesion period). One can see a very low probability (less than 20%) of postural
responses (either correct or incorrect) in any of the six muscles studied. We have not found any
marked difference between various muscles in terms of probability of their activation in the
correct or in the incorrect phase of the tilt cycle, and for that reason grouped all muscles
together when calculating the proportion of different types of responses.

These data are summarized in Fig. 3B that shows the relative number of different types
of EMG responses as a function of post-lesion time for the control group of spinal animals; the
data were averaged for the 1st, 2nd, and 3rd ten-day periods post-lesion. One can see that the
proportion of responding muscles (both correctly and incorrectly phased) was very small in any
period after lesion.

Effects of DOI application combined with SPT

The DOI+SPT group of rabbits (N=3) was subjected to regular (every second day)
intrathecal injection of DOI and to specific postural training (SPT) on the tilting platform, with
~120 tilt cycles in each session (see Methods).

Long-term effects. To characterize the long-term effects of DOI+SPT, the data
obtained in the control tests (before application of activating factors) at different post-lesion
time points were used. Figure 4 shows the control tests in the rabbit 166 performed on day 5 (A)
and day 29 (B) after spinalization. On day 5, the EMG activity was very low, and weak
responses to tilts were observed in Vast-L, Gast-L and Gast R. They were correct in Vast-L and
incorrect in Gast-L and Gast R. On day 29, there was a considerable level of EMG activity in
three out of four muscles, as well as correct responses to tilts in two of them (Gast-R and Vast-
L). The lateral trunk displacements (S) were in anti-phase to tilts, indicating the presence of
postural corrections, but they were very small (less than 1 cm against 4-5 cm in intact rabbits,
These small corrections in rabbit 166 were observed only during one session (day 29), and never observed in the other rabbits.

To characterize the effect of DOI+SPT on the EMG responses in the whole DOI+SPT group of animals, we used the same method as for the control group (see above). For each control test, we calculated for individual muscles the relative number of tilt cycles with responses in each category (correct, incorrect, no response, see Fig. 1F). These data were averaged over all muscles and all rabbits of DOI+SPT group (N=3); the averaging was done separately for the 1st, 2nd, and 3rd ten-day periods post-lesion.

The long-term effects of DOI+SPT are summarized in Fig. 5A-C. For comparison, the data for rabbits of the control group are also shown. One can see that the proportion of correct responses in the DOI+SPT group increased over time, from 28% (in the 1st period) to 37% (in the 3rd period). In any period, this proportion was significantly (three-fold) larger than that in the control group. The proportion of incorrect responses also increased, but to a lesser extent. Correspondingly, the proportion of “no responses” significantly decreased. Thus, the long-term effect of DOI+SPT was a predominant facilitation of the correct EMG responses to tilts, suggesting a considerable enhancement of spinal postural reflexes.

**SHORT-TERM EFFECTS.** To characterize the short-term effects of DOI, the test before DOI application was compared to those after DOI application. Figure 5D shows the proportion of correct, incorrect, and no responses for the control test done before DOI application, as well as for 7 subsequent postural tests. Averaging was done over all animals of the DOI+SPT group and all post-lesion periods. One can see that the proportion of correct responses after DOI application did not change significantly as compared to that before DOI application. By contrast, the proportion of incorrect EMG responses significantly decreased (almost half) during the period of 65 min after DOI application. Correspondingly, the proportion of no responses increased. Thus, the short-term effect of DOI was a considerable reduction of the proportion of incorrect EMG responses.
Effects of EES combined with SPT

The EES+SPT group of rabbits (N=3) was subjected to regular (every second day) epidural electrical stimulation (EES) combined with specific postural training (SPT) on the tilting platform, with ≈200 tilt cycles in each session (see Methods).

LONG-TERM EFFECTS. To characterize the long-term effects of EES+SPT, we used the data obtained in control tests (before application of activating factors) at different post-lesion time points, and used the same method of analysis as for the control and DOI+SPT groups (see above). The long-term effects of EES+SPT treatment are summarized in Fig. 6A-C, which shows the relative number of different types of EMG responses to tilts as a function of post-lesion time, for all animals of the EES+SPT group. For comparison, the corresponding data for the control group are also given. One can see that the proportion of correct responses increased over time, from 25% (in the 1st ten-day period) to 43% (in the 3rd ten-day period). In any period, this proportion was significantly larger than that in the control group. An increase in the number of correct responses was accompanied by a decrease in the number of cases with no response (Fig. 6C). The proportion of incorrect responses in the EES+SPT group was only slightly larger than in the control group. Thus, the long-term effect of EES+SPT was a predominant facilitation of correct EMG responses to tilts, suggesting considerable enhancement of spinal postural reflexes.

SHORT-TERM EFFECTS. To characterize the short-term effects of EES, the proportion of different types of EMG responses to tilt (correct, incorrect, no response) was calculated separately for each of the three parts (before, during, and after EES) of each stimulation test (see Methods). Then data were averaged over all animals of the EES+SPT group, separately for three parts of the stimulation test, and separately for the 1st, 2nd, and 3rd ten-day periods. The short-term effects of EES are summarized in Fig. 6D-F. One can see that, in each ten-day period, the proportion of correct responses during EES decreased as compared to that before and after EES.
The proportion of incorrect responses during EES did not change, but it increased after EES (E). Correspondingly, the proportion of “no responses” increased during EES (F). All these changes were small (less than 20%).

**Effects of SPT**

The SPT group of rabbits (N=4) was subjected to regular (every second day) specific postural training (SPT) on the tilting platform, with ~120 tilt cycles in each session (see Methods).

The long-term effects of regular SPT are summarized in Fig. 7A-C. They are shown together with the data for the control group of rabbits. In the SPT group, the proportion of correct EMG responses in the control tests increased (A) and the proportion of tilt cycles with no response decreased (C) as compared with those in the control group. However, these positive long-term effects on spinal postural reflexes were considerably smaller than in the DOI+SPT and EES+SPT groups (compare Fig. 7A,C with Figs. 5A,C and 6A,C; see also Fig. 10).

**Effects of combined EES, DOI and SPT**

The DOI+EES+SPT group of rabbits (N=3) was subjected to regular (every second day) intrathecal DOI injection and epidural electrical stimulation (EES) combined with specific postural training (SPT) on the tilting platform, with ~440 tilt cycles in each session (see Methods).

**LONG-TERM EFFECTS.** Figure 8A-C shows the relative number of different types of EMG responses in the control tests as a function of post-lesion time for all animals of the DOI+EES+SPT group. For comparison, the corresponding data for rabbits of the control group are also shown. One can see that the proportion of correct responses increased over time, from 13% (in the 1st period) to 22% (in the 3rd period). In any period, this proportion was larger than
that in the control group, but in the 3rd period the difference was not statistically significant. The proportion of incorrect responses in the DOI+EES+SPT group was also larger than in the control group, but in the 3rd period the difference was not statistically significant. Thus, the long-term effect of DOI+EES+SPT was a facilitation of both correct and incorrect EMG responses (and a corresponding reduction in the proportion of cases with no response). The proportion of correct responses in the DOI+EES+SPT group was smaller than in the DOI+SPT group and in the EES+SPT group (compare Fig. 8A with Figs. 5A and 6A; see also Fig. 10).

**Short-term effects.** To characterize the short-term effects of DOI+EES, the proportion of correct and incorrect EMG responses was calculated for the control test (before DOI application), as well as for each of three parts of stimulation test (before, during and after EES) for 7 post-injection stimulations (with intervals of 10 min). Then the data were averaged over all animals of the DOI+EES+SPT group (N=3) during the month of treatment. Fig. 8D-F shows short-term effects of the DOI application combined with EES. One can see that the proportion of correct responses at different time points after DOI application (before EES) did not change considerably as compared to that before DOI application (Fig. 8D). By contrast, the proportion of incorrect EMG responses before EES decreased in the first 3 tests after DOI application (Fig. 8E). However, this decrease was very small (less than 10%). These short-term effects of DOI in the DOI+EES+SPT group were similar to those in the DOI+SPT group (Fig. 5D). After administration of DOI, short-term effects of EES were absent: the proportion of different types of responses during and after EES did not change (Fig. 8D-F).

**Oscillatory EMG activity**

The oscillatory EMG activity in hindlimb muscles was observed in all groups of spinal animals, especially at the later post-lesion stages. Oscillations were often evoked by tilts and superimposed on the ordinary EMG responses (Fig. 2C, Fig. 9A). The oscillations could also be
caused by other sensory stimuli (mechanical stimulation of the tail, flexing or extending the
limb, etc.), or appeared spontaneously. A representative example of the repetitive EMG bursts,
recorded simultaneously from eight hindlimb muscles, is shown in Fig. 9A (rabbit 156 from the
control group, tested on 34 day post-lesion). The oscillations were evoked by tilts of the
platform, and the first few tilts (after the period of unperturbed posture) usually were most
efficient in this respect. In response to tilt, a muscle could generate 1-5 bursts in succession
(burst duration, 0.5-1.0 s; burst frequency, 0.5-1.0 Hz). The bursts generated by different
muscles could be synchronized in phase with each other (as in the cases indicated by frames a, b
and d in Fig. 9A) or could be in anti-phase (indicated by frames c and e). One can see that
agonistic muscles could generate bursts synchronously (see Vast-L and Gast-L, frame b in Fig.
9A) as well as in anti-phase (see Vast-R and Gast-R, frame e in Fig. 9A).

To characterize the development of oscillatory EMG activity in different groups of
rabbits, we calculated the number of bursts generated by all muscles during the control tests, and
divided this value by the number of muscles and by the number of tilt cycles. Figure 9B shows
this value for the control group of rabbits, averaged over all 3 animals at different time-points
after lesion. Oscillations in EMGs appeared soon after spinalization, and then gradually
increased during the month of observation. Figure 9C shows the value of oscillatory EMG
activity in the control tests for different groups of rabbits, averaged separately for each 10-day
post-lesion period. One can see that the rate of development of the oscillatory EMG activity was
significantly lower in the SPT, DOI+SPT, EES+SPT and DOI+EES+SPT groups of rabbits as
compared to the control group. This finding suggests that all three activating factors that
promote recovery of postural reflexes, retard the development of oscillatory EMG activity.

DISCUSSION
The present study has shown that chronic spinal rabbits of the control group (no treatment) are not able to maintain balance on the tilting platform, and their postural functions do not recover over time. Similar results were obtained in spinal cats (Macpherson and Fung 1999), as well as in rabbits with extensive but incomplete SCI – with the ventral hemisection (VHS) or with the ¾-section (VQ) that spared only one ventral quadrant (Lyalka et al. 2005, 2008, 2009), suggesting that ventral pathways (reticulo- and vestibulospinal) are critically important for postural control.

One of the postural deficits observed in spinal rabbits was a considerable reduction of EMG responses to tilts. As a result, the limb muscles developed insufficient force to compensate for the tilts (Fig. 2B,C). The other deficit was instability of these reflex responses – they could spontaneously change their phase in relation to tilts, their value, or disappear altogether. Similar postural deficits were observed in the VHS- and VQ-rabbits (Lyalka et al. 2008, 2009a). A low probability and small magnitude of the correct (properly phased) EMG responses, as well as appearance of incorrect responses can be explained by suggesting that the spinal reflex chains, which are necessary for the generation of postural reactions, are not properly selected and activated in the absence of supraspinal drive. In the control group, the proportion of correct EMG responses increased over time, but this increase was very small (from 7% to 12%, Fig. 3B) and not significant ($p=0.1$). Thus, in the non-treated spinal rabbits, the recovery of postural limb reflexes was practically absent during one month after SCI. It remains unknown if postural limb reflexes would recover over time later, during several months after lesion.

In the present study, we tried to induce long-term plastic changes in the isolated spinal networks, leading to enhancement of postural limb reflexes. In four groups of spinal rabbits, the activating factors (DOI, EES, and SPT) or their combinations were applied every second day during one month. These groups were compared to the control group (no-treatment).

For DOI application, we used intrathecal cannula, and injected the drug at the L5-L6 level. In previous experiments it was shown that after such injection the solution spread over the
whole enlargement (Lyalka et al. 2008). It was recently reported that spinal interneurons, presumably contributing to the generation of postural limb reflexes, are located in L4-L7 (Deliagina et al. 2009). It is also known that the majority of motoneuron pools of limb extensors (the muscular group most involved in postural reactions) are located in the lower lumbar segments (Portal et al. 1991; Romanes 1951; Vanderhorst and Holstege 1997). Thus, we stimulated the whole area presumably involved in the generation of postural limb reflexes.

For EES, we employed the technique previously tested in the acute spinal rabbits (Musienko et al. 2010). We used the characteristics of stimulation (frequency 1-3 Hz; current 100-500 µA; sites of stimulation in L6-L7), which most effectively restored postural limb reflexes in those rabbits.

The main result of this study is that, in the DOI+SPT and EES+SPT groups of animals, the activating factors produced considerable, long-term effects on the postural EMG responses to tilts, and the proportion of correctly phased responses increased significantly as compared to the control group (Figs. 5A, 6A, 10). This increase was observed in each ten-day period after lesion. It seems likely that the effects of DOI+SPT and EES+SPT were cumulative during the month of treatment, resulting in a progressive increase of the proportion of correct responses. The proportion of incorrect responses also increased, but to a much lesser extent (Figs. 5B and 6B). Thus, regular application of DOI+SPT or EES+SPT causes specific, long-term plastic changes, i.e., a gradual increase of excitability in the spinal networks generating postural limb reflexes.

In the SPT group, the proportion of correct responses also increased as compared to the control group (Fig. 7A), but to a much lesser extent than in the DOI+SPT and EES+SPT groups (Fig. 10). There was also some increase in the proportion of incorrect responses (Fig. 7B). A relatively small efficacy of SPT (when this factor was used alone) suggests that a high efficacy of the combinations DOI+SPT and EES+SPT was mainly due to the additive effects of the two factors. It is known that some factors exert additive long-term effects when activating locomotor
mechanisms in spinal animals. In particular, application of serotonergic drugs increased the efficacy of specific locomotor training (Fong et al. 2005; de Leon and Acosta 2006), as well as the efficacy of EES (Courtine et al. 2009; Gerasimenko et al. 2007).

In the present study, however, the long-term effects of the combination of the three factors (DOI, EES and SPT) were not additive. In the DOI+EES+SPT group, the proportion of correct responses increased as compared to the control group (Fig. 8A), but to a much lesser extent than in the DOI+SPT and EES+SPT groups; this increase was similar to that in SPT group (Fig. 10). One possible explanation of this result is that the influences of DOI and EES counteract each other at the level of spinal networks, which results in the reduction of their positive effects. Such mutual influences between different spinal mechanisms, both synergistic and antagonistic, were earlier suggested to be responsible for the recovery of locomotor functions in spinal animals (Edgerton et al. 2006). Another possible explanation is that a decrease in the proportion of correct EMG responses in the DOI+EES+SPT group results from the phenomenon of “over-training” (Purvis et al. 2010). Animals of this group were subjected to many more tilts in each experimental session (~ 440 tilts) as compared to the DOI+SPT group (~120 tilts) or to the EES+SPT group (~190 tilts).

Though in the present study the regular application of the activating factors (DOI+SPT and EES+SPT) significantly increased the proportion of correct EMG responses to tilts, the normal postural corrective movements did not re-appear (except for a few cases of very weak corrections, Fig 4B). One of the possible reasons for a low efficacy of spinal postural mechanisms could be their insufficient activation by the factors used. Since one month of treatment led to progressive enhancement of postural limb reflexes, it seems likely that prolongation of treatment could result in the further increase of the proportion of correct EMG responses, leading to the restoration of postural corrections. Another reason for the recovery limit could be a relatively small role in the postural control, which is played by the spinal mechanisms alone, without support from the supraspinal mechanisms. This contrasts to the
important role of spinal mechanisms in the control of locomotion, which allows a considerable
restoration of locomotor functions in SCI subjects (e.g. Barrière et al. 2008).

In contrast to the long-term effects produced by DOI and by EES (in combination with
SPT), their short-term effects were very small or absent (Figs. 5D, 6D-F, 8D,E). Only DOI had
some short-term positive effect on the spinal postural mechanisms: in the DOI+SPT and
DOI+EES+SPT groups, during one hour after DOI application, a small but statistically
significant gradual decrease in the proportion of incorrect EMG responses was observed (Figs.
5D, 8E), suggesting that DOI specifically decreases the excitability of spinal circuits mediating
incorrect EMG responses.

It is interesting that these short-term effects of EES and the serotonergic drug (DOI) in
chronic spinal rabbits differed from the effects observed in acute spinal rabbits, in which both
EES and application of the serotonergic drug (Quipazine) had positive and additive short-term
effects: they significantly enhanced postural limb reflexes (Musienko et al. 2010). Results of the
present study also differed from those obtained in the study of spinal locomotion in rats, which
demonstrated positive and additive short-term effects of serotonergic drugs and EES during
long-term treatment (Gerasimenko et al. 2007).

In all groups of spinal rabbits, the oscillatory activity in the hindlimb EMGs was
observed. Repetitive EMG bursts were usually caused and modulated by tilts, but they could
also be caused by other sensory stimuli or appeared spontaneously. One could simultaneously
observe several rhythms of oscillations, with complex patterns of synchronization between
different muscles in one limb or in two limbs (Fig. 9A). Such oscillations (but at higher
frequencies, 5-8 Hz) are characteristic for clonus, i.e., the symptom observed in SCI-patients
and presumably caused by the central generating mechanisms subjected to somatosensory
influences (Beres-Jones et al. 2003). One can suggest that unwanted activation of rhythm-
generating networks in the spinal cord perturbs the normal operation of postural mechanisms.
Oscillatory EMG activity appeared soon after spinalization, and then gradually increased during
the month of observations, suggesting enhancement of excitability in the spinal rhythm-generating networks. The rate of this increase in the control group of rabbits was considerably greater than in the other groups (Fig. 9C). This finding suggests that the factors promoting gradual recovery of spinal postural reflexes simultaneously prevent the development of oscillatory EMG activity, which presumably reflects the oscillatory activity in spinal locomotor networks. Antagonistic interactions between the spinal locomotor and postural mechanisms during specific training in SCI subjects were earlier demonstrated by De Leon et al. (1999).

To conclude, the present study has shown that, in chronic spinal rabbits, the regular SPT combined with the application of DOI or EES causes gradual plastic changes in the spinal networks, which result in the facilitation of spinal postural reflexes evoked by lateral tilts, as well as in the reduction of oscillatory EMG activity. Both outcomes promote the recovery of postural functions, but they appear insufficient for generating normal postural corrections. The goal of future studies would be a search for the activating factors whose regular application would increase the efficacy of spinal postural reflexes to such extent that SCI-subjects could use them for the maintenance of lateral stability.

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FIG. 1. Experimental design. A: position of the intrathecal cannula for DOI injection, the
electrodes for epidural stimulation, and the level of spinal cord transection. A-D: testing of
postural reactions to tilts. The animal was standing on two platforms, one under the forelimbs
(P1) and one under the hindlimbs (P2). Platform P2 could be tilted in the transverse plane (α is
the platform tilt angle). The sagittal plane of the animal was aligned to the axis of platform
rotation. Mechanical sensor S, positioned at the half-height of the body, measured lateral
displacements of the caudal part of the trunk in relation to the P2 platform. C: normal postural
reaction to tilt. D: no postural reaction to tilt. E: schematic representation of the trajectory of the
tilt angle (α), corrective movements of the caudal trunk (S), and EMG responses in right (R) and
left (L) flexor (Flex) and extensor (Ext) limb muscles. F: types of EMG responses in the right
extensor and left flexor muscles observed in spinal animals: Correct response (Ext EMG is
timed to the ipsilateral tilt, and Flex EMG to the contralateral tilt); Incorrect response (opposite
phase relations); No response. Shaded columns in this and following Figures highlight half of
the tilt cycles (right tilt) to facilitate comparison between curves.

FIG. 2. Example of postural tests before and after spinalization in the rabbit from the control
group. A: kinematical and EMG responses to tilts before spinalization. B: responses on day 6
after spinalization. C: responses on day 27 after spinalization. Abbreviations: α, tilt angle of the
P2 platform; S, lateral displacement of the caudal part of the trunk in relation to the P2 platform.
The EMGs of the following muscles are presented: left (L) and right (R) vastus (Vast) and
gastrocnemius (Gast). Black and white arrows in C indicate onsets of correct and incorrect
responses.
FIG. 3. Summary of EMG responses to tilts in animals of the control group. A: example of evaluation of EMG responses to tilts (rabbit 154). All EMG responses in individual muscles recorded in 5 postural tests performed in the 2nd ten-day period post-lesion were classified into three categories – correct responses (extensor EMG is timed to the ipsilateral tilt, flexor EMG is timed to the contralateral tilt), incorrect responses (opposite phase relations), and no response. Then relative number of responses (%) in each category was calculated for individual muscles – left (L) and right (R) biceps femoris (Bic), gastrocnemius (Gast) and vastus (Vast)]. B: relative number of different types of EMG responses (mean ± SE) as a function of post-lesion time for the control group of spinal animals (N=3); the data were averaged for the 1st, 2nd, and 3rd ten-day post-lesion periods.

FIG. 4. Example of postural tests in a rabbit from the DOI+SPT group. A,B: kinematical and EMG responses to tilts on day 5 (A) and on day 29 (B) after spinalization, recorded in the control tests (before DOI injection and SPT). Abbreviations and designations as in Fig. 2.

FIG. 5. Summary of long-term effects of DOI+SPT and short-term effects of DOI on the postural EMG responses. A-C: long-term effects of DOI+SPT. Proportion of different types of responses was calculated in the control tests (before DOI injection and SPT) for the DOI+SPT group of animals. Data for the 1st, 2nd, and 3rd ten-day period post-lesion are presented separately (mean ± SE), averaging over all recorded muscles in all animals of the group (N=3). For comparison, the corresponding data for the control group are presented. D: short-term effects of DOI. Here are shown the proportions of different types of responses in the control test (before DOI injection) and in 7 post-injection tests (with intervals of 10 min), averaged over all muscles, all ten-day periods, and all 3 animals of the DOI+SPT group. Note a gradual decrease in the proportion of incorrect responses as compared to the pre-injection value. In this and
following Figures, a level of significance of the difference between two means is indicated: one asterisk, \( p < 0.05 \); two asterisks, \( p < 0.01 \); three asterisks, \( p < 0.001 \).

FIG. 6. Summary of long-term effects of EES+SPT and short-term effects of EES on the postural EMG responses. \( A-C \): long-term effects of EES+SPT. Proportion of different types of responses was calculated in the control test (before EES and SPT) for the EES+SPT group of animals. Data for the 1st, 2nd, and 3rd ten-day period post-lesion are presented separately (mean ± SE); averaging over all recorded muscles in all animals of the group (\( N=3 \)). For comparison, the corresponding data for the control group are presented. \( D-F \): short-term effects of EES. Proportion of different types of responses was calculated in 3 parts of the stimulation test, i.e., before EES, during EES, and after EES (see Methods), and averaged over all muscles and all rabbits of the EES+SPT group (\( N=3 \)). Averaging was performed separately for three ten-day periods post-lesion. In \( D \) and \( F \), for each ten-day period, the difference between the proportion of responses before and during EES was statistically significant. In \( E \), this difference was significant in 2nd and 3rd periods.

FIG. 7. Summary of long-term effects of SPT on the postural EMG responses. \( A-C \): proportion of different types of responses was calculated in the control tests (before SPT) for the SPT group of animals. Data for the 1st, 2nd, and 3rd ten-day period post-lesion are presented separately (mean ± SE); averaging over all recorded muscles in all animals of the group (\( N=4 \)). For comparison, the corresponding data for the control group are presented.

FIG. 8. Summary of long-term effects of DOI+EES+SPT and short-term effects of DOI+EES on the postural EMG responses, found in the DOI+EES+SPT group. \( A-C \): long-term effects of DOI+EES+SPT. Proportion of different types of responses was calculated in the control test (before DOI+EES+SPT). Data for the 1st, 2nd, and 3rd ten-day period post-lesion are presented.
separately (mean ± SE); averaging over all recorded muscles in all animals of the group (N=3).

For comparison, corresponding data for the control group are presented. D,E: short-term effects of DOI+EES. There is shown the proportion of correct (D) and incorrect (E) responses, as well as the proportion of tilt cycles with no responses (F) recorded in the control test (before DOI injection), as well as in 3 parts (before EES, during EES, after EES) of 7 post-injection stimulations (with intervals of 10 min). The data obtained in the control tests and in each post-injection stimulation test during the month of treatment were averaged over all muscles in all animals of the DOI+EES+SPT group (N=3).

FIG. 9. Development of oscillatory EMG activity in spinal rabbits. A: example of repetitive EMG bursts caused by tilts (rabbit 156 from the control group, 34 days after lesion). Note that oscillations in different muscles could be synchronized in phase with each other (indicated by frames a, b and d), or in anti-phase (indicated by frames c and e). B: development of oscillatory EMG activity after spinalization in the rabbits of the control group (N=3). Abscissa, time post-lesion; ordinate, the number of oscillations generated by all muscles during the trial, divided by the number of muscles and the number of tilt cycles. C: development of oscillatory EMG activity in different groups. Bars show the same values as in B for different groups. For the SPT, DOI+SPT, EES+SPT, DOI+EES+SPT groups, data were obtained in the control test, for the control group – by testing each 3rd day. The data were averaged separately for consecutive ten-day periods. The difference between the oscillatory EMG activity in the control group and in the other groups was significant in the 2nd and 3rd periods.

FIG. 10. Comparison of long-term effects of regular application of different factors on the proportion of correct postural EMG responses, averaged for the 1st, 2nd, and 3rd ten-day period after spinalization. In each period, the difference between the control group and the treated groups was statistically significant. The statistically significant difference was also found
between the following groups: DOI+EES+SPT and DOI+SPT (in period 1); SPT and DOI+SPT (in period 1); DOI+EES+SPT and EES+SPT (in period 2); DOI+EES+SPT and EES+SPT (in period 3); SPT and EES+SPT (in period 3); SPT and DOI+SPT (in period 3).
Fig. 1
A  Rab 154, intact
Gast-L
Gast-R
Vast-L
Vast-R
S
α

B  Rab 154, spinal 6 days
Gast-L
Gast-R
Vast-L
Vast-R
S
α

C  Rab 154, spinal 27 days
Gast-L
Gast-R
Vast-L
Vast-R
S
α

Fig. 2
Fig. 3
Fig. 4
Fig. 5

A Correct responses

B Incorrect responses

C No responses

D

Response:
- Correct
- Incorrect
- No response

Groups:
- DOI+SPT
- Control

Percent of responses

Ten-day periods

Before DOI

Time after DOI application (min)
Fig. 6
Fig. 7
Fig. 8

A  Correct responses

B  Incorrect responses

C  No responses

D  Correct responses

E  Incorrect responses

F  No responses
Fig. 9
Fig. 10

Correct responses

Groups:
- EES+SPT
- DOI+SPT
- EES+DOI+SPT
- SPT
- Control

Percent of responses

Ten-day periods

1 2 3