Epidural Spinal Cord Stimulation Plus Quipazine Administration Enable Stepping in Complete Spinal Adult Rats


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Gerasimenko YP, Ichiyama RM, Lavrov IA, Courtine G, Cai L, Zhong H, Roy RR, Edgerton VR. Epidural spinal cord stimulation plus quipazine administration enable stepping in complete spinal adult rats. J Neurophysiol 98: 2525–2536, 2007. First published September 12, 2007; doi:10.1152/jn.00836.2007. We hypothesized that epidural spinal cord stimulation (ES) and quipazine (a serotonergic agonist) modulates the excitability of flexor and extensor related intraspinal neural networks in qualitatively unique, but complementary, ways to facilitate locomotion in spinal cord–injured rats. To test this hypothesis, we stimulated (40 Hz) the S1 spinal segment before and after ES and quipazine (a serotonergic agonist) administration will differ in adult spinalized rats. We hypothesized that epidural spinal cord stimulation after quipazine administration would enable stepping in complete spinal adult rats.

INTRODUCTION

Epidural spinal cord stimulation (ES) applied to the dorsal surface of the lumbar spinal cord can induce locomotor-like movements of the hindlimbs in decerebrated and acute spinal cord transected cats (Gerasimenko et al. 2001; Iwahara et al. 1991), as well as in chronically spinalized cats (Gerasimenko et al. 2003) and rats (Ichiyama et al. 2005). These studies showed that ES can activate the intraspinal neural networks that coordinate and recruit the motoneuronal pools with the precision required for stepping.

The features of the stepping pattern induced by ES in decerebrated, and in acute and chronically spinalized animals, however, differ substantially. In decerebrated cats, ES elicits a well-organized stepping pattern with weight-bearing and plan-...
three groups: 1) control, n = 4; after collection of control data, one rat (3) was spinalized and assigned to group 3, 2) spinal cord transected, nontrained (ST, n = 4), and 3) ST, step trained with ES and quipazine (Q) treatment (ST-Tr, n = 2). All rats were implanted with intramuscular EMG electrodes in selected hindlimb muscles. Rats in the ST and ST-Tr groups were implanted with epidural electrodes at the L_2 and S_1 spinal segments. The rats in the ST-Tr group were step-trained for ~6 wk while being stimulated in the presence of quipazine during each training session. All procedures followed the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Use Committee at the University of California, Los Angeles.

**ST and electrode implantation procedures**

All surgical procedures were performed under aseptic conditions. The rats were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally and maintained at a deep level of anesthesia with supplemental doses of ketamine as needed. The spinal cord was exposed by a partial laminectomy at ~T_8–T_9 vertebral level, the dura was incised longitudinally, and the spinal cord was completely transected at ~T_9 spinal cord level using microscissors as described previously (Talmadge et al. 2002). Gelfoam was inserted into the gap created by the transection as a coagulant and to separate the cut ends of the spinal cord. Partial laminectomies were performed to expose spinal cord segments L_2 and S_1 for implantation of epidural stimulating electrodes. The stimulation electrodes were made by removing a small portion (~1-mm notch) of the Teflon coating to expose the stainless steel wire (AS632, 50-μm gauge; Cooner Wire, Chatsworth, CA) on the surface facing the spinal cord and secured in position as close to the midline as possible by suturing the wire to the dura mater above and below the segment of interest as described previously (Ichiyama et al. 2005). Proper location of the epidural electrodes was verified postmortem.

Bipolar intramuscular EMG electrodes were inserted into the following muscles bilaterally: rectus femoris (RF), vastus lateralis (VL), semitendinosus (St), medial gastrocnemius (MG), and tibialis anterior (TA) as described previously (Roy et al. 1991). The electrodes for recording EMG signals were made by removing ~0.5–1.0 mm of insulation from each wire, and each wire was secured with a suture at its entry and exit from the muscle. A common ground electrode (a wire with ~1–2 cm of Teflon insulation removed at the distal end) was inserted subcutaneously in the mid-back region. All wires were attached to a headplug secured firmly on the skull as described previously (Lavrov et al. 2006). Proper location of the EMG electrodes was verified postmortem. Postsurgical care and maintenance procedures were similar to those described previously for spinal cord–injured cats and rats (Lavrov et al. 2006; Roy et al. 1992).

**Stimulation and recording procedures**

ES was achieved through wires chronically implanted at the L_2 and S_1 spinal cord segments using a Grass S88 stimulator. Rectangular pulses (200-μs duration) were delivered through a constant voltage stimulus isolation unit (Grass SIU5) at 40 Hz (Ichiyama et al. 2005). The intensity of stimulation ranged from 1 to 13 V. EMG signals were differentially amplified (AM System, model 1700) using a band-pass filter.
filter of 30 Hz to 10 kHz. Playback data were digitized at 2 kHz using a National Instrument A/D board and analyzed with computer programs developed with the LabView package. A four-camera system with retro-reflective markers placed on bony landmarks at the antero-posterior border of the iliac crest, greater trochanter, lateral condyle, lateral malleolus, and the metatarsal joint of the fifth digit on both legs was used to record the kinematics of the hip, knee, and ankle joints (Gerasimenko et al. 2006a; Ichiyama et al. 2005).

Data analysis

Video analysis of the kinematics of the stepping movements was performed using a Peak Motion Analysis System (Peak Performance Co.). This arrangement allowed us to perform a three-dimensional (3-D) reconstruction of the synchronized movements of both legs. Stick diagrams and trajectories of knee ankle were calculated using Motus (Peak System) software.

The raw EMG signals were passed through a butterworth band-pass filter with a low cut-off of 30 Hz and high cut-off of 500 Hz and rectified. Cycle period was calculated as the time between the onset of an EMG burst and the onset of the following EMG burst. Burst duration was determined as the time between the onset and the offset of an EMG burst. Plots of the relationship between the burst duration of a flexor (TA) and an extensor (MG) muscle versus cycle period at each of 3 stimulation intensities. Values are means ± SE. Muscle abbreviations are the same as in Fig. 1.

Quipazine treatment

We performed a dose–response study (0.1–1 mg/kg) and found that the optimum dose of quipazine in combination with ES to facilitate coordinated, alternating plantar stepping on a treadmill belt was 0.3 mg/kg delivered intraperitoneally (unpublished observations). As noted previously, we observed that quipazine facilitated stepping beginning ~5 min after administration and that this effect lasted for at least 1 h. Based on these results, this dose of quipazine was admin-

FIG. 2. Amplitude of EMG bursts (A) and duration of cycle period (CP) and EMG bursts (B) during stepping at 3 ES stimulation intensities after quipazine administration. C: relationship between burst duration and cycle period at each of 3 stimulation intensities. Values are means ± SE. Muscle abbreviations are the same as in Fig. 1.

FIG. 3. Differences in initiation of locomotor-like activity by ES alone (A) and in the presence of quipazine (B). Vertical arrows, initiation of ES. Abbreviations are the same as in Fig. 1.

2527EPIDURAL STIMULATION INDUCED STEPPING IN SCI RATS

J Neurophysiol • VOL 98 • NOVEMBER 2007 • www.jn.org
istered 10 min before each step training or testing session in ST and ST-Tr rats.

Training and testing procedures

The rats in the control group were tested while stepping bipedally on a motor-driven rodent treadmill belt at varying speeds (9–21 cm/s). A cylindrical tube made of soft fabric was secured to a body weight support device (Ahn et al. 2005). The rats climbed into the tube such that the head and forelimbs remained inside the cylinder. The cylinder was connected to a weight-supporting device that was positioned over a treadmill belt and used to maintain the rat’s body at ~45° relative to the surface of the treadmill belt. Using this system, the rats were allowed to bear the maximum amount of their body weight while maintaining relatively normal bipedal stepping kinematics. The duration of each training session was between 15 and 20 min.

For the rats in the ST groups, a body harness support system was used during bipedal treadmill stepping. An automated body weight support system provided the specific amount of body weight support that each rat needed to perform the bipedal locomotor task (de Leon et al. 2002). The rats in the ST-Tr group were administered quipazine 10 min before each stepping session and were step trained daily (7 days/wk, for 20 min) for 6 wk. In addition, continuous bipolar ES at 40 Hz was applied to either the L2 or S1 spinal cord segment during the stepping sessions, i.e., at the frequency of epidural stimulation that has been shown to be the most effective for inducing stepping movements (Ichiyama et al. 2005). At the beginning of each session, the cathode was located at L2 and the anode at S1. After 10 min, the polarity was reversed, i.e., the cathode was located at S1 and the anode at L2. All stimulation for testing was done with the cathode at S1. Recording sessions to evaluate the locomotor ability of all spinal rats were performed weekly for ~6 wk, starting ~1 wk after surgery. During these sessions, the hindlimb stepping patterns induced by ES at S1 were determined before and 10 min after quipazine administration. The last recording of EMG activity in all spinal rats with ES and quipazine treatment, i.e., the periodic bursting was clearly sustained in the MG and TA but not the RF and St (Fig. 1C).

The hindlimb locomotor activity occurring in ST-Tr rats after ES alone (Fig. 3A) and with ES combined with quipazine administration (Fig. 3B) differed markedly. In the absence of quipazine administration, ES applied at S1 initially evoked rhythmic EMG bursting activity that was seen most clearly in the RF, St, MG, and TA, showing a centrally derived rhythmic EMG bursting activity in a locomotor rhythm with short interburst intervals in all hindlimb muscles examined (see response at 5 V in Fig. 1B).

During low-intensity ES (4 V), the TA EMG bursts occurred between consecutive MG EMG bursts (see expanded EMG bursts at the bottom of Fig. 1C). With ES at 5 V, two EMG bursts became more apparent in the TA with an initial low-amplitude burst and a second high-amplitude burst (marked by asterisks in the bottom traces in Fig. 1C). In contrast, only relatively short, high-amplitude TA EMG bursts were observed when stimulated at high intensity (6 V). At the 6-V intensity, the bursting pattern in the MG and TA remained distinct compared with the St and RF, i.e., the periodic bursting was clearly sustained in the MG and TA but not the RF and St (Fig. 1C).

FIG. 4. Comparisons of mean CP and MG and TA EMG burst durations (A) and MG and TA EMG burst amplitudes (B) in control, ST (ES + Q), and ST-Tr (ES + Q) groups during bipedal stepping at 9 cm/s. Bars, SE. Abbreviations are the same as in Fig. 1. *Significantly different from control at P < 0.05.
the extensor muscle (MG) (Fig. 3A). When ES was combined with quipazine, the initial response was in the flexor motor pools, with the St and TA evoking two, and sometimes three, synchronous relatively high-amplitude, short EMG bursts (Fig. 3B). After these initial short bursts, locomotor-like EMG burst activity occurred in all muscles. The MG EMG bursting activity, in general, appeared later and frequently the first EMG burst was short and of a low amplitude. In contrast, the high-amplitude flexor bursts observed initially in the presence of quipazine were followed by consistent bursting with a lower amplitude and longer cycle period. An additional difference between the effects of ES on the initiation of stepping in the presence, compared with that in the absence, of quipazine was a much clearer on/off bursting pattern among the flexor motor pools in the presence of quipazine. The patterns of flexor and extensor activation shown in Fig. 3 were typical for the two rats that had received step training.

After conditioning control rats to step bipedally, they could generate consistent stepping patterns during the testing sessions. The mean step cycle duration at a treadmill speed of 9 cm/s was 1.40 ± 0.03 (SE) s in control rats (Fig. 4A). The mean EMG burst duration was 0.73 ± 0.02 s for the MG and 0.30 ± 0.01 s for the TA. The mean EMG burst amplitudes were similar for the two muscles (Fig. 4B).

In ST rats, the stepping pattern was different from control rats, having a shorter cycle period (1.1 ± 0.02 s) and shorter MG (0.60 ± 0.03 s) and TA (0.20 ± 0.01 s) EMG burst durations (Fig. 4A). The mean EMG amplitude of the MG was similar and that of the TA higher in ST compared with control rats. In summary, the rhythm of the stepping pattern in ST-Tr rats was slower than in control and ST rats and the activity of the flexor muscle (TA) during stepping was dominant.

The effects of quipazine in ST and ST-Tr rats with ES on cycle period duration and EMG burst duration and amplitude are shown in Fig. 5. The mean cycle period duration was >5 times longer after than before quipazine administration in the ST-Tr compared with ST rats. Similarly, the increase in EMG burst duration of the MG was longer after than before quipazine in the ST-Tr compared with ST rats (Fig. 5, A and B). Only after quipazine administration could a TA burst be identified in ST rats (Fig. 5C). As shown in Fig. 4, the effect of quipazine on burst amplitude was greater in the TA than the MG in the ST-Tr rats (Fig. 5D).

The footfall pattern is shown from a typical trial at 21 cm/s for a rat in the ST-Tr group prelesion (Fig. 6A) and at 6 wk after lesion (Fig. 6B) in response to quipazine and ES. Each box indicates the time during which a given hindlimb is in contact with the ground (stance phase), and the empty spaces correspond to the swing phase. The darkest portions of the boxes represent the occurrence of foot dragging at the end of stance. Compared with spontaneous bipedal walking in normal rats, bipedal stepping in the spinal rat under ES and quipazine influence was characterized by 1) a significant increase in the cycle period duration (Fig. 6C), 2) a relative decrease in the

**FIG. 5.** Comparisons of quipazine effect on mean EMG burst duration (A and B) and amplitude (C and D) between ST (A and C) and ST-Tr (B and D) rats. All data were obtained during bipedal stepping at 9 cm/s with ES at 40 Hz. Bars, SE. Abbreviations are the same as in Fig. 1.
duration of the stance phase (Fig. 6D), and 3) a dramatic increase in foot dragging at the beginning of the swing phase (Fig. 6, B and D). In contrast, the interlimb coupling was not altered, i.e., the left and right hindlimbs moved out of phase both pre- (184 ± 30°; Fig. 6A) and postlesion (Fig. 6B; 178 ± 3°).

Figure 7 displays the EMG activity of selected hindlimb muscles (Fig. 7A) and representative stick diagrams (Fig. 7B) of hindlimb movements during both the stance and swing phases of gait while stepping bipedally on the treadmill at 21 cm/s prelesion (left) and 6 wk postlesion (right) in the same rat and under the same conditions as in Fig. 6. There was an approximate twofold increase in cycle duration (postlesion compared with prelesion) that consequently was accompanied by a dramatic increase in stride length (Fig. 7B) at a similar treadmill belt speed. Spinal rats had difficulty in properly initiating the swing phase, resulting in increased plantarflexion at the end of stance (Fig. 7, B–D), dragging of the foot along the treadmill belt during the beginning of the swing phase (Fig. 7, B and C), and exaggerated compensatory foot elevation during the subsequent swing phase (Fig. 7B). The joint kinematics were very consistent from step to step when locomotion was elicited in the ST-Tr rat with the variability in joint trajectories mostly restricted to the period of dragging (Fig. 7C, ankle traces). Typical features of the joint kinematics included more pronounced yields at the ankle and knee at the beginning of stance, reduced excursion at the hip joint, and a marked increase in ankle angular motion at the stance-to-swing transition caused by foot drag postlesion compared with prelesion. These changes in joint angular excursions were associated with an alteration in the shape of the angle-angle plots (Fig. 7D). Nevertheless, compared with prelesion the timing of hindlimb joint coordination was generally preserved in ST-Tr rats when ES and quipazine were used to facilitate stepping postlesion.

Effects of sensory input on the locomotor activity induced by ES and quipazine administration in spinal rats

In general, the step cycle period and the duration and amplitude of extensor (MG) and flexor (TA) EMG bursts changed similarly as a function of the treadmill speed in control (Fig. 8, A and D), ST (Fig. 8, B and E), and ST-Tr (Fig. 8, C and F) rats. Cycle period and MG EMG burst duration decreased as the treadmill speed increased from 9 to 21 cm/s in all three groups (Fig. 8, A–C). At the same time, the EMG burst duration of the TA was unaffected in control (Fig. 8A) and ST (Fig. 8B) rats, and increased in the ST-Tr (Fig. 8C) rats. Note the relatively large EMG amplitudes in the ST-Tr (Fig. 8F) rats compared with control (Fig. 8D) and ST (Fig. 8E) rats, consistent with that shown in Fig. 4.

Spectral characteristics of EMG activity during locomotion induced by ES and quipazine administration in spinal rats

The differences in the spectral characteristics of the EMG between flexor and extensor motor pools during stepping in the presence of ES and quipazine were readily apparent (Fig. 9B). To examine this quantitatively, we used FFT analysis. Between six and eight EMG bursts for each MG and TA muscle were analyzed by FFT for each condition in each rat. The typical spectral composition for an MG and TA burst are shown in Fig. 9. In control rats, we did not observe dominant spectral peaks in either the TA or MG. The spectral composition of EMG bursts from both the MG and TA ranged from 1 to 500 Hz.

Figure 9A shows the EMG bursts recorded in the hindlimb muscles of a ST-Tr rat during ES and after cessation of ES. Comparisons of the spectral analysis with and without ES derived for typical MG EMG bursts 1 and 3 and for typical TA EMG bursts 2 and 4 were made. The spectral characteristic of the MG and TA EMG bursts in ST-Tr rats during ES (and in the presence of quipazine) and after the cessation of ES when the rat continues to step for several seconds (poststimulation effect) are shown (Fig. 9B). The FFT of MG EMG burst 1 shows spectral peaks at integer harmonics of the fundamental frequency of 40 Hz (consistent with the frequency of ES) and shows that the MG activation was a response to the stimulation. Similar results were observed in ST rats (data not shown). In contrast, no dominant peak in the range of 40 Hz was observed for the TA (EMG burst 2). In this muscle, there were no predominant spectral peaks in the range from 1 to 500 Hz. Also, in control rats, which did not receive ES, the spectral characteristic of EMG burst during stepping was evenly distributed in both the TA and MG. After cessation of ES in ST-Tr rats, the spectral characteristics of the MG and TA EMG bursts (bursts 3 and 4, respectively) were similar, showing no dominant peaks (Fig. 9B). The spectral compositions of these poststimulation EMG bursts were similar to that observed in control rats which received no stimulation.
In ST-Tr rats, ES (at 40 Hz) alone elicited bursting activity in both the extensor (MG) and flexor (TA) muscles (Fig. 10A). After quipazine administration, the burst durations of both muscles were prolonged compared with the durations during ES alone (Fig. 10B). During ES alone, the spectral peaks of MG activity were integer harmonics of the fundamental frequency of 40 Hz (Fig. 10C). After quipazine administration, the spectral composition of the MG did not change, i.e., the dominant spectral peak was still harmonics of the 40-Hz signal, although it was approximately two- to fourfold larger than that observed during ES alone (Fig. 10C). In contrast, quipazine administration had a significant effect on the spectral composition in the TA muscle. During ES in the presence of quipazine, facilitation of the responses was evident in the higher frequency range (i.e., >75 Hz; Fig. 10D).

The averaged potentials from the individually evoked potentials within a single MG EMG burst before (Fig. 10A, box 1) and after quipazine (Fig. 10B, box 2) are shown in Fig. 10E. These potentials averaged from the MG EMG burst during ES (40 Hz) have a latency of ~5 ms from the stimulus, indicating a monosynaptic response (Gerasimenko et al. 2006a; Lavrov et al. 2006). The amplitude of the average potential after quipazine was higher compared with the potential before quipazine (Fig. 10E). This also suggests that there was monosynaptic modulation of the responses in the MG as indicated by the high-amplitude bursts after quipazine (cf. Fig. 10, A, burst 1, and B, burst 2). The mean amplitude of the averaged potentials from MG EMG bursts in ST (0.51 ± 0.04 mV) and in ST-Tr (3.17 ± 0.30 mV) rats after quipazine administration increased to 1.40 ± 0.11 mV in ST and to 4.43 ± 0.42 mV in ST-Tr rats, respectively (Fig. 10G). The averaged potentials of the individually evoked responses within the EMG burst of the TA before (burst 3) and after (burst 4) quipazine are shown in Fig. 10F. Before quipazine administration, the amplitude of the potential was ~10 times smaller than the amplitude of the potential averaged from MG EMG burst. After quipazine administration, this potential almost disappeared (Fig. 10F). Thus it seems that the polysynaptic activity in the TA EMG burst inhibits the monosynaptic component of the evoked potentials.

DISCUSSION

In this study, we showed that ES combined with quipazine (a nonspecific 5HT agonist) administration is an effective tool for facilitating spinal locomotion and can promote the generation of rhythmic, coordinated motor patterns between extensor and flexor hindlimb muscles in otherwise paralyzed spinal adult rats. This effect seems to be augmented after step training with the aid of ES and quipazine being administered chronically, which greatly facilitates regulation of spinal locomotion. The contributions of each of these treatments in regaining locomo-
tion are considered in the following sections. These data also show fundamental differences in the characteristics of the spinal circuits that control flexor compared with extensor motor pools.

Changing the physiological state of the spinal cord with quipazine facilitates weight-bearing stepping induced by ES in spinal rats

We previously showed that ES applied to different spinal cord segments of the lumbar enlargement can induce rhythmic alternating activity in hindlimb muscles in chronically spinalized adult rats (Ichiyama et al. 2005). However, these stepping-like movements had poor plantar foot placement and weight-bearing capabilities. A similar pattern of stepping of the hindlimbs was observed in spinal cats during ES (Gerasimenko et al. 2002). Although these data show that ES can facilitate locomotor-like movements in spinal animals, it seems that ES alone is not sufficient for generating weight-bearing stepping in rats as occurs in decerebrated cats (Gerasimenko et al. 2001; Iwahara et al. 1991). Recently, it was shown that bilateral locomotion in spinal cats can be induced by intraspinal microstimulation (Barthélemy et al. 2007; Guervemont et al. 2006).

Two of the neurotransmitter systems involved in supraspinal control of locomotion are the serotonergic and noradrenergic systems. Evidence suggests that descending 5-HT fibers can influence the electrophysiological properties of γ- and α-motoneurons of flexor and extensor muscles (Ahlman et al. 1971; Myslinski and Anderson 1978). Although in chronic spinal cats the 5-HT agonist quipazine can modulate the locomotor pattern on a moving treadmill belt (Barbeau and Rossignol 1990), at moderate dosages, it may facilitate rather than induce locomotion. Jacobs and Fornel (1993) hypothesized that 5-HT neurons can facilitate central pattern generation and the activation of α-motoneurons and inhibit sensory information processing. Each of these studies is consistent with the conclusion that 5-HT is an important, although complex, neuromodulator of locomotion, probably involving a variety of populations of neurons and of 5-HT receptor subtypes (Schmidt and Jordan

Fig. 8. Comparisons of CP duration and EMG burst durations (A–C) and amplitudes (D–F) at treadmill speeds of 9 and 21 cm/s are shown for control (A and D), ST (B and E), and ST-Tr (C and F) groups. Bars, SE. Abbreviations are the same as in Fig. 1.
2000). The present data suggest that a combination of ES and quipazine administration provides conditions that are sufficient for generating weight-bearing stepping in spinal animals.

**Differential features of flexor and extensor activation in response to ES and quipazine administration in spinal rats**

An increase in step length and in the EMG amplitude of hindlimb extensors and flexors in spinal cats after the administration of 5-HT agonists has been observed (Barbeau and Rossignol 1990). Similarly, treatment with quipazine results in coordinated rhythmic movements when pinching the tail of spinal rats (Antri et al. 2002; Feraboli-Lohnherr et al. 1999). Weak extension during stepping in spinal animals has been attributed to a loss of the bistable properties of the motor units in the extensor muscles involved in postural control (Lee and Heckman 1998). In this study, we did not observe this extensor deficit when ES was combined with quipazine and step training. Instead, we found the EMG activity of both the extensors and flexors to be robust with flexor activity being more dominant. This response may reflect a similar effect as the facilitation of the flexor reflex by quipazine in spinal rats observed by Maj et al. (1976). Barbeau and Rossignol (1990) also reported that 5-HT agonists increase flexor motoneuron output and the excitability of cutaneous pathways resulting in a greater flexion withdrawal in spinal cats. These findings are consistent with our observations of an enhanced flexor EMG amplitude during stepping after quipazine administration in spinal rats.

The spectral composition of the EMG burst signals of the MG and TA muscles differed in response to ES at 40 Hz. When ES was applied to the $S_1$ segment to induce a locomotor pattern, the spectral composition of the EMG burst signals for the MG and TA muscles differed. The dominant spectral peaks in the MG were integer harmonics of the fundamental frequency of 40 Hz, indicating the MG activation was a response to the stimulation. The potential averaged from the MG EMG burst during ES had a latency of ~5 ms from the stimulus onset and was likely a monosynaptic reflex (Gerasimenko et al. 2001; Minassian et al. 2006a; Lavrov et al. 2006). After quipazine administration, the spectral composition of the MG caused by ES did not change, i.e., the dominant spectral peaks were still at integer harmonics of the 40-Hz frequency (Fig. 10). There was, however, an increase in the power of these peaks and in the amplitude of the potentials that were averaged from the MG EMG burst, i.e., there was a facilitatory effect of quipazine on the output properties of the monosynaptic neural circuit associated with the MG motor pool during ES-elicited stepping.

In the flexor muscles, the spectral composition of the bursts during ES was different from that in the extensor muscles, i.e., there were no predominant spectral peaks (Fig. 10). We suggest that the EMG bursting activity of flexors during ES reflects amplitude modulation of mainly polysynaptic and monosynaptic pathways, although to a lesser extent. A similar effect has been observed during ES in paraplegic patients (Gerasimenko et al. 2001; Minassian et al. 2001, 2004).

The differences in the spectral characteristics noted above suggest some fundamentally different mechanisms in the generation of EMG bursting activity in extensor and flexor muscles during ES-elicited stepping in spinal rats. In extensor muscles, this process seems to be dominated by modulation at a common frequency. The flexor EMG modulation reflects a broader range of frequencies and has a peak frequency at 40 Hz. In ST-Tr and ST rats, quipazine administration increased monosynaptic excitability in both extensor and flexor muscles. However, a more substantial quipazine effect was observed in the flexor muscle in the high-frequency range of the spectrum. This part of the spectrum might reflect a greater role of interneurons and polysynaptic reflex systems in flexors compared with extensors.
Significance of sensory input in the regulation of locomotor activity during ES and quipazine administration in spinal rats

We showed that sensory input played a critical role in the regulation of spinal locomotion induced by ES and quipazine administration in ST-Tr rats. For example, a coordinated stepping pattern could be elicited only when the limbs were placed on the moving treadmill belt. In other words, the levels of ES and quipazine used were insufficient to evoke rhythmic movements when the legs were suspended in a non-weight-bearing position. In addition, a change in treadmill speed during locomotion in the presence of ES and quipazine administration resulted in an appropriate change in stepping pattern, i.e., a change in cycle duration and in the amplitude and temporal features of extensor and flexor muscle activity.

Shik et al. (1966) showed that the locomotor pattern of a decerebrated cat could be changed from stepping to galloping by increasing the intensity of stimulation of the mesencephalic locomotor region and/or increasing treadmill speed (Shik et al. 1966). We also could change the frequency of the locomotor pattern and the duty cycle of extensor/flexor recruitment in spinal rats by increasing the intensity of ES at S1 and/or by quipazine administration, while maintaining a constant treadmill speed (9 cm/s). Alternating activity of the TA and MG was observed even during high-intensity stimulation (6 V) when the cycle duration was 0.62 ± 0.02 s and MG and TA burst durations were 0.27 ± 0.01 and 0.26 ± 0.01 s, respectively (Fig. 1). These data show the flexibility in the spinal circuitry and its responsive to sensory input, i.e., the spinal circuitry itself can produce a high-frequency stepping rhythm, but the details of the stepping pattern is the prerogative of sensory input.

Most successful spinal locomotion induced by ES and quipazine administration was observed in step-trained rats

In this study, the effects of ES and quipazine treatment on the stepping performance of the two spinal rats that were step trained were marked. The ST-Tr rats showed excellent bipedal stepping with partial weight bearing in the presence of ES and quipazine administration. In contrast, the stepping movements in ST rats were not typically weight bearing, and the cycle period duration (at a treadmill speed of 9 cm/s) was signifi-
cantly shorter than in the ST-Tr rats. Similarly, the mean duration of the TA EMG bursts was more than two times longer in the ST-Tr than ST rats. Also, the amplitude differences were much more dramatic in ST-Tr than ST rats. Although we did not determine the effects of step training alone in this study, we observed without exception that adult complete spinal rats cannot be trained to generate plantar-placed steps consistently without some additional facilitating intervention. This poor plantar placement despite step training differs from observations in adult spinal cats (de Leon et al. 1998, 2002; Edgerton et al. 1991, 2004; Lovely et al. 1986, 1990) and in neonatally spinalized rats (Kubasak et al. 2005; Stelzner et al. 1975).

Pharmacological treatment of spinal rats by daily administration of a 5-HT$_2$ agonist for 1 mo also indicated a positive effect in locomotor recovery (Ferabolli-Lohnherr et al. 1999; Antri et al. 2002, 2005). Even after 5-HT$_2$ treatment, however, the spinal rats were able to perform coordinated stepping movements only when the tail was pinched. Also the stepping pattern was described as abnormal because of weak activity of the extensor muscles. Fong et al. (2005) showed that quipazine administration before each robotically assisted step training session improved stepping in adult spinal mice. Both of these observations suggest that a general increase in the excitability of the spinal circuitry can facilitate stepping and promote chronic alterations in the spinal circuit properties when administered in association with use-dependent factors. The results of this study are consistent with this concept in that improvement in stepping can be observed when ES is combined with quipazine treatment and step training, particularly in the ability to perform weight-bearing plantar-placed steps.

In summary, these data showed a clear, selective, and qualitatively different response between flexor and extensor motor pools to ES and quipazine, particularly in step-trained spinal rats. We hypothesize that the differential responses reflect muscle-specific peripheral afferent activity during stepping and the associated processing of locomotor-related proprioreceptive information that selectively projects to flexor and extensor motor pools. These differential effects of ES and quipazine provide the opportunity to use two different, but largely complementary, mechanisms to enhance locomotor potential in spinal animals. These two mechanisms, in turn, enable further complementary effects to be manifested when they are combined with locomotor training.

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