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Initiation of Locomotor Activity in Decerebrate and Spinal Cats Using Noninvasive Transcutaneous Electrical Stimulation of the Spinal Cord

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Spinal neural networks activated by epidural electrical stimulation of the spinal cord (ESSC) are known to be able to take part in generating the stepping EMG pattern and controlling locomotor behavior. We show here that noninvasive transcutaneous stimulation of the spinal cord (TES) in the lumbosacral enlargement area can initiate locomotor activity in decerebrate and spinal animals. Comparison of motor responses in ESSC and TES showed them to have similar reflex mechanisms, as well as similarities in the properties of the locomotor patterns. Our data support the view that TES is an effective approach for further studies of locomotor control in acute and chronic experiments. Considering the noninvasive nature and relative simplicity of using TES, this method may be suitable for further use in clinical practice in the rehabilitation of patients with vertebrospinal pathology.

Keywords: spinal cord, locomotion, cat, decerebration, spinalization, electrical stimulation of the spinal cord.

Epidural electrical stimulation (ESSC) is an effective method for activating the spinal neural networks responsible for controlling locomotor behavior. This method has been used successfully in neurophysiological investigations in a variety of experimental models [4, 18, 23]. Use of ESSC has yielded new data on the neural control of locomotor activity [4, 14]. Sensorimotor [6], neuropharmacological [24], supraspinal [7], and spinal mechanisms [10] regulating locomotor behavior initiated by ESSC have been studied. A series of experiments using different animals demonstrated the efficacy of ESSC in restoring locomotor abilities after spinal cord injury [6, 10, 14, 17]. ESSC has also been used successfully in clinical practice for the motor rehabilitation of patients with spinal cord injury [3, 11, 15, 25]. However, implantation of epidural electrodes in the spinal cord requires surgical intervention, which is associated with both intraoperative technical difficulties (surgical trauma, possible damage to the roots and spinel elements in the vertebral canal, surgical infection) and post-operative complications (wound inflammation, implant rejection of implants with epidural electrodes, bleeding). Therefore, the search for new, low-trauma, and safer approaches to spinal cord stimulation is an extremely important task. Recent studies have shown that cutaneous electrical stimulation of the lumbar enlargement can be used to activate the dorsal roots of the spinal cord and induce reflex responses in the lower limb muscles in healthy subjects [9, 22] and spinal patients [12]. Electrical stimulation of the spinal cord using stimuli of complex shape filled with a high-frequency of 5–10 Hz initiated involuntary stepping movements in healthy subjects in conditions of external support of the legs [5]. The aims of the present work were to: 1) study the reflex mechanisms of action of TES as compared with ESSC and 2) to study the effectiveness of using TES to elicit locomotor activity in decerebrate and spinal cats.

Methods

Experiments using ESSC and TES were performed in acute conditions on decerebrate ($n = 4$) and in chronic expe-

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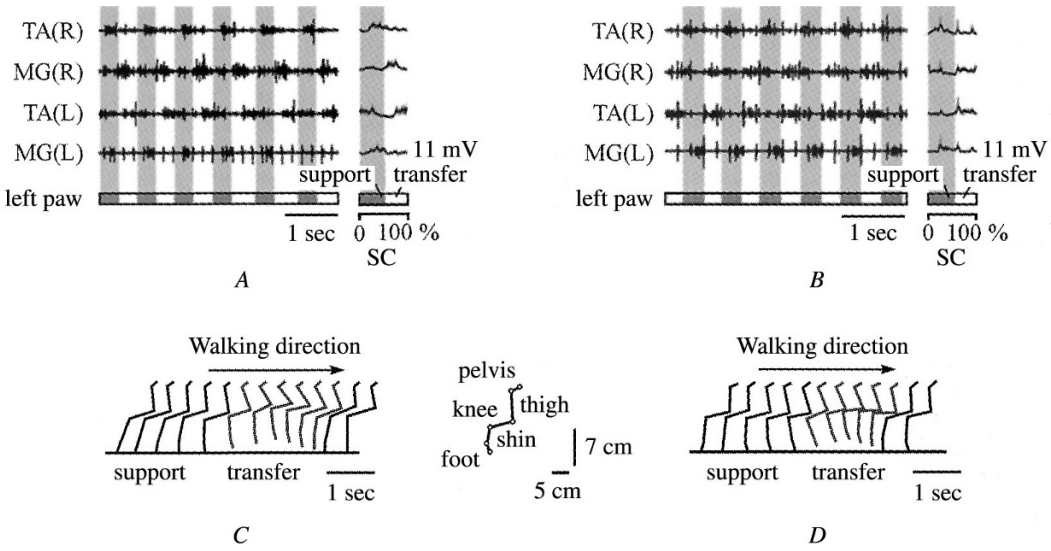

Fig. 1. Locomotor activity elicited by TES and ESSC in decerebrate cats. *A*) locomotor activity elicited by TES. EMG activity in the flexors (TA – tibialis anterior) and extensors (MG – gastrocnemius medialis) of the right and left limbs. Movement of the left limb forward during the transfer phase and backward during the support phase. Averaged (1 SEM in the gray zone close to the curve, $n = 8–10$ stepping cycles(SC)) and rectified myographic signals during the support and transfer phases of the stepping cycles are shown at right; *B*) locomotor activity elicited by ESSC; *C*) movement kinematics during walking elicited by TES; *D*) movement kinematics during walking elicited by ESSC.

riments on spinal cats ($n = 4$) weighing 2.5–3 kg. All experiments were conducted in compliance with the "Regulations for Studies Using Experimental Animals" (USSR Ministry of Health Decree No. 755 of August 12, 1977) and the law "Protection of Animals from Cruel Treatment," Chap. 4, Art. 10, 4679/11 GK of December 1, 1999.

Decerebrate cats were placed in a stereotaxic apparatus where the head and pelvis were fixed, as was the spine in the thoracic and lumbar areas. The fore- and hindlimbs were in contact with a treadmill band. ESSC was performed after laminectomy at the T12-L4 levels. Monopolar stimulation of the spinal cord was used. The active stainless steel wire (AS632, Cooner Wire, Chatsworth, CA) electrode was sutured to the dura mater at the level of spinal segment L5 at the posterior surface of the spinal cord in the midline. The indifferent electrode was implanted in the paravertebral muscles. In the chronic experiments, cats underwent spinalization at the T5-6 level. Apart from regular (twice daily) medical care of the paralyzed animals (including irrigation of the urinary bladder and intestine, kneading and massage of the lower limbs), locomotor training was also performed on a treadmill for 2–3 weeks before recording of experimental data [21]. ESSC was performed using an A-M System model 210 stimulator with 0.5-msec current impulses of amplitude 10–200 μA at frequencies of 0.3 and 5 Hz. We have previously provided a detailed description of this method of epidural stimulation in acute experiments on decerebrate cats [2, 6]. TES in cats in the acute and chronic experiments was performed in the same preparations. Cutaneous electrical stimulation of the spinal cord was performed using a Kulon (GUAP, St. Petersburg) stimulator. Stimulus intensity was 10–100 mA [5]. The electromyographic activity (EMG) of the hindlimb muscles was recorded using a bipolar method with wire electrodes sutured to the study muscles. EMG signals were amplified using a differential amplifier (A-M Systems model 1700) over the range 30 Hz to 10 kHz and sampled at a frequency of 10 kHz with an analog-to-digital converter (National Instrument) followed by analysis in LabView. The kinematic characteristics of hindlimb stepping movements were recorded on video using two digital video cameras at left and right synchronized with the EMG activity trace. Kinematic movement parameters were analyzed in terms of changes in the position of light-reflective markers positioned on the skin in the projections of the hip, knee, and ankle joints and on the fifth toe. Recording of EMG traces and kinematic stepping parameters were synchronized. The mean period of the stepping cycle and the amplitudes of angular displacements of the leg joints were measured for 10–12 cycles. Quantitative characteristics (mean ± standard error) were calculated using standard statistical programs. Statistically significant differences were identified using Student's t test at $p < 0.05$.

Results

Locomotor activity evoked by TES and ESSC in decerebrate cats. We observed that TES in the lumbosacral enlargement elicits locomotor activity in all decerebrate cats tested. Comparison of the motor responses to ESSC and TES demonstrated similarities in the properties of the

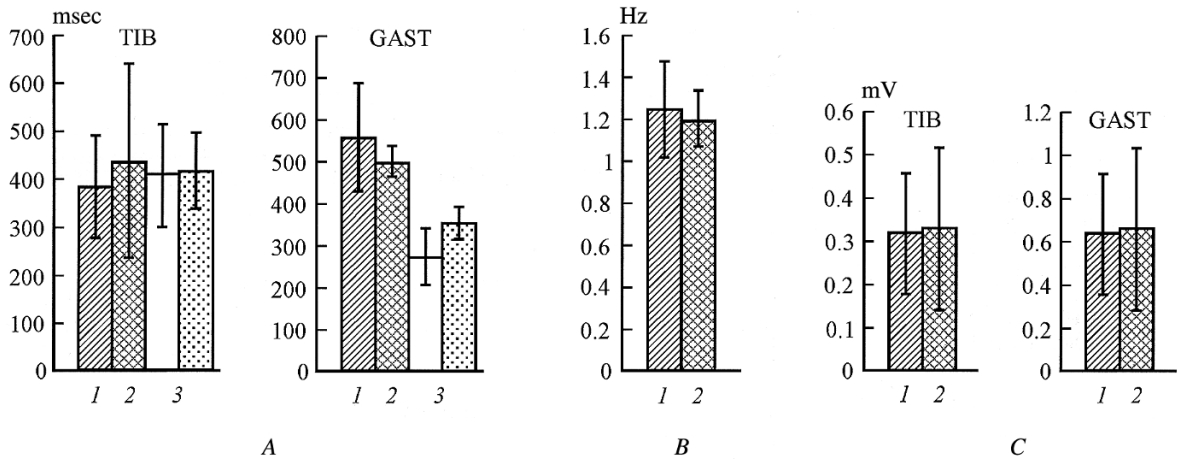

Fig. 2. Parameters of locomotor patterns in ESSC and TES in decerebrate cats. *A*) Duration of locomotor volleys in different muscles in ESSC (*1*) and TES (*2*) and intervolley interval durations (*3*). Averaged for four cats (7–10 steps in each cat); *B*) stepping frequency on walking by cats elicited by ESSC (white) and TES (gray). Averaged for four cats (7–10 steps in each cat); *C*) amplitude of locomotor volleys in the flexor (TIB) and extensor GAST) muscles. Averaged for four cats (7–10 steps in each cat).

evoked locomotor patterns (Fig. 1). In ESSC, stepping movements were initiated at current amplitudes of 50–200 μA, while higher currents, of 30–80 mA, were needed for TES. Locomotor activity in TES was characterized by poorer coordination and stability, which can probably be explained in terms of the insufficiently stable positioning of the electrodes on the skin surface and small changes in electrode position during active walking by the animal. However, the overall locomotor pattern and the reciprocity of activation of antagonist muscles and fellow muscles in the left and right limbs were similar in TES and ESSC (Fig. 1). The optimum frequency for inducing stepping movements in TES, like ESSC, was 5 Hz.

The patterns of formation of volley activity were analogous for both stimulation methods. Electromyographic volley activity formed as a result of modulation of the early and late reflex responses (Figs. 3 and 4) to electrical stimulation [6, 7]. The dynamics of the appearance of reflex responses to increases in stimulation intensity during locomotor activity were similar in both types of stimulation with the one difference that TES could also produce direct responses with latencies of 3–6 msec. At higher intensities, optimum for initiating locomotion, amplitude-modulated early responses with latency 7–12 msec were produced, along with late responses with latency greater than 12 msec. With time, the late responses acquired their own stimulation frequency-independent rhythm taking part in forming the EMG volley activity determining the moment of initiation of stepping movements. In both cases, with ESSC and TES, the appearance of the late responses provided evidence of activation of the locomotor neural network (the spinal stepping generator) and was decisive for triggering locomotion. Analysis of the locomotor patterns evoked by ESSC and TES demonstrated similarities in their main characteristics, i.e., the durations of EMG volleys and intervolley intervals in the flexor and extensor muscles, volley amplitudes, and stepping frequencies. These parameters, averaged for all animals, are shown in Fig. 2.

Comparison of reflex responses in TES and ESSC. Comparison of the amplitude-time characteristics of the individual components of electromyographic responses to single stimuli (stimulation frequency 0.3 Hz, impulse duration 0.5 msec) also demonstrated similarities in these responses in TES and ESSC. As shown in Fig. 3, *A*, the reflex response to ESSC of spinal cord segment L5 consisted of an early component with latency 7–12 msec and a late component with latency 20–30 msec. The early component was dominant on threshold stimulation. The characteristic feature of TES was the presence of a direct response with latency 3–6 msec (Fig. 3, *A*, *B*). This occurred in the reflex muscle responses in all animals tested using TES, though it was generally absent with ESSC. Apart from this, as with ESSC, reflex responses in TES showed an early and a late response whose latencies corresponded to the responses in ESSC (Fig. 3, *C*). Increases in TES intensity, as in ESSC, increased the amplitudes of all responses. There was also an increase in the overall number of responses for all muscles. Thus, while responses to low-intensity stimulation were seen in five of eight muscles, responses to stimuli of greater intensity were seen in all muscles. The numbers of early and late responses increased, as did response duration; early responses frequently became so prolonged that they merged with late responses. Initially, each response corresponded to a stimulus, after which sequences of late responses with intervals of 50–100 msec could be seen, though these were only observed in some cases.

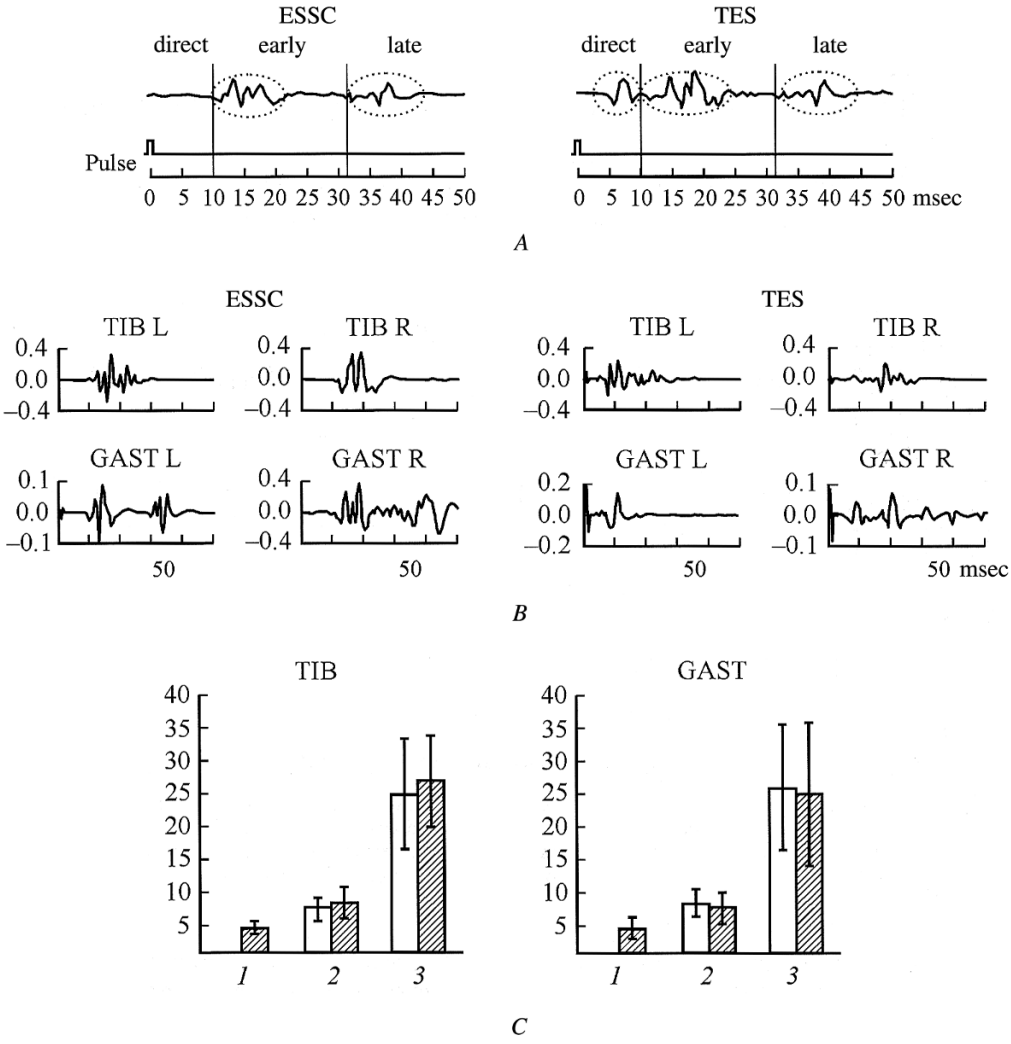

Fig. 3. Comparison of reflex responses with TES and ESSC. *A*) Basic components of responses in ESSC and TES: direct, early, and late; *B*) reflex responses with ESSC and TES (submaximal stimulation) in different muscles, averaged for five responses; *C*) latencies of the direct (*1*), early (*2*), and late (*3*) responses with ESSC (white) and TES (gray) in the flexor (TIB) and extensor (GAST) muscles with submaximal stimulation. Averaged for four cats (5–10 reflex responses for each cat).

Locomotor activity evoked by TES and ESSC in spinal cats. The next series of experiments were chronic experiments in spinal cats using ESSC and TES to elicit locomotor activity without supraspinal influences. Experiments on all four cats showed that TES, like ESSC, was an effective approach for activating spinal neural networks and restoring locomotor function after spinal cord injury, though it should be noted that coordination in locomotor activity in spinal animals was significantly worse than that in decerebrate cats with the spinal cord intact.

The optimum frequency for eliciting stepping movements in TES in spinal cats was 3 Hz, compared with use of a frequency of 5 Hz in ESSC. Locomotor patterns after spinalization using ESSC and TES were characterized by a essentially similar structure, with reciprocity in the functioning of the extensor and flexor muscles (Fig. 4, *A*). As in decerebrate cats, electromyographic volley activity formed as a result of the modulation of early and late reflex responses (Fig. 4, *B*) to electrical stimulation.

In spinal animals, locomotor patterns were less stable, and stepping frequency could vary even during a single trace. The statistical characteristics of movements in TES and ESSC in spinal cats are shown in Fig. 5. In terms of stepping frequency, the durations of EMG volleys and intervolley intervals and mean intravolley amplitudes in the flexors and extensors were not significantly different for the two types of stimulation (Fig. 5, *A–C*). It should be noted that stepping frequency in spinal cats was greater than that in decerebrate cats, averaging 1.65 Hz for ESSC and 1.9 Hz for TES.

Analysis of EMG volley activity (Fig. 5) showed that shortening of the stepping cycle occurred mainly as a result

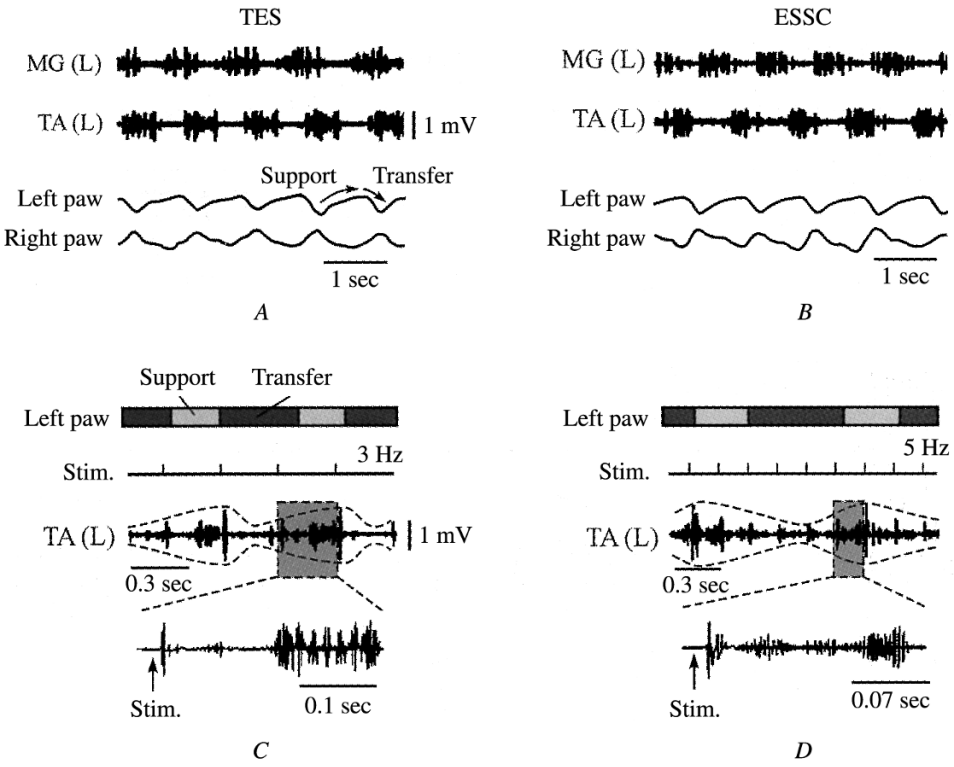

Fig. 4. Locomotor activity elicited by TES and ESSC in spinal cats. *A*, *B*) Locomotor activity evoked by TES and ESSC. EMG of activity in the flexors (TA – tibialis anterior) and extensors (MG – gastrocnemius medialis) of the right and left limbs. Movement of the left and right limbs forward during the transfer phase and backward during the support phase; *C*, *D*) modulation of the early and late responses to stimulation during locomotor activity evoked by TES and ESSC.

of a decrease in intervolley interval duration, while volley duration showed no particular change as compared with the patterns in decerebrate cats. This effect was more marked in the flexor muscles (tibialis anterior), which suggests that shortening of the stepping cycle in spinal cats occurred as a result of contraction of the transfer phase. A possible cause of this is the absence of supraspinal inhibition in spinal animals, leading to a change in the structure of the stepping cycle as compared with that in decerebrate animals, whose locomotor patterns were close to normal.

Discussion

We describe here a new noninvasive method for stimulating the spinal neural networks – transcutaneous electrical stimulation of the spinal cord. Despite the positioning of the electrode quite a large distance from the spinal cord, this stimulation reached its target, passing through the intermediate soft tissues and bony structures of the spine to evoke motor responses linked with the direct and indirect activation of sensory fibers in the dorsal roots and neural apparatus of the spinal cord.

What are the mechanisms of action of TES and in what way do they differ from those of ESSC? Comparative analysis of locomotor patterns and reflex responses showed that in TES, as in ESSC, there are two major components to responses to electrical stimuli – early and late (Fig. 3), which, undergoing modulation, make their contributions to forming locomotor EMG volleys during walking. Our previous studies [4, 6] showed that the appearance of the early component with a latency of 7–12 msec in ESSC is linked with the electrical activation of low-threshold afferents of the dorsal roots and monosynaptic activation of motoneurons. The late component appears in conditions of submaximal stimulation at high currents, which is evidence for activation of higher-threshold fibers within the electrical field. The latency of late responses, varying over the range 20–60 msec, is evidence for excitation of the polysynaptic neural networks of the spinal cord (Fig. 3). In TES, each of these components is also present with similar latency and dynamics during increases in the electrical field on threshold and submaximal stimulation. Studies by other authors have also shown that single cutaneous electrical stimuli at vertebrae T11-12 in humans can elicit responses in the foot muscles with a latent period corresponding to a monosynaptic reflex [9]. The monosynaptic nature of these reflexes was confirmed by their suppression on paired-pulse stimulation and the similarity in the modulation of the classical monosynaptic H-reflex and reflex responses in TES during performing of stepping movements [9, 12].

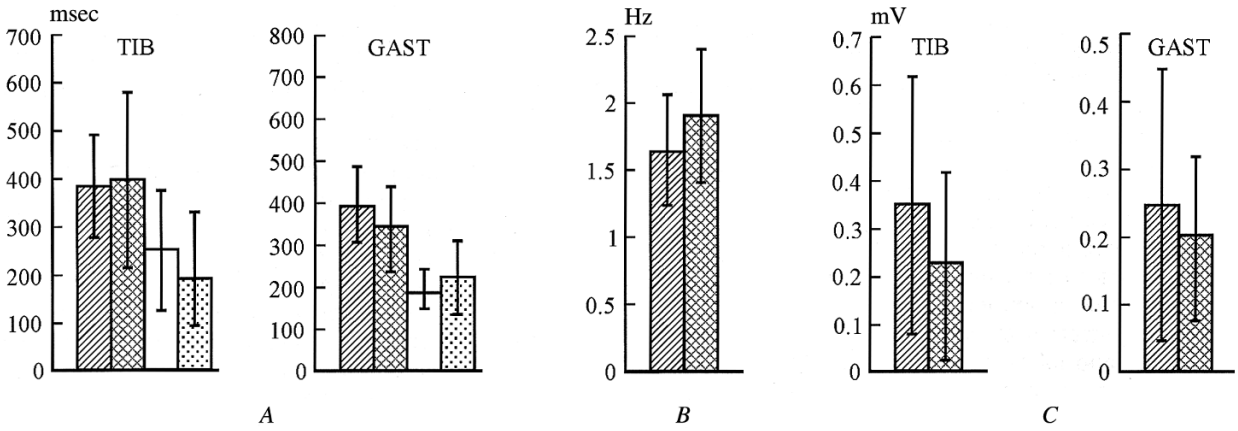

Fig. 5. Parameters of locomotor patterns with ESSC and TES in spinal cats. *A*) Duration of locomotor volleys in different muscles in ESSC (white) and TES (gray) and intervolley interval durations (crosshatched). Averaged for four cats (10–20 steps in each cat); *B*) stepping frequency on walking by cats elicited by ESSC (white) and TES (gray). Averaged for four cats (10–20 steps in each cat); *C*) amplitude of locomotor volleys in the flexor (TIB) and extensor GAST) muscles. Averaged for four cats (10–20 steps in each cat).

TES, however, was characterized by suppression of the earlier response at submaximal and, more rarely, threshold stimulation (Fig. 3), which is evidently linked with direct activation of motoneuron axons at high currents (1–100 mA), the area of the active electrode, and the electrical field required for effective stimulation of the spinal cord through the skin, soft tissues, and bony structures of the spine. A similar picture was seen with magnetic stimulation of the lumbar segments of the spinal cord [1]. In ESSC of the dorsal surface of the spinal cord at segment L5, a significantly smaller current (10–200 μA) was used and the induced electrical field probably propagated more locally to the dorsal structures of the spinal cord and root. ESSC of the more caudal segments (L7-S1) induced all three components of the response [13], probably because of a different anatomical configuration and the closer position of the ventral roots to the epidural electrode. In TES and ESSC, the current propagates perpendicular to the spinal cord and has a high density beneath the paravertebral electrode [26], evidently primarily activating the dorsal roots rather than spinal cord neurons, which have significantly lower conductivity [16]. It is also logical to suggest that in TES, activity sequentially involves afferents of groups Ia and Ib, which have the greatest diameters and, thus, the lowest thresholds, then afferents of group II and spinal interneurons, which mediate polysynaptic reflexes. The similarity in the reflex responses and basal characteristics of locomotor activity in TES and ESSC lead to the conclusion that the evoked motor effects of these two approaches have common mechanisms.

Previous studies have shown that electrical stimulation of the spinal cord using electrodes implanted on the dura mater provides an effective method for evoking locomotion after complete transection of the spinal cord [6, 19, 20]. Recent years have seen clinical trials of the use of implanted spinal electrodes for restoration of motor functions in patients with severe vertebrospinal trauma [15]. The development of noninvasive methods of stimulation with effects similar to those of ESSC and effectively activating the neural apparatus of the spinal cord after injury is a relevant objective which may provide significant simplification of the protocols for stimulatory approaches and widen the potentials for their employment in clinical practice. In this sense, the results of our studies on chronically spinal animals provide all the grounds for achieving these aims.

We have demonstrated that TES is an effective method for increasing the excitability of the spinal networks underlying the spinal locomotor control apparatus in cats. Passing through the underlying tissues, the electrical fields induced by TES reach spinal cord structures and the afferent fibers of the dorsal roots [8, 14], activating locomotor networks. Thus, the results of our experiments, demonstrating the effectiveness of TES in regulating the locomotor behavior of decerebrate and chronically spinal animals, as well as the non-invasive nature and relatively simplicity of use, allow it to be recommended for the rehabilitation of patients with vertebrospinal pathology.

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