

## The Excitability of Spinal Motor Neurons after Spinal Injuries and Spinal Cord

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**Abstract:** In experiments on models of spinal injuries and spinal cord in 79 laboratory rats, we found changes in reflexes revealed by electromyographic evaluation of spinal cord function during the recovery process. It is established that the parameters of calf muscle reflex responses change immediately after spinal cord injury and that the severity of changes in motor responses in the late posttraumatic period depends on the degree of damage. These data suggest a restriction of supraspinal control caused by spinal cord injury. There is a gradual recovery of the reflex excitability of the motor neurons of spinal motor centers, but the state of the peripheral part of the neuro-motor system is deteriorated. This work may be useful for the formation of ideas about the mechanisms of motor disorders and their correction in patients with a spinal cord injury with changing and descending influences on spinal afferent motor centers. This research was supported by the Russian Foundation for Basic Research 13-04-01746 a.

**Key words:** Anxiety • Motor neuron • Spinal centers • Spinal cord injury • Spinal cord injury  
• Electroneuromyography

### INTRODUCTION

List of abbreviations used: Amax - the maximum amplitude of the electrical muscle response during transcranial magnetic stimulation; ASIA - American Spinal Injury Association; Hmax - maximum amplitude of the reflex (H) response; Mmax, - the maximum amplitude of the motor (M) – reply; LP - the latent period; TMS - transcranial magnetic stimulation.

Spinal cord injury in the form of its compression, crushing, partial or complete rupture is one of the most pressing medical and social problems [1;2]. With a prevalence range from 11 to 112 people per one million inhabitants. Its consequences are manifested paresis, flaccid or spastic paralysis of the limbs and pelvic organ dysfunction. Various rehabilitation activities improve the outcome of injury and improve the quality of life of victims, but cannot eliminate the severe neurological deficits. Surgical procedures are effective in the acute phase of injury, but not in achronic period of traumatic disease of the spinal cord [3]. The aim of this study was to evaluate the state of spinal motor centers in the rat with spinal cord injuries of varying severity.

**Objects, Materials and Methods:** In the course of the experiment 79 laboratory rats weighing 200-240 grams were examined. In 40 rats under ketamine anesthesia (5.5 mg / kg), a mid-back laminectomy of the L1 segment. An L1 spinal cord injury was then made in the transverse direction to the left. In 25 animals, transection of the spinal cord was performed (at/between) the L1-L2 segments.

The conduction of spinal cord function was evaluated using complex "Neyrotest" (Novosibirsk), the magnetic stimulus was applied to the projection area of the motor cortex using a magnetic coil with a gradual increase in stimulus intensity from 0 to a value that causes the maximum amplitude of the motor response of the gastrocnemius muscle on the right and left, which were recorded by steel needle electrodes. When processing the results we analyzed the following parameters caused by responses to TMS: the threshold of response, expressed in % of maximum stimulator output of 4 T (the value of the stimulus that causes minimal response), latent period (LP) - the time of applying stimulus to the first (negative or positive) deflection wave and the maximum amplitude of the response (Amax). Stimulation of the sciatic nerve and

increased electrical responses of muscle electromyogram was performed by the firm "Medicor" (Hungary), the intensity of stimulation ranged from 0.35 to 60 V and 0.5 ms duration. Recording of responses of gastrocnemius muscle, as well as electrical stimulation of the nerve, was made with steel needle electrodes, with the stimulating needle electrodes introduced into the projection of the sciatic nerve in the hip. The studied parameters were the threshold, latency and maximum amplitude of the detected H-and M-responses, as was determined the ratio of maximum amplitudes of these responses ( $H_{\max}/M_{\max}$ ). The soleus reflex excitability of the motor neuron is usually tested by the quantification of the "H reflex", in the electromyogram elicited by an electrical stimulation of the sciatic nerve. In the control series (intact animals, 14 animals) under ketamine anesthesia, within 10-15 minutes after the fixation of the animal was carried out, the functional state of the motor centers of spinal electromyographic techniques and evaluation of conductive spinal cord function were assessed by TMS.

## RESULTS

After surgery the rats exhibited movement disorders, neurological status of animals is estimated at 5 points on a scale of Bagley, which corresponds to the least severe spinal cord injury.

All intact animals (control) responses were recorded in the gastrocnemius muscle TMS motor cortex. The threshold of response to TMS during the preoperative examination averaged  $38 \pm 2\%$  of the maximum charge capacity of the magnitude of 4 Tesla. No significant changes in the threshold of the muscle response, after surgery occur (Table 1). The latent period (LP) responses of rat gastrocnemius muscle to TMS before surgery was equal to an average of  $7,2 \pm 0,1$  ms, Amax responses in the control series averaged  $2,8 \pm 0,2$  mV. Significant changes in these parameters recorded in the 21st day (Table 1): LP responses increased by 7% compared with the preoperative index, Amax response was higher than the control level by an average of 25% (Table 1).

With stimulation of the sciatic nerve in all rats before the operation, we recorded M-and H-responses of gastrocnemius muscle. The threshold of the M-response in the gastrocnemius muscle on the first and third day after L1 spinal cord injury increased on average by 46%, then a decrease in the threshold of the M-response, on the twenty-first day, reached the preoperative values (Fig. 1 (black columns).) On the twenty-first day after surgery, there was a recorded an increase in LP M-response in the gastrocnemius muscle by 25%. On the first day after spinal cord injury, the maximum amplitude of the M-response of rat gastrocnemius muscle was reduced by 7%, then during the 14th through the 21st days it increased to that of the original preoperative values (Fig. 1 (open columns).) On the 1st day after spinal cord injury, the threshold of the H-response of rat gastrocnemius muscle increased on average by 2.4 times; on the third day, it averaged 118% of control level, followed by a gradual decrease in this parameter and the threshold response, reaching the control values on the 21st day (Fig. 1 (black columns).) On the twenty-first day after surgery, there was a recorded an increase in the PL H-response in the gastrocnemius muscle by 20%. The amplitude of the maximum H-response of gastrocnemius on the 1st day after surgery decreased on average by 50%, on the third - an average of 47%, after which, the amplitude of the reflex response gradually recovered (Fig. 1 (open columns).) On the 1st and 3rd day after spinal cord injury there was a recorded decrease in the ratio  $H_{\max}/M_{\max}$  an average of 6.0%, with subsequent surveys  $H_{\max}/M_{\max}$  was close to the control level. Following surgery all animals exhibited lower paraplegia, urinary retention, loss of deep pain sensitivity, motor function in the future is not restored, the neurological status of animals is estimated at 1 point on a scale of Bagley, which corresponds to the most severe spinal cord injury. In conditions of chronic spinal cord injury, the threshold of gastrocnemius muscle M-response on the third day decreased significantly and the average was 52% of control level of this index ( $p < 0.05$ ), then there was an observed recovery of this parameter

Table 1: Parameters of motor responses of rat gastrocnemius muscle by transcranial magnetic stimulation. After spinal cord injury at L1.

Parameters of muscle responses	Parameters of muscle responses after surgery (days), % of control				
	1 st	3rd	7st	14st	21st
Threshold, %	89,5±5,9	94,7±5,5	86,8±12,1	97,4±5,4	94,7±11,1
LP,ms	103,8±2,7	93,1±3,0	98,6±2,8	100±2,8	106,9±2,6 *
A max, mV	96,4±11,1	82,1±21,7	96,4±11,1	103,6±6,9	125,0±8,6 *

\* $p < 0.05$ , significant differences of parameters compared with controls.

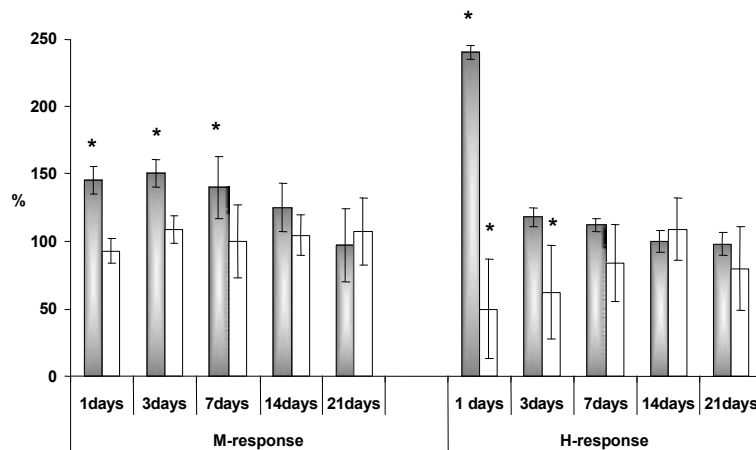


Fig. 1: Changing the motor (M) and reflex (H) responses of rat gastrocnemius muscle to stimulation of the sciatic nerve after spinal cord injury L1. Abscissa - time survey, a day after spinal cord injury; the vertical axis - the threshold value (dark columns) and maximum amplitude (white columns), in% of control (before trading) level. Here and throughout the figures: \* -  $p < 0.05$ , significant differences of parameters compared with controls.

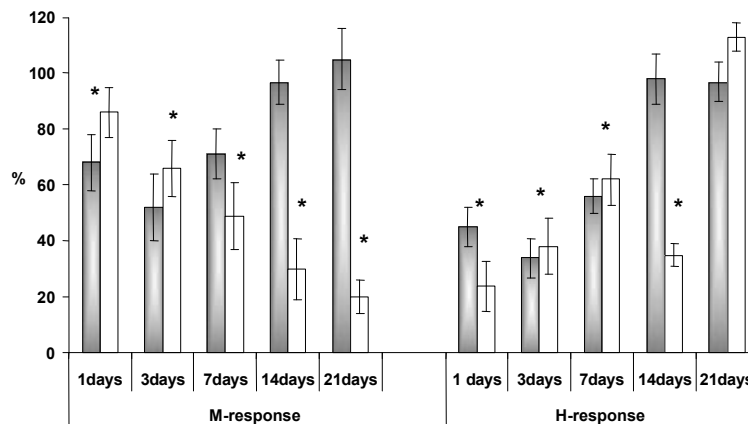


Fig. 2: Changing the motor (M) and reflex (H) responses of rat gastrocnemius muscle to stimulation of the sciatic nerve after section at L1 - L2.

Abscissa - time survey, a day after spinal cord injury; the vertical axis - the threshold value (dark columns) and maximum amplitude (white columns), in% of control (before trading) level.

(Fig. 2 (black columns)). The maximum amplitude of the M-response of rat gastrocnemius muscle was reduced in the postoperative period and at 3, 7 and 14 days after spinal cord injury, averaged 66%, 49%, 30% respectively of the level of this index before surgery ( $p < 0.05$ ). By the 21st day, the amplitude of the M-response decreased 5-fold compared with controls ( $p < 0.01$ ) (Fig. 2 (white columns).) On the third day after surgery, the threshold of the H-response of rat gastrocnemius muscle decreased by 3 times and averaged 34% of control level. The 21st day, the H-response threshold reached control values (Fig. 2 (black columns)). On the 1st day after operation, the amplitude of the maximal H-response of gastrocnemius

decreased and averaged 24% of control level ( $p < 0.05$ ). On the 7th day after surgery it decreased by 30% compared with control values (Fig. 2 (open columns)). On the 14th day after surgery, a further reduction in the the amplitude of the H-response was observed. By the 21st day, there was an increase of the amplitude to 113% of the control level.

## DISCUSSION

After surgery the neurological status of animals corresponded to the least severe spinal cord injury. In all animals, there were significant changes in parameters of

muscle responses when TMS after surgery did not occur. Thus, in experimental injury of the vertebral body, uncomplicated by spinal cord injury, there is no substantial change wiring of spinal cord function. However, a slight increase in PL and amplitude responses of gastrocnemius muscle on the 21st day indicates a change in the functional state of the motor centers of the spinal cord. The increase in latency of motor responses to transcranial stimulation of the muscles associated with demyelination of rapidly conducting fibers of cortico-spinal tract [4, 5, 6] and an increase in the amplitude of motor response in patients with incomplete spinal cord break is estimated as a functional adaptation of the cortico-spinal system that occurs after spinal cord injury [7]. It is shown that the spinal injuries disrupted supraspinal control of presynaptic inhibition of neurons [8]. And as you know, the reflex excitability of the centers of the spinal cord, determined to change the amplitude of the H-response, depends on the alpha motor neurons and the level of presynaptic inhibition of 1a afferents [9]. Thus, the observed increase in reflex excitability is probably the result of amplification transmission system 1a afferents [10]. The existing classification estimates the severity of spinal cord injury patients with vertebral-spinal cord injury ASIA, adopted by the International Society of paraplegia, uncomplicated fractures of the spine refers to the degree of D and E and treats a mild degree of spinal cord injury. We have previously shown with patients having a severity of injury of the appropriate degree of D on a scale of ASIA, that the parameters of any activity and motor (M) soleus response was not significantly different from the levels of these parameters in healthy subjects, as well as parameters of response a short extensor toe on transcranial magnetic stimulation. However, there were changes in reflex excitability identified spinal motor neurons: N-latency response in patients with mild spinal cord injury exceeded the control level by an average of 23%, the ratio of  $H_{max}/M_{max}$  was on average 64% of the level of this indicator of healthy subjects [11]. In the present study, we have shown that changes in the parameters of the electrical muscle response observed in patients with mild spinal cord injury are found in animals with similar situations in the simulation experiment. Apparently, the increase in reflex excitability of spinal trauma centers has a universal character, which can be used for diagnostic purposes. Neurological status of animals was estimated at 1 point on a scale of Bagley, which corresponds to the most severe spinal cord injury. Under these conditions, there was a significant reduction in the parameters of calf muscle responses induced in rats, demonstrating the formation of degenerative changes in

the neuronal system of the spinal cord and within muscles. It is known that damage to the spinal cord tissue is not limited to the scope of the destructive force; and, seizing the primary areas of intact, leads to the formation of more extensive damage [12]. Apoptosis of neurons leads to increased losses of active neurons and glial cells to apoptosis widespread ascending and descending degeneration, there is demyelination of nerve agents and loss of axons [13, 14]. After spinal cord injury, the amplitude of the M-responses is reduced, indicating muscle atrophy, which usually occurs in unused muscles and atony leading to paralysis of the extremities [15]. There is also evidence to support an important role in the regulation of muscle tone [16]. Another factor is the reduction of atony proprioceptive activity associated with a reduction in the tone of the gamma-fibers [17]. Thus, electroneuromyographic research functions of the spinal cord of rats showed that after section at L1 - L2 there is a progressive deterioration of peripheral neuromotor system. The amplitude of the H-response one day after spinal cord injury is also decreased (average 24% of control level), indicating a decrease in reflex excitability of the motor center of the gastrocnemius muscle. In subsequent surveys, the amplitude of the H-response increased and in 21 days reached the control level, despite the lack of recovery of effector monosynaptic reflex arc. The nature of increased excitability is unknown, but it is assumed that such a change may result from changes in activity of the interneuron circuit when removing the supraspinal inhibitory control and one of the possible mechanisms of increased excitability after SCI may be the formation of a plateau potential in spinal neurons [18]. It is believed that interneurons can alter behavior in the form of a plateau-building, including the long-latency facilitation of [19], as well as the plateau potential may occur in motor neurons and contribute to the formation of motor spasticity [20]. The literature indicates that there is no significant difference between light, moderate and severe spinal cord injury during the first days after injury [21]. In our experiments, after severe brain damage, the suppression of reflex responses was more pronounced (the amplitude of reflex responses averaged 24% against the same indicator of healthy animals).

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