Neuroendocrinology Letters No.4 August Vol.26, 2005
 ISSN 0172–780X **www.nel.edu**

ORIGINAL ARTICLE

The effect of motor cortex stimulation in deafferentated rats

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Submitted: June 30, 2005 *Accepted: July 14, 2005*

Key words: **motor cortex stimulation; deafferentation; pain; rats; plantar test; tail-flick**

Neuroendocrinol Lett 2005; **26**(4):283–288 **PMID:** 16136014 NEL260405A06

OBJECTIVES: The aim of the study was to describe the effect of motor cortex stimulation (MCS) on pain thresholds in deafferentated rats.
SETTINGS AND DESIGN: The effect of MCS was studied in 18 deafferentated and 14 intact laboratory rats, using a standardised plantar test and tail-flick latencies. Two inoxious stimulation electrodes were implanted subdurally over the cerebral cortex and a C5 – Th1 dorsal root rhizotomy was performed on the left side. Pain thresholds were measured before and after cortical stimulation. The data were analysed with ANOVA for repeated measures.
RESULTS: MCS in intact animals evoked no changes in pain thresholds except for the contralateral forelimb, in which the pain threshold increased after MCS. Following deafferentation, pain thresholds increased in both plantar test and tail-flick in comparison to baseline values. When MCS was applied to the deafferentated animals, the pain thresholds returned to baseline levels. The effect of MCS disappeared within 24 hours.
MAIN FINDINGS: 1. MCS in intact animals evoked hypoesthesia in the corresponding contralateral forelimb; 2. deafferentation itself increased pain thresholds in the unaffected limbs; 3. under MCS, pain thresholds in deafferentated rats were not different from pre-dafferentation values; 4. the effect of MCS disappeared in 24 hours and oscillated.
CONCLUSIONS: Our results show a similar effect of the stimulation in man and experimental animals despite the differences in the organisation of the cerebral cortex. The use of laboratory animals is promising for further studies in the field of involved antalgic mechanisms of MCS.

Introduction

Motor cortex stimulation (MCS) has become an important part of the treatment of resistant, intolerable pain mainly of neuropathic origin. Its effect has been tested for thalamic, postherpetic, trigeminal, deafferentation and phantom pain [9,12,13,24,25,26,28].

In general, stimulating electrodes are placed over the motor pre-central cortex. The localization of electrodes somatotopically corresponds with the painful region of the body. Perioperative verification of the localization is based on somatosensory evoked potentials, where the central sulcus (sulcus centralis Rolandi) corresponds with the site of a phase reversal of N20. To confirm the precise location of the electrodes, the presumably placed contacts are stimulated at a relative high intensity to induce muscle twitch in the painful area. The stimulation is intermittent, because the MCS exhibits not only immediate – acute analgesic effect, but also post-stimulatory maintenance of analgesia for hours up to a few days [28].

As far as the authors know, two papers dealing with the effect of cortex stimulation in rats have been published so far. Electrodes were placed over the somatosensory area and stimulation evoked weak antinociception in formalin test [18] and the antinociception was suppressed by spinal administration of NOS inhibitors [19]. The effect of motor cortex stimulation on pain and nociception in experimental models of chronic neuropathic pain has not yet been described.

An extensive dorsal rhizotomy at the cervicothoracical level (C5–Th1) in the rat is used as a model of central pain [1,2,4] which often develops after brachial plexus avulsion in man [3,7,23,27,29]. Physiological changes following the rhizotomy in rats implicate development of a chronic pain syndrome in the ipsilateral limb. The dorsal rhizotomy model differs from other chronic pain models due to the localization of the nerve lesion, which is proximal to the neuronal body. It prevents any further afferent firing that occurs in other models – sciatic nerve transection/ligation [6,15,21,34] – in which lesions are localized in the distal part of the axon (related to the neuronal body). In the rhizotomy model any observed changes are to be of central nervous system origin.

The aim of our study was to explore the effect of motor cortex stimulation on pain thresholds in deafferentated and intact laboratory rats.

Methods

Animals. The experiments were carried out on 32 adult Long Evans rats of either sex (body weight 200–350 g). The rats were housed with free access to food and water and maintained under a regime with 12 h of light and 12 h of darkness per day. The mean temperature was 22 ± 2 °C and the relative humidity equalled 55% ± 10%. The acclimation period was 5 days long. This experiment was approved by the Expert Committee for Animal Care and Use of the 3rd Faculty of Medicine, Charles University, Prague, and conducted according to the guidelines of the Ethics Committee of the International Association for the Study of Pain [36].

Pain testing. Nociception in rats was determined according to the latency (s) of avoidance reaction of the forelimbs, the hindlimbs and the tail to the noxious thermal stimulation (Plantar test; Ugo Basile, Comerio, Italy; Tail Flick Analgesia Meter; Life Sciences, USA). In one session every reaction was tested three times following one-minute break, and average values were used for statistical analysis. In intact animals, the effect of the motor cortex stimulation on plantar test was tested on ipsilateral (right) and contralateral (left) sides to the stimulation.

Implantation of the electrodes. Two stimulation electrodes made of inox wire were implanted subdurally over the forelimb representation area of the cerebral cortex under ketamine-xylazin anaesthesia (100 mg.kg^{-1}, 16 mg.kg^{-1}) and fixed with dental enamel. The stereotaxic coordinates were: negative electrode 1mm posterior and 3mm right, positive 1mm anterior and 3.5 mm right related to bregma. Their appropriate location was verified by the stimulation of the left forelimb and recording of somatosensory evoked potentials through the electrodes. Electrical stimulation with duration 0.2 ms of square-wave pulses at a frequency of 25Hz was delivered for five hours in five consecutive days. Subthreshold intensity was set as 80% of the intensity that evoked left limbs myoclonus.

Deafferentation. Under ketamine-xylazin anaesthesia (100 mg.kg^{-1}, 16 mg.kg^{-1}) the rhizotomy of dorsal roots C5 – Th1 was performed on the left side as described previously in details (Vaculin and Rokyta 2004).

Experimental design. 18 rats were included in the first group. Following recovery from the electrodes implantation, the pain thresholds were measured before and after cortical stimulation.

14 rats of the second group were implanted and their pain thresholds were measured before the deafferentation and these were considered as baseline values. Then the rats were deafferentated as described above. 4–6 weeks after the deafferentation the pain thresholds were measured before and after the stimulation in five consecutive days. The deafferentated forelimb was excluded from the measurements.

Sex differences in analysed thresholds were found non-significant; therefore the data from males and females were spooled together.

Statistics. Data were analysed with ANOVA for repeated measures. In the first group, two-way ANOVA has been used for the tail-flick test. Latency obtained before and after the stimulation represented the factor *stimulation*, five consecutive days of stimulation represented the factor *trend*. The plantar test was analysed separately for forelimbs and hindlimbs by three-way ANOVA where the third factor (*side*) compared nociception on right and left side.

In the second group, the main effects of deafferentation and stimulation were tested by one-way ANOVA with three repeated measures which included baseline values before deafferentation, mean values after deaf-

ferentation and after the stimulation. Stability of stimulation effect was analysed by two-way ANOVA with factors *stimulation* comparing pre- and post-stimulation latencies and factor *trend* represented five days of stimulation. In the plantar test of hindlimbs, the factor side was also included in the analysis. Within-subject variability consists of eleven repeated measurements of nociception with the first baseline value and pairs of values obtained before and after the stimulation in five consecutive days. Bonferroni test was used for post hoc comparison. Data are expressed as means ± standard deviations, $p < 0.05$ was considered significant.

Results

The effect of the stimulation in intact animals

In the intact group, repeated motor cortex stimulation did not affect the pain thresholds in plantar test of hindlimbs ($F(1,17) = 2.563$, $p = 0.127$) as well as in tail flick test ($F(1,17) = 2.382$, $p = 0.141$). Stimulation had the same effect on both hindlimbs; the factor side was non-significant ($F(1,17) = 2.147$, $p = 0.161$).

Different results were obtained on forelimbs. The motor cortex stimulation increased latencies from 3.23 s ± 0.49 to 3.41 s ± 0.52 (the main effect of stimulation was $F(1,17) = 12.09$, $p=0.0029$), however, significant interaction of factors side x stimulation $F(1,17) = 6.82$, $p = 0.0182$ showed that this effect was lateralized mainly to the contralateral side to the stimulation (post hoc Bonferroni test $p=0.0108$). Moreover, the magnitude of stimulation-induced analgesia of forelimbs was fluctuating during the observed period (interaction of factors trend x stimulation $F(4,68) = 2.67$, $p = 0.0391$) with higher latencies observed after the 3rd ($p = 0.0265$) and the 5th stimulation ($p = 0.0427$).

The effect of the deafferentation on nociception

After the deafferentation the pain thresholds were increased in plantar test as well as in tail-flick test in comparison with the baseline values. The baseline value for the right forelimb (contralateral to the deafferentated forelimb) was 3.55 ± 0.35 s, six weeks after the deafferentation it increased to 4.22 ± 0.51 s ($t = 4.04$, $p = 0.0014$, t–test for dependent samples). The pain thresholds of the left hindlimb (ipsilateral to the deafferentated forelimb) before and after the deafferentation were 3.58 ± 0,34 and 4.2 ± 0,39, respectively ($t = 4.25$, $p = 0,0009$). The pain thresholds of the right hindlimb (contralateral to the deafferentated forelimb) before and after the deafferentation were 3.55 ± 0,38 and 4.43 ± 0.59, respectively ($t = 5.79$, $p =0.00006$). The tail-flick latencies before and after the deafferentation were 3.61 ± 0,5 and 4.26 ± 0,43, respectively ($t = 3.41$, $p = 0.0046$).

The effect of stimulation in deafferentated animals

One-way ANOVA for repeated measures showed significant differences between compared conditions, i.e. between baseline latency, latency after the deafferentation and latency after the stimulation in all nociceptive tests (for forelimb $F(2,26) = 8.38$, $p = 0.0015$, and for the tail $F(2,26) = 7.38$, $p= 0.0029$) Results are shown in *Fig. 1*. In all tested areas, the stimulation decreased withdrawal latencies to the baseline levels. The same applies to hindlimb ($F(2,26) = 27.53$, $p <0.000001$). The comparison of latencies on right and left hindlimb showed also significant effect of side ($F(1,13) = 5.63$, $p = 0.0337$) with longer latencies observed on the contralateral side to the deafferentation. Latency on the right hindlimb was higher than on the left hindlimb after deafferentation as well as after the stimulation (interaction of factors side x conditions $F(2,26) = 3.5$, $p = 0.045$).

The effect of repeated stimulation

Stability of the magnitude of repeated stimulation was tested from pre- and poststimulation values obtained during the five-day interval. The effect of repeated stimulation on nociception of hindlimbs was highly significant ($F(1,13) = 75.7$, $p = 0.000001$). The changes in withdrawal latencies were stable across the whole observation period of intermittent stimulation, the effect of factor trend was non-significant ($F(4,52) = 0.077$, $p = 0.989$). The differences between pre and poststimulation latencies were in every trial significant (*Fig. 2*). Repeated stimulation confirmed the lateralization effect of deafferentation with the higher both pre- and poststimulation latency on the contralateral side to the deafferented limb ($F(1,13) = 9.96$, $p = 0.0076$).

The cortex stimulation decreased also latencies of forelimb ($F(1,13) = 73.55$, $p = 0.000001$). However, the stimulation-induced decrease was different in different days of stimulation (interaction of factors trend x stimulation $F(4,52) = 2.67$, $p = 0.04226$). The effect of stimulation was significant after the 1st, the 3rd and the 5th day of stimulation (Bonferroni post hoc tests $p = 0.000057$, $p = 0.004385$ and $p = 0.00028$ respectively).

Similar results were obtained in the tail flick test. Stimulation decreased the tail flick latencies ($F(1,13)=21.63$), $p = 0.00045$) and this effect was stable across the five day stimulation. Although the interaction of trend x stimulation was marginally significant $F(4,52) = 2.22$, $p = 0.0793$), similarly to results obtained from forelimb, the observed differences between pre- and poststimulation latencies were only significant after the 1st and the 5th stimulation (Bonferroni post hoc tests $p = 0.0135$ and $p = 0.00057$ respectively).

Discussion

The main findings in our study are as follows: first, motor cortex stimulation in intact animals did not evoke any changes in pain thresholds except contralateral forelimb; second, deafferentation increases withdrawal latencies from the painful stimulation; third, the motor cortex stimulation returned increased latencies to the baseline levels; fourth, the effect of stimulation disappeared during 24 hours; fifth, the effect of stimulation oscillated. The results will be discussed in the abovementioned order.

Motor cortex stimulation in intact animals did not evoke any changes in pain threshold in both hindlimbs

and right forelimb. However, pain threshold in the contralateral (left) forelimb increased after MCS. It corresponds well with the location of electrode implantation, which is located in the representative motor cortex area of the left forelimb. It parallels the increase of cold pain threshold reported in man after a high frequency repetitive transcranial magnetic stimulation [32]. This type of stimulation is known to evoke similar effects, and it is used as a test before the electrode implantation for MCS [22].

The deafferentation resulted in increase of paw withdrawal latencies to thermal noxious stimulation in three innervated limbs. The transient increase of the thresholds after the deafferentation in rats was described by Kayser et al. [14], who tested mechanical nociceptive threshold after deafferentation. The authors concluded, that the loss of tonic afferent input from the deafferentated limb results in a decrease in the background level of firing in whole spinal cord and thus a greater input from the intact limbs is required to drive the second neuron. According to our results, the increase of the thresholds is rather to be long lasting. However, pain threshold should not be stable in rats. During 10–week observation period after unilateral deafferentation Kříž et al. [16] found simultaneous oscillations in self-mutilation behavior and in tail-flick latencies, tail-flick latencies were the lowest during self-mutilation attacks.

In accordance with Kayser et al. [14], we also observed higher pain threshold on hindlimb contralateral to the side of deafferentation. This effect was stable and was present prior to as well as after the motor cortex stimulation. From our experiment we could not conclude whether this effect was predominantly maintained through the different activity of dorsal horn neurons, which are under the descendent antinociceptive control, or through the activity of motor neurons in ventral horns, which should be affected by dorsal rhizotomy too [10].

In man, deafferentation caused by brachial plexus avulsion results in chronic pain [23,27,29]. From clinical studies, there are conflicting views about the effect of chronic pain on pain thresholds of unaffected sites. Whereas Yang et al. [35] and Peters et al. [30] described increase of pain thresholds in patients with chronic pain, Langemark et al. [20] and Bendtsen et al. [5] found decrease of the thresholds in patients with chronic pain. The thresholds seem to be dependent on the type of pain. Autotomy reported to develop after extensive dorsal rhizotomy [4] has not been observed in our study because of the ketamine anaesthesia used for the deafferentation [33].

The main result in present study is that pain thresholds being impaired by the deafferentation recover transiently and repeatedly to the pre-deafferentation values after the motor cortex stimulation. The effect of the stimulation disappeared in one day, which corresponds well with findings from clinical studies, where transience of the effect is described [28]. These results are more interesting when differences in anatomy of cerebral cortex between humans and rats are considered. The man is gyrencephalon and motor and somatosensory areas in the cortex are strictly divided by the central sulcus, whereas the rat is lisencephalon and the representative fields of both sensory and motor areas are mixed up.

There is only one study dealing with the effect of MCS on pain thresholds in man [11]. In patients with partial sensory loss, the threshold to the nociceptive warm stimulation in contralateral side decreased after MCS, however in non-significant manner. Despite that and the fact, that the baseline thresholds were not known in that study, the results support hypothesis about the similar effect of MCS in man and animals.

The detailed analysis of the effect of repeated MCS on nociception showed that the degree of pain suppression in forelimb and tail oscillates within two-day

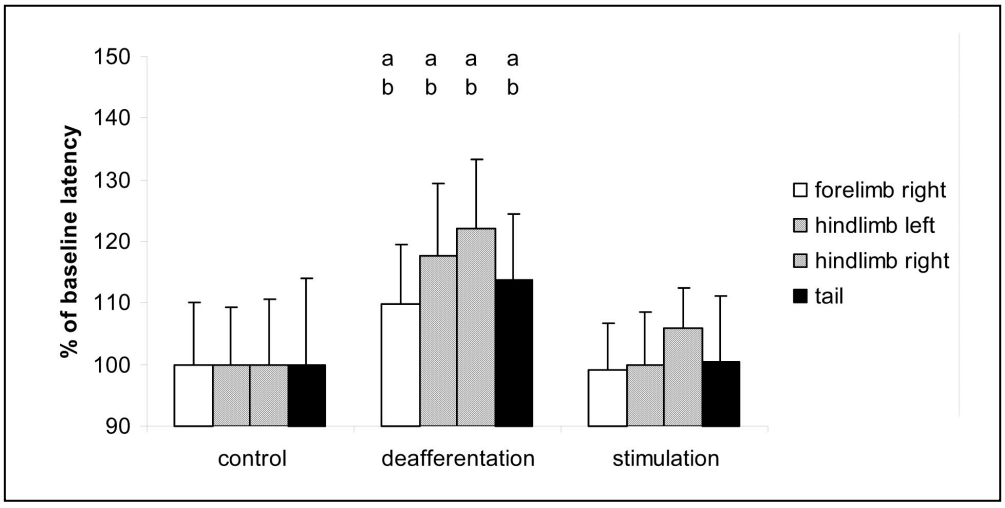

Figure 1: Comparison of changes in nociception on tested areas after the deafferentation and after the stimulation expressed relatively to the baseline latencies obtained prior to the deafferentation. "a" significant differences between deafferentation and control, "b" significant differences between deafferentation and stimulation.

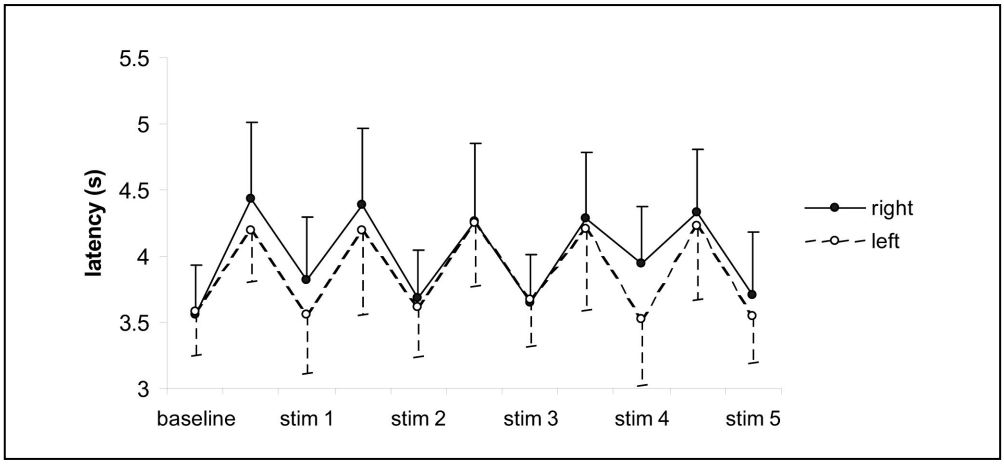

Figure 2: The effect of five-hour stimulation repeated for five days on nociception of right and left hindlimb in the plantar test. The pain thresholds decreased after the stimulation to the baseline values. The effect of the stimulation disappeared in 24 hours and pain thresholds increased to the pre-stimulation levels. The next stimulation resulted in decrease of the thresholds again. After the deafferentation, longer latencies were observed on the side contralateral to the deafferentation.

intervals. The suppression was significant after the first, the third and the fifth stimulation. Similar results were observed after repeated cocaine or repeated morphine exposure on shock-induced hypoalgesia [8,17].

One-day interval of stimulation with subthreshold intensity used in our experiment is close to kindling paradigm, although the duration of the stimulation and the stimulated structure were different. It is hypothesized that pain evolution also should possess kindling-like phenomenon [31].

In conclusion, we considered the increase of pain threshold after the deafferentation as an impact of deafferentation on pain processing, thus afterwards we interpreted observed changes in the thresholds during MCS as the effect of MCS on pain. In this respect, our results show a similar effect of the stimulation in man and experimental animals despite the differences in the organisation of the cerebral cortex. Thus, the usage of laboratory animals is promising for further studies in the field of involved antalgic mechanisms of MCS.

Acknowledgements

The study was supported by grant IGA NF 7406–3/2003.

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