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Extremely Low Frequency Magnetic Field Exposure Attenuates Pain By Inactivation Of Descending Facilitatory System In Complete Spinal Cord Injured Rats.

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ABSTRACT

Background: To investigate the effect of chronic exposure to extremely low frequency magnetic field (ELF- MF) on pain modulation status of completely spinal cord injured (SCI) in rats. **Methods:** Male Wistar rats were divided into Sham (Laminectomy), SCI (complete transection of T13 spinal cord) and SCI+MF (ELF-MF: 17.96 µT, 50 Hz, 2h/day exposure to SCI rats) groups. Pain was studied by utilizing threshold of tail flick (TTF), forepaw lick latency (FPL) and its modulation by temporal summation (TS) and diffuse noxious inhibitory control (DNIC). These tests were performed before surgery (week 0), and after surgery (weeks 4 and 8). Locomotor function was assessed by BBB score at post-SCI weeks 1,3,5,7 and 8. At the end of week 8, spinal cords were collected for histological analysis. **Results:** Data revealed post-SCI significant decrease in TTF and FPL. The amplitude of TS response was increased, while TTF response was not disappeared after pressure pain application in DNIC paradigm. SCI rats also revealed a significant lower BBB score. However, MF exposure to SCI rats significantly restored the above parameters. **Conclusion:** Our observations suggested reduction in post-SCI hyperalgesia by inactivation of descending facilitatory system after MF exposure to SCI rats.

Keywords: Magnetic field, Pain, Spinal cord injury.

INTRODUCTION

Pain is one of the most debilitating sequelae of spinal cord injury (SCI) apart from sensori-motor deficit. Hyperalgesia and allodynia have been reported in SCI rats irrespective of the injury mode (compression, contusion, laser, surgical, chemical), study parameters (mechanical, thermal) and time of evaluation after SCI (2-30 weeks).[1-4] Previous studies from our laboratory have also reported hyperalgesia to phasic whereas, hypoalgesia to tonic pain stimuli in both hemi and complete transection models of SCI rats.[5,6]

The underlying mechanism for post-SCI pain is believed to be hyper responsiveness of neurons; increase in blood flow; increase in spontaneous activity; presence of abnormal burst activity of spinal and supraspinal neurons in brain stem, limbic areas, arcuate, hypothalamus, anterior cingulate cortex besides alteration in neurotransmitter/ modulator profile favouring hyperalgesia.[3,7-9] These brain areas exert their influence on the perception, processing and response to noxious stimuli via descending modulatory system that include independent facilitatory and inhibitory systems.[9] We are ignorant regarding the status of these pain modulatory systems in the agonising pain after SCI. We, therefore experimentally investigated the spinal and supraspinal involvement in post-SCI hyperalgesia.

The genesis and progression of post -SCI sequelae is still in experimental stage and therefore the current management is not targeted towards their cause but remains symptomatic.[10] It is apparent that there is a dire need for an ideal strategy focused towards the initial causative factors that trigger secondary injury processes to curtail all the sequalae of SCI. Recent reports suggest that extremely low frequency magnetic field (ELF-MF; 0.3 to 100 Hz) is beneficial in restoration of physiological functions in SCI also. Besides, it is a non-invasive, non-pharmacological, simple to administer therapeutic strategy. The beneficial effects of ELF-MF (50 Hz, 17.96 µT, 2 h per day for 8 weeks) have been reported by several researchers.[6,11,12] More over, ELF-MF in rats has been reported from our laboratory to improve the quality of life after SCI by significantly improving locomotor recovery, autonomic control of urinary bladder, sensorimotor function, osteoporosis and eating behavior.[5,11,13,14] Recently, we have reported the facilitation of neuroregenerative processes also

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by it after SCI.[14] However, we are still ignorant about the influence of ELF-MF on pain modulation status after SCI.

Therefore, the present study was proposed to investigate the effect of daily (2h for 8weeks) exposure to ELF-MF (17.96 µT, 50 Hz) on nociceptive responses and pain modulation status in spinalised rats.

MATERIALS AND METHODS

Animals

The study was approved by Ethical Committee of All India Institute of Medical Sciences, New Delhi, India and was carried out in accordance with guidelines provided by the World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research. Adult male Wistar rats (n=92) weighing 250–300 g were obtained from Experimental Animal Facility of the Institute and were individually housed in specific pathogen-free conditions. They were maintained at 24 ± 2°C and 14:10 h light–dark cycle and provided with laboratory food pellets and fresh tap water ad-libitum (Ashirwad Industries, Chandigarh, India). The rats (n=20) were divided into Sham-SCI (n=7); SCI (n=7) and SCI+MF (n=6) groups for studying their behavioral response to thermal noxious stimulus (forepaw lick latency; FPL); threshold for stimulation of nociceptive afferents (TTF); pain modulation status (temporal summation (TS) and diffuse noxious inhibitory control (DNIC) and locomotion (Basso Beatie Bresnahan score (BBB score). The rats were then sacrificed at week 8.

Surgical procedures

Spinal cord injury

On the day of surgery, the rat was anesthetized with a combination of ketamine hydrochloride and xylazine (60 and 10mg/kg body weight; respectively). After skin incision and laminectomy, the spinal cord (SC) was completely transected at T13 level with a fine micro scissor under surgical microscope and the gap was filled with a sterile, haemostatic, absorbable gelatin base foam (2mm long; Abgel, Sri Gopal Krishna Pvt. Ltd, Mumbai, India) placed in alignment with the cut stumps of SC (SCI group), while only laminectomy without disrupting the dura was performed in Sham-SCI group. [14,15] The muscles and skin were sutured in layers with silicon thread (Ethicon, Johnson and Johnson Ltd, India) and topical antibiotic cream (Providon Iodine, Glide Chem Pvt Ltd, Rampur Ghat, India) was applied. Intra and post operative body temperature was maintained by placing the rat on a controlled heating pad (CMA-150, CMA Microdialysis, North Chelmsford, MA, USA). Manual expression of urinary bladder was performed thrice daily until spontaneous voiding was achieved. The rats were monitored for any urinary tract infection by daily examination of urine sample under microscope.

Magnetic field (MF) exposure chamber

MF exposure chamber is a modified Helmholtz coil.[14] Briefly, it consists of four coils (two outer and two inner coils with 18 and 8 turns; respectively) mounted on a stand, a movable platform for the rat cage and a current regulator for maintaining constant current through the coils. The coils were connected in series to provide uniform MF on the central movable platform,[16] where six rats were placed in a specially designed polypropylene cage. This cage has six compartments to house rats individually during exposure. MF was precisely adjusted to17.96 µT before exposing rats by utilising magnetometer (Walker Scientific Inc. Auburn Hills, MI, USA) and current regulator.

Assessment of nociceptive beahvioural responses

Fore-paw lick latency (FPL): FPL was determined utilising hot plate analgesia meter (Ominitech, U.S.A), that was maintained at 52±1°C by adjusting the inbuilt thermostat. The rat was placed on the hot plate and the timer was simultaneously started by the pedal switch. The time taken to lick either of the fore-paw(s) got frozen on the monitor, which was recorded as FPL. The cut-off duration was preset at 30 s to circumvent thermal injury to the plantar surface of the paws during the test.

Threshold of tail flick (TTF): TTF was determined by stimulating nociceptive afferents in the tail muscles (Extensor caudae dorsalis) of the rat. The rat was conditioned in plexiglass restrainer for 30 min before the placement of intra dermal electrodes (12 x 0.40 mm; 0.5 x 27G; Ambu, Neuroline, Noblesville, USA). The stimulating electrodes were placed rostral (Anode: 4.5 cm, Cathode: 2.5 cm) and the recording electrodes for EMG were placed caudal (1cm, 2.5cm) to the reference point; i.e, caudal-12 tail vertebra. Brief burst of electric current (1 msec pulse width, 40 Hz, 0.1- 3mA; SEN-3301, Nihon Kohden, Japan) was applied to the tail at 3 min interval and was gradually increased in steps of 0.01mA until the tail flick occurred. This procedure was repeated 3 times at the interval of 5 minutes and the average value was considered for statistical analysis.[17,18] The occurrence of tail flick was confirmed by the corresponding EMG record on oscilloscope.

Assessment of pain modulation status

Temporal summation (TS): TS consists of a progressive increase in the neuronal or reflex response to a constant level of noxious or sub noxious stimulation applied repetitively to the same area of the body. It was assessed by applying electrical stimuli to tail muscles. A train of 15 sub threshold (80% of threshold) electrical stimuli

(biphasic square pulses of 1 ms duration pulse width; interstimulus interval of 500 ms) was applied to tail muscles after the placement of intra dermal electrodes (as described for TTF) on the tail. After 1 sec, the threshold stimulus was given again. The tail flick was noted and its EMG record was obtained. The latency and amplitude of the last response was considered for analysis of TS.[19]

Diffuse noxious inhibitory control (DNIC): After determination of TTF (as described above), the right hind paw of the rat was clamped with a mouth gauge to produce tonic pressure pain. Threshold stimulus was applied to the stimulating electrode and EMG was recorded every 10 seconds. The mouth gauge was removed soon after the abolition of tail flick response and the time taken for the reappearance of the EMG response was noted. The time taken from the disappearance to reappearance of EMG response was considered for analysis.[20]

Assessment of locomotion

Basso Beattie and Bresnahan (BBB) locomotor rating score: The hind limb locomotor function was evaluated by BBB score. Behaving rat in open field arena (Video path analyzer, Coulbourn Instruments, USA) was video graphed and observed for BBB score criteria such as extent of joint movements, weight support ability and stepping/walking behaviour of the hind limbs for 5 min as described elsewhere.[14,21]

Assessment of neuronal activity

c-Fos immunohistochemistry

Fos-immunoreactive (Fos-ir) profile in different areas of the brain was determined to assess their neuronal activity. The procedure of c-Fos immunohistochemistry is described elsewhere.[14] Three sections per rat (6µm thickness; n=6 in each group) were selected for the analysis. They were studied through florescent microscope (Eclipse 80i, Nikon, Tokyo, Japan), and Fos-ir cells were counted by using software (NIS ELEMENTS, Version AR3, Nikon). Cells were considered c-Fos immunoreactive, if they contained dark, punctuate, nuclear immunostaining.

Study plan

The rats were divided in to 3 groups (Sham, SCI and SCI+MF). SCI was performed after obtaining baseline behavioral data of nociceptive behavioural responses and pain modulation status. SCI+MF rats started receiving daily exposure to MF from day 1 of SCI. FPL, TTF, TS and DNIC were performed at wks 0, 4 and 8 post-surgery; while, BBB score was performed at wk 0,1,3,5,7 and 8 in all the rat groups. Rats were sacrificed at the end of week 8 under deep anesthesia and their brains were collected for histological analysis.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences version 16 software (SPSS Inc, Chicago, IL, USA). All values are presented as mean±SD.

Inter group comparison of behavioural parameters was performed at each week utilising one-way analysis of variance (ANOVA) with post hoc analysis using Mann-Whitney U test with Bonferroni correction and intra group changes were compared using generalised estimated equation (GEE. The P value <0.05 was considered statistically significant.

RESULTS

Assessment of responses to noxious stimulus

Fore paw lick latency (FPL)

During wk 4-8, the FPL progressively decreased ($p<0.001$) in SCI versus Sham rats, whereas it gradually recovered in SCI+MF rat versus SCI rats. However it was lesser than sham rats [Figure 1.A].

Threshold of tail flick (TTF)

The TTF progressively decreased ($p<0.001$) in SCI rats versus Sham rats fromwk4-8. The SCI+MF rats showed higher TTF ($p<0.001$) versus SCI rats during wk 8, while it was lower versus Sham rats during wk 4 and 8 [Figure 1.B].

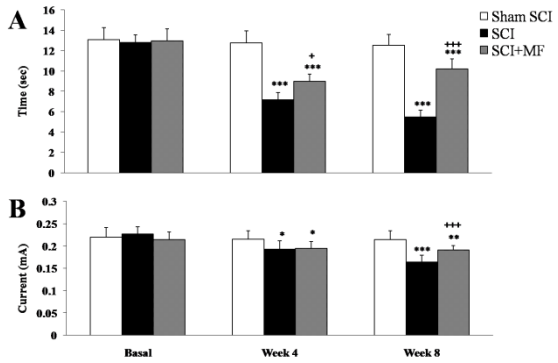

Figure 1: Effect of MF on FPL (A) and TTF (B) in spinalised rats. Bars represent mean values ± SD. '*' indicates comparison with Sham group, '+' indicates comparison with SCI group. (Single symbol denotes P<0.05, double symbol denotes P<0.01 and triple symbol denotes P<0.001).

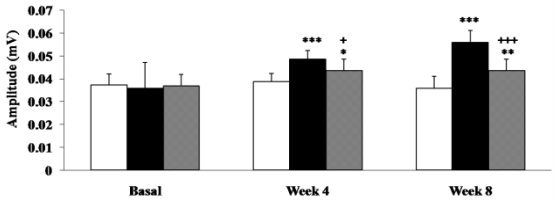

Figure 2: Effect of MF on TS in spinalised rats. Bars represent mean values ± SD. '*' indicates comparison with Sham group, '+' indicates comparison with SCI group. (Single symbol denotes P<0.05, double symbol denotes P<0.01 and triple symbol denotes P<0.001). Groups: Sham (□): , SCI (■) and SCI+MF (▒).

Assessment of pain modulation status:

Temporal summation (TS)

Latency of EMG response: The latency of EMG responses to temporal summation did not vary in any group at all study points.

Amplitude of EMG response: SCI rats vs Sham showed higher amplitude of EMG at wks 4 and 8. SCI+MF rats showed lower amplitude versus SCI rats, while it remained higher than Sham rats during wks 4 and 8 [Figure 2].

Diffuse Noxious Inhibitory Control (DNIC)

There was no intragroup variation in the time taken for reappearance of the response after its disappearance due to pressure pain at wk 4 and 8 in Sham rats; while in SCI and SCI+MF rats, the tail flick response did not disappear after application of clamp during wks 4 and 8 [Figure 3].

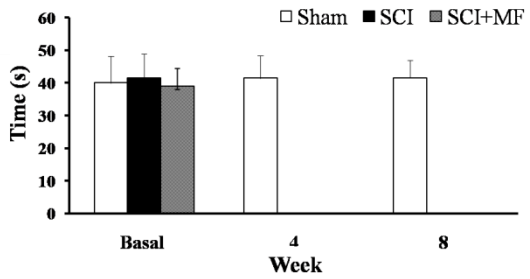

Figure 3: Effect of MF on DNIC in spinalised rats. Bars represent mean values of time taken from the disappearance to reappearance of EMG response ± SD. '*' indicates comparison with Sham group, '+' indicates comparison with SCI group.

Basso Beattie and Bresnahan (BBB) score

Baseline BBB score was 21±0.0 in all rat groups and it did not vary in Sham rats at all study points. However, there was gradual spontaneous recovery from nadir BBB score to 2.86±0.38 in SCI rats, while 8±0.63 in SCI+MF rats during wk 8 [Figure 4].

Intergroup comparison of BBB score between Sham and SCI revealed a significant lower BBB score in SCI rats from wk1-8, while between SCI and SCI+MF rats, it was more in SCI+MF rats from wk 1-8.

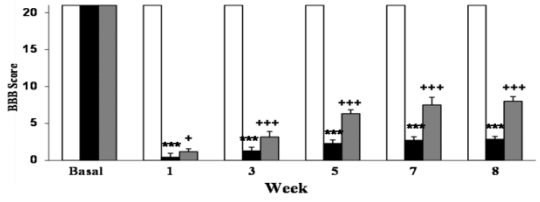

Figure 4: Figure shows BBB score in Sham (□), SCI (■) and SCI+MF (▩) rats. MF exposure significantly improved BBB score from wk 1-8 as compared with SCI rats; + sign indicates comparison with SCI;+++ sign indicates p ≤0.001, + sign indicates p≤0.05.

c-Fos immunohistochemistry

Robust c-Fos expression in PAG, ACC, AMY of SCI rats were observed when compared with Sham rats. In SCI+MF rats, c-Fos expression was reduced in PAG and AMY while restored in ACC [Figure 5,5a,6,6a,7 and 7a].

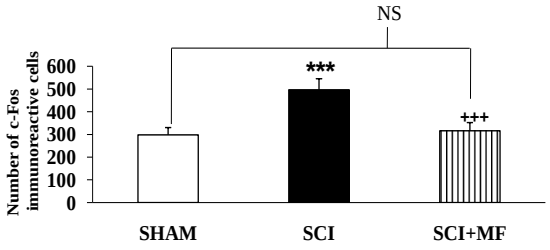

Figure 5: Effect of MF on c-Fos expression in ACC. Bars represent the values in mean ± SD. '*' indicates comparison with Sham rats, '+' indicates comparison with SCI rats. 'NS' indicates: not significant.

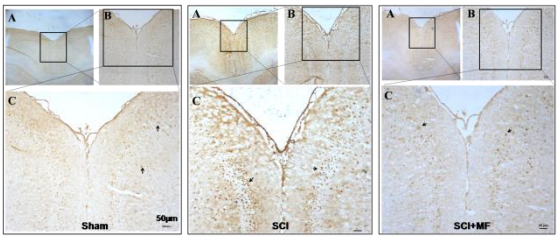

Figure 5a: Representative photomicrographs showing c-Fos expression in ACC of Sham, SCI and SCI+MF rats.

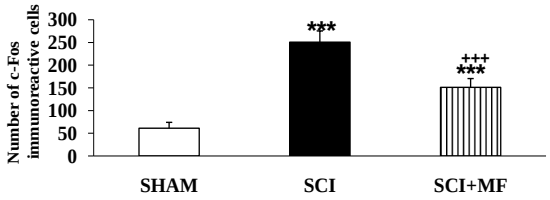

Figure 6: Effect of MF on c-Fos expression in PAG. Bars represent the values in mean ± SD. '*' indicates comparison with Sham rats, '+' indicates comparison with SCI rats. 'NS' indicates: not significant.

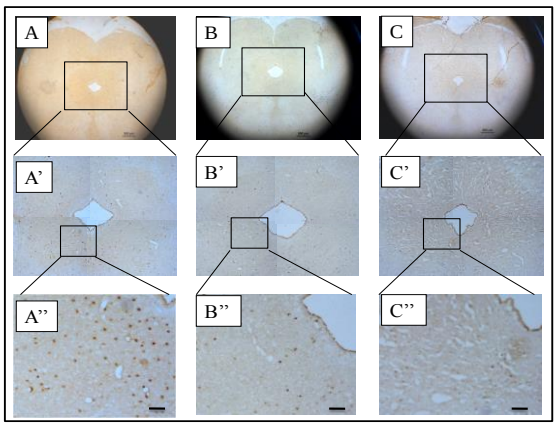

Figure 6a: Representative photomicrographs showing c-Fos expression in PAG of Sham (A), SCI (B) and SCI+MF (C) rats. A', A''; B', B''; C' and C'' are magnified images from A, B and C respectively. (Scale bar 100µm)

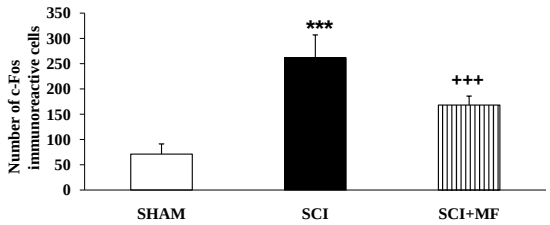

Figure 7: Effect of MF on c-Fos expression in AMY. Bars represent the values in mean ± SD. '*' indicates comparison with Sham rats, '+' indicates comparison with SCI rats. 'NS' indicates: not significant.

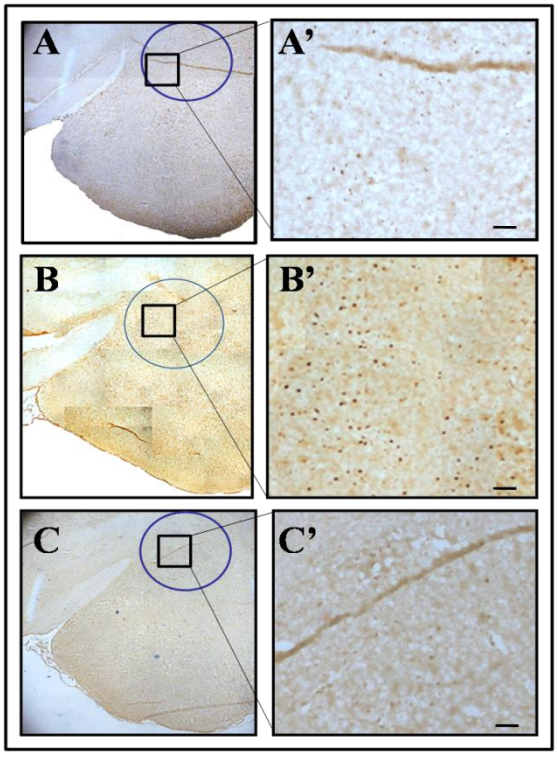

Figure 7a: Representative photomicrographs showing c-Fos expression in AMY of Sham (A), SCI (B) and SCI+MF (C) rats. A', B'and C' are magnified images from A,B and C respectively. Scale bar: 50µm

DISCUSSION

The present study confirms the previous reports of significant hyperalgesia to phasic noxious stimuli after complete transection injury of T13 spinal cord in adult male rats as indicated by marked reduction in their FPL and TTF. The relative contribution of facilitatory and inhibitory components of pain modulation system (PMS) in SCI induced hyperalgesia were experimentally studied by assessing EMG response to temporal summation (TS) and diffuse noxious inhibitory control (DNIC) paradigms, respectively. The data of SCI rats revealed a significant increase in the amplitude of EMG during TS paradigm, while there was absence of disappearance of EMG response after application of pressure pain in DNIC paradigm. It can be inferred from these observations, that hyperalgesia observed in our SCI rats is possibly due to activation of descending facilitatory system simultaneous with attenuation of descending inhibitory system.

Activity of PMS is under the influence of various supra-spinal structures namely; ACC, insular cortex, primary and secondary somatosensory cortices, prefrontal cortex, AMY, PAG and hippocampus that are involved in the genesis of central neuropathic pain.[8,9,22] Present study explored the involvement of some of these areas in spinalised rats by utilizing c-Fos immunoreactivity. c-Fos has been extensively used as a marker of neuronal activity in animal models,[14,23,24] while functional MRI, quantitative EEG and regional blood flow techniques are selected for in-vivo patient/human studies. c-Fos is rapidly and specifically expressed only in nuclei. It is a robust and highly objective technique for determination of neuronal activity in animal models.[23] Our study revealed greater c-Fos immunoreactivity in AMY and ACC of SCI rats when compared to intact rats (Sham-SCI). These areas exert their control on the lower brain areas primarily through PAG located in the upper brain stem, locus ceruleus (LC) located in the dorsal brain stem and nucleus raphe magnus (NRM) and the nucleus reticularis gigantocellularis located in medulla.[25] PAG along with RVM bi-directionally modulates the peripheral nociceptive transmission at the spinal cord level via descending inhibitory and facilitatory system.[26-32] The PMS involves the opioid system associated with the release of endorphins; the adrenergic system associated with the release of NE and the serotonergic system associated with the release of serotonin. The interaction of these systems allows the nociceptive information to be sieved and either permitted or not permitted to further proceed.[25]

In our SCI rats, greater c-Fos immunoreactivity was seen in PAG besides ACC and AMY that is indicative of activation of DNIC pathway. However the expected behavioural analgesia is absent probably because of the injury to descending pathway to spinal cord dorsal horn.

There are several possible approaches for management after SCI in the experimental stage. These strategies are primarily directed towards promoting repair of the damage and/or minimising the secondary injury processes.[33] Besides several invasive and pharmacological management strategies, we selected to study the influence of ELF-MF exposure in SCI rats. ELF-MF has the advantage of being non-invasive, non- pharmacological and simple to administer management strategy. Previous reports from our laboratory have revealed attenuation of hyperalgesia; increased neuronal regeneration and myelination at the lesion site; increased osteogenesis; improvement in locomotion, food intake, water intake and body weight; restoration of tonic pain and the related neurotransmitter concentration in the brain of SCI rats.[6,11,13,14]

Exposure to ELF- MF perse decreased tonic pain in intact rats that is predominantly opioid mediated since naloxone pretreatment reversed the effect.[34] MF exposure improved 5 HT concentration in all parts of the brain in SCI rats which possibly restore the prevailing local biochemical milieu, that is of greater serotonin and lower opioid concentration and thereby restoring nociceptive responses in the MF exposed SCI rats. ELF-MF (55.6 Hz, 8.1 mT) is also reported to produce analgesia that is associated with increased concentrations of hypothalamus beta-endorphin, substance P and brainstem serotonin (5-HT) and are involved in determining the nociceptive status.[35,36]

MF exposure to our SCI rats significantly although not completely restored FPL and TTF, thereby suggesting a beneficial effect of MF on pain state. Moreover, reduction in amplitude of EMG response to TS in MF exposed SCI rats strongly indicates attenuation in the activation of facilitatory system. In the present study, the DNIC continued to remain attenuated even after MF exposure, thereby suggesting that the beneficial effect of MF in our rats can principally attributed primarily to a reduction in the activation of facilitatory system. This is supported by the reduction in c-Fos immunoreactivity observed in the supraspinal structures namely; ACC, PAG and AMY in our MF exposed SCI rats. This reduction in their neuronal activity significantly contributes to the SCI Inducted hyperalgesia. Therefore it is clear from this study that MF exposure reduced the pain possibly by reducing the activity of higher pain processing centers via PAG which have reduced the activation of facilitatory areas.

In our present study the ELF-MF exposure was given post-SCI day1 through day 56 so as to circumvent the cascade effect of the injurious factors after SCI. We assume that our daily exposure of MF on SCI rats possibly induced plastic changes at the site of injury and provided conducive environment for restoration of pain status besides improvement in locomotor function.

This study, for the first time, provides valuable insight into mechanism of beneficial effect of ELF MF on post-SCI pain and open new avenues for the understanding of SCI induced pain. Further studies are necessary to explore the exact mechanisms of MF action in more detail.

CONCLUSION

SCI leads to hyperagesia which has been found to be reduced due to ELF-MF exposure by inactivation of descending facilitatory system.

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