Top-Down Effect of Direct Current Stimulation on the Nociceptive Response of Rats

Luiz Fabio Dimov, Adriano Cardozo Franciosi, Ana Carolina Pinheiro Campos, André Russowsky Brunoni, Rosana Lima Pagano

1 Laboratory of Neuromodulation and Experimental Pain, Hospital Sírio Libanês, Rua Prof Daher Cutait, 69, São Paulo, SP, 01308–060, Brazil, 2 Service of Interdisciplinary Neuromodulation (SIN), Department and Institute of Psychiatry, Faculty of Medicine of University of São Paulo, Laboratory of Neuroscience (LIM27), Department and Institute of Psychiatry, University of São Paulo, Rua Doutor Ovidio Pires de Campos, 785, São Paulo, SP, 05403–000, Brazil, 3 Center for Clinical and Epidemiological Research & Interdisciplinary Center for Applied Neuromodulation (CINA), University Hospital, University of São Paulo, São Paulo, Avenida Professor Lineu Prestes 2565, ext. 3, São Paulo, SP, 05508–000, Brazil

* rosana.lpagano@hsl.org.br

Abstract

Transcranial direct current stimulation (tDCS) is an emerging, noninvasive technique of neurostimulation for treating pain. However, the mechanisms and pathways involved in its analgesic effects are poorly understood. Therefore, we investigated the effects of direct current stimulation (DCS) on thermal and mechanical nociceptive thresholds and on the activation of the midbrain periaqueductal gray (PAG) and the dorsal horn of the spinal cord (DHSC) in rats; these central nervous system areas are associated with pain processing. Male Wistar rats underwent cathodal DCS of the motor cortex and, while still under stimulation, were evaluated using tail-flick and paw pressure nociceptive tests. Sham stimulation and naive rats were used as controls. We used a randomized design; the assays were not blinded to the experimenter. Immunoreactivity of the early growth response gene 1 (Egr-1), which is a marker of neuronal activation, was evaluated in the PAG and DHSC, and enkephalin immunoreactivity was evaluated in the DHSC. DCS did not change the thermal nociceptive threshold; however, it increased the mechanical nociceptive threshold of both hind paws compared with that of controls, characterizing a topographical effect. DCS decreased the Egr-1 labeling in the PAG and DHSC as well as the immunoreactivity of spinal enkephalin. Altogether, the data suggest that DCS disinhibits the midbrain descending analgesic pathway, consequently inhibiting spinal nociceptive neurons and causing an increase in the nociceptive threshold. This study reinforces the idea that the motor cortex participates in the neurocircuitry that is involved in analgesia and further clarifies the mechanisms of action of tDCS in pain treatment.

Citation: Dimov LF, Franciosi AC, Campos ACP, Brunoni AR, Pagano RL (2016) Top-Down Effect of Direct Current Stimulation on the Nociceptive Response of Rats. PLoS ONE 11(4): e0153506. doi:10.1371/journal.pone.0153506

Editor: Andrea Antal, University Medical Center Goettingen, GERMANY

Received: December 8, 2015
Accepted: March 30, 2016
Published: April 12, 2016

Copyright: © 2016 Dimov et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This research was supported by the Hospital Sírio Libanês and São Paulo State Foundation (FAPESP). LFD, RLP and ARB were funded by FAPESP (11/21631-3, 09/50772-4 and 12/20911-5, respectively). ARB is supported by NARSAD Young Investigator from the Brain & Behavior Research Foundation (Grant Number 20493) and Brazilian National Council for Scientific and Technological Development (CNPq, Grant Numbers 303197 and 470904). The funders had no
Introduction

Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique with promising clinical outcomes for various conditions, including Parkinson’s disease [1], stroke [2], multiple sclerosis [3], schizophrenia [4], major depression [5] and pain [6]. This approach is relatively safe, well tolerated, affordable and easily deployable and consists of the application of a weak electric current to the scalp via two electrodes (anode and cathode); its efficacy depends critically on parameters such as electrode position and current strength [7]. The clinical effects of tDCS have been attributed to modulation of neuronal plasticity and synaptic connections [8–11], an increase in cortical excitability beyond the stimulation period [7,12] and the consolidation of this neuroplasticity, which is regulated by several mechanisms including acetylcholine, dopamine, serotonin, GABA, Na+ channel and NMDA-receptor activity [8,13–17]. Additional studies have shown a prolonged facilitation or inhibition of subcortical neuron activation, both in humans and in animals [18,19].

The analgesia induced by tDCS when applied over the primary motor cortex (M1) has been attributed to modulation of areas associated with pain processing, including the anterior cingulate, insula, thalamic nuclei and upper brainstem [9,20–25], as well as to regulation of glutamate, GABA and opioid activity, which results in the activation of descending analgesic pathways [25–28]. Evidence suggests that anodal tDCS increases the excitability of the underlying motor cortex, whereas cathodal tDCS diminishes cortical excitability [12,29,30]. Anodal tDCS over M1 produces long-lasting therapeutic effects in chronic pain conditions, including fibromyalgia [31–33], central pain [34,35], and phantom limb pain [36]. Experimental anodal direct current stimulation (DCS) in rats has been shown to induce thermal antinociceptive effects [37] and to reverse inflammatory chronic pain [38] and chronic stress-induced pain via the inhibition of hippocampal TNFα and spinal BDNF levels [39,40]. In addition, anodal DCS has been found to reverse mechanical hyperalgesia and the behavioral alterations in neurogenic rats, which is accompanied by changes in the BDNF and cytokine levels in different areas of the central nervous system [41,42]. Cathodal tDCS over M1 also induces analgesia in patients with chronic pain [43,44]. Both anodal and cathodal tDCS are known to increase the nociceptive threshold in healthy subjects [22,44–47]; however, until now, no experimental studies have been conducted to evaluate the cathodal DCS-induced antinociception in naive rats to better understand the neurocircuitry that mediates this response.

The expression of inducible transcription factors (ITFs) or immediate early genes, including egr-1 (zif-268, krox-24, or zenk), c-fos and c-jun, has been widely used to map neuronal activation under different physiological and non-physiological conditions [48–50]. ITFs are induced in neurons in response to extracellular stimuli, including depolarization, growth factors and neurotransmitters such as glutamate and substance P [51–56]. ITF expression can be elicited by various types of noxious stimuli, including chemical, thermal and mechanical stimuli, in different areas of the brain and the spinal cord. These factors thus become important tools for the study of the neural circuitry underlying nociception [50,57–61]. A direct correlation between decrease in ITF expression and antinociception in the DHSC has been suggested [62–64]. Few studies have evaluated the relationship between cortical stimulation, analgesia and ITFs. Using Egr-1 and c-Fos immunoreactivity (IR), epidural motor cortex stimulation has been shown to induce analgesia in naive and neuropathic rats, inhibiting the spinal neurons and activating the PAG [65–67]. Although a top-down effect of tDCS has been hypothesized, which would lead to activation of the descending analgesic pathway and consequently an increase in the nociceptive threshold, its inhibitory action on spinal nociceptive neurons has yet to be demonstrated.

The present study was conducted to investigate the effects of cathodal DCS on the thermal and mechanical pain sensitivity of healthy rats and to evaluate the pattern of neuronal
activation using Egr-1 detection in the midbrain periaqueductal gray (PAG), which is a key component of the descending analgesic pathway, and in the dorsal horn of the spinal cord (DHSC), which is the region where nociceptive information is received, integrated, and relayed to higher brain areas. In addition, to evaluate the involvement of the spinal opioid system, enkephalin labeling in the DHSC was also investigated.

**Materials and Methods**

Experimental design

Adult rats were evaluated with nociceptive tests (described in the Measuring nociceptive threshold section below), and subsequently, under anesthesia, epicranial electrodes were implanted over their left motor cortex involving the functional area of the right hind limb. After 5 days, the nociceptive tests were performed again in the animals while they were awake. Next, a group of rats received a single session of 15 minutes of DCS, after which, they were re-evaluated on the nociceptive tests while still under stimulation. Rats that were submitted to the surgical procedures but were not electrically stimulated (sham) and rats that did not receive any surgical procedure (naive) were also evaluated. After 1 hour of the last nociceptive evaluation, the animals were anesthetized, perfused, and their tissue fragments were processed for immunohistochemical analysis to evaluate the neuronal activation pattern in the PAG and DHSC and enkephalin expression in the DHSC (Fig 1).

**Animals**

Twenty-five male Wistar rats (180–220 g) were housed in acrylic boxes (three rats per cage) for at least two days before the initiation of the experimental procedures. The boxes were kept at a constant ambient room temperature with a controlled temperature (22±2°C) and a light/dark cycle (12/12 h), and they had wood shavings and free access to water and rat chow pellets. All procedures were in accordance with the guidelines on the ethical use of animals in research involving pain and nociception [68] and were approved by the Ethics Committee on the Use of Animals at Hospital Sírio Libanês (Sao Paulo, Brazil) under protocol number CEUA 2011/24.

**Fig 1. Experimental design.** Rats were habituated to the paw pressure test in the day 1. In the second day, they were evaluated in the paw pressure and tail flick tests, and subsequently, under anesthesia, epicranial electrodes were implanted over their left motor cortex. After 5 days (day 7), the nociceptive tests were performed again. A group of rats received a single session of 15 minutes of DCS (250 μA), after which, they were re-evaluated on the nociceptive tests while still under stimulation. Rats that were submitted to the surgical procedures but were not electrically stimulated (sham) and rats that did not receive any surgical procedure (naive) were also evaluated. After 1 hour of the last nociceptive evaluation, the animals were anesthetized, perfused, and their tissue fragments were processed for immunohistochemical analysis.

doi:10.1371/journal.pone.0153506.g001
Electrode implantation and electrical stimulation

Mimicking the clinical protocol that induces analgesia in healthy humans, we decided to use cathodal DCS on naive rats in our study. The electrode fixation and the stimulation protocol were applied according to an earlier study [69]. Briefly, rats were deeply anesthetized with ketamine/xylazine (50/20 mg/kg, intraperitoneal) as well as with a local scalp injection of 2% lidocaine (100 μL/animal, subcutaneous). Then, under stereotactic guidance using a map developed by our group [70], an epicranial electrode that consisted of a tubular plastic jacket was fixed onto the skull over the left primary motor cortex (M1) in the functional area of the right hind limb (1 mm caudal to bregma; 1 mm left to midline). Rats were treated with ketoprofen (10 mg/kg, subcutaneous) for three days after surgery. Five days after implantation of the electrode, the epicranial jacket was filled with a conductive gel (Electron Plus<sup>®</sup>, Hal, SP, Brazil), and the cathode was inserted. The animals were dressed in vests that placed a large rubber plate on the ventral thorax, which served as the counter electrode (anode). The electrodes were connected to an electrical stimulator (Striat<sup>®</sup>, IBRAMED, SP, Brazil) with wire leads, which allowed the animals to move freely in the cage (S1 Fig). The experimental group received a single DCS session of 15 minutes (250 μA over an area of 2.27 mm<sup>2</sup>), and the final measurements during the nociceptive tests were recorded while the rats were still under stimulation. The sham group was subjected to the same conditions but did not receive stimulation. The rats were randomly divided into the sham and stimulated groups. In total, the animals were divided into three groups: rats with no surgical procedure (naive, n = 7), rats with an epicranial electrode and false stimulation (sham, n = 7), and stimulated rats (n = 7). Four animals were excluded from the study because they removed their implants before the final nociceptive tests.

Measuring nociceptive threshold

Nociceptive tests were conducted prior to the electrode implantation and five days after, before (initial measurement) and during DCS (final measurement). Naive and sham rats were also evaluated. On the day of the test, the animals were brought into a separate quiet room 1 hour before the nociceptive tests to allow them to habituate to the environment. The thermal nociceptive threshold was determined using the tail-flick test, which has been previously described [71]. Briefly, radiant heat was focused on the lower third of their tail using an analgesiometer (EEF 300, Insight<sup>®</sup>, SP, Brazil). Movement of the tail activated a photocell, thereby turning off both the light and a reaction timer. The nociceptive threshold was defined as the time necessary to induce the tail-flick response. The mechanical nociceptive threshold was determined using a pressure apparatus on the right hind paw (EEF-440, Insight<sup>®</sup>), which has also been previously described [72]. Briefly, the mass (in grams) required to induce a withdrawal response represented the nociceptive threshold. The results were analyzed by comparing between the initial and final measurements. In order to reduce animal stress, the rats were handled by only one experimenter and were habituated to the paw pressure test the day preceding the electrode implantation. Both nociceptive tests were performed before electrode implantation (basal measurement) to evaluate if the nociceptive threshold could be changed after surgical procedure.

Immunohistochemistry

One hour after the last nociceptive test, the rats were deeply anesthetized with ketamine and xylazine and then subjected to transcardiac perfusion with saline solution followed by 4% paraformaldehyde (PFA) dissolved in 0.1 M phosphate buffer (PB). The animals were perfused 1 hour after the last nociceptive stimulus because the peak expressions of ITF proteins occur approximately 1 hour after the stimulus and fade by 3 to 4 hours post-stimulation [50]. The brain and lumbar spinal cord (L2-L5 segments) were collected and post-fixed in PFA for 4
hours, followed by incubation with 30% sucrose solution in PB for 48 hours at 4°C. Tissue sections (30 μm) were cut on a freezing microtome, washed in PB, and incubated for 12 to 16 hours at 4°C with rabbit anti-Egr-1 (Zif268, 1:1000, C-19; Santa Cruz Biotechnology, CA, USA) or mouse anti-enkephalin (ENK, 1:1000, MAB350, Millipore, CA, USA) primary antibodies that were diluted in 0.3% of Triton X-100 containing 5% normal donkey serum (Jackson ImmunoResearch, ME, USA). Then, sections were incubated for 2 hours at room temperature with biotinylated secondary antibodies (Jackson ImmunoResearch, ME, USA) and incubated with an avidin-biotin complex (1:100, ABC Elite kit, Vector Labs, CA, USA) for 2 hours at room temperature. The sections were visualized with 0.05% diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich, MO, USA) and 0.03% (final concentration) hydrogen peroxide in PB. The sections were then mounted on glass slides, air-dried, dehydrated, and coverslipped with Permount (Fisher Scientific, PA, USA). The samples were washed between each step (3 x 10 min). The immunoreactivity was analyzed using a light microscope (E1000, Nikon, NY, USA) and ImageJ software (National Institute of Health, MD, USA; http://rsbweb.nih.gov/ij/). Figures were assembled using Adobe Photoshop (Adobe Systems, CA, USA); the images were converted to black and white and optimized using contrast and brightness only. Quantitative analysis was performed to determine the density of nuclei showing positive immunoreactivity (IR) for Egr-1 in the rostral portion of the PAG (dorsomedial, dorsolateral, lateral and ventrolateral columns) and DHSC (laminae I-IV) and the IR of ENK in the DHSC (laminae I-VI). Measurements were taken from 7 different sections for each analyzed animal. The regions of interest were identified based on a stereotaxic atlas [73] and an atlas of the spinal cord [74].

Statistical analysis

Data are presented as the mean ± standard error of the mean. Statistical analyses were conducted with GraphPad Prism 5.0 software (GraphPad Software Inc; La Jolla, CA, USA). The results of the nociceptive tests were analyzed using two-way repeated measures analysis of variance (ANOVA) followed by the Bonferroni post hoc test. Immunohistochemistry data were analyzed using one-way ANOVA followed by the Bonferroni post hoc test. In all cases, p < 0.05 was considered statistically significant.

Results

Cathodal DCS did not change the thermal nociceptive threshold in the tail of the animals (Factor Time x Factor Treatment, F(2,9) = 0.08030, p = 0.92) (Fig 2A). However, it was able to increase the mechanical nociceptive threshold of the left (Factor Time x Factor Treatment, F(2,27) = 15.37, p < 0.0001) and right (Factor Time x Factor Treatment, F(2,27) = 32.60, p < 0.0001) hind paws (53% and 73% increases, respectively) compared with that of the naive and sham animals (Fig 2B). The results obtained in the nociceptive tests, before electrode implantation in the basal measurement (data not showed), were equal to the data observed after 5 days of the surgical procedure, showing that the increase of nociceptive threshold after the DCS was a consequence of the electrical stimulation.

The immunohistochemistry analysis showed a bilateral decrease in Egr-1-positive neurons in the PAG after DCS (F(2,11) = 12.10, p = 0.0028, left hemisphere; F(2,12) = 15.30, p = 0.0009, right hemisphere), primarily in the ventrolateral PAG (Fig 3), compared with the results of the control groups. Furthermore, our results showed that DCS bilaterally decreased (F(2,9) = 17.05, p = 0.0020, left side; F(2,9) = 28.93, p = 0.0004, right side) the Egr-1-positive neurons in the DHSC (Fig 4) when compared with control animals.
Cathodal DCS bilaterally decreased \( F(2,11) = 11.06, p = 0.0038, \) left side; \( F(2,11) = 5.503, p = 0.0275, \) right side) the spinal ENK immunoreactivity compared with that of the naive group (Fig 5).

Discussion

tDCS has been used in the treatment of various chronic pain disorders [31–36]; however, its mechanism of action remains poorly understood. We showed here that cathodal DCS, applied over the functional area of the hind limb, did not interfere with the thermal nociceptive threshold in the tail. Evidence indicates that the analgesic effect induced by cortical stimulation depends on the electrode position in the cortical somatotopy representation of pain perception [75,76]. In this regard, antinociception induced by epidural motor cortex stimulation in healthy rats was shown to have a topographic relationship with the stimulated area of the M1 [67]. Our results confirm this somatotopic analgesic effect induced by cortical stimulation, as cathodal DCS did not alter the nociceptive threshold in the tail but induced a bilateral increase of the mechanical nociceptive threshold in the hind paws of rats. In contrast with our findings, anodal DCS over the M1 has been shown to increase the nociceptive threshold in naive rats in the tail-flick test [37]. However, our results are consistent with findings obtained in healthy humans who were subjected to cathodal tDCS of the motor cortex; this procedure resulted in an increase in the cold and mechanical detection thresholds but not in the heat threshold, suggesting that cathodal tDCS temporarily reduces sensitivity to the A-fibers mediating the somatosensory inputs [46]. Interestingly, similar to the bilaterality that was observed in our results for the mechanical threshold, the aforementioned study also demonstrated bilateral changes in the cold detection threshold [46].

Another difference that could account for the differential antinociception observed in this study is the polarity-specific alteration in cortical blood flow (CBF) induced by tDCS. Cathodal tDCS results in a decrease in CBF in the cortical area, whereas anodal tDCS results in an increase [77]. In addition to this localized effect, the CBF decrease induced by cathodal tDCS spreads to target-adjacent areas [78]. This widespread modulation could, in turn, account for the widespread effect (namely, the bilateral antinociception) that was observed in our results.
Additionally, another hypothesis considers diffuse noxious inhibitory control (DNIC) activation, which refers to the supraspinally mediated inhibitory process that occurs in the pain-signalning neurons of the DHSC in response to heterogenic noxious stimuli [79, 80]. In fact, tDCS displays synergistic effects when combined with a DNIC approach [22]. As suggested, a plausible mechanism could be that tDCS elicits activation of the same neural circuits that are associated with DNIC, particularly the endogenous descending analgesic pathways [22].

The most prominent neurocircuitry involved in the descending control of pain is the PAG, as activating the rostral ventromedial medulla and the locus ceruleus, which project their serotonergic and noradrenergic descending fibers, respectively, to the DHSC, results in the specific inhibition of the firing of spinal nociceptive neurons [81–83]. To evaluate whether the cathodal DCS-induced antinociception in naive rats was mediated by activation of the descending analgesic system, we evaluated the neuronal activation in the PAG and DHSC. DCS bilaterally decreased the Egr-1 immunolabeling in the PAG, primarily in the ventrolateral and lateral subdivision, and in the superficial laminae of the DHSC. Based on anatomic and functional patterns, the PAG has been subdivided into four columns: the dorsomedial, dorsolateral, lateral and ventrolateral [84]. Antinociception effects of different types are mediated by the lateral...
and ventrolateral PAG [85–87]; our findings corroborate this idea, as the changes in the activation pattern that occurred in these subdivisions were more evident. As previously shown, epidural motor cortex stimulation induces antinociception in healthy rats by enhancing the neuronal firing rate and Fos immunoreactivity within the PAG and, by simultaneously decreasing GABA and Egr-1 labeling; this suggests an inhibition of local GABAergic interneurons with subsequent activation of the excitatory neurons responsible for descending analgesic control [66]. Similarly, França et al. [67] also showed an increase in Fos immunolabeling in the different subdivisions of the PAG of naive rats after epidural stimulation; however, this increase was also observed in Egr-1. Further investigation of the PAG neurocircuitry involved in the tDCS-induced analgesic effect is needed to clarify which populations of neurons are modulated by this therapeutic intervention. Our results also suggest that cathodal DCS induces a disinhibition of the PAG with resulting activation of the descending analgesic pathways, increasing the nociceptive threshold through a top-down modulation mechanism, as previous proposed [22,26,40]. The immunohistochemistry results observed in this study demonstrate that the results of behavioral studies are consistent with both altered activation of the PAG, which is the main region of the midbrain descending analgesic pathway, and a decrease in the activation of spinal nociceptive neurons, which are the main input for ascending nociceptive information. As the expression of Egr-1 in the DHSC is directly related to the activity of nociceptive neurons [59], our results suggest that DCS leads to a decrease in the transmission of ascending nociceptive information. These results are supported by others who have demonstrated, after epidural cortical stimulation, a relationship between analgesia and decreased spinal Egr-1 in naive and neuropathic rats [65,67].

![Fig 4. DCS and neuronal activation in the DHSC. Quantification of the Egr-1-positive nuclei in the DHSC of naive (without surgical intervention), sham (with epicranial electrode without stimulation) and stimulated (DCS, 250 μA, 15 min) rats. Values represent the mean ± SEM (n = 7 animals per group). *p < 0.05, when compared to the naive group (A). Photomicrographs show the Egr-1-positive nuclei staining in the superficial laminae of the DHSC of naive (B, C) and stimulated (D, E) rats. Sections of the left (B, D) and right (C, E) DHSC. Scale bars: 50 μm. DCS: direct current stimulation; DHSC: dorsal horn of the spinal cord; LS: left side; RS: right side.](doi:10.1371/journal.pone.0153506.g004)
A growing body of evidence has indicated the participation of the opioid system in the analgesia induced by cortical stimulation [27,28,88–91]. In an attempt to evaluate the involvement of the spinal opioid system in the antinociception induced by cathodal DCS in healthy rats, we evaluated enkephalin (ENK) labeling in the DHSC. Enkephalins are endogenous opiates that play an important role in the modulation of nociceptive information by mediating synaptic transmission in several areas of the central nervous system [92,93]. Most ENK present in the DHSC originates from local interneurons, although a few enkephalinergic fibers may descend from the brainstem [94–96] or originate from dorsal root ganglion neurons [97, 98]. In the DHSC, ENK is highly concentrated in the neurons located in the superficial laminae I and II and is sparsely distributed in the deep laminae V–VII and around the central canal [99, 100]. Additionally, ENK binds to both μ and δ opioid receptors with similar affinities but is inactive at κ opioid receptors [101]. Spinal ENK inhibits neurotransmitter release by the nociceptive primary afferents and the spinothalamic projection neurons in response to noxious peripheral stimuli [102–104]. Indeed, a positive correlation between an increase in spinal ENK and analgesia in chronic pain conditions has been shown [105–107]. Nevertheless, in the absence of persistent pain, spinal opioid release has been shown to be inhibited by activation of the descending analgesic system [108,109], which could serve to shut down the spinal opioid system whenever the descending serotonergic and noradrenergic pathways are active, thus preventing undesirable analgesia under normal conditions. Our findings are consistent with this process; our results show that during the DCS-induced antinociception in healthy rats, a decrease in spinal ENK concomitant with an inhibition of nociceptive neurons in the DHSC occurs, likely due to activation of the descending analgesic system.

Fig 5. DCS and enkephalin immunostaining in the DHSC. Quantification of the enkephalin (ENK) immunoreactivity (IR) in the DHSC of naive (without surgical intervention), sham (with epicranial electrode without stimulation) and stimulated (DCS, 250 µA, 15 min) rats. Values represent the mean ± SEM (n = 7 animals per group). *p < 0.05, when compared to the naive group (A). Photomicrographs show the ENK-IR in the DHSC of naive (B, D, E) and stimulated (C, F, G) rats. Figs B and C show higher magnification insets of laminae I-II of naive and stimulated animals, indicated by asterisks in figs E and G, respectively. Sections of the left (D, F) and right (B, C, E, G) DHSC. Scale bars: 50 µm (B and C) and 100 µm (D-G). Laminae I-VI of the DHSC are represented in figs D-G. DCS: direct current stimulation; DHSC: dorsal horn of the spinal cord; LS: left side; RS: right side.
The brainstem serotonergic and noradrenergic fibers are the main components of the descending analgesic pathways, as the release of their biogenic amines in the DHSC result in inhibition of spinal nociceptive neurons [82]. However, although the majority of serotonergic fibers act directly on the spinal nociceptive neurons, the noradrenergic fibers have been shown to act indirectly through the secondary release of opiate-like substances [110,111]. As DCS is a modulatory intervention that targets the endogenous neural circuits, it could induce a differential activation of such parallel pathways, resulting in enhanced serotonergic fiber-mediated and reduced noradrenergic fiber-mediated effects and a subsequent decrease in ENK release. Despite this differential modulation, the net effect is still the activation of the descending analgesic system culminating with inhibition of the spinal nociceptive neurons. This hypothesis also explains our results, which showed a decrease in both ENK and Egr-1 immunoreactivity in the DHSC.

Altogether, the present findings suggest that DCS induces disinhibition of the midbrain descending analgesic pathway with a subsequent inhibition of spinal nociceptive neurons, leading to an increase in the nociceptive threshold. This study reinforces the idea that the motor cortex is involved in the neural circuits that control the nociceptive response and highlights the importance of further studies evaluating the clinical use of tDCS as an alternative for pain control.

Supporting Information

S1 Fig. Cathodal DCS treatment. The epicranial electrode was fixed onto the skull over the left primary motor cortex (1 mm caudal to bregma; 1 mm left to midline). Before DCS, the epicranial jacket was filled with a conductive gel and the cathode was inserted. The animals were dressed in vests that placed a large rubber plate on the ventral thorax, which served as the counter electrode (anode). The electrodes were connected to an electrical stimulator with wire leads, which allowed the animals to move freely in the cage. Afterward, rats received a single DCS session of 15 minutes.

(TIF)

Acknowledgments

This research was supported by the Hospital Sírio Libanês and São Paulo State Foundation (FAPESP). LFD, RLP and ARB were funded by FAPESP (11/21631-3, 09/50772-4 and 12/20911-5, respectively). ARB is supported by NARSAD Young Investigator from the Brain & Behavior Research Foundation (Grant Number 20493) and Brazilian National Council for Scientific and Technological Development (CNPq, Grant Numbers 303197 and 470904).

Author Contributions

Conceived and designed the experiments: RLP ARB. Performed the experiments: LFD. Analyzed the data: LFD ACPC. Contributed reagents/materials/analysis tools: LFD ACF ACPC.

Wrote the paper: LFD ACF ACPC ARB RLP.

References

1. Benninger DH, Lomarev M, Lopez G, Wassermann EM, Li X, Considine E, et al. Transcranial direct current stimulation for the treatment of Parkinson’s disease. J Neurol Neurosurg Psychiatry. 2010; 81:1105–11. doi:10.1136/jnnp.2009.202556 PMID: 20870863
2. Sohn MK, Jee SJ, Kim YW. Effect of transcranial direct current stimulation on postural stability and lower extremity strength in hemiplegic stroke patients. Ann Rehabil Med. 2013; 37:759–65. doi: 10.5535/arm.2013.37.6.759 PMID: 24466510
3. Ferrucci R, Vergari M, Cogiamanian F, Bocci T, Ciocca M, Tomasini E, et al. Transcranial direct current stimulation (tDCS) for fatigue in multiple sclerosis. NeuroRehabilitation. 2012; 34:121–7.

4. Brunoni AR, Shiozawa P, Truong D, Javitt DC, Elkis H, Fregni F, et al. Understanding tDCS effects in schizophrenia: a systematic review of clinical data and an integrated computation modeling analysis. Expert Rev Med Devices. 2014; 11:383–94. doi: 10.1586/17434440.2014.911082 PMID: 24754366

5. Shiozawa P, Fregni F, Benseñor IM, Lotufo PA, Berlim MT, Daskalakis JZ, et al. Transcranial direct current stimulation for major depression: an updated systematic review and meta-analysis. Int J Neuropsychopharmacol. 2014; 17:1443–52. doi: 10.1017/S1461145714000418 PMID: 24713139

6. Fregni F, Freedman S, Pascual-Leone A. Recent advances in the treatment of chronic pain with non-invasive brain stimulation techniques. Lancet Neurol. 2007; 6:188–91. PMID: 17239806

7. Nitsche MA, Fricke K, Henschke U, Scheltert A, Liebetanz D, Lang N, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol. 2003; 553:293–301. PMID: 12949224

8. Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain. 2002; 125:2238–47. PMID: 12244081

9. Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, et al. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? Eur J Neurosci. 2005; 22:495–504. PMID: 16045502

10. Nitsche MA, Seeber A, Frommank K, Klein CC, Rochford C, Nitsche MS, et al. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol. 2005; 568:291–303. PMID: 16002441

11. Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: State of the art 2008. Brain Stimul. 2008; 1:206–23. doi: 10.1016/j.brs.2008.06.004 PMID: 20633386

12. Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol. 2000; 527:633–9. PMID: 10990547

13. Nitsche MA, Grundey J, Liebetanz D, Lang N, Tergau F, Paulus W. Catecholaminergic consolidation of motor cortical neuroplasticity in humans. Cereb Cortex. 2004; 14:1240–5. PMID: 15142961

14. Nitsche MA, Jaussky W, Liebetanz D, Lang N, Tergau F, Paulus W. Consolidation of human motor cortical neuroplasticity by D-cycloserine. Neuropsychopharmacology. 2004; 29:1573–8. PMID: 15199378

15. Nitsche MA, Liebetanz D, Scheltert A, Henschke U, Fricke K, Frommank K, et al. GABAergic modulation of DC-stimulation-induced motor cortex excitability shifts in humans. Eur J Neurosci. 2004; 19:2720–6. PMID: 15147306

16. Kuo MF, Grosch J, Fregni F, Paulus W, Nitsche MA. Focusing effect of acetylcholine on neuroplasticity in the human motor cortex. J Neurosci. 2007; 27:14442–7. PMID: 18160552

17. Terney D, Bergmann I, Poreisz C, Chaieb L, Boros K, Nitsche MA, et al. Pergolide increases the efficacy of cathodal direct current stimulation to reduce the amplitude of laser-evoked potentials in healthy humans. J Pain Symptom Manage. 2008; 36:79–91. doi: 10.1016/j.jpainsymman.2007.08.014 PMID: 18358692

18. Di Lazzaro V, Ziemann U, Lemon RN. State of the art: physiology of transcranial motor cortex stimulation. Brain Stimul. 2008; 1:345–62. doi: 10.1016/j.brs.2008.07.004 PMID: 20633393

19. Brunoni AR, Fregni F, Pagano RL. Translational research in transcranial direct current stimulation (tDCS): a systematic review of studies in animals. Rev Neurosci. 2011; 22:471–81. doi: 10.1515/RNS.2011.042 PMID: 21819264

20. Holsheimer J, Nguyen JP, Lefaucheur JP, Manola L. Cathodal, anodal or bifocal stimulation of the motor cortex in the management of chronic pain? Acta Neurochir Suppl. 2007; 97:57–66.

21. Zaghi S, Heine N, Fregni F. Brain stimulation for the treatment of pain: a review of costs, clinical effects and mechanisms of treatment for three different central neuromodulatory approaches. J Pain Manag. 2009; 2:339–52. PMID: 20063574

22. Reidler JS, Mendonca ME, Santana MB, Wang X, Lenkinski R, Motta AF, et al. Effects of motor cortex modulation and descending inhibitory systems on pain thresholds in healthy subjects. J Pain. 2012; 13:450–8. doi: 10.1016/j.jpain.2012.01.006 PMID: 22515945

23. DaSilva AF, Mendonca ME, Zaghi S, Lopes M, DosSantos MF, Spierings EL, et al. tDCS-induced analgesia and electrical fields in pain-related neural networks in chronic migraine. Headache. 2012; 52:1283–95. doi: 10.1111/j.1526-4610.2012.01411.x PMID: 22912348

24. DaSilva AF, Truong DQ, DosSantos MF, Toback RL, Datta A, Bikson M. State-of-art neuroanatomical target analysis of high-definition and conventional tDCS montages used for migraine and pain control. Front Neuroanat. 2015; 9:89. doi: 10.3389/fnana.2015.00089 PMID: 26236199
25. Foerster BR, Nascimento TD, Deboer M, Bender MA, Rice IC, Truong DQ, et al. Excitatory and inhibitory brain metabolites as targets of motor cortex transcranial direct current stimulation therapy and predictors of its efficacy in fibromyalgia. Arthritis Rheumatol. 2015; 67:576–81. PMID: 25371883

26. Lima MC, Fregni F. Motor cortex stimulation for chronic pain: systematic review and meta-analysis of the literature. Neurology. 2008; 2329:37–70.

27. DosSantos MF, Love TM, Martikainen IK, Nascimento TD, Fregni F, Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Oliveira C, Scarabelot VL, et al. Transcranial direct current stimulation and nociception. Brain Stimul. 2015; 8:622–74. doi: 10.15226/2132-1932.BS.2015.02

28. DosSantos MF, Love TM, Martikainen IK, Nascimento TD, DeBoer MD, Schambra HM, et al. Building up analgesia in humans via the endogenous opioid system by combining placebo and active tDCS: a preliminary report. PLoS One. 2014; 9:e102350. doi: 10.1371/journal.pone.0102350 PMID: 25029273

29. Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology. 2001; 57:1899–901. PMID: 11723286

30. Wassermann EM, Grafman J. Recharging cognition with DC brain polarization. Trends Cogn Sci. 2005; 9:503–5. PMID: 16182596

31. Fregni F, Gimenes R, Valle AC, Ferreira MJ, Rocha RR, Natallie L, et al. A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia. Arthritis Rheumatol. 2006; 54:3988–98.

32. Fagerlund AJ, Hansen OA, Aslaksen PM. Transcranial direct current stimulation as a treatment for patients with fibromyalgia: a randomized controlled trial. Pain. 2015; 156:62–71. doi: 10.1016/j.pain.2014.09.006 PMID: 25599302

33. Castillo-Saavedra L, Gebodh N, Bikson M, Diaz-Cruz C, Brandao R, Coutinho L, et al. Clinically effective treatment of fibromyalgia pain with high-definition transcranial direct current stimulation: Phase II placebo-controlled, proof of concept study. J Pain. 2015; 16:657–65. doi: 10.1016/j.jpain.2015.03.013 PMID: 25863170

34. Nekhendzy V, Fender CP, Davies MF, Lemmens HJ, Kim MS, Bouley DM, et al. The antinociceptive effect of transcranial electrostimulation with combined direct and alternating current in freely moving rats. Anesth Analg. 2004; 98:730–7. PMID: 14980928

35. Mehta S, McIntyre A, Guy S, Teasell RW, Loh E. Effect of transcranial direct current stimulation on pain and function in fibromyalgia: a randomized, double-blind, sham-controlled, proof of concept study. Pain. 2010; 148:9–16. doi: 10.1016/j.pain.2009.07.010 PMID: 19837677

36. Spezia Adachi LN, Quevedo AS, de Souza A, Scarabelot VL, Rozisky JR, de Oliveira C, et al. Exogenous induced brain activation regulates neuronal activity by top-down modulation: conceptualized model for electrical brain stimulation. Exp Brain Res. 2015; 233:1377–89. doi: 10.1007/s00221-015-4212-1 PMID: 26565871

37. Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. Brain Stimul. 2015; 8:195–202. doi: 10.1016/j.brs.2014.12.002

38. Fagerlund AJ, Hansen OA, Aslaksen PM. Transcranial direct current stimulation as a treatment for chronic pain: results of a sham-controlled, proof of principle study. Pain. 2015; 156:62–71. doi: 10.1016/j.pain.2015.03.013 PMID: 26193817

39. Castillo-Saavedra L, Gebodh N, Bikson M, Diaz-Cruz C, Brandao R, Coutinho L, et al. Clinically effective treatment of fibromyalgia pain with high-definition transcranial direct current stimulation: Phase II placebo-controlled, proof of concept study. J Pain. 2015; 16:657–65. doi: 10.1016/j.jpain.2015.03.013 PMID: 25863170

40. Nekhendzy V, Fender CP, Davies MF, Lemmens HJ, Kim MS, Bouley DM, et al. The antinociceptive effect of transcranial electrostimulation with combined direct and alternating current in freely moving rats. Anesth Analg. 2004; 98:730–7. PMID: 14980928

41. Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. Brain Stimul. 2015; 8:195–202. doi: 10.1016/j.brs.2014.12.002

42. Filho PR, Vercelino R, Cioato SG, Medeiros LF, de Oliveira C, Scarabelot VL, et al. Transcranial direct current stimulation (tDCS) reverts behavioral alterations and brainstem BDNF level increase induced by neuropathic pain model: Long-lasting effect. Prog Neuropsychopharmacol Biol Psychiatry. 2016; 64:44–51. doi: 10.1016/j.pnpbp.2015.08.016 PMID: 26160898

43. Villamar MF, Wiatravongvana P, Patumanond J, Bikson M, Truong DQ, Datta A, et al. Focal modulation of the primary motor cortex in fibromyalgia using 4x1-ring high-definition transcranial direct current stimulation (HD-tDCS): immediate and delayed analgesic effects of cathodal and anodal stimulation. J Pain. 2013; 14:371–83. doi: 10.1016/j.jpant.2012.12.007 PMID: 23415877
44. Vaseghi B, Zoghi M, Jaberzadeh S. A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain. PLoS One. 2015; 10:e0123873. doi: 10.1371/journal.pone.0123873. PMID: 25978673

45. Boggio PS, Zaghi S, Lopes M, Fregni F. Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. Eur J Neurol. 2008; 15:1124–30. doi: 10.1111/j.1468-1331.2008.02270.x. PMID: 18717717

46. Bachmann CG, Muschinsky S, Nitsche MA, Rolke R, Magerl W, Treede RD, et al. Transcranial direct current stimulation of the motor cortex induces distinct changes in thermal and mechanical sensory percepts. Clin Neurophysiol. 2010; 121:2083–9. doi:10.1016/j.clinph.2010.05.005. PMID: 20570558

47. Vaseghi B, Zoghi M, Jaberzadeh S. Differential effects of cathodal transcranial direct current stimulation of prefrontal, motor and somatosensory cortices on cortical excitability and pain perception—a double-blind randomized sham-controlled study. Eur J Neurosci. 2015; 42:2426–37. doi:10.1111/ejn.13043. PMID: 26275236

48. Herrera DG, Robertson HA. Activation of c-fos in the brain. Prog Neurobiol. 1996; 50:83–107. PMID: 8971979

49. Beckmann AM, Wilce PA. Egr transcription factors in the nervous system. Neurochem Int. 1997; 31:477–510. PMID: 9307998

50. Herdegen T, Leah JD. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Brain Res Rev 1998; 28:370–490. PMID: 9858769

51. Sheng M, Greenberg ME. The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron. 1990; 4:477–85. PMID: 1969743

52. Chapman V, Buritova J, Honoré P, Besson JM. Physiological contributions of neurokinin 1 receptor activation, and interactions with NMDA receptors, to inflammatory-evoked spinal c-Fos expression. J Neurophysiol. 1996; 76:1817–27. PMID: 8890294

53. Tao YX, Wei F, Zhao ZQ. A contribution of neurokinin-1 receptor to formalin-induced c-fos expression in the rat spinal dorsal horn. Neurosci Lett. 1997; 221:105–8. PMID: 9121675

54. Hiscock JJ, Mackenzie L, Medvedev A, Willoughby JO. Kainic acid and seizure-induced Fos in subtypes of cerebrocortical neurons. J Neurosci Res 2001; 66:1094–100. PMID: 11746441

55. Szakács R, Weiczner R, Mihály A, Krisztin-Péva B, Zádor Z, Zádor E. Non-competitive NMDA receptor antagonists moderate seizure-induced c-fos expression in the rat cerebral cortex. Brain Res Bull. 2003; 59:485–93. PMID: 12576146

56. Soyguder Z. Multiple neurotransmitter receptors contribute to the spinal Fos expression.* Brain Res. 2006; 1033:202–9. PMID: 15694925

57. Hunt SP, Pini A, Evan G. Induction of c-fos-like protein in spinal cord neurons following sensory stimulation. Nature. 1987; 328:632–4. PMID: 3112583

58. Herdegen T, Walker T, Leah JD, Bravo R, Zimmermann M. The KROX-24 protein, a new transcription regulating factor: expression in the rat central nervous system following afferent somatosensory stimulation. Neurosci Lett. 1990; 120:21–4. PMID: 2293086

59. Herdegen T, Kovary K, Leah J, Bravo R. Specific temporal and spatial distribution of JUN, FOS, and KROX-24 proteins in spinal neurons following noxious transsynaptic stimulation. J Comp Neurol. 1991; 313:178–91. PMID: 1761754

60. Lanteri-Minet M, Isnardon P, de Pommery J, Menetrey D. Spinal and hindbrain structures involved in visceral nociception as revealed by the expression of Fos, Jun and Krox-24 proteins. Neuroscience 1993; 55:737–53. PMID: 8413935

61. Harris JA. Using c-fos as a neural marker of pain. Brain Res Bull. 1998; 45:1–8. PMID: 9434195

62. Morgan JI, Curran T. Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. Trends Neurosci. 1989; 12:459–62. PMID: 2479148

63. Gogas KR, Presley RW, Levine JD, Basbaum AI. The antinociceptive action of supraspinal opioids results from an increase in descending inhibitory control: correlation of nociceptive behavior and c-fos expression. Neuroscience. 1991; 42:617–28. PMID: 1659673

64. Buritova J, Honoré P, Besson JM. Indomethacin reduces both Krox-24 expression in the rat lumbar spinal cord and inflammatory signs following intraplantar carrageenan. Brain Res. 1995; 674:211–20. PMID: 7796099

65. Pagano RL, Assis DV, Clara JA, Alves AS, Dale CS, Teixeira MJ, et al. Transdural motor cortex stimulation reverses neuropathic pain in rats: a profile of neuronal activation. Eur J Pain. 2011; 15:268.e1–14. doi: 10.1016/j.ejpain.2010.08.003. PMID: 20817578
66. Pagano RL, Fonoff ET, Dale CS, Ballester G, Teixeira MJ, Britto LR. Motor cortex stimulation inhibits thalamic sensory neurons and enhances activity of PAG neurons: possible pathways for antinociception. Pain. 2012; 153:2359–69. doi: 10.1016/j.pain.2012.08.002 PMID: 23017297

67. França NR, Toniolo EF, Franciosi AC, Alves AS, de Andrade DC, Fonoff ET, et al. Antinociception induced by motor cortex stimulation: somatotopy of behavioral response and profile of neuronal activation. Behav Brain Res. 2013; 250:211–21. doi: 10.1016/j.bbr.2013.05.019 PMID: 23692698

68. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983; 16:109–10. PMID: 6877845

69. Liebetanz D, Klinker F, Hering D, Koch R, Nitsche MA, Potschka H, et al. Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. Epilepsia. 2006; 47:1216–24. PMID: 16886986

70. Fonoff ET, Pereira JP Jr, Camargo LV, Dale CS, Pagano RL, Ballester G, et al. Functional mapping of the motor cortex of rat using transdural electrical stimulation. Behav Brain Res. 2009; 202:138–41. doi: 10.1016/j.bbr.2009.03.018 PMID: 19447290

71. D’Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharm Exp Ther. 1941; 72:9.

72. Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther. 1957; 111:409–19. PMID: 13471093

73. Paxinos GW, Watson C. The rat brain in stereotaxic coordinates. San Diego: Academic Press

74. Molander C, Xu Q, Grant G. The cytoarchitectonic organization of the spinal cord in the rat. I. The lower thoracic and lumbarosacral cord. J Comp Neurol. 1984; 230:133–41. PMID: 6512014

75. Canavero S, Bonicalzi V. Cortical stimulation for central pain. Journal of Neurosurgery 1995; 83:1117. PMID: 7490637

76. Nguyen JP, Lefaucheur JP, Decq P, Uchiyama T, Carpentier A, Fontaine D, et al. Chronic motor cortex stimulation in the treatment of central and neuropathic pain. Correlations between clinical, electrophysiological and anatomical data. Pain. 1999; 82:245–51. 2005. PMID: 10486675

77. Wachter D, Wrede A, Schulz-Schaeffer W, Taghizadeh-Waghefi A, Nitsche MA, Kutschenko A, et al. Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat. Exp Neurol. 2011; 227:322–7. doi: 10.1016/j.expneurol.2010.12.005 PMID: 21147105

78. Bandler R, Shipley MT. Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci. 1994; 17:379–89. PMID: 7817403

79. Basbaum AI, Fields HL. Endogenous pain control mechanisms: review and hypothesis. Ann Neurol. 1978; 4:451–62. PMID: 216303

80. Millan MJ. Descending control of pain. Prog Neurobiol. 2002; 66:355–74. PMID: 12034378

81. Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. Brain Res Rev. 2009; 60:214–25. doi: 10.1016/j.brainresrev.2008.12.009 PMID: 19146877

82. Keay KA, Bandler R. Distinct central representations of inescapable and escapable pain: observations and speculation. Exp Physiol. 2002; 87:275–9. PMID: 11856957

83. Benarroch EE. Periaqueductal gray: an interface for behavioral control. Neurology. 2012; 78:210–7. doi: 10.1212/WNL.0b013e31823f81a5c PMID: 22294946

84. Fonoff ET, Dale CS, Pagano RL, Paccola CC, Ballester G, Teixeira MJ, et al. Antinociception induced by epidural motor cortex stimulation in naive conscious rats is mediated by the opioid system. Behav Brain Res. 2009; 196:63–70. doi: 10.1016/j.bbr.2008.07.027 PMID: 18718490

85. de Andrade DC, Mhalla A, Adam F, Texeira MJ, Bouhassira D. Neuropharmacological basis of rTMS-induced analgesia: the role of endogenous opioids. Pain. 2011; 152:320–6. doi: 10.1016/j.pain.2010.03.002 PMID: 21146300
90. Maarrawi J, Peyron R, Mertens P, Costes N, Magnin M, Sindou M, et al. Differential brain opioid receptor availability in central and peripheral neuropathic pain. Pain. 2007; 127:183–94. PMID: 17137714
91. Maarrawi J, Peyron R, Mertens P, Costes N, Magnin M, Sindou M, et al. Brain opioid receptor density predicts motor cortex stimulation efficacy for chronic pain. Pain. 2013; 154:2563–8. doi: 10.1016/j.pain.2013.07.042 PMID: 23900133
92. Hökfelt T, Elde R, Johansson O, Terenius L, Stein L. The distribution of enkephalin-immunoreactive neuron bodies in the rat central nervous system. Neurosci Lett. 1977; 5:25–31. PMID: 19604966
93. Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. Annu Rev Neurosci. 1984; 7:309–38. PMID: 5145267
94. Hökfelt T, Terenius L, Kuypers HG, Dann O. Evidence for enkephalin immunoreactive neurons in the medulla oblongata projecting to the spinal cord. Neurosci Lett. 1979; 14:55–60. PMID: 394028
95. Zhang Y, Zhao S, Rodriguez E, Takatoh J, Han BX, Zhou X, et al. Identifying local and descending inputs for primary sensory neurons. J Clin Invest. 2015; 125:3782–94. doi: 10.1172/JCI81156 PMID: 26426077
96. Bowman BR, Goodchild AK. GABA and enkephalin tonically alter sympathetic outflows in the rat spinal cord. Auton Neurosci. 2015; 193:84–91. doi: 10.1016/j.autneu.2015.08.006 PMID: 26329875
97. Senba E, Shiosaka S, Hara Y, Inagaki S, Sakanaka M, Takatsuki K, et al. Ontogeny of the peptidergic system in the rat spinal cord: immunohistochemical analysis. J Comp Neurol. 1982; 208:54–66. PMID: 6181101
98. Pohl M, Collin E, Bourgoin S, Conrath M, Benoliel JJ, Nevo I, et al. Expression of preproenkephalin A gene and presence of Met-enkephalin in dorsal root ganglia of the adult rat. J Neurochem. 1994; 63:1226–34. PMID: 7931276
99. Bennett GJ, Ruda MA, Dubner R. Enkephalin immunoreactive stalked neurons and lamina IIb islet neurons in cat substantia gelatinosa. Brain Res. 1982; 240:162–6. PMID: 6121374
100. Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI, et al. Pharmacological characterization of the cloned κ-, δ-, and μ-opioid receptors. Molecular Pharmacology. 1993; 45:330–4.
101. Beaudry H, Dubois D, Gendron L. Activation of spinal μ- and δ-opioid receptors potently inhibits substance P release induced by peripheral noxious stimuli. J Neurosci. 2011; 31:13068–77. doi: 10.1523/JNEUROSCI.1817-11.2011 PMID: 21917790
102. Ruda MA. Opiates and pain pathways: demonstration of enkephalin synapses on dorsal horn projection neurons. Science. 1982; 215:1523–5. PMID: 6121374
103. George A, Marziniak M, Schäfers M, Toyka KV, Sommer C. Thalidomide treatment in chronic constriction neuropathy decreases endoneurial tumor necrosis factor-alpha, increases interleukin-10 and has long-term effects on spinal cord dorsal horn met-enkephalin. Pain. 2000; 88:267–75. PMID: 11068114
104. Liu RJ, Zhang RX, Qiao JT, Dafny N. Interrelations of opioids with monoamines in descending inhibition of nociceptive transmission at the spinal level: an immunocytochemical study. Brain Res. 1999; 830:183–90. PMID: 11068114
105. Giuliani A, Fernandez M, Farinelli M, Baratto L, Capra R, Rovetta G, et al. Very low level laser therapy attenuates edema and pain in experimental models. Int J Tissue React. 2004; 26:29–37. PMID: 15573690
106. Borgeat A, Levy M, Marzin G, Mottet-Chabrol B, Neyroud F, Sagnard P, et al. Antinociceptive effects of RB101(S), a complete inhibitor of enkephalin-catabolizing enzymes, are enhanced by (+)-HA966, a functional NMDA receptor antagonist: a c-Fos study in the rat spinal cord. Eur J Pain. 2003; 7:241–9. PMID: 12725847
107. Chen W, Song B, Marvizón JC. Inhibition of opioid release in the rat spinal cord by serotonin 5-HT(1A) receptors. Brain Research. 2007; 1158:57–62. PMID: 17555728
108. Song B, Chen W, Marvizón JC. Inhibition of opioid release in the rat spinal cord by serotonin 5-HT(1A) receptors. Brain Research. 2007; 1158:57–62. PMID: 17555728
109. Chen W, Song B, Marvizón JC. Inhibition of opioid release in the rat spinal cord by alpha2C adrenergic receptors. Neuropharmacology. 2008; 54:944–53. doi: 10.1016/j.neuropharm.2008.02.002 PMID: 18343461
110. Liu RJ, Zhang RX, Qiao JT, Dafny N. Interrelations of opioids with monoamines in descending inhibition of nociceptive transmission at the spinal level: an immunocytochemical study. Brain Res. 1999; 830:183–90. PMID: 11068114
111. Ma J, Qiao JT, Dafny N. Opiate-like substances mediate norepinephrine-induced but not serotonin-induced antinociception at spinal level: reevaluation by an electrophysiological model of formalin test in rats. Life Sci. 2001; 69:969–76. PMID: 11488409