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Research Article

Effects of Scalp Acupuncture on Functional Deficits Induced by Early Sensorimotor Restriction

Angela K. Zanella [1], Jessié M. Gutierres [2], Felipe Stigger [3,*]

[1] *Departamento de Fisioterapia, Universidade Federal do Pampa, Uruguaiana, RS, Brazil*
[2] *Programa de Pós-Graduação em Patologia, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil*
[3] *Departamento de Fisioterapia, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS, Brazil*

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Abstract
The aim of this study was to investigate the effects of scalp acupuncture and electrostimulation, combined or not, in a disuse model consisted of early sensorimotor restriction in rats. Male Wistar pups received sensorimotor restriction from the second postnatal day (P2) until P28. Animals were divided into five different groups ($n = 6$): control (CT), sensorimotor restricted (SR), acupuncture (AC), electrostimulation (EL), and electroacupuncture (AC+EL). Experimental animals received sham, acupuncture, or electrical stimulation, combined or not, of two scalp regions for 7 days (P29-P35). Before treatment period (P29) and after treatment (P36), animals were evaluated with the narrow suspended bar, horizontal ladder, and stride length tests. SR animals had worse performance in the narrow suspended and horizontal ladder tasks compared with SR animals at P29 ($p \leq 0.005$). Significant improvements were observed in both tasks in AC, EL, and EL+AC groups comparing P29 and P36 ($p < 0.001$). Also, at P35, all treated animals performed significantly better motor tasks compared with SR group ($p < 0.05$). There was no difference between treated groups. Finally, acupuncture and electrical stimulation, combined or not, have beneficial effect on motor performance following early developmental disuse.

* Corresponding author. Universidade Federal de Ciências da Saúde de Porto Alegre No. 245 Sarmento Leite Street, Porto Alegre, Rio Grande do Sul, Brazil.
E-mails: angelakemelzanella@gmail.com (A.K. Zanella), jessiegutierres@hotmail.com (J.M. Gutierres), stigger@ufcspa.edu.br (F. Stigger).

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1. Introduction

Cerebral palsy (CP) is a common neurodevelopmental disorder and the main cause of physical impairment of childhood [1]. Children with CP and other developmental neurological disorders are subjected to physical hypoactivity and limited interactions with their environment during early developmental period affecting postural organization and motor control [2].

In the last years, animal studies including sensorimotor restriction (SR) were conducted aiming to clarify the mechanisms related to developmental disuse and its implications to conditions that impair movement, such as CP [3—8]. SR used in those experiments mimics the immobility imposed by the pathological motor conditions and allows the understanding of mechanisms related to neural adaptations that occur in response to different treatment approaches.

Acupuncture has progressively been integrated into pediatric health care, and exploring its effects has been a hot topic in the research of alternative and complementary medicine. Acupuncture has been clinically demonstrated as an effective alternative and/or complementary therapeutic intervention for improving motor performance in children with CP [9], as well as to improve their quality of life [10].

In this context, considering the deleterious impacts of movement deprivation at early stages of development on functional performance in childhood and that acupuncture treatment has received increased attention by researchers with promising effects on children with CP, our goal was to investigate the effects of acupuncture and electrical stimulation treatment, combined or not, on motor behavior deficits induced by early SR in young rats.

2. Materials and methods

2.1. General

All experiments and animal use have been carried out in accordance with the guidelines laid down by NIH Guide for the Care and Use of Laboratory Animals [11] and followed the recommendations of the Brazilian Society for Neuroscience, Committee of the School of Veterinary Surgery, University of Buenos Aires, and the International Brain Research Organization. Animals were housed in standard boxes, with controlled temperature and humidity, under a light/dark cycle of 12h, with food and water available *ad libitum*. Approval from the local ethics committee for animal use (CEUA-UNIFRA) was obtained for procedures conducted in this research. All efforts were done to minimize animal suffering as well as to reduce the number of animals.

2.2. Experimental procedures

After birth, 30 male Wistar pups were randomly divided into five experimental groups ($n = 6$): (1) control rats not exposed to experimental procedures (CT); (2) rats subjected to transient hind limb immobilization from P2 to P28 for 16 h/day, thus producing early SR (SR); (3) animals subjected to SR and received scalp acupuncture for 15 min/day from P29 to P35 (AC); (4) animals subjected to SR and received electrical stimulation for 15 min/day from P29 to P35 (EL); and (5) animals subjected to SR and received AC combined to EL (EL+AC). Fig. 1 shows the timeline of all experimental procedures.

2.2.1. Sensorimotor restriction

The early SR model used in this study was based on Strata et al. [3], which consists in causing disuse of the lower limbs by restricting movements in early development. An medical tape was used to bound together the feet of SR pups. Then, size adaptable casts made of hand-moldable epoxy (Henkel, Brazil) was used to immobilize and maintain hindlimbs in extended position. Casts were well tolerated by the pups, not disturbing urination, defecation, and maternal care.

2.2.2. Acupuncture and electrical stimulation

Briefly, after sterilizing with 75% ethanol, sterile disposable acupuncture needles sized 0.10×15 mm (Acupuncture Dongbang Inc.®, Korea) were inserted accurately and rapidly in two points of cerebral motor area of scalp acupuncture, bilaterally, for 15 min/day. Accordingly to Yamamoto New Scalp Acupuncture, Chinese Scalp Acupuncture is concerned with more directly influencing the brain's motor centers by placing stimulating needles directly over motor and premotor cortexes [12]. For electrical stimulation, animals received electrical stimulation through the Electro EL 608 (NKL Electronic Products®, Brusque, Santa Catarina, Brazil) with 100Htz often with a power of 10 mA, according to the animal tolerance by square waves via silicone-carbon transcutaneous electrodes sized 1.0×1.0 cm and coupling gel. Acupuncture and electrical procedure consisted in the combination of both procedures. All experimental animals were anesthetized intraperitoneally with a prepared mixture of ketamine and xylazine as previous described for acupuncture practice in rats by Figueiredo et al. [13]

2.2.3. Narrow suspended bar, horizontal ladder, and stride length

The animals' hind limb sensorimotor function was evaluated after immobilization period on P29 and P36 using the narrow suspended bar (NSB) and the horizontal ladder (HL) tests. The NSB is a 100×2.5 cm rectangular bar positioned 30 cm from the floor. The HL has the same length but is 5 cm in width, with parallel metal rungs (2 cm apart). Both tests were based on the ability to walk on those apparatus. Scores were given to the animals at the end of the NSB and HL task in accordance with the motor ability (for details see Strata et al. [3]).

In the same period, animals underwent assessment of stride length (SL). Rats walked with their painted hind feet along a 100-cm-long, 8.5-cm-wide track covered with a white sheet of paper. SL of each animal was obtained from the mean values of three consecutive footprints each side (for details see Marcuzzo et al. [5]).

2.3. Statistical analysis

NSB, HL, and SL measurements (from SR, AC, EL, and AC+EL groups) were analyzed using two-way repeated

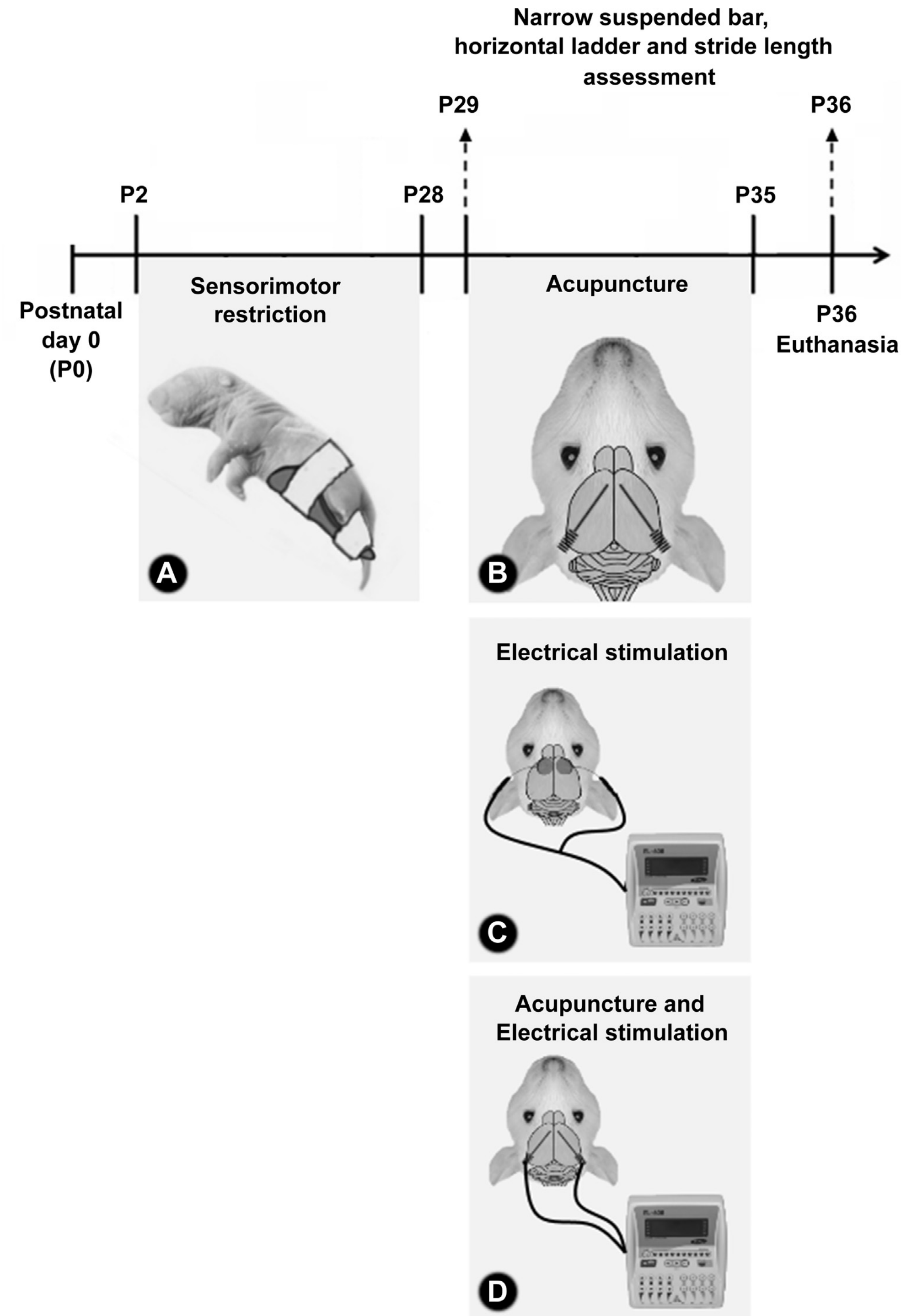

Figure 1 Timeline of experimental procedures. (A) The rats were exposed to sensorimotor restriction between P2-P28, for 16 h/day. (B) During 7 days, from P29-P35, rats were exposed to acupuncture. (C) During 7 days, from P29-P35, rats were exposed to electrical stimulation. (D) During 7 days, from P29-P35, rats were exposed to both treatments. Narrow suspended bar, horizontal ladder, and stride length tests were assessed at P29 and P36.

measures analysis of variance (ANOVA) with acupuncture and electrical stimulation as independent variables and time evaluations days P29 and P36) as repeated measure. All analysis were followed by Tukey's post hoc test. Differences between CT and SR animals in all motor measurements were analyzed using Student *t* test for independent samples. Data were expressed as means $\pm$ standard error of the mean. Probability values less than 5% were considered significant. Statistical analysis was performed using the Statistica® 10.0 for Windows software package.

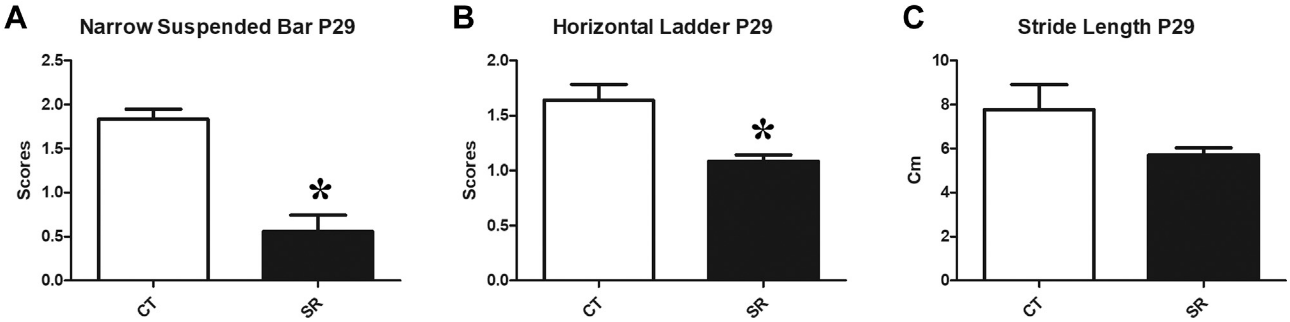

Figure 2 Motor skill evaluation off control (CT) and sensorimotor restriction (SR) groups before treatment procedure to SR animals at P29. (A) On narrow suspended bar. (B) On horizontal ladder. (C) On stride length tests. *Represents $p \leq 0\ .005$ compared with CT. Data were expressed as mean ± SEM. Student *t* test for independent samples. SEM = standard error of the mean.

3. Results

After 26 days of SR, SR rats performed significantly worse than CT in NSB and HL ($p \leq 0.005$—Fig. 2).

For the NSB test, two-way repeated measures ANOVA showed a significant effect AC [$F(1,20) = 9.04$, $p < 0.05$], EL [$F(1,20) = 10.04$, $p < 0.005$], a significant interaction of AC and EL [$F(1,20) = 10.04$, $p < 0.005$], and a high significant effect of time [$F(1,20) = 103.25$, $p < 0.0001$]. Considering the HL, two-way repeated measures ANOVA showed a high significant effect of time [$F(1,20) = 185.18$, $p < 0.0001$] and a significant interaction of time with AC [$F(1,20) = 10.66$, $p < 0.005$], EL [$F(1,20) = 21.40$, $p < 0.005$], and AC+EL [$F(1,20) = 12.51$, $p < 0.005$]. No differences between groups were observed before acupuncture, electrical stimulation, or combination of both treatments in both tests (P29). After treatment period, no differences were observed comparing results of SR rats from P29 and P36. It was possible to observe improvements on the ability to perform the NSB test in AC, EL, and AC+EL groups comparing P29 and P36 ($p \leq 0.001$). Also, compared with SR animals, AC, EL, and AC+EL rats performed significantly better the NSB test ($p < 0.05$). Similar results were found in HL test (Fig. 3).

Two-way repeated measures ANOVA for SL showed a significant effect of AC and EL [$F(1,20) = 4.59$, $p < 0.05$] and a high significant effect of time [$F(1,20) = 36.13$, $p < 0.0001$]. Although was possible to observe a time-dependent increase in SL in all experimental groups, probably because of developmental growth, SL increase from P29 to P36 was significant in EL group ($p < 0.05$). SR, AC, EL, and AC+EL at P36 presented similar SL (Fig. 4).

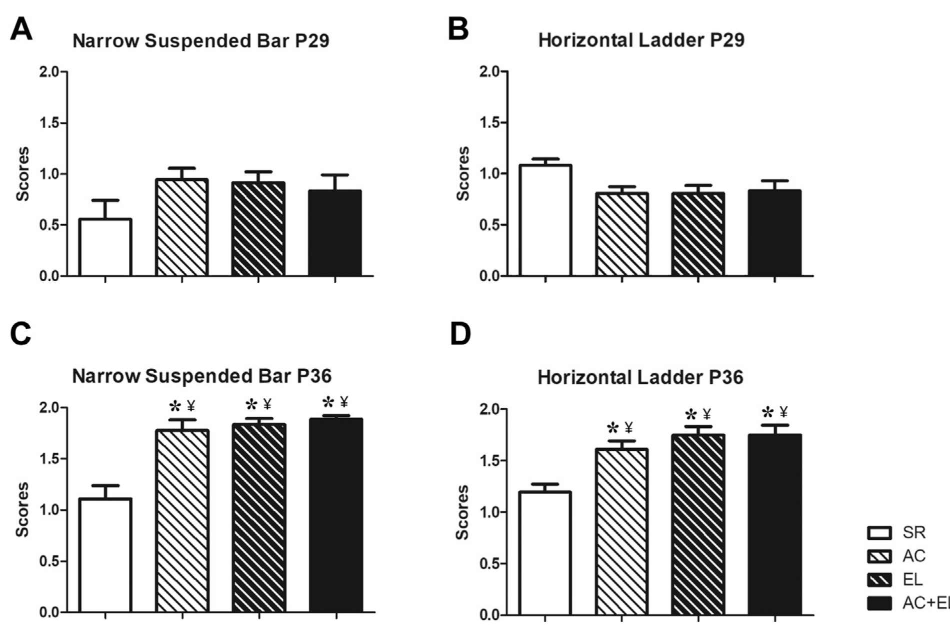

Figure 3 Motor skill evaluation off sensorimotor restriction (SR), acupuncture (AC), electrical stimulation (EL), and electro-acupuncture (AC+EL) groups. (A) On narrow suspended bar at P29. (B) On horizontal ladder tests at P29.(C) On narrow suspended bar at P36.(D) On horizontal ladder tests at P36. Two-way repeated measures ANOVA followed by Tukey's post hoc test. Columns represent means ± SEM. *Different from SR, $p < 0.05$ in same period. ¥Different from respective group in P29, $p \leq 0.001$. ANOVA = analysis of variance; SEM = standard error of the mean.

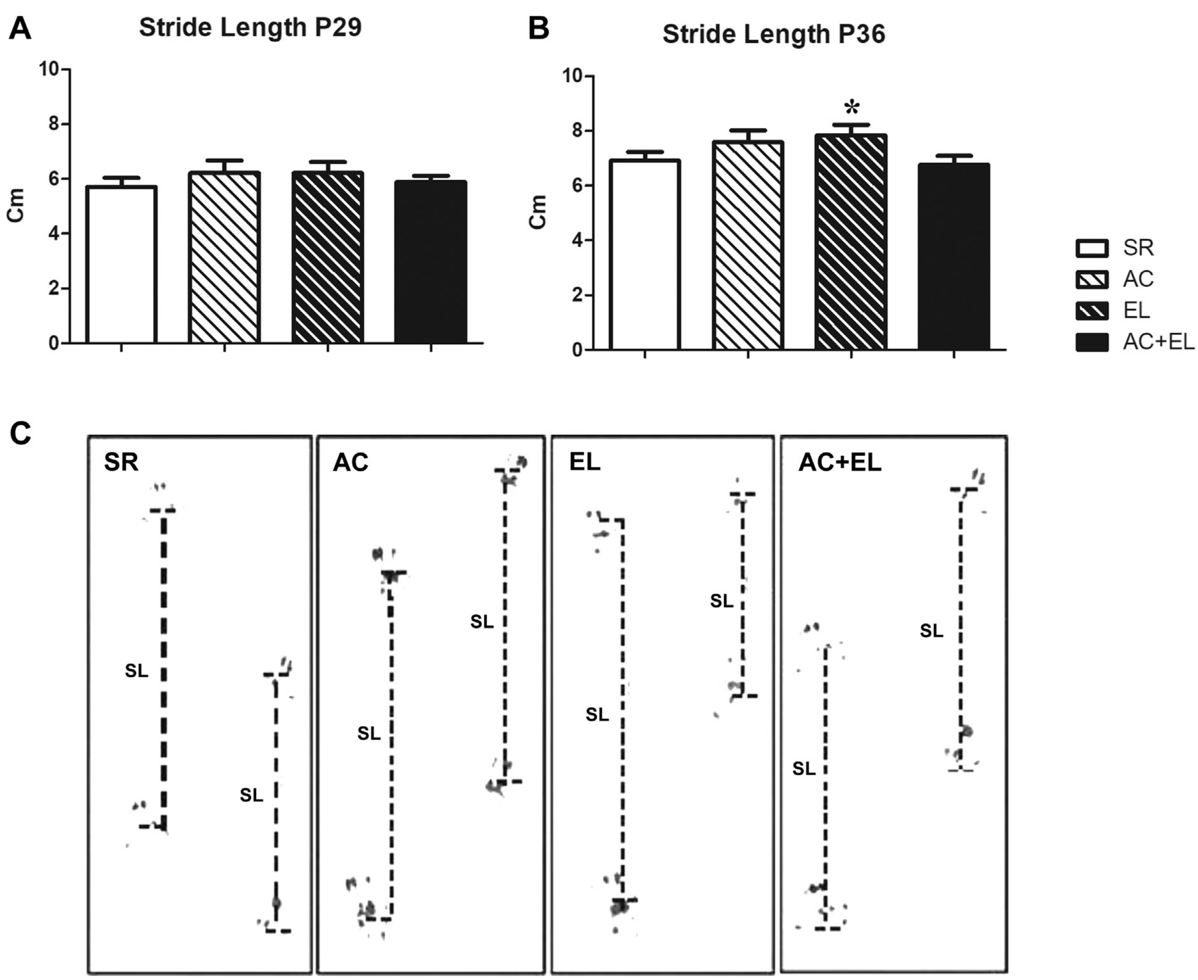

Figure 4 Stride length results of sensorimotor restriction (SR), acupuncture (AC), electrical stimulation (EL) and electroacupuncture (AC+EL) groups. (A) At P29. (B) At P36. Two-way repeated measures ANOVA followed by Tukey's post hoc test. Columns represent means ± SEM. *Different from respective group in P29, $p < 0.05$. (C) Representative images of stride lengths (SL) groups in the last day of the treatment. The figure shows how the measurement was made (distance from metatarsus to metatarsus of the same limb). ANOVA = analysis of variance; SEM = standard error of the mean.

4. Discussion

The underlying factors that contribute to CP motor deficits are diverse. Previous studies suggested that movement deprivation at early developmental period can reproduce clinically relevant features of CP in rat. Our study has demonstrated the effects of chronic hind limb disuse induced by SR, as well as, the effects of acupuncture, electrostimulation, and electroacupuncture on motor behavior of male Wistar rats. Similar to Strata et al. [3], Marcuzzo et al. [6], and Stigger et al. [8], our results demonstrated that SR animals performed significantly worse NSB and HL tasks. Also, our results showed that functional performance of SR rats is ameliorated following acupuncture, electrostimulation, and electroacupuncture.

SR during early development is a rodent model of disuse previously used to understand the effects of movement deprivation on motor disturbance observed in children with CP and other developmental conditions that induces inactivity [3, 6–8]. It is known that information sent by the primary somatosensory cortex contributes to the elaboration of responses by the primary motor cortex; [14] thus, alterations of the somatosensory pathway of movement-restricted rats might impact the cortical control of hindlimb muscles and subsequent aberrant motor behavior. Actually, following developmental disuse induced by SR, reduced or anomalous proprioceptive feedback from the hind limbs affects cortical structural and functional organization [3, 4, 6, 15].

In the present study, the AC, EL, and AC+EL groups presented similar results in behavioral evaluations. Corroborating to our findings, several basic and clinical evidence suggests that acupuncture promotes functional improvements in developmental conditions that induces dysfunction in motor function [9, 16–21]. Scalp acupuncture therapy can significantly improve the cerebral microcirculation [22], release of extracellular nucleotides [23], promote changes in cortical patters of activation of motor, premotor, and supplemental motor cortex [24], and have sustained effects on brain resting state connectivity [25].

Also, a complex somatosensory stimulation is reported after application of electroacupuncture demonstrating that somatosensory neurons regeneration occurs after electrical stimulation [26—28] and electroacupuncture [29]. Although in this study we did not measure such parameters, it seems that those benefits clarify the hypothesis that acupuncture or electrostimulation itself had a positive influence on SR animals.

From another perspective, our results could be related to the fact that neurotrophins apparently implies in cortical changes because of hypoactivity. For example, it is known that insulin-like growth factor 1 prevents cortical shrinkage in rat's brain following SR induced by hindlimb unloading [30]. Thus, acupuncture or electroacupuncture therapy could improve motor function of SR rats by upregulating important neurotrophins in rat brain such as glial line—derived neurotrophic factor, brain-derived neurotrophic factor, and synaptophysin [20, 31, 32]. Also, growth-associated protein 43, a protein linked to neural and synaptic plasticity, has been already demonstrated to be upregulated following acupuncture treatment [20].

Additionally, glial line—derived neurotrophic factor and brain-derived neurotrophic factor has been shown to inhibits the activation of caspase-3 [33, 34], a critical factor in the apoptotic pathway [35]. After acupuncture treatment, Zhang et al. [32] observed a downregulation of activated caspase 9 and caspase 3 levels consistent with terminal deoxynucleotidyl transferase dUTP nick end labeling staining in the rat's hippocampus. Thus, we might speculate that acupuncture could also protect cells in other brain regions from apoptosis induced by disuse. Another hypothesis that seems to clarify the biological mechanisms involved in the acupuncture treatment of neuronal damage and consequently motor behavior is related to inflammatory pathways. Acupuncture treatment can reduce inflammatory cytokines expression in both children [36] and animal with CP [37]. Also, inhibited upregulation of the levels of tumor necrosis factor-alpha and interleukin 1beta mRNA were observed following electroacupuncture [38].

A better understanding of the physiological mechanisms leading to improvements in functional performance following acupuncture treatment of developmental conditions is still necessary. Considering basic and clinical data, acupuncture can be a safe strategy to modulate the activity-dependent disorder imposed by developmental conditions alone or considering that acupuncture combined with exercise therapy can improve motor function with therapeutic effect better than that of simple exercise therapy [39], as a complementary therapy.

5. Summary

The present study demonstrates that acupuncture and electrical stimulation, alone or combined, in two points of motor area of the scalp acupuncture improved motor function in early sensorimotor restricted animals. Such findings provide new possibilities in the treatment of children with CP and other developmental conditions that induces inactivity. Further studies comprising specific mechanisms by which acupuncture promote motor gains should be addressed.

Disclosure statement

The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jams.2019.03.002.

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