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Stimulation of the *Po-shen* and *Shen-hun* scalp-acupuncture bands modifies levels of inhibitory and excitatory amino acids in the immature rat brain

Lucia Estrella Franco-Santana [a,b], Sergio Torres-Castillo [b], María Eva González-Trujano [c], Misael González-Ramírez [a,*]

[a] *Unidad de Investigación Médica en Enfermedades Neurológicas, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, IMSS, Av. Cuauhtémoc 330, Col. Doctores, C.P. 06720 México D.F., Mexico*

[b] *Universidad Estatal del Valle de Ecatepec, Av. Central esquina Leona Vicario S/N, Col. Valle de Anáhuac, C.P. 55210 Edo. de México, Mexico*

[c] *Dirección de Investigación en Neurociencias del Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Calz. México-Xochimilco 101, Col. Sn Lorenzo Huipulco, C.P. 14370 México D.F., Mexico*

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ABSTRACT

Objective: In the present study, the effect of stimulation of the *Po-shen* and *Shen-hun* scalp-acupuncture bands on tissue amino acid concentrations in several brain regions in awake and pentobarbital-sedated immature rats was evaluated.

Materials and methods: Sprague–Dawley rats (aged 15 days) were organized in four groups of at least eight animals: control groups received saline solution 0.9% or sodium pentobarbital at 30 mg/kg dosage via intraperitoneal. Experimental groups received saline solution or sodium pentobarbital plus stimulation in *Po-shen* and *Shen-hun* scalp-acupuncture bands for one continuous hour during 10 sessions by using scalp-acupuncture.

Results: As compared to rats receiving saline solution, scalp-acupuncture produced significant changes in amino acid concentrations, depending on the analyzed region, as follows: in inhibitory amino acids, a GABA increase was observed in amygdala and hippocampus (491 and 184%, respectively), but a decrease in the substantia nigra (80%); glycine showed decrease in all the analyzed regions, except for an increase in brainstem(78%); glutamine presented an increase in hippocampus and cortex (42 and 149%, respectively). In the case of excitatory amino acids, glutamate decreased in all the analyzed regions; whereas aspartate decreased in substantia nigra and brainstem (77.08 and 35%, correspondingly) but increased in hippocampus and cortex (32 and 54%, respectively). The combined treatment of scalp-acupuncture and a GABAergic depressant drug like pentobarbital resulted in almost all changes induced in amino acids for scalp-acupuncture alone being significantly reverted.

Conclusion: Stimulation of the *Po-shen* and *Shen-hun* scalp-acupuncture bands by using scalp-acupuncture alone might produce depressant activity by changes in amino acids, but the combination with a GABAergic tranquilizer like sodium pentobarbital can interfere with this response.

1. Introduction

The word acupuncture derives from the Latin "*Acus*", meaning needle, and "*Pungue*", meaning to penetrate. According to the World Health Organization (WHO), acupuncture consists of the strategic penetration and stimulation through the skin of certain points in the body, specifically of points that have a low electrical resistance and high conductivity. Stimulation of these points can also be achieved via moxibustion, magneto-therapy, electro-stimulation, laser, ultrasound, cupping glasses, tacks, small bullets or acupressure; to choose the most suitable method, one must consider a patient's group of symptoms and her/his diagnosis (WHO, 2002). Scalp-acupuncture was modeled after traditional acupuncture by Jiao Shiunfa in China in 1970. Shiunfa theorized that points of acupuncture could exist in the "brain" (i.e., in the scalp), just as they do in the back, thorax and abdomen. The first supporting case for scalp-acupuncture is the case of an elderly woman who suffered spasmodic pains in her arms and legs caused by arteriosclerosis. The woman inserted needles into her scalp, considering the sensorial area. By the following day, the pain had subsided considerably (Gaynor, 2000). Scalp-acupuncture is now

* Corresponding author. Tel./fax: +52 55 5578 0240.
E-mail addresses: sakura_3280@hotmail.com (L.E. Franco-Santana), sergiotorcas@yahoo.com.mx (S. Torres-Castillo), evag@imp.edu.mx (M.E. González-Trujano), gonzalez.misael@imss.gob.mx, misael.gonzalez@imss.gob.mx (M. González-Ramírez).

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used extensively in China and in many other countries to treat a variety of pathologies. It has been shown to be particularly effective in treating consequences of cerebral events and infantile cerebral paralysis (González, 1995; Marié 1998).

Scalp-acupuncture is a therapeutic procedure in which specific cortical areas, i.e., those involved in motor functions, speech, sensation, vision, audition, vertigo, and balance are punctured (Smiley and Falchier, 2009; Zilles and Wree, 1995). These areas are stimulated with the insertion of a needle below the hair, where it remains for at least 30 minutes. The therapist can additionally stimulate the site by manually turning the needle clockwise or counterclockwise or by electrically stimulating the handle (coil) of the needle using an electro-stimulating device to regulate the frequency and intensity of the electric current (Mayer et al., 1977).

Scalp-acupuncture is applied to well-defined "bands" or locations of the scalp to obtain specific therapeutic results (Rapson, 1984). A cun is a unit of measure of the width and length of the fingers; each cun depends on the physical constitution of each patient. The *shen-hun* band begins at 5 cun posterior to the implantation of hair and 1.5 cun outside of the midline the left side (Tongtian BL-7); it finishes at 6.5 cun posterior implantation of hair and 1.5 cun outside the mean line of the right side (Luoque BL-8), crossing through vertex (Baihui GV-20) (Maciocia, 2009; Zhang and Zheng, 2003). Stimulation of the *shen-hun* acupuncture band is used as an anxiolytic and sleep inducer to treat attention deficit disorders, neuroses, and memory impairments that arise from emotional stress; it is also used to promote long term memory. The *Po-shen* band begins 2 cun posterior to the implantation of hair (*Xinhui* GV-22) and 1.5 cun lateral to the median line, starting from right to left and at 0.1 cun above is from right to left horizontally (Maciocia, 2009; Zhang and Zheng, 2003). The *Po-shen* band activates the *po* function on *shen*: stimulation of the *Po-shen* band inhibits mental activity in order to increase attention and focus and is used to treat attention deficit disorders, palpitations, and anxiety (fear of punishment, sensation of failure) (Maciocia, 2009; Zhang and Zheng, 2003).

The purpose of the present study was to determine the effects of scalp-acupuncture stimulation of the *Po-shen* and *Shen-hun* bands on GABA, glycine, glutamine, glutamate and aspartate levels in the cortex, amygdala, hippocampus, substantia nigra, and brainstem. The effects were measured in immature rats also treated with the sedative sodium pentobarbital or with saline solution.

2. Materials and methods

2.1. Animals

All experimental procedures were carried out according to a protocol approved by the Local Animal Ethics Committee and in compliance with national (NOM-062-ZOO-1999) and international rules stated in the National Institutes of Health Guide for Care and Use of Laboratory Animals. The experiment was performed on fifteen-day-old male and female Sprague–Dawley rats. At birth, each pup was assigned at random to one of four mothers. Each mother had 8 rats (4 males and 4 females). All the animals were housed in a temperature and light controlled room under a 12 h light-dark cycle (lights on at 7:00 a.m.) with water and food *ad libitum*. The number of experimental animals was kept to a minimum. Animals were sacrificed immediately after the experiment. All of the experimental sessions took place between 10:00 h and 12:00 h to avoid circadian variations.

2.2. Experimental groups

Rats were assigned to either control group (saline solution 0.9%, i.p.) or an experimental group (sodium pentobarbital, 30 mg/kg, i.p.) without scalp-acupuncture stimulation. Other rats received saline solution or sodium pentobarbital plus one continuous hour stimulation in *Po-shen* and *Shen-hun* acupuncture bands for each of 10 daily sessions starting at age 15 days. Thirty minutes after saline solution or pentobarbital, animals were immobilized by being wound on a flannel adjusted with tweezers, and then their *Po-shen* and *Shen-hun* scalp-acupuncture bands were stimulated using a sterile disposable 0.17 mm diameter needle (CW-SOOJI). For the placement of two needles into the *Po-Shen* scalp-acupuncture band, an imaginary line from the external angle of the eye of the rat was taken into account to insert one needle from right to left and the other from left to right. The band width comprises the distance between the inner corners of the eyes of the rats (Fig. 1). For the placement of the needle into the *Shen-hun* scalp-acupuncture band it is necessary to consider two angles as follows: for the first angle, two imaginary lines are delineated to form a 90° angle, one is directed from the inner angle of the eye to the posterior side of the head to converge with other line directed from the beginning of one ear to the other one (angle 1, Fig. 1A). For angle 2, two imaginary lines forming a "T" are delineated: the first is demarcated from one ear to the other one and the second line is directed from the tip of the nose to the first line, then the needle is inserted between these two angles (Fig. 1B). Immediately after the 10th session, all animals were sacrificed by decapitation and the brainstem, cortex, amygdala, hippocampus, and substantia *nigra* were obtained from the sacrificed animals. Brain regions were frozen immediately with liquid nitrogen and stored at −70 °C until the amino acid and protein analyses were performed.

2.3. Amino acid analyses

Each brain region was weighed and homogenized in a solution of perchloric acid (0.1 M), (J.T. Baker, Pittsburgh, New Jersey, USA) and sodium methabisulphyte (4 mM) (J.T. Baker, Pittsburgh, New Jersey, USA) at 30 μl/10 mg of tissue using a Thomas homogenizer with Teflon emboli at a speed of 1000 rpm for 60 s. The samples were centrifuged at 12,000 rpm for 15 min and then the protein concentration was determined in the pellet by the Folin potein method, as described by Lowry et al. (1951). Brain tissue was resuspended in 200 μl double-distilled water and diluted 40 times; 100 μl were taken from this dilution. The reaction began when 500 μl of solution A were added, solution A contained 500 μl of copper sulphate (0.04 M) (Sigma Aldrich) and 500 μl of sodium and potassium tartrate (0.07 M) (Sigma Aldrich) in 50 ml of sodium carbonate (0.19 M) (Sigma Aldrich) diluted in sodium hydroxide (1 M) (Sigma Aldrich). The mixture was vigorously shaken and left to rest at room temperature. After 10 min, 50 μl of solution B were added (Folin diluted 1:2 (Sigma Aldrich) with double-distilled water). The samples were incubated for 30 min, and the colorimetric reaction was analyzed using a spectrophotometer (Perkin Elmer Lambda 25 UV/VIS including wavelengths to 700 nm). This procedure enabled data obtained from the HPLC analysis to express the amino acid concentrations in ng/mg of protein.

The supernatant was used to analyze GABA and glycine (GLY) as inhibitory amino acids, and glutamate (GLU) and aspartate (ASP) as excitatory amino acids; as well as glutamine (GLN), in the obtained tissue. The off-line procedure was performed as described by Forster and Marsden, (2001). A high performance liquid chromatography (HPLC) system was used. The entire procedure was performed with HPLC grade water (Mallinkrodt Baker Pittsburgh, New Jersey, USA), in 4 °C temp and under light protection. The system was integrated with Empower software and an electrochemical detector (model 2465; Waters of México) operated at a cell voltage of +540 mV (with a range of 5 nA/V). The detector potential used was 0.80 V, the flow-rate was 0.30 ml min^{-1}, and the column temperature was 30 °C, with a working electrode surface of carbon