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Paravertebral fascial massage promotes brain development of neonatal rats *via* the insulin-like growth factor 1 pathway☆

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Abstract

Massage in traditional Chinese medicine can promote body and brain development of premature and normal newborn infants. In the present study, neonatal rats (1 day old) underwent paravertebral fascial massage (15 consecutive days), followed by subcutaneous injection of insulin-like growth factor 1 receptor antagonist, JB1 (9 consecutive days). Paravertebral fascial massage significantly increased insulin-like growth factor 1 expression and cell proliferation in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus. However, JB1 inhibited this increase. Results suggest that paravertebral fascial massage can promote brain development of neonatal rats via the insulin-like growth factor 1 pathway.

Key Words

paravertebral fascial massage; insulin-like growth factor 1; neurogenesis; immunohistochemistry; neural regeneration

Abbreviations

IGF-1, insulin-like growth factor 1; BrdU, 5-bromodeoxyuridine

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INTRODUCTION

Massage is an established treatment in traditional Chinese medicine, which over the past few decades has grown in popularity on account of unraveling its scientific methodology[1-2]. Understanding the biological mechanisms promoted by massage, allows targeted patient intervention. In recent years, controversy has surrounded research into the biological mechanisms of massage in determining whether regulation occurs through myofascial or neurophysiologic pathways[3-5]. It has been previously proposed that the fascia is the provides the morphological basis of channels (meridians) and collaterals[6-7] which permit the therapeutic benefits of traditional massage, which might be key to understanding its biological mechanism. Massage up-regulates expression of insulin-like growth factor 1 (IGF-1) in premature and normal newborn infants, promoting their growth and development. Furthermore, IGF-1 can pass through the blood-brain-barrier and promote brain development[8-10]. Growth hormone/IGF-1 deficiency is prevalent in children with cerebral palsy[11-12]. Physical therapy and hormone replacement therapy, with exogenous recombinant growth hormone/IGF-1, have been found to be beneficial to recovery of motor function in children with cerebral palsy, suggesting IGF-1 in the blood circulation can promote recovery of brain function[13]. Serous IGF-1 is an important medium in brain activity and

plays a leading role in brain function[14]. In the present study, changes in IGF-1expression and neural stem cell proliferation were observed following paravertebral fascial massage. Using IGF-1 receptor antagonist, we aimed to determine whether IGF-1 play a major role in brain development of neonatal rats.

RESULTS

Quantitative analysis of animals

A total of 60 rats (1 day old) were randomly assigned to five groups: massage (paravertebral fascial massage for 15 days), maternal separation (no massage), control (living with mother with no massage), massage (9 days) plus antagonist JB1 and massage (9 days) plus subcutaneous injection (days 1–9) of saline. Twelve rats were used for each group. All 60 rats were included in the final analysis.

IGF-1 expression in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus

Immunohistochemical staining revealed that after day 15 of paravertebral fascial massage, the number of IGF-1- and 5-bromodeoxyuridine (BrdU)-positive cells was significantly increased in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus in the massage group compared with the maternal separation and control groups ($P < 0.05$; Figures 1, 2).

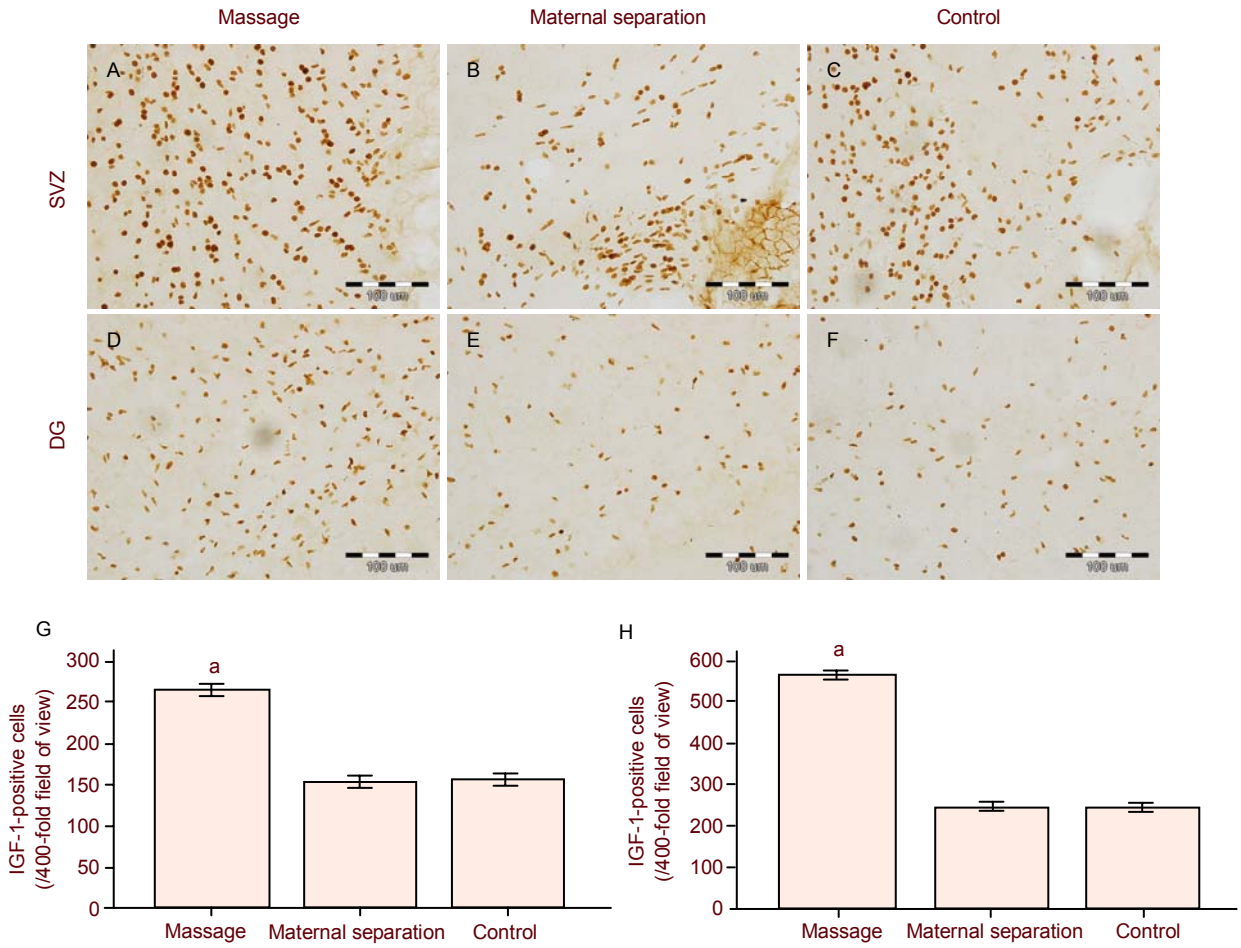

Figure 1 Representative immunohistochemistry images of insulin-like growth factor 1 (IGF-1)-positive cells (brown stain) and graphs showing the number of IGF-1-positive cells in the subventricular zone (SVZ) of the lateral ventricle and dentate gyrus (DG) of the hippocampus 15 days after massage (immunohistochemical staining).

IGF-1-positive cells were observed in the SVZ of the lateral ventricle (A–C) and DG of the hippocampus (D–F) in the massage, maternal separation and control groups. Original magnification: × 400. Scale bars: 100 μm.

The number of IGF-1-positive cells in the SVZ of the lateral ventricle (G) and DG of the hippocampus (H) are shown for the massage, maternal separation and maternal non separation groups. Data are expressed as mean ± SD ($n = 12$). $^aP < 0.05$, *vs.* the other two groups.

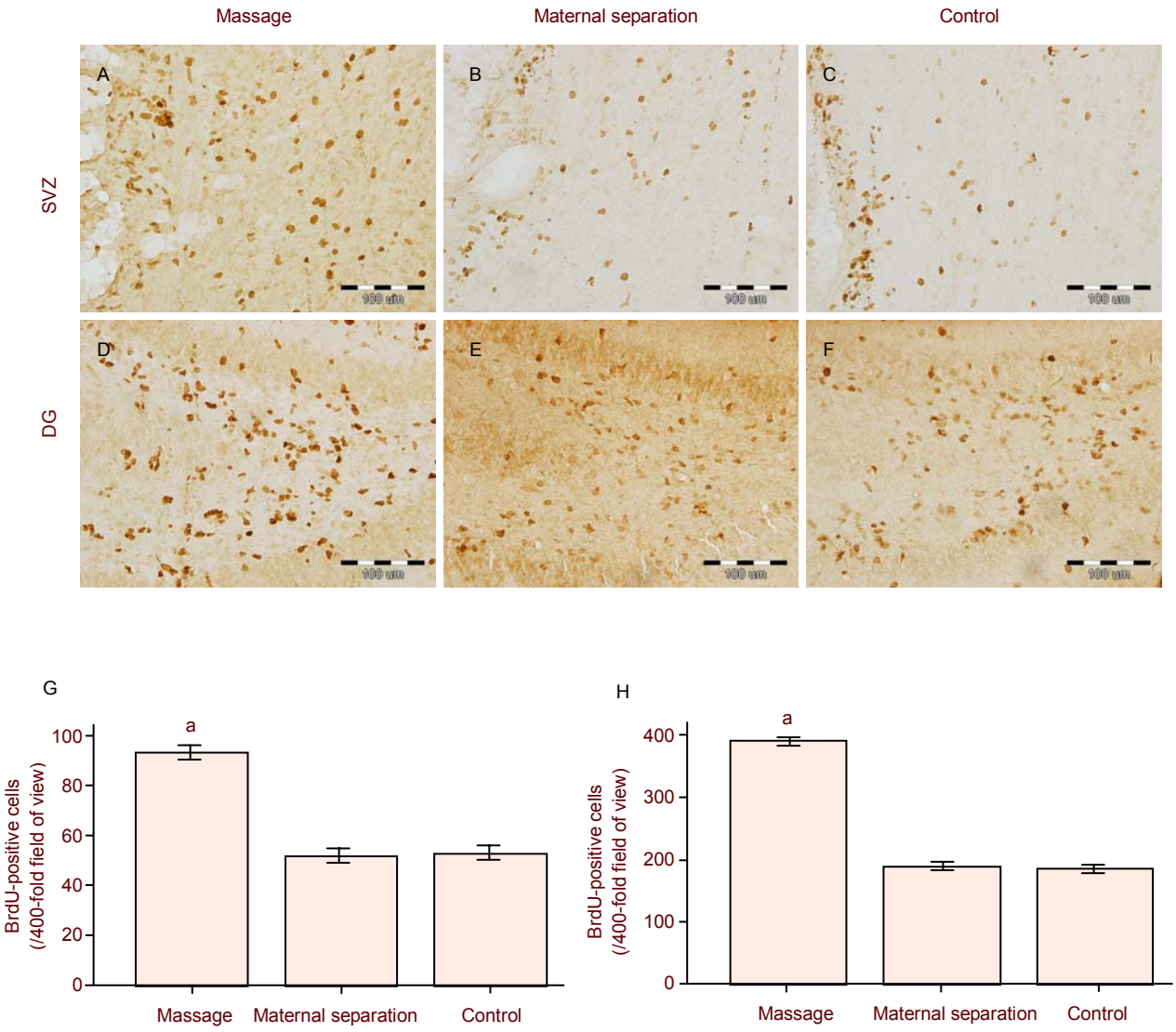

Figure 2 Representative immunohistochemistry of 5-bromodeoxyuridine (BrdU)-positive cells (brown stain) and graphs showing the number of BrdU-positive cells in the subventricular zone (SVZ) of the lateral ventricle and dentate gyrus (DG) of the hippocampus 15 days after massage.

BrdU-positive cells were observed in the SVZ of lateral ventricle (A–C) and in the DG of the hippocampus (D–F) in massage, maternal separation and control groups. Original magnification: × 400. Scale bars: 100 μm.

The number of BrdU-positive cells in the SVZ of the lateral ventricle (G) and DG of the hippocampus (H) are shown for the massage, maternal separation and control groups. Data were expressed as mean ± SD (n = 12). $^{a}P < 0.05$, *vs.* the other two groups.

JB1 suppressed IGF-1 expression in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus

Immunohistochemical staining revealed that after day 9 of paravertebral fascial massage, increased IGF-1 expression in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus was blocked by JB1. In contrast, IGF-1 expression levels were normal in the massage plus saline group. The number of BrdU-positive cells in the massage plus JB1 group significantly decreased in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus compared with the massage plus saline group ($P < 0.05$; Figures 3, 4).

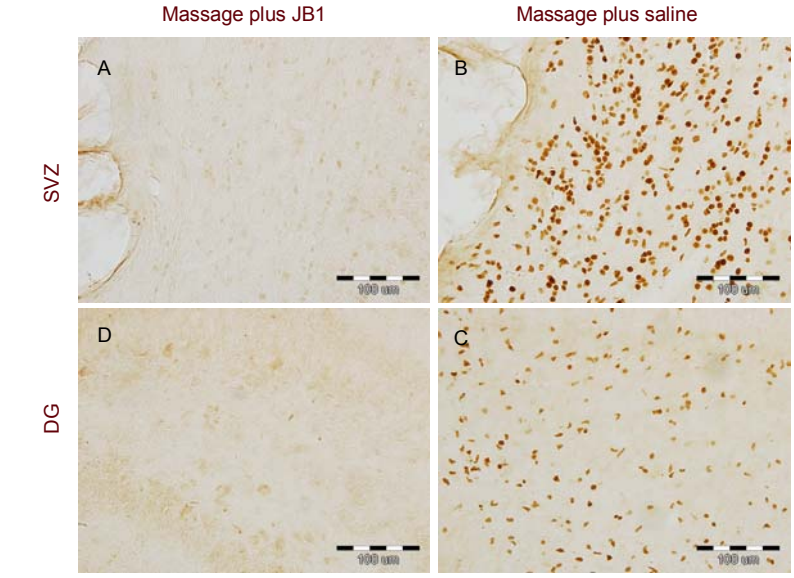

Figure 3 Representative immunohistochemistry of insulin-like growth factor 1 (IGF-1)-positive cells (brown stain) in the subventricular zone (SVZ) of the lateral ventricle and dentate gyrus (DG) of the hippocampus, 9 days after massage in the massage plus JB1 and massage plus saline groups.

IGF-1-positive cells were observed in the SVZ of the lateral ventricle (A, B) and DG of the hippocampus (C, D) in the massage plus JBI and massage plus saline groups. Original magnification: × 400. Scale bars: 100 μm.

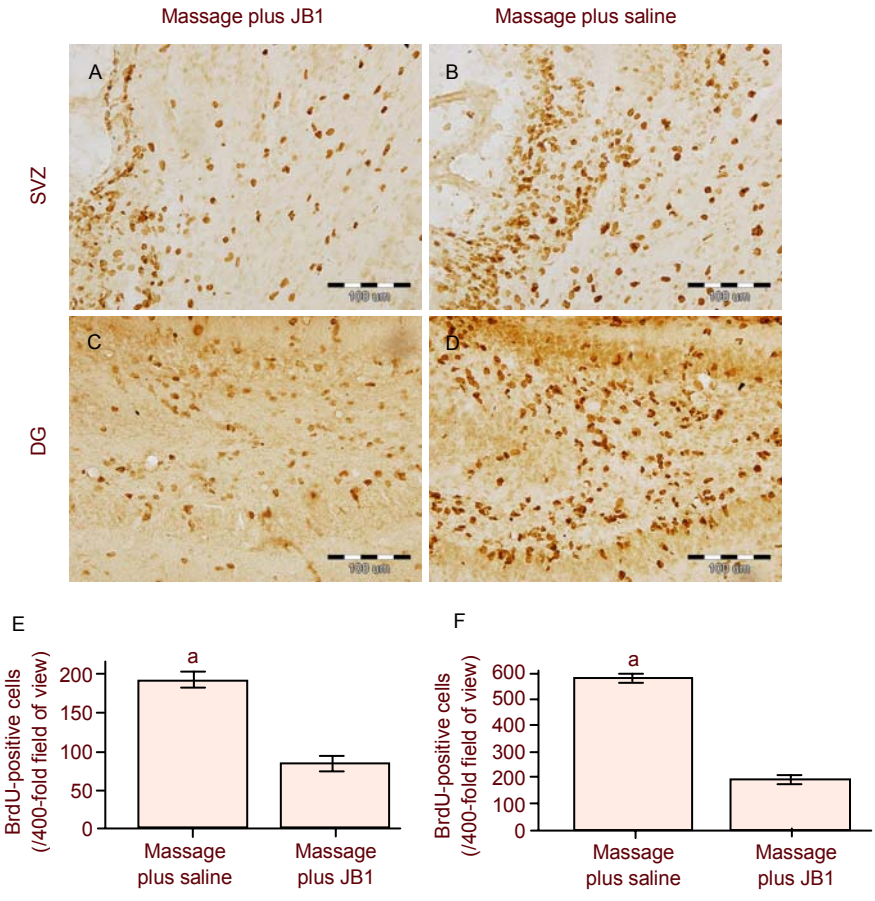

Figure 4 Representative immunohistochemistry images of 5-bromodeoxyuridine (BrdU)-positive cells (brown stain) and graphs showing the number of BrdU-positive cells in the subventricular zone (SVZ) of the lateral ventricle and dentate gyrus (DG) of the hippocampus, 9 days after massage, in the massage plus JB1 and massage plus saline groups.

Brdu-positive cells were observed in the SVZ of lateral ventricle (A, B) and DG of the hippocampus (C, D) in massage plus JB1 and massage plus saline groups. Original magnification: × 400. Scale bars: 100 μm.

The number of BrdU-positive cells in the SVZ of the lateral ventricle (E) and DG of the hippocampus (F) are shown for the massage plus JB1 and massage plus saline groups. Data are expressed as mean ± SD (n = 12). $^aP < 0.05$, *vs.* massage plus saline group.

DISCUSSION

Massage is beneficial to the growth and development of the neonatus. A possible underlying mechanism includes induction of vagal activity, which causes gastric motility and increased insulin and IGF-1 levels[9, 15-17]. However, this mechanism fails to explain how massage improves motor function in cerebral palsy[18] and brain function in autistic children[19-20]. This study aimed to determine how

massage promotes brain development and whether it is regulated by the myofascial pathophysiologic or neurophysiologic function, or a combination of both. This study also addressed whether the fascia regulates a specific region of the brain. In this study, high IGF-1 expression and newly generated cells were observed in the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus, after manipulating the paravertebral fascia of neonatal rats. IGF-1 is an important neuroprotective peptide, which can promote brain development in normal physiological conditions. Massage can increase serous IGF-1, likely produced by the fascia tissue of liver, adipose, muscle, kidney and spleen, whereby it is secreted into the blood circulation where it elicits endocrine effects on target tissues[21-23]. Alternatively, IGF-1 can act directly on microglia, astrocytes and neurons of the neurogenesis region and elicit paracrine effects on target tissues[8]. IGF-1 can pass the blood-brain-barrier and migrate to neurogenic regions, like the subventricular zone of the lateral ventricle and dentate gyrus of the hippocampus, activating the mitogen-activated protein kinase (MAPK) signaling pathway in neural stem cells and promoting their proliferation[24]. In the present study, subcutaneous injection of IGF-1 receptor antagonist, JB1, resulted in an absence of IGF-1 expression in neurogenic regions, including the subventricular zone of the lateral ventricle and the dentate gyrus of the hippocampus. Furthermore, the neurogenic capacity was obviously decreased. A possible mechanism for this involves the competition of JB1 with the IGF-1 receptor, which inhibits serous IGF-1 from crossing the blood-brain-barrier[25], causing IGF-1 to lose its biological function by blocking the mitogen-activated protein kinase signaling in neural stem cells[8, 24]. Alternatively, the biological mechanism could involve inhibition of IGF-1 by JB1 to enhance other growth factors, which in turn act on the biological function of neural stem cells[26-27], significantly decreasing their proliferation. In conclusion, the present study shows manipulation of the paravertebral fascia promotes neurogenic stem cell proliferation within specific neurogenic regions. The underlying mechanism of the fascia-brain interaction remains unknown, however the following hypotheses are proposed: (1) moderate pressure massage elicits a parasympathetic nervous system response[17], which affects the hypothalamus-pituitarium axis, stimulating the pituitary gland to secrete growth hormone, which then enters the liver. With the assistance of antigen presenting macrophages in the fascia, the liver produces IGF-1, which is released into the blood circulation, passing through the blood-brain barrier and promoting adult neural stem cell proliferation within a specific area of brain; (2) massage acts directly on the fascia, connecting all body organs together *via* the fascia framework, promoting adipose, bone, muscle, kidney and spleen tissues to produce autocrine IGF-1[28], which is secreted into the blood circulation to cross the blood-brain-barrier, affecting a specific region of brain tissue; (3) massage acts on the nerve endings in the fascia, which feed back to a specific region of brain tissue *via* neurophysiologic regulation. For example, microglia, astroglia or neurons produce paracrine IGF-1[8], which directly affects neurogenic areas, promoting proliferation of adult neural stem cells. Despite these proposed mechanisms, limitations in this study exist. The impact of massage on the level of IGF-1 in subcutaneous fascia tissue and the blood circulation was not determined. Therefore, the results cannot completely explain the relationship between the fascia and the brain.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

The experiment was performed at the Laboratory of Anatomy, Southern Medical University, Guangzhou, China, from March 2010 to July 2011.

Materials

Ten litters of Sprague-Dawley rats were housed at 21°C in a 12-hour light/dark cycle with free access to food and water. Parturition was checked for on a daily basis and the day of birth was considered postnatal day 0 (P0). The mother and its litter were housed in 26 × 42 × 18 cm^3 Plexiglas cages. All rats were provided by the Laboratory Animal Center of the Southern Medical University (certificate No. SCXK (Yue) 2006-0015). All experiments were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[29].

Methods

Massage and JB1 injection

In the massage group, neonatal rats were separated from their mother twice daily (8:00 a.m. and 4:00 p.m.) from P1 to P15 and received massage. Massage therapy was carried out as follows; the neonatal rat was stationed and the subcutaneous tissue was grabbed on both sides of the coccygeal vertebra with the thumb and forefinger of both hands. The forefinger and middle finger moved ahead alternatively; the thumb pressed down and pushed forward, loosed then hard pressed, keeping the subcutaneous fascia lifted and held in the fingers. The

subcutaneous fascia was manipulated from tail to neck, nine times for one treatment. This technique is called Knead Ridge Therapy in traditional Chinese medicine[30].
In the maternal separation group, neonatal rats were separated from their mother at the same period of day and for the same amount of time, but did not receive massage.
Control group neonatal rats were not massaged and remained with their mother.
In the massage plus JB1 group, JB1 (Bachem, batch: 1035419, Bubendorf, Switzerland) was subcutaneously injected into the rats from P1 to P9. For each rat, a single injection per day was performed following massage (10 a.m.) over 9 consecutive days. JB1 concentration was 50 ng/μL and the volume of injection was weight adjusted for a dose of 18 ng/g[8, 31].
The control massage plus saline group underwent treatment with subcutaneous injection of identical volume with normal saline for 9 consecutive days.
BrdU (sigma; 50 mg/kg) was injected intraperitoneally at 2, 4 and 6 hours pre-cardiac perfusion to detect cell proliferation.

Tissue preparation

Rats were anesthetized with diethyl ether and perfused transcardially with phosphate buffer (100 mM, pH 7.4) followed by ice-cold 4% paraformaldehyde. The rats were decapitated. The brain was removed and postfixed overnight in phosphate buffer saline (50 mM, pH 7.4) containing 4% paraformaldehyde, prior to immersion in 30% sucrose solution (in 50 mM phosphate buffer saline) and storage at 4°C until sectioning. Frozen sections were prepared in the coronal plane (30 μm thick) from −1.5 mm bregma to 2.52 mm interaural using a cryostat (Leica, Nussloch, Germany) and stored at 4°C.

Immunohistochemistry for IGF-1 and BrdU

Immunohistochemical staining for IGF-1 or BrdU was conducted using rabbit anti-IGF-1 (1:200) polyclonal antibody (Santa Cruz, SC9013, USA), or mouse anti-BrdU (1:1 000) monoclonal antibody (Millipore, MAB3424, Massachusetts, USA) respectively at 4°C overnight. Secondary antibody (SP-9000 Histostain TM-Plus Kits, source: Goats) was applied at room temperature for 2 hours, using the free-floating avidin biotin complex method as previously described[32]. To detect BrdU-labeled cells, free-floating sections were rinsed extensively with phosphate buffered saline (0.05 M, pH 7.4) and incubated in HCl (2N) at 37°C for 30 minutes to denature the DNA. Slides were then developed with nickel-intensified diaminobenzidine.

Cell quantification

The number of immune-positive cells in the dentate gyrus was quantified bilaterally in each animal from six dorsal hippocampal sections, separated by six section intervals. The results were expressed as a mean value. The dentate gyrus, including the hilus, subventricular zone of lateral ventricle and the inner third of the granular-cell layer were observed for BrdU positive cells. The number of immunopositive cells in the dorsolateral angle of the subventricular zone of the lateral ventricle was quantified from one of five random visions at high magnification (× 400) in each animal from six dorsal hippocampal sections, separated by six section intervals.

Statistical analysis

All data were statistically processed using SPSS 13.0 software (SPSS, Chicago, IL, USA) and were expressed as mean ± SD. All intergroup group differences were compared using one-way analysis of variance. Comparisons between groups was performed using the *t*-test. A value of $P < 0.05$ was considered statistically significant.

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Author contributions: Zhongqiu Wen and Wenqin Zeng guided this study and wrote the manuscript. Lin Yuan and Jingxing Dai designed the study. Xin Zhou, Chun Yang, Fuhua Duan, Yufeng Liu and Huiying Yang conducted the experiment and analyzed the data.
Conflicts of interest: None declared.
Ethical approval: This experiment received permission from the Animal Care and Research Committee of Southern Medical University, China.

REFERENCES

[1] Smith JM, Sullivan SJ, Baxter GD. A descriptive study of the practice patterns of massage new zealand massage therapists. Int J Ther Massage Bodywork. 2011;4(1): 18-27.
[2] Tang HL, Pang J, Gao LF, et al. Systematic evaluation on treatment of sub-health with acupuncture-moxibustion and Massage in clinical researches. Zhongguo Zhen Jiu. 2010; 30(8):699-704.
[3] Roberts L. Effects of patterns of pressure application on resting electromyography during massage. Int J Ther Massage Bodywork. 2011;4(1):4-11.
[4] Minasny B. Understanding the process of fascial unwinding. Int J Ther Massage Bodywork. 2009;2(3): 10-17.

[5] Finando S, Finando D. Fascia and the mechanism of acupuncture. J Bodyw Mov Ther. 2011;15(2):168-176.
[6] Wang J, Wang CL, Shen BL, et al. Explanation of essence and substance basis of channels and collaterals with fasciology. Zhongguo Zhen Jiu. 2007;27(8):583-585.
[7] Wang J, Dong WR, Wang CL, et al. From meridians and acupoints to self-supervision and control system: a hypothesis of the 10th functional system based on anatomical studies of digitized virtual human. Nan Fang Yi Ke Da Xue Xue Bao. 2007;27(5):573-579.
[8] Guzzetta A, Baldini S, Bancale A, et al. Massage accelerates brain development and the maturation of visual function. J Neurosci. 2009;29(18):6042-6051.
[9] Field T, Diego M, Hernandez-Reif M, et al. Insulin and insulin-like growth factor-1 increased in preterm neonates following massage therapy. J Dev Behav Pediatr. 2008; 29(6):463-466.
[10] Nishijima T, Piriz J, Duflot S, et al. Neuronal activity drives localized blood-brain-barrier transport of serum insulin-like growth factor-I into the CNS. Neuron. 2010; 67(5): 834-846.
[11] Hamza RT, Ismail MA, Hamed AI. Growth hormone deficiency in children and adolescents with cerebral palsy: relation to gross motor function and degree of spasticity. Pak J Biol Sci. 2011;14(7):433-440.
[12] Kuperminc MN, Gurka MJ, Houlihan CM, et al. Puberty, statural growth, and growth hormone release in children with cerebral palsy. J Pediatr Rehabil Med. 2009;2(2): 131-141.
[13] Reimunde P, Rodicio C, Lopez N, et al. Effects of recombinant growth hormone replacement and physical rehabilitation in recovery of gross motor function in children with cerebral palsy. Ther Clin Risk Manag. 2010; 6:585-592.
[14] Torres-Aleman I. Toward a Comprehensive Neurobiology of IGF-I. Dev Neurobiol. 2010;70(5):384-396.
[15] Field T, Diego M, Hernandez-Reif M. Preterm infant massage therapy research: a review. Infant Behav Dev. 2010;33(2):115-124.
[16] Kulkarni A, Kaushik JS, Gupta P, et al. Massage and touch therapy in neonates: the current evidence. Indian Pediatr. 2010;47(9):771-776.
[17] Diego MA, Field T. Moderate pressure massage elicits a parasympathetic nervous system response. Int J Neurosci. 2009;119(5):630-638.
[18] Glew GM, Fan MY, Hagland S, et al. Survey of the use of massage for children with cerebral palsy. Int J Ther Massage Bodywork. 2010;3(4):10-15.
[19] Lee MS, Kim JI, Ernst E. Massage therapy for children with autism spectrum disorders: a systematic review. J Clin Psychiatry. 2011;72(3):406-411.
[20] Silval MT, Schalock M, Ayres R, et al. Qigong massage treatment for sensory and self-regulation problems in young children with autism: a randomized controlled trial. Am J Occup Ther. 2009;63(4):423-432.
[21] Hamrick MW, Mcneil PL, Patterson SL. Role of muscle-derived growth factors in bone formation. J Musculoskelet Neuronal Interact. 2010;10(1):64-70.
[22] Wei X, Zhao L, Zhong J, et al. Adipose stromal cells-secreted neuroprotective media against neuronal apoptosis. Neurosci Lett. 2009;462(1):76-79.
[23] Yakar S, Liu JL, Stannard B, et al. Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci U S A. 1999;96(13): 7324-7329.
[24] Choi YS, Cho HY, Hoyt KR, et al. IGF-1 receptor-mediated ERK/MAPK signaling couples status epilepticus to progenitor cell proliferation in the subgranular layer of the dentate gyrus. Glia. 2008;56(7):791-800.
[25] Carro E, Nunez A, Busiguina S, et al. Circulating insulin-like growth factor I mediates effects of exercise on the brain. J Neurosci. 2000;20(8):2926-33.
[26] Ding Q, Vaynman S, Akhavan M, et al. Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. Neuroscience. 2006; 140(3):823-833.
[27] Landi S, Ciucci F, Maffei L, et al. Setting the pace for retinal development: environmental enrichment acts through insulin-like growth factor 1 and brain-derived neurotrophic factor. J Neurosci. 2009;29(35): 10809-10819.
[28] Wu Y, Sun H, Yakar S, et al. Elevated levels of insulin-like growth factor (IGF)-I in serum rescue the severe growth retardation of IGF-I null mice. Endocrinology, 2009 ;150(9): 4395-4403.
[29] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.
[30] Zhou YF, Jiao F, Lei Y. Mechanism discussion of knead ridge therapy in treating children with spleen and stomach weakness. Zhongyi Xuebao. 2011;26(6):760-761.
[31] Ciucci F, Putignano E, Baroncelli L, et al. Insulin-like growth factor 1 (IGF-1) mediates the effects of enriched environment (EE) on visual cortical development. PLoS One. 2007;2(5):e475.
[32] Lee S, Kim DH, Yoon SH, et al. Sub-chronic administration of rimonabant causes loss of antidepressive activity and decreases doublecortin immunoreactivity in the mouse hippocampus. Neurosci Lett. 2009;467(2):111-116.

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