

EXPERIMENTAL STUDY

Effects of electroacupuncture at Guanyuan (CV 4) or Sanyinjiao (SP 6) on hypothalamus-pituitary-ovary axis and spatial learning and memory in female SAMP8 mice

Wang Jing, Cheng Kai, Qin Zhuo, Wang Yanping, Zhai Lijing, You Min, Wu Juanjiao

Wang Jing, Cheng Kai, Qin Zhuo, Wang Yanping, Zhai Lijing, You Min, Wu Juanjiao, School of Acupuncture, Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China

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Correspondence to: Prof. Cheng Kai, School of Acupuncture, Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China. kevin@chengs.cn.

Telephone: +86-13601106820

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Abstract

OBJECTIVE: To investigate the effects of electroacupuncture (EA) at the Guanyuan (CV 4) or Sanyinjiao (SP 6) acupoints on the hypothalamus-pituitary-ovary (HPO) axis and spatial learning and memory in female mice.

METHODS: Nine-month-old female mice with senescence-accelerated mouse prone 8 (SAMP8) were divided into three groups: the disease model, EA-Guanyuan and EA-Sanyinjiao groups. Concurrently, 9-month old female mice with senescence-accelerated mouse resistance 1 (SAMR1) were set as the control model group. The two treatment groups were given the same pattern of EA stimulation. Gonadotropin-releasing hormone, luteinizing hormone, follicle-stimulating hormone (FSH) and Serum estradiol levels in the Hypothalamus-pituitary-ovary axis were assessed by enzyme-linked immunosorbent assay to determine

the HPO axis function level. Spatial learning and memory were assessed by the Morris Water Maze (MWM) test.

RESULTS: (a) HPO axis: compared with the control model group, the disease model group displayed a decrease in E2 levels ($P < 0.01$), and an increase in GnRH, LH and FSH levels ($P < 0.01$). E2 levels were increased in EA treatment groups compared with the disease model group ($P < 0.05$). In contrast, GnRH and LH and FSH levels were reduced ($P < 0.05$). EA-Sanyinjiao group was superior than EA-Guanyuan group on increasing E2 and declining GnRH levels ($P < 0.01$). (b) The MWM test demonstrated that the response latency in the EA-Sanyinjiao treatment group declined from day 2 to day 5 compared with the disease model group ($P < 0.05$), whereas the EA-Guanyuan treatment group showed no significant difference.

CONCLUSION: EA can regulate hormone (E2, FSH, LH, GnRH) levels in the HPO axis and the spatial learning and memory ability in female SAMP8 mice. Moreover, this effect may have been more pronounced in the EA-Sanyinjiao group than the EA-Guanyuan group. The underlying mechanism of the EA-induced changes may be related to gonadal hormone shifts in the HPO axis, followed by an improvement in spatial learning and memory.

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Key words: Alzheimer disease; Electroacupuncture; Point CV 4 (Guanyuan); Point SP 6 (Sanyinjiao); Spatial learning; Memory; Hypothalamus-pituitary-ovary axis

INTRODUCTION

Alzheimer's disease (AD) is a progressive, degenerative central nervous system disease. The clinical manifestations of AD include gradual worsening of memory impairment, loss of cognitive skills, personality changes, and abnormal mental and behavioral activities. With the aging of the global population, the incidence of AD is increasing yearly. Age has been considered the primary risk factor for AD. However, in decade years ago, some researchers have found that the prevalence of AD is much higher in women than men of the same age,^{1,2} and this trend becomes stronger with age. The incidence of female is 2-3 times to male.³ The increased risk is mainly caused by the hypothalamus – pituitary – ovary axis-related hormone disorders resulting from a decline of peri-menopausal ovary function.^{4,7} Many studies of the mechanisms of AD have considered the estrogen level decline theory,^{8,9} and estrogen is thought to have a close relationship with the learning and memory ability.¹⁰ Estrogen has the function of the neurons protection.¹¹ When menopausal women lose the protection of estrogen, moderate supplementation of estrogen may lower the risk of developing AD.^{12,13} Nevertheless, there are many side effects of estrogen therapy and limitations with using estrogen in clinical practice and animal studies.¹⁴ Therefore, it is important to find a way to improve both female gonadal hormone levels and HPO axis function. The Guanyuan (CV 4) and Sanyinjiao (SP 6) acupoints are widely used to treat climacteric syndrome. Previous studies confirmed that electroacupuncture (EA) at these acupoints is beneficial for HPO axis function in ovariectomized rats,^{15, 16} and EA at Sanyinjiao (SP 6) had benign adjustment to HPO axis in perimenopausal rats.¹⁷ Based on these prior findings, the current study used 9-month-old female SAMP8 mice that had reduced learning and memory abilities, and displayed menopausal symptoms as an AD model.¹⁸ EA at Sanyinjiao (SP 6) and Guanyuan (CV 4) were conducted on female AD model mice to investigate the effects on the HPO axis as well as on learning and memory.

MATERIALS AND METHODS

Animal selection and grouping

Both SAMP8 and SAMR1 mice are types of senescence-accelerated mice (SAM), a murine model developed at Kyoto University in 1975. Compared with SAMR1 mice, the life span of SAMP8 mice is shorter, reduction of neurons, nerve cell atrophy, pathological changes in neurotransmitter metabolism. SAMP8 mice display learning and memory impairments from 5 months of age. Hormone levels in the HPO axis and the estrous cycle were observed in mice of different ages. The results demonstrated that 9-month-old and 11-month-old SAMP8 female mice, and 11-month-

old SAMR1 female mice, have irregular estrous cycle and sex hormone levels abnormal. Furthermore, 9-month-old female SAMP8 mice display more marked abnormalities in hormone levels than SAMR1 mice. Therefore, 9-month-old female SAMP8 mice were selected as the most suitable type of model animal.

Instruments and reagents

New Xinglin acupuncture needles (Tian Yu Heng Science and Technology Co., Ltd., Beijing, China, 0.17 mm × 7 mm), a low-temperature centrifuge (ZK380, Hermle, Germany), microscope (Olympus Medical Co., Tokyo, Japan), and HANSLH202 EA device (Peking University Institute of Science Nerve and Beijing HuaWei Industrial Development Company, Beijing, China). ELISA kits (Dingguochangsheng Biotechnology Co., Ltd., Beijing, China) were used to detect the levels of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and Serum estradiol (E2).

EA manipulation

In the EA-Guanyuan and EA-Sanyinjiao groups, treatment was performed for 20 min, once every 2 days for 30 days total. These acupoints were identified in accordance with the "Laboratory Animal Acupuncture Atlas," developed by the National Acupuncture Society for Experimental Research. The needle handle was connected to the EA device to emit sparse waves at 2 Hz, 2 V and 1 mA. Mice in the model control group and disease model group were restrained to ensure that treatment was conducted under the same rearing conditions.

Tissue handling

Mice were decapitated quickly, and the hypothalamus and pituitary apoplexy were removed and placed on bags of ice. The ovaries were removed by laparotomy and placed in liquid nitrogen after being wiped clean with filter paper. The tissues were precisely weighed on an electronic scale; physiological saline was added at 1:9 dilution; the tissues were then pulverized and placed into an Eppendorf tube at a low temperature (4 °C) for centrifugation at 1132.6 × g for 15 min. Next, the supernatant was extracted and stored in a refrigerator (– 80 °C) until analysis.

Estrous cycle

Histological analysis of exfoliated vaginal cells is a simple and effective method to identify estrous levels at various stages. epithelial tissues were taken from the vagina regularly on a daily basis at the same time (9:00 am), followed by dyeing with alkaline Meilan stain, using distilled water to flush the dye solution after 20 min. Histological changes in stained tissues were observed under a microscope. The observation was carried out during three estrous cycles (15 days). Female SAMP8 mice with estrous axis disorder, as measured by cell type (white blood cells, keratinocyte, nucleated cells) and quantity, were excluded.

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