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Electro-acupuncture improves the social interaction behavior of rats

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HIGHLIGHTS

- Single EA increased OXT and AVP mRNA levels in the SON.
- Repeated EA improved the social behavior of low-socially interacting rats.
- Repeated EA elevated expressions of OXT and AVP in the SON.
- Activation of OXT/AVP systems may contribute to the pro-social effect of repeated EA.

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ABSTRACT

Oxytocin (OXT) and arginine-vasopressin (AVP) are two closely related neuropeptides and implicated in the regulation of mammalian social behaviors. A prior clinical study in our laboratory suggested that electro-acupuncture (EA) alleviated social impairment in autistic children accompanied by changes of peripheral levels of OXT and AVP. However, it remains unclear whether EA stimulation had an impact on central OXT and AVP levels. In the present study, rats were subjected to a single session of EA (sEA) or repeated sessions of EA (rEA). Following the stimulation, mRNA levels and peptide levels of OXT/AVP systems were determined. The results showed that sEA led to region-specific up-regulation of OXT and AVP mRNA levels in the hypothalamus where the peptides were produced, without affecting the content of OXT and AVP in the hypothalamus and peripheral blood. The rEA of 5 sessions in 9 days was given to the low socially interacting (LSI) rats. LSI rats that underwent rEA showed significant improvement of social behavior characterized by spending more time investigating the strange rats in the three-chamber sociability test. The improved sociability was accompanied by an up-regulation of mRNA and the peptide levels of OXT or AVP in SON of the hypothalamus as well as a significant increase of the serum level of AVP. It is concluded that activation of OXT/AVP systems may be associated with the pro-social effect caused by EA stimulation.

1. Introduction

The neuropeptides oxytocin (OXT) and arginine-vasopressin (AVP), mainly synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, play a facilitatory role in a variety of social interactions as has been confirmed both in animal and human studies. In rodents, administration of exogenous OXT or AVP enhanced social proximity [1] and helped to overcome social defeat-induced social avoidance [2]. Additionally, OXT, OXT receptor or AVP receptor knockout mice displayed impaired social communication and social preference [3–5]. In clinical trials, intranasal OXT administration has been shown to affect many aspects of human sociability including empathy, in-group trust and cooperation [6–8]. Intranasal AVP application revealed similar effects on social recognition and emotion encoding [9–10]. However, animal studies demonstrated that a single administration of OXT could adversely affect the endogenous OXT system and other systems in the developing brain [11]. Additionally, chronic intranasal OXT has detrimental effects on social behaviors [12] accompanied by a reduction of the OXT receptor (OXTR) in various brain regions of mice [13]. Therefore, it is of great interest to find a therapy which can activate the intrinsic AVP and/or OXT systems in the brain in order to enhance the social interaction.

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Acupuncture, an important component of the traditional Chinese medicine, has been used as a therapeutic option for a wide range of clinical conditions. It has been suggested that acupuncture or electroacupuncture (EA) stimulation with unique frequencies facilitates the release of frequency-specific neurochemicals in the central nervous system (CNS) and elicited profound physiological effects [14]. A previous study has shown that 2 Hz EA significantly increased OXT levels in both plasma and cerebrospinal fluid of rats [15]. Results of several clinical studies indicated that long-term acupuncture treatment improved social communication, social interaction and emotional response in autistic patients characterized by social behavior impairment [16–18].

We hypothesized that EA may promote the function of central OXT/AVP systems thus influencing sociability. Two series of experiments were performed in SD rats. Normal SD rats were given a single session of 2/15 Hz EA stimulation for 30 min and the rats were then sacrificed. Tissues were harvested immediately for detection of the expressions of OXT and AVP in SON and PVN of the hypothalamus. Blood was also collected to measure the serum levels of the peptides. To explore whether the pro-social effect of EA is associated with an increased turnover rate of the brain social neuropeptides OXT and AVP, we carried out another experiment using the natural occurring low socially interacting (LSI) rats that were selected from the normal SD rat population with a three-chamber test. These LSI rats were subjected to 30 min 2/15 Hz EA every other day for a total of 5 sessions. Then the three-chamber sociability test was conducted at the termination of the stimulation. Changes in the capability of social interaction of the rats were coped with the alteration of the levels of OXT and AVP in the hypothalamus and in the serum, as well as the mRNA level of their receptors in the amygdala.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats weighing 250 g were obtained from the Department of Experimental Animal Sciences, Peking University Health Science Center. The rats were housed 5 per cage and maintained on a 7 AM and 7 PM light–dark cycle with free access to food and water. All animal experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, USA and were approved by the Peking University Animal Care and Use Committee.

2.2. Single EA stimulation

Thirty-two male rats were used in this experiment. They were randomly and evenly distributed into two groups. One group received a single session of EA (sEA group), and the other served as the control group [19–22]. The unrestrained EA method was adopted, allowing rats to move freely in the cage during EA stimulation instead of immobilization in a plastic tube. In brief, rats were briefly restrained in plastic tubes with the hindlimbs extending through two holes. To avoid the spontaneous detachment of inserted acupuncture needles from the rat body, we used hook-shaped needles (0.35 mm in diameter) as previously reported [23]. Hook-shaped needles were swiftly inserted into the skin and underneath tissues at bilateral acupoint Zusanli (ST-36) which is located at the lower lateral to the anterior tubercle of the tibia. The use of ST-36 was supported by previous studies [18,24]. The needle insertion procedure typically lasted about 1 min and caused little distress. These rats were then released from tubes and allowed to move freely in the cages with the inserted needles in the bilateral acupoints connected to a Han's Acupoint Nerve Stimulator (HANS, LH series, manufactured at Nanjing Jesen Company, Nanjing) and stimulated by rectangular dense-disperse pulses of 2/15 Hz (pulse width: 0.6 ms in 2 Hz, and 0.4 ms in 15 Hz, each lasting for 3 s). The delivery of appropriate intensity of stimulation was confirmed by observing light local muscle twitches to reflect the activation of muscle–nerve afferents. The sEA lasted for 30 min with intensities increased in a stepwise manner at 1.0, 1.5 and 2.0 mA with 10 min for each step. The animals in the control group were briefly restrained in the same manner as the EA treatment without needle insertion. Then these rats were released into a cage and allowed to move freely for 30 min.

2.3. Three-chamber sociability test

The sociability of animals was assessed in the three-chamber apparatus according to prior studies with minor modification [25]. The apparatus was a rectangular, three-chambered Plexiglas box (40 cm × 34 cm × 24 cm) with the side chambers each connected to the middle chamber by a corridor. The test began with a 5 min habituation and allowed the subject rat to explore the whole apparatus freely. This rat was then encouraged into the center chamber with bilateral corridors closed by the side doors. An unfamiliar stranger (a sex-matched SD rat) was locked in a small cage made of stainless-steel wires, and placed in one of the side chambers. At the same time, an identical but empty cage was placed in the other side chamber. The side doors were then opened simultaneously and the subject rat was allowed to access both chambers for 10 min. Time spent in each of the three chambers was recorded automatically. To minimize the impact from residual rat odors, the entire apparatus was thoroughly cleaned by 70% ethanol at the beginning of each trial. All experimental rats were tested during their dark cycle.

2.4. Repeated EA intervention

Thirty-eight low socially interacting (LSI) rats were selected based on their social interaction time spent in the three-chamber test from a total of ninety-six rats. The LSI rats were randomized into two groups: the rEA group and control group. Animals in the rEA group were subjected to 30 min 2/15 Hz EA treatment every other day (on the 1st, 3rd, 5th, 7th and 9th day) for a total of 5 sessions. The EA procedure used in each session of rEA was the same as described for sEA. The rats in the control group went through the same procedure as rats in the rEA group without having needles inserted into the tissue.

2.5. Brain tissue collection

Normal rats were euthanized by decapitation immediately following sEA for tissue collection and biochemical assessment. LSI rats underwent a social behavior test after completion of rEA before decapitation. The brain was quickly removed and frozen in liquid nitrogen with embedding medium for 20 s. The frozen brains were stored at −80 °C until further processed. Bilateral micropunches of 1.5 mm in diameter (1 mm for paraventricular nucleus) were taken using a freezing microtome from the following regions: the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, basolateral nucleus of the amygdala (BLA), central nucleus of the amygdala (CeA) and medial nucleus of the amygdala (MeA). These brain regions were identified based on the Paxinos and Watson Rat Brain Atlas (PVN: plates 38–49, SON: plates 37–47, BLA: plates 47–63, CeA: plates 47–58, MeA: plates 47–63). The unilateral micropunch of PVN or SON was used to determine OXT/AVP mRNA levels. The contralateral micropunch was used to detect the peptide contents.

2.6. Determination of mRNA levels of OXT, AVP and their receptors

Total mRNA was extracted from the brain tissue micropunches using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. For each sample, 1 μg RNA was reverse-transcribed by a PrimeScript reverse transcription reagent kit (TaKaRa, Dalian, China). The expression level of the target genes (OXT, AVP, OXT receptor (OXTR) and AVP receptor 1a (V1aR)) and the internal