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Electro-acupuncture improves the social interaction behavior of rats

Hong-Feng Zhang [a,b,c,1], Han-Xia Li [a,b,c,1], Yu-Chuan Dai [a,b,c], Xin-Jie Xu [a,b,c], Song-Ping Han [d], Rong Zhang [a,b,c,*], Ji-Sheng Han [a,b,c,*]

[a] Neuroscience Research Institute, Peking University, Beijing, PR China
[b] Department of Neurobiology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, PR China
[c] Key Laboratory for Neuroscience, Ministry of Education/National Health and Family Planning Commission, Peking University, Beijing, PR China
[d] School of Basic Medical Sciences, Peking University, Beijing, PR China

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.

* Corresponding authors at: Neuroscience Research Institute, Peking University, 38 Xue Yuan Road, Beijing 100191, PR China.
E-mail addresses: zhangrong@bjmu.edu.cn (R. Zhang), hanjisheng@bjmu.edu.cn (J.-S. Han).
[1] Hong-Feng Zhang and Han-Xia Li contributed equally to this work.

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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 control gene (β-

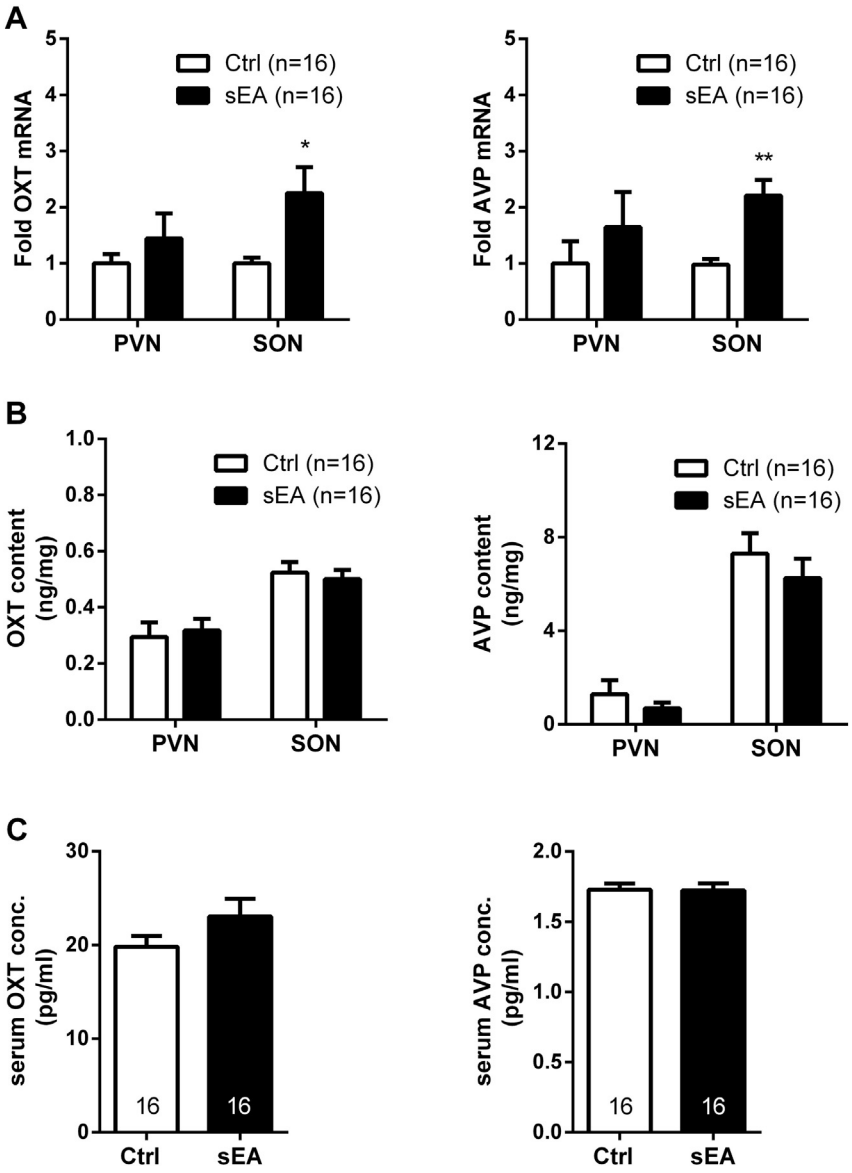

Fig. 1. Effects of 30 min sEA on OXT and AVP levels. (A) OXT and AVP mRNA levels were significantly up-regulated in SON, but not in PVN, after the sEA administration. (B) OXT or AVP content in either PVN or SON was not changed. (C) No difference was found in serum OXT or AVP concentrations between control and sEA groups. N = 16 in each group, data are expressed as mean ± SEM, $^{*}P < 0.05$, and $^{**}P < 0.001$. OXT: oxytocin; AVP: arg-vasopressin; PVN: paraventricular nucleus; SON: supraoptic nucleus; Ctrl: control group; and sEA: single EA group.

actin) was estimated by TaqMan® Gene Expression Assays (assay ID: OXT — Rn00564446_g1, AVP — Rn00690189_g1, OXTR — Rn00563503_m1, V1aR — Rn00583910_m1, β-actin — Rn00667869_m1) on a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) under standard amplification conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 1 min at 60 °C. All samples were assayed in duplicate in an optical 96-well reaction plate. Data were transformed using the ΔΔCT method with β-actin as the reference gene, and further normalized to the control samples in the control group for comparison.

2.7. Detection of peptide levels of OXT and AVP

The tissue micropunches were sonicated for 1 min in ice-cold RIPA buffer (Beyotime, Hangzhou, China) containing a 1:100 protease inhibitor, and then centrifuged for 15 min at 12,000 ×*g*. After centrifugation, the supernatant was removed and frozen at −80 °C. The total protein concentration of each sample was determined by a BCA Protein Assay kit (Beyotime, Hangzhou, China) and read by a BIO-RAD iMark™ microplate reader.

OXT content and AVP content were assayed in protein extracts of the PVN and SON using an Oxytocin ELISA kit (Enzo Life Sciences, PA, USA) and Arg-Vasopressin ELISA kit (Enzo Life Sciences, PA, USA), respectively. Non-extracted samples were diluted to 100 μl and assayed according to the product manual supplied by the manufacturer. The OXT and AVP content for each sample was corrected by the total protein concentration of that sample. These two ELISA kits were highly sensitive (detecting limit: 15.0 pg/ml for OXT and 3.39 pg/ml for AVP) with very little cross-reactivity with other related compounds. The percent cross-reactivity between OXT and AVP was less than 0.02%. The intra-assay CV for OXT and AVP was 10.2% and 5.9%, respectively.

2.8. Determination of OXT and AVP concentrations in the serum

Trunk blood (5 ml) was collected rapidly following decapitation into a clean tube containing Aprotinin (500 KIU/ml of blood). The blood samples were kept at room temperature for 30 min, and then centrifuged at 1600 ×*g* for 15 min at 4 °C. The serum was isolated and divided into aliquots of 650 μl and stored at −80 °C. In order to accurately determine serum OXT and AVP concentrations, all samples were subjected to prior

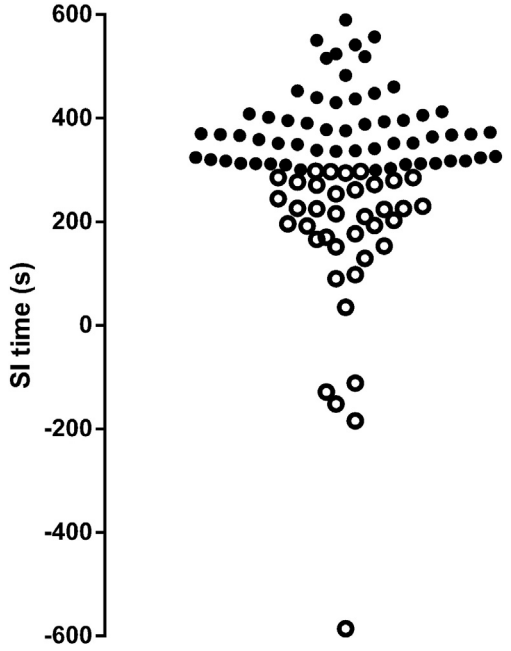

Fig. 2. Scatter-plot showing SI times of 96 normal SD rats. Thirty-eight LSI rats (empty circle) were selected from the rat population. SI time: social interaction time.

extraction with acetone and petroleum ether. The final serum levels of the two peptides were detected using the same OXT and AVP ELISA kits as mentioned above.

2.9. Statistics

Statistical analyses were performed using IBM SPSS Statistics 19 (SPSS, Inc., an IBM Company) and graphs were generated by GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The comparisons in mRNA levels and peptide levels of OXT/AVP systems between the control and EA-treated groups were analyzed by an unpaired *t* test. For those data with non-normal distribution, non-parametric tests (Mann–Whitney U test) were used for the comparisons. A paired *t* test was applied to analyze the data of social behavior before and after the intervention. Social behavior between control and rEA groups was evaluated by the Mann–Whitney U test. The Spearman correlation coefficient was used to assess the relationship between OXT level and AVP level in SON or PVN, as well as correlation between OXT/AVP systems and social interaction time. All results were expressed as mean ± SEM and $P < 0.05$ (two-tailed) was considered as reaching the statistical significance.

3. Results

3.1. The influence of sEA stimulation on OXT and AVP levels

As displayed in Fig. 1A, 30 min sEA stimulation led to a significant increase of both OXT and AVP mRNA levels in SON ($P = 0.0189$ and $P < 0.0001$, respectively), suggesting increased expressions of the genes. However, no such changes were observed in the PVN where the same two neuropeptides were also synthesized. Enzyme immunoassay showed that sEA failed to influence the peptide levels of OXT or AVP in PVN, SON (Fig. 1B) or peripheral blood (Fig. 1C).

3.2. Selection of low socially interacting rats

The three-chamber sociability test was used to estimate the natural variation in social interaction of normal SD rats. Social interaction time (SI time) was defined as the time spent in the stranger side minus the time spent in the empty cage side ($time_{stranger} - time_{empty}$) [23–24]. The average SI time of the 96 normal rats was 294.8 s with the standard deviation of 168 s (Fig. 2). The bottom 40 percentile of the rats (thirty-eight rats) was defined as low socially interacting (LSI) rats. The SI times of the LSI rats were presented by the empty circles in Fig. 2.

3.3. rEA stimulation resulted in a pro-social effect in LSI rats

In order to assess the influence of rEA on social behavior, the LSI rats in the rEA group were subjected to 2/15 Hz EA stimulation for 30 min every other day for 5 sessions. During this experiment, one rat in the rEA group died because of head injuries by a cage cover squeeze accidentally. After 9 days of treatment, the sociability of the two groups was tested again using the three-chamber apparatus. Compared with the behavior of pre-rEA rats, post-rEA rats spent longer time interacting with the strange rat (paired *t* test, $P = 0.009$, Fig. 3A) and shorter time in the empty cage side (paired *t* test, $P = 0.012$, Fig. 3A) resulting in a significant increase of SI time (paired *t* test, $P = 0.0172$, Fig. 3B). For the control group, a 9 day time lapse did not lead to marked changes of the time spent in each chamber (Fig. 3C), or the SI time (Fig. 3D). These data indicate that rEA may have a pro-social effect. In addition to longitudinal intra-group comparisons of social behavior, the sociability of control and rEA groups was also compared after the intervention. Contrasted with the control group, rats in the rEA group spent approximately 39% more time in the chamber containing an unfamiliar conspecific ($P = 0.048$, Fig. 3E) and about 49% less time in the chamber with the empty cage ($P = 0.049$, Fig. 3E). SI time also showed a tendency of an increase following rEA treatment ($P = 0.059$, Fig. 3F). These behavioral data suggested that rEA intervention led to a significant improvement in social behavior of LSI rats.

3.4. Changes of OXT/AVP systems following rEA treatment

To investigate the underlying mechanisms of the EA-elicited pro-social effect, the alterations of OXT/AVP systems in LSI rats were analyzed after the social behavior test. The OXT mRNA level in either PVN or SON in the rEA group was not changed as compared to the control group (Fig. 4A). However, the AVP mRNA level was nearly doubled in SON but not in PVN following rEA ($P = 0.007$, Fig. 4B). In contrast to sEA, rEA significantly elevated OXT content ($P = 0.03$, Fig. 5C) and led to a tendency of an increase of AVP content ($P = 0.074$, Fig. 5D) in SON. The Serum level of OXT remained unchanged after the stimulation (Fig. 5E). In accordance with the elevation of the AVP level in the SON, rats exposed to rEA also showed a significant increase of serum AVP ($P = 0.042$, Fig. 5F). The mRNA levels of OXTR and V1aR were additionally measured in three sub-regions of the amygdala, which has been implicated as one of the key regions mediating neuronal actions of OXT and AVP on social behaviors. OXTR mRNA levels were not changed in all three sub-regions of the amygdala (Fig. 4G). Intriguingly, the V1aR mRNA level was decreased in BLA ($P = 0.029$, Fig. 4H) in response to rEA intervention.

3.5. The relationship between OXT/AVP systems and social behavior

We made a correlation analysis between rEA-elicited changes in social behavior (SI time) and changes in mRNA or peptide levels in brain regions collected in the study. Spearman correlation analysis revealed that the SI time of rats in control and rEA groups showed positive correlations with mRNA levels of OXT and AVP in SON (Fig. 5A and 5B), which suggested that a longer SI time was associated with higher mRNA levels of OXT and AVP in SON. No other correlations were observed between SI time and OXT/AVP systems (Table 1).

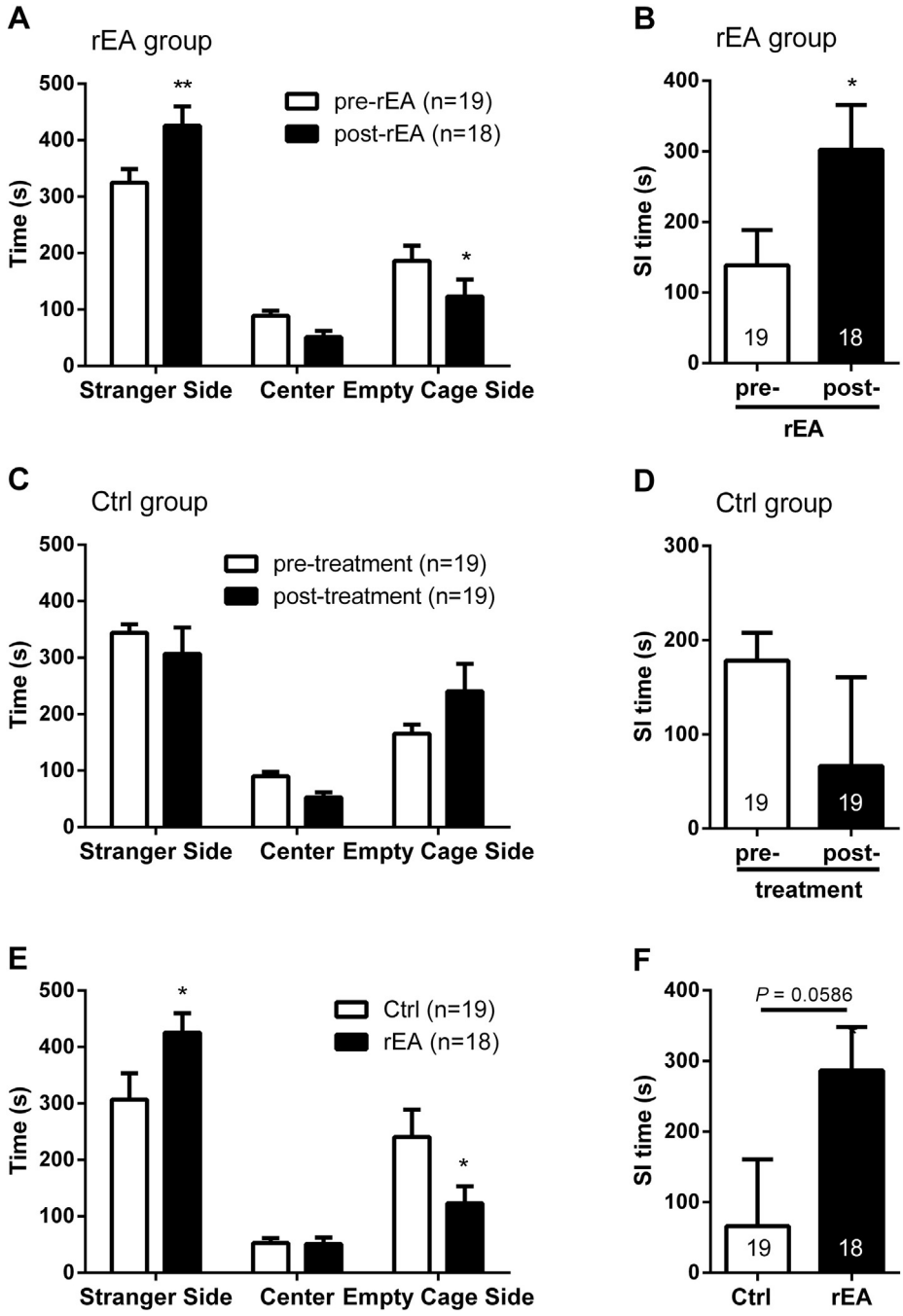

Fig. 3. LSI rats spent more time in close proximity to conspecifics following rEA stimulation. (A, B) Longitudinal comparison of sociability between pre-rEA and post-rEA. Rats spent more time in the cage with a strange rat and less time in empty cage side after the rEA. Accordingly, the SI time was markedly increased following the stimulation. (C, D) No such differences were seen in the control group. (E, F) Comparison of social interaction between control and rEA groups. Rats subjected to rEA showed longer time in proximity to the stranger and shorter time in the empty cage. The difference of SI time between the two groups was nearly significant. N = 18–19 in each group, data are expressed as mean ± SEM, $^{*}P < 0.05$, and $^{**}P < 0.001$. Ctrl: control group; rEA: repeated EA group; and SI time: social interaction time.

3.6. Correlations between OXT and AVP levels in the hypothalamus

As revealed by the Spearman correlation analysis, a positive correlation between OXT and AVP mRNA levels was observed in PVN ($r = 0.716$, $P < 0.0001$, Fig. 6A) and SON ($r = 0.678$, $P < 0.0001$, Fig. 6B) in LSI rats. Peptide levels of OXT and AVP were also positively correlated with each other within PVN ($r = 0.774$, $P < 0.0001$, Fig. 6C) and SON ($r = 0.612$, $P < 0.0001$, Fig. 6D). These data suggest that the expressions of the two peptides may be similarly regulated.

4. Discussion

In the present study, we showed for the first time that 30 min sEA treatment significantly increased the expressions of OXT and AVP genes in the SON of normal rats. No such alteration was seen in the content of the two neuropeptides in SON or PVN. The differential changes between the OXT/AVP mRNA and peptide levels in response to sEA led us to hypothesize that the interval between stimulation and tissue collection might be too short to allow the mRNA to be translated into

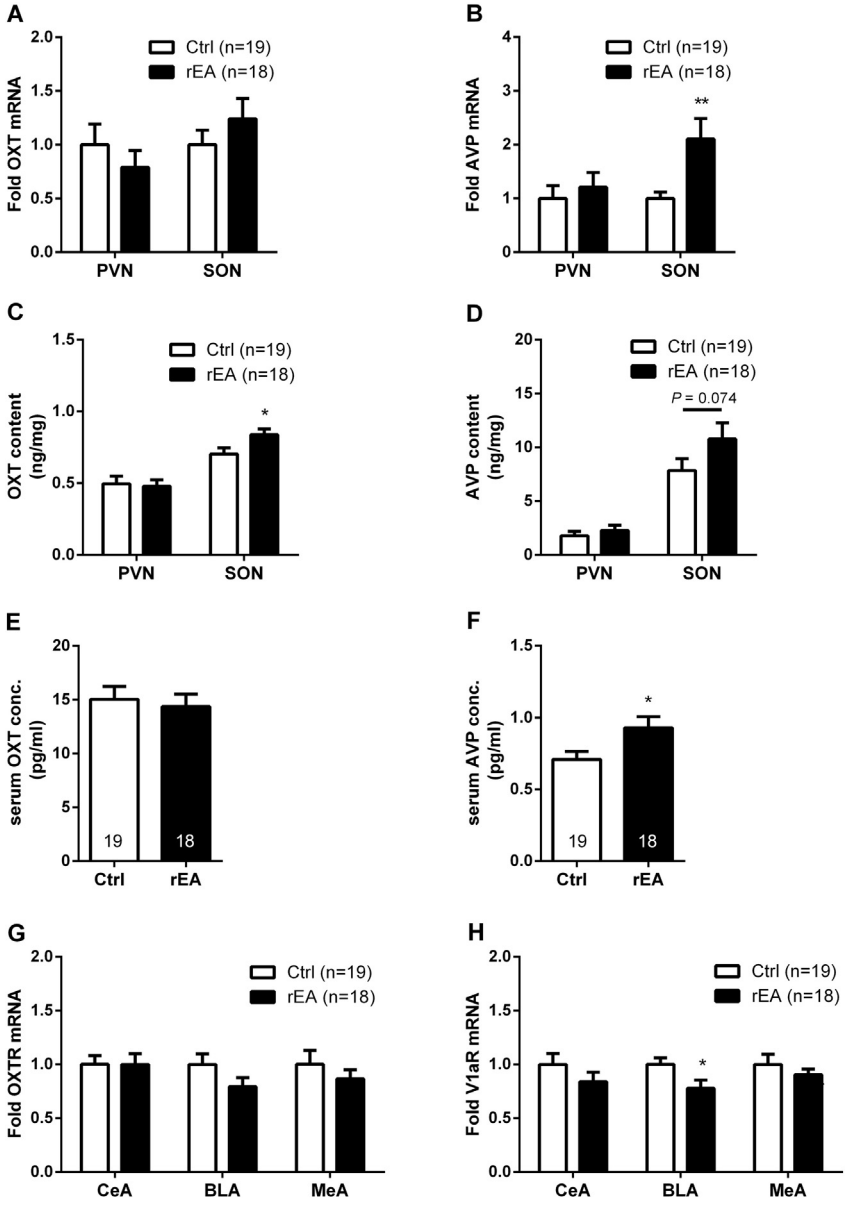

Fig. 4. Effect of rEA on OXT/AVP systems of LSI rats. (A, B) rEA treatment failed to affect the level of OXT mRNA in PVN or SON, but significantly increased AVP mRNA level in SON. (C, D) A significant increase of peptide level of OXT and a marginal increase of AVP level were found in SON in rEA group as contrast to control group. (E) No significant difference was found in serum OXT concentration between control and rEA groups. (F) Serum AVP concentration in rEA group was higher than that in control group. (G, H) rEA stimulation did not alter OXTR mRNA level in all three sub-regions of amygdala but selectively reduced V1aR mRNA level in BLA sub-region. N = 18–19 in each group, data are expressed as mean ± SEM, $^{*}P < 0.05$, and $^{**}P < 0.001$. OXT: oxytocin; AVP: arg-vasopressin; OXTR: OXT receptor; V1aR: AVP receptor 1a; PVN: paraventricular nucleus; SON: supraoptic nucleus; CeA: central nucleus of the amygdala; BLA: basolateral nucleus of amygdala; MeA: medial nucleus of the amygdala; Ctrl: control group; and rEA: repeated EA group.

proteins. LSI rodents not only manifested poor social interaction behavior but also had low mRNA levels of OXT and AVP [26], which appear to be ideal biomarkers to verify the efficacy of EA. We provided evidence that rEA stimulation resulted in greater preference for the stranger of LSI rats in the social approach test and simultaneously enhanced the function of OXT/AVP systems by up-regulating mRNA and peptide levels of OXT or AVP in SON, as well as an increase in peripheral AVP release. In addition, the OXT and AVP mRNA levels in SON were found to be positively correlated to the rat's social behavior, indicating that the prosocial effect induced by rEA stimulation may in part be mediated through activation of oxytocinergic and vasopressinergic transmission.

In the majority of EA experiments, rodents are placed into small tubes or bags during the EA stimulation. However, this restraining procedure may lead to stress-like behaviors such as vocalization, urination and hindlimb flinches [27]. Additionally, it is well known that OXT/AVP systems could be affected by acute or chronic restraint stress [28–29], as well as other types of stressors such as forced swimming [30], dehydration [31] or electric footshock [32]. To minimize the abovementioned potential interference and to investigate the therapeutic effects of electrical stimulation, we adopted the unrestrained EA method which allowed rats to move freely in the cage during EA intervention. This method seemed to cause less stress and maintained the physiological effects as produced in restrained rats [27].

There is no completely satisfactory control in acupuncture research in human or animal studies. Some investigators inserted needles into control rats without having electric current [33–34]. Others put the control animals under same handling and restriction procedures without having needles inserted into the acupoints [20–22,35]. Similarly, the control rats in our study also underwent the same process as EA rats except needle penetration and electrical stimulation. We believe that electro-acupuncture includes procedures of needle insertion and the subsequent electrical stimulation. Insertion of needles into the

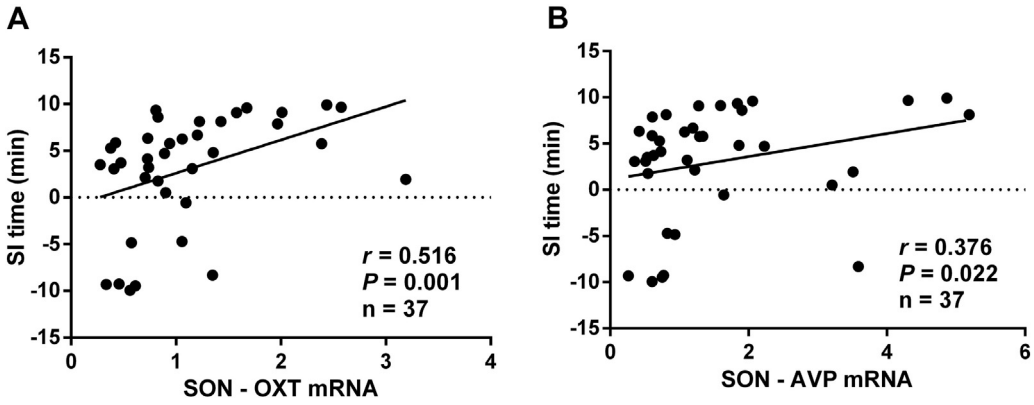

Fig. 5. Correlations of SI time with OXT mRNA and AVP mRNA levels in SON. Both OXT (A) and AVP (B) mRNA levels in SON were positively correlated with SI time. The correlation value was 0.516 ($P = 0.001$) for OXT mRNA and 0.376 ($P = 0.022$) for AVP mRNA. OXT: oxytocin; AVP: arg-vasopressin; SON: supraoptic nucleus; and SI time: social interaction time.

acupoints is an important part of EA intervention. At the same time, a retained needle at a specific acupoint could produce biological effects even with no electric stimulation [36].

It is well recognized that peripheral electrical stimulation facilitates the release of certain neuropeptides in the CNS [14]. The types of neurochemical substances released by peripheral stimulation are dependent on the parameters of stimulation such as frequency [37]. Results from prior studies indicated that EA stimulation with 2 Hz or 10–20 Hz in frequency could influence brain OXT and AVP levels. Uvnas-Moberg et al. [15] reported that OXT levels in the plasma and cerebrospinal fluid of SD rats were both increased following 2 Hz EA intervention. Yang and colleagues [38–39] have shown that 10–20 Hz EA stimulation for 30 min altered OXT and AVP contents in the hypothalamus in rats. In order to maximize the effect of EA on hypothalamic OXT and AVP, EA with alternating frequencies between 2 Hz and 15 Hz (midpoint between 10 and 20 Hz) was employed in this study. As expected, both sEA and rEA with 2/15 Hz in frequency increased functional activities of OXT/AVP systems in this study.

OXT and AVP are two closely related nonapeptides with a high level of homology in the primary sequence, differing only in two amino acids at positions 3 and 8 [40]. Their genes, located within the same locus on chromosome [41–42], are thought to have arisen by duplication of a common ancestral gene [43]. For rEA experiments, OXT mRNA and AVP mRNA were positively correlated with each other within the same nucleus (PVN or SON). This correlation was also observed between OXT and AVP at peptide levels. All these findings point to a direction that these two neuropeptides may share the same mechanisms of regulation of their synthesis and content. These findings support the notion that these two peptides may share same or similar biological functions in the central nervous system.

SON and PVN are the two main regions synthesizing OXT and AVP. The two nuclei do share common features, but they differ in several dimensions, such as locations within the hypothalamus, neuronal structures and certain physiological functions [44]. The PVN is composed of a lateral magnocellular subdivision and a more medial parvocellular subdivision. In contrast, the SON is composed of all magnocellular neurons, without a parvocellular subdivision [44–45]. Additionally, PVN and SON neurons manifest different responses when confronting certain physical or emotional stimulations, such as hyperosmotic challenge and social stress. For example, sodium loading increased the amount of AVP mRNA in the SON nearly twofold, while the increase in the PVN was much less [46]. Similarly, the increase in AVP release within the PVN in response to social defeat was not accompanied by similar changes in AVP release within the SON [47]. In our study, the AVP and/or OXT level was elevated in SON but not in PVN following sEA and rEA administration. This selective elevation of AVP or OXT in the hypothalamus suggests that OXT/AVP-neurons in SON may be more easily motivated than that in PVN in response to EA intervention.

There are three different receptors for AVP: V1a, V1b, and V2 in mammals. The V1a receptor is the predominant form in the brain. The amygdala, a brain nucleus richly expressing OXTR and V1aR, was regarded as one of the key nuclei mediating central actions of OXT and AVP on social behaviors in humans [6,48] and rodents [49–50]. Upon sustained stimulation, OXTR can undergo desensitization and internalization [51–52] resulting in physiological tolerance. In the present study, rEA but not sEA stimulation led to decreased expression of the gene encoding V1aR in BLA. We suspect that elevated expression of AVP induced by rEA may result in a long-lasting higher level of the peptide in the brain. So the down-regulation of the V1aR mRNA level probably was a negative feedback in response to the enduring higher AVP neurotransmission.

Our findings showed that rEA stimulation significantly increased the social time of LSI rats with the unfamiliar conspecific. Whether the regulatory effect of EA on social behavior is a general phenomenon is currently unknown. Therefore, studying the effects of EA on other types of social deficient models, such as genetically- or chemically-induced autistic animal models, will be valuable. OXT and AVP systems were still in the developing stage during early postnatal life in rodents [53], which implies that OXT or AVP productions may be vulnerable to environmental changes in the early stage of life. Environmental alterations during the early postnatal state in life have been known to influence the function of central OXT systems and social interaction skills in

Table 1
The relationship of OXT/AVP systems with SI time.

OXT/AVP systems		Regions	SI time	
			r	*P* value
mRNA level	OXT	PVN	0.025	0.882
		SON	0.516	0.001**
	AVP	PVN	0.131	0.445
		SON	0.376	0.022*
		CeA	0.015	0.931
	OXTR	BLA	−0.089	0.601
		MeA	−0.260	0.120
		CeA	−0.090	0.597
	V1aR	BLA	0.015	0.932
		MeA	−0.165	0.328
Peptide level	OXT	PVN	−0.166	0.326
		SON	0.146	0.388
	AVP	PVN	−0.074	0.665
		SON	−0.048	0.778
	OXT	Serum	0.006	0.973
	AVP	Serum	0.011	0.957

SI time: social interaction time; PVN: paraventricular nucleus; SON: supraoptic nucleus; OXTR: OXT receptor; V1aR: AVP receptor 1a; CeA: central nucleus of the amygdala; BLA: basolateral nucleus of amygdala; and MeA: medial nucleus of the amygdala. n = 37.
** $P < 0.01$.
* $P < 0.05$.

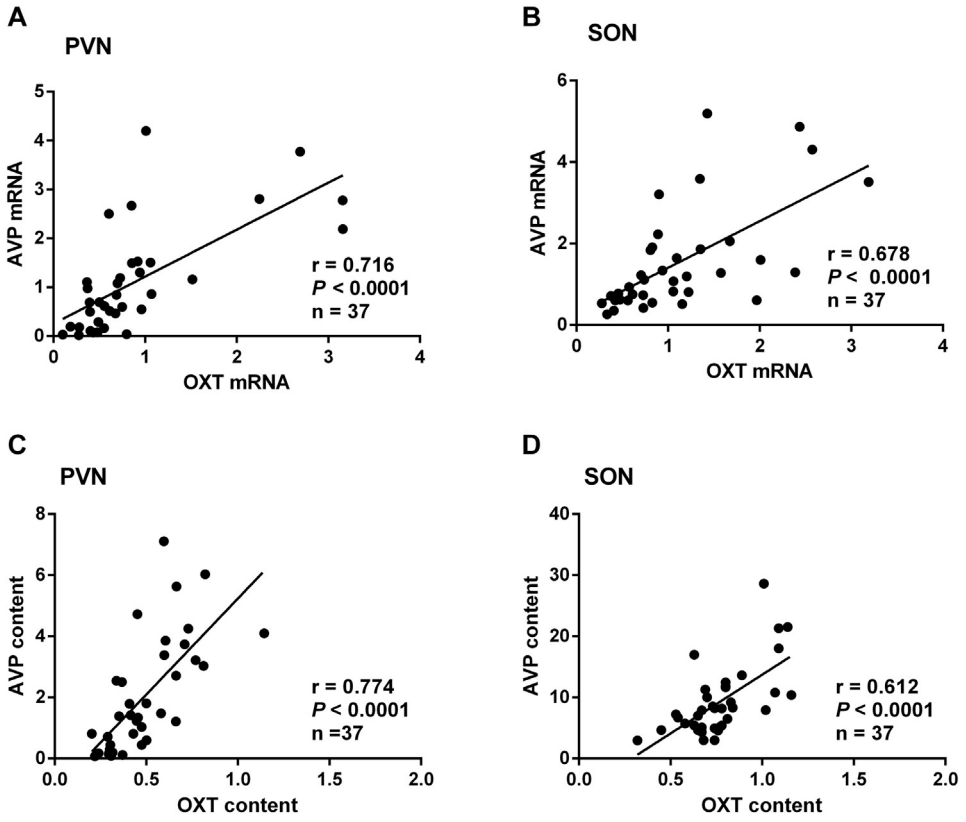

Fig. 6. Scatter-plot showing correlation between OXT and AVP levels in hypothalamic nuclei in rEA experiment. OXT mRNA and AVP mRNA were positively correlated with each other within PVN (A) and SON (B). There were also positive relationships of OXT content with AVP content in PVN (C) and SON (D). OXT: oxytocin; AVP: arg-vasopressin; PVN: paraventricular nucleus; and SON: supraoptic nucleus.

adult life [54–55]. Therefore, EA subjected to juvenile or adolescent rats may be more likely to change the activity of brain OXT/AVP systems and to improve social behavior than in the adult rats. In addition, the central release of OXT/AVP from SON and PVN following EA into the CSF or targeted brain regions should not be neglected.

5. Conclusions

EA stimulation led to a selective increase of neuronal expressions of OXT and AVP in the hypothalamus and elevated OXT and AVP content in the CNS and periphery. There was a concomitant augment of social interaction time in LSI rats. Moreover, the rat's social behavior was positively correlated with mRNA levels of OXT and AVP in the hypothalamus, indicating that the pro-social response observed following EA may be, at least partially, mediated by activation of brain oxytocinergic and vasopressinergic mechanisms.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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