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Transcutaneous electrical acupoint stimulation in children with autism and its impact on plasma levels of arginine-vasopressin and oxytocin: A prospective single-blinded controlled study☆

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ABSTRACT

Acupuncture increases brain levels of arginine-vasopressin (AVP) and oxytocin (OXT), which are known to be involved in the modulation of mammalian social behavior. Transcutaneous electrical acupoint stimulation (TEAS) is often used clinically to produce a similar stimulation to that of acupuncture on the acupoints. In the present study, TEAS was applied to children with autism to assess its therapeutic efficacy. Seventy-six autistic children receiving rehabilitation training were divided into 2 groups: a treatment group receiving TEAS 30 min per day, 5 days per week for 12 weeks ($n = 37$) and a control group without TEAS treatment ($n = 39$). A series of rating scales was used in outcome assessment. Plasma levels of AVP and OXT were determined by enzyme immunoassay (EIA) before and after treatment. The TEAS group showed a significant improvement over the control in their emotional response, fear or anxiety, level/consistency of intellective relations and general impressions on the Childhood Autism Rating Scale (CARS) as well as improvements in the sensory and related factors in the Autism Behavior Checklist (ABC). In addition, the varieties of accepted food increased after TEAS treatment. It appears that TEAS was effective in autistic children who showed passive and aloof behavior, but not in those who were active but odd. The plasma level of AVP was significantly higher in the TEAS group than in the control group after the intervention. In addition, the change in the plasma AVP level paralleled the improvement of some of the behavior factors in CARS, including adaptation to environmental change, listening response, perceptive response and fear or anxiety. It is concluded that TEAS is effective for the treatment of autistic children with a passive and aloof social interaction style. Changes in plasma levels of AVP and possibly OXT may be involved in mediating the therapeutic effect of TEAS.

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1. Introduction

Autism is a mental development disorder characterized by various degrees of impairment in language, communication and social skills and repetitive and stereotypic patterns of behavior (Hughes, 2008). Autism has received more attention around the world in recent years due to its relatively high prevalence rate, poor prognosis and absence of effective therapeutic means (Mulvihill et al., 2009). Among the available methods for the treatment of autism, rehabilitation training is helpful but has poor efficacy in influencing individuals' biological characters (Nienke, Robert, Hubert, & Peter, 2011). Antipsychotic drugs can relieve some of the symptoms but have no effect on social interaction or language abilities. It is also known that antipsychotics may produce serious adverse effects (Posey, Stigler, Erickson, & McDougle, 2008).

Several lines of evidence suggest that changes in some neurotransmitter systems in the central nervous system may be involved in the etiology of autistic spectrum disorders (ASDs). For example, arginine-vasopressin (AVP) and oxytocin (OXT) are produced in the brain and play a central role in modulating mammalian social behavior (Harony & Wagner, 2010). AVP and OXT are also known to play important roles in social behavior and cognition, including facial recognition (Rimmele, Hediger, Heinrichs, & Klaver, 2009; Domes et al., 2010), anxiety (Bosch, 2010; Kessler, Bosch, Bunck, Landgraf, & Neumann, 2010) and trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005; Bartz et al., 2010) in social interactions. In addition, lower plasma levels of OXT were found in autistic children (Modahl et al., 1998). The administration of OXT improved their social behaviors (Hollander et al., 2003; Andari et al., 2010; Guastella et al., 2010). Therefore, it is hypothesized that AVP and OXT may be involved in the development of ASDs and should be considered as potential therapeutic targets for treatment (Green & Hollander, 2010; Kuehn, 2010).

Acupuncture is one of the crucial components of traditional Chinese medicine. It appears to be effective in the treatment of many symptoms and/or disorders by regulating the functions of the autonomic nervous system and neuroendocrine system (Han, 2003). It has been reported that manual acupuncture (Jun, 1992) and 10–20 Hz electroacupuncture stimulation (Wu et al., 2005; Yang et al., 2007) increased the levels of AVP and OXT in certain areas of the brain in rats. Therefore, it is rational to posit that acupuncture or electroacupuncture may improve the social behaviors of autistic children by enhancing the function of the AVP and/or OXT systems in the brain.

Several lines of evidence indicate that acupuncture may be effective in the treatment of autism. Zhang (1996) first reported the use of acupuncture on autistic children in 1996. Among the 12 studied cases, they observed an "obvious effect" in 4 cases, found the treatment "effective" in 4 cases and found "no effect" in 4 cases. The article did not describe the specific symptoms which were improved. Ma, Yuan, & Rui (2006) reported that "Jin's three-needling" treatment combined with behavior intervention provided improvements in several areas, such as body movement, social communication and the language factor in the Autism Behavior Checklist (ABC) in children with autism. Yan, Wei, Chen, & Chen (2007) revealed that acupuncture treatment over 60–90 sessions had marked effects on the development, imitation and oral cognition of autistic children. There were also reports showing that treatment with electroacupuncture significantly enhanced sensation, association, body ability of self-care (Wang, Liu, Wei, & Li, 2007) and the language comprehension domain of the Functional Independence Measure for Children (WeeFIM) (Wong & Chen, 2010) in autistic children. Overall, earlier therapeutic efficacy studies in autistic children with acupuncture presented some encouraging results. However, these studies suffered from a lack of systemic assessment of autistic syndromes, appropriate control groups or detailed descriptions of the methods used in the acupuncture or electroacupuncture treatments. These weaknesses made it difficult, if not impossible, to interpret the results or replicate the studies. In addition, the mechanisms of action of the therapeutic effect of acupuncture in the treatment of autism are poorly understood.

To increase the reproducibility of acupuncture-like stimulation and to reduce the invasiveness of the therapeutic procedure, skin electrodes were placed on the acupoints instead of piercing the skin with needles. In addition to the assessment of clinical autistic symptoms, observations of food choices and sleep status were made, and the plasma concentrations of AVP and OXT were determined before and after the TEAS intervention.

2. Materials and methods

2.1. Participants

The prospective controlled trial was conducted from November of 2009 to January of 2011 at Wucailu Center for Children with Autism, which is an autism rehabilitation center in Beijing where children with ASD received an applied behavior analysis (ABA)-based early intervention program. Parents were invited to attend a public lecture about the purpose of the project to provide information for them to make decisions.

2.1.1 The inclusion criteria included the following: (1) being diagnosed as autistic, based on the Diagnostic and Statistical Manual of Mental Disorders, fourth version (DSM-IV) (Volkmar, 1996) and the Child Autism Rating Scale (CARS) (Rellini, Tortolani, Trillo, Carbone, & Montecchi, 2004) and (2) being between the ages of 2 and 7 years.

2.1.2 The exclusion criteria included the following: (1) children with disorders other than autism in the autism spectrum and (2) children with prior acupuncture experience, taking antipsychotic drugs, or receiving stem cell therapy.

2.2. Setting and design

The project was completed in 3 consecutive runs. Each child was assigned to one of the 3 runs. The course of treatment lasted for 3 months in each run. In the first run, only clinical symptoms, food choice and sleep status were evaluated. In the second and third runs, a venous blood sample was collected for AVP and OXT analysis. All parents were informed in written form about the aims and the details of the study. They all expressed willingness to participate via a signed agreement. This clinical trial was approved by the Peking University Institutional Review Board (Approval No. IRB00001052-09017) and registered at the Chinese Clinical Trial Registry, a World Health Organization International Clinical Trial Registration Platform (http://www.chictr.org; Registration No. ChiCTR-TNC-11001299).

2.3. Blinding

All the participating children were evaluated before and after intervention by the same experienced psychiatrist (Dr. Mei-Xiang Jia), who was blinded for the treatment assignment.

2.4. ABA training

The children in Wucailu Center received at least 5 h of discrete trial training weekly. In this program, teachers and students work one-on-one to teach functions such as imitation, cooperation and speech. Based on ABA principles and procedures, the training also has various exercises designed to improve a child's weakest areas to help develop coordination and movement. Children with no speech ability were trained to use the Picture Exchange System to communicate with others.

2.5. TEAS treatment

TEAS treatment was applied in the afternoon, immediately after the completion of the rehabilitation training session, 5 days a week on weekdays. The following acupoints were used: LI 4 (Hegu) and PC 6 (Neiguan) on one side and ST 36 (Zusanli) and SP 6 (Sanyinjiao) on the other side. The stimulation was delivered via self-adhesive skin electrodes (2.9 mm × 2.9 mm). An alternating 2/15 Hz frequency was used (2 Hz with a 0.6 ms pulse width alternating automatically with 15 Hz with a 0.3 ms pulse width, each lasting for 3 s). The stimulation was 30 min per session. The intensity of the stimulation was kept low in the first week (3 mA in the upper limb and 5 mA in the lower limb) and was increased gradually in the following weeks to achieve the final intensity of 10 mA and 15 mA, respectively. A total of 60 TEAS sessions were administered over 12 weeks. The TEAS was operated by the same researcher in each run to ensure the consistency of the procedure.

2.6. Classification of the subject's social interaction style

Autistic children were classified and assigned to the following three groups based on their daily social interaction styles, according to Wing and Gould's description: (1) "Active-but-odd individuals", who displayed and pursued inappropriate or peculiar approach behaviors; (2) "Passive individuals", who accepted social approaches but would not make contact spontaneously; and (3) "Aloof individuals", who had no interest in social contact.

2.7. Main outcome measures

The following instruments were used to assess the treatment outcomes: the CARS, ABC, Social Adaptive Development Quotient Scale (ADQ), Sleep Status and Food Choice Questionnaires. These evaluation instruments are all used routinely by psychiatrists in China. The evaluation was performed twice for each participant; the first was completed within one week before the intervention, and the second was completed within one week after the intervention.

2.7.1. CARS

The scale assesses behaviors in 14 domains that are generally affected by severe autism, plus one category of general impressions of autism. The 15 items in the scale are the following: relating to people, imitative behavior, emotional response, body use, object use, adaptation to change, visual response, listening response, perceptive response, fear or anxiety, verbal communication, non-verbal communication, activity level, level and consistency of intellective relations, and general impressions (Rellini et al., 2004).

2.7.2. ABC

This scale describes a series of typical autistic behaviors to assess the presence of these behaviors in a given subject. The assessment form consists of 57 items, each corresponding to a single score referring to a single symptomatological area. Five areas are considered: sensory, relating, stereotypes and object use, language, and self-help and social (Rellini et al., 2004).

2.7.3. ADQ

This scale was made by Yao & Gong (1993). The 8 items in the scale are motor, daily life, language development, personal orientation, social responsibility, time and space, labor skills, and economic activity. In this study, only the total scores were analyzed.

2.7.4. Sleep status questionnaire

A self-made scale referring to the Pittsburgh Sleep Quality Index (PSQI) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) was used. The major areas of investigation are sleep latency and sleep time per day.

2.7.5. Food choice questionnaire

This is a self-made scale consisting of six species of food and a total of 113 types of common foods consumed in daily life in China: cereal and potato (14 types), beans (8 types), meats (9 types), vegetables (31 types), fruits (17 types), and snacks (34 types). The parents were asked to indicate the food preference of the child by selecting "accepted", "never tried" or "rejected" for each of the 113 types of common foods. For each type of food, only one choice can be made.

2.7.6. Parental report

A standardized, self-devised parental report was provided for parents to record changes in their children's behavior. After treatment with TEAS, the following choices can be made regarding its efficacy: (1) improvement, (2) no change or (3) worsening. The report was filed once each week during the intervention.

2.8. Blood sample collection procedures and biochemical analyses for AVP and OXT

The blood sample collection took place in the morning between 9 and 11 AM in the autism rehabilitation center (TEAS group $n = 18$; control group $n = 11$). The same pediatric nurse completed the standardized blood drawing procedures before and after the intervention to ensure consistency. The parents were present when the blood was taken. Blood samples of 10 cubic centimeters were obtained from the antecubital foci and collected into EDTA (1 mg/mL blood) tubes containing aprotinin (500 KIU/mL of blood). The samples were kept on ice for 15 min and then centrifuged at 1600 g for 15 min at 4 °C. The plasma was separated into two tubes and immediately frozen at −80 °C until assayed. Plasma AVP and OXT levels were determined by enzyme immunoassay (EIA) (Assay Designs, Ann Arbor, MI) by the same individual who was blinded for the treatment assignment. The EIAs were highly sensitive (minimal detection levels are 4 pg/mL AVP assay and 15 pg/mL for the OXT assay). The cross-reactivity of OXT with related compounds (Arg^8-vasopressin-SH, Lys^8-vasopressin-SH, Ser^4, Ile^8-oxytocin, thyrotropin-releasing hormone (TRH), somatostatin, Met-enkephalin, VIP, Lys^8-vasopressin, Arg^8-vasopressin, α-atrial natriuretic polypeptide (α-ANP), growth hormone, tocinoic acid, and melanostatin) was <0.6%. The cross-reactivity of AVP with related compounds (oxytocin, TRH, VIP, Leu-enkephalin, Met-enkephalin, mesotocin, somatostatin, vasotocin,-desmopressin, Arg^8-vasotocin, Ser^4, Ile^8-oxytocin) was <0.001%. The cross-reactivity between AVP and OXT was <0.2%. All of the samples were run at the same time, and the *r*-value of standard curves was greater than 0.99 for both assays.

2.9. Statistical methods

Data were analyzed with Statistical Package for the Social Sciences 11.5.1 (SPSS). The initial analysis examined the demographic and baseline characteristics of children in the two groups with an unpaired *t* test (age), χ^2 analysis (gender) and Mann–Whitney *U* test (CARS). The differences in symptoms observed between the TEAS and control groups with different outcome measures (CARS, ABC and ADQ) were analyzed using the Mann–Whitney *U* test. A paired *t* test was used to analyze the sleep and food choice data before and after the intervention. An unpaired *t* test was used to compare changes in the plasma AVP or OXT levels between TEAS and the control group. Linear regression analysis was used to detect the correlation of the plasma levels of AVP vs OXT as well as improvement in symptoms vs changes in AVP. All statistics were completed with two-tailed tests and $P < 0.05$ was considered to indicate the statistical significance.

3. Results

3.1. Study design and demographics

The trial is completed in three consecutive runs, with each run lasting 3 months. The data were pooled for analysis. The flow chart of enrollment is shown in Fig. 1. Of the 132 children receiving rehabilitation training, 96 met the inclusion criteria and were assigned either to the TEAS group ($n = 48$) or to the control group ($n = 48$) based on their own choices. Fifteen children terminated prematurely (7 in the control group and 6 in the TEAS group) for different reasons: two children in the TEAS group stopped treatment due to their high sensitivity to electric stimulation, and 2 children in the control group and 3 children in the TEAS group received other medical therapies. Seventy-six subjects (39 in control, 37 in TEAS group) completed the three-month treatment as well as the pre- and post-treatment assessments. No significant differences were found in the baseline characteristics between the two groups, including age [4.09 ± 1.66 (mean ± SD) in the control; 4.65 ± 1.51 in TEAS, unpaired *t* test, $t = 1.527$, $P = 0.131$], gender (34 males and 5 females in the control; 34 males and 3 females in

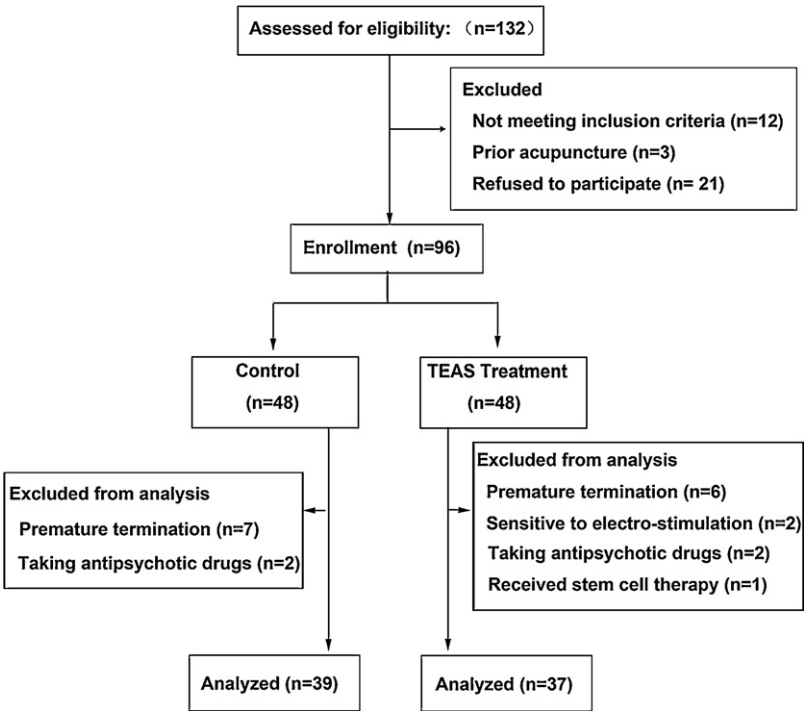

Fig. 1. Flow chart of inclusion/exclusion of study participants.

TEAS, χ^2 analysis, $\chi^2 = 0.448$, $P = 0.503$), or the severity of autistic syndrome based on a CARS evaluation [37.00 (4.75): median (quartile interval) in the control; 37.00 (7.13) in TEAS, the Mann–Whitney U test, $z = -0.930$, $P = 0.352$].

3.2. Outcome measures

Table 1 shows a significant improvement after TEAS treatment over the control group in the following areas: emotional response ($P = 0.037$), fear or anxiety ($P = 0.008$), level and consistency of intellective relations ($P = 0.023$), general impressions ($P = 0.030$) and total score ($P = 0.042$) of CARS. There are additional significant improvements in the areas of sensory ($P = 0.006$) and relating ($P = 0.013$) compared to the control group in ABC. In contrast, the total score in ADQ did not change after the TEAS intervention. No difference was found in sleep latency or total sleep time per day between the two groups (Table 2). Our results also show that the number of accepted food varieties increased after TEAS treatment ($P = 0.046$), but not in the control group (Table 2). The major changes found in the food choices in the TEAS group were in the categories of vegetables, fruits and snacks (data not shown).

3.3. Changes in the plasma levels of AVP and OXT following TEAS intervention

The plasma levels of AVP increased significantly in the TEAS group ($P = 0.007$) compared with that in the control. The change in the plasma level of OXT was quite different from that of AVP. It decreased in both the control and TEAS groups vs the pretreatment values, but the decrement was significantly less in the TEAS group than that of the control ($P = 0.041$) (Fig. 2A). The plasma levels of OXT and AVP showed a positive correlation in the control group before any intervention, which persisted after 3 months of rehabilitation, except for a net decrease in the OXT content (Fig. 2B). In the TEAS group (Fig. 2C), the plasma levels of AVP and OXT were also highly correlated. The slope of the linear regression curves remained essentially the same before and after the TEAS intervention. The curve was shifted upward dramatically after TEAS treatment, indicating a net increase in the AVP content.

3.4. Distinguishing the responders from the nonresponders to TEAS based on their behavioral characteristics

It was observed in the present study that TEAS intervention did not work equally well for all autistic children. It is well accepted that autistic children can be classified into three subtypes according to their behavioral characteristics (Wing & Gould, 1979). In the present study, we made an effort to determine whether a certain subtype responds better to TEAS intervention. All the subjects were classified into three subtypes according to their general behavior: active-but-odd, passive, or aloof (Wing & Gould, 1979). Of a total of 76 children, 21 (27.6%) belonged to the active-but-odd group, 44 (57.9%) to the passive group, and 11 (14.5%) to the aloof group. Because the effects of TEAS on children with passive and aloof styles were

Table 1
Effects of TEAS on primary outcome measures.

	Control	TEAS	*Z*	*P*
Childhood Autism Rating Scale (CARS)				
Number of cases	36	34		
Relating to people	0.00 (1.00)	−0.25 (1.00)	−0.702	0.483
Imitative behavior	0.00 (1.26)	0.00 (1.00)	−0.927	0.354
Emotional response	0.00 (1.50)	−0.50 (1.00)	−2.087	0.037
Body use	0.00 (0.00)	0.00 (1.00)	−1.580	0.114
Object use	0.00 (0.00)	0.00 (1.00)	−1.573	0.116
Adaptation to change	0.00 (0.88)	0.00 (1.00)	−1.801	0.072
Visual response	−0.50 (0.88)	−0.25 (1.00)	−0.376	0.707
Listening response	0.00 (0.59)	0.00 (1.00)	−0.176	0.860
Perceptive response	0.00 (1.00)	0.00 (1.00)	−0.862	0.389
Fear or anxiety	0.00 (1.00)	−1.00 (1.00)	−2.641	0.008
Verbal communication	0.00 (0.50)	0.00 (1.00)	−0.252	0.801
Non-verbal communication	0.00 (0.50)	0.00 (1.00)	−1.272	0.203
Activity level	0.00 (0.50)	0.00 (1.00)	−1.198	0.231
Level/consistency of intellective relations	0.00 (0.50)	−0.50 (0.50)	−2.268	0.023
General impressions	0.00 (0.88)	−0.50 (1.00)	−2.164	0.030
Total score	−2.00 (7.38)	−4.75 (10.26)	−2.029	0.042
Autism Behavior Checklist (ABC)				
Number of cases	33	33		
Sensory	0.00 (9.00)	0.00 (7.00)	−2.765	0.006
Relating	0.00 (10.50)	−4.00 (10.00)	−2.474	0.013
Stereotypes and object use	−1.00 (10.50)	0.00 (6.00)	−0.084	0.933
Language	−2.00 (5.50)	−2.00 (8.00)	−0.321	0.748
Self-help and social	−2.00 (8.00)	−2.00 (6.50)	−0.727	0.468
Total score	−4.00 (27.50)	−10.00 (28.50)	−1.828	0.068
Social Adaptive Development Quotient Scale (ADQ)				
Number of cases	26	31		
Total score	8.50 (30.00)	7.00 (32.00)	−0.735	0.462

Note. Numbers are median (quartile interval). The Mann–Whitney *U* test was used to analyze data in CARS, ABC and ADQ.

similar, the data from these two groups were pooled. Interestingly, the results show that children with passive and aloof styles demonstrated a better response to the TEAS treatment (CARS total score change, $P = 0.042$; ABC total score change, $P = 0.024$), while this treatment was not effective at all on the children with active-but-odd style (Fig. 3A and B). Similarly, the increase of plasma AVP ($P = 0.002$) in response to TEAS was noted only in the passive- and aloof-style children but not in the active-but-odd children (Fig. 3C). There was no difference in OXT levels before and after the TEAS treatment in the active-but-odd children (Fig. 3D). The OXT level after treatment seems to be higher in the children with passive and aloof behaviors than in the active-but-odd children, although the difference did not achieve a significant difference level ($P = 0.063$) (Fig. 3D).

Table 2
Effects of TEAS on sleep status and food choice.

Sleep status		Control	TEAS
Number of cases		33	32
Sleep latency (min)	Before	27.03 (16.45)	23.91 (14.18)
	After	25.31 (15.96)	22.50 (11.85)
	t	0.622	0.557
	P	0.539	0.581
Sleep time (h/day)	Before	8.84 (1.05)	8.82 (0.92)
	After	8.81 (0.82)	8.74 (0.71)
	t	0.178	0.605
	P	0.860	0.549
Food choice (total: 113 types)			
Number of cases		33	33
Accepted kinds	Before	72.88 (20.95)	70.2 (21.62)
	After	70.64 (23.46)	75.7 (19.73)
	t	0.621	−2.074
	P	0.539	0.046

Note. Numbers are means (SD). A paired *t* test was used in the statistical analysis. *P* values indicate significance levels vs. levels before intervention.

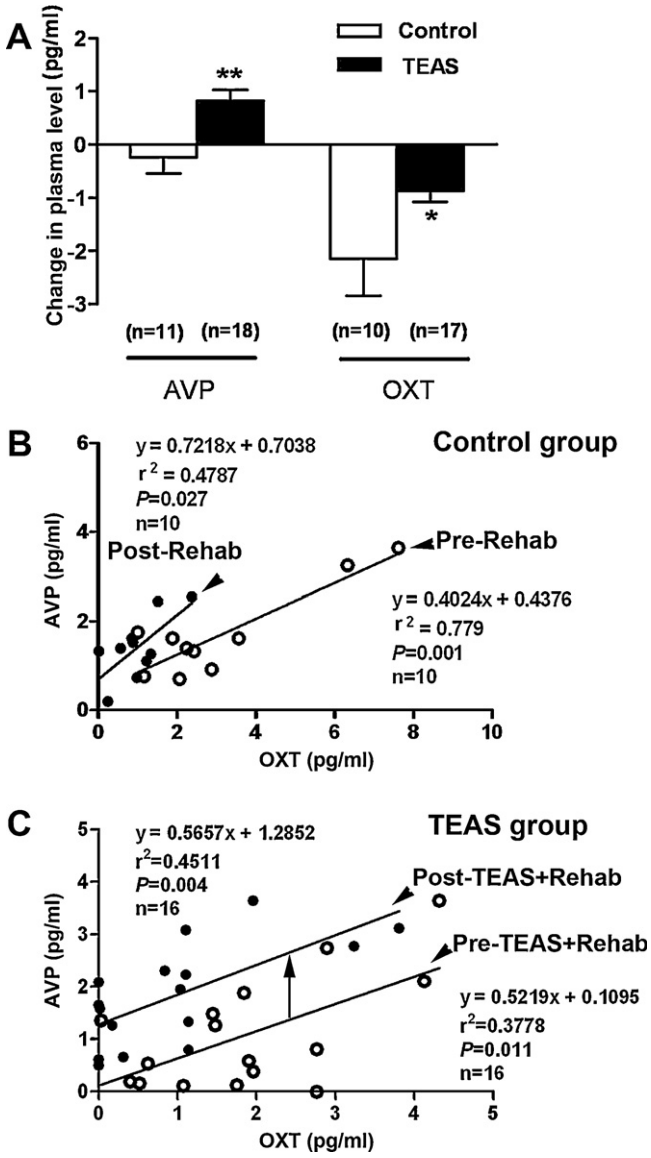

Fig. 2. Changes in plasma AVP and OXT levels and linear regression analysis. The TEAS intervention increased the plasma AVP levels and prevented the reduction of plasma OXT levels observed in the control group (A). In the control group, a positive correlation was shown between the plasma levels of OXT and AVP before and after 3 months of rehabilitation (rehab) (B). In the TEAS group, the regression line between OXT and AVP was shifted upward after 3 months of TEAS treatment (C). Columns and bars represent the mean ± SE. The data were analyzed with unpaired *t* test with * and ** representing $P < 0.05$ and $P < 0.01$, respectively. Linear regression analysis was used to determine the significance level in the correlation analysis between the plasma levels of AVP and OXT before (○) and after (●) treatment.

3.5. Correlation between changes in plasma AVP and improvements in behavior in children with passive and aloof styles

Linear regression analysis showed that in autistic children with passive- and aloof-style behaviors, the increase in the plasma AVP level (%) positively correlates with improvements (%) in the following factors in CARS: adaptation to change ($P = 0.021$), listening response ($P = 0.023$), perceptive response ($P = 0.010$) and fear or anxiety ($P = 0.026$) (Table 3). These observations suggest that the changes in plasma AVP levels may play a causal role in behavioral improvements in autistic children following the TEAS intervention.

3.6. Parental report on the potential adverse effects of TEAS

To assess the safety of TEAS treatment, all of the parents were interviewed once per week on the potential adverse effects of TEAS during the entire treatment period of 12 weeks. Changes in the children's behavior as a result of these adverse effects were recorded in a structured format.

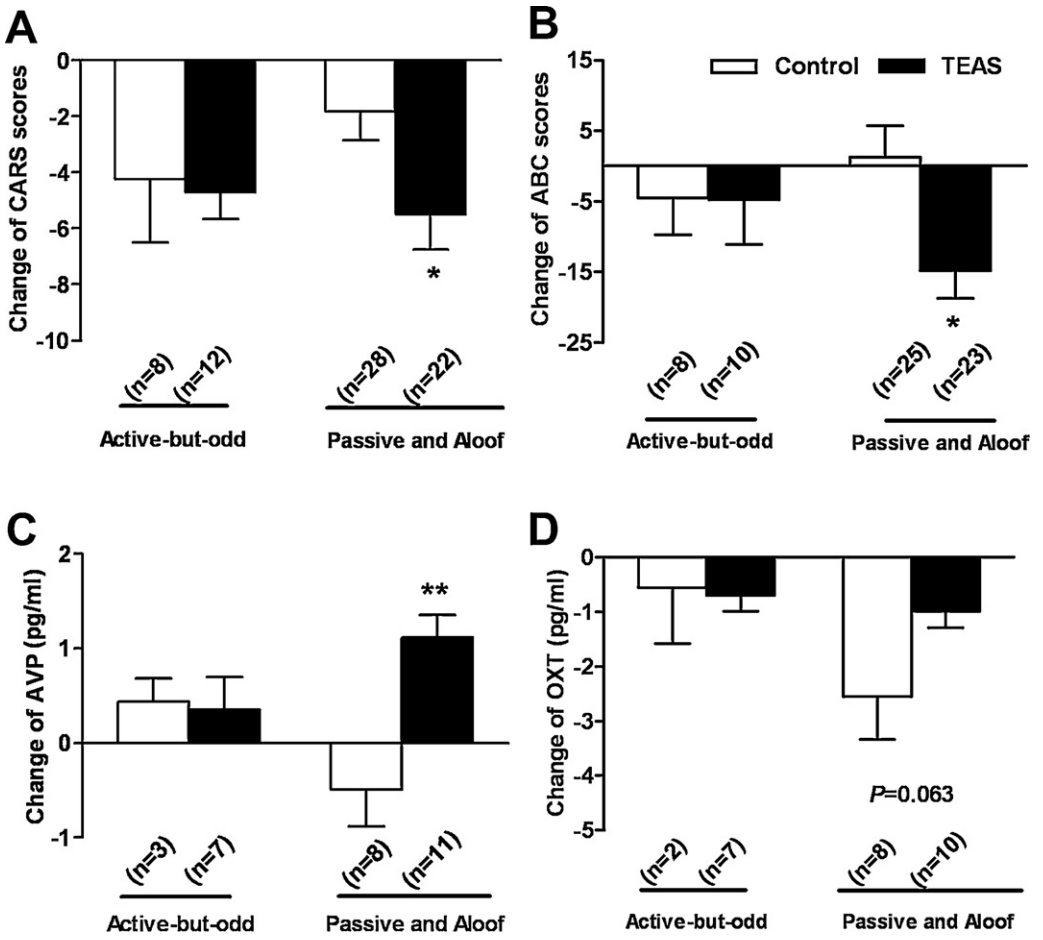

Fig. 3. Social interaction style and behavioral and neurochemical responses to TEAS. The CARS (A) and ABC (B) total scores decreased significantly in autistic children with passive and aloof, but not active-but-odd, social interaction styles after TEAS treatment. The plasma AVP level increased in children with passive and aloof, but not active-but-odd, social interaction styles after TEAS (C). No significant changes were observed in the OXT level after TEAS treatment (D). Columns and bars represent the mean ± SE. The Mann–Whitney *U* test was used to compare changes in the scores in A and B; an unpaired *t* test was used to compare changes in the plasma AVP and OXT levels in C and D; $^{*}P < 0.05$, $^{**}P < 0.01$.

Transitory excitatory syndrome was reported by parents in some of the autistic children in both the control group (36%) and the TEAS group (38%) during the period of the study. The excitatory syndrome manifested in more frequent laughing and singing during the day and occasional waking up during the night. It does not appear that the transitory excitatory syndrome is related to the TEAS intervention. No aggravation of the autistic syndrome was reported in either group, with or without the presence of temporary excitatory syndrome.

4. Discussion

In the present study, we recruited 96 Chinese children with autism in one rehabilitation center. Most of the children were boys aged 2–7 years. Other types of ASDs were excluded from the present study. All children in the study also received rehabilitation training based on ABA in an attempt to improve their socially significant behaviors and to identify the variables responsible for changes in behavior (Baer, Wolf, & Risley, 1968). Because this is a preliminary study, the assignment of children into the two treatment groups was not randomized. The decision was made based on the parents' choice. Fortunately, the demographics (number of participants, age, gender, severity of autistic syndrome) of the two groups were very similar. In addition, the parents were not blinded in the study, although CARS assessment was performed by a blinded psychiatrist. The assessments of the parents (ABC) and the psychiatrist (CARS) both showed a significant improvement in the TEAS group over the control group. The improvements observed in CARS included emotional response, fear or anxiety, level/consistency of intellective relations and general impressions factors. Improvements were also observed in ABC, including sensory and relating factors (Table 1). It is not clear why most parents in the TEAS group observed noticeable progress in language over the control group, as this observation was not confirmed by the results from the assessment scales mentioned above. Additionally, no difference between the TEAS and control groups was observed in ADQ, which was designed to assess

Table 3
Correlation between changes in plasma AVP (%) and behavioral improvements (%) in children with passive and aloof style after TEAS intervention.

		R^2	P
CARS $N = 9$	Relating to people	0.3177	0.090
	Imitative behavior	0.0889	0.403
	Emotional response	0.0063	0.827
	Body use	0.0724	0.452
	Object use	0.1814	0.220
	Adaptation to change	0.5050	0.021
	Visual response	0.3445	0.075
	Listening response	0.4953	0.023
	Perceptive response	0.5867	0.010
	Fear or anxiety	0.4801	0.026
	Verbal communication	0.0418	0.571
	Non-verbal communication	0.0009	0.932
	Activity level	0.0095	0.789
	Level and consistency of intellective relations	0.0099	0.784
	General impressions	0.0003	0.962
	Total score	0.2089	0.184
ABC $N = 9$	Sensory	0.0041	0.571
	Relating	0.0406	0.576
	Stereotypes and object use	0.0038	0.866
	Language	0.0034	0.872
	Self-help and social	0.3173	0.090
	Total score	0.0629	0.485

Note. Values are R^2 or P values. Linear regression analysis was used to detect the significance level in the correlation analysis between the percentage of change in plasma AVP level and behavioral improvements.

social adaptive development abilities. Patterns of behavioral improvements produced by TEAS in the present study were similar, but not identical, to those reported by authors using other acupuncture-like methods (Ma et al., 2006; Wang et al., 2007; Yan et al., 2007; Wong & Chen, 2010), which obtained much more notable therapeutic effects. In addition, unlike the acupuncture studies mentioned above, we have made systemic assessments of improvements in behavior as well as food choice and sleep status. Most importantly, in the present study, we observed the concomitant changes in plasma levels of AVP and OXT in response to TEAS. The degree of changes in these neurochemical substrates was found to be positively correlated to the extent of the behavior changes, suggesting that the concomitant changes in AVP and OXT may reflect the underlying mechanism of action.

Many researchers observed abnormal eating behaviors in autistic children, which manifest as very limited dietary choices (Dominick, Davis, Lainhart, Tager-Flusberg, & Folstein, 2007; Martins, Young, & Robson, 2008; Schreck & Williams, 2006; Schreck, Williams, & Smith, 2004). This might be related to their stereotyped behavior and abnormal sensory sensitivity (Cermak, Curtin, & Bandini, 2010) rather than serious gastrointestinal abnormalities (Levy et al., 2007). In our study, we found an increase in the varieties of accepted food after the TEAS treatment, especially in the categories of vegetables, fruits and snacks, which was not observed in the control group (Table 2). The parents reported no increases in the amount of food being consumed in a given period of time; therefore, we hypothesize that TEAS may have improved the stereotyped eating behavior rather than causing an increase in appetite.

Regarding sleeping behavior, most parents reported better sleep status at the early stage of the TEAS intervention, although no statistically significant change in the structured scales was achieved at the end of the 3-month study (Table 2).

To explore whether there are responders and nonresponders to the TEAS therapy, we compared the effects of TEAS on children with different social interaction styles. It was found that TEAS was effective only in children with passive and aloof styles, but not in those with the active-but-odd style (Fig. 3A and B). Because 72.4% of autistic children exhibit the passive and aloof styles, TEAS appears to be useful to most autistic patients.

Concerning the mechanisms of action of TEAS, it has been reported that electroacupuncture increased the production and release of AVP and OXT in the central nervous systems of rats (Wu et al., 2005; Yang et al., 2007). To our knowledge, no studies have been conducted to measure the changes in AVP or OXT levels in relationship to the therapeutic effects of intervention in autistic children.

In this study, we found that in the control group, a significant decrease in plasma OXT occurred within a period of three months, with no changes found in the concentration of plasma AVP. In contrast, the TEAS group showed a significant increase in the plasma level of AVP and a much smaller decrease in the plasma OXT after 3 months of TEAS intervention (Fig. 2). The increase in AVP levels and the improvement of behavior occurred only in children with passive and aloof styles of social interaction, but not in those with the active-but-odd interaction style (Fig. 3C). The positive correlation between the changes in the AVP concentration and the degree of improvements in behavior (adaptation to change, listening response, perceptive response and fear or anxiety factors in CARS) (Table 3) may indicate a causal relationship between these two variants. In this study, we have demonstrated that TEAS produced a solid increase in the peripheral level of endogenous AVP, suggesting that

AVP might be a target for the treatment of autism. Currently, we do not yet know whether the levels of AVP and OXT in the plasma would parallel those in the brain; however, the strong correlation between the improvement in symptoms and the increase in the plasma AVP level seems to support the notion that AVP may play an important role (Table 3) in mediating the therapeutic effects of TEAS. Further studies are underway to assess the effect of TEAS on the release of AVP and OXT in various brain areas in rats.

One of the most puzzling observations in this study was that the plasma level of OXT decreased markedly during the 3 months of behavior rehabilitation, which seemed, at least partially, to be prevented by TEAS. We have analyzed the relationship between age and the level of OXT or AVP in the pretreated and pooled plasma samples. We found no correlation between AVP levels and age. A trend of negative correlation seemed to exist between OXT and age ($P = 0.063$), suggesting that in contrast to an increase in OXT with age in the normal population (Modahl et al., 1998), there might be a trend of a decrease in OXT with age in autistic patients.

The novel finding that 2/15 Hz TEAS was effective only in children with a passive or aloof style of social behavior would certainly open up a new area of research if it is confirmed in further studies.

Our decade-long research on acupuncture-induced analgesic effects demonstrated that peripheral electric stimulation can induce the release of a host of neurotransmitters and neuropeptides in the central nervous system. The types of neurochemical substances released by peripheral stimulation are frequency dependent (Han, 2003, 2011). A remarkable example is that low-frequency (2 Hz) stimulation triggers the release of enkephalins and endorphins in the brain, while high-frequency (100 Hz) stimulation triggers the release of dynorphins in the spinal cord. Our working hypothesis is that the effect of TEAS on autistic children is due to the release of endogenous AVP and OXT by 2/15 Hz stimulation. More studies should be conducted to explore whether the symptoms of autistic children with the active-but-odd style of social interaction can be improved using different parameters of TEAS. This endeavor would certainly be greatly facilitated if new animal models of ASD mimicking various social activity deficits become available.

It is worth noting that there are limitations on this study, and thus, the results derived from this study should be interpreted with care. (a) In this preliminary study, patients were not assigned into the two study groups randomly. Instead, the assignment was based on the parents' preference. This would inevitably introduce a certain degree of expectation (placebo effect) or qualms. (b) The dropout rate of the study is relatively high. One of the reasons for this is that in some cases, the rehabilitation treatment started before the TEAS treatment started. They terminated the TEAS treatment when the rehabilitation training was completed. (c) The most appropriate assessment measures for autistic behaviors are still unavailable in China, especially in the area of language development. These need to be developed in future studies. (d) The time period of 3 months seems insufficient for the acupuncture-like treatment to reach its full therapeutic efficacy. In fact, most improvements appeared at a late stage (8–12 weeks) of treatment. (e) The patient population was limited to the ages of 2–7. It would be interesting to investigate whether TEAS would be effective for older autistic patients.

5. Conclusions

TEAS treatment is beneficial for autistic children with passive and aloof social interaction styles. Its therapeutic effect may be related to the increase in plasma levels of AVP. Encouraged by the preliminary beneficial effects of TEAS with no obvious adverse effects, a larger-scale, randomized multi-center clinical trial is warranted. Meanwhile, an exploration of its precise mechanisms of action is desirable.

Conflicts of interest and financial disclosures

Ji-Sheng Han is the inventor of the transcutaneous electrical nerve stimulator used in the present study. Song-Ping Han is the provider of the stimulators and the technical consultant. The content of this article has been used to declare an invention patent at the Chinese intellectual property office (No. 2010090600343360) and at the world intellectual property organization (No. PCT/CN2011/001508).

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References

Andari, E., Duhamel, J. R., Zalla, T., Herbrecht, E., Leboyer, M., & Sirigu, A. (2010). Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 4389–4394.

Baer, D. M., Wolf, M. M., & Risley, T. R. (1968). Some current dimensions of applied behavior analysis. *Journal of Applied Behavior Analysis, 1*, 91–97.

Bartz, J., Simeon, D., Hamilton, H., Kim, S., Crystal, S., & Braun, A. (2010). Oxytocin can hinder trust and cooperation in borderline personality disorder. *Social Cognitive and Affective Neuroscience* .

Bosch, O. J. (2010). Maternal nurturing is dependent on her innate anxiety: The behavioral roles of brain oxytocin and vasopressin. *Hormones and Behavior, 59*, 202–212.

Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Research, 28*, 193–213.

Cermak, S. A., Curtin, C., & Bandini, L. G. (2010). Food selectivity and sensory sensitivity in children with autism spectrum disorders. *Journal of the American Dietetic Association, 110*, 238–246.

Domes, G., Lischke, A., Berger, C., Grossmann, A., Hauenstein, K., & Heinrichs, M. (2010). Effects of intranasal oxytocin on emotional face processing in women. *Psychoneuroendocrinology, 35*, 83–93.

Dominick, K. C., Davis, N. O., Lainhart, J., Tager-Flusberg, H., & Folstein, S. (2007). Atypical behaviors in children with autism and children with a history of language impairment. *Research in Developmental Disabilities, 28*, 145–162.

Green, J. J., & Hollander, E. (2010). Autism and oxytocin: new developments in translational approaches to therapeutics. *Neurotherapeutics, 7*, 250–257.

Guastella, A. J., Einfeld, S. L., Gray, K. M., Rinehart, N. J., Tonge, B. J., & Lambert, T. J. (2010). Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biological Psychiatry, 67*, 692–694.

Han, J. S. (2003). Acupuncture: Neuropeptide release produced by electrical stimulation of different frequencies. *Trends Neuroscience, 26*, 17–22.

Han, J. S. (2011). Acupuncture analgesia: Areas of consensus and controversy. *Pain, 152*, S41–S48.

Harony, H., & Wagner, S. (2010). The contribution of oxytocin and vasopressin to mammalian social behavior: Potential role in autism spectrum disorder. *Neuro-Signals, 18*, 82–97.

Hollander, E., Novotny, S., Hanratty, M., Yaffe, R., DeCaria, C. M., & Aronowitz, B. R. (2003). Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. *Neuropsychopharmacology, 28*, 193–198.

Hughes, J. R. (2008). A review of recent reports on autism: 1000 studies published in 2007. *Epilepsy & Behavior, 13*, 425–437.

Jun, Y. (1992). Effect of acupuncture on the contents of vasopressin and oxytocin in the rat. *Zhen Ci Yan Jiu, 17*, 217–220.

Kessler, M. S., Bosch, O. J., Bunck, M., Landgraf, R., & Neumann, I. D. (2010). Maternal care differs in mice bred for high vs. low trait anxiety: Impact of brain vasopressin and cross-fostering. *Social Neuroscience, 6*, 156–168.

Kosfeld, M., Heinrichs, M., Zak, P. J., Fischbacher, U., & Fehr, E. (2005). Oxytocin increases trust in humans. *Nature, 435*, 673–676.

Kuehn, B. M. (2010). Scientists probe oxytocin therapy for social deficits in autism, schizophrenia. *Journal of the American Medical Association, 305*, 659–661.

Levy, S. E., Souders, M. C., Ittenbach, R. F., Giarelli, E., Mulberg, A. E., & Pinto-Martin, J. A. (2007). Relationship of dietary intake to gastrointestinal symptoms in children with autistic spectrum disorders. *Biological Psychiatry, 61*, 492–497.

Ma, R. L., Yuan, Q., & Rui, J. (2006). Effect of acupuncture combined behavior intervention on children with autism. *Zhongguo Zhong Xi Yi Jie He Za Zhi, 26*, 419–422.

Martins, Y., Young, R. L., & Robson, D. C. (2008). Feeding and eating behaviors in children with autism and typically developing children. *Journal of Autism and Developmental Disorders, 38*, 1878–1887.

Modahl, C., Green, L., Fein, D., Morris, M., Waterhouse, L., & Feinstein, C. (1998). Plasma oxytocin levels in autistic children. *Biological Psychiatry, 43*, 270–277.

Mulvihill, B., Wingate, M., Kirby, R. S., Pettygrove, S., Cunniff, C., & Meaney, F. J. (2009). Prevalence of autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, United States, 2006. *MMWR. Surveillance Summaries, 58*, 1–20.

Nienke, P. S., Robert, D., Hubert, K., & Peter, S. (2011). A meta-analytic study on the effectiveness of comprehensive ABA-based early intervention programs for children with Autism Spectrum Disorders. *Research in Autism Spectrum Disorders, 5*, 60–69.

Posey, D. J., Stigler, K. A., Erickson, C. A., & McDougle, C. J. (2008). Antipsychotics in the treatment of autism. *The Journal of Clinical Investigation, 118*, 6–14.

Rellini, E., Tortolani, D., Trillo, S., Carbone, S., & Montecchi, F. (2004). Childhood Autism Rating Scale (CARS) and Autism Behavior Checklist (ABC) correspondence and conflicts with DSM-IV criteria in diagnosis of autism. *Journal of Autism and Developmental Disorders, 34*, 703–708.

Rimmele, U., Hediger, K., Heinrichs, M., & Klaver, P. (2009). Oxytocin makes a face in memory familiar. *The Journal of Neuroscience, 29*, 38–42.

Schreck, K. A., & Williams, K. (2006). Food preferences and factors influencing food selectivity for children with autism spectrum disorders. *Research in Developmental Disabilities, 27*, 353–363.

Schreck, K. A., Williams, K., & Smith, A. F. (2004). A comparison of eating behaviors between children with and without autism. *Journal of Autism and Developmental Disorders, 34*, 433–438.

Volkmar, F. R. (1996). Brief report: diagnostic issues in autism: results of the DSM-IV field trial. *Journal of Autism and Developmental Disorders, 26*, 155–157.

Wang, C. N., Liu, Y., Wei, X. H., & Li, L. X. (2007). Effects of electroacupuncture combined with behavior therapy on intelligence and behavior of children of autism. *Zhongguo Zhen Jiu, 27*, 660–662.

Wing, L., & Gould, J. (1979). Severe impairments of social interaction and associated abnormalities in children: epidemiology and classification. *Journal of Autism and Developmental Disorders, 9*, 11–29.

Wong, V. C., & Chen, W. X. (2010). Randomized controlled trial of electro-acupuncture for autism spectrum disorder. *Alternative Medicine Review, 15*, 136–146.

Wu, S., Jia, M., Ruan, Y., Liu, J., Guo, Y., & Shuang, M. (2005). Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. *Biological Psychiatry, 58*, 74–77.

Yan, Y. F., Wei, Y. Y., Chen, Y. H., & Chen, M. M. (2007). Effect of acupuncture on rehabilitation training of child's autism. *Zhongguo Zhen Jiu, 27*, 503–505.

Yang, J., Yang, Y., Chen, J. M., Liu, W. Y., Wang, C. H., & Lin, B. C. (2007). Effect of oxytocin on acupuncture analgesia in the rat. *Neuropeptides, 41*, 285–292.

Yao, S. Q., & Gong, Y. X. (1993). Constructing the Chinese adaptive behavior rating scale for 3–12 year old children. *Psychological Science, 16*, 38–42.

Zhang, S. K. (1996). Clinical observation of effect of acupuncture on 12 autistic cases. *Zhe jiang Journal of Traditional Chinese Medicine,* 270.