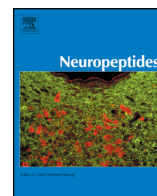




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Neuropeptides

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Effects of chronic restraint stress on social behaviors and the number of hypothalamic oxytocin neurons in male rats

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ARTICLE INFO

Article history:

Received 9 March 2016

Received in revised form 22 July 2016

Accepted 2 August 2016

Available online xxxx

Keywords:

Chronic homotypic restraint stress

Social behavior

Oxytocin

Paraventricular nucleus

ABSTRACT

Oxytocin (OXT) and vasopressin (AVP) are considered to be related to mammalian social behavior and the regulation of stress responses. The present study investigated the effects of chronic homotypic restraint stress (CHRS) on social behaviors and anxiety, as well as its repercussions on OXT- and AVP-positive neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) nuclei in rat. Male Sprague-Dawley rats receiving CHRS were exposed to repeated restraint stress of 30 min per day for 10 days. Changes in social approach behaviors were evaluated with the three-chambered social approach task. Changes in anxiety-like behaviors were evaluated in the light-dark box test. The number of neurons expressing oxytocin and/or vasopressin in PVN and SON were examined by immunohistochemistry techniques. The results demonstrated that social approach was increased and anxiety was decreased following 10-day exposure to CHRS. Furthermore, the number of OXT-immunoreactive cells in PVN was increased significantly, whereas no change in SON was seen. The number of AVP immunoreactive cells either in PVN or SON was unaffected. The results of this study suggest that certain types of stress could be effective in the treatment of social dysfunction in persons with mental disorders such as autism, social anxiety disorder. The therapeutic effects may be mediated by changes in the function of OXT neurons in PVN.

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1. Introduction

The neuropeptides oxytocin (OXT) and arginine vasopressin (AVP) are evolutionarily highly involved in the regulation of various aspects of mammalian social behaviors (Lukas and Neumann, 2013; Meyer-Lindenberg et al., 2011; Harony and Wagner, 2010). Studies provide evidence that the function of these neuropeptides is impaired in mental disorders that are characterized by severe social deficits

(Harony and Wagner, 2010; Caronna et al., 2008; Aspe-Sanchez et al., 2015). However, despite the neurobiological mechanisms of these disorders are largely unknown, researches in rodents and humans suggest OXT and AVP might be promising targets for such disorders.

Several lines of evidence suggest that the two peptides are crucial for regulating social behaviors impaired in autism spectrum disorders (ASD), mainly including affiliative behavior, social cognition, and social approach (Harony and Wagner, 2010; Caronna et al., 2008; Strathearn, 2009). Modahl et al. (1998) reported that plasma OXT levels were lower in children with ASD than in Control group. Gene association studies showed a link between polymorphisms in the OXT and/or AVP receptors in patients with ASD (Lauritsen et al., 2006; McCauley et al., 2005; Kim et al., 2002). There was also evidence that administration of OXT improves social cognition and reduces stereotypic movements in adults with ASD (Hollander et al., 2003). Some studies in social anxiety disorder (SAD) showed that OXT administration improved speech performance (Guastella et al., 2009) and attenuated the heightened amygdala reactivity to fearful faces in patients with SAD (Labuschagne et al., 2010).

The activity of paraventricular (PVN) and supraoptic (SON) nuclei of the anterior hypothalamus might be stimulus-dependent (Briski and

Abbreviations: OXT, oxytocin; AVP, vasopressin; HPA, hypothalamus-pituitary-adrenal; CHRS, chronic homotypic restraint stress; PVN, paraventricular nucleus; SON, supraoptic nucleus; CRH, corticotropin-releasing factor; ASD, autism spectrum disorders; SAD, social anxiety disorder.

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<http://dx.doi.org/10.1016/j.npep.2016.08.011>

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Please cite this article as: Li, J., et al., Effects of chronic restraint stress on social behaviors and the number of hypothalamic oxytocin neurons in male rats, *Neuropeptides* (2016), <http://dx.doi.org/10.1016/j.npep.2016.08.011>

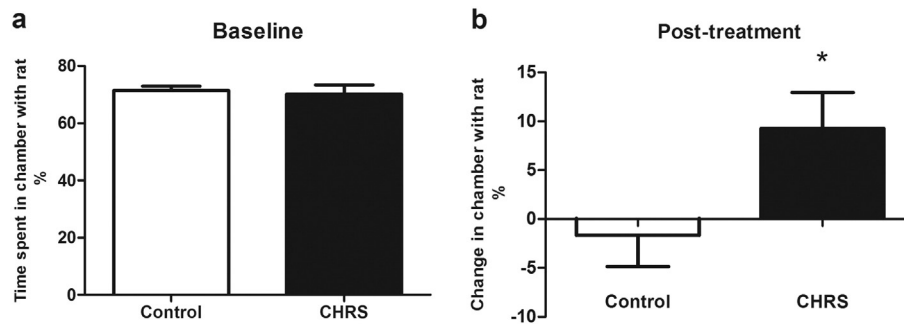


Fig. 1. Effects of CHRS on social approach in male adult rats.

Gillen, 2001; Palkovits, 2000). Previous studies showed that PVN and SON are activated by osmotic and reproductive stimuli as well as during stress (Engelmann and Ludwig, 2004; Wotjak et al., 1998; Engelmann et al., 1999). OXT and AVP are nonapeptides synthesized in PVN and SON of the hypothalamus (Sofroniew, 1983). Besides being involved in regulating mammalian social behaviors, they also play an important role in the regulation of stress response (Nishitani et al., 2004; Pirnik et al., 2004; Pirnik and Kiss, 2005; Wang et al., 2009).

OXT attenuates stress-induced activation of the hypothalamus-pituitary-adrenal (HPA) axis, thus modulates stress response in rodents (Windle et al., 1997; Neumann, 2002). Stress exposure potentiates OXT secretion into the peripheral circulation (Lang et al., 1983; Kastig, 1988) and within the brain as reflected by increased OXT concentration in the cerebrospinal fluid (Ivanyi et al., 1991). It has also been reported that OXT is released within the hypothalamus in response to shaker (Nishioka et al., 1998) and forced swimming (Wotjak et al., 1998) stress in rat. Study of oncogene expression has demonstrated that restraint stress activates oxytocinergic neurons in PVN and SON (Miyata et al., 1995). OXT administration also attenuates the increase in gene expression of corticotropin-releasing factor (CRH) in PVN in response to acute restraint stress in rats (Windle et al., 2004). On the other hand, repeated experiences with the same stressor may produce habituation or reduction of behavioral responses (Zheng et al., 2010; Yoshimoto et al., 2012).

In contrast to OXT, fewer studies have been carried out to investigate the role of AVP in stress responses. AVP acts as an important modulator of adrenocorticotrophic hormone release in response to stress by potentiating the effect of CRH (Antoni, 1993). Acute stress induces rapid and concomitant release of CRH and AVP into the pituitary portal circulation from parvocellular neurons of PVN (Kovacs and Sawchenko, 1996). Immunohistochemical studies have shown that following repeated immobilization stress CRH stores remain unchanged, but there are progressive increases in AVP stores as well as the number of CRH nerve endings containing AVP (de Goeij et al., 1991).

Other studies have shown that exposure to chronic stress increases OXT content in PVN in rat (Zheng et al., 2010; Yoshimoto et al., 2012). Exposure to repeated immobilization also increased oxytocin mRNA levels in the hypothalamus (Zheng et al., 2010; Babygirija et al., 2010). However, relatively few studies have directly examined the repeated restraint stress on social behaviors.

The present studies were therefore designed to investigate the effects of chronic homotypic restraint stress (CHRS) on social behaviors (social approach and anxiety) of male rats. It has been well accepted that the abnormal social behavior and excessive anxious reactions are the core symptoms of mental disorders like ASD and SAD. It is not clear how stress therapy helps to improve the abnormal behavior in the symptoms of such disorders. OXT and/or AVP neurons in hypothalamus have been shown to be involved in mediating stress responses and social behavior. In addition, these neurons have also been suggested to be at least partially responsible for the etiology of autism. We hypothesized that the therapeutic effect of chronic stress is mediated by changes in the function of hypothalamic OXT and/or AVP neurons.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats weighing 220–250 g, aged 65–85 d at the beginning of the experiment were obtained from experimental animal department of Peking University Health Science Center. They were housed in a 12:12 h light/dark cycle (lights on at 07:00 h) with food and water ad lib. The room temperature was maintained at 24 ± 1 °C and relative humidity at 50%. Twenty rats were evenly divided into two groups: the CHRS group and the Control group. Animals were housed 4 to 5 per cage (590 mm \times 380 mm \times 200 mm). In order to reduce the stress due to the novel environment, animals were individually acclimated for 5 min/two times daily for 2 days on the day before the experiment. Then we performed a baseline behavioral testing. The

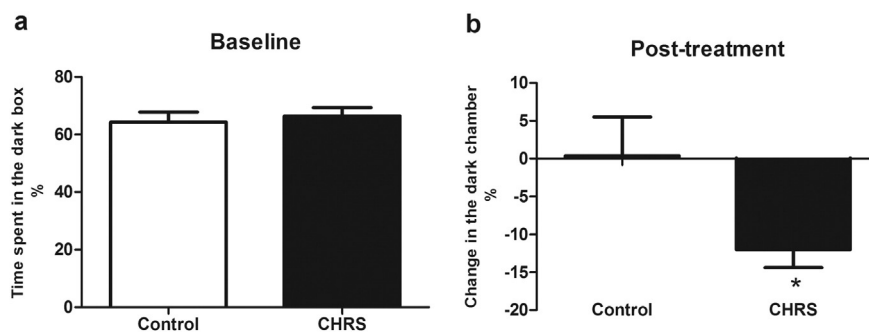


Fig. 2. Effects of CHRS on anxiety level in male adult rats. The percent of time spent in dark box reflects anxiety level, CHRS reduced anxiety behavior in the light-dark box. Data represent mean \pm SEM ($n = 10$). * $P = 0.042$, an unpaired t -test was used to compare changes in anxiety-like behaviors (con vs stressed) in Panel b.

study protocols were approved by the university's Research Ethics Committee (LA 2013-80) and were in accordance with the NIH guide for experimental animal research.

2.2. Chronic homotypic restricted stress (CHRS) loading

After baseline behavioral testing, for CHRS loading, rats were placed in a specially designed holder as described previously with modifications (Zheng et al., 2009). Their trunks were wrapped in a confining harness for 30 min per session, one session per day for ten consecutive days. The animal was able to move the hind legs and tail but not the trunk. The rats without CHRS were not exposed to restraint stress, and were not removed from their cages.

2.3. Behavioral testing

2.3.1. Social approach test

After 10-day exposure to CHRS, the social approach test was designed according to Nadler et al. (2004) with slight modifications. The apparatus consisted of three plastic boxes (34 cm × 40 cm × 24 cm) namely A, N and B. The three boxes were connected by two corridors (10 cm × 10 cm × 15 cm), and there was an infrared detector at each corridor to record the number of entries into each chamber and the duration of time stayed in each chamber. The procedure for our social test included 2 phases: habituation and testing. Phase I, Habituate rat to all three chambers for 15 min one day before the test. Phase II, Test for sociability. Subject rat was placed in the center box N. In box A, an

a OXT

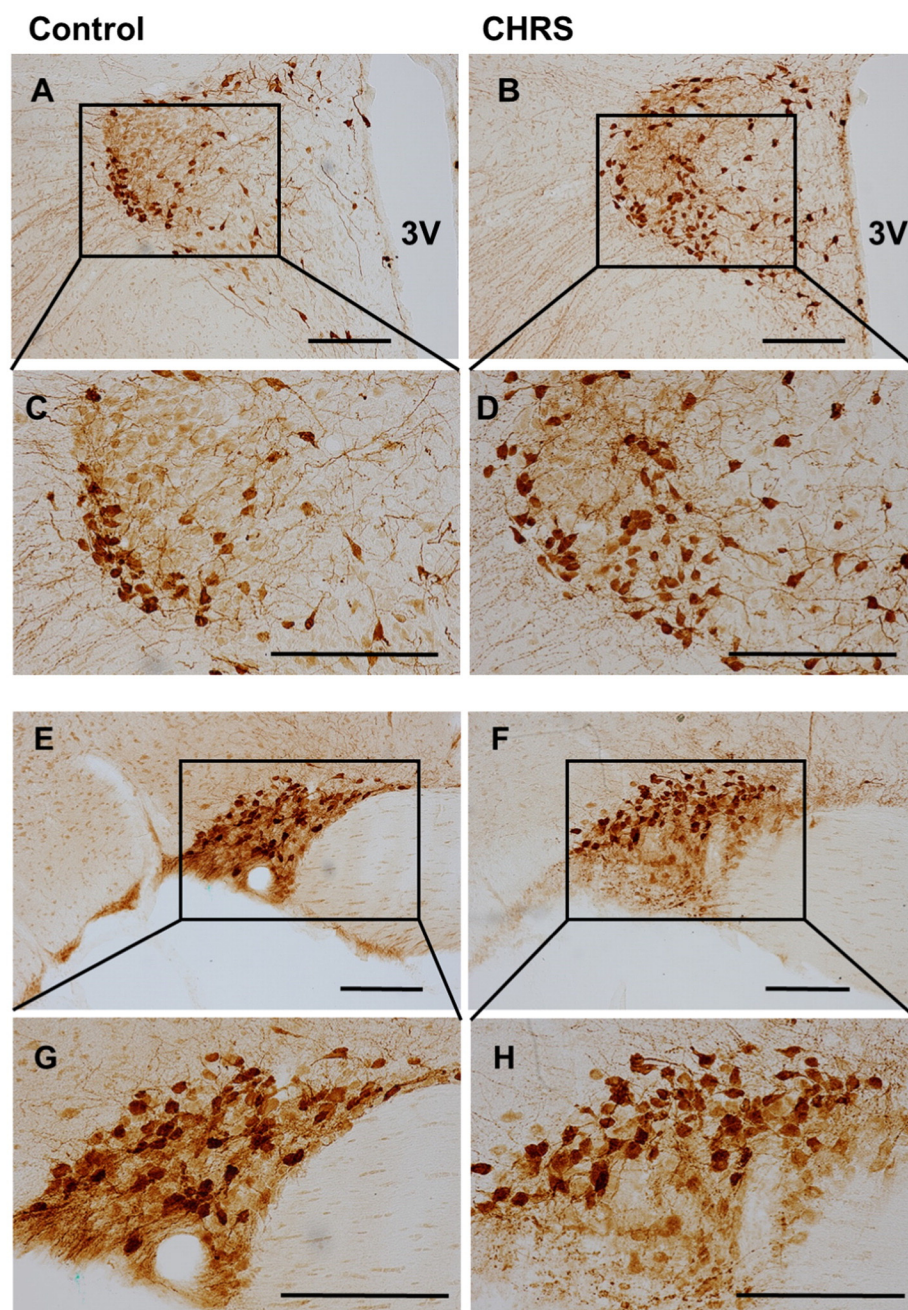


Fig. 3. Representative photomicrograph of OXT-positive cells in male adult rats. Immunohistochemistry of OXT in PVN (A & C, Control; B & D, CHRS) and in SON (E & G, Control; F & H, CHRS). Compared to control rats, an increased number of OXT-immunoreactive cells in PVN was observed in CHRS rats (scale bar = 200 μ m; 3V = third ventricle).

unfamiliar rat (we used different model rats during the baseline test and post-treatment test) was kept in a small cage; and an empty cage was placed in box B. The time spent by the testing animal in box A was thought to be an indication of the rat's tendency to participate in social approach. Each test lasted for 15 min. At the end of the test, rats were returned to their home cage and the apparatus was thoroughly cleaned to remove the smell from the previous one. Animals used as strange rats were male with the same age without previous contact with the testing rats.

2.3.2. Light-dark exploration test

After the social approach test, we performed light-dark exploration test. The test apparatus consisted of an enclosed dark box and a bright box with a transparent ceiling (34 cm × 40 cm × 24 cm). A small

corridor (10 cm × 10 cm × 15 cm) allowed rats to move freely between boxes. There was also an infrared detector placed at the corridor detecting crossing objects. At the beginning of the test, the testing rat was placed into the light box and the time spent in each box was recorded for 15 min. Cumulative time spent in the dark box was calculated as an indication of anxiety-related behavior. At the end of each test, rats were returned to their home cages and the apparatus were thoroughly cleaned to remove the smell from the previous one.

2.4. Immunohistochemical analysis of OXT- and AVP-containing cells in the PVN and SON

After the behavioral tests, rats were anesthetized with 10% chloral hydrate (300 mg/kg body weight) administered intraperitoneally.

b AVP

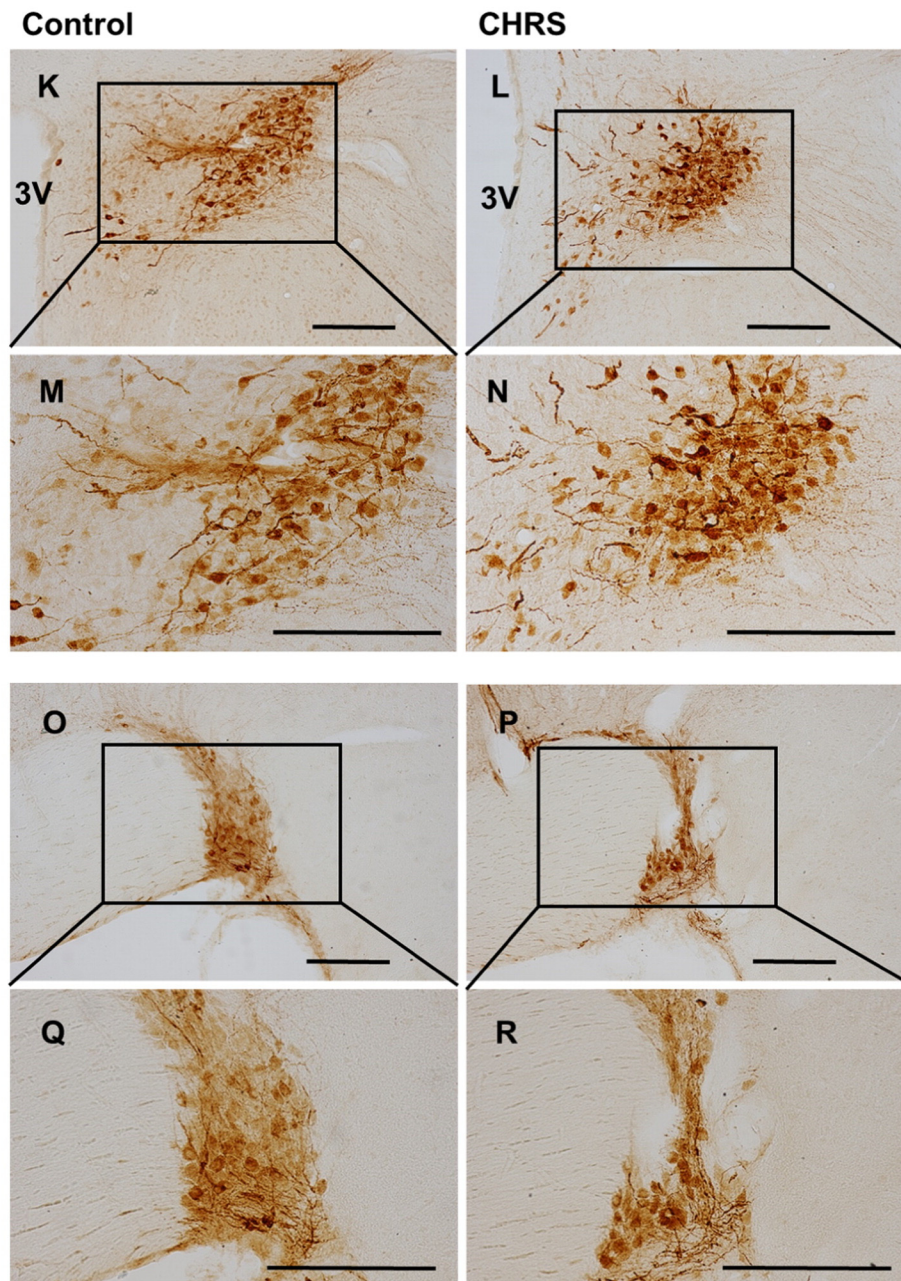


Fig. 4. Representative photomicrograph of AVP-positive cells in male adult rats. Immunohistochemistry of AVP in PVN (K & M, Control; L & N, CHRS) and in SON (O & Q, Control; P&R, CHRS). (Scale bar = 200 μ m; 3 V = third ventricle).

They were then transcardially perfused with saline solution and buffered 4% paraformaldehyde (pH 7.4) using a peristaltic pump. Brains were removed, post-fixed in the same fixative solution and cryoprotected in 15% and 30% sucrose in phosphate buffered saline (PBS). Serial sections (30 μm each) were collected and stored at -20°C in anti-freezing solution. The immunoreactivity of OXT and AVP was detected by dual ABC (avidin-biotin-peroxidase complex) immunohistochemical technique. The procedures were as follows: Free floating sections were rinsed in 0.01 mol/L PBS (pH 7.2) and then floated in 4% formaldehyde for 10 min, then rinsed in 0.01 mol/L PBS, followed by a preincubation in 0.3% H_2O_2 for 30 min at room temperature to eliminate endogenous peroxidase activity. After rinsing in 0.01 mol/L PBS, the sections were incubated with blocking buffer, containing 10% normal goat serum and 0.3% Triton X-100 in 0.01 mol/L PBS for 30 min in a thermostat at 37°C , followed by incubation with rabbit anti-OXT antibody (Abcam, USA, 1:2000 dilution in 0.01 mol/L PBS containing 1% normal goat serum and 0.3% TritonX-100) for 2 h at 37°C and then -4°C for 48 h. The sections were then washed three times (15 min each) in PBS prior to incubation for 30 min at 37°C with the secondary biotinylated goat anti-rabbit IgG (Vectastain, USA, 1:200 dilution). The slices were then rinsed three times with PBS followed by incubation with streptavidin-biotin-horseradish peroxidase complex (Vectastain, USA, diluted 1:200) for 45 min at 37°C . Immunoreactivity was visualized by incubating each slice in a medium containing 0.05 M Tris-HCl (pH 7.6) with 0.5 mg/mL diaminobenzidine (DAB) and 0.03% H_2O_2 for 10 min. This method produces a brown nuclear reaction product. Lastly, the free-floating sections were air-dried overnight, dehydrated in a series of alcohols, cleared in xylene and placed under a coverslip with gum.

2.5. Cell counting and statistical analysis

The immunoreactive cells were counted and photographed under an Olympus microscope with a Canon camera. OXT and AVP were identified as brown immunoreactive products deposited in the nuclei in PVN and SON. The total number of OXT- and AVP-immunoreactive cells was counted bilaterally in the PVN and SON on the sections

between -0.8 and -2.1 mm from the Bregma. The selected section showed the most immunoreactive products. The identification of neurons in the PVN and SON of hypothalamus was performed, as previously reported (Sawchenko and Swanson, 1982).

2.6. Statistics

The statistical analysis was performed with SPSS 13.0 software. The results were expressed as the mean \pm SEM and the differences between unpaired variables were determined by *t*-test. *P* value < 0.05 was considered statistically significant.

3. Results

3.1. Social approach test

The results of three-chambered sociability test were shown in Fig. 1. There was no difference between groups in social behavior during the baseline period (Fig. 1a). Fig. 1b illustrated the change of time percent spent in chamber A (with stranger rat). A significant difference was observed between groups on the time spent exploring the stranger rat (9.286 ± 3.662 for the CHRS and -1.647 ± 3.208 for the Control group). Fig. 1b showed that CHRS rats spent more time exploring the stranger rat than control rats ($P < 0.05$).

CHRS increased exploratory behavior in the three-chambered social approach task. (a) The baseline of percent time spent in chamber A with strange rat between both groups, (b) Chronic stress increased social recognition in male rats. Data represent mean \pm SEM ($n = 10$). $*P = 0.038$, an unpaired *t*-test was used to compare changes in social approach behaviors (con vs stressed) in Panel b.

3.2. Light-dark exploration test

Fig. 2 showed the percentage of time the rats spent in the dark box which reflects the anxiety level. There was a significant decrease in post-treatment phase in CHRS rats (-12.02 ± 2.372) compared to

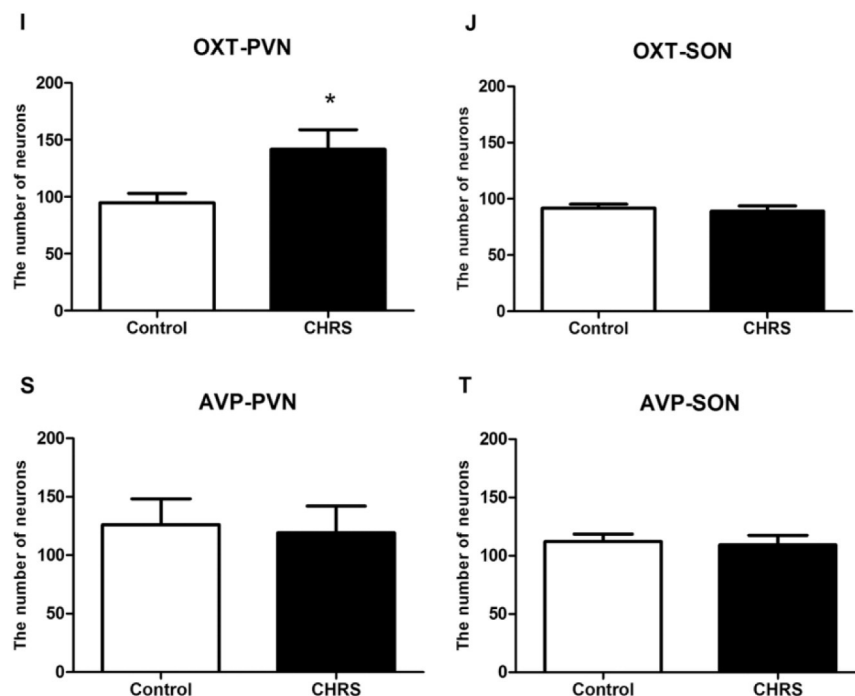


Fig. 5. Effects of CHRS on number of OXT-immunoreactive neurons and AVP-immunoreactive neurons in the PVN and SON in adult rats. The numbers of cells were averaged (mean \pm SEM) in group. Results were compared between groups with unpaired *t*-test. Number of animals 8–10 in each group; $*P = 0.036$.

the Control group (0.383 ± 5.131) on percentage of time spent in the dark box ($P < 0.05$, Fig. 2b).

3.3. Immunohistochemistry analysis for OXT- and AVP-positive neurons in PVN and SON

In response to chronic homotypic restraint stress, the number of OXT-immunoreactive cells in PVN was significantly increased from 94.75 ± 8.33 to 141.8 ± 17.05 ($P < 0.05$, Fig. 5I). There was no significant change in OXT-immunoreactive cells in SON following CHRS (Fig. 5J). Nor was there any significant difference between the groups in the number of AVP-immunoreactive cells in PVN (Fig. 5S) or SON (Fig. 5T).

Representative photomicrographs of OXT and AVP-positive neurons from PVN and SON nuclei were showed in Figs. 3 and 4, respectively.

4. Discussion

The results showed that chronically stressed rats spent significantly more time exploring the strange rat in the three-chambered test, and significantly less time in the dark environment following CHRS versus baseline. It suggests that 10-day exposure to chronic homotypic restraint stress may increase social approach and decrease anxiety. Furthermore, the number of OXT-immunoreactive cells in the PVN, but not SON, was found to be increased after 10 days of CHRS exposure, whereas AVP-immunoreactive cells in PVN or SON was not affected by CHRS. These data suggest that OXT may play an important role in PVN in mediating the behavioral changes following CHRS.

Previous study (Sandi and Haller, 2015) showed that there has been a vast increase in research on how various forms of stressors administered in different phases of the lifespan affect individuals' interest in and reactions towards conspecifics—including social motivation, social recognition and aggression. Animal studies have shown that the effects of stress on social behaviors cover a range of neurodevelopmental periods. Their long-term effects on the HPA axis are different. Prenatal stress, adult acute and chronic stress lead to increased HPA-axis activity, while peripubertal stressors lead to decreased HPA axis activity (Toth et al., 2011). In one study, juvenile social stressors (post-weaning social isolation) increased adult social interactions (Shimozuru et al., 2008). Our results showed that social approach was significantly improved in chronically stressed rats.

Babygirija et al. (Babygirija et al., 2012) have recently demonstrated that the stressed rats received more amicable behaviors from their partners than the non-stressed rats. In our current study, CHRS rats displayed a higher tendency to participate social approach compared with control rats. As positive social approach implies receiving and giving more empathy, it is conceivable that subjects may display more affiliative behaviors toward others in response to stress. It is important to point out that in both studies the hypothalamic OXT system was up-regulated.

It is believed that OXT and AVP are promising targets for mental disorders characterized by social dysfunction such as autism and anxiety (Lukas and Neumann, 2013; Aspe-Sanchez et al., 2015). In particular, some clinical studies have shown that intranasal administration of OXT might improve social behaviors in people with ASD (Baron-Cohen et al., 2001; Andari et al., 2010). OXT has also been described as a key regulator of the stress response for both physiological and behavioral aspects (Neumann, 2002). Waldherr and Neumann reported that mating releases OXT in the PVN and facilitates a reduction in anxiety-like behavior during psychological stress in male rats (Waldherr and Neumann, 2007).

In our study, the elevated level of social approach found in rats chronically exposed to restraint stressor was accompanied by corresponding alterations in the level of hypothalamic neuropeptides. The number of OXT-immunoreactive cells in the PVN was significantly increased following CHRS. It is likely that alterations in social behaviors are resulted from activation of the brain OXT system by immobilization

stress. Several lines of evidence support our supposition. For instance, a number of recent studies have shown that restraint stress induces c-fos expression in oxytocinergic neurons in PVN in rats (Miyata et al., 1995; Zheng et al., 2010; Yoshimoto et al., 2012). According to Zheng et al. (2010), the oxytocin mRNA expression in PVN was significantly increased following chronic homotypic stress, suggesting that the up-regulation of oxytocin gene expression in the hypothalamus may serve as an adaptive response to chronic stress. Lukas et al. (2011) reported that OXT immunoreactive neurons in PVN may facilitate positive social approach. Their study also showed that icv administration of OXTR antagonist impaired social approach while central administration of synthetic OXT reversed social avoidance.

Our present study showed that 10-day exposure to chronic stress decreased anxiety of the rats. However, one must be aware that the mechanisms underlying the effect of chronic stress on anxiety related behaviors are not well understood and results from different laboratories are sometimes contradictory. In contrast to our results, a number of acute or repeated stressors have been shown to increase the level of anxiety-related behavior (Matuszewich et al., 2007; Buwalda et al., 2005; Vyas and Chattarji, 2004). In addition, it has been reported that exposure of rats to 14 days of restraint stress did not change anxiety levels in the elevated-plus maze (Poulin et al., 2014). Thus, it is possible that the differences in the behavioral effects of chronic stress are likely to rely on (1) the species studied, (2) the type and duration of the stressor, and (3) the tests used to estimate the effect on social behaviors (Reber and Neumann, 2008). It is worth considering that we did not see the reduced exploration times in the dark chamber in control animals at the second time when they were placed into the chamber. Actually one would expect to see habituation to the environmental settings and therefore increased light chamber exploration times during the second test. One of the reasons for this may be that the interval between baseline and post-treatment light-dark testing is a bit long (nearly 15 days), the rat's adaptability to the light chamber could therefore not be shown.

Given the importance of OXT/AVP in the regulation of social behaviors and stress responses, we assumed that these behavioral alterations observed in the CHRS group were associated with OXT/AVP system activation. Our immunohistochemistry results showed obvious morphological changes in the number of OXT positive neurons in PVN. More work is needed to clarify whether the increased expression of OXT in PVN is related to reduced release or enhanced synthesis of this neuropeptide.

There are several limitations ought to be mentioned. We used normal adult rats in our research, they did not have social deficits and have few relevance with human children. In order to verify the relation between chronic restraint stress and different kinds of neural disorders, we consider to use rat models of autism or anxiety in the next work. Additionally, the Control group has not undergone any handling or other disturbances. Effects such as cohort removal (sequential removal of rats from the cage) may act as a rapid and potent stressor for the remaining rats (Cook et al., 1972), repeated brief separations from the mother followed by handling during the neonatal period may induce a reduction in affiliative social behavior among adult males (Todeschin et al., 2009). All these factors may cause some confounding effect on the interpretation of the results.

5. Conclusions

In summary, chronic homotypic restraint stress increased social approach and decreased anxiety in male adult rats. Furthermore, the number of OXT-immunoreactive cells in PVN was found to be increased concomitantly following CHRS group. Our study may help to better understand that moderate and controllable stress may be helpful in ameliorating mental disorders such as autism, social anxiety disorder.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by the research special fund for public welfare, Department of Health of China (201302002) to Jisheng Han.

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. *Proc. Natl. Acad. Sci. U. S. A.* 107, 4389–4394.
- Antoni, F.A., 1993. Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. *Front. Neuroendocrinol.* 14, 76–122.
- Aspe-Sanchez, M., Moreno, M., Rivera, M.I., Rossi, A., Ewer, J., 2015. Oxytocin and vasopressin receptor gene polymorphisms: role in social and psychiatric traits. *Front. Neurosci.* 9, 510.
- Babygirija, R., Zheng, J., Bülbül, M., Cerjak, D., Ludwig, K., Takahashi, T., 2010. Sustained delayed gastric emptying during repeated restraint stress in oxytocin knockout mice. *J. Neuroendocrinol.* 22, 1181–1186.
- Babygirija, R., Cerjak, D., Yoshimoto, S., Gribovskaja-Rupp, I., Bülbül, M., Ludwig, K., Takahashi, T., 2012. Affiliative behavior attenuates stress responses of GI tract via up-regulating hypothalamic oxytocin expression. *Auton. Neurosci.* 169, 28–33.
- Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., Plumb, I., 2001. The “Reading the Mind in the Eyes” Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J. Child Psychol. Psychiatry* 42, 241–251.
- Briski, K., Gillen, E., 2001. Differential distribution of fos expression within the male rat preoptic area and hypothalamus in response to physical vs. psychological stress. *Brain Res. Bull.* 55, 401–408.
- Buwalda, B., Kole, M.H., Veenema, A.H., Huininga, M., de Boer, S.F., Korte, S.M., Koolhaas, J.M., 2005. Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning. *Neurosci. Biobehav. Rev.* 29, 83–97.
- Caronna, E.B., Milunsky, J.M., Tager-Flusberg, H., 2008. Autism spectrum disorders: clinical and research frontiers. *Arch. Dis. Child.* 93, 518–523.
- Cook, D.M., Kendall, J.W., Greer, M.A., Kramer, R.M., 1972. The effect of acute or chronic ether stress on plasma ACTH concentration in the rat. *Endocrinology* 93, 1019–1024.
- de Goeij, D.C., Kvetnansky, R., Whitnall, M.H., Jezova, D., Berkenbosch, F., Tilders, F.J., 1991. Repeated stress-induced activation of corticotropin-releasing factor neurons enhances vasopressin stores and colocalization with corticotropin-releasing factor in the median eminence of rats. *Neuroendocrinology* 53, 150–159.
- Engelmann, M., Ludwig, M., 2004. The activity of the hypothalamo-neurohypophysial system in response to acute stressor exposure: neuroendocrine and electrophysiological observations. *Stress* 7, 91–96.
- Engelmann, M., Ebner, K., Landgraf, R., Holsboer, F., Wotjak, C.T., 1999. Emotional stress triggers intrahypothalamic but not peripheral release of oxytocin in male rats. *J. Neuroendocrinol.* 11, 867–872.
- Guastella, A.J., Howard, A.L., Dadds, M.R., Mitchell, P., Carson, D.S., 2009. A randomized controlled trial of intranasal oxytocin as an adjunct to exposure therapy for social anxiety disorder. *Psychoneuroendocrinology* 34, 917–923.
- Harony, H., Wagner, S., 2010. The contribution of oxytocin and vasopressin to mammalian social behavior: potential role in autism spectrum disorder. *Neurosignals* 18, 82–97.
- Hollander, E., Novotny, S., Hanratty, M., Yaffe, R., DeCaria, C.M., Aronowitz, B.R., Mosovich, S., 2003. Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. *Neuropsychopharmacology* 28, 193–198.
- Ivanyi, T., Wiegant, V.M., de Wied, D., 1991. Differential effects of emotional and physical stress on the central and peripheral secretion of neurohypophysial hormones in male rats. *Sci. Life* 48, 1309–1316.
- Kasting, N.W., 1988. Simultaneous and independent release of vasopressin and oxytocin in the rat. *Can. J. Physiol. Pharmacol.* 66, 22–26.
- Kim, S.J., Young, L.J., Gonen, D., Veenstra-VanderWeele, J., Courchesne, R., Courchesne, E., Lord, C., Leventhal, B.L., Cook Jr., E.H., Insel, T.R., 2002. Transmission disequilibrium testing of arginine vasopressin receptor 1A (AVPR1A) polymorphisms in autism. *Mol. Psychiatry* 7, 503–507.
- Kovacs, K.J., Sawchenko, P.E., 1996. Sequence of stress-induced alterations in indices of synaptic and transcriptional activation in parvocellular neurosecretory neurons. *J. Neurosci.* 16, 262–273.
- Labuschagne, I., Phan, K.L., Wood, A., Angstadt, M., Chua, P., Heinrichs, M., Stout, J.C., Nathan, P.J., 2010. Oxytocin attenuates amygdala reactivity to fear in generalized social anxiety disorder. *Neuropsychopharmacology* 35, 2403–2413.
- Lang, R.E., Heil, J.W., Ganten, D., Hermann, K., Unger, T., Rascher, W., 1983. Oxytocin unlike vasopressin is a stress hormone in the rat. *Neuroendocrinology* 37, 314–316.
- Lauritsen, M.B., Als, T.D., Dahl, H.A., Flint, T.J., Wang, A.G., Vang, M., Kruse, T.A., Ewald, H., Mors, O., 2006. A genome-wide search for alleles and haplotypes associated with autism and related pervasive developmental disorders on the Faroe Islands. *Mol. Psychiatry* 11, 37–46.
- Lukas, M., Neumann, I.D., 2013. Oxytocin and vasopressin in rodent behaviors related to social dysfunctions in autism spectrum disorders. *Behav. Brain Res.* 251, 85–94.
- Lukas, M., Toth, I., Reber, S.O., Slattery, D.A., Veenema, A.H., Neumann, I.D., 2011. The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. *Neuropsychopharmacology* 36, 2159–2168.
- Matuszewich, L., Karney, J.J., Carter, S.R., Janasik, S.P., O'Brien, J.L., Friedman, R.D., 2007. The delayed effects of chronic unpredictable stress on anxiety measures. *Physiol. Behav.* 90, 674–681.
- McCauley, J.L., Li, C., Jiang, L., Olson, L.M., Crockett, G., Gainer, K., Folstein, S.E., Haines, J.L., Sutcliffe, J.S., 2005. Genome-wide and ordered-subset linkage analyses provide support for autism loci on 17q and 19p with evidence of phenotypic and interlocus genetic correlates. *BMC Med. Genet.* 6, 1.
- Meyer-Lindenberg, A., Domes, G., Kirsch, P., Heinrichs, M., 2011. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat. Rev. Neurosci.* 12, 524–538.
- Miyata, S., Itoh, T., Lin, S.H., Ishiyama, M., Nakashima, T., Kiyohara, T., 1995. Temporal changes of c-fos expression in oxytocinergic magnocellular neuroendocrine cells of the rat hypothalamus with restraint stress. *Brain Res. Bull.* 37, 391–395.
- Modahl, C., Green, L., Fein, D., Morris, M., Waterhouse, L., Feinstein, C., Levin, H., 1998. Plasma oxytocin levels in autistic children. *Biol. Psychiatry* 43, 270–277.
- Nadler, J.J., Moy, S.S., Dold, G., Trang, D., Simmons, N., Perez, A., Young, N.B., Barbaro, R.P., Piven, J., Magnuson, T.R., Crawley, J.N., 2004. Automated apparatus for quantitation of social approach behaviors in mice. *Genes Brain Behav.* 3, 303–314.
- Neumann, I.D., 2002. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. *Prog. Brain Res.* 139, 147–162.
- Nishioka, T., Anselmo-Franci, J.A., Li, P., Callahan, M.F., Morris, M., 1998. Stress increases oxytocin release within the hypothalamic paraventricular nucleus. *Brain Res.* 781, 57–61.
- Nishitani, S., Moriya, T., Kondo, Y., Sakuma, Y., Shinohara, K., 2004. Induction of Fos immunoreactivity in oxytocin neurons in the paraventricular nucleus after female odor exposure in male rats: effects of sexual experience. *Cell. Mol. Neurobiol.* 24, 283–291.
- Palkovits, M., 2000. Stress-induced expression of co-localized neuropeptides in hypothalamic and amygdaloid neurons. *Eur. J. Pharmacol.* 405, 161–166.
- Pirnik, Z., Kiss, A., 2005. Fos expression variances in mouse hypothalamus upon physical and osmotic stimuli: co-staining with vasopressin, oxytocin, and tyrosine hydroxylase. *Brain Res. Bull.* 65, 423–431.
- Pirnik, Z., Mravec, B., Kiss, A., 2004. Fos protein expression in mouse hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei upon osmotic stimulus: colocalization with vasopressin, oxytocin, and tyrosine hydroxylase. *Neurochem. Int.* 45, 597–607.
- Poulin, J.F., Laforest, S., Drolet, G., 2014. Enkephalin downregulation in the nucleus accumbens underlies chronic stress-induced anhedonia. *Stress* 17, 88–96.
- Reber, S.O., Neumann, I.D., 2008. Defensive behavioral strategies and enhanced state anxiety during chronic subordinate colony housing are accompanied by reduced hypothalamic vasopressin, but not oxytocin, expression. *Ann. N. Y. Acad. Sci.* 1148, 184–195.
- Sandi, C., Haller, J., 2015. Stress and the social brain: behavioural effects and neurobiological mechanisms. *Nat. Rev. Neurosci.* 16, 290–304.
- Sawchenko, P.E., Swanson, L.W., 1982. Immunohistochemical identification of neurons in the paraventricular nucleus of the hypothalamus that project to the medulla or to the spinal cord in the rat. *J. Comp. Neurol.* 205, 260–272.
- Shimozuru, M., Kikusui, T., Takeuchi, Y., Mori, Y., 2008. Effects of isolation-rearing on the development of social behaviors in male Mongolian gerbils (*Meriones unguiculatus*). *Physiol. Behav.* 94, 491–500.
- Sofroniew, M.V., 1983. Morphology of vasopressin and oxytocin neurones and their central and vascular projections. *Prog. Brain Res.* 60, 101–114.
- Strathearn, L., 2009. The elusive etiology of autism: nature and nurture? *Front. Behav. Neurosci.* 3, 11.
- Todeschin, A.S., Winkelmann-Duarte, E.C., Vianna Jacob, M.H., Cerpa Aranda, B.C., Jacobs, S., Fernandes, M.C., Marques Ribeiro, M.F., Sanvitto, G.L., Lucion, A.B., 2009. Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. *Horm. Behav.* 56, 93–100.
- Toth, M., Mikics, E., Tulogdi, A., Aliczki, M., Haller, J., 2011. Post-weaning social isolation induces abnormal forms of aggression in conjunction with increased glucocorticoid and autonomic stress responses. *Horm. Behav.* 60, 28–36.
- Vyas, A., Chattarji, S., 2004. Modulation of different states of anxiety-like behavior by chronic stress. *Behav. Neurosci.* 118, 1450–1454.
- Waldherr, M., Neumann, I.D., 2007. Centrally released oxytocin mediates mating-induced anxiolysis in male rats. *Proc. Natl. Acad. Sci. U. S. A.* 104, 16681–16684.
- Wang, L., Martinez, V., Larauche, M., Taché, Y., 2009. Proximal colon distension induces Fos expression in oxytocin-, vasopressin-, CRF- and catecholamines-containing neurons in rat brain. *Brain Res.* 1247, 79–91.
- Windle, R.J., Shanks, N., Lightman, S.L., Ingram, C.D., 1997. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology* 138, 2829–2834.
- Windle, R.J., Kershaw, Y.M., Shanks, N., Wood, S.A., Lightman, S.L., Ingram, C.D., 2004. Oxytocin attenuates stress-induced c-fos mRNA expression in specific forebrain regions associated with modulation of hypothalamo-pituitary-adrenal activity. *J. Neurosci.* 24, 2974–2982.
- Wotjak, C.T., Ganster, J., Kohl, G., Holsboer, F., Landgraf, R., Engelmann, M., 1998. Dissociated central and peripheral release of vasopressin, but not oxytocin, in response to repeated swim stress: new insights into the secretory capacities of peptidergic neurons. *Neuroscience* 85, 1209–1222.

Yoshimoto, S., Cerjak, D., Babygirija, R., Bulbul, M., Ludwig, K., Takahashi, T., 2012. Hypothalamic circuit regulating colonic transit following chronic stress in rats. *Stress* 15, 227–236.

Zheng, J., Dobner, A., Babygirija, R., Ludwig, K., Takahashi, T., 2009. Effects of repeated restraint stress on gastric motility in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1358–R1365.

Zheng, J., Babygirija, R., Bülbül, M., Cerjak, D., Ludwig, K., Takahashi, T., 2010. Hypothalamic oxytocin mediates adaptation mechanism against chronic stress in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 299, G946–G953.