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Expression of glial fibrillary acidic protein in astrocytes of rat supraoptic nucleus throughout estrous cycle

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Abstract

OBJECTIVE: Oxytocin (OXT) could facilitate preovulatory luteinizing hormone surge in animals and humans while brain OXT production depends on glial fibrillary acidic protein (GFAP)-associated astrocytic plasticity. Here, we examined if GFAP expressions in OXT-producing hypothalamic supraoptic nucleus (SON) correlate to special estrous stages.

METHODS: 38 adult female rats were classified into diestrus, proestrus, estrus, and metestrus groups determined by vaginal smear. Rats were decapitated and the SON was dissected for detecting Fos and GFAP levels by Western Blot and immunohistochemistry.

RESULTS: The result showed that Fos expression was significantly high at proestrus compared to other stages in Western blotting. No significant difference in total GFAP expression was observed between rats at different stages of the estrous cycle; however, at proestrus GFAP level at the dorsolateral portion of the SON (a region filled with OXT neurons) was significantly lower than that at the ventromedial portion in immunohistochemistry.

CONCLUSION: There is a functional correlation between supraoptic neuron activity and proestrous OXT peak during estrous cycle; it is likely that a plastic change in GFAP expression in astrocytes selectively occurs around OXT neurons at proestrus and facilitates OXT release.

Abbreviations:

GFAP	- Glial fibrillary acidic protein
GnRH	- gonadotropin-releasing hormone
IHC	- immunohistochemistry
LH	- luteinizing hormone
OXT	- oxytocin
PVN	- paraventricular nucleus
SON	- supraoptic nucleus

ORIGINAL ARTICLE

INTRODUCTION

The estrus cycle is critically regulated by periodic gonadotropin-releasing hormone (GnRH) secretion from the medial basal hypothalamus in vertebrates. This regulatory effect of GnRH is mainly through two pituitary hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Plant 2015). The GnRH pulse generator is under the influence of multiple neural, hormonal and environmental inputs to the hypothalamus (Maggi *et al.* 2015). Among them, OXT is apparently involved in the follicle development and ovulation.

Experimental evidence suggests that the effect of OXT on reproduction can be achieved via gonadotropin release from the anterior pituitary. OXT was present in the portal blood of rats at high concentration (Eckland *et al.* 1988) and administration of OXT in adult female rats during early proestrus advances the spontaneous LH surge and markedly increases peripheral LH levels through activation of OXT receptor on the anterior pituitary cells (Evans *et al.* 1989; Evans & Catt 1989). The anterior pituitary which was pre-exposed to OXT had an increased LH response to GnRH (Evans *et al.* 1995). Moreover, OXT can act on the hypothalamus to increase GnRH release (Selvage *et al.* 2001; Evans *et al.* 2012). Interestingly, the raised levels of OXT in portal blood occur specifically at proestrus (Sarkar *et al.* 1984). Thus, clarification of the regulatory mechanisms underlying proestrus OXT secretion is particularly meaningful for understandings of the neuroendocrine control of the ovulation through the hypothalamic pituitary-ovary axis.

Brain OXT is synthesized in OXT neurons in the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus and released into blood from the posterior pituitary during ovulatory cycle (Amico *et al.* 1981; Mitchell *et al.* 1981; Kumaresan *et al.* 1983) and into the brain from somatodendritic sites (Neumann 2007). The activity of OXT neurons is modulated by their adjacent astrocytes. In the SON, changes in astrocyte morphology and function can dramatically alter OXT neuronal activity (Wang & Hatton 2009). In this process, glial fibrillary acidic protein (GFAP) in astrocytes plays a critical role (Wang & Zhu 2014). Reduction of GFAP filament or retraction of astrocyte processes can increase OXT neuron excitability in the SON (Hatton 1997). It is likely that the proestrus increase in OXT release results from a retraction of astrocytic processes in the SON. However, experimental evidence is not available yet. To obtain such evidence, we detected GFAP level of the SON in female cycling rats. The results suggest a possible involvement of astrocytic plasticity in the SON in proestrus OXT secretion.

MATERIAL & METHODS

All procedures in the animal experiments were in accordance with the Guidelines on the Use and Care of Laboratory Animals set by National Institutes of Health and approved by the Institutional Animal Care and Use Committee of Harbin Medical University.

Thirty-eight healthy adult female Sprague-Dawley rats were used for this study. The stages of their estrous cycle, i.e., diestrus, proestrus, estrus, and metestrus, were identified through vaginal smear (Figure 1A). Rats with a clear stage were decapitated in the afternoon; brains were quickly removed and placed in an ice-cold oxygenated, artificial cerebrospinal fluid for ~1 min.

The methods for immunohistochemistry (IHC) and Western blot referred to those that were described previously (Wang *et al.* 2007). The primary antibody include polyclonal rabbit antibody against Fos (sc-7202, 1:250) monoclonal mouse antibody against GFAP (sc-33673, 1:250) and polyclonal goat antibody against OXT-neurophysin (SC-7810, 1:400). Immunostained sections were examined with a Fluorescence microscope (Eclipse FN1, Nikon) through a CCD camera (DS Ri2, Nikon).

For Western blot, the methods were also described recently with details (Wang *et al.* 2013b; Wang *et al.* 2013a). Monoclonal mouse antibody against β-Actin (sc-47778, 1:250) was used as loading control and was the product of Santa Cruz Biotechnology (Shanghai) Co., Ltd.

All measures were expressed as means ± SE relative to no-secondary control in IHC and to β-actin loading controls in Western blot and as arbitrary units of the diestrus. Comparisons between groups were performed by using One-Way ANOVA analysis or paired t-test of the SPSS 17 software. The significance level was set at $p<0.05$.

RESULTS

Among 38 virgin females, 10 rats were in diestrus, 10 in proestrus, 9 in estrus and 9 in metestrus. Fos expression together with GFAP at rats of different stages was examined first in IHC in our preliminary study to determine if cellular activity in the SON is correlated with the proestrus OXT peak. The result showed that Fos expression was dramatically high at proestrus, which was accompanied with a dramatic reduction of GFAP, compared to other stages (Figure 1B). This finding is consistent with previously reported OXT peaks at the proestrus (Evans *et al.* 2003; Salonia *et al.* 2005; Moscovice & Ziegler 2012). However, the low power of initial imaging study did not allow us to quantitate these changes accurately. Thus, we performed Western blots with SON specimens from four rats in each stage. As shown in Figure 1C, Fos protein levels were significantly higher in the proestrus than that in other stages ($p<0.05$ by one-way ANOVA).

We next examined expressions of GFAP level in SON lysates from 22 rats to link astrocytic plasticity to preovulatory OXT peak. In Western blot, there was no significant difference in GFAP level among the four estrous stages (n=22, F=0.407, $p=0.750$ by one-way ANOVA) despite the presence of a trend of lower GFAP level in proestrus rats (Figure 2A).

To resolve the contradiction between the observed GFAP levels in whole SON and the hypothesized contribution of astrocytic/GFAP plasticity to OXT secretion, we further examined GFAP expression in IHC in different stages with four rats in each stage. In quantitation of GFAP expression, we assayed the total intensity of GFAP in the SON first, which did not yield a significantly value despite all rats in the proestrus showed lower levels of GFAP staining (Figure 2Ba and 2Bb1). Thus we further quantitated GFAP intensity in OXT neurons-dominant dorsolateral zone (Mason *et al.* 1984), and compared with that in vasopressin neurons-dominant ventromedial zone of the SON. The result revealed that in the proestrus, the ratio of GFAP intensity in the dorsolateral versus ventromedial portion was significantly lower than the ratio in other stages ($p<0.05$, compared to DE or ME by paired t-test, two-tailed, Figure 2Bb2). This finding supports the hypothesis that a plastic astrocytic/GFAP change selectively occurs around OXT neurons at proestrus.

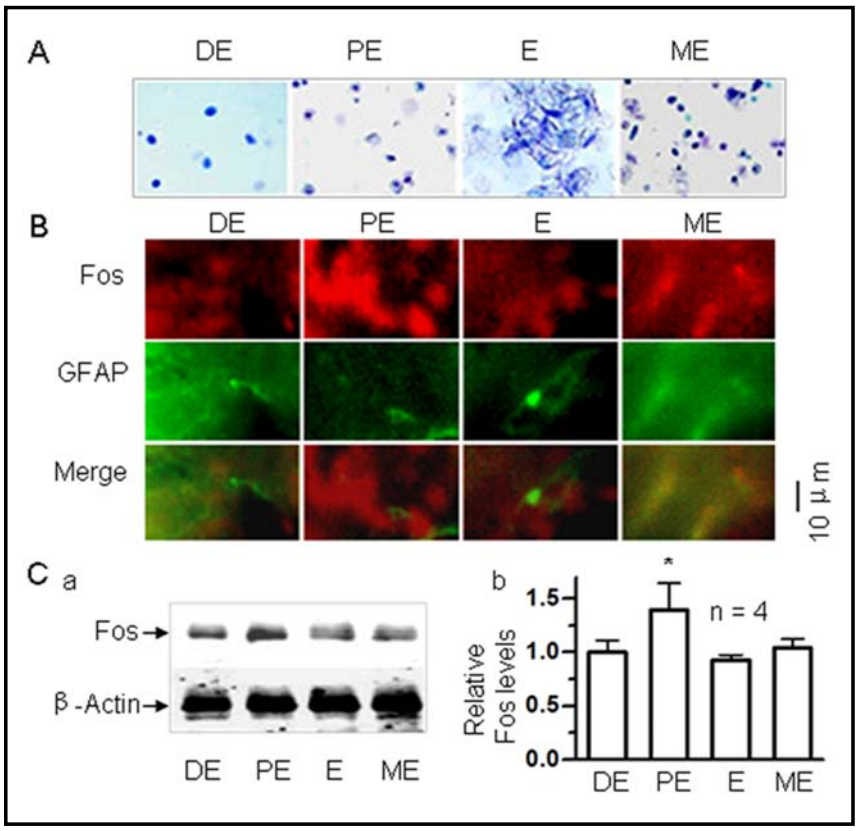

Fig. 1. Expressions of Fos protein in the supraoptic nucleus (SON) from rats in different stages of estrous cycle. A. Vaginal smears of female rats in diestrus (DE), proestrus (PE), estrus (E) and metestrus (ME). B. Representative immunohistochemical images of Fos protein in red, glial fibrillary acidic protein (GFAP) in green and their merges in fluorescent microscopy at different stages of the estrous cycle. C. Western blotting bands of Fos protein at the different stages (a) and their summary (b). Note that, actin was used as loading control; *, $p<0.05$ in ANOVA.

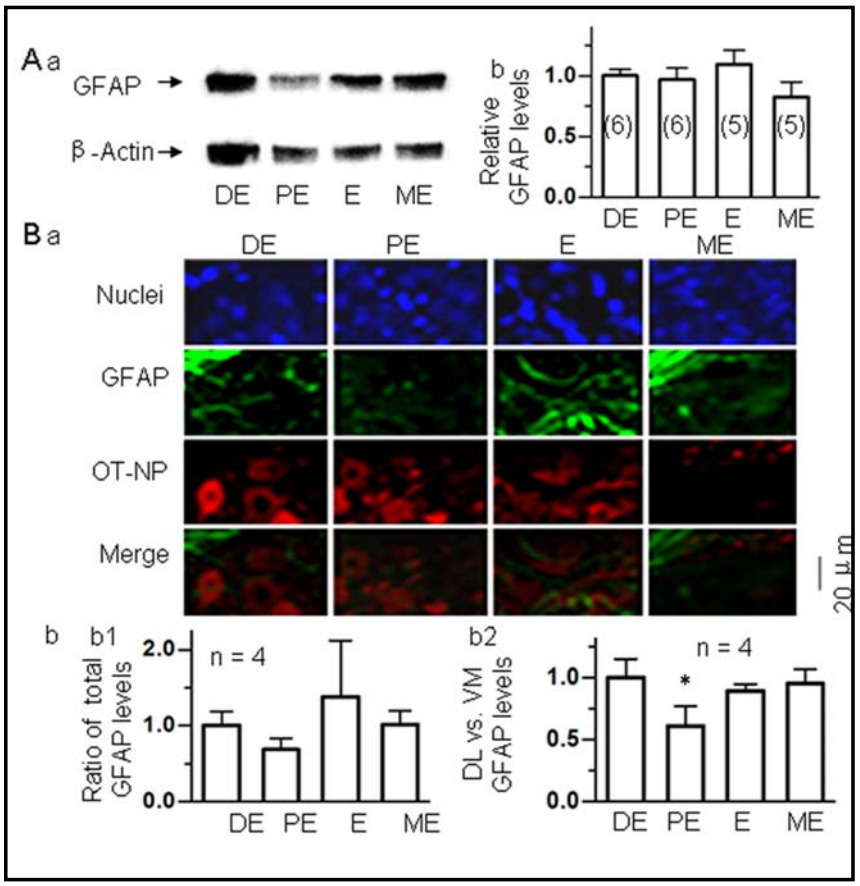

Fig. 2. GFAP expression at different stages of estrous cycle in the SON. A. Exemplary Western blotting bands of GFAP (a) and the summary in bar graph (b). The numbers in the parentheses represent the number of rats used in each stage. B. Images of GFAP with OXT neurophysin (OT-NP) immune-staining. Ba. Representative images showing nuclear staining with Hoechst, GFAP and OT-NP, respectively. Bb. Bar graph summarizing total GFAP intensity (Bb1) and the ratio of GFAP in dorsolateral section (DL) versus ventromedial section (VM) of the SON (Bb2). Other annotations refer to Figure 1.

DISCUSSION

Astrocytes can modulate the activity and neurosecretion of OXT neurons in the SON through their morphological and functional plasticity (Wang *et al.* 2009). GFAP plasticity is pivotal in the interaction between astrocytes and OXT neurons (Wang & Zhu 2014). Moreover, circulating OXT increases at proestrus (Sarkar & Gibbs 1984) and can trigger the LH surge (Evans *et al.* 1989; Evans & Catt 1989) that determines the ovulation and appearance of estrus (Plant 2015). Therefore, we hypothesized that OXT neuron-associated GFAP plasticity is temporally correlated with different stages of estrous cycle. Our result reveals that increased SON cellular activity is correlated with proestrus stage and GFAP reduction at OXT neuron-dominant region in the SON could partially account for the proestrus OXT peak despite the insignificant reduction of GFAP proteins in whole SON.

The reason why we failed to obtain expected GFAP reduction from Western blot is likely due to the following reasons. First, in the SON, astrocytes are not only associated with OXT neurons, but also with their partner cells, vasopressin neurons (Ni *et al.* 2014). Thus, if selective changes in GFAP-associated astrocytic plasticity occur around OXT neurons, this change could be averaged by "no change" around vasopressin neurons in the Western blotting. Second, GFAP plastic changes involve polymerization and depolymerization, assembling and dissembling and spatial distribution of GFAP (Yang & Wang 2015). Either depolymerization, dissembling or somatic distribution can appear as glial retraction that is known to increase neuronal interactions and excitation (Hatton 1997). Western blot is used to detect GFAP monomer and large fragments but not its filament that can be detected by IHC. Thus, the failure in Western blotting to show GFAP reduction during activation of SON cells in proestrus could simply reflect a depolymerization without dramatic GFAP dissembling or decomposition. Thus, we could conclude that there is a correlated reduction in astrocytic processes/GFAP around OXT neurons at proestrus.

OXT is a neuropeptide and best known for its roles in female reproduction among a variety of physiological functions (Yang *et al.* 2013). OXT can control the estrous cycle length by influencing follicle luteinization and ovarian steroidogenesis in the ovary through increasing the release of GnRH (Selvage *et al.* 2001). In female baboons OXT level is higher during their periovulatory period (Moscovice *et al.* 2012). Similarly, in ovulating women, plasma OXT is significantly lower during the luteal phase compared with both of the follicular and ovulatory phases (Saloniva *et al.* 2005). The high level of OXT before luteal phase is correlated with LH surge and, conversely, OXT receptor antagonists restrain the full production of the LH surge in non-pregnant women (Evans *et al.* 2003). Therefore, OXT can modulate the activity of hypothalamo-pituitary-gonad axis and the ensuing ovulation. Thus, the involvement of GFAP in OXT regulation of the hypothalamic pituitary-ovary axis have broad biological implication in vertebrate reproduction.

In conclusion, GFAP-associated glial retraction in the SON during proestrus is temporally associated with the previously observed excitatory effect on OXT neurons and OXT release (Wang & Hatton 2009; Hatton 1997), which in turn stimulates GnRH (Selvage *et al.* 2001) and LH (Evans *et al.* 1989; Evans & Catt 1989) release, thereby influencing follicle luteinization and ovarian steroidogenesis in the ovary (Pitzel *et al.* 1993).

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REFERENCES

1 Amico JA, Seif SM, Robinson AG (1981). Elevation of oxytocin and the oxytocin-associated neurophysin in the plasma of normal women during midcycle. J Clin Endocrinol Metab. **53**: 1229–1232.
2 Eckland DJ, Todd K, Lightman SL (1988). Immunoreactive vasopressin and oxytocin in hypothalamo-hypophysial portal blood of the brattleboro and long-evans rat: Effect of adrenalectomy and dexamethasone. J Endocrinol. **117**: 27–34.
3 Evans JJ, Anderson GM (2012). Balancing ovulation and anovulation: Integration of the reproductive and energy balance axes by neuropeptides. Hum Reprod Update. **18**: 313–332.
4 Evans JJ, Catt KJ (1989). Gonadotrophin-releasing activity of neurohypophysial hormones: Ii. The pituitary oxytocin receptor mediating gonadotrophin release differs from that of corticotrophs. J Endocrinol. **122**: 107–116.
5 Evans JJ, Hurd SJ, Mason DR (1995). Oxytocin modulates the luteinizing hormone response of the rat anterior pituitary to gonadotrophin-releasing hormone *in vitro*. J Endocrinol. **145**: 113–119.
6 Evans JJ, Reid RA, Wakeman SA, Croft LB, Benny PS (2003). Evidence that oxytocin is a physiological component of lh regulation in non-pregnant women. Hum Reprod. **18**: 1428–1431.
7 Evans JJ, Robinson G, Catt KJ (1989). Gonadotrophin-releasing activity of neurohypophysial hormones: I. Potential for modulation of pituitary hormone secretion in rats. J Endocrinol. **122**: 99–106.
8 Hatton GI (1997). Function-related plasticity in hypothalamus. Annu Rev Neurosci. **20**: 375–397.
9 Kumaresan P, Kumaresan M, Hossini M, Arellano C, Vasicka A (1983). Human ovulation and plasma oxytocin. Int J Gynaecol Obstet. **21**: 413–418.
10 Maggi R, Cariboni AM, Montagnani Marelli M, Moretti RM, Andre V, Marzagalli M, Limonta P (2015). GnRH and GnRH receptors in the pathophysiology of the human female reproductive system. Hum Reprod Update. 2015 Dec 29. pii: dmv059. [Epub ahead of print].

11 Mason WT, Ho YW, Hatton GI (1984). Axon collaterals of supraoptic neurones: Anatomical and electrophysiological evidence for their existence in the lateral hypothalamus. Neurosci. **11**: 169–182.
12 Mitchell MD, Haynes PJ, Anderson AB, Turnbull AC (1981). Plasma oxytocin concentrations during the menstrual cycle. European J Obstet Gynecol Reprod Biol. **12**: 195–200.
13 Moscovice LR, Ziegler TE (2012). Peripheral oxytocin in female baboons relates to estrous state and maintenance of sexual consortships. Horm Behav. **62**: 592–597.
14 Neumann ID (2007). Stimuli and consequences of dendritic release of oxytocin within the brain. Biochem Soc Trans. **35**: 1252–1257.
15 Ni RJ, Shu YM, Wang J, Yin JC, Xu L, Zhou JN (2014). Distribution of vasopressin, oxytocin and vasoactive intestinal polypeptide in the hypothalamus and extrahypothalamic regions of tree shrews. Neurosci. **265**: 124–136.
16 Pitzel L, Jarry H, Wuttke W (1993). Effects and interactions of prostaglandin f2 alpha, oxytocin, and cytokines on steroidogenesis of porcine luteal cells. Endocrinology. **132**: 751–756.
17 Plant TM (2015). 60 years of neuroendocrinology: The hypothalamo-pituitary-gonadal axis. J Endocrinol. **226**: T41–54.
18 Salonia A, Nappi RE, Pontillo M, Daverio R, Smeraldi A, Briganti A, Fabbri F, Zanni G, Rigatti P, Montorsi F (2005). Menstrual cycle-related changes in plasma oxytocin are relevant to normal sexual function in healthy women. Horm Behav. **47**: 164–169.
19 Sarkar DK, Gibbs DM (1984). Cyclic variation of oxytocin in the blood of pituitary portal vessels of rats. Neuroendocrinology. **39**: 481–483.
20 Selvage D, Johnston CA (2001). Central stimulatory influence of oxytocin on preovulatory gonadotropin-releasing hormone requires more than the median eminence. Neuroendocrinology. **74**: 129–134.
21 Wang YF, Hamilton K (2009). Chronic vs. Acute interactions between supraoptic oxytocin neurons and astrocytes during lactation: Role of glial fibrillary acidic protein plasticity. Scientific World J. **9**: 1308–1320.
22 Wang YF, Hatton GI (2009). Astrocytic plasticity and patterned oxytocin neuronal activity: Dynamic interactions. J Neurosci. **29**: 1743–1754.
23 Wang YF, Hatton GI (2007). Interaction of extracellular signal-regulated protein kinase 1/2 with actin cytoskeleton in supraoptic oxytocin neurons and astrocytes: Role in burst firing. J Neurosci. **27**: 13822–13834.
24 Wang YF, Sun MY, Hou Q, Hamilton KA (2013a). Gabaergic inhibition through synergistic astrocytic neuronal interaction transiently decreases vasopressin neuronal activity during hypoosmotic challenge. Eur J Neurosci. **37**: 1260–1269.
25 Wang YF, Sun MY, Hou Q, Parpura V (2013b). Hyposmolality differentially and spatiotemporally modulates levels of glutamine synthetase and serine racemase in rat supraoptic nucleus. Glia. **61**: 529–538.
26 Wang YF, Zhu H (2014). Mechanisms underlying astrocyte regulation of hypothalamic neuroendocrine neuron activity. Sheng Li Ke Xue Jin Zhan. **45**: 177–184.
27 Yang HP, Wang L, Han L, Wang SC (2013). Nonsocial functions of hypothalamic oxytocin. ISRN Neurosci. **2013**: 179272.
28 Yang Z, Wang KK (2015). Glial fibrillary acidic protein: From intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci. **38**: 364–374.