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Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids

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Abstract

Oxytocin has been linked to social behavior, including social recognition, pair bonding and parenting, but its potential role in promoting neuronal growth has not been investigated. We show here that oxytocin, but not vasopressin, stimulates both cell proliferation and adult neurogenesis in the hippocampus of rats. Oxytocin is also capable of stimulating adult neurogenesis in rats subjected to glucocorticoid administration or cold water swim stress. These findings suggest that oxytocin stimulates neuronal growth and may protect against the suppressive effects of stress hormones on hippocampal plasticity.

Keywords

cell proliferation; hippocampus; HPA axis; plasticity; vasopressin

INTRODUCTION

Oxytocin is a neuropeptide, released both peripherally and centrally, that has been linked to social behaviors such as mating and parenting (Carter et al., 2008; Neumann, 2009; Shelley et al., 2006; Young and Wang, 2004). Oxytocin has also been shown to buffer against some of the negative effects of stress (Cohen et al., 2010; Windle et al., 2004; 2006) – its actions include a reduction in hypothalamic-pituitary adrenal (HPA) axis activity and diminished stress-induced activation of brain regions associated with the HPA axis, including the hippocampus.

Oxytocin enhances hippocampal synaptic plasticity and cognitive functions of mother rats (Tomizawa et al., 2003), but its overall influence on this brain region remains unexplored. The hippocampus is a site of structural plasticity, undergoing neurogenesis throughout life – evidence has linked cognitive, anxiety and stress regulation functions of the hippocampus to adult neurogenesis (Leuner and Gould, 2010). Oxytocin neurons in the hypothalamic paraventricular nucleus project to various brain regions, including the hippocampus (Bujis and Swaab, 1979; Sofroniew, 1983). Oxytocin receptors are present in the hippocampus where they are more concentrated in the ventral than dorsal region and subject to modulation by stress and glucocorticoids (Liberzon and Young, 1997).

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Oxytocin has been shown to affect cell proliferation in peripheral systems, exhibiting stimulatory effects on blood cells, osteoblasts and some types of tumor cells (Macciò et al., 2010; Petersson, 2008) as well as inhibitory effects on prostate and mammary cells (Petersson, 2008; Sapino et al., 1998; Whittington et al., 2007), but its ability to influence neuronal growth in the brain remains unexplored. Since stress and glucocorticoids can be potent regulators of cell proliferation and adult neurogenesis in the hippocampus (Leuner et al., 2010; Mirescu and Gould, 2006) and oxytocin is known to attenuate stress responses (Windle et al., 2004; 2006), we investigated whether oxytocin alters adult neurogenesis and whether this effect occurs in the presence of elevated glucocorticoids. Here we show that peripheral and central oxytocin, but not the closely related neuropeptide vasopressin, enhances cell proliferation and adult neurogenesis. The stimulatory effect of oxytocin on cell proliferation occurred even in rats treated with glucocorticoids or exposed to a stressor. These findings raise the possibility that oxytocin acts to protect the hippocampus from the damaging effects of elevated glucocorticoids by promoting neuronal growth.

MATERIALS AND METHODS

Animals

Adult (≥ 60 days of age) male Sprague-Dawley rats (Taconic; Germantown, NY) were provided unlimited access to food and water and maintained on a 12:12 light-dark cycle (lights on at 7:00 a.m.). Rats were group-housed (2–3/cage) and all rats within a cage were included in the same experimental group. Procedures were conducted in accordance with Princeton University IACUC and The National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Peripheral oxytocin

Rats ($n = 6–11$ /group) were injected i.p. with oxytocin (1 mg/kg or 10 mg/kg; Bachem Americas, Inc., King of Prussia, PA; doses based on Ring et al., 2006) or an equal volume of saline. Thirty min later, rats were injected i.p. with the DNA synthesis marker, bromodeoxyuridine (BrdU; 200 mg/kg; Sigma, St. Louis, MO), a dose that labels the maximal number of S phase cells in the dentate gyrus (Cameron and McKay, 2001). Rats were perfused 2 hr later, a post-BrdU survival time that is sufficient to label cells in S-phase but not to allow the labeled cells to divide, thus providing a measure of cell proliferation. Additional groups of rats ($n = 10–11$ /group) were injected i.p. with 1 mg/kg oxytocin, 30 min later injected i.p. with BrdU and perfused after one wk or three wks. At one wk, the number of BrdU-labeled cells is at its maximum and most express markers of immature neurons (Gould et al., 1999). By three wks post-BrdU labeling, the majority of new cells in the dentate gyrus express the mature neuronal marker NeuN. A final group of rats ($n = 6–9$ /group) were injected i.p. with 1 mg/kg oxytocin or an equal volume of saline once daily for seven consecutive days. On the last day, rats were injected with BrdU 30 min following the oxytocin or saline injection and perfused three wks later.

Peripheral vasopressin

Rats ($n = 4–5$ /group) were injected i.p. with arginine vasopressin (10 μ g/kg; Sigma) or an equal volume of saline. This dose was chosen based on previous studies of i.p. injections of

vasopressin in rats (Izquierdo et al., 1988). Thirty min later, all rats were injected with BrdU and perfused after 2 hrs.

Central oxytocin

Rats (n = 11) were anesthetized with Nembutal and restrained in a stereotaxic apparatus. Using a 26 gauge Hamilton syringe, rats received hippocampal injections of oxytocin (1ng/1 μ L; Sigma; dose based on Melis et al., 1997; Ring et al., 2006) in one hemisphere and an equal volume of saline (1 μ L) in the other hemisphere (stereotaxic coordinates: AP, -5.3 from Bregma; LM, \pm 4.0; DV, -3.5 from surface of brain; Paxinos and Watson, 1998). Prior studies have shown that injection volumes of \leq 2 μ l do not produce degeneration in the granule cell layer (Gould and Tanapat, 1997). The injections in each hemisphere were counter-balanced and the hemispheres were analyzed separately. Thirty min later, rats were injected with BrdU and perfused after a two hr survival time.

Oxytocin and corticosterone

Rats (n = 12) were given an i.p. injection of oxytocin (1mg/kg; Bachem) and thirty min later were subcutaneously injected with 40 mg/kg corticosterone (Sigma), a dose that has been previously shown to diminish cell proliferation in the dentate gyrus (Cameron and Gould, 1994). An additional group of rats (n = 14) were injected with saline followed by corticosterone. One hr later, both groups of rats were injected with BrdU and perfused after a two hr survival time.

Oxytocin and cold swim stress

Rats (n = 11) were given i.p. injections of oxytocin (1mg/kg; Bachem) and then thirty min later exposed to a cold water (2–6° C) swim stress for three min. This stressor has been shown to diminish cell proliferation in the dentate gyrus (Stranahan et al., 2006). An additional group of rats (n = 13) were exposed to the cold water swim stress following treatment with saline. This procedure was repeated once daily for seven consecutive days. On the last day, rats were injected with BrdU one hr following the oxytocin or saline injection and perfused after a two hr survival time.

Perfusion

All animals were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and postfixed for at least two days.

Immunohistochemistry

Forty μ m coronal sections throughout the entire rostrocaudal extent of the dentate gyrus were cut with a Vibratome into a bath of 0.1 M phosphate-buffered saline (PBS). For peroxidase staining, a 1:12 series of sections were mounted onto slides, dried, heated in 0.1 M citric acid, rinsed in PBS, digested in trypsin, rinsed, denatured in 2N HCl:PBS and rinsed. Slides were incubated overnight at 4° C in mouse monoclonal antibody against BrdU (1:200; BD Biosciences, San Jose, CA) then rinsed, incubated with biotinylated horse anti-mouse (1:200; Vector, Burlingame, CA), rinsed, incubated with avidin-biotin complex

(Vector), rinsed and reacted in 0.01% diaminobenzidine (Sigma) with 0.003% H₂O₂. Slides were counterstained with cresyl violet, dehydrated, cleared with Citrisolv (Fisher Scientific) and coverslipped with Permount (Fisher Scientific).

For assessment of cell phenotype, tissue from rats with a three wk post-BrdU survival time was processed for double-labeling immunofluorescence for BrdU and the neuronal marker NeuN or the astroglial marker GFAP. A 1:12 series of free-floating sections were denatured in 2 M HCl:tris buffered saline (TBS), rinsed in TBS, and incubated with rat anti-BrdU (1:200; Accurate, Westbury, NY) plus mouse anti-NeuN (1:500; Chemicon, Temecula, CA) or guinea pig anti-GFAP (1:1000; Advanced Immunochemical, Long Beach, CA) for 2 days at 4° C. Sections were then rinsed, incubated with biotinylated anti-rat (Chemicon), rinsed, and incubated with streptavidin-conjugated Alexa 568 (1:1000; Molecular Probes, Eugene, OR) for BrdU, and with goat anti-mouse or goat anti-guinea-pig Alexa 488 (1:500; Molecular Probes) for NeuN or GFAP. After rinsing, sections were mounted onto slides and coverslipped using glycerol:TBS (3:1).

Data Analysis

Slides were coded prior to quantitative analysis. For BrdU peroxidase analysis, the number of BrdU-labeled cells in the dentate gyrus (subgranular zone, granule cell layer and hilus) was counted on every 12th section of tissue at 100× on an Olympus BX-50 light microscope. For experiments with a two hr survival, BrdU-labeled cells throughout the entire rostrocaudal extent of the dentate gyrus (2.20 to 7.20 mm; Paxinos and Watson, 1998) were counted. For experiments with one wk or three wk survival times, BrdU-labeled cells in the dorsal and ventral dentate gyrus were counted separately (Banasr et al., 2006). For experiments involving peripheral injections of oxytocin or vasopressin, half brains were analyzed. Counts were multiplied by 24 to obtain bilateral estimates of the number of BrdU-labeled cells per brain. For the central oxytocin study, counts were multiplied by 12 because both sides of the brain (saline and oxytocin) were analyzed separately. Cavalieri's principle (Gundersen et al., 1988) was used for determination of dentate gyrus volume (hilus and granule cell layer) from cross-sectional area measurements obtained with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD).

For purposes of comparison, the density of BrdU-labeled cells was also determined in the subventricular zone (SVZ). BrdU-labeled cells in the SVZ present on every twelfth coronal section throughout the dentate gyrus were counted, the volume of the analyzed region determined, and the data expressed as densities (number of BrdU-labeled cells/mm³).

For BrdU immunofluorescence, the percentage of BrdU-labeled cells in the granule cell layer and subgranular zone expressing NeuN or GFAP was determined using a Zeiss Axiovert confocal laser scanning microscope (510 LSM; lasers, Argon 458/488 and HeNe 543; Zeiss, Oberkochen, Germany). Dorsal and ventral dentate gyrus were analyzed separately as described above. For each brain, marker, and subregion, 15–25 randomly selected BrdU-labeled cells were analyzed. Optical stacks of 1-μm thick sections were obtained through putative double-labeled cells. To verify double labeling throughout their extent, cells were examined in orthogonal planes.

Statistical analyses

Unpaired t-test or one-way ANOVA followed by Newman-Keuls post-hoc comparisons were used to analyze differences in the number of BrdU-labeled cells in the dentate gyrus (total, dorsal, ventral), BrdU-labeled cells in the subventricular zone, the volume of the dentate gyrus, and the volume of damage in the granule cell layer caused by the central injections of oxytocin and saline. When animals were given central injections, paired t-tests were used because the other side of the brain (saline-injected) served as a control for the oxytocin-injected side.

RESULTS

Acute peripheral oxytocin administration enhances cell proliferation in the ventral dentate gyrus

To assess the effects of acute oxytocin on cell proliferation, animals were given a single peripheral injection of oxytocin, injected with BrdU 30 min later and perfused after a 2 hr survival time. A single injection of oxytocin enhanced cell proliferation in the dentate gyrus ($F(2,23) = 4.49 - p < 0.05$). Analysis of dorsal and ventral dentate gyrus separately revealed that the increase was evident only in the ventral dentate gyrus ($F(2,23) = 10.02 - p < 0.005$; Figure 1a). Rats given 1 mg/kg oxytocin had more BrdU-labeled cells (than rats given saline or a higher dose of oxytocin (10 mg/kg). Groups given saline or 10 mg/kg oxytocin did not differ in dorsal, ventral or dorsal-ventral combined ($p > 0.05$) To determine whether these effects are specific to oxytocin, an additional group of animals was injected with the closely related neuropeptide vasopressin (AVP), injected with BrdU after 30 min and perfused 2 hr later. Unlike oxytocin, AVP had no significant effect on the number of proliferating cells in the dorsal (Saline: 1824 ± 249.6 , AVP: $2298 \pm 181.0 - p > 0.05$) or ventral (Saline: 1982 ± 256.1 , AVP: $2178 \pm 418.4 - p > 0.05$) dentate gyrus. Furthermore, the effects of oxytocin on cell proliferation were regionally specific since there was no difference in the density of BrdU-labeled cells in the SVZ 2 hrs after BrdU administration ($p > 0.05$; Figure 2). Lastly, no differences in the dorsal, ventral or dorsal-ventral combined volume of the granule cell layer were detected among the groups (combined dorsal-ventral Saline: 10.24 ± 0.50 , Oxytocin 1 mg/kg: 11.40 ± 0.48 , Oxytocin 10 mg/kg: $10.0 \pm 0.41 - p > 0.05$; dorsal Saline: 5.30 ± 0.25 , Oxytocin 1mg/kg: 5.50 ± 0.30 , Oxytocin 10mg/kg: $5.52 \pm 0.30 - p > 0.05$; ventral Saline 4.93 ± 0.49 , Oxytocin 1mg/kg: 5.65 ± 0.35 , Oxytocin 10mg/kg: $4.47 \pm 0.31 - p > 0.05$).

Using the same single dose of oxytocin (1mg/kg) that enhanced cell proliferation, we found that the number of BrdU labeled cells was no longer increased at later time points after BrdU injection. One week and three weeks after BrdU administration, there was no difference in the number of BrdU-labeled cells in the dorsal, ventral or dorsal-ventral combined dentate gyrus in groups treated with saline or 1 mg/kg oxytocin (p values > 0.05).

Repeated peripheral oxytocin increases the number of new neurons

We next examined whether more sustained exposure to oxytocin would produce a lasting enhancement in the number of BrdU labeled cells by using a repeated oxytocin injection paradigm. Male rats were given seven days of oxytocin injections, a BrdU injection on the last day, and perfused three weeks later. Compared to saline-treated controls, rats treated

with oxytocin had more BrdU-labeled cells in the ventral dentate gyrus ($t(12) = 2.18 - p < 0.05$; Figure 3a). The number of BrdU-labeled cells in the dorsal dentate gyrus did not differ between saline and oxytocin treated groups ($p > 0.05$). Most (~88%) BrdU-labeled cells expressed the marker of mature neurons, NeuN (Figure 3b). A small percentage (~5%) expressed the astroglial marker, GFAP (Figure 3c). There was no difference in the proportion of BrdU-labeled cells expressing these markers across groups or subregions (p values > 0.05). No differences in the total volume of the granule cell layer were detected among the groups (Saline: 11.78 ± 0.24 , Oxytocin: $11.87 \pm 0.49 - p > 0.05$). No differences were detected in the volume of the granule cell layer at dorsal (Saline: 6.25 ± 0.41 , Oxytocin: $5.68 \pm 0.22 - p > 0.05$) or ventral (Saline: 5.53 ± 0.28 , Oxytocin: $6.19 \pm 0.30 - p > 0.05$) levels. Taken together, these data suggest that repeated oxytocin injection enhanced the number of adult-generated cells for at least 3 weeks, including both neurons and glia.

Hippocampal oxytocin enhances cell proliferation

We next evaluated whether oxytocin acts directly within the hippocampus to alter cell proliferation. Rats received hippocampal microinfusions of oxytocin in one hemisphere and saline in the other, allowing the other side of the brain of each animal to serve as its own control. Thirty min after the infusion, rats were injected with BrdU and perfused after a two hr survival time. We found that the hemisphere treated with oxytocin had a significantly greater number of BrdU-labeled cells in the ventral dentate gyrus than the saline-treated hemisphere ($t(10) = 2.69, p < 0.05$ Figure 4). The number of BrdU-labeled cells in the dorsal dentate gyrus was unaffected by OT ($p > 0.05$). These regional differences were not due to variations in the area of damage in the dentate gyrus at the injection site which was similar for the saline and oxytocin treated hemispheres ($p > 0.05$).

Oxytocin enhances cell proliferation in animals treated with corticosterone or exposed to swim stress

We next examined whether oxytocin could enhance cell proliferation under conditions associated with increased levels of glucocorticoids. Rats were treated with oxytocin 30 min before receiving a single corticosterone injection. After one hr, rats were injected with BrdU and perfused after a 2 hr survival. Corticosterone treated rats that were also given oxytocin had more BrdU-labeled cells in total dentate gyrus than animals that did not receive oxytocin ($t(24) = 2.44, p < 0.05$). This difference was concentrated in the ventral hippocampus which showed a significant increase in oxytocin treated animals ($t(24) = 3.52, p < 0.005$; Table 1). In the dorsal dentate gyrus, no difference in the number of BrdU-labeled cells was detected between CORT and OT \pm CORT groups ($p > 0.05$, Table 1). To evaluate the effects of oxytocin on cell proliferation in animal undergoing repeated stress, rats were treated daily with oxytocin and 30 min later exposed to cold water swim stress. On the seventh day, rats were injected with BrdU one hr after the stressor and perfused after a 2 hr survival. Rats that were treated with oxytocin and exposed to cold water swim stress for seven days showed an increase in BrdU labeled cells in the total dentate gyrus ($t(22) = 2.13 = 2.13 - p < 0.05$). However, there was no statistically significant difference in BrdU cell number in either the dorsal ($t(22) = 1.90 - p = 0.07$) or ventral ($t(22) = 1.87 - p = 0.08$) dentate gyrus (Table 1).

DISCUSSION

Here we show that acute administration of oxytocin, either centrally or peripherally, stimulates cell proliferation in the dentate gyrus. Although the proliferative effects of acute oxytocin treatment did not result in an increase in neurogenesis at one week or three weeks, more prolonged treatment of oxytocin (i.e. one week of daily peripheral oxytocin injections) increased neurogenesis, an effect that appears to be specific to the ventral portion of the dentate gyrus. In addition, we show that the stimulatory effect of oxytocin on cell proliferation occurred even in rats treated with glucocorticoids or exposed to stress. Taken together, these results suggest that oxytocin stimulates neuronal growth and may protect against the suppressive effects of stress hormones on hippocampal plasticity.

Although we tested two doses of peripheral oxytocin on cell proliferation, only the lower dose was found to stimulate cell proliferation. In this regard it should be noted that effects of neuropeptides such as oxytocin are often dose-dependent but not always linear. For example, the effects of peripheral oxytocin on social recognition follow an inverted U-shaped dose response curve where moderate doses facilitate, and high doses attenuate social recognition (Popik et al., 1992). Because we didn't examine doses lower than 1 mg/kg or higher than 10 mg/kg, the exact dose response relationship between oxytocin and cell proliferation remains to be determined.

Only small amounts of oxytocin given peripherally pass through the blood brain barrier (Ermisch et al., 1985; Engelmann et al., 1996). Nonetheless, a number of studies have shown that either subcutaneous or intraperitoneal administration of OT can trigger many of the same responses that occur following the release of, or administration of, OT within the CNS (Arletti et al., 1992; McCarthy et al., 1992; Petersson et al., 1996). We also found that like peripheral oxytocin, acute central administration of oxytocin into the hippocampus similarly increased cell proliferation. Taken together, these data suggest that oxytocin is acting within the hippocampus to increase the number of new cells. Oxytocin receptors are known to be expressed in the hippocampus (Liberzon and Young, 1997; Gimpl and Farrenholz, 2001; Tomozawa et al., 2003) further indicating that the hippocampus is a likely target for oxytocin. However, it remains unclear whether the effects of oxytocin on cell proliferation and neurogenesis are mediated via direct activation of oxytocin receptors on precursor cells within the dentate gyrus. Alternatively, it's possible that oxytocin modulates these processes indirectly by acting on oxytocin-responsive cells within brain regions that provide afferent input to the dentate gyrus such as the amygdala (Gimpl and Farrenholz, 2001) or through its influence on other hormonal or neurotransmitter systems that have been shown to alter cell proliferation.

Oxytocin is known to diminish HPA axis responsiveness and lower levels of circulating glucocorticoids (Cohen et al., 2010; Windle et al., 2004; 2006). Another possible interpretation of our findings demonstrating that oxytocin stimulates cell proliferation and neurogenesis is that it does so by lowering levels of corticosterone. Numerous studies have shown that elevated corticosterone, applied either exogenously or through exposure to aversive stressors, suppresses cell proliferation and neurogenesis (Cameron and Gould, 1994; Mirescu and Gould, 2006; Tanapat et al., 2001). Conversely, lowering glucocorticoid

levels by adrenalectomy enhances cell proliferation and neurogenesis (Gould et al., 1992). Our results show that oxytocin enhances cell proliferation even after elevating corticosterone levels acutely by injection. These findings suggest that the stimulatory effects of oxytocin on cell proliferation and neurogenesis in the dentate gyrus are unlikely to be mediated only indirectly through oxytocin-induced reductions in glucocorticoid levels. These findings further suggest the possibility that oxytocin may be capable of overriding some of the negative effects of elevated glucocorticoids, such as those on neuronal growth inhibition. It should be noted, however, that our stress and oxytocin findings were less conclusive than the corticosterone injection study. That is, we observed a statistically significant increase in the number of BrdU labeled cells in the stress plus oxytocin condition only in the overall dentate gyrus but not within the dorsal or ventral subregions. The lack of a region-specific increase in cell proliferation in oxytocin treated stressed rats suggests that different mechanisms may be operating under this condition compared with following corticosterone injection. In this regard, we cannot rule out some potential involvement of the HPA axis in mediating these effects without additional experimentation.

Nonetheless, it remains possible that oxytocin is responsible for promoting neuronal growth under some circumstances where glucocorticoids are naturally elevated. For example, sexual experience is rewarding yet elevates corticosterone levels and paradoxically promotes cell proliferation and adult neurogenesis (Leuner et al., 2010). Sexual experience also stimulates oxytocin release in the periphery, as well as in the brain (Carmichael et al., 1987; Stoneham et al., 1985; Waldherr and Neumann, 2007). Taken together with the present paper, this work suggests that oxytocin may buffer the brain from elevated corticosterone levels during mating by preventing growth inhibition and stimulating neurogenesis. Similarly, oxytocin is released during physical activity (Michelini, 2001), another rewarding experience which also paradoxically causes an increase in glucocorticoids and an increase in adult neurogenesis (Stranahan et al., 2006; van Praag et al., 1999). The release of oxytocin during these experiences could provide the mechanism by which the hippocampus is protected from the rise in glucocorticoids.

Although acute peripheral oxytocin injection rapidly increases cell proliferation in the dentate gyrus, the additional granule cells are lost during the ensuing period of cell death. After daily injection with oxytocin, however, a lasting effect on adult neurogenesis is observed. This increase is specific to the ventral portion of the dentate gyrus. The ventral hippocampus is connected with the amygdala, the medial prefrontal cortex and HPA axis structures (Fanselow and Dong, 2010; Sahay and Hen, 2007) and is also involved in some types of learning and memory (Dalla et al., 2009; Snyder et al., 2009a), though it is more generally thought to play a role in anxiety and stress regulation (Bannerman et al., 2004; Herman et al., 1995). Studies have shown that oxytocin receptors are more abundant in the ventral portion of the hippocampus (Liberzon and Young, 1997). Thus, the effects of oxytocin on neurogenesis in the ventral hippocampus are consistent with the release of oxytocin in response to certain stressors and the role of the ventral hippocampus in stress regulation.

Although the specific role of adult-born neurons is not yet fully understood, there is a wide body of evidence that adult-born cells start to become integrated into the neuronal circuitry

shortly after their production (Hastings and Gould, 1999; Ide et al., 2008; Snyder et al., 2009b) and that these cells are important for hippocampal function, especially anxiety regulation and learning. A reduction in adult-generated neurons in the dentate gyrus produces deficits in hippocampus-dependent learning (Leuner et al., 2006; Shors et al., 2001; Snyder et al., 2009b), and learning other hippocampus-dependent tasks is impaired under circumstances in which adult neurogenesis is diminished, such as after glucocorticoid administration (Bodnoff et al., 1995; Luine et al., 1993) or negative stress paradigms (Luine et al., 1996; Krugers et al., 1997). When the number of new neurons in the hippocampus is enhanced by experiences such as living in an enriched environment (Kempermann et al., 1997; Nilsson et al., 1999) or running (van Praag et al., 1999), performance on these learning tasks is improved. It has also been shown that hippocampal adult neurogenesis is involved in anxiety regulation and the suppression of neurogenesis leads to a potentiated HPA axis response after exposure to a mild stressor (Revest et al., 2009; Schloesser et al., 2009). Therefore oxytocin-induced increases in new neurons in the dentate gyrus could contribute to the reductions in anxiety and enhancement in learning observed with rewarding experiences. These results taken together show that oxytocin has the potential for protecting the hippocampus from the adverse effects of elevated glucocorticoids and that oxytocin released during certain experiences may have beneficial effects in the hippocampus.

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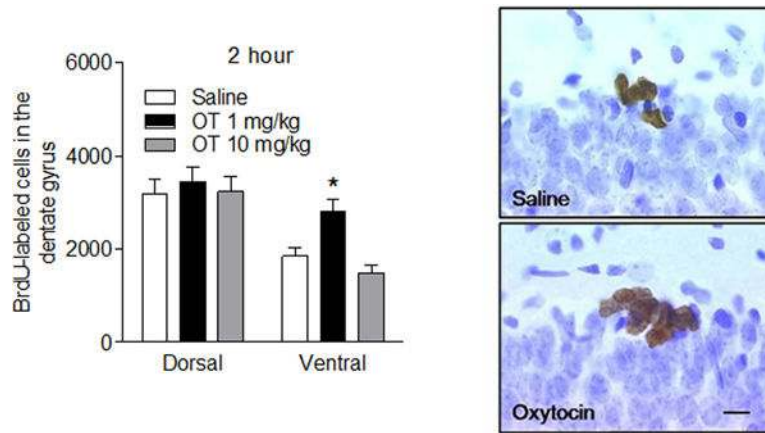


Figure 1. Peripheral oxytocin administration enhances cell proliferation in the ventral dentate gyrus

Adult male rats received an i.p. injection of oxytocin (OT) or an equal volume of saline. All rats were injected with BrdU 30 min later and perfused after 2 hr. Rats treated with 1mg/kg OT had a greater number of BrdU-labeled cells in the ventral, but not dorsal, dentate gyrus compared to those treated with saline or 10 mg/kg OT. Bars represent mean \pm SEM, * $p < 0.05$. Photomicrographs show more BrdU-labeled cells (brown cells) in the ventral dentate gyrus of rats treated with OT. Scale bar, 10 μ m.

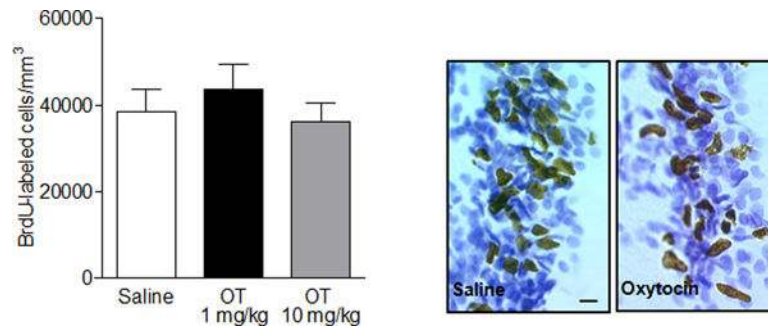


Figure 2. Peripheral OT administration does not alter the density of BrdU-labeled cells in the SVZ

The density of BrdU-labeled cells in the SVZ of rats treated with 1 mg/kg OT or 10 mg/kg OT did not differ from saline-treated rats. Bars represent mean \pm SEM. Photomicrographs show BrdU-labeled cells in the SVZ of saline and oxytocin treated rats. Scale bar, 10 μ m.

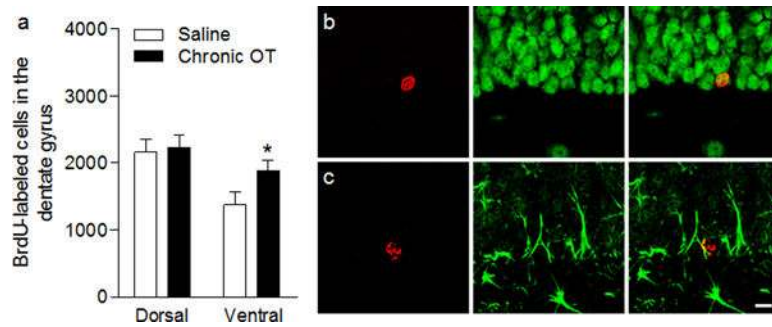


Figure 3. Chronic peripheral oxytocin enhances adult neurogenesis

Rats received an i.p. injection of OT (1mg/kg) or an equal volume of saline once daily for 7 consecutive days. All animals were injected with BrdU 30 min after the last injection and perfused after a 3 wk survival. (a) Chronic OT increased the number of BrdU-labeled cells in the ventral, but not dorsal, dentate gyrus. Bars represent mean \pm SEM, $*p < 0.05$. At this time, most BrdU-labeled cells (red) in the saline and OT groups were co-labeled with (b) the mature neuronal marker NeuN (green) but not with (c) the astroglial marker GFAP (green). Scale bar, 10 μ m.

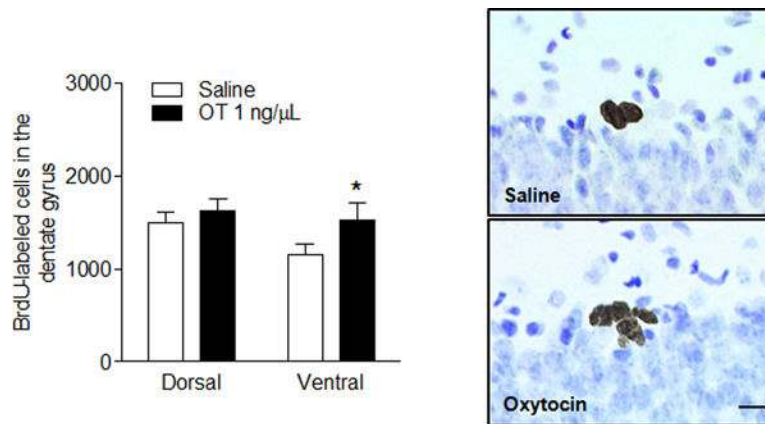


Figure 4. Central administration of oxytocin enhances cell proliferation

Adult male rats received hippocampal injections of OT (1ng/μL) in one hemisphere or an equal volume of saline into the other hemisphere. All animals were injected with BrdU 30 min later and perfused after a 2 hr survival. The hemisphere treated with OT had a greater number of BrdU-labeled cells in the ventral dentate gyrus compared to the saline-treated hemisphere. The number of BrdU-labeled cells in the dorsal dentate gyrus did not differ between the saline and OT treated hemispheres. Bars represent mean ± SEM, * $p < 0.05$. Photomicrographs show more BrdU-labeled cells (brown cells) in the ventral dentate gyrus of rats treated with OT. Scale bar, 10 μm.

TABLE 1

BrdU-Labeled Cells (mean \pm SEM) in the Dorsal and Ventral Dentate Gyrus of Rats Treated With Oxytocin (OT) Under Conditions of Elevated Glucocorticoids as a Result of a Single Corticosterone (CORT) Injection or Seven Days of Cold Water Swim Stress (Stress)

	CORT	OT + CORT	Stress	OT + Stress
Dorsal	4,255 \pm 228	4,424 \pm 340	2,245 \pm 160	2,891 \pm 319
Ventral	2,755 \pm 206	4,206 \pm 376*	2,339 \pm 219	3,135 \pm 384

* $P < 0.05$.

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