International Journal of Neuropsychopharmacology (2017) 20(10): 861–866

doi:10.1093/ijnp/pyx061
Advance Access Publication: July 25, 2017
Brief Report

BRIEF REPORT

Intranasal Oxytocin following Uncontrollable Stress Blocks Impairments in Hippocampal Plasticity and Recognition Memory in Stressed Rats

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Abstract

Background: Nasal pretreatment with the neuropeptide oxytocin has been reported to prevent stress-induced impairments in hippocampal synaptic plasticity and spatial memory in rats. However, no study has asked if oxytocin application following a stress experience is effective in rescuing stress-induced impairments.
Methods: Synaptic plasticity was measured in hippocampal Schaffer collateral-CA1 synapses of rats subjected to uncontrollable stress; their cognitive function was examined using an object recognition task.
Results: Impaired induction of long-lasting, long-term potentiation by uncontrollable stress was rescued, as demonstrated both in rats and hippocampal slices. Intranasal oxytocin after experiencing uncontrollable stress blocked cognitive impairments in stressed rats and in stressed hippocampal slices treated with a perfused bath solution containing oxytocin.
Conclusions: These results indicated that posttreatment with oxytocin after experiencing a stressful event can keep synaptic plasticity and cognition function intact, indicating the therapeutic potential of oxytocin for stress-related disorders, including posttraumatic stress disorder.

Keywords: oxytocin, synaptic plasticity, hippocampus, posttraumatic stress disorder

Introduction

Stress from the external environment causes a variety of physiological challenges to homeostasis maintenance through actions of the sympathetic nervous system via norepinephrine and the hypothalamus-pituitary-adrenal gland axis via glucocorticoids (Shors, 2006). However, if a stressful event persists beyond the buffering capacity of homeostasis, a variety of neurological abnormalities (e.g., learning and memory loss and neuronal dysfunction) can develop (Kim and Diamond, 2002). For example, these symptoms are observed in patients with posttraumatic stress disorder (PTSD), a psychiatric disorder caused by uncontrollable and unpredictable stress and an affective disorder associated with anxiety and fear (Almli et al., 2014; de Quervain et al., 2017). Uncontrollable stress has been reported to alter a series of synaptic and cognitive functions (Bowers and Ressler, 2015).

Received: March 7, 2017; **Revised:** July 4, 2017; **Accepted:** July 24, 2017

Significance Statement

Posttraumatic stress disorder (PTSD) is a serious psychiatric disorder affecting many people worldwide. Currently, however, effective solutions to deal with PTSD are limited. Recently we reported that pretreatment with oxytocin prevents stress-induced synaptic and cognitive dysfunctions in animal PTSD models. Thus, it is interesting to address whether oxytocin treatment has rescue effects after stress has been experienced. In this report, we studied the efficacy of oxytocin to rescue the synaptic and cognitive dysfunctions caused by PTSD-related stress. Our results showed that nasally applied oxytocin rescued stress-induced impairments in long-lasting synaptic plasticity induction and recognition memory. The rescue effect of oxytocin on synaptic dysfunction was also confirmed in hippocampal slices from stressed animals. We believe that our findings answer the call for greater diversification of available treatments for PTSD.

The neuropeptide oxytocin has been shown to have multiple functions (Gimpl and Fahrenholz, 2011), and it regulates a variety of cognitive functions (McCall and Singer, 2012; Clark-Elford et al., 2014). Oxytocin modulates anxiety (Ring et al., 2006), pain sensation (Zunhammer et al., 2016), depression due to neonatal maternal separation (Amini-Khoei et al., 2017), social interactions (Singh et al., 2016), social perceptions (Gordon et al., 2016), cocaine seeking (Zhou et al., 2014), and food craving (Striepens et al., 2016). Specifically, it is well known that oxytocin alleviates many PTSD symptoms (Tomizawa et al., 2003; Koch et al., 2014). Recently, we investigated the mechanisms underlying oxytocin pretreatment to prevent the formation of stress-induced abnormal long-term potentiation, long-term depression, spatial learning, and memory (Lee et al., 2015). Oxytocin pretreatment prevented the onset of PTSD, so it can be effectively employed when a dangerous situation (e.g., combat) is predictable.

However, because PTSD is often caused by unexpected accidents (e.g., traffic accidents, sexual assault), treatment to facilitate the recovery process from stress or to keep individuals in a normal state is more beneficial. Therefore, the present study was conducted to determine whether oxytocin has alleviating effects on stress-induced synaptic and cognitive impairments after a stress has been experienced.

Here we examined the effects of oxytocin posttreatment on stress-induced impairments in hippocampal long-lasting, long-term potentiation (L-LTP) induction and recognition memory. Specifically, the duration of LTP impairment after undergoing uncontrollable stress was determined. Then, LTP was measured in hippocampus slices from stressed rats treated with nasally applied oxytocin and in stressed hippocampal slices treated with bath-perfused oxytocin. In addition, the status of recognition memory was examined in rats that had received nasally applied oxytocin following uncontrollable stress.

Methods

Animals and Stress Paradigm

Male Sprague-Dawley rats weighing 150 to 250 g were used for electrophysiological recordings. Experimental protocols were approved by the Seoul National University (SNU-160718-4-1) and the Konkuk University (KU17094) Institutional Animal Care and Use Committees. Animals were housed in a vivarium with a 12-hour-light/-dark cycle at 50% to 60% humidity. Behavioral stress was evoked while animals were restrained in a Plexiglas tube using 60 tail shocks (1-mA stimulations of 1 second duration with a 30- to 90-second random inter-stimulus interval). The restrained, tail-shock stress procedure was adapted from the learned helplessness paradigm using uncontrollable and uncontrollable aversive stimuli (Seligman and Maier, 1967).

Intranasal Delivery of Oxytocin

Intranasal injection of oxytocin was performed as described previously (van den Berg et al., 2002). Briefly, a 24-gauge i.v. catheter (Angiocath PlusTM, BD Biosciences) was inserted into the rat nasal cavity under anesthesia with isoflurane. A 200-μL volume of oxytocin (1 mg/mL, dissolved in sterile isotonic saline) was injected into each rat's nasal cavity (Lee et al., 2015). To obtain maximal drug absorption in the rat nasal cavity, each rat's head was placed at a supine -70° angle.

Hippocampal Slice Preparation and Electrophysiology

Rat hippocampal slice preparation and electrophysiological recording were performed as described previously (Lee et al., 2015). After rapid extraction of rat hippocampi, 400-μm transverse hippocampal slices were prepared using a vibratome. After a minimum recovery period of 60 to 90 minutes, the slices were continually perfused with oxygenated artificial cerebrospinal fluid solution (28°C–30°C) (117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 11 mM glucose) in a submersion-type recording chamber. Extracellular recordings were performed with an A-M Systems model 1800 amplifier (A-M Systems). Field excitatory postsynaptic potentials (fEPSPs) were recorded in CA1 stratum radiatum using a glass pipette filled with artificial cerebrospinal fluid solution (2–3 MΩ resistance) and stimulation of Schaffer collateral-commissural afferents at 0.066 Hz with a stimulation intensity that yielded a 40% to 60% maximal response with a concentric bipolar stimulating electrode (FHC) (Choi et al., 2015). The responses were digitized and analyzed using the IGOR program (Wave Metrics Inc). Baseline responses were collected with a stimulation intensity that yielded a half-maximal response. L-LTP was induced by theta-burst stimulation (TBS): 10 stimulus trains (4 pulses at 100 Hz) delivered at 5 Hz. The initial (negative) slope of responses was used in the fEPSP analyses. The magnitude of L-LTP was measured between 0 and 180 min after TBS. For statistical comparisons, the L-LTP magnitude was taken as the average of the last 10 minutes recorded.

Object Recognition Task

The object recognition task utilizes the rat's natural tendency to explore novel stimuli. Before the task, handling and habituation to an open-field arena (45 × 4 × 40 cm) were conducted respectively for 5 minutes for 5 consecutive days. The task was conducted 1 hour after intranasal delivery of oxytocin or vehicle, as described previously (Baker and Kim, 2002). All rats were subjected to a single trial consisting of a familiarization phase followed by a test phase. At the beginning of the familiarization, each rat was placed in the empty arena for 1 minute (rehabituation). Afterward, rats

were placed in a holding cage, and 2 identical objects were placed in the 2 corners of the arena. Rats were then placed back in the arena and remained there until they had explored the objects for 30 seconds. Upon reaching the criterion, rats were placed back in their home cages for a delay of 3 hours prior to the test phase. In the test phase, 2 objects were placed in the same position as those in the familiarization phase: one object was identical to those in the familiarization phase and the other was a novel object. After the delay interval, rats were returned to the arena and remained there until they again explored the 2 different objects for 30 seconds. Exploration was scored by a computer-assisted scoring program (in QBASIC) only when the rats directly touched the objects with their snout. Exploration was not scored if the rats raised themselves by placing their forepaw on the objects or if another part of the rat's body touched the objects.

Statistical Analyses

All quantitative data are expressed as mean ± SEM. The responses after LTP induction were analyzed by 2-way ANOVA with a repeated-measure followed by Fisher's least significant difference and the averages of the last 10 minutes of LTP recording by 1-way ANOVA followed by least significant difference. Performances in the object recognition task were analyzed by paired *t* test and 1-way ANOVA. Software was Clampfit 10.2 (Molecular Devices) or JMP (SAS Institute Inc). Differences were considered significant at $P < .05$.

Results

Intranasal Oxytocin Rescued Impaired Hippocampal L-LTP Induction in the Uncontrollable Stress-Treated Rat

To observe the time course of the effects of uncontrollable stress on hippocampal synaptic plasticity, TBS-induced L-LTP was examined by preparing hippocampal slices at 1, 2, and 5 days after uncontrollable stress application. L-LTP induction decreased in rats at day 1 and day 2 after stress, but normal L-LTP induction was observed at 5 days after stress (group, $F_{(3,33)} = 11.86$, $P < .001$; time, $F_{(18,594)} = 324.79$, $P < .001$) (Figure 1A). Thus, we tested the effect of nasal oxytocin application after 1 day, when the stress effect persisted. When hippocampal L-LTP induction was measured 2 hours after applying nasal oxytocin and 22 hours after stress, normal L-LTP induction was observed in the oxytocin-treated animals at the same level as in unstressed controls (group, $F_{(2,32)} = 19.44$, $P < .001$; time, $F_{(18,576)} = 300.69$, $P < .001$) (Figure 1C).

Intranasal Oxytocin Rescued Impaired Recognition Memory in the Uncontrollable Stress-Treated Rat

To examine effects of intranasal oxytocin treatments on stress-induced impairments of recognition memory, we conducted an object recognition task 1 hour after oxytocin treatments following uncontrollable stress. During the familiarization phase, all rats exhibited a comparable amount of time exploring the 2 identical objects, indicating that there was no preference for object location (Figure 1E, left). At the 3-hour delay between the familiarization and test phases, the control rats with vehicle administration (CTL + vehicle) exhibited significantly greater preference for the novel object compared with the familiar object ($t_{(7)} = -8.94$, $P < .001$), whereas the stressed rats with vehicle (STR + Vehicle) failed to engage with the novel object ($t_{(7)} = -1.52$, $P = .17$). However, stressed rats with oxytocin (STR + Oxytocin) spent significantly more time exploring the novel object than the familiar object ($t_{(8)} = -9.39$, $P < .001$) (Figure 1E, right). One-way ANOVA on the amount of time exploring the novel object assessed as a percentage of the total 30-second exploration time revealed the significant group effects ($F_{(3,29)} = 8.68$, $P < .001$) (Figure 1F). Posthoc analysis revealed that the STR + oxytocin rats exhibited significantly better performances than the STR + vehicle rats.

Bath-Applied Oxytocin Rescued Impaired L-LTP Induction in the Hippocampi of Uncontrollable Stress-Treated Rats

To examine the mechanism for the oxytocin effect in stressed rats, we prepared hippocampal slices from stressed animals and applied oxytocin directly to a perfusion bath and then examined synaptic changes. Bath-applied oxytocin (1 μM) had no effect on the input–output relationship of Schaffer collateral-CA1 synapses in both stressed and unstressed animals (Figure 2A). In addition, the paired-pulse response to 2 consecutive stimuli did not show any difference between stressed animals and unstressed control animals (Figure 2B), implying that the rescue effect of oxytocin was not a presynaptic mechanism. However, bath-applied oxytocin successfully increased L-LTP induction in unstressed animals and also restored impaired L-LTP induction in stressed animals to control levels (group, $F_{(3,27)} = 11.69$, $P < .001$; time, $F_{(18,486)} = 158.09$, $P < .001$) (Figure 2C). Based on these results, we conclude that oxytocin rescued the altered synaptic plasticity seen in stressed animals.

Discussion

Our previous study reported that pretreatment with oxytocin prevented uncontrollable stress-induced impairment in synaptic plasticity and cognition, and that oxytocin treatment blocked stress-induced alterations of extracellular signal-regulated kinases (Lee et al., 2015). However, it has not been determined whether oxytocin is also effective in post-stress conditions. Many signaling mechanisms have distinct effects in stress induction and maintenance phases. For example, the signaling pathway turned on by oxytocin may inhibit the induction of stress, or the effect of oxytocin may compensate for the stress effect by acting in parallel with the stress effect. In the current study, we tested the efficacy of oxytocin as a posttreatment after stress induction and found that oxytocin treatments following uncontrollable stress rescued impairments to synaptic plasticity and recognition memory.

We observed that synaptic plasticity reduction induced by uncontrollable stress persisted for 1 to 2 days (Figure 1A–B). Interestingly, the stress-induced reduction that was observed after 1 day was restored to normal levels by nasally applied oxytocin (Figure 1C–D). We also examined the effect of oxytocin perfusion in the recording chamber after obtaining hippocampal slices from stressed rats, which may mimic the condition in which oxytocin is nasally applied after stress. Impaired L-LTP was restored by perfusion-mediated oxytocin treatment, as in the nasally applied oxytocin case (Figure 2C–D). And we also demonstrated that oxytocin treatments given after experiencing a stress event improved impaired recognition memory. These results indicate that oxytocin treatment following stress could act as a compensating mechanism for the stress-output effect rather than influencing the induction of stress susceptibility or stress output.

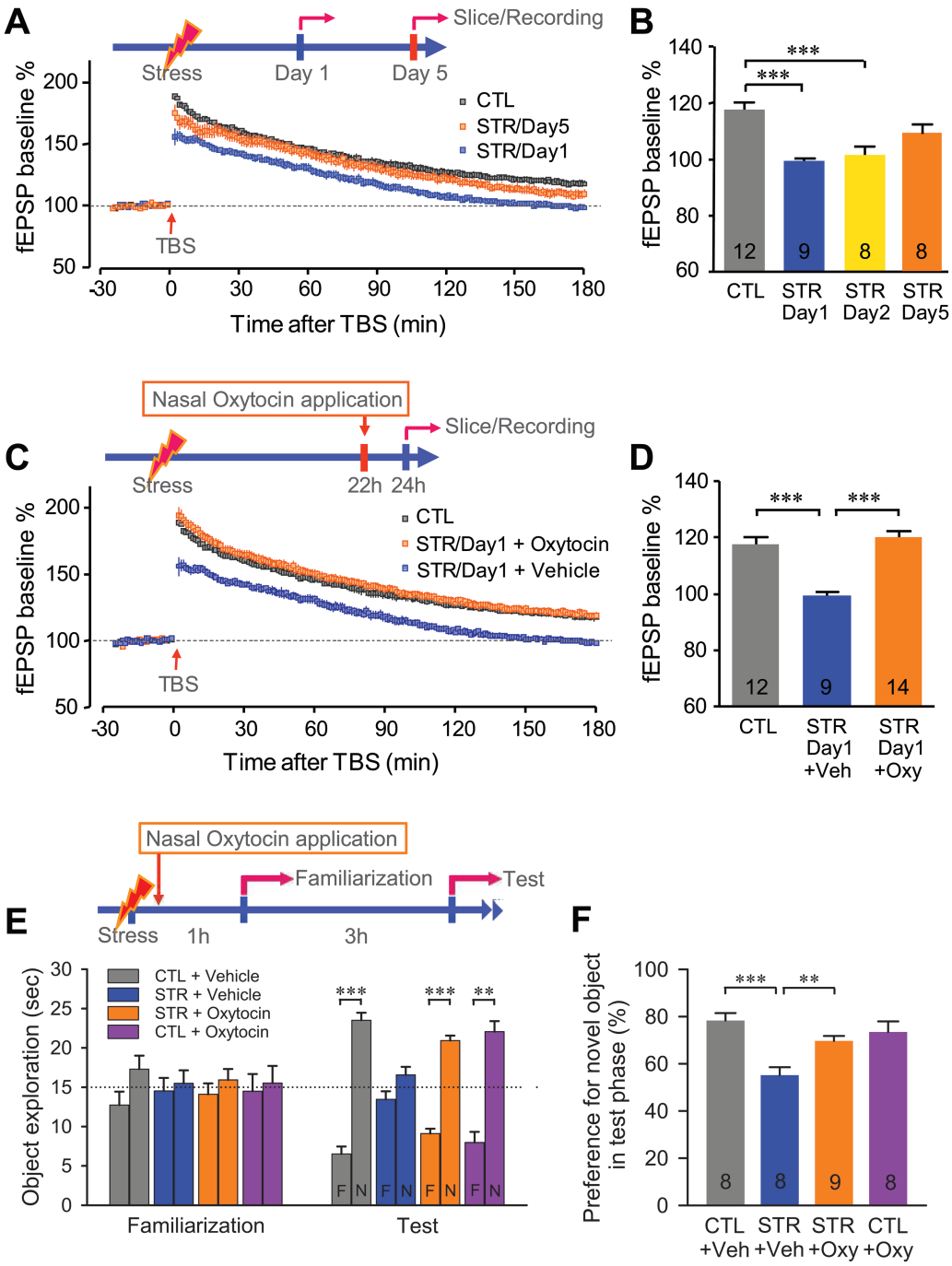

Figure 1. Intranasal administration of oxytocin rescued impaired synaptic plasticity and recognition memory in the uncontrollable stress-treated rat. (A) theta-burst stimulation (TBS)-induced long-lasting, long-term potentiation (L-LTP) induction was monitored by measuring the Field excitatory postsynaptic potentials (fEPSPs) slope in the rat Schaffer collateral-CA1 synapse at 1 day (blue), 2 days (yellow), or 5 days (orange) after receiving uncontrollable stress. (B) Quantification of L-LTP in the stressed rats was calculated from fEPSP responses at 170 to 180 minutes after TBS. (C) Rats were treated with nasally applied oxytocin (1 mg/mL, 200 μL, orange) or vehicle (blue) at 1 day after receiving uncontrollable stress, and 2 hours later. TBS-induced L-LTP induction was monitored. (D) Quantification of L-LTP in the oxytocin-treated rats. (E) (top) Schematic of the experimental design for assessing the effects of oxytocin on stress-induced cognitive impairments. Following the treatment of uncontrollable stress, oxytocin or vehicle was applied to rat's intranasal cavity. One hour later, the object recognition test with a 3-hour delay between the familiarization and test phases was conducted. (bottom) Time exploring the 2 identical objects during the familiarization phase (left) and 3 hours later one previously explored object (F) and one novel object (N) during the test phase (left). (F) Percentage of the preference for the novel object during the test phase (30-second exploration time). n = 8 for CTL + Veh, STR + Veh, and CTL + Oxy, n = 9 for STR + Oxy. All values represent the average ± SEM. *P < .05; *P < .01; ***P < .001.

The findings of the present study provide important implications regarding the possible clinical potential of oxytocin. That is, oxytocin could be used for ameliorating cognitive dysfunction in PTSD patients who have experienced unexpected stress during accidents, war, and disasters. Currently, serotonin uptake inhibitors (e.g., sertraline and paroxetine) are prescribed for the treatment of PTSD (Ipser and Stein, 2012). However, many people diagnosed with PTSD continue to have symptoms and require new drugs for further treatment. Recently, experimental attempts have been made to investigate various candidates for

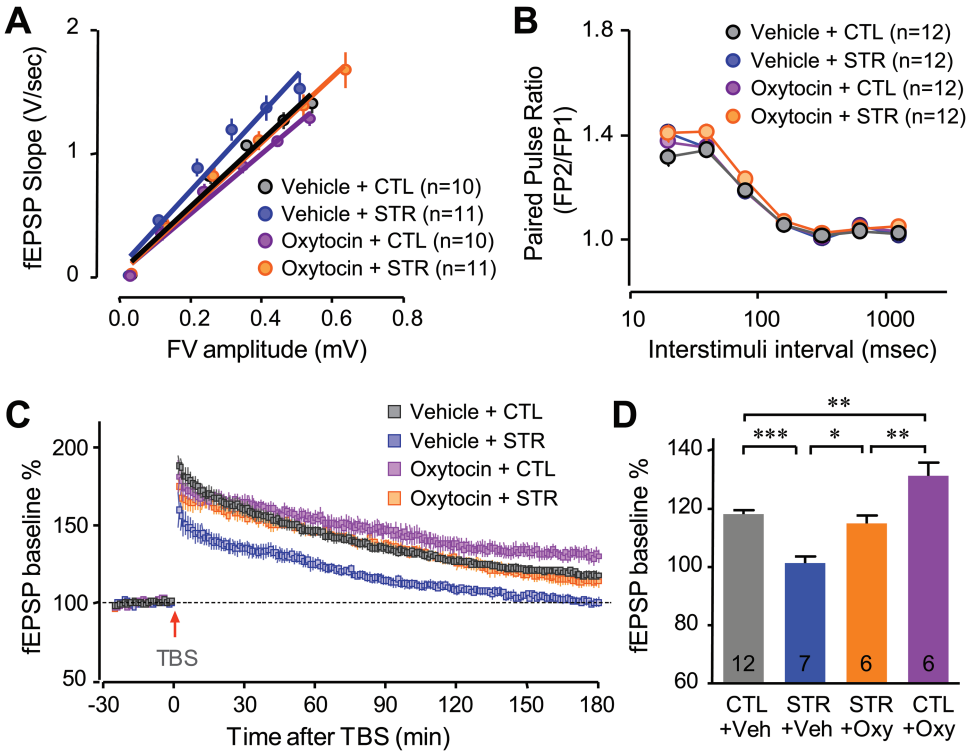

Figure 2. Bath application of oxytocin to the hippocampus from uncontrollable stress-treated rats rescued impaired synaptic plasticity. Hippocampal slices were prepared from rats 1 day after receiving uncontrollable stress. Effects of bath-applied oxytocin (1 μM) on synaptic transmission were examined by measuring the field excitatory postsynaptic potential (fEPSP) slope at Schaffer collateral-CA1 synapses. Vehicle treatment in slices from the control rats (black), vehicle treatment in slices from the stressed rats (blue), oxytocin treatment in slices from the control rats (yellow), oxytocin treatment in slices from the stressed rats (orange). (A) Input–output relationship between the amplitude of the fiber volley (FV) and the slope of the fEPSP with different stimulus intensities. (B) The ratio of paired-pulse-induced responses achieved by 2 stimulation pulses separated by the indicated time intervals. (C) Theta-burst stimulation (TBS)-induced long-lasting, long-term potentiation (L-LTP). (D) Quantification of L-LTP. All values represent the average ± SEM. *P < .05; *P < .01; ***P < .001.

new drug treatments, such as minocycline and catecholamine (Levkovitz et al., 2015; Lin et al., 2016). It is likely that oxytocin alone or in combination with these other candidates will provide more diverse, effective treatments for PTSD.

Acknowledgments

This work was supported by the National Research Foundation of Korea (2016M3C7A1905481, 2016R1A2B4006811, and 2007-313-C00630 to S.-Y.C. and 2015M3C7A1031395 to J.-S.H.)

Statement of Interest

None.

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