Acute Administration of Oxytocin in the Functional Recovery of Neurocognitive and Social Deficits Following Juvenile Frontal Traumatic Brain Injury

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Abstract

Introduction: Juvenile traumatic brain injury (jTBI) is one of the leading causes of death and disability in children. The prefrontal cortex (PFC) is most susceptible to injury which leads to deficits in executive function, social behaviors, and cognitive flexibility. Prior research has shown a significant role of the oxytocin (OXT) system in the modulation of social behaviors, and that intranasal OXT (IN-OXT) is potentially neuroprotective. Therefore, we believe IN-OXT could improve behavioral deficits caused by a PFC injury.

Methods: Animals received a single midline cortical contusion bilaterally damaging the medial PFC (mPFC) and immediately given a single dose of IN-OXT, placebo, or no treatment. Animals were assessed using behavioral and histological measures.

Results: The results indicated that IN-OXT in jTBI animals mildly improved spatial learning but did not improve spatial memory. Additionally, TBI increased social dominance behavior, but IN-OXT did not mitigate those behaviors. IN-OXT did not alleviate neuroinflammation but did elevate OXT receptors and OXT peptide levels. Furthermore, TBI decreased OXT in the SON of the hypothalamus, and IN-OXT returned OXT levels in this region to normal.

Discussion: These results show that IN-OXT increases OXT levels in the brain via pathways originating in the SON and improved spatial learning.

Introduction

Traumatic brain injury (TBI) is a major global health concern (1,2). Young children are particularly susceptible, often due to falls, resulting in 837,000 cases annually (3,4). TBI frequently damages the frontal lobe, leading to executive dysfunctions (5,6) that impact daily functioning and independence (5–8). Children with TBI face challenges in postsecondary education, independent living, and employment, with increased rates of psychiatric disorders and interpersonal difficulties (3,9–13).

Despite the severity of TBI's impact, there are windows during development where injury response and recovery are more favorable (7,14–17). Oxytocin (OXT) exhibits neuroprotective properties, including social neuroprotection and anti-inflammatory effects (18–20). Intranasal OXT (IN-OXT) has shown promise in ameliorating social and cognitive deficits in injury models (18,20–30). Our study investigates the impact of juvenile TBI on the OXT system and inflammation in rats during the acute phase following injury and evaluates the potential efficacy of IN-OXT at a treatment.

Experiment 1

Methods

Experimental procedures were approved by the Southern Illinois University Institutional Animal Care and Use Committee. Thirty animals at post-natal day (PND) 28 were housed in pairs under a 12-h light/dark cycle. Female subjects were selected based on previous findings indicating greater therapeutic potential of OXT treatment in females (28,31).

Surgery

Animals received either a bilateral frontal controlled cortical impact (fCCI) or sham surgery and were sacrificed on post-injury days (PIDs) 1, 14, or 30. For fCCI, a midline incision

was made, followed by a 5 mm diameter craniotomy centered 2 mm anterior to bregma (+4.5 mm to -0.5 mm) and 0 mm from the midline (+2.5mm to -2.5 mm). An electromagnetically activated piston (Leica, St. Louis, MO) attached to a 4 mm impactor probe was used to strike the cortex at 2.25m/s for 500ms after retracting and lowering the probe by 3 mm. Sham surgery replicated the incision procedure without performing the craniotomy.

Transcardial Perfusion

Following intraperitoneal injection of 1 mL 50% urethane in water, subjects underwent transcardial perfusion. The entire brain was then removed and post-fixed in 4% paraformaldehyde at 4□C for 48 hours, followed by immersion in 30% sucrose solution for cryoprotection before sectioning on a cryostat.

Histochemistry

Brains were sectioned into 40 μm coronal sections using a Leica CM 1860 cryostat. Four slices (+4.2 mm to +1.7 mm from bregma) were obtained from each animal, followed by an immunohistochemistry protocol (32). Primary antibodies anti-OXTR (1:100; Alomone Labs #AVR-013), anti-OXT (1:10,000, ImmunoStar #20068) and Anti-Iba-1 (microglia; 1:1000; Wako #019-19741) were used.

Eight images of the pericontrused cortex were taken for each stain at the injury site at 40x magnification (60 μm). Additionally, two images were taken of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus with the anti-OXT stain. Finally, four images were taken of the anterior olfactory nucleus (AON) and the caudate putamen (CP) for the anti-OXT and anti-OXTR stains. These regions were chosen due to their relevance in IN-OXT passage into the brain (33,34) and endogenous OXT production (18,26,35–37). Additionally, previous work only assessed OXT mRNA in the PVN (20) and found no effect of injury.

However, the stain utilized in our study will be used to assess the peptide instead of the mRNA. Furthermore, since the SON also projects to the cortex it was included in our study.

For anti-Iba staining, Iba+ cells were counted. For the anti-OXTR and anti-OXT stains, mean grey values were measured, and for anti-OXTR, normalized by the number of cells in the image.

Statistical Analysis

To assess the microgliosis and the density of OXT and OXTRs, 2 (injury) x 3 (Day: PID 1, 14, and 30) ANOVAs were used to analyze group differences across development. Bonferroni corrected post-hoc tests were then used to identify significant mean differences.

Experiment 1 Results

All data met the assumptions of normality and homogeneity of variance for the ANOVA tests. Nonsignificant results are included in the supplementary material.

Microgliosis

There was a main effect of injury [$F(1, 218) = 1372.677$, $p < 0.001$, $\eta^2 = 0.369$] and injury*day [$F(2, 218) = 632.509$, $p < 0.01$, $\eta^2 = 0.055$] on neuroinflammation (Figure S1). Sham animals exhibited fewer Iba+ cells compared to TBI animals ($p<0.001$), which was consistent across development ($p<0.001$). Moreover, TBI animals showed peak neuroinflammation levels 30 days post-injury (compared to day 1, $p<0.01$, and day 14, $p<0.05$).

Oxytocin System

For OXTR levels at the injury site, injury*day was significant [$F(2, 218) = 5.875$, $p < 0.01$, $\eta^2 = 0.051$] but not injury [$F(1, 218) = 0.564$, $p = 0.453$, $\eta^2 = 0.003$] (Figure S2). Sham animals displayed an inverse U-shaped pattern of development in the mPFC, peaking on day 14 (compared to day 1, $p<0.001$, and day 30, $p<0.001$). Day 30 also showed more OXTRs than day

1 (p<0.05). Regarding OXT levels, there was a main effect of injury [F(1, 218) = 14.719, p < 0.001, η^2 = 0.063], but not injury*day [F(2, 218) = 1.435, p = 0.240, η^2 = 0.013] (Figure 1). TBI animals exhibited decreased OXT levels at the injury site compared to sham animals (p<0.001).

In the AON, there were no significant effects for OXTRs based on injury [F(1, 106) = 0.059, p = 0.809, η^2 = 0.001] or injury*day [F(2, 106) = 2.229, p = 0.113, η^2 = 0.04] (Figure S2). However, a main effect of injury [F(1, 106) = 9.365, p < 0.01, η^2 = 0.081], and injury*day [F(2, 106) = 3.212, p < 0.05, η^2 = 0.057] was observed on OXT levels in the AON (Figure 1). Sham animals displayed higher OT levels than TBI animals (p<0.01) on days 1 (p<0.05) and 14 (p<0.001). Furthermore, TBI animals displayed higher levels of OXT on day 30 compared to days 1 (p<0.01) and 14 (p<0.001).

Similarly, there was a significant main effect of injury*day [F(2, 106) = 4.424, p < 0.05, η^2 = 0.077] but not injury [F(1, 106) = 0.952, p = 0.331, η^2 = 0.009] on OXTR levels in the CP (Figure S2). TBI animals demonstrated more OXTRs in the CP on day 14 compared to day 30 (p<0.01). Regarding OXT levels in the CP, a main effect of injury [F(1, 106) = 4.710, p < 0.05, η^2 = 0.043] was found but not injury*day [F(2, 106) = 1.140, p = 0.324, η^2 = 0.021], with sham animals showing higher OXT levels than TBI animals (p<0.05) (Figure 1).

In the PVN, there were no significant effects for injury [F(1, 50) = 3.566, p = 0.065, η^2 = 0.067] or injury*day [F(2, 50) = 1.956, p = 0.152, η^2 = 0.073] on OXT levels (Figure 1). Conversely, a main effect of injury was observed [F(1, 50) = 7.505, p < 0.01, η^2 = 0.131] for OXT levels in the SON, but not injury*day [F(2, 50) = 0.374, p = 0.690, η^2 = 0.015] (Figure 1). TBI animals displayed decreased levels of OXT compared to sham animals (p<0.01).

Experiment 1 Discussion

As hypothesized, TBI heightened neuroinflammation across development, persisting for at least 30 days post-injury, consistent with previous findings (24,30). Despite OXTR expression on microglia, TBI did not alter OXTR levels at the injury site; however, it did decrease OXT levels, potentially contributing to the unabated microglial response. Similarly, TBI minimally impacted OXTR levels in the AON and CP, while reducing OXT levels. The OXT deficits are likely due to decreased OXT production in the SON not the PVN. Subsequently, we investigated whether acute IN-OXT administration post-injury could restore depleted OXT levels and promote functional recovery following TBI.

Experiment 2

Methods

Surgery

For this experiment, 48 animals at PND 28 were utilized and divided into six groups: receiving either fCCI or sham surgery combined with IN-OXT, vehicle (VEH), or no treatment (NoTx). Identical procedures for fCCI and sham surgeries were employed as in Experiment 1.

IN-OXT Administration

Animals received IN-OXT, (OXT, Cayman Chemical Company, #11799) diluted in sterile Ringer's solution (mM: 147.1 Na^+, 2.25 Ca^{2+}, 4 K^+, 155.6 Cl^-, pH 7.4) to a concentration of 1 μg/ 1 μl (20μL) (18,29,37–40). VEH animals received an equivalent volume of sterile Ringer's solution. IN-OXT was administered bilaterally on the rhinarium immediately after scalp closure and before anesthesia recovery, adapting application methods from existing literature (18,39,40).

Behavior

The behavior tasks included the modified foot fault task (mFFT), the open field (see supplementary material), the Morris water maze (MWM), social preference task (see supplementary material), and social dominance task (SDT). For reference Table 1 shows the post-injury days relative to the animal's age (post-natal day).

Modified Foot Fault Task. The foot fault task is a standard assessment in TBI research, ensuring motor coordination but without any movement incentives, thus potentially masking deficits (41–45). To address this limitation, our study employed a mFFT, which is a combination of the beam task and foot fault task that was developed in our lab. The beam was 120 cm long and consisted of a 2.5 cm^2 plastic grid beam attached to a black box.

Morris Water Maze. The MWM featured a 200 cm (D) x 74 cm (H) polyethylene pool divided into four quadrants, filled with opaque water, colored with non-toxic paint. A 13 cm (D) x 72 cm (H) clear plexiglass platform, submerged 2 cm under the water's surface, was located in the northeast quadrant. ANY-maze video tracking software recorded animal movements. The MWM comprised three phases: training, probe, and reversal training. These phases followed previously established protocols (32).

Platform search strategy was assessed during training and probe trials, focusing on the progression from inefficient to efficient strategies. Animal coordinates from ANY-maze were analyzed using Pathfinder software (46) to assess search strategies for each trial.

Social Dominance Task. The SDT assessed changes in social hierarchy post-TBI and followed a protocol from previous research (47). 'Winning' was defined as reaching the entrance barrier slit at the tube's opposite end, and results were recorded for each target rat. Novel rats were matched in sex and age.

Histochemistry

The same procedure for transcardial perfusion and immunohistochemistry in experiment 1 was used on animals in experiment 2. Animals were sacrificed on PID 30.

Statistical Analysis

We collapsed sham groups into a single sham group, additionally we collapsed NoTx TBI and VEH TBI groups into a single NoTx group. Analysis involved repeated-measures ANOVAs, MANOVAs and Kruskal-Wallis H Tests. Bonferroni corrected post-hoc tests were employed to identify significant mean differences between groups.

Experiment 2 Results

All data meet the assumption of normality and homogeneity of variance for the (M)ANOVA tests. Nonsignificant results are included in the supplementary material.

Modified Foot Fault Task

Significant group [$F(2, 45) = 3.512$, $p<0.05$, $\eta^2=0.135$], and group*day [$F(2.729, 61.409) = 3.494$, $p<0.05$, $\eta^2=0.134$] effects were observed (Figure S3). Sham animals had fewer faults/second than OXT TBI animals ($p<0.05$). Notably, significant group differences were only evident on the final testing day, with sham animals outperforming both NoTx TBI ($p<0.05$) and OXT TBI ($p<0.05$) animals.

Morris Water Maze

Training Phase. Significant group [$F(2, 45) = 25.287$, $p<0.001$, $\eta^2=0.529$], and group*day effects [$F(6.645, 149.517) = 2.471$, $p<0.05$, $\eta^2=0.099$] were observed (Figure 2). NoTx TBI animals exhibited the most spatial learning deficits compared to sham ($p<0.001$) and OXT TBI animals ($p<0.05$). Notably, NoTx TBI animals consistently displayed spatial learning deficits across training days (Day 15, 17-19: $p<0.001$, Day 16: $p<0.01$). Conversely, OXT TBI animals only showed spatial learning deficits compared to sham animals on training days 15

(p<0.05), and 16 (p<0.05), then demonstrated improved performance compared to NoTx TBI animals on training day 19 (p<0.001).

Regarding search strategies for training days, significant effects were found on days 15 [H(2) = 42.499, p<0.001, η^2 = 0.214], 16 [H(2) = 25.964, p<0.001, η^2 = 0.127], 17 [H(2) = 27.619, p<0.001, η^2 = 0.136], 18 [H(2) = 30.231, p<0.001, η^2 = 0.149], and 19 [H(2) = 37.425, p<0.001, η^2 = 0.187] (Figure 2). Sham animals consistently employed more efficient search strategies than NoTx TBI animals on all training days (p<0.001), while being more efficient than OXT TBI animals only on days 15 (p<0.001) and 16 (p<0.05). Subsequently, OXT TBI animals were more efficient than NoTx TBI animals on training days 17 (p<0.05), 18 (p<0.05), and 19 (p<0.01).

Probe Trial. On the probe day, a significant effect was observed for latency to initial platform crossing [F(1, 45) = 124.396, p < 0.001, η^2 = 0.734] and the number of platform zone crossings [F(1, 45) = 63.179, p < 0.001, η^2 = 0.584] (Figure 2). Sham animals exhibited shorter latencies to the platform zone (p<0.001) and more platform zone crossings (p<0.01) compared to NoTx TBI animals.

Regarding search strategies during the probe trial, significant differences were found based on group [H(2) = 16.469, p < 0.001, η^2 = 0.322] (Figure 2). Sham animals showed greater efficiency in their search strategy compared to NoTx TBI animals (p<0.001).

Reversal Day. After the probe day, animals underwent a test for cognitive flexibility, where the platform was relocated to a new position, and the latency to reach the new platform location was recorded. A significant effect of injury was observed [F(2, 45) = 5.216, p < 0.01, η^2 = 0.188] (Figure S4). Sham animals exhibited superior cognitive flexibility compared to NoTx TBI animals (p<0.01).

Social Dominance Task

For the percentage of wins, there was a significant effect of group [H(2) = 13.131, $p<0.001$, $\eta^2 = 0.247$] (Figure 3). Sham animals displayed a lower win percentage than NoTx TBI ($p<0.01$) and OXT TBI ($p<0.05$) animals.

Microgliosis

When measuring inflammation (Figure S5), significance was found for group [H(2) = 79.672, $p<0.001$, $\eta^2 = 0.344$] (Figure S6). Sham animals displayed less neuroinflammation than NoTx TBI ($p<0.001$), and OXT TBI ($p<0.001$) animals.

Oxytocin System

Significance was found for group regarding OXTR levels (Figure S7) at the injury site [$F(2, 229) = 4.586$, $p < 0.05$, $\eta^2 = 0.039$] (Figure S8). Sham animals exhibited fewer OXTRs in this region compared to OXT TBI animals ($p<0.01$). Regarding the effects of IN-OXT treatment on OXT levels at the injury site (Figure 4), significance was found for group [$F(2, 229) = 15.304$, $p < 0.001$, $\eta^2 = 0.118$] (Figure 5). Sham animals displayed higher OT levels than NoTx TBI animals ($p<0.001$).

Regarding OXTR levels in AON (Figure S9), no significant group effect was found [$F(2, 113) = 1.156$, $p =0.318$, $\eta^2 = 0.02$] (Figure S8). However, a significant group effect on OXT levels in the AON (Figure 6) was observed [$F(2, 113) = 7.847$, $p < 0.001$, $\eta^2 = 0.122$] (Figure 5). NoTx TBI animals exhibited lower OXT levels than sham ($p<0.001$) and OXT TBI ($p<0.001$) animals.

Concerning the levels of OXTRs in the CP (Figure S10), no significance was found for group [$F(2, 113) = 2.547$, $p = 0.083$, $\eta^2 = 0.043$] (Figure S8). However, a significant effect on OXT levels in the CP (Figure 7) was observed [$F(2, 113) = 8.138$, $p < 0.001$, $\eta^2 = 0.126$] (Figure

5). NoTx TBI animals displayed lower OXT levels than sham ($p<0.01$) and OXT TBI ($p<0.001$) animals.

No significant effect of group was found on OXT levels in the PVN (Figure 8) [$F(2, 55) = 2.427$, $p = 0.098$, $\eta^2 = 0.081$] (Figure 5) nor the SON (Figure 9) [$F(2, 55) = 0.142$, $p = 0.868$, $\eta^2 = 0.005$] (Figure 5).

Experiment 2 Discussion

Animals with TBI exhibited gross motor deficits, consistent with prior studies (32,42,43), while acute IN-OXT treatment failed to enhance performance in this task. However, IN-OXT did yield modest improvements in spatial learning, as seen in previous research (23), though these benefits did not extend to spatial memory or cognitive flexibility. TBI heightened social dominance, with IN-OXT offering no mitigation. Overall, IN-OXT conferred only mild enhancements in spatial learning.

Histologically, IN-OXT treatment did not alleviate neuroinflammation, despite elevating OXTRs and rectifying OXT deficits at the injury site. Thus, acute IN-OXT proved ineffective on neuroinflammation post-TBI. While IN-OXT did not affect OXTR levels in the AON or CP, it did restore OXT deficits induced by TBI in both regions. Additionally, consistent with Experiment 1 and previous findings (20), TBI had no impact on OXT levels in the PVN. Surprisingly, there were no injury or treatment effects on OXT levels in the SON, diverging from Experiment 1. This suggests that IN-OXT treatment resolves OXT deficits within a month post-injury, returning OXT levels in other brain regions to normal. Further investigation is needed on the acute-phase effect of IN-OXT treatment in the SON.

Discussion

The study revealed that juvenile TBI induces behavioral deficits (motor, memory, social dominance) linked to increased microglia, consistent with prior research (44,45,48–51). A novel finding was the elevation of social dominance in young female animals following a focal CCI injury to the mPFC, contrasting with deficits observed in adulthood (52).

Behaviorally, acute OXT treatment post-TBI led to mild enhancements in spatial learning, aligning with previous findings (23), but failed to improve spatial memory recall in subsequent probe trials. Histologically, despite elevating OXTR and OXT levels at the injury site, acute IN-OXT did not alleviate inflammation. Surprisingly, TBI had no impact on OXT levels in the PVN but induced deficits in the SON, which were restored by IN-OXT. Since IN-OXT solely influenced the SON and resolved OXT deficits without mitigating inflammation, investigating chronic dosing is warranted.

Overall, OXT-treated TBI animals exhibited mild spatial learning improvements, possibly attributed to hippocampal effects (23). However, IN-OXT failed to ameliorate social dominance changes, despite its effects on OXT and OXTR levels in the frontal lobe. Further research into additional brain regions is necessary to elucidate these deficits. Lastly, the SON, not the PVN, seems responsible for OXT system deficits in the frontal lobe. As IN-OXT only impacted the SON and resolved OXT deficits without attenuating inflammation, exploration of chronic dosing is recommended.

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Table 1

Post-Injury Days Relative to Post-Natal Days

Behavior	**Post-Injury Day (PID)**	**Postnatal Day (PND)**
Modified Foot Fault	1, 3, 5	29, 31, 33
Open Field	8-12	36-40
Morris Water Maze	14-21	42-49
Social Preference	22-23	50-51
Social Dominance	24-25	52-53

Figure 1

OXT Stain Intensity Across Development

Note. Graphs show group means ± SEM; *, $p<0.05$, ***, $p<0.001$

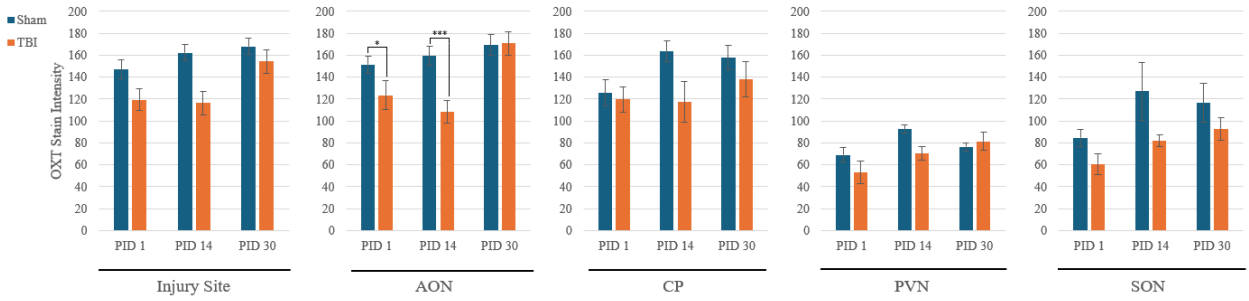


Figure 2

MWM Training and Probe Results

Note. Graphs show group means ± SEM; &, significantly different from sham animals, #, significantly different from NoTx TBI animals.

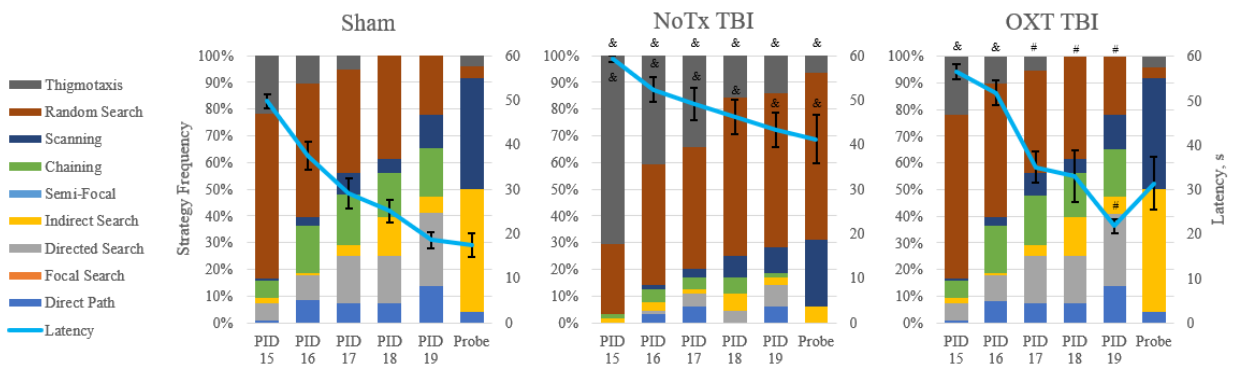


Figure 3

SDT Results

Note. Graphs show group means ± SEM; *, $p<0.05$, **, $p<0.01$.

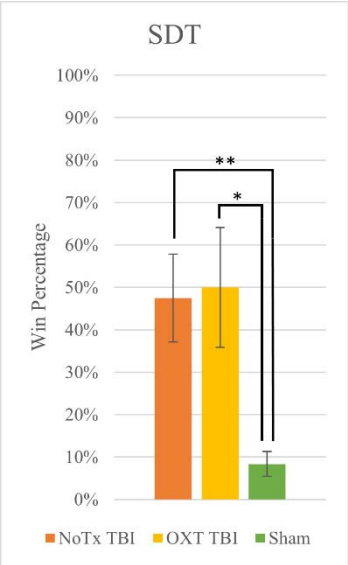


Figure 4

Representative OXT-Stain Images at the Injury Site

Note. Scale bar = 40 μm.

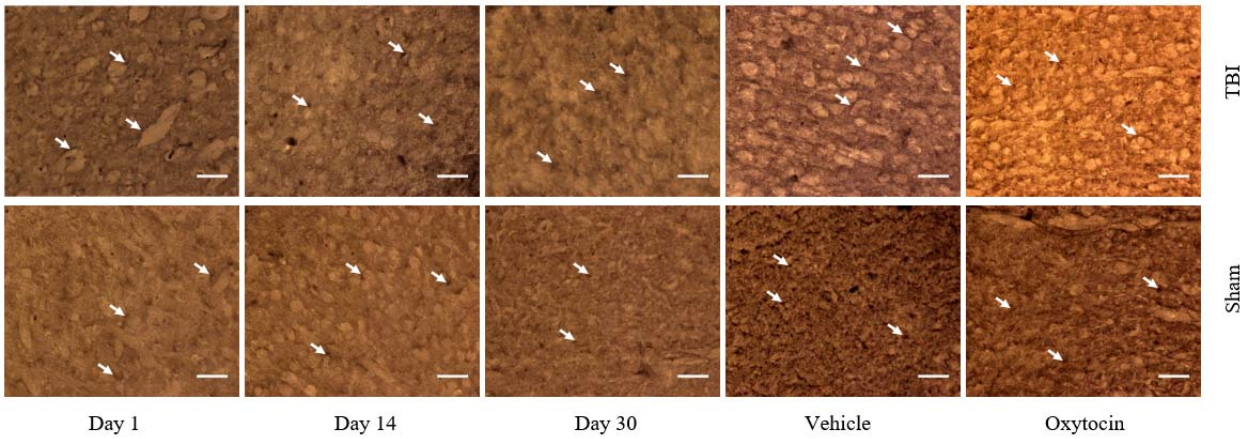


Figure 5

OXT-Stain Results

Note. Graphs show group means ± SEM; **, $p<0.01$; ***, $p<0.001$.

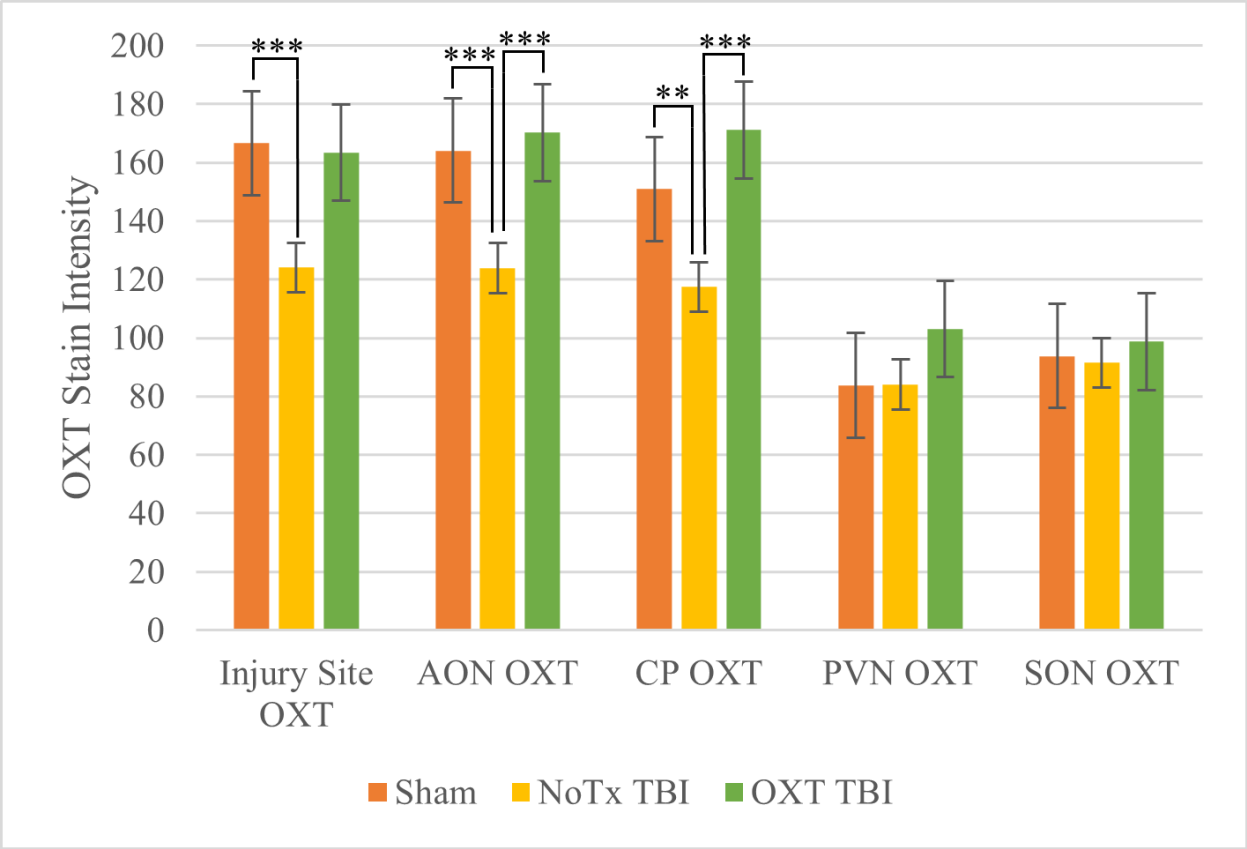


Figure 6

Representative OXT-Stain Images in the AON

Note. Scale bar = 40 µm

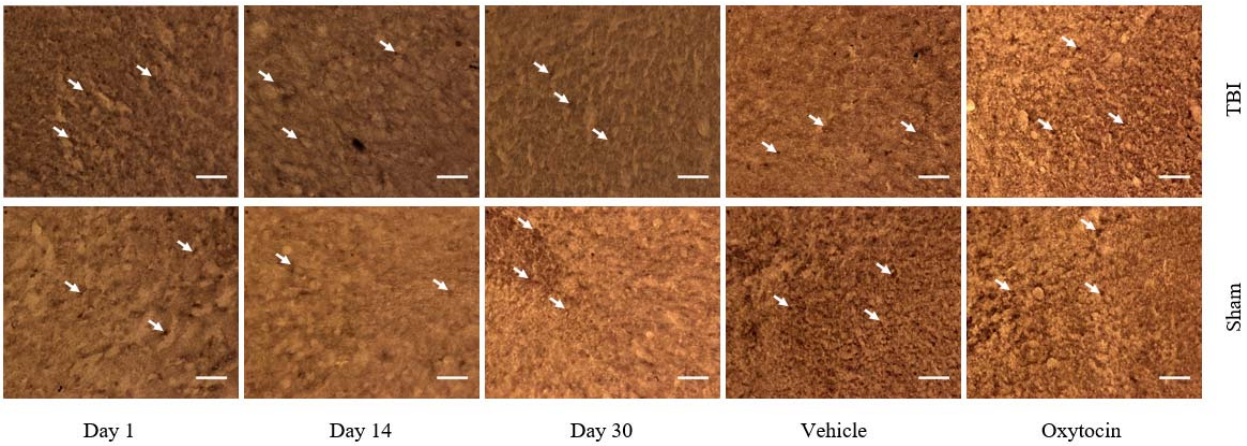


Figure 7

Representative OXT-Stain Images in the CP

Note. Scale bar = 40 µm

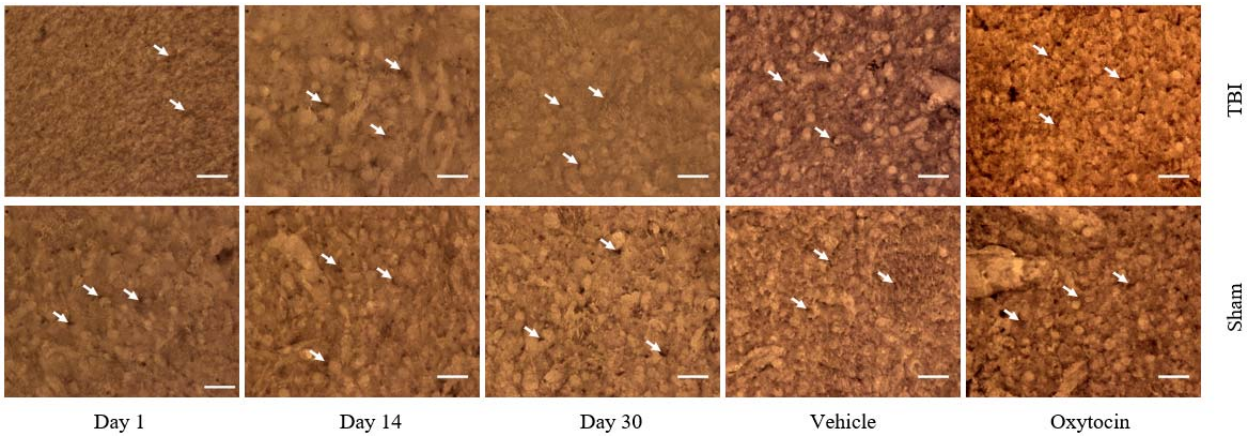


Figure 8

Representative OXT-Stain Images in the PVN

Note. Scale bar = 40 μm

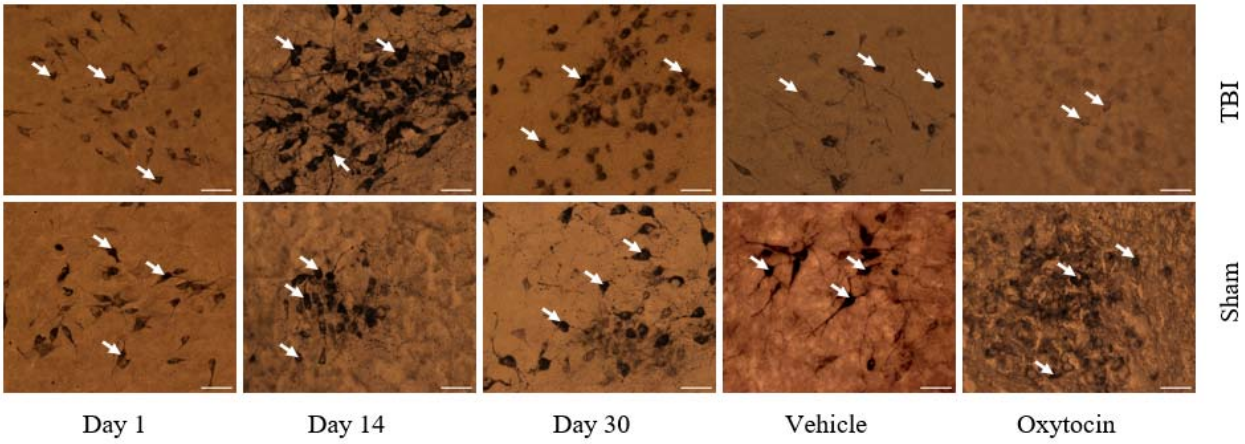
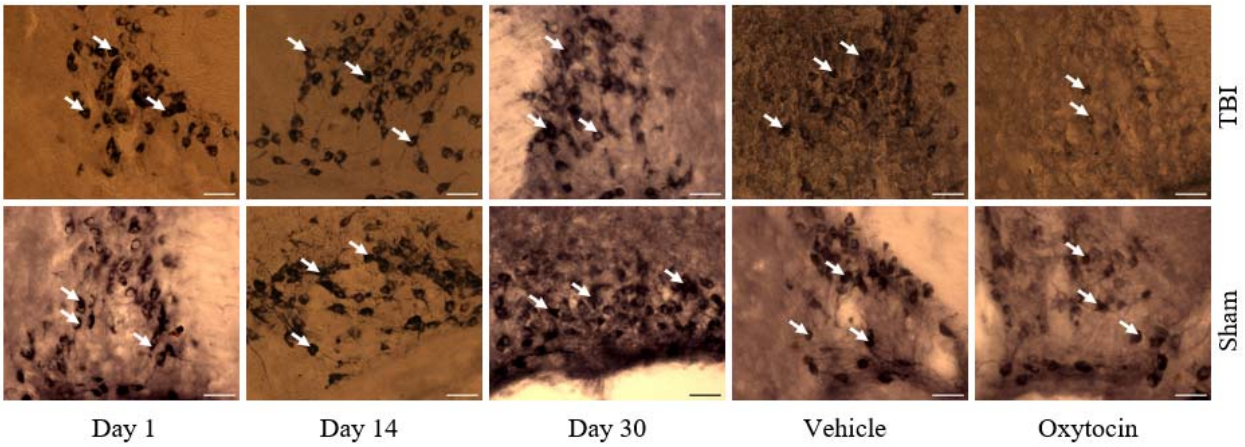


Figure 9

Representative OXT-Stain Images in the SON

Note. Scale bar = 40 μm