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Bladder dysfunction changes from underactive to overactive after experimental traumatic brain injury

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Abstract

Although bladder dysfunction is common after traumatic brain injury (TBI), few studies have investigated resultant bladder changes and the detailed relationship between TBI and bladder dysfunction. The goal of this study was to characterize the effects of TBI on bladder function in an animal model. Fluid-percussion injury was used to create an animal model with moderate TBI. Female Sprague-Dawley rats underwent TBI, sham TBI or were not manipulated (naïve). All rats underwent filling cystometry while bladder pressure and external urethral sphincter electromyograms were simultaneously recorded 1 day, 1 week, 2 weeks, and 1 month after injury. One day after injury, 70% of the animals in the TBI group and 29% of the animals in the sham TBI group showed no bursting activity during urination. Compared to naïve rats, bladder function was mainly altered 1 day and 1 week after sham TBI, suggesting the craniotomy procedure affected bladder function mostly in a temporary manner. Compared to either naïve or sham TBI, bladder weight was significantly increased 1 month after TBI and collagen in the bladder wall was increased. Bladder function in the TBI group went from atonic 1 day post-TBI to overactive 1 month post-TBI, suggesting that TBI significantly affected bladder function.

Keywords

bladder; electromyogram; rat; traumatic brain injury; urethra; urodynamics

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Introduction

Traumatic brain injury (TBI) is a common cause of death and disability (NIH Consensus Statement, 1998; Vaishnavi et al., 2009). TBI presents as both a primary injury and a progression of secondary injury which requires a long period of care and rehabilitation (Masel and DeWitt, 2010). Bladder dysfunction, such as urinary retention and incontinence, are a common symptom after TBI and can further induce chronic voiding dysfunction, urinary tract infection, skin ulcers, stones, and even renal failure (Chua et al., 2003; Chernev and Yan, 2009; Giannantoni et al., 2011). However, bladder dysfunction is less well characterized than other neurogenic complications of TBI.

In current clinical practice, there is no indication to perform urodynamic studies demonstrating underlying bladder function changes after TBI, few studies have investigated the relationship between TBI and bladder dysfunction (Chua et al., 2003; Moiyadi et al., 2007; Chernev and Yan, 2009). Animal models of TBI usually investigate motor function, cognitive function and behavioral assessments (Dixon et al., 1987; Gennarelli, 1994; Cernak, 2005; Schiff et al., 2007), but there is no clear documentation for autonomic organ symptoms such as bladder function. In the present study, we use a well-established fluid percussion injury (FPI) model to induce the TBI in the area that involves the center of micturition control to interrupt supraspinal regulation of bladder function. We hypothesized that the brain injury near the micturition center results in progressive bladder dysfunction. Therefore, if underlying bladder problems are characterized early after TBI, appropriate treatment may prevent pathologic changes and serious complications. Additionally, bladder function could potentially be used to evaluate recovery and efficacy of treatment after TBI.

Materials and Methods

Twenty-three age-matched female Sprague-Dawley rats (Harlan, Inc.) weighing 225–250 g underwent TBI (n=15), sham TBI (n=7), or were not manipulated (naïve, n=6). The mortality rate in the TBI group was 30%. Therefore, 10 animals in the TBI group were able to complete all the tests. All animals received repeated urodynamic study 1 day, 1 week, 2 weeks, and 1 month after injury. All procedures were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic.

Fluid percussion surgery and injury

Animals were surgically prepared 24 hours before injury using standardized procedures slightly modified from those previously published (Dixon *et al.*, 1987). Briefly, rats were anesthetized and placed in a stereotaxic frame. A midline circular craniotomy was performed and a stainless steel screw was placed 1.5-mm caudal to the craniotomy to add support to the modified Leur-Loc syringe hub that was placed over the exposed dura. Rats were anesthetized and connected to the fluid-percussion injury (FPI) device (Dragonfly Research & Development, Inc.) via the Leur-Loc syringe 24 hours after surgical preparation. The hub was removed and the skin was closed after FPI (M=2.0 atm). Rats in the sham TBI group were same procedure except that no fluid pulse was delivered.

Filling cystometric and external urethral sphincter (EUS) electromyographic (EMG) recordings

All rats were anesthetized with ketamine (100mg/kg) and xylazine (10mg/kg) for urodynamic testing. Similar to a previous study (Steward et al., 2010), a polyethylene catheter (PE50) was inserted in the bladder via the urethral orifice and connected to both a pressure transducer and a syringe pump for filling cystometry. For EUS EMG, 50µm diameter teflon-insulated platinum wires were inserted into the urethra bilaterally along the

mid-urethra using a 30-gauge needle from the vagina. The electrodes were connected to an AC amplifier (Model P511, Astro-Med, Inc., Providence, RI) and a recording system (DASH 8X, Astro-Med, Inc.). The wires and catheter were removed from the rat when recordings were completed.

Histology

After the 4th urodynamics study, 1 month after injury, all animals with TBI and sham TBI underwent transcardial perfusion with 0.9% saline followed by 4% paraformaldehyde. The bladder of all animals was dissected and weight wet was recorded. The bladder and urethra were then transferred to 30% sucrose, transversely cryostat sectioned transversely (8 μ m) at the level of mid-bladder and mid-urethra, and stained with Masson's trichrome. The brains were removed from the skulls and fixed with 4% paraformaldehyde for 1 day. Following fixation, the brains were transferred to 30% sucrose and then were sectioned on a cryostat. Transverse sections (30 μ m) were collected over the FPI injured location and then were stained with thionin (Nissl stain) for tissue integrity and morphology.

Data analysis

EUS EMG activity during the middle of the storage phase was taken as a measure of baseline activity. EUS EMG bursting activity was also identified during voiding. Both time points were archived in 1s samples and were filtered and smoothed as previously described (Steward *et al.*, 2010). Pressures from filling cystometry were recorded to quantify voiding contractions and non-voiding contractions (> 5 cmH₂O above baseline in the absence of voiding or urine leakage). For voiding parameters, two-way repeat measures analysis of variance (ANOVA) was used to compare the results in different groups and different time points. One-way ANOVA was used to compare the results of bladder wet weight 1 month after TBI in all three groups. A Student's *t*-test with a Bonferroni correction was utilized for pairwise comparisons of individual groups (Sigma Plot 11.0; Systat, Inc. Chicago, IL).

Results

EUS EMG

One day after injury, 70% of the animals in the TBI group and 29% of the animals in the sham TBI group showed no bursting activity in EUS EMG and no related high frequency pressure oscillations in bladder pressure during voiding. However, one week after injury, all rats in the TBI and sham TBI groups demonstrated bursting during voiding. Tonic EUS EMG activity during filling as well as bursting activity and related high frequency pressure oscillations during voiding were present in naïve rats both during the initial test and in repeated tests (Figs. 1A and 2A).

Compared to sham TBI, both amplitude and firing rate of baseline EUS EMG activity was significantly decreased 1 day and 1 week after TBI. Baseline EUS EMG amplitude remained low at later time points after TBI, although there was no statistically significant difference between groups. Amplitude and firing rate of EUS EMG bursting activity 1 day after TBI were significantly decreased compared to the other 2 groups. Other significance changes between groups or time points are labeled in Fig. 1.

Voiding contractions

The effect of TBI on voiding function depended on the time after TBI (Fig. 2A and B). There were statistically significant differences with time after TBI in peak voiding pressure, voiding frequency, and voiding interval. Peak voiding pressure was significantly decreased 1 day and 1 week after TBI and 1 day after sham TBI compared to naïve rats. Two weeks and 1 month after injury, both injured groups showed no differences in peak voiding pressure

compared to naïve rats. However, 2 weeks and 1 month after TBI, peak voiding pressure was significantly increased compared to 1 day after TBI, while no such differences existed between any timepoints within the sham TBI or naïve groups. Voiding frequency increased significantly 1 day after TBI or sham TBI compared to naïve rats, but no statistically significant differences between TBI and sham TBI rats were found. Voiding duration was significantly decreased 1 day and 1 month after TBI and sham TBI compared to the naïve group.

Non-voiding contractions

All animals demonstrated non-voiding contractions during filling cystometry, although not in every voiding cycle (Fig. 2C and D). Frequency of non-voiding contractions was significantly increased 1 day after sham TBI and decreased with time. In contrast, frequency of non-voiding contractions was significantly decreased 1 day after TBI and increased with time. As a result, frequency of non-voiding contractions 1 day and 1 week after sham TBI was significantly higher than in naïve animals, as was frequency of non-voiding contractions 1 month after TBI. Pressure increase and duration of non-voiding contractions also demonstrated a similarly contrasting pattern after sham TBI and TBI, such that, after sham TBI, increase in pressure and duration of non-voiding contractions continually decreased during recovery. In contrast, pressure and duration of non-voiding contractions continually increased after TBI.

Morphology

Bladder wet weight increased significantly ($p=0.024$) 1 month after TBI compared to either naïve or sham TBI, while bladder weight was not significantly different between naïve and sham TBI rats (Fig. 3). One month after TBI, bladders appeared larger and thickened and ureters were partially distended bilaterally. In addition, qualitative analysis revealed decreased smooth muscle in the bladder and increased collagen in the suburethelium and between muscle fascicles compared to 1 month after sham TBI. Urethral cross-sections demonstrated similar size and distribution of smooth muscle in sham TBI and TBI groups, while the layers of the EUS appeared to decrease and have uneven distribution 1 month after TBI. No morphological changes or signs of tissue damage were identified in both gross anatomical observations and Nissl staining at the FPI site in the brain 1 month after sham TBI or TBI (Fig. 4).

Discussion

In this study, we applied a central FPI to produce moderate TBI in rats to investigate the effects of TBI on the bladder and urethra via repeated urodynamic studies and morphological assessment. The results of this study demonstrate that craniotomy at this location, as done for sham TBI, affects bladder function mostly acutely. In addition, we have demonstrated the progression of bladder dysfunction after TBI by urodynamics, which remains dysfunctional 1 month after injury.

Bladder function demonstrated increased contractile activity 1 day after sham TBI, as evidenced by an increase in non-voiding contractions and increased frequency and decreased duration of voiding. However, this dysfunction mostly recovered with time, although some limited results still remained different at the later time points, suggesting that craniotomy mainly has a temporary effect on bladder function. Craniotomy has previously been shown to disrupt intracranial hemodynamics and cerebrospinal fluid dynamics (Moskalenko et al., 2008), and may have temporarily affected neural control of the bladder by either of these mechanisms.

Bladder contractility and urethral activity were decreased immediately after TBI. FPI in rats can produce transient electroencephalographic suppression and loss of muscle tone, as well as suppression of many reflexes and complexly organized behavior (Dixon *et al.*, 1987). Voiding is associated with activation of the motor cortex, somatosensory cortex, cingulate cortex, retrosplenial cortex, thalamus, putamen, insula, and septal nucleus (de Groat, 1998; Dasgupta *et al.*, 2005; Kavia *et al.*, 2005; Griffiths and Tadic, 2008; Drake *et al.*, 2010). In rats, brain switching circuits could control reflex micturition and involve both the periaqueductal gray and the pontine micturition center according to functional MRI (Tai *et al.*, 2009). In our FPI model of TBI, these regions were likely damaged by diffuse injuries, coup injury from the direct impact, contrecoup injury from the opposite impact, and cerebral spinal fluid pressure transmission to the ventricle, since the location of FPI was between the cerebrum and likely affected cerebrospinal pressure in the pons (Shaw, 2002; Saatman *et al.*, 2008). However, there may also have been an indirect effect of the injury on bladder function. For example, urinary incontinence is a common symptom in normal pressure hydrocephalus, believed to be due to expansion of ventricles which distorts the fibers of the corona radiata (Klassen and Ahlskog, 2011). These speculative possibilities need further investigation to determine the underlying mechanisms of bladder dysfunction from TBI. The mechanism of bladder dysfunction due to TBI is out of the scope of this project but should be the focus of future work.

Clinically, one of most common urinary symptoms after TBI is overactive bladder or urge incontinence (Oostra *et al.*, 1996; Moiyadi *et al.*, 2007), especially in the chronic stage of severe TBI (Giannantoni *et al.*, 2011). Our FPI model demonstrated a significant increase in non-voiding contractions 1 month after TBI, resembling clinical bladder dysfunction after head injury (Giannantoni *et al.*, 2011; Oostra *et al.*, 1996; Moiyadi *et al.*, 2007). Bladder dysfunction after TBI could be due to an increase in bladder contractility and/or to detrusor sphincter dyssynergia (DSD) from the brain injuries of TBI. Histology revealed an increase in bladder size as well as collagen deposits in the bladder wall 1 month after TBI. At the same timepoint, EUS activity and bursting response during voiding remained low, which could further impair bladder function. Although out of the scope of this project, longer time points after TBI should be investigated to determine if bladder and urethral function returns to normal without intervention.

Consistent bladder dysfunction after TBI, such as over distension due to decreased voiding contraction strength, bladder outlet obstruction, or DSD, can cause morphological changes, such as bladder weight increase, distended ureters, and increased collagen deposits (Gabella and Uvelius, 1990). In this study, we did not observe hypertrophy of the bladder smooth muscle layer 1 month after TBI, suggesting bladder dysfunction after TBI is mainly due to decreased contractile ability. We speculate that DSD is not likely to have made a major contribution to the morphological changes since the urethral sphincter was not hypertrophied and the bladder smooth muscle layer can increase over 10 times after a functional bladder outlet obstruction (Gabella and Uvelius, 1990). However, longer duration studies are needed to determine the long term effects of DSD in this model. One month after sham TBI there were no significant changes in bladder morphology, paralleling the functional data to suggest that craniotomy primarily affects bladder function temporarily.

This initial study on an animal model of TBI contains some limitations. Rats underwent ketamine/xylazine anesthesia for repeat bladder function testing since this anesthesia allows survival and repeat studies. Ketamine can affect bladder function; therefore bladder function may have been different than if another anesthesia, such as urethane, had been utilized in the bladder functional studies (Maggi and Conte, 1990; Chang and Havton, 2008). However, all rats underwent the same anesthesia and naïve rats demonstrated highly consistent results. Bladder function was underactive immediately after TBI; however we did not perform

special bladder care such as assisting with bladder emptying after injury. Nonetheless, we observed no cases of urinary infection, stones, and other potentially related complications 1 month after TBI and not palpating some bladders to assist with emptying kept all rats under the same management.

Finally, this initial study did not investigate the detailed mechanisms of bladder dysfunction after FPI. This mechanism could be more complicated than discussed above and needs to be further investigated. We did not observe significant morphological changes of the brain at the injury site 1 month after FPI, so earlier time points after FPI may be needed to assess neuroinflammation or cell death associated with injured tissues since short-term (1 week) damage to the brain after FPI has been demonstrated previously (Hicks et al., 1996). We haven't investigated if different locations of injury can affect bladder function differently. However, central FPI in this study, unlike lateral FPI, may cause bilateral cortical alterations associated with direct axial movement of the lower brainstem (Gennarelli, 1994; Cernak, 2005). We expected this lesion to cause lower urinary tract symptoms since urinary control is primarily in the pons in human, cats, rats, and other animals (Kruse et al., 1990; de Groat et al., 1998; Sasaki, 2002; Tai et al., 2009; Fowler and Griffiths, 2010). The particular location of craniotomy as well as FPI in this study may significantly affect bladder function acutely or chronically, but the mechanisms still need to be determined.

Conclusions

In this study, we examined bladder and urethral function and histology after TBI in rats to characterize the effects of TBI on filling and voiding. Bladder dysfunction changes from underactive to overactive after experimental TBI in rats. Our results suggest that urodynamic testing in those with brain injury may reveal long-term bladder problems and could enable early treatment. Bladder function could be a useful approach for evaluating TBI and efficacy of treatment options.

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References

- Cernak I. Animal models of head trauma. *NeuroRx*. 2005; 2:410–422. [PubMed: 16389305]
- Chang HY, Havton LA. Differential effects of urethane and isoflurane on external urethral sphincter electromyography and cystometry in rats. *Am J Physiol Renal Physiol*. 2008; 295:F1248–F1253. [PubMed: 18753298]
- Chernev I, Yan K. Position-dependent urinary retention in a traumatic brain injury patient: a case report. *Cases J*. 2009; 2:9120. [PubMed: 20062697]
- Chua K, Chuo A, Kong KH. Urinary incontinence after traumatic brain injury: incidence, outcomes and correlates. *Brain Inj*. 2003; 17:469–478. [PubMed: 12745703]
- Dasgupta R, Critchley HD, Dolan RJ, Fowler CJ. Changes in brain activity following sacral neuromodulation for urinary retention. *J Urol*. 2005; 174:2268–2272. [PubMed: 16280802]
- de Groat WC. Anatomy of the central neural pathways controlling the lower urinary tract. *Eur Urol*. 1998; 34(Suppl 1):2–5. [PubMed: 9705544]
- de Groat WC, Araki I, Vizzard MA, Yoshiyama M, Yoshimura N, Sugaya K, Tai C, Roppolo JR. Developmental and injury induced plasticity in the micturition reflex pathway. *Behav Brain Res*. 1998; 92:127–140. [PubMed: 9638955]

- Dixon CE, Lyeth BG, Povlishock JT, Findling RL, Hamm RJ, Marmarou A, Young HF, Hayes RL. A fluid percussion model of experimental brain injury in the rat. *J Neurosurg.* 1987; 67:110–119. [PubMed: 3598659]
- Drake MJ, Fowler CJ, Griffiths D, Mayer E, Paton JF, Birdier L. Neural control of the lower urinary and gastrointestinal tracts: supraspinal CNS mechanisms. *Neurourol Urodyn.* 2010; 29:119–127. [PubMed: 20025025]
- Fowler CJ, Griffiths DJ. A decade of functional brain imaging applied to bladder control. *Neurourol Urodyn.* 2010; 29:49–55. [PubMed: 19412958]
- Gabella G, Uvelius B. Urinary bladder of rat: fine structure of normal and hypertrophic musculature. *Cell Tissue Res.* 1990; 262:67–79. [PubMed: 2257617]
- Gennarelli TA. Animate models of human head injury. *J Neurotrauma.* 1994; 11:357–368. [PubMed: 7837277]
- Giannantoni A, Silvestro D, Siracusano S, Azicnuda E, D'Ippolito M, Rigon J, Sabatini U, Bini V, Formisano R. Urologic dysfunction and neurologic outcome in coma survivors after severe traumatic brain injury in the postacute and chronic phase. *Arch Phys Med Rehabil.* 2011; 92:1134–1138. [PubMed: 21704794]
- Griffiths D, Tadic SD. Bladder control, urgency, and urge incontinence: evidence from functional brain imaging. *Neurourol Urodyn.* 2008; 27:466–474. [PubMed: 18092336]
- Hicks R, Soares H, Smith D, McIntosh T. Temporal and spatial characterization of neuronal injury following lateral fluid-percussion brain injury in the rat. *Acta Neuropathol.* 1996; 91:236–246. [PubMed: 8834535]
- Kavia RB, Dasgupta R, Fowler CJ. Functional imaging and the central control of the bladder. *J Comp Neurol.* 2005; 493:27–32. [PubMed: 16255006]
- Klassen BT, Ahlskog JE. Normal pressure hydrocephalus: How often does the diagnosis hold water? *Neurology.* 2011; 77:1119–1125. [PubMed: 21849644]
- Kruse MN, Noto H, Roppolo JR, de Groat WC. Pontine control of the urinary bladder and external urethral sphincter in the rat. *Brain Res.* 1990; 532:182–190. [PubMed: 2282512]
- Maggi CA, Conte B. Effect of urethane anesthesia on the micturition reflex in capsaicintreated rats. *J Auton Nerv Syst.* 1990; 30:247–251. [PubMed: 2229891]
- Masel BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. *J Neurotrauma.* 2010; 27:1529–1540. [PubMed: 20504161]
- Moiyadi AV, Devi BI, Nair KP. Urinary disturbances following traumatic brain injury: clinical and urodynamic evaluation. *NeuroRehabilitation.* 2007; 22:93–98. [PubMed: 17656833]
- Moskalenko I, Vainshtein GB, Kravchenko TI, Mozhaev SV, Semernia VN, Feilding A, Halvorson P, Medvedev SV. The effect of craniotomy on the intracranial hemodynamics and cerebrospinal fluid dynamics in humans. *Fiziol Cheloveka.* 2008; 34:41–48. [PubMed: 18677946]
- NIH Consens.Statement. Rehabilitation of persons with traumatic brain injury. NIH Consens Statement. 1998; 16:1–41.
- Oostra K, Everaert K, Van LM. Urinary incontinence in brain injury. *Brain Inj.* 1996; 10:459–464. [PubMed: 8816099]
- Saatman KE, Duhaime AC, Bullock R, Maas AI, Valadka A, Manley GT. Classification of traumatic brain injury for targeted therapies. *J Neurotrauma.* 2008; 25:719–738. [PubMed: 18627252]
- Sasaki M. Bladder contractility-related neurons in Barrington's nucleus: axonal projections to the spinal cord in the cat. *J Comp Neurol.* 2002; 449:355–363. [PubMed: 12115671]
- Schiff ND, Giacino JT, Kalmar K, Victor JD, Baker K, Gerber M, Fritz B, Eisenberg B, O'Connell J, Kobylarz EJ. Behavioural improvements with thalamic stimulation after severe traumatic brain injury. *Nature.* 2007; 448:600–603. [PubMed: 17671503]
- Shaw NA. The neurophysiology of concussion. *Prog Neurobiol.* 2002; 67:281–344. [PubMed: 12207973]
- Steward JE, Clemons JD, Zaszczurynski PJ, Butler RS, Damaser MS, Jiang HH. Quantitative evaluation of electrodes for external urethral sphincter electromyography during bladder-to-urethral guarding reflex. *World J Urol.* 2010; 28:365–371. [PubMed: 19680661]

- Tai C, Wang J, Jin T, Wang P, Kim SG, Roppolo JR, de Groat WC. Brain switch for reflex micturition control detected by FMRI in rats. *J Neurophysiol.* 2009; 102:2719–2730. [PubMed: 19741099]
- Vaishnavi S, Rao V, Fann JR. Neuropsychiatric problems after traumatic brain injury: unraveling the silent epidemic. *Psychosomatics.* 2009; 50:198–205. [PubMed: 19567758]

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Highlights

- > Characterization of bladder dysfunction after traumatic brain injury
- > There is no significant tissue damage at target area after one month post injury
- > Bladder control is a potential long term clinical issue after traumatic brain injury

Figure 1.

A: Example baseline EUS EMG activity and bursting activity during voiding in a naïve rat.

B: Amplitude and firing rate of EUS EMG activity during filling and voiding in naïve, sham TBI, and TBI groups. Each bar represents mean $\pm$ standard error of the mean of results from each group. * indicates a significant difference compared to naïve at the same point after injury ($p < 0.05$). † indicates a significant difference compared to sham TBI at the same point after injury ($p < 0.05$). Paired Greek letters (α , β , and γ) indicate a significant difference between two groups ($p < 0.05$). TBI, traumatic brain injury; EUS EMG, external urethral sphincter electromyogram.

Figure 2.

A: Example filling cystometry with voiding contraction in a naïve rat. * indicates bladder pressure high frequency oscillation. **B:** Baseline pressure, peak voiding pressure and duration, frequency, and interval of voiding in naïve, sham TBI, and TBI groups. **C:** Example non-voiding contractions during filling cystometry in a TBI rat. * indicates non-voiding contractions with a pressure increase greater than 5 cmH₂O that were included in the quantitative analysis. **D:** Duration, frequency, and pressure increase of non-voiding contractions in naïve, sham TBI, and TBI groups. Each bar represents mean $\pm$ standard error of the mean of results from each group. * indicates a significant difference compared

to naïve at the same point after injury ($p < 0.05$). † indicates a significant difference compared to sham TBI at the same point after injury ($p < 0.05$). Paired Greek letters (α , β , γ , δ , ϵ , and ζ) indicate a significant difference between two groups ($p < 0.05$). TBI, traumatic brain injury.

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Figure 3.

A: Examples of bladders one month after sham TBI and TBI. **B:** Bladder wet weight. * indicates a significant difference compared to naïve one month after TBI ($p=0.006$). † indicates a significant difference compared to sham TBI one month after TBI ($p=0.024$). Each bar represents mean $\pm$ standard error of the mean of results from each group. **C:** Cross-section of example bladders one month after sham TBI and TBI. Magnification: 2 $\times$, 10 $\times$, and 20 $\times$. Stain: Masson's trichrome. **D:** Cross-section of example urethras one month after sham TBI and TBI. Magnification: 4 $\times$, 10 $\times$, and 40 $\times$. Stain: Masson's trichrome. TBI, traumatic brain injury.

Figure 4.

A: Dorsal view of rat skull indicating location of craniotomy. A 4.0-mm midline circular craniotomy was performed 9.0-mm posterior to bregma using a manual trephine. **B:** No significant tissue damage from gross anatomical observations one month after sham TBI or TBI. Circle indicates the location of craniotomy. **C:** Coronal-section of brain at the craniotomy site one month after sham TBI and TBI. Stain: thionin. TBI, traumatic brain injury. Bar = 1mm.