Experimental Neurology 240 (2013) 57–63

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

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

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ARTICLE INFO

Article history:
Received 31 August 2012
Revised 8 November 2012
Accepted 10 November 2012
Available online 21 November 2012

Keywords:
Bladder
Electromyogram
Rat
Traumatic brain injury
Urethra
Urodynamics

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.

Introduction

Traumatic brain injury (TBI) is a common cause of death and disability (NIH Consens. 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, is a common symptom after TBI and can further induce chronic voiding dysfunction, urinary tract infection, skin ulcers, stones, and even renal failure (Chernev and Yan, 2009; Chua et al., 2003; 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, and few studies have investigated the relationship between TBI and bladder dysfunction (Chernev and Yan, 2009; Chua et al., 2003; Moiyadi et al., 2007). Animal models of TBI usually investigate motor function, cognitive function and behavioral assessments (Cernak, 2005; Dixon et al., 1987; Gennarelli, 1994; 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

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http://dx.doi.org/10.1016/j.expneurol.2012.11.012

able to complete all the tests. All animal 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 and 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 (100 mg/kg) and xylazine (10 mg/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, 50 μm diameter teflon-insulated platinum wires were inserted into the urethra bilaterally along the mid-urethra using a 30-gage 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 μm) 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 μm) 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 1 s 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 cmH_2O 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, 1 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 (Figs. 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 (Figs. 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