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Improved voiding function by deep brain stimulation in traumatic brain-injured animals with bladder dysfunctions

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Abstract

Objective Traumatic brain injury (TBI) is a global scenario with high mortality and disability, which does not have an effectual and approved therapy till now. Bladder dysfunction is a major symptom after TBI, and this study deals with the alleviation of bladder function in TBI rats, with the aid of deep brain stimulations (DBS).

Methods TBI was induced by weight drop model (WDM) and standardized with the experimental subjects with variable heights for weight dropping. The rats survived after TBI were considered for bladder dysfunction observations. DBS with variable stimulation parameters like cystometric analysis and MRI studies were also performed.

Results After experimental studies, TBI 2-m-height crash was determined as suitable parameter due to minimal mortality rate and significant reduction in the voiding efficiency from 67 to 28%, whereas DBS significantly reversed the value of voiding efficiency to 65–84%. MRI studies revealed the severity of TBI impact and DBS localization.

Conclusion The results showed profound therapeutic effect of PnO-DBS on voiding functions and bladder control on TBI rats.

Keywords Deep brain stimulations · PnO · Traumatic brain injury · Weight drop model · Cystometric analysis

Introduction

Traumatic brain injury (TBI) remains a devastating and intractable cause of ailment and mortality around the world. The Centre for Disease Control defined TBI as 'injury to the head (arising from blunt or penetrating trauma or from acceleration–deceleration forces) that is associated with symptoms or signs attributable to the injury: reduced level of consciousness, amnesia, other neurological or neuropsychological abnormalities, skull fracture, diagnosed intracranial lesions, or even death' [1].

Population-based studies of TBI from South Africa, India, and Taiwan suggest higher rates in developing countries, predominantly accounted by road-traffic injuries. Evidently, more data are needed to firmly establish incidence and prevalence; nonetheless, TBI remains a significant public health problem throughout the world [2]. TBI induces a wide range of pathological events, largely resulting in a temporary or permanent neurological deficit [3]. Bladder dysfunctions, such as urinary retention and incontinence, are common symptoms after TBI, and it can further induce chronic voiding dysfunction, urinary tract infection, skin ulcers, stones, and even renal failure [4].

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Persistent electrical stimulation of subcortical structures is known as deep brain stimulation (DBS), and it is a safe and effective neuromodulatory therapy for relieving several motor dysfunctions resulting from neurological disorders such as Parkinson's disease [5, 6]. Besides this, the application of DBS extends tremendously in the management of neurological and psychiatric disorders and is also used to manage pain [7], depression [8], epilepsy [9], and Schizophrenia [10]. DBS is carried out by implanting a slender electrode in the brain which electrically stimulates the targeted area. The main mechanism of DBS treatment is recognized as the regulation of axonal output which overrides pathologic network activity [11, 12]. Numerous studies of DBS in recent years clearly reveal the neuroprotective effects and memory enhancement in animals and humans [13, 14].

Most of research works related to TBI model have concentrated on the behavioural studies and motor functions, and some have investigated the bladder function [15, 16]. According to our knowledge, there are few studies related to the bladder function with DBS in TBI animal model. In the present study, the weight drop model (WDM) was used to establish TBI in rats. Due to TBI the animals developed abnormal bladder functions. DBS with different stimulation parameters were used to alleviate the improper bladder functions. Additionally, the cystometric measurement could potentially be used to evaluate recovery and efficacy of DBS treatment after TBI.

Methods

Experimental animals

Forty-nine male Sprague-Dawley rats (experimental subject $n = 37$; normal control $n = 12$) (BioLASCO Taiwan Co., Ltd, Yilan, Taiwan) weighing between 270 and 320 g were utilized for the present experiment. The experimental procedures used in the present study were approved by the Institutional Animal Care and Use Committee (IACUC) of Taipei Medical University (TMU) and followed by the TMU IACUC guidelines to treat animals humanely and reduce animal suffering by use of appropriate anaesthesia and analgesics (IACUC Approval No. LAC-2013-0199).

Standard procedures were followed for the animal maintenance. Rats were housed under 12-h light/dark cycle with food and water available ad libitum and in a temperature humidity-controlled animal centre until experimental use. Furthermore, at the end of the study, all animals were sacrificed by carbon dioxide inhalation followed by cervical dislocation.

Effects of impact height on mortality rate

All the experiments were performed in the day time. Animals were anaesthetized using tiletamine—zolazepam (50 mg/kg, i.p.; Zoletil, Vibac, France) and xylazine (10 mg/kg; Rompun, Bayer, Leverkusen, Germany) (intraperitoneal injection) 30 min prior to impact in order to reduce the suffering and distress during TBI. Marmarou's impact acceleration model was employed for the induction of TBI with minimal modifications [17, 18]; rats were placed on flexible foam and were secured in place by using two elastic belts. A Plexiglas tube was then positioned vertically, and the lower end of the tube was centred on the rat skull vault between the bregma and lambdoid sutures. TBI was induced using a 450-g brass weight falling from different heights as 0.5, 1, 1.5, 2, and 2.25 m through a vertical transparent plexiglass tube. Normal control (NC) and TBI rats underwent the same surgical procedures, but NC rats did not receive weight drop-induced TBI. Body temperature was maintained at 37.0 ± 0.5 °C using an adjustable heating pad during recovery from anaesthesia, and the body temperature was constantly monitored with a rectal probe throughout surgery.

Effects of impact height on cystometric measurement

After 7 days, in order to investigate the bladder dysfunction due to the effect of impact height, cystometric measurements were taken in TBI ($n = 11$) and NC rats ($n = 12$). The animals were anaesthetized by using urethane (1.25 g/kg, subcutaneous). The anaesthetized animal was placed on a heated pad and the urinary bladder and the distal part of the urethra was exposed through an abdominal incision. A polyethylene (PE) tube 60 (1.0 mm ID and 1.5 mm OD) was inserted into the bladder lumen for bladder pressure measurements. The bladder end of the PE tube was heated to form a collar and then passed through a small incision at the apex of the bladder dome. The tube was secured with a purse-string suture, and the abdominal wall was closed with nylon sutures. The PE tube was, in turn, connected via a 3-way stopcock to an infusion pump for filling with physiological saline with the infusion rate of 0.2 mL/min at room temperature and to a pressure transducer for monitoring the intravesical pressure.

Effects of deep brain stimulation on cystometric measurement

On the basis of the data obtained from the experiments of the effects of impact height on mortality rate and cystometric measurement, an optimal TBI animal group with a fixed impact height for the further DBS experiments was

determined. For the implantation of DBS electrode, each rat was placed in a stereotaxic apparatus and the top of its head was shaved and then the midline of the head was cut and scalped. The connective tissue attached to the bone was scraped, and hydrogen peroxide was used to disinfect and clean the bone surface. The bregma point was exposed, and the PnO (AP, − 8.0 mm; L, + 1.0 mm; and DV, − 7.5 mm from the bregma) was located according to the bregma point [19] as shown in Fig. 1a. Moreover, a hole was drilled into the skull, after which a bipolar stimulating electrode (SS80SNE-100, MicroProbes, Gaithersburg, MD, USA) was implanted in the targeted brain point.

The DBS parameter used in this study was fixed at a frequency of 50 Hz; a variation in the current voltage of 1, 1.5, 2 and 2.5 V; and a pulse width of 182 μs was applied to TBI rats. When the bladder pressure exceeded 30 cm H_2O, the duration of brain stimulation was lasted for 10 s. Since it was a biphasic and low intensity pulse, no lesion or seizure occurrence was observed in this study. The cystometric measurement with concomitant DBS interventions was conducted in TBI animals after 1 h post-surgery. The procedures of cystometric measurement were the same as aforementioned.

Magnetic resonance imaging to assess TBI impact and DBS electrode location

To evaluate the TBI impact severity, MRI was performed the next day after TBI induction. Only live animals were subjected to this study ($n = 12$). Three animals ($n = 3$/height range) from each different height range (i.e. 0.5 m, 1 m, 1.5 m, and 2 m) were selected for the study. NC animals ($n = 3$) were also included for this study. For the MRI experiment, rats were first anaesthetized with tiletamine—zolazepam (50 mg/kg, i.p.) and MRI was performed after the rats were placed in the prone position in a 7T Bruker PharmaScan 70/16 US (Bruker Medical System, Karlsruhe, Germany).

To confirm the precise localization of the DBS electrode, Day 7 MRI was performed ($n = 3$) with TBI-DBS rats and similar anaesthetic procedure was followed as above mentioned. In this study, T2-weighted coronal MRI sequences (repetition time/echo time = 4500/80 ms) were used to localize the tip of DBS electrode. To avoid the substantial image artefacts of the DBS electrode in MRI, the tip was withdrawn from the brain tissue before performing the MRI. All MRI data were processed using Paravision 6.0 software (Bruker Medical System).

Statistical analysis

The cystometric analysis was blinded to the status of the rat. Several cystometric parameters were measured to quantify the effects of DBS on bladder voiding function: (1) the micturition volume threshold (VT), defined as the infused volume of saline sufficient to induce the first voiding contraction; (2) the contraction amplitude (CA), defined as the maximum pressure during voiding; and (3) the bladder contraction duration (CD) during voiding. The voiding efficiency (VE) was the ratio of the voided volume (VV) to the VT. The VV was calculated as a VT less than the residual volume (RV) of saline withdrawn through the intravesical catheter after the final voiding contraction. The inter-contraction interval (ICI) defined as the interval between two bladder contractions. All cystometric parameters were calculated using Acknowledge software (Biopac Systems, Inc.). The computed data were compiled in spreadsheets by using Excel (Microsoft, Redmond, WA, USA).

All data were presented as mean ± standard errors. One-way analysis of variance was used for overall comparisons between NC, TBI, and TBI-DBS rats, followed by the Tukey honest significant difference post hoc test performed using SigmaStat (SPSS, Chicago, IL, USA). Individual comparisons in a group were performed using the Student's t test. For all tests, a P value of < 0.05 was considered as statistically significant.

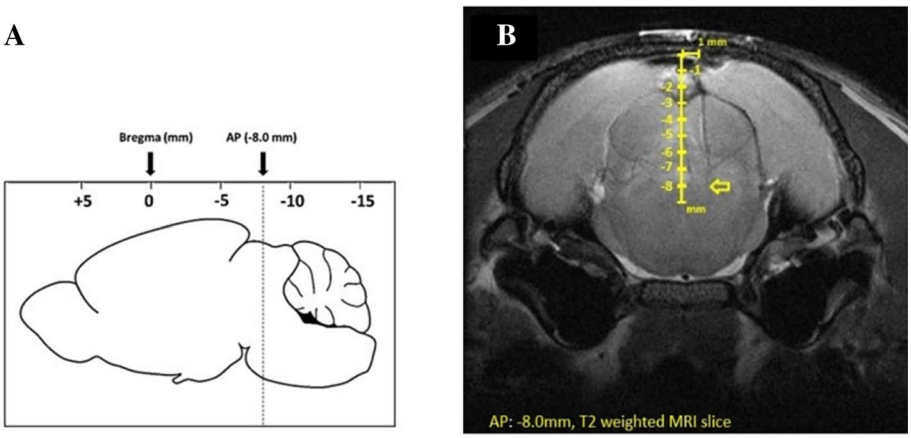

Fig. 1 Example of a T2-weighted coronal image to measure the targeting point of the tip of the deep brain stimulation (DBS) electrode in a TBI rat. **a** The coronal MRI slice at an 8-mm distance posterior to bregma point was acquired. **b** The tip of DBS electrode was exactly located at the rostral pontine reticular nucleus (PnO) (AP, − 8.0 mm; L, + 1.0 mm; and DV, − 7.5 mm). The arrow indicates the electrode tip point

Results

Effects of impact height on mortality rate

The observed results from the Fig. 2 clearly show the effects of impact height on mortality rate in the present experiment. By the WDM for 450 g heavy hammer impact, the mortality rates in the height crash from 0.5 m ($n=4$) and 1 m ($n=4$) level were observed as 0%. Even at the level of 1.5 m ($n=5$), mortality rate of 20% was detected. At the level of 2 m ($n=20$) height crash 45% mortality was observed. Increasing the height beyond this level such as to 2.25 m ($n=4$) resulted in 100% mortality of the rats.

Effects of impact height on voiding efficiency

The effects of height crash reflected on the voiding efficiency of the rats (Table 1). The voiding efficiency gradually decreased when the crash height was increased. From 0.5 m height, the voiding efficiency reduced up to 65% only, whereas at 1.0 m the voiding efficiency reduced to 58%. From the height of 1.5 m, the voiding efficiency was recorded as 56% and no significant difference was observed from the aforementioned values. The height crash from the 2 m showed a significant decrease in the voiding efficiency of 28%. Considering the significance of mortality rates and voiding efficiency, the animals that survived the 2-m-height crash were selected for further study.

Detailed study of cystometric changes in weight drop model

After selecting the 2-m height as the suitable parameter for the WDM, the animals were further subjected for the cystometric study to analyse the changes in the urinary function after TBI. The results tabulated in Table 1 illustrate the comparison between the NC ($n=12$) and 2 m TBI ($n=11$) rats. Since the 2-m TBI rats only demonstrated significant voiding efficiency, other height groups (0.5 m, 1.0 m, and 1.5 m) were not discussed in detail.

The observed results showed that the volume threshold increased (0.67 ± 0.21 c.c) and the contraction amplitude (31.6 ± 5.7 cm-H_2O) decreased after the TBI in rats,

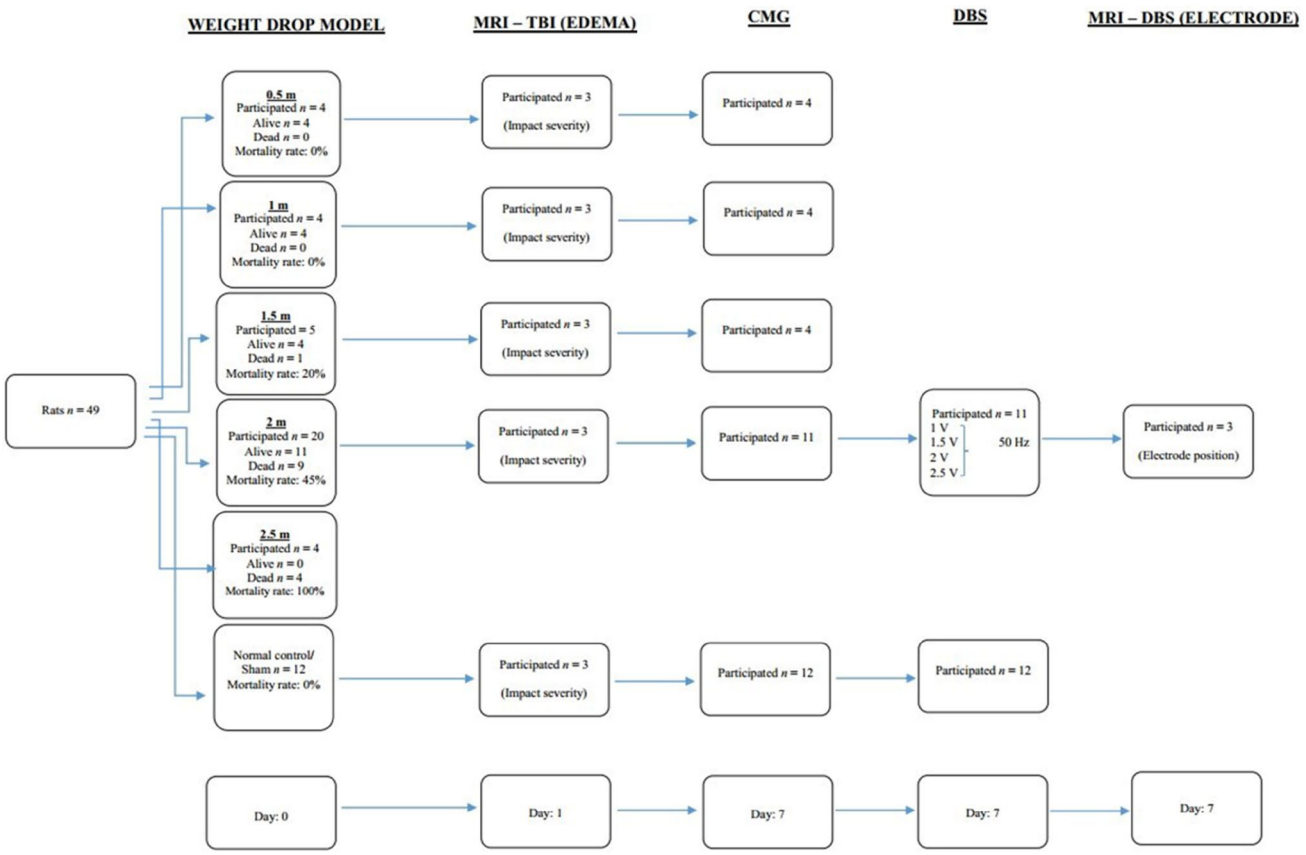

Fig. 2 Showing the study scenario and timescale of the experiments. WDM ($n=49$) was performed on Day 0; cystometric measurement ($n=35$) and DBS ($n=11$) were performed on Day 7. MRI ($n=12$) for TBI (edema); NC ($n=3$) was performed on Day 1 and MRI for TBI-DBS (electrode position) ($n=3$) was performed on Day 7

Table 1 Cystometric measurements between NC rats and TBI rats

	VT (c.c)	CA (cm-H_2O)	CD (sec)	ICI (sec)	RV (c.c)	VV (c.c)	VE %
NC	0.41 ± 0.11	36.4 ± 4.7	22.5 ± 4.3	74 ± 20	0.14 ± 0.04	0.27 ± 0.06	67 ± 2
0.5 m TBI	0.49 ± 0.06	35.1 ± 5.9	22.3 ± 2.0	77 ± 23	0.17 ± 0.04	0.32 ± 0.02	65 ± 4
1.0 m TBI	0.51 ± 0.07	33.2 ± 5.0	21.7 ± 2.0	76 ± 27	0.22 ± 0.07	0.29 ± 0.03	58 ± 8
1.5 m TBI	0.53 ± 0.11	32.4 ± 4.0	20.1 ± 2.1	80 ± 31	0.24 ± 0.07	0.29 ± 0.05	56 ± 4
2.0 m TBI	0.67 ± 0.21	31.6 ± 5.7	17.2 ± 3.8	94 ± 34	0.52 ± 0.03*	0.15 ± 0.02*	28 ± 2*

The symbol "±" represents the standard deviation of variation, and the asterisk (*'s) indicates a significant difference ($p < 0.05$) compared with the NC

NC normal control ($n = 12$), *TBI* traumatic brain injury, TBI: 0.5 m ($n = 04$):TBI: 1.0 m ($n = 04$):TBI: 1.5 m ($n = 04$):TBI 2.0 m ($n = 11$), *VT* volume threshold, *CA* contraction amplitude, *CD* contraction duration, *ICI* inter-contraction interval, *RV* residual volume, *VV* voiding volume, *VE* voiding efficiency = VV/VT

when compared to the NC rats. Besides this, the contraction duration (17.2 ± 3.8 s) also decreased in TBI rats when compared with NC rats. However, no significant difference was observed when compared with the NC rats. The inter-contraction interval (94 ± 34 s) increased after the TBI. Nevertheless, no significance was observed when compared with NC rats. The residual volume level (0.52 ± 0.03 c.c) also increased in TBI rats when compared with the NC rats and there was a significant difference ($p < 0.05$) observed against the NC rats. The voided volume (0.15 ± 0.02 c.c) had remarkably reduced in TBI rats when compared with the NC rats and there was a significant difference ($p < 0.05$) observed against the NC rats. As mentioned above, the voiding efficiency decreased (28 ± 2%) after brain injury in rats and it showed a significant difference ($p < 0.05$) against the NC rats.

Effects of deep brain stimulation on cystometric measurement

DBS was performed in the rats which had underwent the 2-m weight crash and urodynamic analysis. In the present study, DBS was used as a potential tool to improve the efficacy of the bladder function of the TBI rats. Table 2 shows the cystometric measurement changes in PnO-DBS of TBI rats with various electrical stimulation parameters compared with the TBI rats without any stimulation and NC rats. Increased amount of volume threshold (0.67 ± 0.21 c.c) was observed in the TBI rats without stimulation when compared to NC rats (0.41 ± 0.11 c.c). The TBI rats which received serial voltage stimulation also showed an increased volume threshold from 0.70 ± 0.34 c.c to 0.73 ± 0.40 c.c when compared with NC and TBI rats which were not stimulated, and no significance was observed in the volume threshold values in between NC, TBI, and TBI-DBS rats. In TBI rats, the average contraction amplitude (31.6 ± 5.7 cm-H_2O) was reduced when compared to NC rats (36.4 ± 4.7 cm-H_2O). After electrical stimulation in the PnO region of TBI rats, an elevated level of contraction amplitude (35.5 ± 6.9 cm-H_2O to 39.6 ± 5.8 cm-H_2O) with simultaneous increase in electrical stimulation voltage from 1.0 V to 2.5 V was observed, but no significant difference was observed when compared with TBI rats without stimulation and NC rats. The contraction duration was significantly reduced ($p < 0.05$) to about 17.2 ± 3.8 s in TBI rats when compared with NC rats (22.5 ± 4.3 s).

After receiving the electrical stimulations in the PnO region, the TBI rats showed an elevated level of contraction duration of about 23.5 ± 2.2 s to 30.7 ± 3.1 s when compared with the TBI rats which did not receive the electrical stimulation. A significant difference was also observed ($p < 0.05$) when the intensity of stimulation (1.0–2.5 V) increased.

Table 2 Cystometric measurements of PnO-DBS of TBI rats

	VT (c.c)	CA (cm-H_2O)	CD (sec)	ICI (sec)	RV (c.c)	VV (c.c)	VE (%)
50 HZ, 1.0 V	0.73 ± 0.40	35.5 ± 6.9	23.5 ± 2.2*	103 ± 40	0.26 ± 0.02	0.47 ± 0.04	65 ± 3*
50 HZ, 1.5 V	0.70 ± 0.34	38.9 ± 2.5	27.8 ± 4.6*	138 ± 24	0.18 ± 0.03	0.52 ± 0.03	75 ± 1*
50 HZ, 2.0 V	0.64 ± 0.17	39.6 ± 5.8	25.2 ± 5.2*	135 ± 39	0.10 ± 0.03	0.54 ± 0.02	84 ± 3*
50 HZ, 2.5 V	0.67 ± 0.38	39.2 ± 3.6	30.7 ± 3.1*	154 ± 48	0.17 ± 0.03	0.50 ± 0.03	74 ± 1*

The symbol "±" represents the standard deviation of the variation; the pound sign (# 's) indicates a significant difference ($p < 0.05$) from the NC rats; the asterisk (*' s) indicates a significant difference compared to the TBI rats ($p < 0.05$)

VT volume threshold, *CA* contraction amplitude, *CD* contraction duration, *ICI* inter-contraction interval, *RV* residual volume, *VV* voiding volume, *VE* voiding efficiency = VV/VT

The inter-contraction interval in TBI rats was increased to about 94 ± 34 s when compared with NC rats 74 ± 20 s, and the inter-contraction interval time increased in TBI-DBS rats about 103 ± 40 s to 154 ± 48 s and no difference was observed between NC, TBI and TBI-DBS rats. The residual volume was remarkably increased (0.52 ± 0.03 c.c) in TBI rats when compared with NC (0.14 ± 0.04 c.c). In the TBI-DBS rats, residual volume was decreased according to the serial pattern of stimulation parameters (0.26 ± 0.02 c.c to 0.10 ± 0.03 c.c), but there was no significance observed when compared with NC and TBI rats without stimulation. The voided volume was strikingly decreased in TBI rats (0.10 ± 0.03 c.c) when compared to NC rats (0.27 ± 0.06 c.c). The TBI-DBS rats showed remarkable increase in the voided volume according to the serial stimulation pattern (1.0–2.5 V) from 0.47 ± 0.04 c.c to 0.54 ± 0.02 c.c, and no noteworthy difference was observed when compared with NC and TBI rats without stimulation. Voiding efficiency of about 28 ± 2% had significantly reduced in the TBI rats, when compared with NC (67 ± 2%) and it had increased to about 65 ± 3% to 84 ± 3% in TBI rats with PnO electrical stimulation. There was a significant difference observed in each parameter of electrical stimulation from 1 V to 2.5 V. From the observed results, it can be concluded that DBS with 50 Hz, 2 V showed promising values for the voiding efficiency when compared to all other stimulation parameters.

Magnetic resonance imaging (MRI) to assess TBI impact and electrode position

The obtained MRI results clearly show the typical scenario of the TBI with different height ranges. The results were compared with the NC rats ($n = 3$) as shown in Fig. 4a. From the height range of 0.5–2 m, the respective Fig. 3b–e clearly depicts the cerebral edema and intracranial haemorrhage at the areas near to cerebral cortex due to heavy hammer impact of TBI induction. Thus, these MRI results indicated that the WDM can be feasible for the induction of severe TBI in rats from 2-m height.

To further confirm whether the DBS electrode had precisely reached the stimulation targets accurately and consistently, MRI examinations were additionally conducted in TBI-DBS rats ($n = 3$). The MRI results of NC (Fig. 4b) T2-weighted coronal image show that the tip of DBS electrode was exactly located at PnO point (AP, − 8.0 mm; L, + 1.0 mm; and DV, − 7.5 mm from the bregma) in all three TBI rats, and thus the accuracy rate for reaching the PnO was 100%. Thus, our stereotaxic coordinate technique was reliable for localization of the stimulation targets.

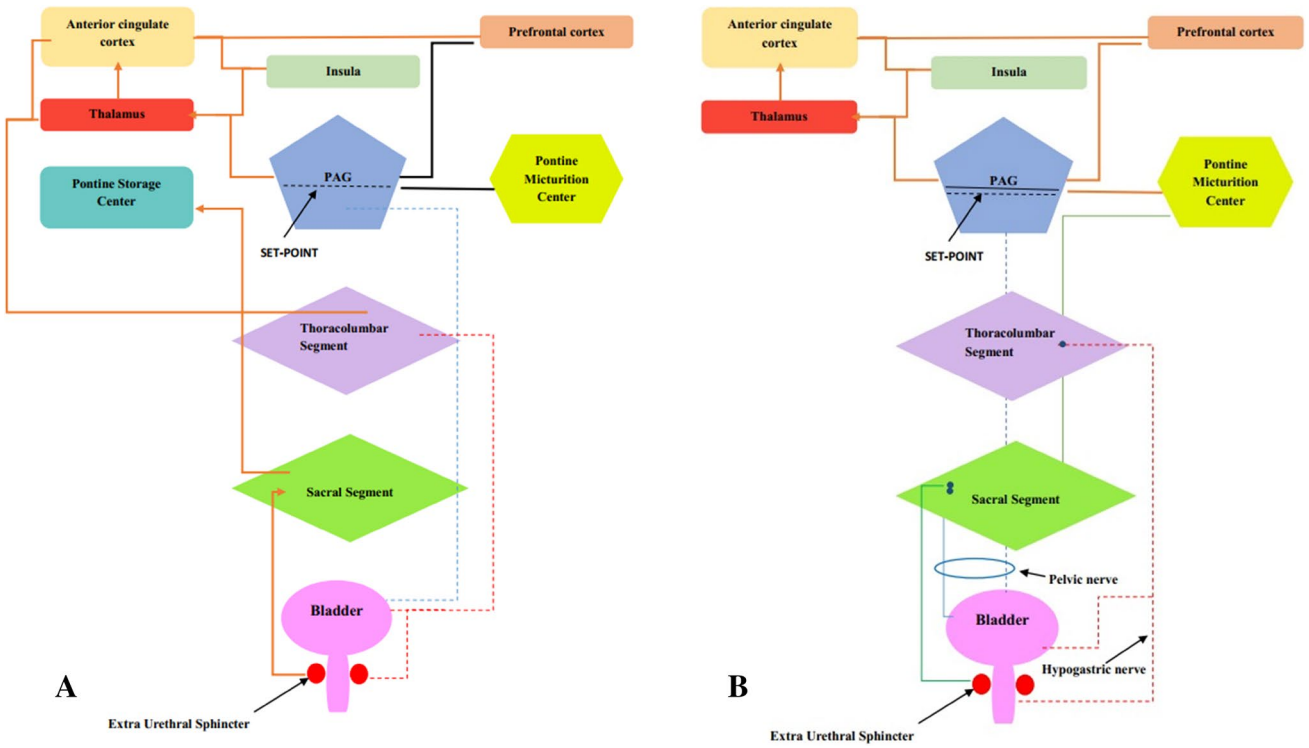

Fig. 3 Micturition circuit and the interlink between the central nervous system and lower urinary tract. **a** The schematic representation of late storage mode, thick lines show the excitatory state of signals. **b** The schematic representation of early voiding state, the dotted lines show the inhibitory state of signals

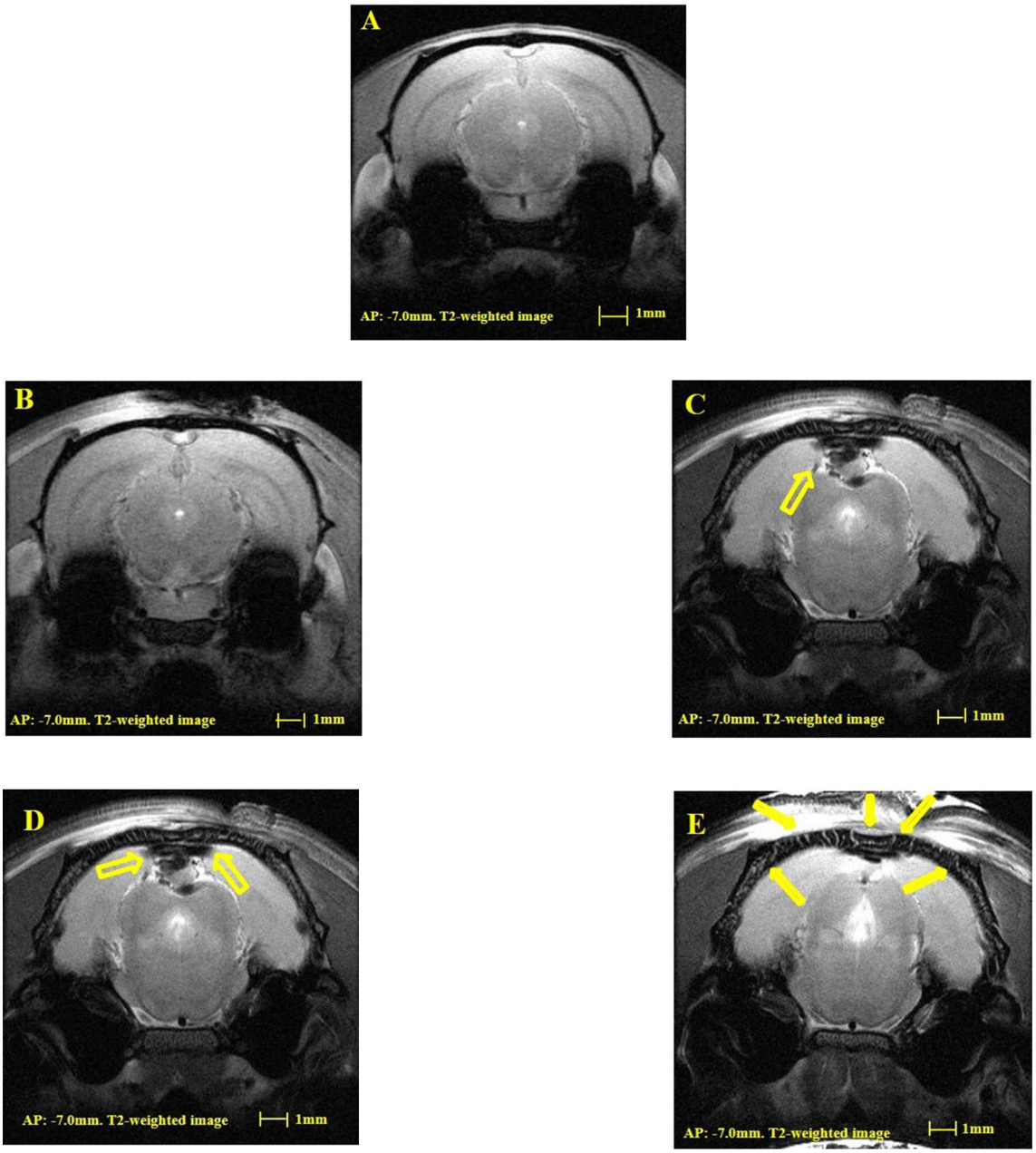

Fig. 4 **a** T2-weighted MRI of normal control (NC), **b** T2-weighted MRI of TBI from 0.5 m, **c** T2-weighted MRI of TBI from 1 m, **d** T2-weighted MRI of TBI from 1.5 m. **e** T2-weighted MRI of TBI from 2 m. Arrows shows the severity of TBI and cerebral edema

Discussion

The weight drop-induced TBI in rats has been extensively involved in various neurological studies [20]. Furthermore, the literature pertaining to the time-course variations in neurobehavioral functions and pathophysiological processes following TBI may provide a vision into the fundamental pathophysiology of the disease for imminent diagnostic purposes and therapeutic applications. The detailed relationship between the severity of brain damage and bladder dysfunction along with DBS has not been clearly understood. According to the literature, very few studies have been performed related to TBI and voiding dysfunction. The present study slightly deviated from our previous study [6] in modifying the TBI induction by introducing different range of height and ensuring animal survival with significant voiding dysfunction for the study. In addition, the DBS parameters and stimulation strategies in the present study showed a better understanding when compared to other studies. Previous studies have demonstrated that the TBI model

was successful, [17, 18]; however, we have improvised and added a range of heights to determine the sustainability and low mortality rate in TBI model. It has been reported that the impact velocity will cause the change in injury at different levels and types [21, 22].

To determine the suitable model for TBI, WDM was used from different heights range from 0.5 to 2.25 m. Hence, it is evident from Fig. 5 that the increase in the height is directly proportional to the decrease in the voiding efficiency. Likewise, from the height range of 0.5 and 1 m shows a mortality rate of 0% and no significant change in voiding efficiency. At the height of 1.5 m, 20% mortality rate was observed but no significant change in voiding efficiency was observed. Only at the height range of 2 m, 45% of mortality rate was observed; besides this a significant decrease in voiding efficiency was observed. Beyond this level at the height range of 2.25 m, 100% of mortality rate was observed. It is evident that dropping weight from a height of 2 m and above caused a severe level of TBI [18]. The position of the weight drop causes a unilateral skull fracture and a cortical cavity encircled by a pericontusional injury. Modifying the height of the fall will result in variable degrees of injury.

Even though less controlled compared to other models, this pattern has proven easily accessible [21, 23] and it can be reproducible in various laboratories and can be used for preclinical testing of therapeutics. Variety of experimental techniques have been developed for TBI in various literatures [24–29], even though the present study had proved that the 2-m height induced the controlled damage to the experimental subject and resulted in increased survival rate of the animal along with a significant range of urinary dysfunction.

The micturition circuit entirely depends on the central nervous system, and any damage to the brain or spinal cord will definitely affect the entire urinary system. In normal conditions, the bladder switches automatically from storage phase to voiding phase mainly according to the signal

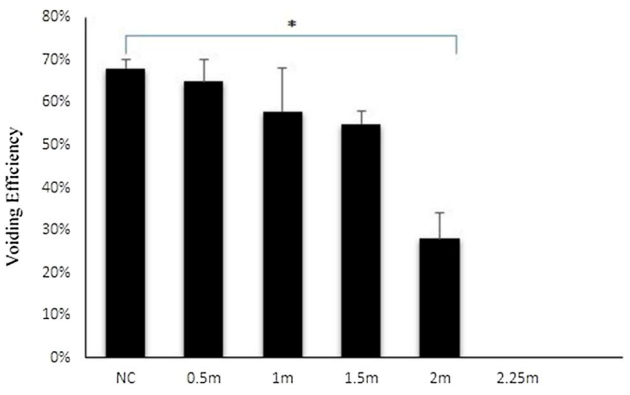

Fig. 5 Normal control rats (NC) ($n=12$) and TBI ($n=23$) rats challenged with 450 g of weight in variable heights showing the voiding efficiency (*$p<0.05$)

received from the central nervous system which includes brain and spine. In the micturition circuit (Fig. 3a, b), at the storage phase, afferent signals are sent to the spinal cord, and from here the signals ascend to periaqueductal grey area (PAG). PAG distributes the signal to higher brain centre called insula thru thalamus. The condition of the bladder filling is encoded here, and then, insula sends the signal to anterior cingulate cortex (ACC). ACC interprets the accurate state of the bladder condition, and it also controls the desire to void and emotional response to bladder filling. The desire to void does not instantly lead to urination, as it is controlled by prefrontal cortex (PFC). It usually suppresses the voiding behaviour until the socio-emotional situation is acceptable for voiding. Pontine storage centre (PSC) also plays a major role to stimulate striated urethral sphincter activity. Collectively these signals elicit the spinal reflex to inhibit voiding. After the bladder is full, the final decision will be mediated by medial PFC (mPFC) to void. In the default mode, PFC suppresses voiding, but in the bladder filled condition, mPFC is deactivated with decreased signal to PAG. The decreased signal to PAG signals from bladder to elicit the activation of Pontine Micturition Center (PMC) located in the medial part of the pons. PMC inhibits the sympathetic outflow of the bladder thru parasympathetic nucleus located in the spinal cord. Voiding can be initiated by input to the bladder detrusor muscle contraction and urethral outlet relaxation mediated by the parasympathetic nerve. The urine flow is constantly sustained through the urethra by a secondary reflex in spinal cord, and it uninterruptedly increases further bladder emptying [30].

Rodents are commonly used in the cystometric studies [31, 32]. Numerous specific techniques have been developed and employed, including acute and subacute suprapubic and acute transurethral catheterization. Micturition in rodents is due to serial activation of reflexes whose coordination is essential for efficient voiding. Cystometric recording techniques in awake or anaesthetized rats provide valuable tools for analysing the neural control of the urinary bladder [33]. Clinically, the major and common urinary symptoms after TBI are overactive bladder or urge incontinence [34]. An important centre with an inhibitory action on the bladder is found beneath the frontoparietal cortex. When this centre is no longer intact, detrusor hyperreflexia occurs. Two pontine micturition centres were distinguished, one for bladder contraction and one for bladder relaxation, which have a reciprocally inhibitory effect. In addition, they also affect the anterior horn cells of the sphincter and pelvic floor, so that the pons regulates coordination between detrusor and sphincter [35, 36].

In the present study, initially cystometric measurements were taken between NC rats and TBI rats (Table 1) and in that 2-m-height crash rats showed a better survival rate and significant voiding efficiency, so 2-m TBI animals

were compared with NC animals and discussed. From the observed results, it was obvious that the volume threshold values increased and the contraction amplitude values decreased after TBI in rats when compared to NC rats This condition was mainly due to the TBI damage of the supra-pontine region of the rats so, the sphincter and detrusor continued to react in a coordinated fashion, i.e. detrusor contraction was accompanied by sphincter relaxation, resulting in incontinence [37, 38]. From the current observations, a decrease in the contraction duration was observed in the TBI rats against the NC rats but no significance was observed against the NC animals. The development of urinary retention after TBI showed that the normal functioning system failed to respond to the increasing bladder volume. Impaired detrusor contractility (IDC) may also be a reason for this, which resulted in a large dilated bladder condition called as an atonic bladder and thus reduced voiding function due to innervation or chronic obstruction. In this condition, the bladder could not achieve complete emptying within a normal time span due to loss of strength and/or duration which resulted in prolonged bladder emptying [39]. Besides this, due to the intact sphincter tone, urinary retention persisted until larger bladder volumes were reached. After reaching certain level the bladder pressure and the bladder wall stress overcame the obstacle to maintain the urinary flow which was made by the sphincter muscle [3].

In the present study, the TBI rat shows that the residual volume was remarkably and significantly increased in TBI rats when compared with NC rats. Similar condition was observed in the patients with spinal cord injury, and it was documented that this condition was mainly due to increased risk of upper urinary tract dilation and renal insufficiency [40]. The urinary excretion efficiency was decreased after brain injury in rats, and this showed a significant difference against the NC rats. This specific urodynamic scenario was characterized by loss of voluntary control over micturition, disturbed bladder perception, and poor sphincter control [34].

The significant decrease in the voided volume of the TBI rats when compared with the NC rats showed that the bladder activity and urethral activity were remarkably decreased instantly after TBI. This condition was mainly due to electroencephalographic suppression and loss of muscle tone. Besides this voiding was associated with several parts of the brain such as motor cortex, somatosensory cortex, cingulate cortex, retrosplenial cortex, thalamus, putamen, insula, and septal nucleus [4]. Heavy impact of the TBI caused a damage in this area and that might have directly affected the bladder function and reduced the voiding volume in the rats instantly after the TBI.

A number of electrical neuromodulations have been established to treat patients with chronic voiding dysfunction. It has been reported that the sacral nerve root neuromodulation has been successfully used in spinal cord-injured patients with neurogenic voiding dysfunction. It was mainly used to increase bladder capacity and compliance and improve the voiding efficiency [41, 42]. In 1987 Benabid et al. [43] discovered the modern era of DBS through his pioneer works in the field of Parkinson's disease. They reported that electrical stimulation of functional targets was able to elicit a greater response for movement disorders [44] While the fact of fundamental therapeutic mechanism of DBS still remains mysteriously divisive, it has been extensively used for the management of a large spectrum of neurological disorders [45]. The underlying pathophysiology of TBI bladder dysfunction is often multifactorial, including damage to the brain areas that coordinate normal micturition, communication deficits, and cognitive impairment. The location and extent of the brain injury also correlated with the type and severity of bladder dysfunction. The rats received various stimulation intensities of DBS, and the results were interesting (Table 2) compared with the TBI rats which did not receive the stimulations and NC rats. The TBI rats which received serial voltage stimulation also showed an increased volume threshold when compared with NC and TBI rats which were not stimulated and no significance was observed in the volume threshold values in between the NC, TBI, and TBI-DBS rats. The volume threshold was mainly determined by the peripheral neurogenic afferent activity under CNS control. The micturition reflux was triggered by the increasing volume in the bladder. Usually in a condition of mechanical trauma of the afferent receptors and nerves provide the desire to void. Eventually, insufficient afferent activation in the central nervous system efferent nerve fibres will end in impaired detrusor contractility [46]. Perhaps the DBS voltage variations might be insufficient to activate the above described mechanism which results in increased volume threshold in the TBI-DBS rats.

According to the cystometric results of our TBI model, the decrease in the voiding efficiency in TBI rats can be attributed to the decrease in the contraction duration and contraction amplitude (Table 2) this was a symptom of IDC, which resulted in atonic bladder and thus reduced voiding function. IDC as a bladder contraction of reduced strength and/or duration resulted in prolonged bladder emptying and/or failure to achieve complete bladder emptying within a normal time span [47]. In contrast, the conduction of DBS in TBI rats significantly increased the VE, respectively, to the increase in the voltage because of an improvement in the detrusor contraction force, which was evident by an increase in the cystometric parameters of the contraction duration and contraction amplitude consistently at higher stimulation intensities of 1.0–2.5 V. It was also observed that in the off condition while DBS showed a typical sign of detrusor

hyperreflexia presenting as an "overactive bladder" with a markedly reduced initial desire to void and reduced bladder capacity [48].

The inter-contraction interval of the TBI PnO stimulated rats showed and elevated patterns of readings according to the DBS with the differential type of voltage (1.0–2.5 V) when compared with the NC and TBI rats which did not receive the stimulations. The increase in the inter-contraction interval indicated the efficient bladder evacuation which was interrelated to the bladder contraction or contraction duration. Similar results were observed in the pulsed radio frequency pelvic nerve stimulated rats [49]. Furthermore, to substantiate the above results subthalamic DBS stimulations showed a promising result in neural regulation of bladder function which could normalize the non-voiding function [48].

In contrast, in the TBI-DBS rats, the residual volume was decreased according to the serial pattern of stimulation parameters when compared with the non-stimulated TBI rats and was in very close range to the NC rats even though no significance was observed when compared with NC and TBI rats. Usually urine flow attained its maximum level during the plateau period of the bladder contraction under high frequency stimulations. The burst of sphincter activity reflected back in to the bladder as oscillations in bladder pressure and this triggered the firing of bladder afferents and made the bladder emptying process most efficient [50]. Due to the efficient bladder emptying process, the residual volume of the TBI-DBS rats showed reduced levels of residual volume.

Interestingly the voided volume decreased in TBI rats compared to NC rats, and the TBI-DBS rats showed remarkable increase in the voided volume according to the serial stimulation pattern. However, no significance was observed when compared with NC and TBI rats without stimulation. Studies of functional magnetic resonance tomography had revealed that micturition was accompanied by an activation of prefrontal and cingular cortex areas, and these areas were modulated during the urinary storage and voiding. The subthalamic nucleus stimulations inhibited the cortical areas. The blockage of this disinhibition would enhance the bladder control more efficiently. DBS on the subthalamic region acted on bladder function through descending pontine pathways [48]. Nevertheless, this hypothesis was determined by further studies. Despite the fact that the DBS might be the major reason for the increased voided volume in the TBI rats. Obtained results from the present study show that 50 Hz with 2 V contributes the best voiding efficiency of 85% when compared to other TBI-DBS animals.

Numerous studies have reported that DBS induced multiphasic responses on target neurons, including excitation and inhibition and the outcome of DBS mainly depended on the competition of excitatory and inhibitory signals that were generated [6]. Increasing the stimulation amplitude of DBS may alter the balance of excitatory and inhibitory signals. Therefore, the augmentation of the detrusor voiding reflex through DBS at the PnO site might be attributable to the direct or indirect modulation of the neural circuit from the central brain nucleus to the pelvic efferent pathway [51].

Induction of the TBI is the major limitation in the present study. We have used the WDM for the induction the TBI instead of lateral fluid percussion impact model (LFPI) [3]. Even though LFPI model rapidly induced severe and transient lower urinary tract dysfunctions, we chose WDM due to the favourable results of the MRI showing significant brain damage over the cerebral cortex area. Furthermore, present study focused on TBI which could mimic fall or motor cycle road accidents. Apart from this, the present study mainly emphasized voltage dependent stimulations connected with neuromodulatory effects of bladder dysfunction. So, we have not incorporated the study in to cellular level. Certainly there must be a difference in the voiding efficiency between anaesthetized rats and free moving rats. However, the obtained results from stimulated rats showed significant effects on voiding efficiency when compared to NC rats. Hence forth, further studies should be warranted to overcome the current limitations in the present study.

Conclusions

This study is a novel attempt in WDM with variable height ranges to establish a typical TBI model with a significant voiding dysfunction. This is the first voltage dependent study according to our knowledge that proved the fact that Pno-DBS had a significant effect on the voiding function in TBI rats. Our study results showed deep insights of DBS on the neural regulation of voiding function leading to normalization of these abnormal urodynamic values. This might indicate feasibility for a profound therapeutic effect of PnO-DBS on voiding functions.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

Ethical approval This work was approved and conducted under the ethical guidance of Institutional Animal Care and Use Committee

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Research involving human and animal participants This article does not contain any studies with human participants performed by any of the authors.

References

1. Finan DJ (2018) Biomechanical simulation of traumatic brain injury in the rat. Clin Biomech. https://doi.org/10.1016/j.clinbiomech.2018.01.015
2. Corrigan DJ, Selassie WA, Orman AJ (2010) The epidemiology of traumatic brain injury. J Head Trauma Rehabil 25:72–80. https://doi.org/10.1097/HTR.0b013e3181ccc8b4
3. Moody JB, Liberman C, Zvara P, Smith PP, Freeman K, Zvarova K (2014) Acute lower urinary tract dysfunction (LUTD) following traumatic brain injury (TBI) in rats. Neurourol Urodyn 33:1159–1164. https://doi.org/10.1002/nau.22470
4. Jiang HH, Kokiko-Cochran NO, Li K, Balog B, Lin CY, Damaser SM, Lin V, Cheng JY, Lee YS (2013) Bladder dysfunction changes from underactive to overactive after experimental traumatic brain injury. Exp Neurol 240:57–63. https://doi.org/10.1016/j.expneurol.2012.11.012
5. Miocinovic S, Lempka FS, Russo SG, Marks BC, Buston CR, Sakaie KE, Vitek JL, Mclntyre CC (2009) Experimental and theoretical characterization of the voltagedistribution generated by deep brain stimulation. Exp Neurol 216(1):166–176. https://doi.org/10.1016/j.expneurol.2008.11.024
6. Jen E, Lin WC, Hsieh HT, Chiu YC, Lu TC, Chen SC, Chen MC, Peng CW (2007) Prototype deep brain stimulation system with closed-loop control feedback for modulating bladder functions in traumatic brain injured animals. J Med Biol Eng 38(3):337–349. https://doi.org/10.1007/s40846-017-0326-1
7. Kringelbach ML, Jenkinson N, Green AL, Owen SLF, Hansen PC, Cornelissen PL, Holliday IE, Stein J, Aziz TZ (2007) Deep brain stimulation for chronic pain investigated with magnetoencephalography. Neuro Rep 18(3):223–228
8. Anderson RJ, Frye MA, Abulseoud OA, Lee KH, McGillivray JA, Berk M, Tye SJ (2012) Deep brain stimulation for treatment-resistant depression: efficacy, safety and mechanisms of action. Neurosci Biobehav Rev 36:1920–1933. https://doi.org/10.1016/j.neubiorev.2012.06.001
9. Salanova V (2018) Deep brain stimulation for epilepsy. Epilepsy Behav. https://doi.org/10.1016/j.yebeh.2018.06.041
10. Frederick C, Nucifora E, Woznica BJ, Lee N, Cascella A, Sawa (2018) Treatment resistant schizophrenia: clinical, biological, and therapeutic perspectives. Neuro Biol Dis. https://doi.org/10.1016/j.nbd.2018.08.016
11. Montgomery EB Jr, Baker KB (2000) Mechanisms of deep brain stimulation and future technical developments. Neurol Res 22(3):259–266. https://doi.org/10.1080/01616412.2000.11740668
12. Vitek JL (2002) Deep brain stimulation for Parkinson's disease a critical re-evaluation of STN versus GPi DBS. Stereotact Funct Neurosurg 78:119–131. https://doi.org/10.1159/000068959
13. Wallace BA, Ashkan K, Heise CE, Foote KD, Torres N, Mitrofanis J, Benabid AL (2007) Survival of midbrain dopaminergic cells after lesion or deep brain stimulation of the subthalamic nucleus in MPTP-treated monkeys. Brain 130(8):2129–2145. https://doi.org/10.1093/brain/awm137
14. Veerakumar A, Berton O (2015) Cellular mechanisms of deep brain stimulation: activity-dependent focal circuit reprogramming? Curr Opin Behav Sci 1(4):48–55. https://doi.org/10.1016/j.cobeha.2015.02.004
15. Schiff ND, Giacino JT, Kalmar K, Victor JD (2007) Behavioural improvements with Thalamic stimulation after severe traumatic brain injury. Nature 448:600–603. https://doi.org/10.1038/nature06041
16. Cernak L (2005) Animal models of head trauma. NeuroRX 2(3):410–422. https://doi.org/10.1602/neurorx.2.3.410
17. Marmarou A, Foda MAAE, van den Brink W, Campbell J, Kits H, Demetriadou K (2010) A new model of diffuse brain injury in rats part I: pathophysiology and biomechanics. J NeuroSurg 112(2):291–300
18. Foda MAAE, Marmarou A (1994) A new model of diffuse brain injury in rats: part II: morphological characterization. J Neurosurg 80(2):301–313
19. Noto H, Roppolo J, Steers W, De Groat W (1989) Excitatory and inhibitory influences on bladder activity elicited by electrical stimulation in the pontine micturition center in the rat. Brain Res 492(1):99–115. https://doi.org/10.1016/0006-8993(89)90893-7
20. Goddeyne C, Nichols J, Wu C, Anderson T (2015) Repetitive mild traumatic brain injury induces ventriculomegaly and cortical thinning in juvenile rats. J Neurophysiol 113:3268–3280. https://doi.org/10.1152/jn.00970.2014
21. Hsieh TH, Kang JW, Lai JH, Huang YZ, Rotenberg A, Chen YK, Wang JY, Chan SY, Chen SC, Chiang YH, Peng CW (2017) Relationship of mechanical impact magnitude to neurologic dysfunction severity in a rat traumatic brain injury model. PLoS ONE 12(5):e0178186. https://doi.org/10.1371/journal.pone.0182300
22. Chen QJ, Zhang CC, Lu H, Wang W (2014) Assessment of traumatic brain injury degree in animal model. Asian Pac J Trop Med 7(12):991–995. https://doi.org/10.1016/S1995-7645(14)60174-7
23. Shohami E, Ginis I, Hallenbeck JM (1999) Dual role of tumor necrosis factor alpha in brain injury. Cytokine Growth Fact Rev 10(2):119–130. https://doi.org/10.1016/S1359-6101(99)00008-8
24. Denny-Brown DE, Russell RW (1941) Experimental concussion. Proc R Soc Med 34(11):691–692. https://doi.org/10.1152/physrev.1945.25.2.296
25. Lindgren S, Rinder L (1996) Experimental studies in head injury. Biophysik 3(2):174–180. https://doi.org/10.1007/BF01191611
26. Ommaya KA, Grennarelli AT (1974) Cerebral concussion and traumatic unconsciousness correlation of experimental and clinical observations on blunt head injuries. Brain 97(1):633–654. https://doi.org/10.1093/brain/97.1.633
27. Sullivan GH, Martinez J, Becker PD, Miller JD, Griffith R, Wist AO (1976) Fluid-percussion model of mechanical brain injury in the cat. J Neurosurg 45:520–534
28. Nilsson B, Ponten U, Voigt G (1977) Experimental head injury in the rat Part 1: mechanics, pathophysiology, and morphology in an impact acceleration trauma mode. J Neurosurg 47:241–251
29. Dixon EC, Lyet GB, Povlishock TJ, Findling RL, Hamm RJ, Marmarou A, Young HF, Hayes RL (1989) A fluid percussion model of experimental brain injury in the rat. J Neurosurg 67:110–119
30. Jang HJ, Kwon MJ, Cho KO (2018) Central regulation of micturition and its association with epilepsy. Int Neurourol J. https://doi.org/10.5213/inj.1836040.020
31. McMurray G, Casey HJ, Naylo MA (2006) Animal models in urological disease and sexual dysfunction. Brit J Pharmacol 147(S2):S62–S79. https://doi.org/10.1038/sj.bjp.0706630
32. Fry CH, Meng E, Young JS (2010) The physiological function of lower urinary tract smooth muscle. Auton Neurosci Basic Clin 154(1–2):3–13. https://doi.org/10.1016/j.autneu.2009.10.006
33. Andersson EK, Soler R, Füllhase C (2011) Rodent models for urodynamic investigation. Neurol Urodyn 30(5):636–646. https://doi.org/10.1002/nau.21108

34. Oostra K, Everaert K, Laere V, M (1995) Urinary incontinence in brain injury. Brain Inj 10(6):459–464. https://doi.org/10.1080/026990596124313
35. Khan Z, Hertanu J, Yang WC, Melman A, Leiter E (1981) Predictive correlation of urodynamic dysfunction and brain injury after cerebrovascular accident. J Urol 126(1):86–87. https://doi.org/10.1016/S0022-5347(17)54392-X
36. Khan Z, Starer P, Yang WC, Bhola A (1990) Analysis of voiding disorders in patients with cerebrovascular accidents. Urology 35(3):265–270. https://doi.org/10.1016/0090-4295(90)80048-R
37. Wyndaele JJ, Meyer MJ, Sy AY, Claessens H (1986) Intracavernous injection of vasoactive drugs, an alternative for treating impotence in spinal cord injury patients. Paraplegia 24:271–275. https://doi.org/10.1038/sc.1986.37
38. Blaivas GJ (1982) The neurophysiology of micturition: a clinical study of 550 patients. J Urol 127(5):958–963. https://doi.org/10.1016/S0022-5347(17)54147-6
39. Kadow TB, Tyagi P, Chermansky JC (2015) Neurogenic causes of detrusor underactivity. Cur Blad Dysfunct Rep 10(4):325–331. https://doi.org/10.1007/s11884-015-0331-6
40. Kelly EC (2004) Evaluation of voiding dysfunction and measurement of bladder volume. Rev Urol 6(Suppl 1):S32–S37
41. Krasmik D, Krebs J, Ophoven VA, Pannek J (2014) Urodynamic results, clinical efficacy, and complication rates of sacral intradural deafferentation and sacral anterior root stimulation in patients with neurogenic lower urinary tract dysfunction resulting from complete spinal cord injury. Neurourol Urodyn 33:1202–1206. https://doi.org/10.1002/nau.22486
42. Chew DJ, Zhu L, Delivopoulos E, Minev IR, Musick KM, Mosse CA, Craggs M, Donaldson N, Lacour SP, McMahon SB, Fawcett JW (2013) A microchannel neuroprosthesis for bladder control after spinal cord injury in rat. Sci Transl Med. https://doi.org/10.1126/scitranslmed.3007186
43. Benabid AL, Pollak P, Louveau A, Henry S, De Rougemont J (1987) Combined (thalamotomy and stimulation) stereotactic surgery of the VIM thalamic nucleus for Bilateral Parkinson disease. Appl Neurophysiol 50:344–346. https://doi.org/10.1159/000100803
44. Benabid AL, Pollak P, Gao D, Hoffmann D, Limousin P, Gay E, Payen I, Benazzouz A (1996) Chronic electrical stimulation of the ventralis intermedius nucleus of the thalamus as a treatment of movement disorders. J Neuro Surg 84:203–214
45. Chen CS, Grill MW, Fan JW, Kou YR, Lin YS, Lai CH, Peng CW (2011) Bilateral pudendal afferent stimulation improves bladder emptying in rats with urinary retention. Br J Urol 109(7):1051–1058. https://doi.org/10.1111/j.1464-410X.2011.10526.x
46. Klevmark B (2002) Volume threshold for micturition. Infuence of filling rate on sensory and motor bladder function. Scand J Urol Nephrol 210:6–10. https://doi.org/10.1080/003655902320765890
47. Kadow TB, Tyagi P, Chermansky JC (2015) Neurogenic causes of detrusor underactivity. Curr Blad Dysfunct Rep 10(4):325–331. https://doi.org/10.1007/s11884-015-0331-6
48. Seif C, Herzog J, Horst CV, Schrader B, Volkmann J, Deuschl G, Juenemann, Braun PM (2004) Effect of subthalamic deep brain stimulation on the function of the urinary bladder. Ann Neurol 55(1):118–120. https://doi.org/10.1002/ana.10806
49. Jen E, Heish HT, Lu CT, Chen CM, Lee FJ, Lin TC, Chen SC, Chu PY, Peng CW, Lin CW (2016) Effects of pulsed radio frequency neuro modulations on the rat with over reactive bladder. Neurol Urodynamic 36(7):1734–1741. https://doi.org/10.1002/nau.23183
50. Kruse NM, Noto H, Roppollo JR, Groat de W C (1990) Pontine control of the urinary bladder and external urethral sphincter in the rat. Brain Res 532(1–2):182–190. https://doi.org/10.1016/0006-8993(90)91758-9
51. Fan WJ, Li TY, Chen JJJ, Chen SC, Lin YS, Kou YR, Peng CW (2013) Sexually dimorphic urethral activity in response to pharmacological activation of 5-HT1A receptors in the rat. Am J Physiol Renal Physiol 305(9):F1332–F1134. https://doi.org/10.1152/ajprenal.00261.2013