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Improvement of lower urinary tract function by a selective serotonin 5-HT_{1A} receptor agonist, NLX-112, after chronic spinal cord injury

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Abstract

Spinal cord injury (SCI) above the lumbosacral level results in lower urinary tract dysfunction, including (1) detrusor hyperreflexia, wherein bladder compliance is low, and (2) a lack of external urethral sphincter (EUS) control, leading to detrusor-sphincter dyssynergia (DSD) with poor voiding efficiency. Experimental studies in animals have shown a dense innervation of serotonergic (5-HT) fibers and multiple 5-HT receptors in the spinal reflex circuits that control voiding function. Here, we investigated the efficacy of NLX-112 (a.k.a. befiradol or F13640), in regulating lower urinary tract function after T8 contusive SCI in rats. NLX-112 is a very potent, highly-selective, and fully efficacious 5-HT_{1A} receptor agonist, which has been developed for the treatment of L-DOPA-induced dyskinesia in Parkinson's disease patients. We performed urodynamics tests and external urethral sphincter electromyogram recordings to assess lower urinary tract function while NLX-112 was infused through the femoral vein in rats with chronic complete SCI or contusive SCI. The dose response studies indicated that NLX-112 was able to improve voiding behavior by regulating both detrusor and EUS activity. These included improvements in voiding efficiency, reduction of detrusor hyperactivity, and phasic activity of EUS during the micturition period. In addition, the application of a selective 5-HT_{1A} receptor antagonist, WAY100635, reversed the improved detrusor and EUS activity elicited by NLX-112. In summary, the current data suggest that pharmacological activation of 5-HT_{1A} receptors by NLX-112 may constitute a novel therapeutic strategy to treat neurogenic bladder after SCI.

Keywords

spinal cord injury; bladder; external urethral sphincter; 5-HT_{1A} receptor agonist

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1. Introduction

Depending upon the level of the injury, spinal cord injury (SCI) results in functional deficiencies, with differing consequences for the autonomic system, including lower urinary tract (LUT) dysfunction (de Groat and Yoshimura, 2006). Urine storage and release are highly dependent upon central nervous system (CNS) pathways, which are modulated by multiple excitatory and inhibitory neurotransmitters including norepinephrine, gamma-aminobutyric acid (GABA), glutamate, serotonin (5-HT), enkephalin, and corticotrophin releasing factor (Yoshimura et al., 2014). Thus, neurologic mechanisms are an important consideration in the diagnosis and treatment of voiding disorders. Along with other parts of the autonomic nervous system, for example heart, vasculature, and airways (Ramage, 2001), central 5-HT receptors have been implicated in the control of micturition (de Groat, 2002). The 5-HT_{1A} receptor is the most plastic and most highly-characterized spinal 5-HT receptor, and it modulates a number of different cellular processes in the spinal cord. 5-HT_{1A} receptors can increase spinal motor neuron excitability pre-synaptically in neonatal rats by modulating the amount of transmitter released (Wu et al., 2002), while cell excitability can be decreased post-synaptically by inhibiting adenylate cyclase activity through a G_i protein in fetal mouse spinal cord ganglion explants (Makman et al., 1988). In adult human spinal cord, there is evidence showing dense expression of 5-HT_{1A} receptors in both thoracic and lumbar segments (Perrin et al., 2011). In spinally-intact rats, 5-HT_{1A} receptor agonists increase and 5-HT_{1A} receptor antagonists decrease urinary frequency through actions at both the supraspinal and spinal levels (Kakizaki et al., 2001; Testa et al., 1999). In addition, the 5-HT_{1A} receptor agonist 8-OH-DPAT can improve voiding efficiency by increasing micturition volume, decreasing residual volume, and increasing the bursting activity of external urethral sphincter (EUS) in urethane-anesthetized rats with complete SCI (Chang et al., 2007; Cheng and de Groat, 2010; Dolber et al., 2007). However, activation of 5-HT_{1A} receptors by 8-OH-DPAT can significantly suppress reflex bladder activity in both conscious and α -chloralose-anesthetized cats after complete SCI (Tai et al., 2006), indicating that there are species-dependent differences in the influence of 5-HT_{1A} receptors on micturition.

NLX-112 (also known as befiradol or F13640) is a small molecule novel chemical entity that exhibits “full” agonist efficacy for activation of 5-HT_{1A} receptors (Colpaert et al., 2002; Newman-Tancredi et al., 2017). It has high binding affinity *in vitro* at 5-HT_{1A} receptors (pK_i = 9) and exceptional selectivity (>1000x) versus other targets (Colpaert et al., 2002).

NLX-112 is potently active in analgesic, anti-dyskinetic, and antidepressant tests in rodent (typical doses: 0.04 – 0.63 mg/kg p.o.) (Colpaert, 2006; Iderberg et al., 2015) and acts by modulating the activity of several neurotransmitter systems (Llado-Pelfort et al., 2012). Thus, NLX-112 potently inhibits 5-HT neuron electrical activity in the raphe nucleus and stimulates glutamatergic pyramidal neuron firing in the prefrontal cortex (Llado-Pelfort et al., 2012). NLX-112 inhibits 5-HT release in terminal regions (e.g. hippocampus and frontal cortex) and increases cortical dopamine release (Llado-Pelfort et al., 2012). In addition, NLX-112 has a promising pharmacokinetic and tolerance profile. In rat, it is rapidly absorbed (plasma C_{max} at 15 min after i.p. administration), has >50% bioavailability, and its plasma exposure is linear with dose. NLX-112 is also safe and well-tolerated upon both acute (LD₅₀ > 800 mg/kg p.o.) and chronic administration (13 weeks) in 3 species (rat, dog,

and non-human primate). Furthermore, NLX-112 has been tested in humans (over 500 subjects) in the context of Phase I and Phase II clinical trials for another indication (diabetic neuropathy). Principal adverse events were mild (dizziness and headache), and they mainly occurred at the start of treatment and resolved quickly. Although NLX-112 is a clinically-available compound with beneficial effects in models of other neurological disorders, its effects in micturition behavior after SCI are still unclear. In the present study, we hypothesized that pharmacological activation of 5-HT_{1A} receptors with NLX-112 could improve LUT function after SCI in adult rats. We assessed the LUT by comprehensive urodynamics and external urethral sphincter (EUS) electromyogram (EMG) recordings. We also determined whether pharmacological application of a 5-HT_{1A} receptor antagonist, WAY100635, could reverse the effects of NLX-112 in these experiments.

2. Materials and Methods

2.1. Animal groups

Thirty adult female Sprague-Dawley rats (220–250g; Harlan Laboratories, Madison, WI, USA) were assigned randomly into three groups: (1) spinally-intact group (laminectomy only; N=10); (2) T8 complete transection group; N=10 (2) T8 spinal cord contusive injury group; N=10. Rats were housed in standard laboratory cages under 12:12-hour light-dark cycle conditions with standard rodent chow and water available *ad libitum*. All experiments were performed during the light cycle. All animal procedures were approved by the Cleveland Clinic Institutional Animal Care and Use Committee. The spinal cord surgery was performed under ketamine/xylene anesthesia and LUT functional assessments were prepared under isoflurane with urethane anesthesia, and all efforts were made during post-operation care to minimize suffering. All procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

2.2. T8 spinal cord laminectomy, complete transection, and contusive spinal cord injury surgery

The spinal cord transection and contusion surgical procedures were described in detail in our previous studies (DePaul et al., 2017; Lee et al., 2013). Briefly, animals had operations performed while they were under general anesthesia using intraperitoneal administration of ketamine (60 mg/kg) and xylazine (10 mg/kg). Rectal temperature was monitored and maintained using a heating pad underneath the animal, which automatically adjusts the temperature based upon feedback from the rectal probe. A sterile eye lubricant (paralube) was applied to prevent dehydration. The musculature was cut from T7–T9 and the dorsal surface of T8 was exposed by laminectomy. The spinally-intact group did not receive injury procedures. For the complete transection group, a complete cut was made by scissors at T8, resulting in a separated gap (2–3 mm) between two stumps. A high-magnification surgical microscope was used to confirm that there was no remaining neural tissue between the two stumps of cord. For the contusive injury group, the vertebral column was stabilized by clamping the T7 and T9 vertebral bodies with forceps fixed to the base of the Infinite Horizon Impact Device. The animal was situated on the platform and a 2.5 mm stainless steel impactor tip was positioned over the midpoint of T8 and impacted with a 250 kDyne

force. The overlying musculature was sutured closed and the skin was closed using 4–0 black monofilament sutures. A force/displacement graph was used to monitor impact consistency and any animals that exhibited an abnormal impact graph or greater than 10% deviation from 250 kDynes were immediately excluded from the study.

2.3. Urodynamics, EUS EMG recordings, and pharmacological interventions

Two months after complete SCI or four months after contusive SCI, animals were anesthetized under 2.5% isoflurane with oxygen to perform surgeries, and then cystometrography (CMG) and EUS EMG recording were performed under urethane (1.0 mg/kg) anesthetic conditions. The urinary bladder was exposed by an incision at the midline abdomen. A catheter (polyethylene 50) was inserted into the bladder through a small incision at the apex of the bladder dome. The bladder end of a PE catheter was heated to form a collar and was secured with a purse-string suture on the opening of the detrusor. A normal saline-filled femoral vein catheter was inserted into the femoral vein for drug delivery. The bladder catheter exited from the rostral edge of the abdominal wound, which was closed in layers. The skin above the vascular catheters was also closed. Fine (50µm diameter) teflon-insulated platinum wire electrodes (A-M Systems; Sequim, WA, USA) were inserted percutaneously on both sides of the mid-urethra using a 27-gauge needle for subsequent measurements of EUS EMG activity. Next, rats were placed in a restraining cage to perform physiological tests. Following a one-hour recovery period from isoflurane anesthesia, continuous CMGs were collected using constant infusion (6 ml/hr) of saline by an infusion pump (World Precision Instruments; Sarasota, FL, USA) through the catheter into the bladder to elicit repetitive voids. The electrodes were connected to an AC amplifier (AstroNova, Inc.; West Warwick, RI, USA) with high- and low-pass frequency filters at 10 Hz and 1 kHz and a recording system (DASH 8X, AstroNova, Inc.) at a sample frequency of 10 kHz. Continuous CMG at a flow rate of 6 ml/hr for 1 hour prior allowed the lower urinary tract to adjust to the flow rate. After an hour, the pump was stopped and the bladder was emptied by aspiration through the bladder catheter via a stopcock port. The pump was restarted and after at least three to five reproducible fillings, CMGs were obtained as a baseline and the bladder was emptied again. Three dosages of NLX-112 (0.05 mg/kg; 0.5 mg/kg; 2.5 mg/kg, i.v. (Neurolix Inc.; Wilmington, DE, USA)) were intravenously administered through the femoral vein and then another filling CMG was performed, from which measurements were taken for three to five filling cycles. The interval between each was not shorter than 30 minutes. The bladder was emptied at the end of each dose study prior to starting the next one. Following the delivery of 2.5 mg/kg NLX-112, the 5-HT_{1A} receptor antagonist, WAY100635 (2 mg/kg, Sigma) was intravenously administered through the femoral vein and then another filling CMG was performed, from which measurements were taken for three to five filling cycles. WAY100635 solution was administered in a volume of 0.2 ml followed by a 0.2 ml flush of saline. The testing dosage was selected in accordance with previous studies (Kakizaki et al., 2001).

2.4. Quantification of CMG and EUS EMG patterns

For each of the individual treatments in the CMG and EUS EMG recordings, 3 to 5 consecutive and stabilized micturition events were segmented for analysis as described in our previous study (Lee et al., 2013). Segmented data included the full duration between the

completions of two consecutive voiding contractions. A MATLAB (The MathWorks, Inc., Natick, MA, USA) script was written to measure various CMG/EMG parameters. CMG voiding parameters include: the time to the first void (ttfV) (sec), peak pressure (cmH₂O), volume-per-void (ml), and voiding efficiency. Non-voiding contractions (NVCs) were defined as contractions with amplitudes exceeding 5 cm H₂O that were not associated with any successful micturition. In SCI animals, the series of NVCs preceding each voiding event were analyzed together to match their respective voiding events. Measured CMG non-voiding parameters include: amplitude (peak pressure – baseline pressure) (cmH₂O) and the number of non-voids. The EMG parameters include (during the bursting phase): the number of EMG bursts, EMG bursting duration (sec), and EMG burst interval (sec). EMG bursts were qualified as coordinated when they were timed in sync with the CMG high-frequency pressure oscillations. Data are presented as group mean $\pm$ S.E.M. and were analyzed using a one-way ANOVA with a Tukey's *post hoc* test using GraphPad Prism 8 (GraphPad, San Diego, CA, USA). The significance level was set to 0.05 for all comparisons.

2.5. Statistical analysis

All animal-related procedures, data collection, and data analyses were performed in a blinded fashion. Functional assessments were compared using repeated measures ANOVAs. Following significant main effects, Tukey *post hoc* tests were used to determine specific group and/or treatment differences. Significant differences between groups are indicated by a significant * (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$)

3. Result

3.1. Both complete SCI and contusive SCI negatively impact LUT function

The assessment of LUT function included both detrusor and EUS activity by CMG and EUS EMG recordings in all three animal groups. In spinally-intact animals, continuous infusion of saline (6 ml/hour) into the bladder elicited reflex-bladder contractions with a very regular interval in CMG recordings. There was no bladder contraction during the filling phase before the micturition event and EUS EMG showed tonic activity, indicating the closure of the urethral outlet. During the voiding period, bursting activity in the EUS EMG was observed, indicating the rhythmic opening and closing of the urethral outlet to generate a pulsatile flow of urine, which is normal in rodents (Figs. 1A and 1B). The EUS bursting activity included active and silent periods on the EMG recording. The silent period indicated the outflow of urine. The urethaneanaesthetized rats with complete lesion SCI or contusive SCI exhibited different types of detrusor contractions and EUS EMG activities. The detrusor exhibited hyperactivity showing many non-voiding contractions prior to the voiding event (Figs. 1C and 1E). The non-voiding contractions with no fluid coming out from the urethral outlet exhibited gradually-increasing intra-vesicle pressure waves prior to the micturition event. The EUS EMG exhibited tonic activity (Figs. 1D and 1F) instead of the clear phasic activity that was shown in the spinally-intact animals (Fig. 1B). In addition, the non-voiding contractions associated with the highlighted EUS EMG activity indicate detrusor sphincter dyssynergia following SCI.

3.2. The dose-dependent effects of NLX-112 in LUT function after chronic complete SCI

Next, we investigated the effects of NLX-112 in regulating LUT after chronic T8 complete SCI. In this dose-dependent study, the delivery of NLX-112 showed alterations in both detrusor activity and EUS EMG patterns. The CMG recordings clearly indicated a reduction in peak voiding pressure during micturition and the intra-vesicle pressure of non-voiding during the filling phase at both 0.5 mg/kg and 2.5 mg/kg NLX-112 application, but not 0.05 mg/kg NLX-112 (Fig. 2A). The peak voiding pressure of 0.5 mg/kg and 2.5 mg/kg NLX-112 were 45.6 ± 2.3 cm-H₂O and 42.2 ± 3.6 cm-H₂O, respectively, which were significantly lower than SCI without NLX-112 (baseline, 54.8 ± 3.8 cm-H₂O) and with 0.05 mg/kg NLX-112 application (52.3 ± 3.6 cm-H₂O) (Fig. 3A). The average intra-vesicle pressure of non-voiding contractions in each voiding cycle at 0.5 mg/kg and 2.5 mg/kg NLX-112 were 13.3 ± 3.1 cm-H₂O and 9.6 ± 2.8 cm-H₂O, respectively, which were significantly lower than SCI without NLX-112 (21.4 ± 2.9 cm-H₂O) and with 0.05 mg/kg NLX-112 application (19.7 ± 3.2 cm-H₂O) (Fig. 3B). Significantly reduced numbers of non-voiding contractions were also observed with 2.5 mg/kg NLX-112 (2.4 ± 0.7) as compared to with 0.5 mg/kg (5.3 ± 1.2), 0.05 mg/kg (7.6 ± 0.4) NLX-112, and without NLX-112 (baseline, 8.9 ± 0.6). Voiding efficiencies were significantly improved by 0.5 mg/kg ($54.3 \pm 1.2\%$) and 2.5 mg/kg ($68 \pm 3.7\%$) of NLX-112 when compared to both baseline ($13.5 \pm 2.2\%$) and 0.05 mg/kg NLX-112 ($18.2 \pm 2.9\%$) (Fig. 3B). Micturition (the first-time voiding volume after the saline infusion start) was not significantly affected by 0.05 mg/kg NLX-112 (0.25 ± 0.08 ml), but was increased in 0.5 mg/kg (0.51 ± 0.1 ml) and 2.5 mg/kg (0.59 ± 0.08 ml) NLX-112 when compared to baseline (0.18 ± 0.05 ml). With the higher dosage of NLX-112, the volume of first-time voiding became larger due to the improvement of voiding efficiency. The residual volumes in both 0.5 mg/kg (0.43 ± 0.09 ml) and 2.5 mg/kg (0.18 ± 0.03 ml) NLX-112 were significantly lower than what in both 0.05 mg/kg NLX-112 (0.92 ± 0.25 ml) and baseline (1.0 ± 0.17 ml). Moreover, 2.5 mg/kg NLX-112 showed further significant improvement in both voiding parameters (voiding efficiency and residual volume) and non-voiding parameters (number and pressure of non-voiding) compared to 0.5 mg/kg NLX-112.

In the EUS EMG patterns, both 0.5 mg/kg and 2.5 mg/kg NLX-112 showed phasic activity instead of tonic activity, while tonic activity was observed at both baseline and 0.05 mg/kg NLX-112 during the voiding period (Fig. 3C). The number of bursts was significantly increased in 0.5 mg/kg (18.5 ± 2.3) and 2.5 mg/kg (24.8 ± 3.1) NLX-112 than at baseline (2.3 ± 2.7) or in 0.05 mg/kg NLX-112 (3.1 ± 2.6). The duration of EMG bursting was also significantly increased in 0.5 mg/kg (5.9 ± 1.4 sec) and 2.5 mg/kg (7.8 ± 0.8 sec) NLX-112 compared to baseline (0.2 ± 0.6 sec) or 0.05 mg/kg NLX-112 (0.3 ± 0.1 sec). The interval of EUS burst (EMG silent period) was significantly increased in 0.5 mg/kg (0.18 ± 0.02 sec) and 2.5 mg/kg (0.2 ± 0.01 sec) NLX-112 compared to baseline (0.01 ± 0.05 sec) or 0.05 mg/kg NLX-112 (0.02 ± 0.04 sec). The 2.5 mg/kg NLX-112 group showed a significantly greater increase than the 0.5 mg/kg NLX-112 group in the number and duration of bursts ($p < 0.05$), but not the burst interval. With the application of the antagonist WAY100635 (2 mg/kg), both the CMG and EUS effects of NLX-112 (2.5 mg/kg) were significantly reversed (Fig. 4). The measured parameters of voiding, non-voiding, and EUS EMG were analyzed and shown in Figure 5.

3.3. The dose-dependent effects of NLX-112 in LUT function after chronic contusive SCI

We then investigated the effects of NLX-112 in regulating LUT after chronic T8 contusive SCI. Similar to the previous observation in T8 complete SCI animals, intravenous delivery of NLX-112 demonstrated dose-dependent alterations in both CMG parameters (Fig. 6A) and EUS EMG activity (Fig. 6B). Both 0.5 mg/kg and 2.5 mg/kg NLX-112 (42.4 ± 1.2 cm-H₂O, 39.9 ± 2.2 cm-H₂O, respectively) significantly decreased peak voiding pressure compared to baseline (52.3 ± 3.6 cm-H₂O) and 0.05 mg/kg NLX-112 (47.9 ± 2.8 cm-H₂O) (Fig. 7A). With the increasing doses of NLX-112, the voiding efficiency significantly improved from $16.8 \pm 2.5\%$ (baseline) and $23.9 \pm 4.8\%$ (0.05 mg/kg NLX-112) to $70.3 \pm 5.6\%$ (0.5 mg/kg) and $82.1 \pm 2.2\%$ (2.5 mg/kg) (Fig. 7A). The micturition volume significantly increased from 0.25 ± 0.08 ml (baseline) and 0.29 ± 0.1 ml (0.05 mg/kg NLX-112) to 0.71 ± 1.6 ml (0.5 mg/kg NLX-112) and 1.02 ± 2.9 ml (2.5 mg/kg NLX-112). Residual volume significantly decreased from 1.08 ± 0.2 ml (baseline) and 0.92 ± 0.15 ml (0.05 mg/kg NLX-112) to 0.41 ± 0.27 ml (0.5 mg/kg NLX-112) and 0.12 ± 0.04 ml (2.5 mg/kg NLX-112) (Fig. 7A). The number of non-voiding contractions was dramatically decreased for both 0.5 mg/kg NLX-112 (0.9 ± 0.02) and 2.5 mg/kg NLX-112 (0.7 ± 0.02) compared to baseline (7.8 ± 2.6) and 0.05 mg/kg NLX-112 (6.8 ± 2.4) (Fig. 7B). Non-voiding pressure was also dramatically decreased in both 0.5 mg/kg NLX-112 (5.7 ± 1.3) and 2.5 mg/kg NLX-112 (4.9 ± 1.6) compared to baseline (8.8 ± 1.5) and 0.05 mg/kg NLX-112 (8.1 ± 1.7) (Fig. 7B). In addition, the 2.5 mg/kg NLX-112 showed significantly greater improvement over 0.5 mg/kg NLX-112 in voiding parameters (voiding efficiency and residual volume), but not in non-voiding parameters (number and pressure of non-voiding).

In EUS EMG recordings, the dose-dependent effects of NLX-112 can also be observed in the number, duration, and intervals of EUS burst activities (Fig. 7C). Both 0.5 mg/kg NLX-112 (22.8 ± 3.1) and 2.5 mg/kg NLX-112 (27.6 ± 2.4) demonstrated significant increases in the number of EUS bursts as compared to baseline (2.8 ± 1.7) and 0.05 mg/kg NLX-112 (4.9 ± 1.2). The duration of EUS bursts was significantly increased from baseline (0.8 ± 0.2 sec) and 0.05 mg/kg NLX-112 (1.2 ± 0.4 sec) to the higher doses of 0.5 mg/kg NLX-112 (5.8 ± 1.6 sec) and 2.5 mg/kg NLX-112 (8.8 ± 2.3 sec). The interval of EUS bursts were also significantly increased in the higher doses of 0.5 mg/kg NLX-112 (0.19 ± 0.04 sec) and 2.5 mg/kg NLX-112 (0.27 ± 0.09 sec) compared to baseline (0.1 ± 0.08 sec) and 0.05 mg/kg NLX-112 (0.13 ± 0.1 sec). The 2.5 mg/kg NLX-112 group showed significantly greater increases than 0.5 mg/kg NLX-112 in the number and duration bursts ($p < 0.05$). Importantly, application of the antagonist WAY100635 (2 mg/kg, i.v.) significantly reversed both CMG and EUS effects of NLX-112 (2.5 mg/kg), similar to the observation from the complete transection animals (Fig. 8). All measured voiding, non-voiding, and EUS EMG parameters were analyzed and shown in Figure 9.

4. Discussion

The main findings of the present study suggest that systematic delivery of the selective 5-HT_{1A} receptor full agonist, NLX-112 can improve LUT function in urethane-anesthetized rats after chronic SCI. The efficacy of the improvement of LUT function, including detrusor

and EUS activity, can be observed in both complete and incomplete SCI models, while most of the assessments of pharmacological interventions were performed in the complete SCI model. In addition, pharmacological intervention with the 5-HT_{1A} receptor selective antagonist WAY 100635 reversed these actions of NLX-112 treatment. This reversed observation is unlikely from the degradation of NLX-112 in this study. The half-life of NLX-112 in plasma of rat following IV administration is about 2 hours (unpublished data by Neurolix Inc.). In brain NLX-112 is present and bound to 5-HT_{1A} receptors for more than 2 hours as shown by using PET imaging of 18F labeled NLX-112 in rat, cat, macaque (Vidal et al., 2018) and human (Colom et al., 2020).

In our experimental setting, it usually takes less than 30 minutes after NLX-112 application to apply WAY100635. The time (30 minutes) taken is much less than the half-life of NLX-112, which support that the reverse effects of WAY100635 do not come from the degradation of NLX-112. Furthermore, the pharmacokinetics (PK) effects of NLX-112 in rat plasma and brain following IP administration (unpublished data by Neurolix Inc.) further support that NLX-112 still worked while WAY100635 was given.

SCI-induced LUT dysfunctions above the lumbosacral level include hyperactivity of detrusor, detrusor-sphincter dyssynergia (DSD), and poor relaxation of urethra, leading to low voiding efficiency and large residual volume. Regulation of normal micturition requires the involvement of supraspinal pathways and becomes reflex activity (lack of voluntary control) after SCI. In the present study, both complete and incomplete (moderate contusive) injury showed similar LUT dysfunctions from both CMG and EUS EMG recordings. This suggests that there is little contribution of spared fibers from supraspinal inputs in the regulation of LUT function in the more clinically-relevant contusive injury model, while in previous contusive SCI studies, LUT disorders could be attributable to the severity of injury (Pikov and Wrathall, 2001) or the level of the injury (David and Steward, 2010).

Beneficial effects of activating 5-HT_{1A} receptors via NLX-112 to improve LUT function were observed in both complete and contusive SCI models. There were several different aspects of these actions between the two models. NLX-112 showed more efficacy in the reduction of detrusor hyperactivity at the lower dose (0.05mg/kg versus 0.5 mg/kg) in the contusive SCI model. This could be due to less severe overactivity of detrusor (lower non-voiding pressure and number) in the baseline recordings from the contusive injury model. In addition, the relaxation period with NLX-112 treatment in contusive injury is larger than in the complete injury model, leading to greater improvement of voiding efficacy in the contusive injury model. Unlike the response of detrusor activity, we did not see a dramatic change of EUS bursting activity at the lower dose (0.05mg/kg versus 0.5 mg/kg) between contusive SCI and complete SCI. In addition, NLX-112 treatment was more effective in reducing DSD during the bladder storage phase in the contusive injury model than in the complete SCI model. This may due to reductions in the number and pressure of non-voiding contractions and/or improved relaxation of EUS.

Drugs acting on serotonergic pathways in the central nervous system have been considered as interventions to treat bladder hyperactivity (de Groat, 2002; Khaled and Elhilali, 2003). LUT control by activity of serotonergic pathways generally facilitates the sympathetic reflex

pathway to provide urine storage and inhibits the parasympathetic voiding pathway (Khaled and Elhilali, 2003). In spinally-intact animals, suppression of detrusor activity can be achieved by activating 5-HT_{2C} receptors or inhibiting 5-HT_{1A} receptors. The activation of 5-HT_{2C} receptors by its agonists ((2S)-1-(6-chloro-5-fluoroindol-1-yl)propan-2-amine fumarate (Ro 60-0175)) could produce a depression of reflex bladder contractions (Mbaki et al., 2012). An inhibitory effect of 5-HT_{1A} receptor antagonists (WAY100635 or NAD-299) on micturition has been shown in conscious (Pehrson et al., 2002; Testa et al., 1999) and anaesthetized (Kakizaki et al., 2001; Pehrson et al., 2002) rats. In chronic SCI rats, intrathecal administration of exogenous 5-HT showed inhibitory effects on detrusor activities. There is very little information regarding whether activation of serotonergic receptors can reduce hyperactivity of neurogenic bladder after SCI. Importantly, the present study is the first to show detrusor hyperactivity was reduced by activation of 5-HT_{1A} receptors (by NLX-112) following both complete transection and contusive SCI. This observation is different from the spinally-intact animals, while the detrusor activity is inhibited by blocking 5-HT_{1A} receptor. This may indicate plasticity of 5-HT_{1A} receptors in regulating detrusor function after SCI. Besides the reduction of detrusor hyperactivity, the reduction of bladder peak voiding pressure by NLX-112 could be due to more relaxation of EUS, which has less urethral resistance during bladder contractions to push fluid out. The lower peak voiding pressure can also reduce the risk of kidney damage during micturition. Moreover, NLX-112 intervention could improve voiding efficiency, reduce peak voiding pressure, and residual volume, similar to a previous study using 8-OH-DPAT (a 5-HT_{1A/7} receptor agonist) in urethane-anesthetized rats with complete SCI (Dolber et al., 2007).

EUS activity plays significant roles in LUT function in both urine storage (tonic activity) and voiding period (minimal activity in cats or phasic/bursting activity in rats) (Kruse and de Groat, 1993; Thor et al., 2002). Patterns of EUS activity become more prominently tonic, reduction periods of bursting activity are reduced, and relaxation periods during bursting are shortened after SCI in rats. In the present study, EUS EMG activity showed prominent tonic activity in both complete and contusive SCI under urethane anesthesia, consistent with previous studies (Chang et al., 2007; Dolber et al., 2007; Pikov and Wrathall, 2001). In addition, alterations in EUS EMG activity could be attributable to the level of anesthesia in animals with SCI (Cheng and de Groat, 2004) or in spinally-intact animals (Chang and Havton, 2008). Although EUS EMG can show bursting activity in unanesthetized SCI rats, it remains abnormal with poor voiding efficiency (Cheng and de Groat, 2004). Similarly, patients with SCI show abnormal or tonic EUS EMG, leading to the occurrence of DSD and inefficiency voiding (Fowler et al., 2008). Activation of 5-HT_{1A} receptors by NLX-112 can significantly increase EUS EMG bursting activity and voiding efficiency in a dose-dependent manner, which is consistent with the observations from a previous study using 8-OH-DPAT (Dolber et al., 2007). The silent period of EUS activity in humans or EUS bursting activity in rodents presumably represents urethral opening to allow urine to flow through (Fowler et al., 2008). In the present study, we demonstrated that NLX-112 could increase the interval of EUS bursting activity (silent period) during the micturition period in both complete and contusive SCI rats. This observation supports the findings of improved voiding efficiency with NLX-112 treatment. The presence of EUS bursting after complete or incomplete SCI (Leung et al., 2007) demonstrated the existence of spinal circuits that can

serve as EUS pattern generators to trigger phasic activity of EUS. The exact interneuronal population(s) and their location have not been identified yet. It has been considered in caudal to L1 and possible location in L3/L4 segment of spinal cord in animals with complete SCI in different level of injury (Chang et al., 2007; LaPallo et al., 2017). EUS pattern generator activity may be inhibited by the level of anesthesia, but the activation of 5-HT_{1A} receptor by using 8-OH-DPAT (Dolber et al., 2007). It is very likely that the actions of NLX-112 on EUS activity occur through similar mechanisms after SCI. In addition to targeting the 5-HT_{1A} receptor, modulation of EUS activity can be achieved by neuromuscular blockers or adrenoceptor blockers to relax the urethra after SCI (Ishida et al., 2016; Yoshiyama et al., 2000). Overall, the present pharmacological data support using NLX 112 as a potential therapeutic strategy to treat bladder dysfunction after SCI.

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References

- Chang HY, Cheng CL, Chen JJ, and de Groat WC., 2007 Serotonergic drugs and spinal cord transections indicate that different spinal circuits are involved in external urethral sphincter activity in rats. *Am J Physiol Renal Physiol.* 292:F1044–1053. [PubMed: 17047164]
- Chang HY, and Havton LA., 2008 Differential effects of urethane and isoflurane on external urethral sphincter electromyography and cystometry in rats. *Am J Physiol Renal Physiol.* 295:F1248–1253. [PubMed: 18753298]
- Cheng CL, and de Groat WC., 2004 The role of capsaicin-sensitive afferent fibers in the lower urinary tract dysfunction induced by chronic spinal cord injury in rats. *Exp Neurol.* 187:445–454. [PubMed: 15144870]
- Cheng CL, and de Groat WC., 2010 Role of 5-HT_{1A} receptors in control of lower urinary tract function in anesthetized rats. *Am J Physiol Renal Physiol.* 298:F771–778. [PubMed: 20042459]
- Colom M, Costes N, Redouté J, Dailler F, Gobert F, Le Bars D, Billard T, Newman-Tancredi A, and Zimmer L., 2020 18 F-F13640 PET Imaging of Functional Receptors in Humans. *Eur J Nucl Med Mol Imaging.* 47:220–221. [PubMed: 31414208]
- Colpaert FC, 2006 5-HT(1A) receptor activation: new molecular and neuroadaptive mechanisms of pain relief. *Curr Opin Investig Drugs.* 7:40–47.
- Colpaert FC, Tarayre JP, Koek W, Pauwels PJ, Bardin L, Xu XJ, Wiesenfeld-Hallin Z, Cosi C, Carilla-Durand E, Assie MB, and Vacher B., 2002 Large-amplitude 5-HT_{1A} receptor activation: a new mechanism of profound, central analgesia. *Neuropharmacology.* 43:945–958. [PubMed: 12423664]
- David BT, and Steward O., 2010 Deficits in bladder function following spinal cord injury vary depending on the level of the injury. *Exp Neurol.* 226:128–135. [PubMed: 20713043]
- de Groat WC, 2002 Influence of central serotonergic mechanisms on lower urinary tract function. *Urology.* 59:30–36. [PubMed: 12007520]
- de Groat WC, and Yoshimura N., 2006 Mechanisms underlying the recovery of lower urinary tract function following spinal cord injury. *Prog Brain Res.* 152:59–84. [PubMed: 16198694]
- DePaul MA, Lin CY, Silver J, and Lee YS., 2017 Combinatory repair strategy to promote axon regeneration and functional recovery after chronic spinal cord injury. *Sci Rep.* 7:9018. [PubMed: 28827771]
- Dolber PC, Gu B, Zhang X, Fraser MO, Thor KB, and Reiter JP., 2007 Activation of the external urethral sphincter central pattern generator by a 5-HT(1A) receptor agonist in rats with chronic spinal cord injury. *Am J Physiol Regul Integr Comp Physiol.* 292:R1699–1706. [PubMed: 17204596]

- Fowler CJ, Griffiths D, and de Groat WC., 2008 The neural control of micturition. *Nat Rev Neurosci.* 9:453–466. [PubMed: 18490916]
- Iderberg H, McCreary AC, Varney MA, Kleven MS, Koek W, Bardin L, Depoortere R, Cenci MA, and Newman-Tancredi A., 2015 NLX-112, a novel 5-HT1A receptor agonist for the treatment of L-DOPA-induced dyskinesia: Behavioral and neurochemical profile in rat. *Exp Neurol.* 271:335–350. [PubMed: 26037043]
- Ishida H, Yamauchi H, Ito H, Akino H, and Yokoyama O., 2016 alpha1D-Adrenoceptor blockade increases voiding efficiency by improving external urethral sphincter activity in rats with spinal cord injury. *Am J Physiol Regul Integr Comp Physiol.* 311:R971–R978. [PubMed: 27605559]
- Kakizaki H, Yoshiyama M, Koyanagi T, and De Groat WC., 2001 Effects of WAY100635, a selective 5-HT1A-receptor antagonist on the micturition-reflex pathway in the rat. *Am J Physiol Regul Integr Comp Physiol.* 280:R1407–1413. [PubMed: 11294761]
- Khaled SM, and Elhilali M., 2003 Role of 5-HT receptors in treatment of overactive bladder. *Drugs Today (Barc).* 39:599–607. [PubMed: 14566383]
- Kruse MN, and de Groat WC., 1993 Spinal pathways mediate coordinated bladder/urethral sphincter activity during reflex micturition in decerebrate and spinalized neonatal rats. *Neurosci Lett.* 152:141–144. [PubMed: 8515867]
- LaPallo BK, Wolpaw JR, Yang Chen X, and Carp JS., 2017 Spinal Transection Alters External Urethral Sphincter Activity during Spontaneous Voiding in Freely Moving Rats. *Journal of neurotrauma.* 34:3012–3026. [PubMed: 28467736]
- Lee YS, Lin CY, Jiang HH, Depaul M, Lin VW, and Silver J., 2013 Nerve regeneration restores supraspinal control of bladder function after complete spinal cord injury. *J Neurosci.* 33:10591–10606. [PubMed: 23804083]
- Leung PY, Johnson CS, and Wrathall JR., 2007 Comparison of the effects of complete and incomplete spinal cord injury on lower urinary tract function as evaluated in unanesthetized rats. *Exp Neurol.* 208:80–91. [PubMed: 17822702]
- Llado-Pelfort L, Assie MB, Newman-Tancredi A, Artigas F, and Celada P., 2012 In vivo electrophysiological and neurochemical effects of the selective 5-HT1A receptor agonist, F13640, at pre- and postsynaptic 5-HT1A receptors in the rat. *Psychopharmacology (Berl).* 221:261–272. [PubMed: 22147258]
- Makman MH, Dvorkin B, and Crain SM., 1988 Modulation of adenylate cyclase activity of mouse spinal cord-ganglion explants by opioids, serotonin and pertussis toxin. *Brain Res.* 445:303–313. [PubMed: 3370465]
- Mbaki Y, Gardiner J, McMurray G, and Ramage AG., 2012 5-HT 2A receptor activation of the external urethral sphincter and 5-HT 2C receptor inhibition of micturition: a study based on pharmacokinetics in the anaesthetized female rat. *Eur J Pharmacol.* 682:142–152. [PubMed: 22374259]
- Newman-Tancredi A, Martel JC, Cosi C, Heusler P, Lestienne F, Varney MA, and Cussac D., 2017 Distinctive in vitro signal transduction profile of NLX-112, a potent and efficacious serotonin 5-HT1A receptor agonist. *J Pharm Pharmacol.* 69:1178–1190. [PubMed: 28612503]
- Pehrson R, Ojteg G, Ishizuka O, and Andersson KE., 2002 Effects of NAD-299, a new, highly selective 5-HT1A receptor antagonist, on bladder function in rats. *Naunyn Schmiedebergs Arch Pharmacol.* 366:528–536. [PubMed: 12444493]
- Perrin FE, Gerber YN, Teigell M, Lonjon N, Boniface G, Bauchet L, Rodriguez JJ, Hugnot JP, and Privat AM., 2011 Anatomical study of serotonergic innervation and 5-HT(1A) receptor in the human spinal cord. *Cell Death Dis.* 2:e218. [PubMed: 21993394]
- Pikov V, and Wrathall JR., 2001 Coordination of the bladder detrusor and the external urethral sphincter in a rat model of spinal cord injury: effect of injury severity. *J Neurosci.* 21:559–569. [PubMed: 11160435]
- Ramage AG, 2001 Central cardiovascular regulation and 5-hydroxytryptamine receptors. *Brain Res Bull.* 56:425–439. [PubMed: 11750788]
- Tai C, Miscik CL, Ungerer TD, Roppolo JR, and de Groat WC., 2006 Suppression of bladder reflex activity in chronic spinal cord injured cats by activation of serotonin 5-HT1A receptors. *Exp Neurol.* 199:427–437. [PubMed: 16488413]

- Testa R, Guarneri L, Poggesi E, Angelico P, Velasco C, Ibba M, Cilia A, Motta G, Riva C, and Leonardi A., 1999 Effect of several 5-hydroxytryptamine(1A) receptor ligands on the micturition reflex in rats: comparison with WAY 100635. *J Pharmacol Exp Ther.* 290:1258–1269. [PubMed: 10454502]
- Thor KB, Katofiasc MA, Danuser H, Springer J, and Schaus JM., 2002 The role of 5-HT(1A) receptors in control of lower urinary tract function in cats. *Brain Res.* 946:290–297. [PubMed: 12137933]
- Vidal B, Fieux S, Colom M, Billard T, Bouillot C, Barret O, Constantinescu C, Tamagnan G, Newman-Tancredi A, and Zimmer L., 2018 18 F-F13640 Preclinical Evaluation in Rodent, Cat and Primate as a 5-HT 1A Receptor Agonist for PET Neuroimaging. *Brain Struct Funct.* 223:2973–2988. [PubMed: 29730825]
- Wu X, Kushwaha N, Albert PR, and Penington NJ., 2002 A critical protein kinase C phosphorylation site on the 5-HT(1A) receptor controlling coupling to N-type calcium channels. *J Physiol.* 538:41–51. [PubMed: 11773315]
- Yoshimura N, Miyazato M, Kitta T, and Yoshikawa S., 2014 Central nervous targets for the treatment of bladder dysfunction. *Neurourol Urodyn.* 33:59–66. [PubMed: 23832777]
- Yoshiyama M, deGroat WC, and Fraser MO., 2000 Influences of external urethral sphincter relaxation induced by alpha-bungarotoxin, a neuromuscular junction blocking agent, on voiding dysfunction in the rat with spinal cord injury. *Urology.* 55:956–960. [PubMed: 10840125]

Highlights

- NLX-112 differentially improves LUT function after chronic SCI in a dose-dependent manner

Fig. 1.

Urodynamic and EUS activity recordings in a representative spinally-intact rat (A, B), T8 spinal cord complete injury rat (C, D), and T8 contusive spinal cord injury rat (E, F). (A, C, E) The top tracing is CMG indicating bladder pressure, and the bottom tracing is EUS EMG for three micturition cycles. The black asterisks indicate voiding contractions. Both SCI models show significant DSD (C, E, double arrows) in the bladder-filling phase of CMG. (B, D, F) Magnified EUS EMG recordings (A, C, E, red asterisk) during the voiding period show bursting activity of EUS. Both SCI models (D, F) showed tonic EUS activities, while spinally-intact animals showed phasic EUS activity (B), under urethane anesthesia.

Fig. 2.

Dose-response effects of NLX-112 on bladder function in chronic T8 complete SCI animals (N=10). (A) Representative voiding cycles of CMG and EUS EMG recordings from NLX-112 at three dosages (0.05 mg/kg; 0.5 mg/kg.; 2.5 mg/kg, i.v.). The black asterisks indicate voiding contractions. Note the reduction of non-voiding activity of detrusor with increasing dosage of NLX-112 (upper tracing in A). The red asterisks in the EMG tracings indicate EUS activity corresponding to the voiding contractions in the black asterisks. (B) Magnified EUS EMG recording (A, red asterisk) was the period with the decreasing bladder pressure but high frequency oscillation during the voiding event of each dosage showing that both 0.5 mg/kg and 2.5 mg/kg, but not 0.05 mg/kg, improved bursting activity of EUS when compared to the baseline showing tonic EUS activity.

Fig. 3.

NLX-112 improves urodynamics and bladder/EUS coordination two months after complete SCI. Statistical analysis of parameters of voiding (A), non-voiding (B), and EUS EMG (C) activity in a NLX-112 pharmacological dose-response study in chronic SCI rats. The voiding volume shown is for the first-time voiding amount after the saline infusion start. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ when compared to spinally-intact animals; ^c, $P < 0.05$; ^{cc}, $P < 0.01$; ^{ccc}, $P < 0.001$; ^{cccc}, $P < 0.0001$ when compared to baseline; ⁺, $P < 0.05$; ⁺⁺, $P < 0.01$; ⁺⁺⁺, $P < 0.001$; ⁺⁺⁺⁺, $P < 0.0001$ when compared to 0.05 mg/kg NLX-112 treatment; [^], $P < 0.05$ when compared to 0.5 mg/kg NLX-112 treatment. N=10 per group.

Fig. 4.

The effects of a 5-HT_{1A} receptor antagonist (WAY100635, 2 mg/kg, i.v.) on both detrusor and EUS EMG activities generated by NLX-112 (2.5 mg/kg, i.v.) in chronic T8 complete SCI animals (N=10). (A) WAY100635 treatment reversed the reduction of hyperactive bladder by NLX-112. Note the time to the first-time voiding has also been extended after WAY100635 treatment. The black asterisks indicate voiding contractions. (B) WAY100635 treatment reversed the improved EUS bursting activity by NLX-112.

A. voiding **B. non-voiding** **C. EUS EMG** **Fig. 5.**

The effects of a 5-HT_{1A} receptor antagonist (WAY100635, 2 mg/kg, i.v.) on NLX-112 improvement of both urodynamics and bladder/EUS coordination two months after complete SCI. Statistical analysis of parameters of voiding (A), non-voiding (B), and EUS EMG (C) activity showing WAY100635 counteracting the effects of NLX-112 on micturition in chronic SCI rats. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ when compared to baseline; ++, $P < 0.01$; +++, $P < 0.001$; +++++, $P < 0.0001$ when compared to 2.5 mg/kg NLX-112 treatment. N=10 per group.

Fig. 6.

Dose-response effects of NLX-112 on bladder function in chronic T8 contusive injured animals (N=10). (A) Representative voiding cycles of CMG and EUS EMG recordings from NLX-112 at three dosages (0.05 mg/kg; 0.5 mg/kg.; 2.5 mg/kg, i.v.). The black asterisks indicate voiding contractions. Note the remarkable reduction of non-voiding activity of detrusor with 0.5 mg/kg and 2.5 mg/kg, but not 0.05 mg/kg, when compared to baseline showing typical hyperactive bladder after SCI. (B) Magnified EUS EMG recordings (A, red asterisk) during the voiding period of each dosage showing both 0.5 mg/kg and 2.5 mg/kg, but not 0.05 mg/kg, improved bursting activity of EUS when compared to the baseline showing little to no EUS bursting activity.

Fig. 7.

NLX-112 improves urodynamics and bladder/EUS coordination four months after contusive SCI. Statistical analysis of parameters of voiding (A), non-voiding (B), and EUS EMG (C) activity in a NLX-112 pharmacological dose-response study in chronic SCI rats. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ when compared to spinally-intact animals; ^c, $P < 0.05$; ^{cc}, $P < 0.01$; ^{ccc}, $P < 0.001$; ^{cccc}, $P < 0.0001$ when compared to baseline; +, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$; +++, $P < 0.0001$ when compared to 0.05 mg/kg NLX-112 treatment; ^, $P < 0.05$ when compared to 0.5 mg/kg NLX-112 treatment. N=10 per group.

Fig. 8.

The effects of a 5-HT_{1A} receptor antagonist (WAY100635, 2 mg/kg, i.v.) on both detrusor and EUS responses generated by NLX-112 (2.5 mg/kg) in chronic T8 contusive-injured animals (N=10). (A) WAY100635 treatment reversed the reduction of hyperactive bladder by NLX-112. The black asterisks indicate voiding contractions. (B) WAY100635 treatment reversed the improved EUS bursting activity by NLX-112.

Fig. 9.

The effects of a 5-HT_{1A} receptor antagonist (WAY100635, 2 mg/kg, i.v.) on NLX-112 improvement of both urodynamics and bladder/EUS coordination four months after contusive SCI. Statistical analysis of parameters of voiding (A), non-voiding (B), and EUS EMG (C) activity showing WAY100635 counteracting NLX-112 micturition effects in chronic SCI rats. **, P < 0.01; ***, P < 0.001; ****, P < 0.0001 when compared to baseline; ++, P < 0.01; +++, P < 0.001; +++, P < 0.0001 when compared to 2.5 mg/kg NLX-112 treatment. N=10 per group.