



Proof-of-Concept of Acute Drug-Elicited Defecatory Behaviors in Spinal Cord-Transected Animals: A Model for Identifying Centrally-Acting Prokinetic Agents

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Authors' contributions

This work was carried out in collaboration between all authors. Author PAG designed the study and wrote the protocol. Author IS wrote the first draft of the manuscript. Author PAG managed the literature searches. Authors IS and MG conducted analyses where as author MG performed all experiments. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

No cure and no safe or acceptable treatment exist yet against bowel problems and chronic constipation in spinal cord-injured (SCI) patients. Although some non-central nervous system (CNS)-acting drugs have already been identified and used clinically as symptomatic treatment, most have been associated with significant side effects and deleterious complications. To ease basic research aimed at identifying new drug candidates against bowel control problems, we developed a standardized approach and corresponding assays for quantitatively measuring prokinetic and acute defecatory effects in paraplegic animals. Following a period of acclimation, a single subcutaneous injection of 0.5 ml of vehicle (sterile water) was performed in normal animals or in early chronic (> 7

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days post-surgery) low-thoracically (Th9/10)-transected (Tx) mice. A 30 minute-period of observation of freely moving animals using a transparent Plexiglas arena was used to subsequently measure timing (latency of each episode), amounts (fecal pellets in mg) and frequency (number of episodes/30 min). Residual activity levels, clearly determined in control animals, were used as baseline level to determine statistically greater effects induced by compounds of potential interest. Tests with SR57227 (5-HT₃ receptor agonist) and quipazine (5-HT_{2/3} receptor agonist) revealed that only quipazine acutely elicited significantly greater amounts of fecal pellets in Tx mice. Using this straightforward and reliable method, future drug screening experiments that may yield the identification and development of new potent and safe centrally acting-drug treatments (e.g., upon the Lumbosacral Defecation Center) for potent 'on-demand' facilitation or induction of reflex bowel control and acute episodes of defecation in patients with SCI.

Keywords: Paraplegia; spinal cord injury; transaction; voiding; bladder problems; rodents; mice.

1. INTRODUCTION

Spinal Cord Injury (SCI) generally leads to an immediate and irreversible loss of sensation and voluntary motor control below the level of injury as well as to a rapid development of severe health problems such as osteoporosis, muscle atrophy, immune deficiency, hormonal dysregulation, infertility, autonomic dysreflexia, diabetes, obesity, cardiovascular complications, sexual dysfunction, bladder and bowel problems [1-3].

Given that defecation is controlled by neurons located in the lumbo-sacral segments of the spinal cord, most SCI patients injured cervically or thoracically generally experience both bladder and bowel problems [4]. Defecation may be improved to some extent using different methods and devices. However, most are also associated, when repeatedly used, with irritation, stress, rectal tissue damage, dependency, and bowel accident [3-6].

The region of the spinal cord between L1 and S3 (the levels being slightly different between species) contains a defecation centre [7]. In the rat, the centre is located primarily at L6–S1 [8-10]. Reflexes through this centre can be initiated by irritation or distension of the rectum, persist after transection of the more rostral spinal cord, but are eliminated by section of the sacral outflows or the pelvic nerves [10]. Although, that center is normally under descending inhibitory and facilitatory control by neurons in the cortex and pons [11], it has been shown to exhibit intrinsic control capabilities over colorectal contractions [12]. Indeed, its existence and specific reactivation have been demonstrated clearly using selective lesions and pharmacological administration locally (intrathecal) of Ghrelin receptor agonist,

CP464709, in rhizotomized rodents elicited propulsive contractions that empty the colorectum [12].

However, potent and safe brain-permeable compounds active upon clinically relevant route of administration (s.c. or p.o) remains to be identified. Although different assays and animal models exist (i.e. colon-cannulated anesthetized animals, colon motility measurements, etc.), none are ideally suited for extensive drug screening studies on large cohorts of animals.

To facilitate and accelerate basic research, drug screening experiments and the development of new potent treatments, we developed a standardized approach and related assays that can simply and reliably assess the potential of systemically administered brain permeable drugs in eliciting or facilitating periodically bowel control recovery and fecal pellet production in paraplegic animals.

2. MATERIALS AND METHODS

2.1 Animal Model and Surgical Procedures

All experimental procedures were conducted in accordance with the Canadian Council on Animal Care guidelines and accepted by the Laval University Animal Care and Use Committee. Data from adult male CD1 mice (Charles River, Montreal, QC) initially weighing 30-35g prior to surgery were used in this study. In brief, animals were housed 4 or 5 per cage in a controlled-temperature environment maintained under a 12 h light/dark cycle with free access to food (Teklab global 18% protein rodent diet 2018, Harlan Teklab, Madison, WI) and water. Pre-operative care included injections of lactate Ringer's solution (1.0 ml, s.c.) and of an analgesic

(buprenorphine; 0.1 mg/kg, s.c.; Schering-Plough, Pointe-Claire, QC) 30 min prior to surgery. For surgery, mice were completely anesthetized using isoflurane (2.5%) as described elsewhere [13,14]. The spinal cord transection (Tx) was performed inter-vertebrally using microscissors inserted between the 9th and 10th thoracic vertebrae. To ensure that complete transection was achieved, the inner vertebral walls were entirely scrapped with scissor tips in order to sever all remaining spinal fibers. The corresponding skin area was closed with clips and mice were placed for a few hours on a heating pad. Post-operative care included daily injections of lactate-Ringer's solution (2x 1.0 ml, s.c.), buprenorphine (2x 0.1 mg/kg, s.c.), and Baytril (5 mg/kg, s.c.; Bayer, Toronto, ON) for four days. Bladders were also manually expressed twice a day until testing. Complete transection was confirmed behaviorally (flaccid paralysis of the hindlimbs for one week) and histologically (post-mortem examination using luxol blue and cresyl violet coloration).

2.2 Acclimation

Once mice purchased from Charles River Laboratory (St-Constant, Quebec) arrived in the animal care facility, they were housed as groups of 3 or 4 animals per cage with free access to food and water. Basic acclimation began with 4 days in these conditions. Four (4) days before experiments, we place each group in the Plexiglas arena (circular shape, diameter of 60 cm, Fig. 4) for 30 minutes. Generally, first 10 minutes were characterized by intense exploration (locomotion) by the animals which tended to rapidly decline subsequently. On the day of testing, each group was acclimated again for 10 minutes in the Plexiglas arena prior to injections. Acclimation is critically important to reduce as much as possible the effects of stress on fecal pellet production which, otherwise, would lead to false positive results and lack of reproducibility.

2.3 Quantitative Assays

Animals were tested separately. Upon injection (vehicle in this case was sterile water), each animal was placed in the arena for observation and data collection during 30 minutes. Subcutaneous administration (see below) of sterile water, 3 mg/kg SR57227 (5-HT₃ receptor agonist) or 5 mg/kg quipazine (5-HT_{2/3} receptor agonist) was used for testing. Fecal pellets were removed from the arena using forceps as they

were being produced. Number of episodes were assessed using a counter, timing (latency) of these episodes was assessed (either during the first 15 min or the last 15 min of the period of observation, and total amount of pellets were weighed (in mg) at the end (i.e., fecal pellets were collected with forceps and placed aside in a plastic box until weighing). Incidence, defined as the number of animals in which fecal pellets were found, was also calculated subsequently to assess potency.

2.4 Statistical Analysis

Comparisons between groups were performed using unpaired T tests with Graph Pad Prism 5.0. Data passed the Shapiro-Wilk normality test. All results were expressed as mean \pm SEM. P values < 0.05 were considered statistically significant.

3. RESULTS

We first assessed the total amount of dejections occurring within 30 minutes post-injection of 0.5 ml water. Comparisons (unpaired T tests) between control (non-Tx) and Tx mice revealed a significant difference ($P = 0.0015$) in the total amount (in mg) of fecal pellets expressed spontaneously in 30 minutes. Fig. 1 shows that 135.0 ± 9.5 mg of feces/animal on average were collected from intact mice (dark grey bar on the left; incidence of 100%, pale grey bar on the left). In Tx mice, significantly ($P < 0.05$) less fecal pellets (44.8 ± 11.2 mg/animal, dark grey bar on the right) were found compared with intact mice. When examining number of pellets, a non-statistically significant ($P = 0.14$) difference of 20% was found (less in Tx mice). Non-Tx mice displayed on average 4.2 ± 0.3 pellets/animal whereas Tx mice showed 3.4 ± 0.5 pellets/animal in 30 minutes (Fig. 2).

From testing previously done with a large variety of ligands (monoaminergic, dopaminergic, glutamatergic) in other series of experiments (for drug-induced locomotion or drug-induced micturition) [13], we chose to examine the effects of two serotonin receptor agonists for which some evidence suggested potential prokinetic effects. We administered (s.c.) a single dose of 5 mg/kg quipazine (5-HT_{2/3} agonist) or 3 mg/kg SR57227 (5-HT₃ agonist) in Tx mice. Fig. 2 shows that quipazine (56.4 ± 8.4 mg), but not SR57227A (46.2 ± 11.1 mg), significantly ($P < 0.05$) increased fecal pellet production compared with control (44.8 ± 11.2 mg). Incidence values

(percentages of animals expressing some defecation, pale grey bars) above 85% reveal that most mice expressed at least some defecation. This provided first proof-of-concept

(PoC) data in vivo showing that systemic administration of specific small molecules can potentially elicit on-demand defecatory activities in Tx mice.

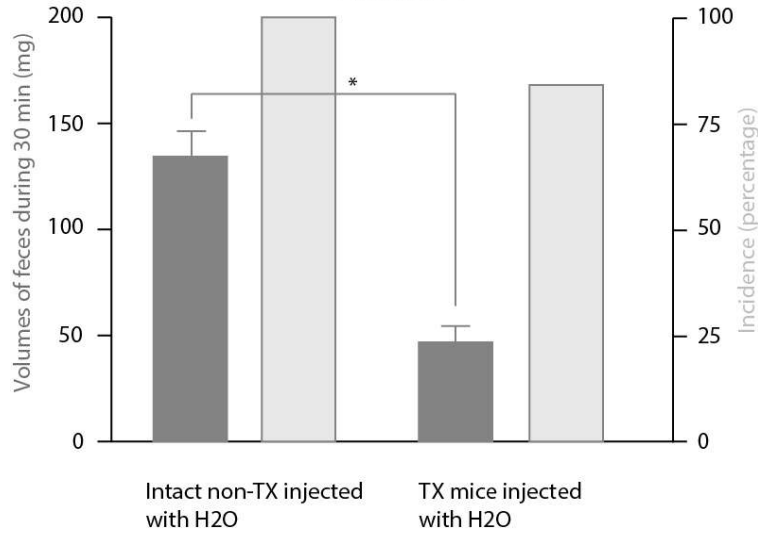


Fig. 1. Total amount of feces spontaneously expressed within 30 min post-H2O injection (s.c.) in intact (non-Tx) and Tx mice

Amounts reported as dark grey bars show that significantly less feces were found on average in Tx mice. Incidence values (right axis) and pale grey bars reveal that nearly all mice expressed at least some defecatory activity in these experimental conditions attributed to stress-related autonomic actions upon peripheral organs. Ten (10) Tx mice and 10 non-Tx received water

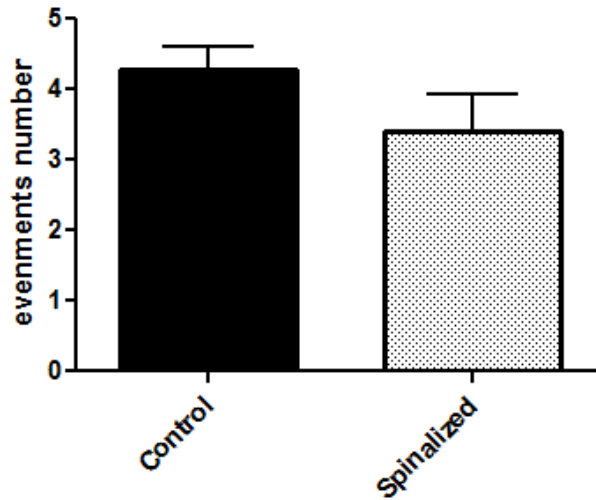


Fig. 2. Number of fecal pellets spontaneously expressed within 30 min post-H2O injection (s.c.) in intact (controls are non-Tx) and Tx (spinalized) mice

Non-significant differences were found suggesting that smaller pellets (or with reduced moisture) were produced in SCI animals in these experimental conditions. Ten (10) Tx mice and 10 non-Tx received water

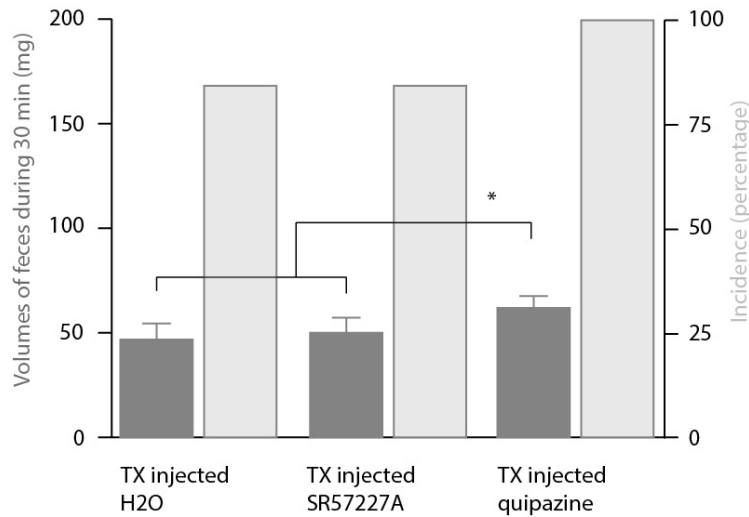


Fig. 3. Amount of fecal pellets expressed within 30 min post-injection (s.c.) of 5-HT receptor agonists in Tx mice

Incidence values (percentages of animals expressing some defecation, pale grey bars) show that most mice expressed at least some defecation. However, dark grey bars (amounts) reveal that only quipazine was found to elicit significantly greater defecatory effects. Ten (10) Tx mice received quipazine, 10 Tx mice received SR57227A and 10 other Tx mice received water. For references regarding dosing, see articles 21 and 22

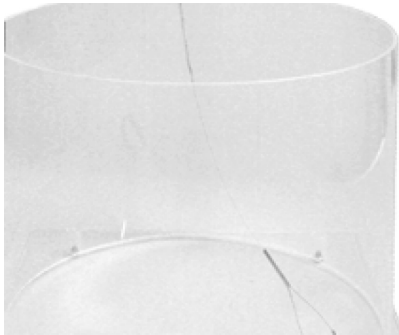


Fig. 4. Open-field circular arena

A Plexiglas circular structure of 60 cm in diameter was used for acclimation and all experiments.

4. DISCUSSION AND CONCLUSION

The results essentially reveal that Tx animals display a large reduction in defecation that may be associated, to some extent, with comparable problems such as chronic constipation in SCI patients. It is shown also that despite extensive acclimation procedures aimed at reducing stress levels in these experimental conditions, a basic level (threshold) remained spontaneously found even in SCI animals. Given the small but significant effect of quipazine on acutely-elicited defecation in this animal model, the results provide also clear PoC data in vivo demonstrating the simplicity, efficacy, and

feasibility of this quantitative approach to identify and compare prokinetic compounds that may be clinically relevant for CNS trauma-related chronic constipation.

Other compounds are known to promote defecation such as cholinesterase inhibitors and 5-HT4 agonists [15,16]. However, Guttman and Walsh have shown in 1971 (earlier case reports also exist) that cholinesterase inhibitors such as prostigmine and neostigmine could also elicit spontaneous erection in SCI men and dysreflexia at higher doses (e.g., above 2 mg/kg/day) although in lower dose range, its safety and efficacy has been approved for use in adults and children with myasthenia gravis [17,18]. 5-HT4 agonists such as mosapride can only moderately enhance rectorectal reflexes in peripheral nerve injured models although effects in SCI model remain to be seen [16]. In other words, it remains unclear from their experiments, whether effects induced by 5-HT4 agonists would be maintained if descending tracts from the brain were to be severed (such as in our spinal-transected model) or if LDC neurons were impaired?

Other technologies can promote defecation in SCI patients such as peripherally acting methods or molecules and digital stimulation, manual removal of feces, abdominal massage, suppositories (e.g. Dulcolax), mini-enemas and other laxatives. However, none of these products

efficiently and safely treat chronic constipation problems in SCI patients. In fact, they are often associated with complications including irritation, stress, rectal tissue damage, dependency (Dulcolax), bowel accident (laxatives), etc (www.apparelyzed.com/bowel-complications.html) [3-6].

The idea of acting upon central (spinal) center activity in order to improve or perhaps more specifically (i.e., than peripherally-acting drugs) trigger episodes of defecation is not entirely new in itself. It has been indeed proposed previously by others scientists who tested direct activation of LDC neurons (i.e., lumbosacral defecation center that centrally controls defecation [9,12,19]) with intrathecal administration of capromorelin (ghrelin receptor agonist) or indirectly using electrical stimulation of sacral nerves [12,19,20]. Although, invasive and/or unsafe (reported safety concerns that would impair approval), those earlier experimental approaches have been instrumental to reveal the great potential of LDC neurons as a new target for future safer clinically-relevant drug therapies [21,22].

We thus strongly believe that drugs and specifically small molecules active upon systemic administration that could increase fecal pellet production above threshold level (near 50 mg) will constitute lead compounds of interest for the development of defecation-inducing treatments against chronic constipation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rouleau P, Ayoub E, Guertin PA. Traumatic and non-traumatic spinal cord-injured patients in Quebec, Canada: Part 1 – Epidemiological, clinical and functional characteristics. *Open Epidemiol J.* 2011;4:131-7.
2. Bauman WA, Spungen AM. Metabolic changes in persons after spinal cord injury. *Phys Med Rehabil Clin N Am.* 2000;11:109-140. [PubMed: 10680161].
3. Coggrave M, Norton C, Wilson-Barnett J. Management of neurogenic bowel dysfunction in the community after spinal cord injury: A postal survey in the United Kingdom. *Spinal Cord.* 2009;47:323–330 [PubMed: 19015665].
4. Liu CW, Huang CC, Chen CH, Yang YH, Chen TW, Huang MH. Prediction of severe neurogenic bowel dysfunction in persons with spinal cord injury. *Spinal Cord.* 2010;48:554–559. [PubMed: 20065986].
5. Furlan JC, Urbach DR, Fehlings MG. Optimal treatment for severe neurogenic bowel dysfunction after chronic spinal cord injury: A decision analysis. *Br J Surg.* 2007;94:1139-50. [PubMed: 17535012].
6. Yim SY, Yoon SH, Lee IY, Rah EW, Moon HW. A comparison of bowel care patterns in patients with spinal cord injury: Upper motor neuron bowel vs lower motorneuron bowel. *Spinal Cord.* 2001;39:204–207 [PubMed: 11420735].
7. Gonella J, Bouvier M, Blanquet F. Extrinsic nervous control of motility of small and large intestines and related sphincters. *Physiol Rev.* 1987;67:902-61 [PubMed: 3299412].
8. Nadelhaft I, Booth AM. The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish peroxidase study. *J Comp Neurol.* 1984;226:238-45. [PubMed: 6736301].
9. Vizzard MA, Brisson M, de Groat WC. Transneuronal labeling of neurons in the adult rat central nervous system following inoculation of pseudorabies virus into the colon. *Cell Tissue Res.* 2000;299:9-26 [PubMed: 10654066].
10. De Groat WC, Krier J. The central control of the lumbar sympathetic pathway to the large intestine of the cat. *J Physiol.* 1979;289:449-68 [PubMed: 472721].
11. Nagano M, Ishimizu Y, Saitoh S, Okada H, Fukuda H. The defecation reflex in rats: Fundamental properties and the reflex center. *Auton Neurosci.* 2004;111:48-56. [PubMed: 15109938].

12. Shimizu Y, Chang EC, Shafton AD, Ferens DM, Sanger GJ, Witherington J, Furness JB. Evidence that stimulation of ghrelin receptors in the spinal cord initiates propulsive activity in the colon of the rat. *J Physiol.* 2006;76:329-38. [PubMed: 16873401].
13. Guertin PA. Preclinical evidence supporting the clinical development of central pattern-modulating therapies for chronic spinal cord-injured patients. *Front Hum Neurosci.* 2014;8:272 [PubMed: 24910602].
14. Guertin PA. A technological platform to optimize combinatorial treatment design and discovery for chronic spinal cord injury. *J. Neurosci Res.* 2008;86:3039-51. [PubMed: 18615646].
15. Korsten MA, Rosman AS, Nq A, Cavusoglu E, Spungen AM, Radulovic M, Wecht J, Bauman WA. Infusion of neostigmine-glycopyrrolate for bowel evacuation in persons with spinal cord injury. *Am J Gastroenterol.* 2005;100: 1560-5. [PubMed: 15984982].
16. Kojima Y, Nakagawa T, Katsui R, Fujii H, Nakajima Y, Takaki M. A 5-HT4 agonist, mosapride, enhances intrinsic rectorectal and rectoanal reflexes after removal of extrinsic nerves in guinea pigs. *Am J Physiol Gastrointest Liver Physiol.* 2005;289:351-60. [PubMed: 15817810].
17. Guttmann L, Walsh JJ. Prostigmine assessment test of fertility in spinal man. *Paraplegia* 1971;9:39-51. [PubMed: 5171244].
18. Biering-Sorensen F, Sonksen J. Sexual function in spinal cord lesioned men. *Spinal Cord.* 2001;39:455-70. [PubMed: 11571657].
19. Ferens DM, Habgood MD, Saunders NR, Tan YH, Brown DJ, Brock JA, Furness JB. Stimulation of defecation in spinal cord-injured rats by a centrally acting ghrelin receptor agonist. *Spinal Cord.* 2011;49:1036-41. [PubMed: 21625243].
20. Rasmussen MM, Kutzenberger J, Krogh K, Zepke F, Bodin C, Domurath B, Christensen P. Sacral anterior root stimulation improves bowel function in subjects with spinal cord injury. *Spinal Cord.* 2015;53:297-301. [PubMed: 25600307].
21. Guertin PA, Steuer I. Ionotropic 5-HT3 receptor agonist-induced motor responses in the hindlimbs of paraplegic mice. *J Neurophysiol.* 2005;94:3397-405. [PubMed: 16049141].
22. Ung RV, Landry ES, Rouleau P, Lapointe NP, Rouillard C, Guertin PA. Role of spinal 5-HT2 receptor subtypes in quipazine-induced hindlimb movements after a low-thoracic spinal cord transection. *Eur J Neurosci.* 2008;28:2231-42. [PubMed: 19019202].

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