ORIGINAL RESEARCH—BASIC SCIENCE

A Pivotal Role of Lumbar Spinothalamic Cells in the Regulation of Ejaculation via Intraspinal Connections

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ABSTRACT

Introduction. A population of lumbar spinothalamic cells (LSt cells) has been demonstrated to play a pivotal role in ejaculatory behavior and comprise a critical component of the spinal ejaculation generator. LSt cells are hypothesized to regulate ejaculation via their projections to autonomic and motor neurons in the lumbosacral spinal cord.

Aim. The current study tested the hypothesis that ejaculatory reflexes are dependent on LSt cells via projections within the lumbosacral spinal cord.

Methods. Male rats received intraspinal injections of neurotoxin saporin conjugated to substance P analog, previously shown to selectively lesion LSt cells. Two weeks later, males were anesthetized and spinal cords were transected. Subsequently, males were subjected to ejaculatory reflex paradigms, including stimulation of the dorsal penile nerve (DPN), urethrogenital stimulation or administration of D3 agonist 7-OH-DPAT. Electromyographic recordings of the bulbocavernosus muscle (BCM) were analyzed for rhythmic bursting characteristic of the expulsion phase of ejaculation. In addition, a fourth commonly used paradigm for ejaculation and erections in unanesthetized, spinal-intact male rats was utilized: the ex copula reflex paradigm.

Main Outcome Measures. LSt cell lesions were predicted to prevent rhythmic bursting of BCM following DPN, urethral, or pharmacological stimulation, and emissions in the ex copula paradigm. In contrast, LSt cell lesions were not expected to abolish erectile function as measured in the ex copula paradigm.

Results. LSt cell lesions prevented rhythmic contractions of the BCM induced by any of the ejaculatory reflex paradigms in spinalized rats. However, LSt cell lesions did not affect erectile function nor emissions determined in the ex copula reflex paradigm.

Conclusions. These data demonstrate that LSt cells are essential for ejaculatory, but not erectile reflexes, as previously reported for mating animals. Moreover, LSt cells mediate ejaculation via projections within the spinal cord, presumably to autonomic and motor neurons. **Staudt MD, Truitt WA, McKenna KE, de Oliveira CVR, Lehman MN, and Coolen LM. A pivotal role of lumbar spinothalamic cells in the regulation of ejaculation via intraspinal connections. J Sex Med 2012;9:2256–2265.**

Key Words. Ejaculation; Spinal Cord; Spinal Ejaculation Generator; Sexual Behavior; Copulation; Motor Neurons

Introduction

Male sexual behavior consists of many components, including pursuit of the female, mounts, and intromissions, accumulating to ejaculation [1,2]. Ejaculation is a physiological process that is comprised of two distinct phases: emission and expulsion [3,4]. In emission, seminal fluids are released through contraction of the accessory sex glands, including the seminal vesicles, prostate, and vas deferens [5], and the external urethral sphincter and bladder neck are

closed to prevent retrograde ejaculation [4]. The expulsion phase, which empties the contents of the urethra and consists of the ejection of semen from the urethral meatus [6] is characterized by rhythmic coordinated contractions of the striated perineal muscles, primarily the bulbocavernosus muscle (BCM) [7–9]. The visceral organs involved in the emission and expulsion phases are under the control of sympathetic and parasympathetic autonomic innervation. In the rat, sympathetic preganglionic neurons are located in the lower thoracic and upper lumbar regions of the spinal cord, within the intermediolateral cell column (IML) and the central autonomic nucleus (CAN) [10,11]. Parasympathetic preganglionic neurons are located in the lower lumbar and upper sacral regions of the spinal cord, within the sacral parasympathetic nucleus (SPN) [12,13]. The motor component of ejaculation (expulsion phase) is coordinated by pudendal motorneurons located in the spinal nucleus of the bulbocavernosus (SNB) [14].

Ejaculation is a complex reflex, controlled by a central pattern generator in the lumbosacral spinal cord [15], referred to as spinal ejaculation pacemaker [16], or spinal ejaculation generator [6]. This ejaculation generator coordinates the autonomic and motor outflow to induce emission and expulsion [6], and integrates this outflow with sensory inputs related to the summation of sexual activity and that are required to trigger ejaculation. The primary sensory nerve considered to be responsible for the mating-related activation of ejaculation is the sensory branch of the pudendal nerve: the dorsal nerve of the penis (DPN) [14,17]. Bilateral transection of the DPN prevent ejaculation in mating rats [18] while stimulation of the DPN has been demonstrated to elicit an ejaculatory reflex in all mammals, including rats [19], primates [20], and humans [21].

The spinal ejaculation generator contains interneurons in the lumbosacral spinal cord that are essential for ejaculation. Previous studies identified neural activation specifically related to ejaculation in this population of interneurons within the central gray of lumbar segments L3-L4, in lamina X and the medial portion of lamina VII [22] that contain galanin, cholecystokinin [23,24], enkephalin [25], and gastrin-releasing peptide (GRP) [26]. Based on their location in the lumbar spinal cord and thalamic projections [23], these cells are referred to as lumbar spinothalamic (LSt) cells. Lesions of the LSt cells using a cell-specific targeted lesion approach, completely eliminated ejaculatory behavior, demonstrating the essential

role of this neural population for ejaculation [27]. Stimulation of the DPN activates LSt cells in similar manner to ejaculation, by inducing phosphorylated ERK (pERK) [28] and phosphorylated NMDA receptor subunit 1 (pNR1) [29]. Moreover, this neural activation of LSt cells is essential for ejaculatory reflexes induced by DPN stimulation as pharmacological blockade of ERK and NMDA receptor activation prevented BCM bursting characteristic of ejaculation following DPN stimulation [28,29]. Finally, LSt cells have direct axonal intraspinal connections with autonomic sympathetic (IML and CAN) and parasympathetic (SPN) neurons within the spinal cord [23,27,30–33] and project to pudendal motorneurons [34]. Thus, LSt cells have been demonstrated to form an essential component of the spinal ejaculation generator. Moreover, it is long hypothesized that LSt cells trigger ejaculation via their intraspinal projections to autonomic and motor neurons within the spinal cord [27], but this hypothesis remains untested.

The spinal ejaculation generator, and/or the autonomic and somatic connections of the spinal ejaculatory network are under influence by supraspinal sites [35,36], including the nucleus paragigantocellularis (nPGi) [37–40] and the medial preoptic area (MPOA) [41–43]. These supraspinal influences complicate investigation of the regulation of ejaculation by the spinal generator in freely moving animals. Therefore, several physiological paradigms or preparations have been developed to study the spinal control of ejaculation in the absence of supraspinal influences [44]. One such paradigm is the urethrogenital (UG) reflex model [45,46], in which the ejaculatory reflex is induced by mechanical stimulation of the urethra in anesthetized and spinalized rats. Ejaculatory reflexes are measured by the rhythmic BCM bursting characteristic of the ejaculatory reflex [46]. In addition, a second paradigm utilizes DPN stimulation in anesthetized and spinalized rats, which elicits rhythmic contractions of the BCM similar to those in a behaving animal [19,47]. A third pharmacological paradigm to study ejaculation utilizes intracerebroventricular administration of D3 dopamine receptor agonist 7-OH-DPAT which elicits rhythmic BCM contractions in anesthetized spinal-intact rats [48]. In addition, systemic administration of 7-OH-DPAT facilitated male rat sexual behavior by decreasing the number of intromissions and latency to ejaculate [49,50]. In the current study, this pharmacological paradigm was used to study rhythmic BCM contractions in anesthetized and spinalized rats. It has previously been demon-

strated that LSt cells are essential for ejaculatory behavior, and hence form an integral part of the spinal ejaculation generator [27]. In the current study, we tested the hypothesis that LSt cells control ejaculation via their intraspinal connections using the three reflex paradigms described above, in isolation from supraspinal influences.

Finally, we tested effects of LSt lesions on sexual reflexes using a fourth paradigm commonly used in spinal intact male rats to investigate erectile function, as well as emissions and ejaculation, namely the ex copula reflex paradigm [17]. In this paradigm, penile responses including erection and seminal emissions are observed in spinal intact male rats in response to mechanical retraction of the prepuce [16,17,26,51–53]. Previously, it was shown that LSt cell lesions in freely mating animals completely abolish ejaculation, whereas erectile function is preserved, based on intact intromissions with a receptive female [27]. These data support the hypothesis that spinal generators for ejaculation and erection consist of different cell populations. Therefore, it was predicted that erectile function as determined by the ex copula paradigm remains intact, whereas LSt lesions were hypothesized to abolish ejaculatory reflexes in all paradigms.

Materials and Methods

Animals

Adult male Sprague Dawley Rats (200–250 g) were obtained from Charles River (Sherbrooke, Quebec, Canada) and housed in pairs in Plexiglas cages on a 12/12 light–dark cycle (lights off at 10 am). Food and water were available *ad libitum*. All procedures were approved by the Animal Care Committee at the University of Western Ontario and conform to guidelines outlined by the Canadian Council on Animal Care.

LSt Cell Lesions

Male rats (N = 48) were anesthetized with a ketamine/xylazine mixture (87/13%, i.p.) and a laminectomy was performed to expose the L3–L4 spinal segment. The selective neurotoxin saporin conjugated to Sar9, Met(O2)11 (SSP), a substance P analog (SSP-SAP; 4 ng/µL; Advanced Targeting Systems, San Diego, CA, USA) was infused bilaterally into the spinal cord as described by Truitt and Coolen [27]. In brief, six injections (separated by approximately 1 µm; 1.5 µm ventral to the dura) were placed in each side for a total of 12 injections (12 × 1 µL) targeting the entire LSt cell population

in L3–L4 spinal levels. After injury, the wound was closed with intramuscular sutures followed by woundclips, and animals were allowed to recover for 2 weeks before further manipulations were performed. This lesion technique has been demonstrated to result in specific lesions of the LSt cells without loss of other surrounding neurons in the spinal cord [27]. Moreover, our previous experiments have demonstrated that due to the technical difficulty of the lesioning surgeries, a portion of the animals have misplaced injections, and thus do not have LSt lesions. Males with misplaced injections have intact ejaculatory behavior and can thus serve as controls [27]. Hence, lesion verification was performed (described below) to identify animals with lesions and misplaced injections, and the latter served as controls. In addition, Sham control animals in the UG reflex (N = 9) and ex copula reflex (N = 9) paradigms received an injection of unconjugated saporin (blank-SAP; 3.68 ng/mL, Advanced Targeting Systems) as described for toxin injections.

Ejaculatory Reflex Paradigms

DPN Stimulation Paradigm. Male rats (N = 18) that received SSP-SAP injections were anesthetized with urethane (1.5 g/kg, i.p.), a laminectomy between thoracic levels T6–T8 was performed, and the spinal cord was completely transected at the same level. Following transection, animals did not receive manipulation for at least 1 hour. The pelvic cavities were then opened and both connective tissue and muscle were dissected away to expose the DPN and BCM. A bipolar stimulating electrode connected to the SD9 Square Pulse Stimulator (Grass Technologies, West Warwick, RI, USA) was placed bilaterally on the DPN. Silver recording electrodes were inserted bilaterally into the BCM and a ground electrode was inserted into the muscle of the right thigh. Recording electrodes were connected to the PowerLab/4SP Data Acquisition System (ADInstruments, Inc., Colorado Springs, CO, USA). Electrical stimulation consisted of square wave pulses of 1 ms duration, 4 V at 30 Hz for 10 seconds. All animals received electrical stimulation of the DPN and BCM EMG activity was recorded. These stimulation parameters have previously been demonstrated to reliable trigger BCM bursting in 100% of control animals [28,29]. In animals in which BCM bursting was not observed, DPN stimulation was repeated at least twice to ensure consistent lack of response.

Urethrogenital Reflex Paradigm. Male rats infused with SSP-SAP (N = 12) or blank-SAP (N = 9)

were anesthetized and spinalized as described above. The pelvic cavities were opened and connective tissue and muscle were dissected away to expose the BCM and pelvic organs. The urethra was cannulated with a PE-50 catheter, which was inserted via the bladder and secured to the bladder neck, as previously described by McKenna and coworkers [45,46]. Urethral pressure was increased to 50 mm Hg via the perfusion of saline (0.9%; 0.4–0.6 mL/minute) through the urethral catheter during simultaneous occlusion of the urethral meatus. Upon release of the occlusion of the urethral meatus, BCM EMG activity was recorded as described above. Urethral stimulation and BCM EMG recordings were conducted three times per animal with increasing urethral pressure (to 100 mm Hg) to ensure consistent lack of response.

7-OH-DPAT Administration. Male rats with SSP-SAP infusions (N = 18) were anesthetized, spinalized, and the BCM was exposed as previously described. All animals received a subcutaneous (s.c.) injection of the preferential dopamine D3 receptor 7-hydroxy-2-(di-N-propylamino) (7-OH-DPAT; 1 mg/kg; H8653; Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada; as reported by Ferrari and Giuliani [49]), and BCM EMG activity was recorded as described above for 10 minutes following drug administration. The s.c. administration of 7-OH-DPAT triggered BCM bursting in 100% of all control animals.

Ex Copula Paradigm. Unanesthetized, spinal-intact male rats infused with SSP-SAP (N = 12) or blank-SAP (N = 9) were placed in a restrainer in supine position, as previously described by Hart [17]. Animals were placed on a piece of Plexiglas, head-first into a piece of downspout tubing (12 inches in length) to above the hip area. Masking tape was used to stabilize the peritoneal region and affix the hindpaws to the plexiglass in an extended position. Animals were allowed to habituate to the tube environment (during three separate habituation sessions), which was defined as 5 minutes without active movement of the animal. During the final test, animals were again placed in this position, the penis was extended following mechanical retraction of the prepuce, and penile responses were observed. Penile reflexes elicited by the ex copula paradigm included erections, including flaring of the glans penis and dorsal flexions of the penis, and seminal emissions (expulsion of a white, viscous fluid or coagulated plug) [16,51,54]. Data are expressed as the percentages of animals that displayed erections or emissions. Several days following testing in this paradigm, these animals were used for the UG reflex paradigm as previously described.

Analysis of BCM Activity. For all animals, for all paradigms (except ex copula paradigm), EMG activity of the BCM was analyzed for 70 seconds following end of stimulation or infusions [29]. BCM EMG activity during the first stimulations in each of the paradigms was analyzed using Power-Lab Chart 7 to quantify the numbers of events, defined as EMG activity spikes above baseline EMG (v7.1.2; ADInstruments, Inc.; see Staudt et al., 2011 for additional details [29]), and numbers of bursts (groups or clusters of events uninterrupted by baseline EMG activity). BCM EMG activity following subsequent stimulations in each of the paradigms was analyzed qualitative for rhythmic bursting patterns by observers blinded to experimental treatments. Student's *t*-test (unpaired, two-tailed) was used to analyze differences in numbers of events or bursts between control and LSt lesioned animals.

Lesion Verification

Immunohistochemistry. At the end of the experiments, animals were anesthetized with sodium pentobarbital and perfused transcardially with 30 mL 0.9% saline solution followed by 500 mL of 4% paraformaldehyde in phosphate buffer (0.1 PB). Spinal cords were removed and post-fixed for 1 hour in the same fixative, after which they were placed in cryoprotective solution (20% sucrose in 0.1 PB with 0.01% sodium azide) until further processing. The spinal tissue was sectioned using a freezing microtome (Thermo Fisher Scientific, Walldorf, Germany) into 12 parallel series of 35 μm coronal sections in cryoprotectant solution (30% sucrose, 30% ethylene glycol in 0.1 M PB with 0.01% sodium azide) and stored at −20°C until further processing.

One series of lumbosacral sections were immunoprocessed for galanin immunoreactivity (used as marker for LSt cells). Free floating sections were rinsed thoroughly with 0.1 M saline buffered sodium phosphate (PBS) between incubations, and all incubations were performed at room temperature with gentle agitation. Sections were blocked with 1% H_2O_2 in PBS for 10 minutes, then incubated in incubation solution (PBS containing 0.1% bovine serum albumin and 0.4% Triton X-100 [BP151-500, Thermo Fisher Scientific, Ottawa, Ontario, Canada]) for 1 hour. Sections were incubated overnight with rabbit anti-galanin (1:60,000 in incubation solution; T-4334; Bachem, Torrance,
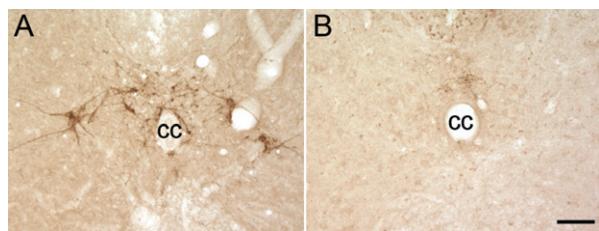
Figure 1 Representative images illustrating the presence of galanin-immunoreactive LSt cells in a male injected with blank-SAP (**A**) and the lack of galanin-immunoreactive LSt cells in a male injected with SSP-SAP (**B**). cc = central canal; Scale bar = 100 μm.

CA, USA), followed by incubations with biotinylated goat anti-rabbit (1:500 in incubation solution, 1 hour; Vector Laboratories, Burlingame, CA, USA), avidin horseradish peroxidase complex (ABC-elite, 1:1,000 in PBS, 1 hour; Vector Laboratories, Burlingame, CA, USA). Next, sections were incubated for 10 minutes in 0.02% diaminobenzidine (DAB) in 0.1 M PB containing 0.012% hydrogen peroxide, resulting in a reddish-brown reaction product. Sections were mounted onto plus charged glass slides and coverslipped with Di-N-Butyl Phthalate in Xylene (DPX; Electron Microcopy Sciences, Hatfield, PA, USA).

LSt Cells Analysis. Numbers of galanin cells were counted in each section containing one or more galanin-immunoreactive (ir) cells by an observer blinded to the experimental treatments and were expressed as numbers of galanin-ir cells per analyzed section for each animal [27]. As previously described in Truitt and Coolen, animals that expressed less than 1/3 of the numbers of galanin-ir cells in intact animals did not display ejaculatory behavior [27]. Therefore, all SSP-SAP injected males with less than 1/3 of the average number of cells in non-surgery intact control animals based on previous published studies (2.3 cells/section [27]) were classified as LSt-lesioned males (Figure 1). All other SSP-SAP (with misplaced injections and more than 1/3 of intact control animals) or blank-SAP infused animals were classified as control animals. LSt-lesioned animals in the DPN stimulation paradigm expressed an average of 0.45 ± 0.1 cells/section (N = 6), whereas control animals expressed an average of 2.01 ± 0.19 cells/section (N = 12). LSt-lesioned animals in the UG reflex and ex copula reflex paradigms expressed an average of 0.31 ± 0.07 cells/section (N = 12), whereas blank-SAP infused control animals expressed an average

of 2.66 ± 0.23 cells/section (N = 9). LSt-lesioned animals in the 7-OH-DPAT injection paradigm expressed an average of 0.18 ± 0.06 cells/section (N = 8), whereas control animals expressed an average of 1.18 ± 0.1 cells/section (N = 10).

Results

DPN Stimulation-Induced BCM Activity was Dependent on LSt Cells

In anesthetized, spinalized control males with intact LSt cells, DPN stimulation reliably evoked rhythmic bursting of the BCM (following the first and each subsequent stimulation), as previously described [28] (Figure 2A). In contrast, in SSP-SAP-treated rats with complete lesions of the LSt cells, DPN stimulation did not elicit rhythmic bursting of the BCM, following any of the repeated stimulations (Figure 2B). Quantitative analysis showed that numbers of events were significantly reduced in LSt-lesioned males compared to control males ($P = 0.017$; Figure 2C), as were the number of bursts ($P < 0.001$; Figure 2D).

UG Reflex-Induced BCM Activity was Dependent on LSt Cells

UG stimulation in anesthetized, spinalized control males with intact LSt cells reliably evoked a rhythmic bursting of the BCM (following first and each subsequent stimulation), as described previously
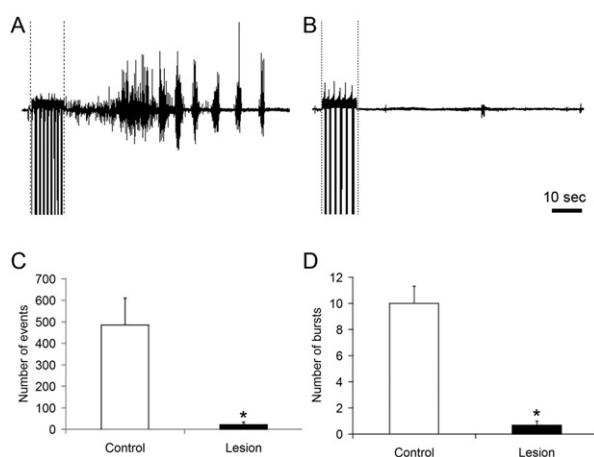

Figure 2 LSt cell lesions eliminated the DPN stimulation-induced ejaculatory reflex. The bursting pattern of the BCM following DPN stimulation is illustrated in representative examples of BCM bursting in control (**A**) and LSt-lesioned (**B**) males. Dashed lines in **A** and **B** depict period of DPN stimulation. Quantitative analysis of BCM EMG is shown as numbers of events (**C**) and bursts (**D**). * indicates significant difference from control animals.
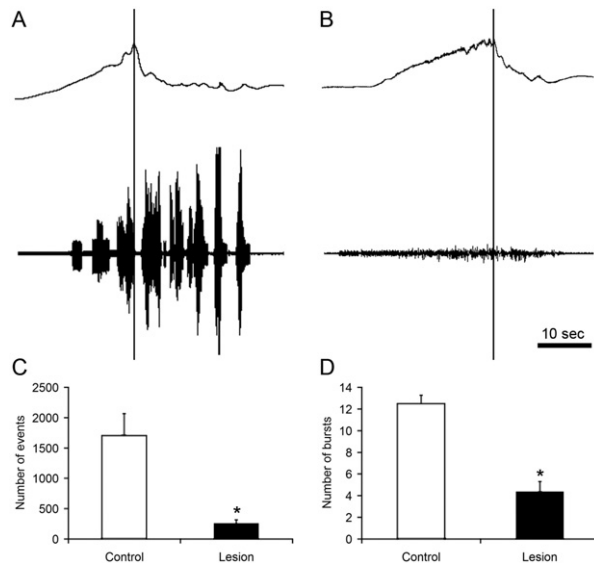
Figure 3 LSt cell lesions eliminated the UG stimulation-induced ejaculatory reflex. The bursting pattern of the BCM is illustrated in representative examples of BCM bursting in control (**A**) and LSt-lesioned (**B**) males. Top traces in **A** and **B** depict rise in urethral pressure, and vertical lines depict release of urethral meatus occlusion (approximately 50 mm Hg). Quantitative analysis of BCM EMG is shown as numbers of events (**C**) and bursts (**D**). * indicates significant difference from control animals.

[45,46] (Figure 3A). In contrast, LSt lesions completely disrupted BCM activity induced by UG stimulation (Figure 3B), following any of the repeated stimulations, even at higher urethral pressures. Quantitative analysis showed that numbers of events ($P < 0.001$; Figure 3C) and bursts ($P < 0.001$; Figure 3D) were significantly reduced in LSt-lesioned males compared to control males.

7-OH-DPAT-Induced BCM Activity was Dependent on LSt Cells

The subcutaneous injections of 7-OH-DPAT used in this study were sufficient to elicit rhythmic BCM contractions characteristic of the ejaculatory reflex in 100% of the anesthetized, spinalized males with intact LSt cells (Figure 4A). The average latency from drug injection to BCM bursting was 134.5 ± 21.9 seconds in control males. In contrast, 7-OH-DPAT did not induce BCM bursting in any of the LSt-lesioned males (Figure 4B), and the number of EMG events ($P < 0.001$; Figure 4C) and bursts ($P < 0.001$; Figure 4D) were significantly reduced compared to control males.

Ex Copula-Induced Penile Reflexes were not Dependent on LSt Cells

LSt lesions did not affect erectile reflexes in the ex copula paradigm, as mechanical retraction of the prepuce elicited erection in 100% of unanesthetized, spinal-intact males with intact LSt cells or with LSt cell lesions. Moreover, LSt lesions did not affect the occurrence of seminal emissions. Emissions were elicited in $25 \pm 25\%$ of control animals, and $37.5 \pm 18.3\%$ of LSt-lesioned animals and there were no significant differences between groups.

Discussion

These studies demonstrate that LSt cells are essential for ejaculatory reflexes induced by sensory or pharmacological stimulation. Cell-specific lesions of LSt cells severely disrupted BCM bursting characteristic of ejaculation upon stimulation of the DPN, urethra, or D3 receptors in anesthetized, spinalized male rats, using stimulation parameters that reliably trigger BCM bursting in all control animals. These data confirm and extend previous findings that LSt cells are essential for ejaculatory behavior in mating animals [27] and provide further evidence for the pivotal role of the LSt cells in control of ejaculation.

Moreover these data provide direct evidence that LSt cells can control ejaculation via intraspinal projections. LSt cells have direct projections within the lumbosacral spinal cord to the
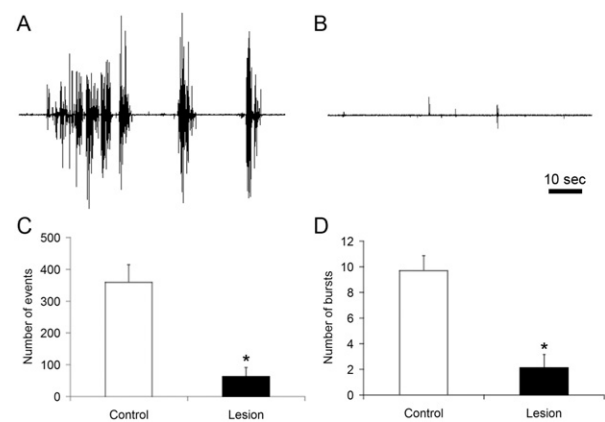

Figure 4 LSt cell lesions eliminated the 7-OH-DPAT-induced ejaculatory reflex. The bursting pattern of the BCM is illustrated in representative examples of BCM bursting in control (**A**) and LSt-lesioned (**B**) males. Quantitative analysis of BCM EMG is shown as numbers of events (**C**) and bursts (**D**). * indicates significant difference from control animals.

sympathetic preganglionic nuclei (CAN, IML), parasympathetic preganglionic nuclei (SPN) [2,27,31,33,49,50], and pudendal motorneurons [34], in addition to projections to the parvocellular subparafascicular nucleus of the posterior intralaminar thalamus [23]. It was long hypothesized that LSt cells regulate ejaculation via the intraspinal projections [27], but this hypothesis remained untested. Alternatively, it was recently proposed that LSt cells trigger ejaculation via the thalamic projections [26,55]. The first evidence supporting the hypothesis that LSt cells regulate ejaculation via intraspinal projections came from the demonstration that electrical stimulation of the area of the LSt cells triggered BCM bursting in anesthetized animals with transections of the spinal cord [56]. The current data further extend these observations and clearly demonstrate the essential role of LSt cells in control of ejaculatory reflexes in spinalized male rats.

The DPN is the primary sensory nerve considered to be responsible for the mating-related sensory activation of ejaculation [14,17,57] as bilateral transection prevents ejaculation in mating rats [18] while stimulation results in rhythmic contractions of the BCM similar to those in a behaving animal [19]. LSt cell lesions blocked ejaculation induced by sensory stimulation of the DPN, either by direct stimulation of the nerve fibers or by recruiting DPN fibers via urethral distension [46]. It is yet unclear how sensory information from the DPN is conveyed to LSt cells. DPN nerve fibers terminate primarily in the lower lumbar and upper sacral levels of the spinal cord, in the general location of the LSt cells, in the dorsal horn and DCG [14,58,59]. However, direct connections of DPN fibers to LSt cells remain to be investigated. We have previously demonstrated that LSt cells are activated by DPN stimulation by activation of NMDA receptors [29] and map kinase signaling [28]. Blockade of either of these events prevents DPN stimulation-induced ejaculatory reflexes [28,29], indicating that LSt cells are essential for conveying sensory information relayed via the DPN to the output centers of the spinal ejaculation generator.

LSt cells were also essential for the effects of D3 receptor agonist 7-OH-DPAT on ejaculation. Previous studies have demonstrated that systemic administration of 7-OH-DPAT facilitated male rat sexual behavior by decreasing the number of intromissions and latency to ejaculation [50]. Subsequent studies have demonstrated that intracerebroventricular administration of 7-OH-DPAT in anesthetized rats elicited rhythmic contractions of the BCM, which was blocked by pre-treatment with a D2/D3 or D3 antagonist [48]. Moreover, injections of 7-OH-DPAT into the medial preoptic area have been shown to trigger ejaculatory reflex, indicating that this D3 agonist acts in this brain area to mediate ejaculation [60]. In the current study, subcutaneous injections of 7-OH-DPAT in spinalized males were sufficient to elicit ejaculatory reflexes, and LSt cell lesions eliminated these reflexes. Therefore, these results suggest that 7-OH-DPAT also acts in the spinal ejaculation generator to regulate ejaculation. The exact sites of D3 receptor expression in the spinal ejaculation generator have not yet been demonstrated. However, the finding that LSt cells are essential for the effects of the D3 receptor agonist suggest that D3 receptors are expressed on LSt cells and/or on spinal neurons that relay sensory information to LSt cells.

An unexpected finding of the current study was that LSt cells do not appear to be essential for emissions as noted in the ex copula paradigm in spinal intact male rats. Emissions in this paradigm were defined based on definitions provided in previous studies as expulsion of a white, viscous fluid or coagulated plug [51]. This finding is in direct contrast to the ample evidence of the role of LSt cells in ejaculation during mating [27] in male rats and for the role of these cells in ejaculatory reflex (expulsion phase) using three different reflex paradigms in spinalized males and. Hence, it is possible that reflexes producing emissions as noted during the ex copula reflex paradigm are not representative for in copula ejaculatory reflexes. Indeed, contradictory findings with the ex copula paradigm have previously been reported, including inhibitory effects of serotonin 1A receptor agonist 8-OH-DPAT, which has inhibitory effects on emissions in the ex copula paradigm [61], while strong facilitative effects on ejaculation latencies in copula [61–65] and induces BCM bursting in the urethrogential reflex model [66]. An alternate explanation for the discrepancy between effects of LSt cell lesions on ejaculatory reflexes in the ex copula paradigm versus the other reflex paradigms used in the present study is that LSt cells may not be essential for emission, but rather for the expulsion phase of ejaculation. Hence, it is possible that emission can still occur (and observed during the ex copula reflex paradigm), but expulsion (as observed in the BCM EMG activity) is disrupted. There is indeed evidence for separate mechanisms mediating

emission and expulsion, as the expulsion phase is not dependent on emission of fluids through the urethra. In particular, anesthesia of the urethra did not prevent ejaculatory motor patterns in mating rats [67], nor did removal of seminal vesicles [68] or sympathetic blockers that prevent emission [67]. Further investigation of the effects of LSt lesions on physiological markers of emission (contractions of vas deferens and seminal vesicle) are needed to address this possibility. Finally, it is possible that supraspinal influences in intact animals can compensate for the loss of LSt cells in the ex copula paradigm. However, LSt cell lesions in spinal intact males completely abolished ejaculation during mating behavior [27]. Hence, such supraspinal compensatory mechanisms mediating reflexes in the ex copula paradigm may not be recruited nor functionally relevant for ejaculatory behavior in mating male rats.

Erectile function as determined in the ex copula reflex test remained intact in male rats with ablations of LSt cells and ejaculatory function, in agreement with previous findings of intact erectile function in the mating animal [27]. Hence, LSt cells are not an essential part of a spinal reflex generator for erectile function [69]. It has been proposed that the neuropeptide GRP is expressed both by LSt cells and by additional spinal lumbar neurons hypothesized to regulate erections [70]. Infusions of GRP agonists and antagonists facilitate or inhibit erectile reflexes in the ex copula reflex paradigm [26], presumably via activation or blockade of GRP receptors in the SPN or pudendal motor neurons.

In conclusion, these data further confirm a pivotal role for LSt cells in control of ejaculatory reflexes and demonstrate that LSt cells regulate ejaculation via intraspinal connections. The exact nature of the LSt intraspinal projections that are required for ejaculation remain unknown. It is currently unclear if LSt cells project to a subset of sympathetic or parasympathetic preganglionic cells, or pudendal motor neurons that are specifically involved in regulating ejaculation. Moreover, the contributions to ejaculatory behavior or reflexes of neuropeptides expressed in LSt axons, including galanin, cholecystokinin [23,24], enkephalin [25], and GRP [26], are currently unknown. Finally, this study provides insight into the validity of paradigms currently used in the study of ejaculation and shows that ejaculation triggered by sensory stimulation of DPN or urethra, or by pharmacological activation of D3 receptors are dependent on LSt cells.

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Statement of Authorship

Category 1

(a) Conception and Design
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(b) Acquisition of Data
William Truitt; Michael Staudt; Cleusa de Oliveira
(c) Analysis and Interpretation of Data
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Category 2

(a) Drafting the Article
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(b) Revising It for Intellectual Content
Michael Staudt; William Truitt; Kevin McKenna; Cleusa de Oliveira; Michael Lehman; Lique Coolen

Category 3

(a) Final Approval of the Completed Article
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