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-