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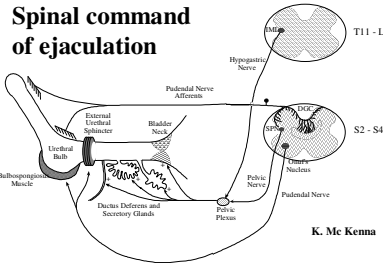
ABSTRACT

Introduction and Objectives: Ejaculation is a complex physiological event which requires coordination of sympathetic, parasympathetic and somatic neural outputs. Timely occurrence of the emission and expulsion of sperm results from an interplay between spinal nuclei directly controlling the sexual accessory glands including the prostate and perineal striated muscles with a key role for the bulbospongiosus muscle (BS). Functional experiments have led to the identification at the L3-L4 spinal level of a group of cells referred as lumbar spinothalamic (LST) neurons as essential for such a coordination in male rats (Science 2002,297:1566-1569). These LST cells are located lateral to the central canal in lamina X and in the medial portion of lamina VII. It has been proposed that these cells constitute the spinal ejaculation generator. LST cells are immunoreactive for galanin and neurokinin-1 receptors (NK-1R). Ablation of the NK-1R immunoreactive LST cells resulted in a complete disruption of ejaculatory behavior. We investigated the anatomical relationships between LST neurons and both the prostate and the BS muscles.

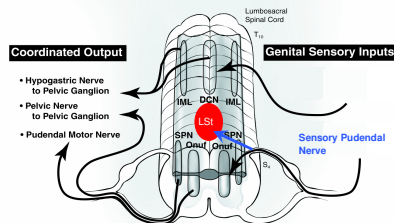
Methods: Pseudorabies virus (PRV) retrograde tracing technique was used combined with immunohistochemistry against galanin or NK-1R. PRV was injected into the BS muscle or the ventral lobe of prostate in respectively 14 and 6 adult SD male rats under anesthesia. Between 3 to 6 days, rats were perfused with 4% paraformaldehyde, the spinal cord was removed and transverse sections (50µm) of the lumbar spinal cord were processed for double immunofluorescence against PRV and galanin or NK1-R. Sections were then examined with confocal laser scanning microscope.

Results: 5 days after injection of PRV in the prostate, double-labeled neurons for PRV and galanin or NK-1R were found at the L3-L4 level lateral to the central canal in lamina X and in lamina VII. 4 days after injection of PRV in BS muscle double-labeled neurons for PRV and galanin or NK-1R were found in the same spinal regions.

Conclusions: Lumbar spinothalamic neurons previously identified as playing a pivotal role in ejaculatory behavior were retrogradely labeled from the prostate and BS muscle. This anatomical finding reinforces the role for these cells in the spinal control of both the emission and the expulsion of sperm.



Spinal generator for ejaculation



METHODS

- Ejaculation is a complex reflex which requires the coordination of sympathetic, parasympathetic, and somatic mechanism
- Ejaculation consists in the succession of distinct physiological events. Closure of the bladder neck, to prevent flow of semen backward in the bladder from the prostatic urethra, precedes and goes with the emission phase, i.e. secretion by seminal vesicles, prostate and ampullary vas deferentia contents into the prostatic urethra of the different sperm components.
- Forceful expulsion of sperm to the urethral meatus is then caused by rhythmic contractions of pelvic and perineal striated muscles, with a primary role for the bulbospongiosus (BS) muscle.
- Neural control of ejaculation likely results from an interplay between spinal nuclei directly controlling the sexual accessory glands i.e. prostate and seminal vesicles, seminal tract, prostatic urethra, bladder neck and pelvi-perineal striated muscles and the supraspinal nuclei modulating their activity.

The role of lumbar spinothalamic (LST) cells for ejaculation has been evidenced in rats. These cells are positioned to relay ejaculation related signals from reproductive organs to the brain, and they express neurokinin-1 receptors. Ablation of these neurons by the selective toxin SSPaparin resulted in a complete disruption of ejaculatory behavior.

Truitt and Coolen, Science 297: 1566-1569, 2002

OBJECTIVES

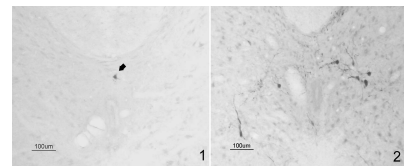
To investigate the anatomical relationships between LST neurons and both the prostate and the BS muscles.

- Male Sprague-Dawley rats (180-220 g, n=44)
- Pseudorabies virus (PRV) retrograde tracing technique was used combined with immunohistochemistry against galanin or NK-1R.
- PRV (Bartha strain of pseudorabies virus (0.65x10⁸ pfu/ml, Dr. A. Jestin, Ploufragan, France) was injected into the BS muscle or the ventral lobe of prostate in respectively 14 and 6 adult SD male rats under anesthesia.
- Between 3 and 6 days, rats were sacrificed and perfused with 4% paraformaldehyde.
- The spinal cord was placed into a Petri dish and the dorsal roots of each spinal segment were identified. Lumbar spinal cord segments were cut off, transverse sections of spinal cord was cut (50µm) with vibratome and collected free-floating in four parallel series.
- One series was used for detecting the infection of PRV with immunohistochemistry by using ABC method.
- The sections from the remaining series were used for double-immunofluorescent detection of NK1 receptor and PRV . Galanin and PRV from BS muscle or prostate or served as a positive control for the various immunohistochemical reactions.
- The following antibodies were used :
 - anti-PRV (RB-133, rabbit polyclonal anti-pseudorabies virus antiserum diluted to final concentration of 1:10,000; kindly provided by L.W. Enquist, Princeton Univ, U.S.A)
 - anti-NK1 receptor (guinea pig polyclonal antibody to neurokinin-1 receptor, 1:40000 Chemicon International, Inc, France)
 - anti-galanin (rabbit anti-galanin, 1: 60,000; Peninsula Labs).

• Sections processed for immunohistochemistry were examined under brightfield illumination (Nikon microscope, Labopho 2) and the number and location of virus-labeled in the spinal cord were recorded for each section. Images were captured at 10 X magnification with Nikon digital camera DXM1200F, qualification of neuron size and the optical density (OD) of NK1 receptor and galanin immunohistochemistry reactive product were performed in a area of 800 X 800 surrounding the central canal with the Image-pro plus image analysis software (Media cybernetics, USA).

• Sections processed for immunofluorescence were examined with a Nikon microscope equipped with epifluorescence. Most of the sections were examined with a LSM 510 / confoCor2 combi confocal laser scanning microscope equipped with a krypton-argon laser, mounted on an inverted microscope Axiovert 200 (ZEISS Germany). Sections were scanned sequentially at a series of optical planed separated by 1 µm with an argon krypton ion laser adjust to 488 nm to Alexa excitation and an argon-ion laser adjusted to 588 nm for Rhodame. Image resolution was 1024x1024 pixels.

RESULTS



Distribution of PRV-immunoreactive neurons in the L1-S1 segment of the rat spinal cord after injection of PRV into the right ventral prostate

Segm enits	Lamina X		DGC	VH	DH	IML(L1-L4) or SPN (L5-S1)	
	Right	Left				Right	Left
L1-L2	13±8(4)	1±1(0)	98±56(0/0)	4±2(1)	111±80(34)	74±24(2/3)	24±9(7)
L3-L4	62±35(18)	38±25(11)	98±36(2/9)	4±1(1)	90±58(2/7)	36±27(1/1)	10±5(0)
L5-L6	5±5(1)	3±3(0)	375±91(50)	15±9(2)	139±87(19)	140±80(19)	73±59(10)

This table displays the mean ± S.E., and percentage (parentheses) of transneurally labeled interneurons in L1-S1 spinal cord after injection of PRV into the right ventral prostate after 5 days of survival (n=6). Labeled neurons in ventral horn did not appear to be motoneurons.

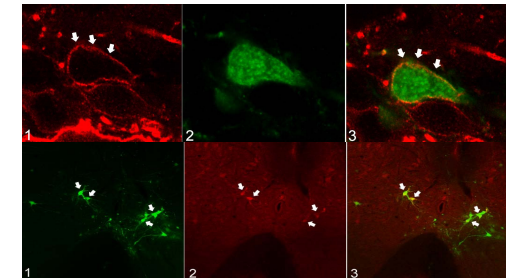
Figure 1. 74 hours after PRV injection in BS muscle, one neuron was immunoreactive for the PRV in the dorsal grey commissure (DGC) of the L3 segment.

Figure 2. 94 hours after PRV injection in BS muscle, number of neurons were immunoreactive for the PRV in lamina X surrounding the central canal of the L4 segment.

Distribution of PRV-immunoreactive interneurons in the L1-S1 segments of rat spinal cord after injection of PRV into the right bulbospongiosus muscle

Segments	Lamina X		DGC	Ventral Horn	Dorsal Horn	IML(L1-L4) or SPN (L5-S1)	
	Right	Left				Right	Left
L1-L2	12±11(9)	2±2(2)	26±3(20)	0±0(0)	11±6(8)	67±28(5/1)	14±5(11)
L3-L4	17±0(26)	3±2(5)	28±2(42)	2±2(3)	3±1(5)	12±9(18)	1±2(2)
L5-L6	0±0(0)	0±0(0)	88±31(4/1)	3±2(1)	12±2(6)	97±30(4/5)	17±9(8)

This table displays the mean ± S.E., and percentage (parentheses) of the transneurally labeled interneurons in L1-S1 spinal cord segments after injection of PRV into the bulbospongiosus muscle after 4 days of survival (n=6). Labeled neurons in ventral horn did not appear to be the motoneurons.



Confocal photograph of PRV immunoreactive neuron enveloped by an NK1 receptor immunoreactive plasma membrane. (1) NK1 receptor immunoreactive product located in plasma membrane (rhodamine); (2) PRV labeled neuron cell body (Alexa 488); (3) Double labeled neuron.

Confocal photograph of PRV and galanin double labeled neurons in lamina X in the L3 segment. (1) PRV labeled neurons. (2) Galanin labeled neurons. (3) Some neurons were double labeled for PRV and galanin.

SUMMARY OF RESULTS

- We have found that subpopulation of interneurons located in the laminae X of the L3-L4 segment of the male rat spinal cord express neurokinin 1 receptor or galanin and project
 - to the motoneurons of the DM nucleus innervating the bulbospongiosus muscle and
 - to the preganglionic neurons of the IML innervating the prostate.
- These interneurons are located in the same area as the LST cells.

CONCLUSION

- LSt neurons previously identified as playing a pivotal role in ejaculatory behavior are likely retrogradely labeled from the prostate and the BS muscle.
- This anatomical finding provide a organisational support for the mandatory coordination between the emission and the expulsion phase of ejaculation.
- The role for LSt cells in the spinal control of ejaculation is reinforced.