electron density from diffraction data collected on a 5-bromouracil derivative (5-bromouracil at positions 11, 23, and 32') was used to confirm the placement of DNA. Helical segments of the solution structure of αCTD (PDB accession number 1COO) were modeled into electron density in $F_{obs} - F_{calc}$ maps, additional residues of CAP and αCTD were modeled into electron density in successive cycles of Fourier refinement, and the structure was refined by using CNS (*53*) with anisotropic initial *B*-factor refinement and bulk solvent correction, incorporating water molecules conservatively, following strict criteria as described (*5*). The completeness of the highest resolution shell (3.2 to 3.1 Å) was 0.4; reflections from the highest resolution shell were included in the refinement. Omit maps, combined with simulated annealing, were used to confirm the placement and conformation of each residue in the structure. The asymmetric unit contains one CAP protomer and two αCTD protomers (2688 nonhydrogen atoms), one DNA half-site (895 nonhydrogen atoms), and 32 water molecules (Fig. 1, B and C). Atomic coordinates have been deposited in the PDB (accession number 1LB2).

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39. Expected positions of first-shell minor-groove-spine water molecules were calculated as described (*55*). The position of the experimental water molecule (cyan in Fig. 2, D and E) corresponds to the expected position of a first-shell minor-groove-spine water molecule. Expected positions of second-shell minor-groove-spine water molecules were calculated analogously (*56*). The position of the Arg^{265} NH1 atom corresponds to the expected position of a second-shell minor-groove-spine water molecule.
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Figs. S1 and S2

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Identification of a Potential Ejaculation Generator in the Spinal Cord

William A. Truitt and Lique M. Coolen*

We tested the significance of a population of lumbar spinothalamic cells for male sexual behavior 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 SSP-saporin resulted in a complete disruption of ejaculatory behavior. In contrast, other components of sexual behavior remained intact. These results suggest that this population of spinothalamic cells plays a pivotal role in generation of ejaculatory behavior and may be part of a spinal ejaculation generator.

Male sexual behavior is a complex behavior dependent on intrinsic and extrinsic factors, including olfactory, somatosensory, and visceral cues (*1*). The pathways that relay somatosensory and visceral sensory information from the reproductive organs to the brain are not well understood. Recent studies have used expression of the protein product Fos of the immediate early gene c-*fos* to map neural activation in the brain related to the expression of ejaculation in male rodents (*2*). Ejaculation-related Fos induction is restricted to a few brain regions, including areas within the medial amygdala, the bed nucleus of the stria terminalis, and a medial portion of the parvocellular subparafascicular nucleus (SPFp) within the posterior thalamus (*3*). The thalamus receives direct sensory inputs from the spinal cord and may thus be an important relay for genital sensory inputs to other areas of the brain important for sexual behavior. Indeed, the SPFp receives unique inputs from a population of spinothalamic neurons located in laminae VII and X in lumbar segments 3 and 4 (L3 and L4) containing galanin, cholecystokinin (*4–6*), and enkephalin (*7*). Here we refer to this population of lumbar SPFp-projecting neurons as LSt (lumbar spinothalamic) cells. These cells are specifically activated with ejaculation but not with other components of male sexual behavior (*5*, *6*). Thus we hypothesized that LSt neurons are involved in the relay of ejaculation-specific information, although the behavioral significance of these neurons is unknown.

To test the behavioral significance of LSt neurons, effects of lesions of the LSt population on sexual behavior were investigated. LSt neurons are sparsely distributed lateral to the central canal in lamina X and in the medial portion of lamina VII of L3 and L4 and are difficult to lesion by traditional methods. We thus identified a membrane target located on the LSt neurons. It was demonstrated that 93.0 ± 1.7% of LSt neurons express neurokinin-1 receptor (NK-1R) and conversely 84.7 ± 2.47% of NK-1R–containing cells in the area surrounding the central canal at L3 and L4 express galanin (Fig. 1). We therefore used the targeted toxin SSP-saporin (SAP), which consists of the toxin SAP conjugated to SSP, a substance P analog with high affinity for NK-1R (*8*). SSP-SAP was infused into the L3 and L4 spinal cord at the location of the LSt cells in sexually experienced or sexually naïve male rats (*9*). Control animals were injected with unconjugated equimolar concentrations of SAP. The doses used selectively ablate NK-1R–containing cells in vivo without producing nonselective lesions (*8*). Sexual behavior was first tested 10 days after lesion surgery, and during five subsequent twice-weekly tests.

After the final behavior test, animals were perfused, and spinal cord tissue was immunoprocessed for galanin, NK-1R, or neuronal marker N (NeuN) (*9*). Labeled cells were counted in a standard area surrounding the central canal of L3 and L4 sections representative of the location of LSt cells (Fig. 2A, area 1).

Of the 19 rats included in the behavioral

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analysis, eight SSP-SAP–treated rats had complete lesions of LSt neurons (SSP-les; Figs. 2B and 3) defined by less than one-third of the number of LSt cells observed in untreated rats (*10*), whereas incomplete or misplaced lesions were observed in four SSP-SAP–treated males (SSP-il), and no lesions were present in SAP-treated males (SAP, $n = 7$). SSP-les animals had significantly fewer galanin immunoreactive (galanin-IR) neurons than SSP-il or SAP animals ($P < 0.001$; Figs. 2C and 3). Cell counts performed in adjacent sections immunostained for NK-1R further confirmed these data. SSP-les rats had a significantly reduced number of NK-1R–containing cells ($P < 0.006$; Figs. 2D and 3), compared with SSP-il or SAP animals. Despite the severe reduction in LSt neurons in SSP-les rats, there was no overall reduction in numbers of NeuN-IR cells ($P > 0.26$; Figs. 2E and 3), indicating the selectivity of the lesions. The lesions were restricted to the area surrounding the central canal and did not affect the number of NK-1R–expressing neurons in the dorsal horn (Fig. 2F). In addition, pain perception was not altered in

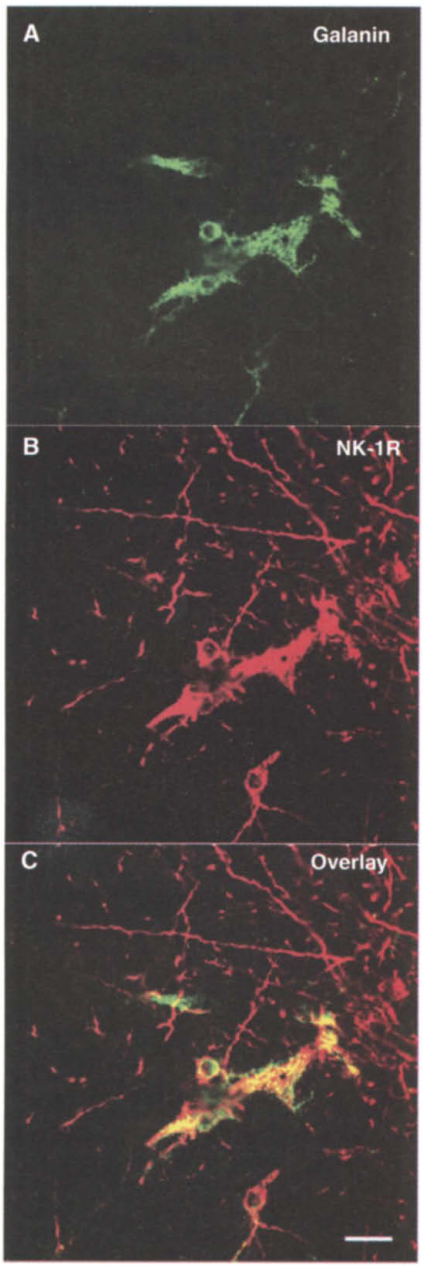

Fig. 1. Fluorescent images illustrating the co-expression of galanin (**A**) and NK-1R (**B**) in LSt neurons (**C**) Overlay of (A) and (B). Scale bar, 50 μm.

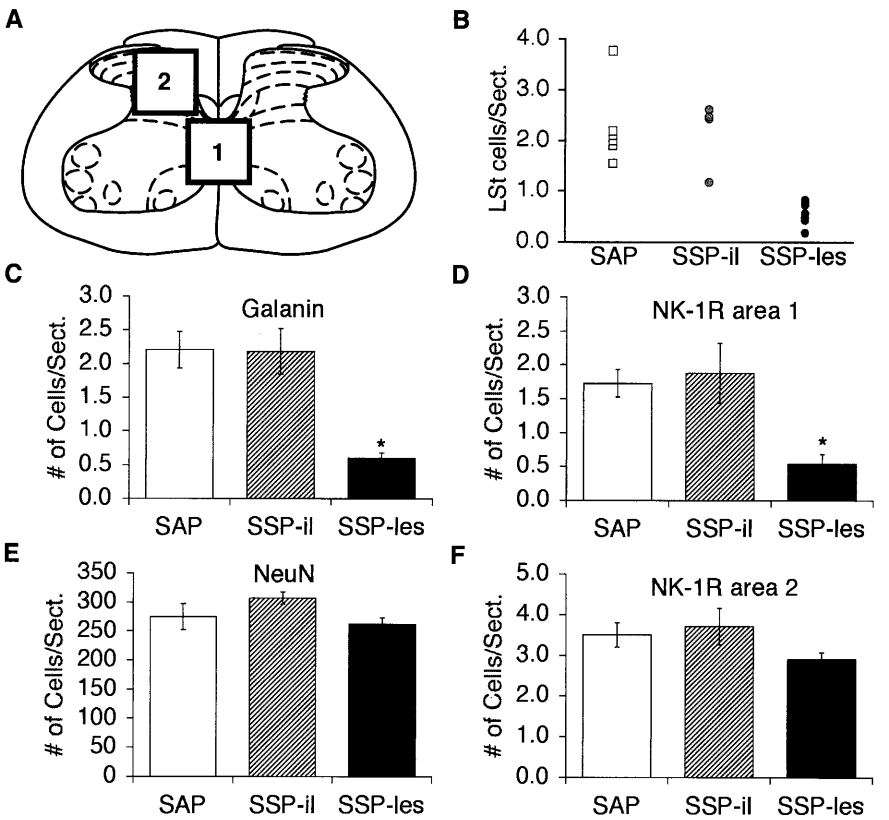

Fig. 2. Quantitative analysis of LSt cells per section (B), galanin-IR (C), NK-1R-IR (D, F), or NeuN-IR (E) cells. Counts were performed in two separate areas in L3 and L4 (**A**) that measured 800 by 800 μm each. (**B** to **E**) Counts were performed in area 1, which encompasses the LSt cell population, i.e., lamina X and the medial portion of lamina VII. (B) The average number of LSt neurons per analyzed tissue section for each animal (average of 15.21 ± 0.99 sections were analyzed per animal), which demonstrates the effectiveness of lesions within the three groups (SSP-les, $n = 8$; SSP-il, $n = 4$; SAP, $n = 7$). In (C) to (**F**), group means ± SEM are shown of the average number of cells per analyzed section per animal. SSP-les rats had significantly fewer LSt neurons evidenced by a reduction in numbers of galanin-IR (C) or NK-1R-IR (D) neurons compared with SSP-il or SAP rats. In contrast, there is no significant reduction in NeuN-IR (E), or NK-1R-IR in the dorsal horn (F) [counts performed in area 2 (A)]. *$P \leq 0.001$ compared with SAP and SSP-il.

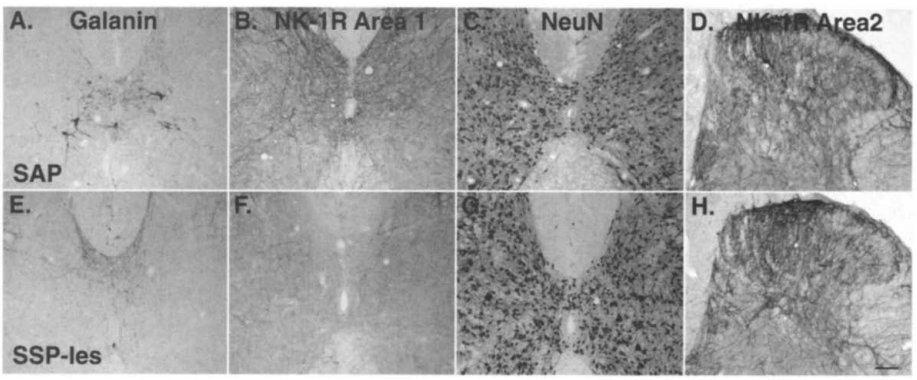

Fig. 3. Photomicrographs of galanin-IR (**A** and **E**), NK-1R-IR (**B** and **F**), and NeuN-IR (**C** and **G**) in area 1 surrounding the central canal. In addition, NK-1R-IR is shown in area 2 [NK-1R-2 (**D** and **H**)] in the dorsal horn. All photomicrographs illustrate L4 of a representative SAP [(A) to (D)] and SSP-les [(E) to (H)] animal. Galanin-IR and NK-1R-IR are visibly reduced, whereas NeuN labeling shows no reduction in SSP-les compared with SAP-treated males. Scale bar, 100 μm.

SSP-les animals compared with SSP-il or SAP controls (*11*).

LSt lesions had a dramatic effect on sexual behavior. Lesions completely disrupted display of ejaculatory behavior in SSP-les males, and examination of the female partner revealed that seminal plugs were uniformly absent throughout the testing session. In contrast, SSP-il and SAP males continued to ejaculate regularly after surgery [Fig. 4A and (*12*)]. Furthermore, ablation of LSt neurons selectively blocked ejaculatory behavior without affecting other components of sexual behavior. SSP-les animals did not differ from the SSP-il or SAP animals in number of mounts ($P > 0.317$) or intromissions ($P > 0.644$; Fig. 4, B and C). Average mount latencies and average intromission latencies also did not significantly differ among groups ($P > 0.492$ and $P > 0.569$, respectively). There were no detectable differences in behavior between animals that were naïve before lesion surgery and those that were sexually experienced (*9*); hence, data were grouped for sexually naïve and experienced animals.

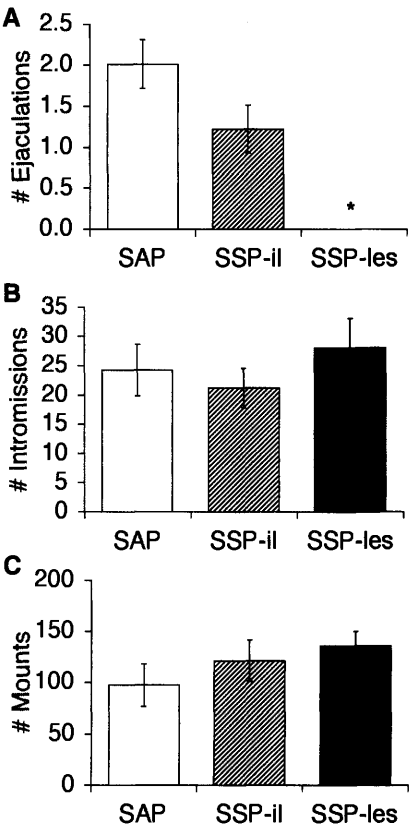

Fig. 4. Quantitative analysis of sexual behavior. LSt neuronal lesioning abolishes ejaculatory behavior [number of ejaculations (**A**)] but has no effect on number of intromissions (**B**) or mounts (**C**). For group means ± SEM, numbers were averaged across six postsurgery trials. *$P \leq 0.001$ compared with SSP-il or SAP. No differences were detected between SAP and SSP-il animals ($P > 0.05$).

It is well established that ejaculation is a reflex and that the central components necessary to complete this reflex are located in the lumbosacral spinal cord (*13*). This spinal ejaculatory neural system is under descending excitatory and inhibitory influence from supraspinal centers in brainstem and hypothalamus (*13*). However, ejaculatory reflexes remain intact when control by supraspinal sites is eliminated, suggesting the existence of a spinal ejaculation generator. In particular, the ejaculatory reflex remains intact in spinalized rats and humans and can be evoked by electrostimulation (*14*, *15*) or intense vibration (*16*) in humans with spinal cord injuries between cervical level 3 and lumbar level 3. Furthermore, the existence of a central spinal generator for ejaculation has been inferred in the rat (*17*). However, the anatomical site of such an ejaculation generator is yet unknown. Here, we provide evidence that a population of lumbar spinothalamic neurons plays a pivotal role in generation of ejaculatory behavior, suggesting that these LSt cells form a critical component of the ejaculation generator. Moreover, LSt cells have additional characteristics that are consistent with their role as a component of an ejaculation generator. Ejaculation consists of two phases, emission (secretion and movement of seminal fluids to the urethra) and expulsion (forceful ejection of urethral contents), which have been demonstrated to follow a highly synchronized series of events in humans (*18*) and in rats (*19*). The ejaculatory reflex is complex and modular in nature and involves multiple afferent and efferent systems. The precise nature of the afferent stimuli inducing ejaculation is unknown and may involve sensory, visceral, proprioceptive, and somatic inputs. It is possible that LSt cells receive stimuli related to onset of ejaculation and, in turn, trigger the ejaculatory reflex. Indeed, LSt cells are activated, i.e., express Fos, with ejaculation (*5*, *6*). On the output side, the ejaculatory reflex involves a complex control of sympathetic, parasympathetic, and somatic efferent systems. It is speculated that these systems are coordinated by a central pattern generator, consisting of interneurons located in the lumbosacral spinal cord, producing a sequential rhythmic bursting of muscles and nerves responsible for the secretion and the external ejection of seminal fluid (*20*). Consistent with this hypothesis, the data in Table 1 indicate that LSt neurons have projections to sympathetic neurons of the intermediolateral column and the central autonomic nucleus, areas that are crucial to the emission phase of ejaculation (*20*), and to the sacral parasympathetic nucleus involved in regulation of epithelial secretion and prostatic activity (*13*), as well as sphincter control (*21*). In summary, LSt neurons are in a position to process stimuli related to onset of ejaculation, as well as regulating ejaculation. In addition, via projections to the thalamus, LSt cells may contribute to the sensation of ejaculation.

Our results demonstrate a potential site of an ejaculation generator in the spinal cord, consisting of a population of LSt cells. Characterization of the afferent and efferent connections of LSt neurons, investigation of the roles of neuropeptides and neurotransmitters in the function of LSt cells, and identification of the reflex components controlled by LSt cells should provide further insight into the spinal mechanisms involved in control of the ejaculatory reflex. Detailed understanding of a spinal ejaculation generator will significantly benefit treatment of sexual dysfunction, in particular related to ejaculation. The practical implications include the possible development of additional treatments for premature ejaculation and ejaculatory function in paraplegic men.

Table 1. Optical density measurements (9) in pixels (×1000) of galanin or NK-1R-IR in the intermediolateral column (IML), central autonomic nucleus (CAN), and sacral parasympathetic nucleus (SPN). In these areas, galanin-IR is present in fibers, whereas NK-1R-IR is located in neurons and fibers. SSP-les animals have significantly reduced galanin-IR, indicating that the galanin-IR fibers in these areas are the axon terminals of LSt neurons. In contrast, NK-1R-IR is not reduced after LSt lesions. Thus, loss of galanin-IR fibers in these areas is not a result of spread of the toxin, but rather reflects the loss of LSt axon terminals. * $P \leq 0.001$ compared with SAP and SSP-il.

	SAP	SSP-il	SSP-les
IML galanin	58.82 ± 12.85	70.87 ± 18.58	20.47 ± 5.61*
CAN galanin	128.84 ± 25.81	118.13 ± 21.25	25.56 ± 9.41*
SPN galanin	137.75 ± 14.99	177.60 ± 26.40	77.64 ± 10.79*
IML NK-1R	209.91 ± 30.92	150.81 ± 71.11	176.83 ± 21.44
CAN NK-1R	380.26 ± 39.06	266.99 ± 159.47	241.75 ± 49.26
SPN NK-1R	392.68 ± 50.65	362.91 ± 101.08	340.97 ± 49.89

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10. Untreated male rats (n = 6) had 2.709 ± 0.174 (mean ± SEM) of galanin-IR cells per L3 or L4 section. Rats with less than 0.906 galanin-IR cells per L3 or L4 section were considered completely lesioned.
11. Mean ± SEM pain thresholds (seconds) accessed by hot plate test were 33.33 ± 3.99, 35.98 ± 2.08, and 32.75 ± 4.70 for SAP, SSP-il, and SSP-les rats, respectively ($P > 0.85$).
12. Mean numbers of ejaculations did not significantly differ between pre- and postoperative tests for SAP and SSP-il males, and seminal plugs were consistently observed with display of ejaculatory behavior. Moreover, latency to first ejaculation and average duration of test did not differ between pre- and postoperative tests for SAP and SSP-il males.
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Materials and Methods

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