Neuropeptidergic control circuits in the spinal cord for male sexual behaviour: oxytocin–gastrin-releasing peptide systems

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Abstract

The neuropeptidergic mechanisms controlling socio-sexual behaviours consist of complex neuronal circuitry systems in widely distributed areas of the brain and spinal cord. At the organismal level, it is now becoming clear that 'hormonal regulations' play an important role, in addition to the activation of neuronal circuits. The gastrin-releasing peptide (GRP) system in the lumbosacral spinal cord is an important component of the neural circuits that control penile reflexes in rats, circuits that are commonly referred to as the "spinal ejaculation generator (SEG)." Oxytocin, long known as a neurohypophyseal hormone, is now known to be involved in the regulation of socio-sexual behaviors in mammals, ranging from social bonding to empathy. However, the functional interaction between the SEG neurons and the hypothalamo-spinal oxytocin system remains unclear. Oxytocin is known to be synthesised mainly in hypothalamic neurons and released from the posterior pituitary into the circulation. Oxytocin is also released from the dendrites of the neurons into the hypothalamus where they have important roles in social behaviours via non-synaptic *volume transmission*. Because the most familiar functions of oxytocin are to regulate female reproductive functions including parturition, milk ejection, and maternal behaviour, oxytocin is often thought of as a 'feminine' hormone. However, there is evidence that a group of parvocellular oxytocin neurons project to the lower spinal cord and control male sexual function in rats. In this report, we review the functional interaction between the SEG neurons and the hypothalamo-spinal oxytocin system and effects of these neuropeptides on male sexual behaviour. Furthermore, we discuss the finding of a recently identified, localised '*volume transmission*' role of oxytocin in the spinal cord. Findings from our studies suggest that the newly discovered 'oxytocin-mediated spinal control of male sexual function' may be useful in the treatment of erectile and ejaculatory dysfunction.

(293/300 words)

KEYWORDS: male sexual function, oxytocin, gastrin-releasing peptide, spinal cord, non-synaptic *volume transmission*.

Introduction

Neuropeptides are the master regulators of neuroendocrine systems controlling socio-sexual behaviours (1, 2). These control mechanisms in the brain and spinal cord are formed and maintained by complicated neural circuits (2). The neurohypophyseal hormones (*neuropeptides*), oxytocin and vasopressin, control a series of behaviours such as territorial behaviour, courtship behaviour, pair bonding, reproductive behaviour, and nurturing behaviour, in addition to the peripheral functions; *e.g.*, antidiuretic and reproductive functions. In addition, the mammalian bombesin-like peptide, gastrin-releasing peptide (GRP) is closely related to autonomic regulation such as appetite (3-5), circadian rhythms (6-8), and fear responses (9-11), via specific G protein-coupled receptor, GRP-preferring receptor (GRPR)-mediated mechanisms (12). Sexual function is also closely related to the autonomic nervous system. Several reports previously demonstrated a functional relationship between GRP and male sexual behaviour (13, 14). Sex steroid hormones such as oestrogens and androgens also regulate various socio-sexual behaviours, including sexual, aggressive, and parental behaviours, as well as food intake, stress responses, mood regulation, social anxiety, and the modulation of somatosensory transmission (15). Considering how these behaviours are regulated at the organismal level, 'hormonal regulations' appear to play an important role in these behaviours in addition to the activation of neuronal circuits. Furthermore, the sexual dimorphism of these nuclei is controlled by the action of sex steroids (16). However, it is not fully understood how and when 'hormones' act on the

nervous system and regulate these behaviours and develop sexual dimorphism in the central nervous system. Oxytocin has a well-established role in social bonding, sexual function, maternal instinct, nursing, and lactation (17, 18). We have demonstrated the roles of oxytocin in male sexual function at the spinal cord level (19, 20). Findings from these studies suggest that oxytocin-mediated control of male sexual function via the spinal cord may be instrumental in treating erectile and/or ejaculation dysfunctions. In this report, we review the neural regulatory mechanisms of the sexually dimorphic functions in the central nervous system and the role of these neuropeptides on male sexual behaviour. Furthermore, we discuss findings on the recently identified localised volume transmission role of oxytocin in the regulation of the spinal GRP system (20).

GRP and its cognate receptor, GRPR

GRP is a 27-amino acid peptide (29-amino acids in rodents) originally isolated from the porcine stomach as the mammalian equivalent of a frog peptide, bombesin (21, 22). Subsequently, neuromedin B (NMB) was also isolated, and these two peptides were considered bombesin family peptides (23). GRP has been identified in many mammalian species, including humans, macaque monkeys, pigs, rats, and mice (24). To date, GRP orthologs have also been identified in birds, reptiles, amphibians, and teleost fishes (24), suggesting the universality of GRP in

vertebrates. Bombesin and GRP were earlier considered orthologous among frogs and mammals. However, it is reported that in frogs, GRP exists separately from bombesin, suggesting that GRP is not a mammalian bombesin (25, 26). GRP is highly expressed in the hippocampus (11), lateral amygdala (11), accessory basal nucleus of the amygdala (27), suprachiasmatic nucleus (8), dorsomedial nucleus of the hypothalamus (28), and dorsal root/trigeminal ganglion neurons (29). Substantial evidence indicates that GRP functions extensively as a neuromodulator that regulates the autonomic nervous system (30). GRPRs are G protein-coupled receptors, known as one of the subtypes of the bombesin receptor family (12). However, NMB-preferring receptors of the bombesin receptor family have a high affinity for NMB but a low affinity for GRP (31). Another member of the bombesin receptor family is the bombesin receptor subtype 3 (BRS-3), which has a low affinity for both GRP and NMB and is known as an orphan receptor in mammals (32). GRPRs are highly expressed in the hippocampus, amygdala (33), and medial preoptic area (POA) (34). GRPR-expressing neurons in the lateral amygdala are known as GABAergic neurons and are involved in conditioned fear responses (11). GRPR-expressing neurons in the spinal dorsal horn and trigeminal sensory nuclei of the brainstem have been reported to be involved in itch transmission, which is completely separated from pain transmission at the spinal cord and brainstem levels (29, 35, 36). Furthermore, it has long been reported that the GRP system acts significantly and play an important role in feeding behaviour

(3-5, 28). In addition to actions in the nervous system, it has been reported that high expression of GRPR promotes not only carcinogenesis and proliferation in prostate cancer (37) but also insulin secretion in pancreatic islet cells (38). Taken together, GRPR may be a useful target for pharmacological treatments of various diseases (30).

The SEG/lumbar spinothalamic (LSt) neurons control male sexual activity

The upper lumbar spinal cord has been shown to contain a neuronal region called the "spinal ejaculation generator" (SEG) because toxins that selectively lesion the galanin-containing neurons there also severely diminish the ability of male rats to ejaculate (39). SEG neurons in the lumbar spinal cord exhibit male-dominant sexual dimorphism in a population of galanin (40)-, cholecystokinin (41)- and enkephalin (42)-expressing neurons and GRP (13)-expressing neurons.. These neurons are situated dorsolateral to the central canal in lamina X within the third and fourth lumbar spinal cord and project to the thalamic region of the brain (43, 44). These so-called lumbar spinothalamic (LSt) neurons are also sexually dimorphic, with males possessing a greater number than females (13, 43-45) (Fig. 1). We have reported that GRP is expressed in SEG neurons, and that levels of immunoreactivity for GRP are regulated by androgens. (13, 46) (Fig. 1). The GRP neuron system is a male-dominant sexual dimorphic system that projects both to the sacral parasympathetic nucleus (SPN) and the spinal nucleus of

126 the bulbocarvenosus (SNB) (Fig. 1). The SNB and the dorsolateral nucleus (DLN) motoneuron
127 populations in the fifth and sixth lumbar segments of rats is known as the representative
128 sexually dimorphic nuclei in the spinal cord, and they innervate the striated perineal muscles
129 attached to the base of the penis (47-49). We reported by using ultra-high voltage electron
130 microscopy (HVEM) techniques and transsynaptic viral tracers that GRP neurons provide
131 synaptic inputs onto both somatic SNB neurons and autonomic SPN neurons (50, 51). GRP
132 neuron fibres also project to the sympathetic nervous system in the thoracic spinal cord (14).
133 Therefore, SEG neurons not only influence preganglionic sympathetic neurons in the thoracic
134 spinal cord (lateral horn) but also form a local intraspinal neural network that regulates the
135 motor and parasympathetic nervous systems controlling erection in the lumbosacral spinal cord
136 and the thoracic sympathetic nervous system involved in ejaculation (14, 52).
137 It is suggested that the spinal GRP neuron system that regulates sexual function is a
138 conserved property in mammals (30, 52). We have previously identified male-dominant sex
139 differences in the lumbar spinal cord of primate Japanese macaques (53), rodent rats (13), mice
140 (54), and Eulipotyphla Asian house musk shrews (suncus) (55). Also, a group of
141 galanin-containing male-dominant SEG neurons might exist in humans (56). The localisation
142 and sex differences in the SEG/LSt cells are conserved across mammals including macaque

143 monkeys, and it is therefore likely that, in humans, the system functions to regulate male sexual
144 function.
145
146 **Sexual dimorphism of the SEG**
147 The SEG neuron system in the spinal cord is also male-dominant sexually dimorphic and
148 expresses androgen receptors (ARs) (13). The testicular feminization mutation (Tfm) rodent
149 model provides a unique model for examining the role of the ARs in the central nervous system
150 and behaviour, because a point mutation in the AR gene renders the protein dysfunctional (57).
151 In the Tfm rat, the number of GRP-expressing neurons is completely female (or
152 hyperfeminized) (13, 46, 58). The expression levels of galanin and cholecystokinin are also
153 female-like in Tfm males (58), suggesting that the number of SEG neurons significantly
154 reduced in Tfm males. Treating female rats with androgens on the day of birth and the next day
155 (2 injections subcutaneously) completely masculinised the spinal GRP-immunoreactive neurons
156 in the spinal cord so that, during adulthood, it resembled the masculinised phenotype of adult
157 males and induced a masculine appearance in females (59). The perinatal androgen surge
158 also plays a key role in masculinisation of the spinal GRP system that controls male
159 sexual activity (59). Furthermore, the sexually dimorphic nucleus of the POA (SDN-POA) is
160 several-fold larger in males than in females (16). The enzyme aromatase, which is abundant in

161 the hypothalamus, converts androgens (*e.g.*, testosterone) into oestrogens (*e.g.*, oestradiol).
162 Oestrogens then bind to oestrogen receptors to induce a masculine SDN-POA; this is the
163 '*aromatase theory*' in rodents (16). Thus, oestrogens appear to play an important role in the
164 development of the masculine SDN-POA (brain), while androgens may play a central role in
165 sexual differentiation in the spinal cord. It is well known that muscles develop in an
166 androgen-dependent manner. Synergistic development of neuromuscular junctions might be
167 essential for the masculine SNB system. It is therefore suggested that testosterone induces the
168 muscles to produce a kind of neurotrophic factors (such as ciliary neurotrophic factor) that
169 preserves the muscles and that either the same factor or an additional factor preserves the motor
170 neurons; the neurotrophic theory in the development of the neuromuscular system (16).
171 The number of GRP-immunoreactive neurons in the lumbosacral spinal cord
172 markedly increases from postnatal day (PND) 30 onward (60) (Fig. 2). From PND 30 to 44 in
173 females, we previously found that few GRP-positive cell bodies were detectable and that GRP
174 staining was not intense (60) (Fig. 2). Vaginal opening occurs around PND 33 in rats (61),
175 suggesting that this is the age of puberty onset in females. After puberty, circulating testosterone
176 increases significantly in males and circulating oestradiol and progesterone in females (Fig. 2).
177 Chronic administration of exogenous progesterone to adult male rats significantly decreases
178 GRP expression in the spinal cord (62). The spinal GRP neurons highly express ARs but not

progesterone receptors. *In vitro* studies further demonstrated that the androgen effect was completely blocked in HEK293 cells (expressing ARs but not progesterone receptors) treated with androgen plus progesterone, suggesting that progesterone acts to inhibit the expression of GRP in the spinal cord via an AR-mediated mechanism (62). These results taken together suggest that the sex difference in the spinal GRP neurons is developed by the androgen surge during the critical period, resulting in a male-biased neuron number. Furthermore, after puberty, it is likely that increasing circulating testosterone induces the expression of GRP in the spinal cord of males, while progesterone inhibits GRP expression in the spinal cord of females, suggesting that progesterone could be an important feminizing factor in the spinal cord of females at least during pubertal development (62).

Neural circuits controlling male sexual function that link the spinal cord to the periphery

As described above, it is accepted that, in rodents, the SNB and DLN are male-dominant sexually dimorphic nuclei that promote penile erection (47). Penile erection occurs by increasing parasympathetic and inhibiting sympathetic activity. Stimulation of the hypogastric nerves in rats does not change the internal pressure of the penile corpus cavernosus, but when the sacral spinal cord is lesioned and parasympathetic innervation is removed, penile erection can be induced by stimulation of the hypogastric nerves (63). Thus, it is shown that many

197 peripheral nerves have a mixture of stimulating (possibly parasympathetic) and inhibitory
198 (possibly sympathetic) effects on a penile erection (64). Spinal GRP neurons project to the
199 lower lumbar and the sacral cord, where GRP might be released locally. SNB neurons that
200 control the erectile reflex (especially cup-like flaring erections of the distal glans) and the sacral
201 parasympathetic nucleus neurons both express GRPRs (13). Pharmacological stimulation of
202 GRPRs in the lumbosacral cord ameliorates penile reflexes and ejaculation in castrated male
203 rats (13). In anesthetised and spinalised male rats, intrathecal administrations of GRP influence
204 ejaculation, while GRPR antagonists prevent ejaculation induced by sensory nerve stimulation
205 (14). It is also reported that severe psychological stress significantly decreases the axonal
206 distribution of GRP in the lumbar spinal cord and attenuates the erectile reflexes. This
207 stress-induced decrease in the activity of the erectile reflex is ameliorated by the treatment of
208 GRPR agonist (45). Immunoelectron microscopy, combined with a retrograde tracing technique
209 using HVEM, was employed for 3-dimensional visualization of synaptic contacts from the GRP
210 system in the lumbar cord onto the SNB motoneurons. HVEM analysis clearly shows that axon
211 terminals containing GRP-immunoreactivity make direct contact with the dendrites of SNB
212 neurons (65). Infection of pseudorabies virus (PRV), a transsynaptic retrograde tracer, from the
213 balbocavernosus muscles and/or levator ani showed that spinal GRP neurons are labelled with
214 PRV (50). These results suggest that axons of GRP neurons project to dendrites of SNB neurons

to form synaptic inputs, and that GRP released locally from GRP terminals promotes penile reflexes (51). The SPN is in the lateral lumbosacral cord and SPN neurons express the neural nitric oxide synthase (nNOS), a maker for preganglionic autonomic neurons (66). Chronic mid-thoracic contusion injury decreased immunoreactivity for GRP (at the cell body level) in the lumbosacral cord of males, but not for galanin (14, 67). Intrathecal administration of galanin or cholecystokinin in the lumbar cord promotes ejaculation in a stimulation-dependent manner in the dorsal penile nerve (DPN), whereas intrathecal administration of GRP induces the emission and expulsion phase of ejaculation without DPN stimulation (14, 68). It is demonstrated that the SEG neurons process sensory inputs and project both intraspinal and to the thalamus (44, 69, 70). Thus, GRP-expressing neurons in the lumbar cord are likely to project both intraspinally and to the thalamus (71). Therefore, information transmitted from the spinal GRP system to the brain appears to play an important role in the control of male sexual activity, *e.g.*, switching between erection and ejaculation. Furthermore, GRP may be important as a neuromodulator in these sexual circuitry systems.

The afferent connection from spinal GRP neurons to the thalamic nuclei of the brain and their mechanisms for control of male sexual activity

232 Truitt and Coolen (39) reported that specific toxin treatments that selectively lesion the
233 galanin-expressing neurons in the lumbar cord completely eliminate ejaculation in rats while
234 other aspects of male sexual behaviour remain unaffected. These galaninergic neurons have
235 been clearly demonstrated to project to the thalamic region, specifically the medial portion of
236 the parvocellular subparafascicular thalamic nucleus (mSPFp) (39, 72) (Fig. 1). As mentioned
237 above, cholecystokinin, enkephalin, and GRP are expressed in SEG neurons in addition to
238 galanin (71). Ejaculation induces c-Fos expression in the mSPFp and the posterior dorsolateral
239 part of the medial amygdala (73). Thus, SEG neurons appear to play an important role in
240 conveying information about the occurrence of ejaculation and, possibly, its pleasant sensation
241 to the thalamus (74, 75). SEG neurons project both to the intermediolateral nucleus (IML;
242 sympathetic preganglionic) at T12-L2 and to the SPN (parasympathetic preganglionic nucleus)
243 at L6-S1 in the spinal cord and are likely to regulate simultaneously the sympathetic and
244 parasympathetic nervous systems to control male sexual activity in rats (74). GRP neurons in
245 lamina X of the L3-L4 level co-express galanin (13, 14, 67). Most GRP fibres projecting to the
246 IML also express galanin, whereas a part of fibres projecting to the SPN express only galanin
247 (13, 14). SEG neurons would use different neuropeptides to function locally in different
248 projection areas. Given that selective lesion of SEG neurons eliminates ejaculation, whereas
249 intrathecal administration of GRP induces the ejaculatory reflex (14, 39) and that

250 galanin-containing fibres projecting from SEG neurons are observed in the mSPFp (69, 72), it is
251 likely that ejaculatory information from the spinal cord is conveyed to mSPFp, suggesting that
252 the mSPFp is involed in controlling the postejaculatory interval (74). Neuropeptides including
253 galanin might act when ejaculation information is transmitted from LSt (SEG) neurons to the
254 mSPFp (73).
255
256 **A volume transmission role of oxytocin controls male sexual activity via the spinal GRP**
257 **system**
258 The neuropeptides oxytocin and vasopressin are mainly synthesised by neurons in the
259 paraventricular nucleus of the hypothalamus (PVN) and supraoptic nucleus and are well-known
260 to be released into the systemic circulation from axon terminals in the posterior pituitary (76,
261 77). They are also now known to be released from dendrites of the neurons into the
262 hypothalamus where they have important roles in socio-sexual behaviours via non-synaptic
263 volume transmission (78-82). Because the most familiar functions of oxytocin are to regulate
264 female reproductive functions including parturition, milk ejection and maternal behaviour,
265 oxytocin is often thought of as a female hormone (77). However, it is reported that, in men,
266 vasopressin is secreted during sexual arousal (induces erection), and there is, subsequently, a
267 selective release of oxytocin at the time of ejaculation (83) . Furthermore, there is evidence that

a group of oxytocin neurons located in the parvocellular part of the PVN project to the lower spinal cord and control penile erection and ejaculation in rats (84-87) (Fig. 3). Chemical lesions of the PVN reduced copulatory plug weight, suggesting a modulatory role by this oxytocin projection in seminal emission, although no effects on penile reflexes or postejaculatory interval were observed in male rats (88). More recently, we have reported the functional interaction between the spinal GRP system and the hypothalamo-spinal oxytocin system in rats (20) (Fig. 3). This unveiled a novel mode of oxytocin release driving this interaction via "*en passant*" release from axonal varicosities in the spinal cord to modulate male sexual activity (Fig. 3). Electron microscopic analysis demonstrated that, in the lumbar spinal cord, oxytocin is secreted by exocytosis from axonal varicosities (not synaptic boutons) and acts in a paracrine fashion—a localized volume transmission—to communicate with neighbouring cells expressing oxytocin receptors (20) (Fig. 3). It is likely that oxytocin-containing neurons extend axon projections to the lumbosacral cord and diffusely release oxytocin into the tissue gap for efficient one-to-many signal transduction (20). Furthermore, we demonstrate that oxytocin directly activates SEG/GRP neurons via oxytocin receptors and influences male sexual function in the rat lumbar spinal cord (20). Oxytocin is, of course, transferred from the brain to various parts of the body by the blood, and transmitted from neuron to neuron through synapses. Overall, the endocrine system, which acts on widespread distant organs via the circulation, resembles a '*broadcasting*

286 *satellite*' communication, whereas synaptic transmission resembles the hard-wired '*ethernet.*'
287 Accordingly, the localised volume transmission of peptides resembles '*Wi-Fi*' communication,
288 since it is a hybrid of both endocrine (*satellite*) and synaptic (*ethernet*) systems and may be the
289 predominant mechanism of oxytocinergic modulation of socio-sexual behaviour and cognition
290 throughout the central nervous system.
291 There is a male-dominant sex difference in oxytocin fibres in the lumbosacral spinal
292 cord (20, 89). It is reported that oxytocin neurons in the PVN express ARs and oestrogen
293 receptors (90, 91). No sex or age differences in oxytocin fibre distribution in the brain have
294 been reported (92). Oxytocin fibre projections to the lumbosacral cord that controls
295 intracavernous pressure would be linked to the development of sex differences in the spinal
296 GRP system in an androgen-dependent manner. It is well known that cell bodies of oxytocin
297 neurons with a male-dominant sex difference in axonal projection at the level of the lumbosacral
298 cord are localised in the posterolateral parvocellular subnucleus of the PVN, involving the
299 autonomic control (18, 20, 87) because both PVN and SON magnocellular neurons appear to
300 project axons only to the posterior pituitary. However, it is unclear when the oxytocin/GRP
301 neuron relationship in the lumbar spinal cord is established during development. The
302 male-biased differentiation mechanism of oxytocin projection in the lumbosacral cord (although
303 the cell bodies are in the hypothalamus) is of interest in understanding developmental

differences generating sexual dimorphism in the brain and spinal cord. Additional studies examining oxytocin fibres in the male lumbar spinal cord after neonatal castration are needed to draw a firm conclusion.

Relationship between the oxytocin-GRP system and other neurotransmitters/neuromodulators

Microinjection of oxytocin into the PVN or into the CA1 region of the hippocampus induces an increase in the number of penile erection and yawning episodes in male rats (93). Electrical stimulation of the DPN activates oxytocin neurons in the PVN (94). We have demonstrated recently, in rats, that a collection of oxytocin neurons in the caudal parvocellular PVN projects to the lumbosacral spinal cord and promotes male sexual function by activating spinal GRP neurons (20). On the other hand, dopamine and glutamate may activate oxytocin neurons in the PVN, induce erection, and may also be involved in the control of male sexual behaviour (95, 96). Glutamate N-Methyl-D-aspartic acid (NMDA) receptors are expressed in SEG neurons, and phosphorylation of the NMDA receptor subunit 1 is essential for the development of ejaculation (97, 98). In addition, it is reported that metabotropic glutamate receptor subtype 7 (mGluR7) knockout mice exhibit ejaculatory disorders, although they have normal sexual motivation (99).

Intrathecal administration of the mGluR7-selective antagonist (MMPIP) into the lumbosacral cord inhibits drug-induced ejaculation, suggesting that mGluR7 in the lumbosacral spinal cord plays an important role in ejaculation (99).

Serotonergic neurons in the locus coeruleus project widely to the forebrain and may act as an inhibitory system, strongly suppressing male sexual activity (100). Conversely, facilitatory effects of serotonin (5-HT) on male sexual activity have also been reported. It is well known that increased 5-HT levels in the central nervous system elevate the ejaculatory threshold, probably via 5-HT_{1B}, and 5-HT_{2C} receptors, whereas depletion of 5-HT decreases the ejaculatory threshold (101). Serotoninergic neurons in the locus coeruleus also project directly to the lumbosacral cord (102), but erections and yawning induced by serotonin receptor ($5HT_{1C}$) agonists are not caused via dopamine or oxytocin neurons. In the lumbosacral cord, it is reported that 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptors are expressed in SPN and SNB neurons (97). Systemic administration of serotonin receptor ($5HT_{2C}$) agonists [1-(3-Chlorophenyl)piperazine (m-CPP) and N-(3-trifluoromethylpiperazine) (TFMPP)] promotes erection similar to oxytocin, suggesting that the cross-talk among oxytocin-dopamine-serotonin neurons is essential for the generation of the erectile reflexes (103). Neuronal activation of the mSPFp is triggered not only by ejaculation but also by subcutaneous administration of 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT), a

340 5-HT_{1A} receptor agonist (104). Rats treated with 8-OH-DPAT also achieve ejaculation with
341 little mounting or intromission and show Fos expression similar to that after ejaculation in
342 saline-treated rats (104). Spinal serotonin fibres may regulate erection via $5HT_{2C}$ and ejaculation
343 via 5-HT_{1A} (possibly expressed in SEG neurons). In human ejaculatory disorders, selective
344 serotonin reuptake inhibitors induces premature ejaculation (105, 106). It is to be hoped that
345 further discoveries can be leveraged to treat a wider range of ejaculatory disorders, although the
346 mechanisms regulating ejaculation are complex.
347
348 **Conclusions**
349 It is to be hoped that new findings concerning the neuropeptidergic control of male sexual
350 functions discussed here might help in the design of drugs that relieve sexual dysfunction. Here,
351 we concentrate on oxytocin and GRP actions in the lumbar spinal cord. Intranasal
352 administration of oxytocin is already widespread, suggesting minimal obstacles to its clinical
353 use. However, the effects of neuropeptides on the central nervous system are varied, and
354 systemic administration should clearly be used with caution.
355
356
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Author Contributions

T.O. and H.S. wrote the paper. H.S. conceived and supervised the whole study. All authors had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests

The authors declare no conflict of interest.

Data availability statement

All relevant data are within the manuscript, and the data that support the findings of this study are available from the corresponding author upon request.

REFERENCES

1. Meyerson BJ. Hormone-dependent socio-sexual behaviors and neurotransmitters. *Prog Brain Res*. 1984; **61**: 271-81.
2. Breedlove SM, Watson NV. *Behavioral neuroscience* Sinauer Associates, 2020.
3. Ladenheim EE, Behles RR, Bi S, Moran TH. Gastrin-releasing peptide messenger ribonucleic acid expression in the hypothalamic paraventricular nucleus is altered by melanocortin receptor stimulation and food deprivation. *Endocrinology*. 2009; **150**(2): 672-8.
4. Ladenheim EE, Taylor JE, Coy DH, Moore KA, Moran TH. Hindbrain GRP receptor blockade antagonizes feeding suppression by peripherally administered GRP. *Am J Physiol*. 1996; **271**(1 Pt 2): R180-4.
5. Yamada K, Wada E, Wada K. Bombesin-like peptides: studies on food intake and social behaviour with receptor knock-out mice. *Ann Med*. 2000; **32**(8): 519-29.
6. Karatsoreos IN, Romeo RD, McEwen BS, Silver R. Diurnal regulation of the gastrin-releasing peptide receptor in the mouse circadian clock. *Eur J Neurosci*. 2006; **23**(4): 1047-53.
7. Okamura H, Ibata Y. GRP immunoreactivity shows a day-night difference in the suprachiasmatic nuclear soma and efferent fibers: comparison to VIP immunoreactivity. *Neurosci Lett*. 1994; **181**(1-2): 165-8.
8. Shinohara K, Tominaga K, Isobe Y, Inouye ST. Photic regulation of peptides located in the ventrolateral subdivision of the suprachiasmatic nucleus of the rat: daily variations of vasoactive intestinal polypeptide, gastrin-releasing peptide, and neuropeptide Y. *J Neurosci*. 1993; **13**(2): 793-800.
9. Merali Z, Bedard T, Andrews N, Davis B, McKnight AT, Gonzalez MI, Pritchard M, Kent P, Anisman H. Bombesin receptors as a novel anti-anxiety therapeutic target: BB1 receptor actions on anxiety through alterations of serotonin activity. *J Neurosci*. 2006; **26**(41): 10387-96.
10. Roesler R, Lessa D, Venturella R, Vianna MR, Luft T, Henriques JA, Izquierdo I, Schwartsmann G. Bombesin/gastrin-releasing peptide receptors in the basolateral amygdala regulate memory consolidation. *Eur J Neurosci*. 2004; **19**(4): 1041-5.
11. Shumyatsky GP, Tsvetkov E, Malleret G, Vronskaya S, Hatton M, Hampton L, Battey JF, Dulac C, Kandel ER, Bolshakov VY. Identification of a signaling network in lateral nucleus of amygdala important for inhibiting memory specifically related to learned fear. *Cell*. 2002; **111**(6): 905-18.

12. Battey J, Wada E. Two distinct receptor subtypes for mammalian bombesin-like peptides. *Trends Neurosci*. 1991; **14**(12): 524-8.
13. Sakamoto H, Matsuda K-I, Zuloaga DG, Hongu H, Wada E, Wada K, Jordan CL, Breedlove SM, Kawata M. Sexually dimorphic gastrin releasing peptide system in the spinal cord controls male reproductive functions. *Nat Neurosci*. 2008; **11**(6): 634-6.
14. Kozyrev N, Lehman MN, Coolen LM. Activation of gastrin-releasing peptide receptors in the lumbosacral spinal cord is required for ejaculation in male rats. *J Sex Med*. 2012; **9**(5): 1303-18.
15. Sakamoto H, Takahashi H, Matsuda K, Nishi M, Takanami K, Ogoshi M, Sakamoto T, Kawata M. Rapid signaling of steroid hormones in the vertebrate nervous system. *Front Biosci*. 2012; **17**: 996-1019.
16. Morris JA, Jordan CL, Breedlove SM. Sexual differentiation of the vertebrate nervous system. *Nat Neurosci*. 2004; **7**(10): 1034-9.
17. Froemke RC, Young LJ. Oxytocin, neural plasticity, and social behavior. *Annu Rev Neurosci*. 2021; **44**: 359-81.
18. Melis MR, Argiolas A. Oxytocin, erectile function and sexual behavior: Last discoveries and possible advances. *Int J Mol Sci*. 2021; **22**(19): 10376.
19. Oti T, Sakamoto T, Sakamoto H. Systemic effects of oxytocin on male sexual activity via the spinal ejaculation generator in rats. *Commun Integr Biol*. 2021; **14**(1): 55-60.
20. Oti T, Satoh K, Uta D, Nagafuchi J, Tateishi S, Ueda R, Takanami K, Young LJ, Galione A, Morris JF, Sakamoto T, Sakamoto H. Oxytocin influences male sexual activity via non-synaptic axonal release in the spinal cord. *Curr Biol*. 2021; **31**(1): 103-14 e5.
21. Anastasi A, Erspamer V, Bucci M. Isolation and structure of bombesin and alytesin, 2 analogous active peptides from the skin of the European amphibians *Bombina* and *Alytes*. *Experientia*. 1971; **27**(2): 166-7.
22. McDonald TJ, Jornvall H, Nilsson G, Vagne M, Ghatei M, Bloom SR, Mutt V. Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue. *Biochem Biophys Res Commun*. 1979; **90**(1): 227-33.
23. Minamino N, Kangawa K, Matsuo H. Neuromedin B: a novel bombesin-like peptide identified in porcine spinal cord. *Biochem Biophys Res Commun*. 1983; **114**(2): 541-8.
24. Sakamoto H. Gastrin-releasing peptide. In: Ando H, Ukena K, Nagata S, eds. *Handbook of Hormones : Comparative Endocrinology for Basic and Clinical Research*. 2nd ed: Academic Press 2021: 333-6.
25. Hirooka A, Hamada M, Fujiyama D, Takanami K, Kobayashi Y, Oti T, Katayama Y, Sakamoto T, Sakamoto H. The gastrin-releasing peptide/bombesin system revisited by a reverse-evolutionary study considering *Xenopus*. *Sci Rep*. 2021; **11**(1): 13315.

26. Nagalla SR, Gibson BW, Tang D, Reeve JR, Jr., Spindel ER. Gastrin-releasing peptide (GRP) is not mammalian bombesin. Identification and molecular cloning of a true amphibian GRP distinct from amphibian bombesin in Bombina orientalis. *J Biol Chem*. 1992; **267**(10): 6916-22.
27. Inoue R, Abdou K, Hayashi-Tanaka A, Muramatsu SI, Mino K, Inokuchi K, Mori H. Glucocorticoid receptor-mediated amygdalar metaplasticity underlies adaptive modulation of fear memory by stress. *eLife*. 2018; **7**: e34135.
28. Imoto D, Yamamoto I, Matsunaga H, Yonekura T, Lee ML, Kato KX, Yamasaki T, Xu S, Ishimoto T, Yamagata S, Otsuguro KI, Horiuchi M, Iijima N, Kimura K, Toda C. Refeeding activates neurons in the dorsomedial hypothalamus to inhibit food intake and promote positive valence. *Mol Metab*. 2021; **54**: 101366.
29. Takanami K, Sakamoto H, Matsuda KI, Satoh K, Tanida T, Yamada S, Inoue K, Oti T, Sakamoto T, Kawata M. Distribution of gastrin-releasing peptide in the rat trigeminal and spinal somatosensory systems. *J Comp Neurol*. 2014; **522**(8): 1858-73.
30. Takanami K, Sakamoto H. The gastrin-releasing peptide receptor (GRPR) in the spinal cord as a novel pharmacological target. *Curr Neuropharmacol*. 2014; **12**(5): 434-43.
31. Wada E, Way J, Shapira H, Kusano K, Lebacq-Verheyden AM, Coy D, Jensen R, Battey J. cDNA cloning, characterization and brain region-specific expression of neuromedin B-preferring bombesin receptor. *Neuron*. 1991; **6**: 421-30.
32. Fathi Z, Corjay MH, Shapira H, Wada E, Benya R, Jensen R, Viallet J, Sausville EA, Battey JF. BRS-3: a novel bombesin receptor subtype selectively expressed in testis and lung carcinoma cells. *J Biol Chem*. 1993; **268**(8): 5979-84.
33. Kamichi S, Wada E, Aoki S, Sekiguchi M, Kimura I, Wada K. Immunohistochemical localization of gastrin-releasing peptide receptor in the mouse brain. *Brain Res*. 2005; **1032**(1-2): 162-70.
34. Wada E, Wray S, Key S, Battey J. Comparison of gene expression for two distinct bombesin receptor subtypes in postnatal rat central nervous system. *Mol Cell Neurosci*. 1992; **3**(5): 446-60.
35. Katayama Y, Miura A, Sakamoto T, Takanami K, Sakamoto H. Footedness for scratching itchy eyes in rodents. *Proc Biol Sci*. 2022; **289**(1985): 20221126.
36. Sun YG, Chen ZF. A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. *Nature*. 2007; **448**(7154): 700-3.
37. Elshafae SM, Hassan BB, Supsavhad W, Dirksen WP, Camiener RY, Ding H, Tweedle MF, Rosol TJ. Gastrin-releasing peptide receptor (GRPr) promotes EMT, growth, and invasion in canine prostate cancer. *Prostate*. 2016; **76**(9): 796-809.

484 38. Hermansen K, Ahren B. Gastrin releasing peptide stimulates the secretion of insulin,
485 but not that of glucagon or somatostatin, from the isolated perfused dog pancreas. *Acta Physiol*
486 *Scand*. 1990; **138**(2): 175-9.
487 39. Truitt WA, Coolen LM. Identification of a potential ejaculation generator in the spinal
488 cord. *Science*. 2002; **297**(5586): 1566-9.
489 40. Newton BW. A sexually dimorphic population of galanin-like neurons in the rat
490 lumbar spinal cord: functional implications. *Neurosci Lett*. 1992; **137**(1): 119-22.
491 41. Phan DC, Newton BW. Cholecystokinin-8-like immunoreactivity is sexually
492 dimorphic in a midline population of rat lumbar neurons. *Neurosci Lett*. 1999; **276**(3): 165-8.
493 42. Nicholas AP, Zhang X, Hokfelt T. An immunohistochemical investigation of the
494 opioid cell column in lamina X of the male rat lumbosacral spinal cord. *Neurosci Lett*. 1999;
495 **270**(1): 9-12.
496 43. Ju G, Melander T, Ceccatelli S, Hokfelt T, Frey P. Immunohistochemical evidence for
497 a spinothalamic pathway co-containing cholecystokinin- and galanin-like immunoreactivities in
498 the rat. *Neuroscience*. 1987; **20**(2): 439-56.
499 44. Truitt WA, Shipley MT, Veening JG, Coolen LM. Activation of a subset of lumbar
500 spinothalamic neurons after copulatory behavior in male but not female rats. *J Neurosci*. 2003;
501 **23**(1): 325-31.
502 45. Sakamoto H, Matsuda K, Zuloaga D, Nishiura N, Takanami K, Jordan C, Breedlove S,
503 Kawata M. Stress affects a gastrin-releasing peptide system in the spinal cord that mediates
504 sexual function: Implications for psychogenic erectile dysfunction. *PLoS ONE*. 2009; **4**(1):
505 e4276.
506 46. Sakamoto H, Takanami K, Zuloaga DG, Matsuda K, Jordan CL, Breedlove SM,
507 Kawata M. Androgen regulates the sexually dimorphic gastrin-releasing peptide system in the
508 lumbar spinal cord that mediates male sexual function. *Endocrinology*. 2009; **150**(8): 3672-9.
509 47. Breedlove SM, Arnold AP. Hormone accumulation in a sexually dimorphic motor
510 nucleus of the rat spinal cord. *Science*. 1980; **210**(4469): 564-6.
511 48. Forger NG, Breedlove SM. Sexual dimorphism in human and canine spinal cord: role
512 of early androgen. *Proc Natl Acad Sci U S A*. 1986; **83**(19): 7527-31.
513 49. Sengelaub DR, Forger NG. The spinal nucleus of the bulbocavernosus: firsts in
514 androgen-dependent neural sex differences. *Horm Behav*. 2008; **53**(5): 596-612.
515 50. Dobberfuhl AD, Oti T, Sakamoto H, Marson L. Identification of CNS neurons
516 innervating the levator ani and ventral bulbospongiosus muscles in male rats. *J Sex Med*. 2014;
517 **13**(3): 664-77.
518 51. Oti T, Satoh K, Saito K, Murata K, Kawata M, Sakamoto T, Sakamoto H.
519 Three-dimensional evaluation of the spinal local neural network revealed by the high-voltage

electron microscopy: a double immunohistochemical study. *Histochem Cell Biol*. 2012; **138**(4): 693-7.

52. Sakamoto H. Sexually dimorphic nuclei in the spinal cord control male sexual functions. *Front Neurosci*. 2014; **8**: 184 eCollection 2014.

53. Ito T, Oti T, Takanami K, Satoh K, Ueda Y, Sakamoto T, Sakamoto H. A sexually dimorphic peptidergic system in the lower spinal cord controlling penile function in non-human primates. *Spinal Cord*. 2017; 56(1): 57-62.

54. Sakamoto H, Saito K, Marie-Luce C, Raskin K, Oti T, Satoh K, Tamura K, Sakamoto T, Mhaouty-Kodja S. Androgen regulates development of the sexually dimorphic gastrin-releasing peptide neuron system in the lumbar spinal cord: Evidence from a mouse line lacking androgen receptor in the nervous system. *Neurosci Lett*. 2014; **558**: 109-14.

55. Tamura K, Kobayashi Y, Hirooka A, Takanami K, Oti T, Jogahara T, Oda S, Sakamoto T, Sakamoto H. Identification of the sexually dimorphic gastrin-releasing peptide system in the lumbosacral spinal cord that controls male reproductive function in the mouse and Asian house musk shrew (Suncus murinus). *J Comp Neurol*. 2017; **525**(7): 1586-98.

56. Chehensse C, Facchinetti P, Bahrami S, Andrey P, Soler JM, Chretien F, Bernabe J, Clement P, Denys P, Giuliano F. Human spinal ejaculation generator. *Ann Neurol*. 2017; **81**(1): 35-45.

57. Zuloaga DG, Morris JA, Jordan CL, Breedlove SM. Mice with the testicular feminization mutation demonstrate a role for androgen receptors in the regulation of anxiety-related behaviors and the hypothalamic-pituitary-adrenal axis. *Horm Behav*. 2008; **54**(5): 758-66.

58. Newton BW, Phan DC. Androgens regulate the sexually dimorphic production of co-contained galanin and cholecystokinin in lumbar laminae VII and X neurons. *Brain Res*. 2006; **1099**(1): 88-96.

59. Oti T, Takanami K, Katayama N, Edey T, Satoh K, Sakamoto T, Sakamoto H. Perinatal testosterone exposure is critical for the development of the male-specific sexually dimorphic gastrin-releasing peptide system in the lumbosacral spinal cord that mediates erection and ejaculation. *Biol Sex Differ*. 2016; **7**: 4.

60. Katayama N, Oti T, Takanami K, Sakamoto T, Sakamoto H. Postnatal development of the gastrin-releasing peptide system in the lumbosacral spinal cord controlling male reproductive function in rats. *Proc Jpn Acad Ser B Phys Biol Sci*. 2016; **92**(2): 69-75.

61. Franssen D, Ioannou YS, Alvarez-real A, Gerard A, Mueller JK, Heger S, Bourguignon JP, Parent AS. Pubertal timing after neonatal diethylstilbestrol exposure in female rats: neuroendocrine vs peripheral effects and additive role of prenatal food restriction. *Reprod Toxicol*. 2014; **44**: 63-72.

62. Oti T, Takanami K, Ito S, Ueda T, Matsuda KI, Kawata M, Soh J, Ukimura O, Sakamoto T, Sakamoto H. Effects of Sex Steroids on the Spinal Gastrin-Releasing Peptide System Controlling Male Sexual Function in Rats. *Endocrinology*. 2018; **159**(4): 1886-96.
63. Dail WG, Walton G, Olmsted MP. Penile erection in the rat: stimulation of the hypogastric nerve elicits increases in penile pressure after chronic interruption of the sacral parasympathetic outflow. *J Auton Nerv Syst*. 1989; **28**(3): 251-7.
64. Giuliano F, Rampin O, Bernabe J, Rousseau JP. Neural control of penile erection in the rat. *J Auton Nerv Syst*. 1995; **55**(1-2): 36-44.
65. Sakamoto H, Arii T, Kawata M. High-voltage electron microscopy reveals direct synaptic inputs from a spinal gastrin-releasing peptide system to neurons of the spinal nucleus of bulbocavernosus. *Endocrinology*. 2010; **151**(1): 417-21.
66. Studeny S, Vizzard MA. Corticotropin-releasing factor (CRF) expression in postnatal and adult rat sacral parasympathetic nucleus (SPN). *Cell Tissue Res*. 2005; **322**(3): 339-52.
67. Wiggins JW, Kozyrev N, Sledd JE, Wilson GG, Coolen LM. Chronic Spinal Cord Injury Reduces Gastrin-Releasing Peptide in the Spinal Ejaculation Generator in Male Rats. *J Neurotrauma*. 2019; **36**(24): 3378-93.
68. Kozyrev N, Coolen LM. Activation of galanin and cholecystokinin receptors in the lumbosacral spinal cord is required for ejaculation in male rats. *Eur J Neurosci*. 2017; **45**(6): 846-58.
69. Coolen LM, Veening JG, Wells AB, Shipley MT. Afferent connections of the parvocellular subparafascicular thalamic nucleus in the rat: evidence for functional subdivisions. *J Comp Neurol*. 2003; **463**(2): 132-56.
70. Sun XQ, Xu C, Leclerc P, Benoit G, Giuliano F, Droupy S. Spinal neurons involved in the control of the seminal vesicles: a transsynaptic labeling study using pseudorabies virus in rats. *Neuroscience*. 2009; **158**(2): 786-97.
71. Sakamoto H. The gastrin-releasing peptide system in the spinal cord mediates masculine sexual function. *Anat Sci Int*. 2011; **86**(1): 19-29.
72. Coolen LM, Veening JG, Petersen DW, Shipley MT. Parvocellular subparafascicular thalamic nucleus in the rat: anatomical and functional compartmentalization. *J Comp Neurol*. 2003; **463**(2): 117-31.
73. Veening JG, Coolen LM. Neural activation following sexual behavior in the male and female rat brain. *Behav Brain Res*. 1998; **92**(2): 181-93.
74. Coolen LM. Neural control of ejaculation. *J Comp Neurol*. 2005; **493**(1): 39-45.
75. Sakamoto H. Brain-spinal cord neural circuits controlling male sexual function and behavior. *Neurosci Res*. 2012; **72**(2): 103-16.

591 76. Donaldson ZR, Young LJ. Oxytocin, vasopressin, and the neurogenetics of sociality.
592 *Science*. 2008; **322**(5903): 900-4.
593 77. Russell JA, Leng G. Sex, parturition and motherhood without oxytocin? *J Endocrinol*.
594 1998; **157**(3): 343-59.
595 78. Chini B, Verhage M, Grinevich V. The action radius of oxytocin release in the
596 mammalian CNS: From single vesicles to behavior. *Trends Pharmacol Sci*. 2017; 38(11):
597 982-91.
598 79. Fuxe K, Borroto-Escuela DO, Romero-Fernandez W, Ciruela F, Manger P, Leo G,
599 Diaz-Cabiale Z, Agnati LF. On the role of volume transmission and receptor-receptor
600 interactions in social behaviour: focus on central catecholamine and oxytocin neurons. *Brain*
601 *Res*. 2012; **1476**119-31.
602 80. Johnson ZV, Young LJ. Oxytocin and vasopressin neural networks: Implications for
603 social behavioral diversity and translational neuroscience. *Neurosci Biobehav Rev*. 2017; **76**(Pt
604 A): 87-98.
605 81. Ludwig M, Leng G. Dendritic peptide release and peptide-dependent behaviours. *Nat*
606 *Rev Neurosci*. 2006; **7**(2): 126-36.
607 82. Young LJ, Wang Z. The neurobiology of pair bonding. *Nat Neurosci*. 2004; **7**(10):
608 1048-54.
609 83. Corona G, Jannini EA, Vignozzi L, Rastrelli G, Maggi M. The hormonal control of
610 ejaculation. *Nat Rev Urol*. 2012; **9**(9): 508-19.
611 84. Argiolas A, Melis MR. Central control of penile erection: role of the paraventricular
612 nucleus of the hypothalamus. *Prog Neurobiol*. 2005; **76**(1): 1-21.
613 85. Swanson LW, McKellar S. The distribution of oxytocin- and neurophysin-stained
614 fibers in the spinal cord of the rat and monkey. *J Comp Neurol*. 1979; **188**(1): 87-106.
615 86. Wagner CK, Clemens LG. Projections of the paraventricular nucleus of the
616 hypothalamus to the sexually dimorphic lumbosacral region of the spinal cord. *Brain Res*. 1991;
617 **539**(2): 254-62.
618 87. Wagner CK, Clemens LG. Neurophysin-containing pathway from the paraventricular
619 nucleus of the hypothalamus to a sexually dimorphic motor nucleus in lumbar spinal cord. *J*
620 *Comp Neurol*. 1993; **336**(1): 106-16.
621 88. Ackerman AE, Lange GM, Clemens LG. Effects of paraventricular lesions on sex
622 behavior and seminal emission in male rats. *Physiol Behav*. 1997; **63**(1): 49-53.
623 89. Uhl-Bronner S, Waltisperger E, Martinez-Lorenzana G, Condes Lara M,
624 Freund-Mercier MJ. Sexually dimorphic expression of oxytocin binding sites in forebrain and
625 spinal cord of the rat. *Neuroscience*. 2005; **135**(1): 147-54.

90. Simonian SX, Herbison AE. Differential expression of estrogen receptor alpha and beta immunoreactivity by oxytocin neurons of rat paraventricular nucleus. *J Neuroendocrinol*. 1997; **9**(11): 803-6.
91. Zhou L, Blaustein JD, De Vries GJ. Distribution of androgen receptor immunoreactivity in vasopressin- and oxytocin-immunoreactive neurons in the male rat brain. *Endocrinology*. 1994; **134**(6): 2622-7.
92. DiBenedictis BT, Nussbaum ER, Cheung HK, Veenema AH. Quantitative mapping reveals age and sex differences in vasopressin, but not oxytocin, immunoreactivity in the rat social behavior neural network. *J Comp Neurol*. 2017; **525**(11): 2549-70.
93. Melis MR, Argiolas A, Gessa GL. Oxytocin-induced penile erection and yawning: site of action in the brain. *Brain Res*. 1986; **398**(2): 259-65.
94. Yanagimoto M, Honda K, Goto Y, Negoro H. Afferents originating from the dorsal penile nerve excite oxytocin cells in the hypothalamic paraventricular nucleus of the rat. *Brain Res*. 1996; **733**(2): 292-6.
95. Clement P, Peeters M, Bernabe J, Denys P, Alexandre L, Giuliano F. Brain oxytocin receptors mediate ejaculation elicited by 7-hydroxy-2-(di-N-propylamino) tetralin (7-OH-DPAT) in anaesthetized rats. *Br J Pharmacol*. 2008; **154**(5): 1150-9.
96. Melis MR, Argiolas A. Central control of penile erection: a re-visitation of the role of oxytocin and its interaction with dopamine and glutamic acid in male rats. *Neurosci Biobehav Rev*. 2011; **35**(3): 939-55.
97. Giuliano F, Clement P. Pharmacology for the treatment of premature ejaculation. *Pharmacol Rev*. 2012; **64**(3): 621-44.
98. Staudt MD, Truitt WA, McKenna KE, de Oliveira CV, Lehman MN, Coolen LM. A pivotal role of lumbar spinothalamic cells in the regulation of ejaculation via intraspinal connections. *J Sex Med*. 2012; **9**(9): 2256-65.
99. Masugi-Tokita M, Tomita K, Kobayashi K, Yoshida T, Kageyama S, Sakamoto H, Kawauchi A. Metabotropic glutamate receptor subtype 7 is essential for ejaculation. *Mol Neurobiol*. 2020; **57**(12): 5208-18.
100. Kiser D, Steemers B, Branchi I, Homberg JR. The reciprocal interaction between serotonin and social behaviour. *Neurosci Biobehav Rev*. 2012; **36**(2): 786-98.
101. de Jong TR, Veening JG, Waldinger MD, Cools AR, Olivier B. Serotonin and the neurobiology of the ejaculatory threshold. *Neurosci Biobehav Rev*. 2006; **30**(7): 893-907.
102. Bancila M, Giuliano F, Rampin O, Mailly P, Brisorgueil MJ, Calas A, Verge D. Evidence for a direct projection from the paraventricular nucleus of the hypothalamus to putative serotoninergic neurons of the nucleus paragigantocellularis involved in the control of erection in rats. *Eur J Neurosci*. 2002; **16**(7): 1240-8.

103. Stancampiano R, Melis MR, Argiolas A. Penile erection and yawning induced by 5-HT1C receptor agonists in male rats: relationship with dopaminergic and oxytocinergic transmission. *Eur J Pharmacol*. 1994; **261**(1-2): 149-55.
104. Coolen LM, Olivier B, Peters HJ, Veening JG. Demonstration of ejaculation-induced neural activity in the male rat brain using 5-HT1A agonist 8-OH-DPAT. *Physiol Behav*. 1997; **62**(4): 881-91.
105. Atmaca M. Selective Serotonin Reuptake Inhibitor-Induced Sexual Dysfunction: Current Management Perspectives. *Neuropsychiatr Dis Treat*. 2020; **16**: 1043-50.
106. Peleg LC, Rabinovitch D, Lavie Y, Rabbie DM, Horowitz I, Fruchter E, Gruenwald I. Post-SSRI Sexual Dysfunction (PSSD): Biological Plausibility, Symptoms, Diagnosis, and Presumed Risk Factors. *Sex Med Rev*. 2022; **10**(1): 91-8.

675
676
677 **Figure Legends**
678
679 **Figure 1**
680 A schematic drawing of the gastrin-releasing peptide (GRP) system, which controls male sexual
681 activity in the lumbosacral spinal cord. A sexually dimorphic spinal cord system of
682 GRP-containing neurons in the lumbar spinal cord (at the L3–L4 level)— 'the spinal ejaculation
683 centre (SEG)'—projects axons both to sympathetic centre (*i.e.*, intermediolateral nucleus [IML];
684 sympathetic preganglionic) at T12-L2 and to the parasympathetic centre (*i.e.*, sacral
685 parasympathetic nucleus [SPN]; parasympathetic preganglionic) and also to the somatic centre
686 (*i.e.*, spinal nucleus of the bulbocavernosus [SNB]) in the lower lumbar and sacral cord. The
687 SEG neurons have also been shown to process sensory inputs and project both intraspinal and to
688 the thalamic region, specifically the medial portion of the parvocellular subparafascicular
689 thalamic nucleus (mSPFp); so-called lumbar spinothalamic (LSt) neurons (44, 69, 70). These
690 centres mediate penile reflexes and trigger ejaculation.
691
692 **Figure 2**
693 Developmental changes in the number of gastrin-releasing peptide (GRP)-immunoreactive
694 neurons in the lumbar spinal cord of male (light blue) and female (orange) rats. Circulating
695 testosterone levels in males (blue) and circulating oestradiol and progesterone levels in females
696 (magenta) are overlaid on the graph. PND, postnatal day.
697
698 **Figure 3**
699 Oti *et al*. (20) show that oxytocin directly activates the spinal ejaculation centre (SEG)/ lumbar
700 spinothalamic (LSt) neurons including gastrin-releasing peptide (GRP) via oxytocin receptors
701 and influences male sexual function in the rat lumbar spinal cord. The release of oxytocin in the
702 lumbar cord is not limited to conventional synapses but acts by diffusion—a localized volume
703 transmission—to reach oxytocin receptors on GRP neurons and facilitate male sexual activity.
704 PVN, paraventricular nucleus; SPN, sacral parasympathetic nucleus; SNB, spinal nucleus of the
705 bulbocavernosus.