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Review

The spinal pattern generator for ejaculation

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ABSTRACT

Ejaculation is the physiological process that describes the expulsion of the semen from the urethra. This physiological response is a remarkably sophisticated phenomenon that requires the participation of several stereotyped motor patterns for its expression and when taking place, it starts a constellation of short- and long-lasting physiological and behavioural processes. Little is known about the neural mechanisms accounting for its activation. It has been recently proposed that a central pattern generator located at the spinal level is involved in the control of ejaculation. The aim of this paper is to review the evidence supporting this notion. Thus, the mechanics of ejaculation, its anatomical substrates and innervation are described. Besides, evidence from behavioural, physiological and pharmacological studies that support the existence of an intraspinal rhythm modulating the ejaculatory response are provided. Data are discussed in the context of the physiology of male sexual function.

Contents

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1. Introduction

The recent advances in the study of male sexual dysfunction have become with time a fascinating topic of interest for the field of neurobiology. Most prominent studies have focused in the understanding of erectile dysfunction. Ejaculatory dysfunctions have received less attention in spite of the fact that they have a higher prevalence and are the most frequent cause of sexual complaints (Jannini and Lenzi, 2005; Wolters and Hellstrom, 2006). Ejaculatory disorders are divided into four categories: premature ejaculation, delayed ejaculation, retrograde ejaculation and anejaculation (Wolters and Hellstrom, 2006). Premature ejaculation is defined as an ejaculation resulting from a lack of ejaculatory control and occurring, depending on the study, within 1, 2 or 3 min following intravaginal penetration (Wolters and Hellstrom, 2006). Premature ejaculation interferes with sexual or emotional well-being in one or both partners and affects from 5 to 40% of the sexually active men (Jannini and Lenzi, 2005). On the other side, delayed ejaculation is characterised by a man's inability to ejaculate in a reasonable period under normal sexual stimulation. Delayed ejaculation incidence ranges from 1 to 4% of the sexually active men (Jannini and Lenzi, 2005). Retrograde ejaculation occurs when the semen intended for propulsion out of the urethral meatus is directed backwards into the urinary bladder. The true incidence of this ejaculatory disorder is difficult to estimate, but it ranges from 0.3% to 2% of patients with sexual dysfunction (Jannini and Lenzi, 2005). Retrograde ejaculation is most commonly due to intrinsic problems with the internal sphincter of the bladder. Finally, anejaculation and anorgasmia are often and mistakenly used as synonyms. Anejaculation specifically refers to the lack of ejaculation that may or may not be coupled with an orgasm (Wolters and Hellstrom, 2006). This dysfunction observes an occurrence of 15% of the sexually dysfunctional men (Jannini and Lenzi, 2005).

Increased interest in the study of ejaculation obeys not only to the importance of ejaculatory dysfunctions, but also to the growing attention of scientists on its physiological consequences. Ejaculation starts a constellation of short- and long-lasting physiological and behavioural processes. Thus, for instance, in naïve male rats the occurrence of one ejaculation is a powerful reinforcing stimulus (Kippin and Pfaus, 2001). On the other hand, in sexually experienced male rats repeated ejaculation produces a progressive inhibition of sexual motivation, featured by an exponential increase in the duration of the post-ejaculatory interval, which triggers the establishment of a long-lasting inhibitory state known as sexual exhaustion (Rodríguez-Manzo and Fernández-Guasti, 1994). Besides, ejaculation induces analgesia (Szechtman et al., 1981; González-Mariscal et al., 1994) and anxiolytic-like effects (Rodríguez-Manzo et al., 1999). It has been suggested that when a mammal faces a downshift in the expected quality or quantity of an appetitive reinforcer, it experiences an anxiety-like frustration response that can be prevented by ejaculation (Freidin et al., 2005). Ejaculation-induced effects also include a long-lasting increased sensitivity to drug action (Martinez-Mota et al., 2005). The immune system is also influenced by ejaculation, since it activates the innate components of the immune response. Thus, increases in the absolute number of leukocytes, in particular natural killer cells, in the peripheral blood are found after ejaculation (Haake et al., 2004). Finally, neuroendocrine changes are also important consequences of ejaculation, such as the post-orgasmic prolactin (Kruger et al., 2002; Brody and Kruger, 2006) and oxytocin peaks (Kruger et al., 2006) and the post-ejaculatory testosterone increase (Kamel et al., 1975).

Ejaculation is a remarkably sophisticated phenomenon that requires the participation of several stereotyped motor patterns for its elicitation. Each of these patterns can be individually visualised and separately studied. The activation of three interacting components is required to set in motion the ejaculatory response: a motor component, an external and an internal genital component (Morali et al., 2003; for review). The motor component mainly involves the pelvic thrusting pattern implicated in penile insertion, the external genital component includes all penile vascular and muscular events needed for penile erection, and the internal genital component comprises the autonomic and somatic activities of various glands involved in seminal emission and ejection (Morali et al., 2003). Thus, ejaculation takes place after the activation of the physiological mechanisms resulting from the interaction among these three components.

Ejaculation is the physiological process that describes the expulsion of the semen from the urethra. This process consists of two different stages, an emissive phase and an ejective phase (Newman et al., 1982; Mitsuya et al., 1976; Watson, 1964). The emissive phase implies the closure of the bladder neck (preventing retrograde ejaculation) and contraction of the sexual accessory glands: seminal vesicles, prostate, vas deferens and coagulant glands. The expulsive phase of ejaculation consists of the forceful ejection of semen from the urethral meatus that is caused by the rhythmic contraction of all genital muscles associated with the genital tract. Traditionally, it has been described that in some mammalian species, such as human and rat, seminal ejection is produced by the coordinated contraction of the urethralis, bulbospongiosus and ischiocavernosus muscles (Kollberg et al., 1972; Gerstenberg et al., 1993; McKenna, 1999; Shafik et al., 2000, 1998; 2005; Shafik, 1997) and supported by the contraction of the anal and urethral sphincters (Shafik, 1997, 1998). From these two components, the ejective phase has been considered as the only indicator of the ejaculatory event (Beckett et al., 1973, 1974; Watson, 1964;

Gerstenberg et al., 1993; Meisel and Sachs, 1994). Successful ejaculation depends on coordinated responses involving autonomic and somatic events. The autonomic component comprises the synchronised activation of visceral accessory structures such as the prostate and of the tunica albuginea (Shafik et al., 2005; Exintaris et al., 2006), while somatic events include the rhythmic contraction of perineal and pelvic striated muscles. This complex pelvic activity encompasses the participation of several reflexes that are all turned on during genital stimulation: the pudendo-pudendal reflex (McKenna and Nadelhaft, 1989), the bulbocavernosus reflex (Vodusek and Janko, 1990) and the glans-vasal, urethromuscular, cavernosourethral (Shafik and El-Sibai, 2000; Shafik et al., 2005) and abdominal-genital reflexes (Shafik et al., 2007); and finally, the ano-cavernosal excitatory reflex (Shafik et al., 2000). All these sexual reflexes imply the interaction among spinal nuclei innervating the seminal tract, the sexual accessory glands and the genital striated muscles.

The neural commands for ejaculation are organised at the spinal level. Traditionally, a sequence of spinal reflexes elicited by sensory genital stimulation was considered as the origin of ejaculation, however behavioural, clinical and experimental studies have suggested the existence of a pattern generator, located at the lumbosacral level, involved in the control of ejaculation (McKenna, 1999; Coolen et al., 2004).

A central pattern generator is defined as a neural circuit that can produce self-sustained patterns of repetitive rhythmic outputs to the muscles involved in the rhythmic behaviour, independently of the sensory input (Grillner, 1985, 2006). The complexity of neural motor networks conforming individual CPGs correlates with the complexity of the movement generated by a given CPG. Thus, most basic CPGs coordinate protective reflexes such as swallowing or coughing. At the next level of complexity are those CPGs that generate rhythmic movements, are active throughout life and are modulated by varying metabolic demands i.e. respiratory CPG. Other CPGs, such as those involved in locomotion, are inactive at rest but can be turned on by signals from higher command centres. These CPGs involve several muscles coordinated with a precise timing. When one considers the saccadic eye movements for instance, another level of complexity is added. In this case, the movements are generated by the superior colliculus which contains a topological map, and within each microregion of this map, the location of activity determines the specific direction and amplitude of the saccade (Grillner, 2003). Finally, the maximum level of CPG complexity is found in those CPGs playing a role in different patterns of goal-directed behaviours that are triggered in response to stimulation of higher neural structures such as the hypothalamus or the periaqueductal gray region (Grillner, 2006). These behaviours include attack (sham rage), sexual behaviour and the search for water (Grillner, 2006). In these cases, a sequence of motor programs becomes activated, and the resulting motor acts are adapted well to the surrounding world.

Interestingly, a large portion of the standard motor repertoire can be generated spontaneously in animals that lack a cerebral cortex or other neural structures, but can be initiated and coordinated with the help of the remaining neural structures

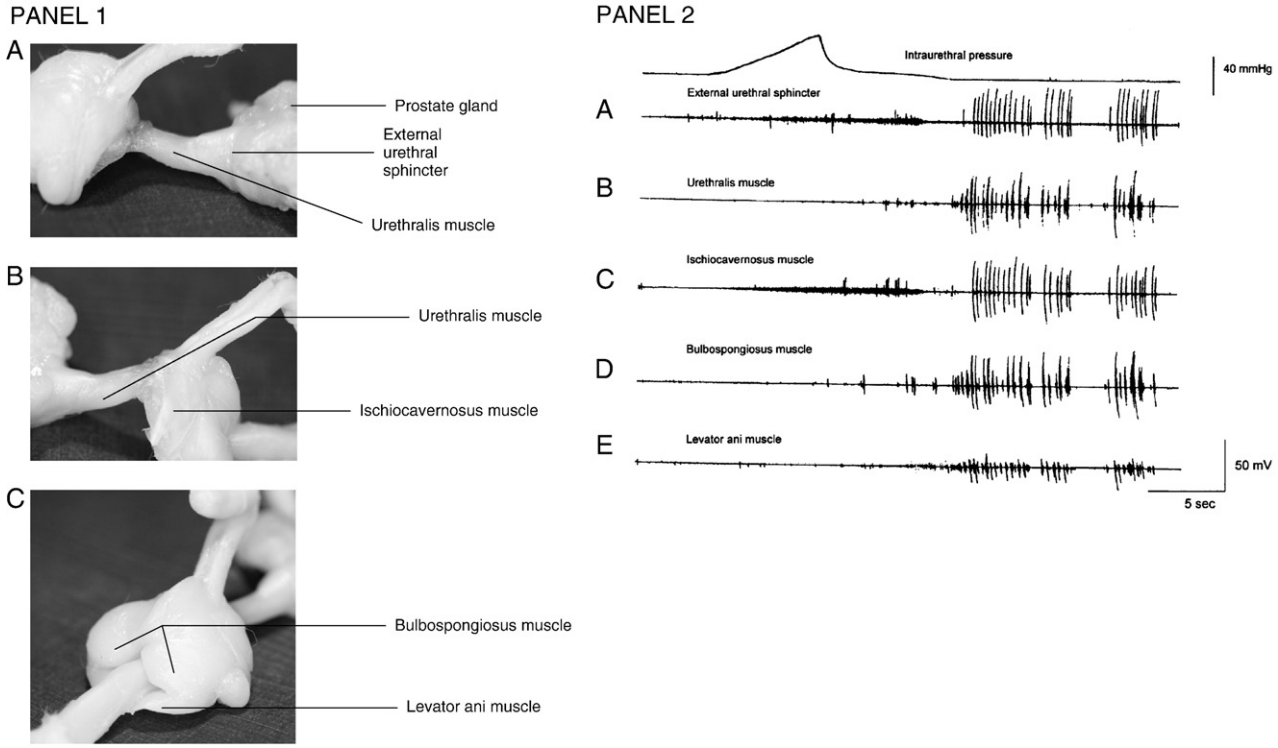

Fig. 1 – The striated genital muscles associated with the genital tract of the male rat. In Panel 1, the anatomical position of the striated muscles around the genital structures such as the urethra and rectum. In Panel 2, the electromyographic response registered during fictive ejaculation in spinal cord transected male rats. Notice the simultaneous contraction of all genital muscles during the expression of the genital rhythmic pattern of ejaculation elicited by urethral stimulation.

(Grillner, 2006). Thus, for instance many pattern generators underlying different sorts of coordinated movements are located at the spinal cord level (Rossignol and Dubuc, 1994). For each category of movement, there is machinery, innate in nature, which can be adapted and perfected by experience. The motor pattern generator circuitry is present early in development, although it may remain silent until adulthood, subsequent to a progressive maturation (Fenelon et al., 1998).

2. Genital muscular apparatus and its activation during ejaculation

2.1. *Striated muscles and the mechanics of ejaculation*

As shown in Fig. 1 (Panel 1), the muscular apparatus in charge of ejaculation is located in the pelvic region and includes muscles located both within and outside the pelvic cavity. Both groups of striated muscles surround the urethra in its prostatic, membranous and penile urethral portions. The prostatic portion of the urethra is completely surrounded by the external sphincter (Fig. 1, Panel 1), while the membranous portion of the urethra (Fig. 1, Panel 1) is supported by the urethralis muscles (Carro-Juárez, 2005). Finally, the external bulbospongiosus muscles and its dorsal portion known as levator ani as well as the ischiocavernosus muscles (Fig. 1, Panel 1) surround the initial portion of the penile urethra (Schmidt and Schmidt, 1993; Holmes et al., 1991). During emission, the presence of seminal secretions with the participation of the internal and external urethral sphincters (Marberger, 1974) and urethralis muscles converts the urethra into a pressure chamber (Fig. 2). It has been suggested that continuous deposition of genital fluids increases urethral pressure at its maximum diameter until ejection is triggered. At this point, urethral contents are forcefully expelled. The ejaculatory pressure chamber needs to resist repetitive and compressive forces at ejection. Outer and inner striated muscle layers of the external urethral sphincter and the urethralis muscles (Fig. 2) contribute to the formation of the pressure chamber for ejaculation (Carro-Juárez, 2005). Thus, for instance, in the case of the urethral sphincter, the striated external fibres that reinforce the internal fibres, considered as the true striated sphincter, appear to constitute the main barrier to prevent retrograde ejaculation. Outer muscular fibres in the urethralis muscle, running the urethra lengthwise (Fig. 2), seem to reinforce the circumferentially organised striated fibres (Fig. 2) in the inner layer (Fig. 2). The penile bulb would finally contribute to the formation of the ejaculatory pressure chamber. A similar anatomical array of fibres has been found in genital structures such as the corpus cavernosum. This type of array is of axial–orthogonal nature (Kelly, 1997) and has been considered to be a hydrostatic system. Hydrostats are mechanical support systems characterised by a central volume of pressurised uncompressible fluid surrounded by a membrane in tension. The striated muscles organised around the urethra, within the pelvic cavity and supporting seminal ejection, structure a hydrostatic system during ejaculation similar to that suggested for the corpus cavernosum (Carro-Juárez, 2005). Once the ejaculatory threshold is achieved and the seminal secretions propelled, this ejaculatory hydrostat would be activated and, finally, the semen would be directed from the penile urethra to the outside by the bulbospongiosus and ischiocavernosus muscles (Schmidt and Schmidt, 1993).

2.2. *Innervation of the genital structures*

2.2.1. *Sensory innervation of genital structures*

In mammals the sensory genital system plays a role in discriminative and motivational functions. For an understanding of the regulation of the ejaculatory response, it is important to know how genital sensory inputs contribute to its expression. The pelvic genital structures involved in ejaculation are innervated by four principal nerves that include the pudendal, the pelvic, the genitofemoral and the hypogastric nerves. Each of these nerves conveys sensory information to different levels of the central nervous system. Thus, retrograde injection of horseradish peroxidase into the scrotal skin unilaterally labels cells, predominantly located in the lumbosacral enlargement, including the L5–S1segments and the L1–S4 segments (Pascual et al., 1992; Taylor et al., 1982). The lumbosacral spinal cord segments provide the trunk that originates the pudendal and pelvic nerves (McKenna and Nadelhaft, 1986). The pudendal nerve has been considered as the purest nerve, particularly the portion corresponding to the dorsal penile nerve, in the sense that it appears to be exclusively of somatic nature. This nerve extends on the penis and has an abundance of free nerve endings. Pudendal nerve afferent pathways contain both slowly and rapidly adapting fibres (Hubscher, 2006). In addition, sensory fibres are conveyed by the pudendal nerve from the urethra, prepuce, scrotum and ventral proximal tail and the coccygeous muscle (Johnson, 2006, for review; Pacheco et al., 1997). Pudendal afferent fibres exhibit a complex pre-spinal convergence (Katagiri et al., 1986) and the dorsal penile nerve

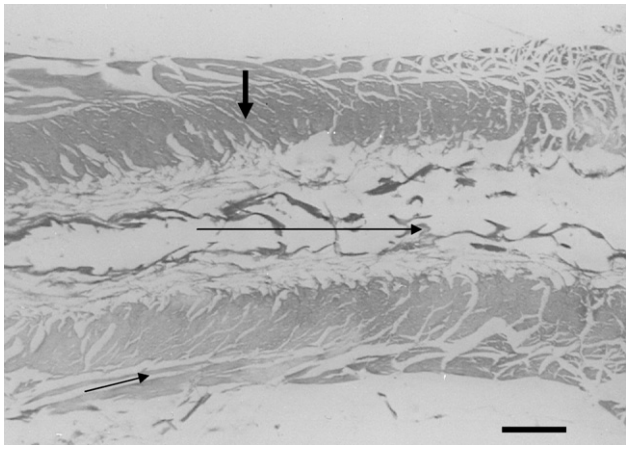

Fig. 2 – The ejaculatory chamber. Notice that the ejaculatorius ductus is completely surrounded by the urethralis muscle. The thin small arrow indicates the external striated muscular fibres and the small tick arrow shows the internal striated muscular fibres of the urethralis muscle. The long tick arrow indicates the urethral ductus where the seminal secretions are deposited to be ejected. Sections were stained with haematoxylin-eosin and were photographed at 4×.

shows monosynaptic contacts in close apposition to motoneurones in lamina IX, VII and X (Nuñez et al., 1986). It has been reported that the pudendal nerve contributes to the expression of ejaculation, since its bilateral transection prevents this response (Lodder and Zeilmaker, 1976).

The genitofemoral nerve innervates, together with the pudendal and pelvic nerves, the penile sheath and the peripenile skin. Lesions of this nerve do not result in any significant effect on ejaculation or other sexual responses (Sachs and Liu, 1992). The pelvic nerve innervates the scrotal and perianal skin (Manzo et al., 1989), however, the reported consequences of its lesion do not allow a conclusion on its participation in the ejaculatory response (Lodder and Zeilmaker, 1976). Besides, the hypogastric nerve also transfers inputs from the genitalia to the central nervous system, since it possesses afferent fibres that enter the spinal cord, via the thoracolumbar dorsal roots, and terminate in the medial dorsal horn (Giuliano and Clement, 2005 for review). Available data do not support a role of the sensory hypogastric components in the ejaculatory process, but only in the autonomic control of the seminal tract tone (Clement et al., 2006a,b).

2.2.2. *Peripheral innervation of genital striated muscles*

The genital tract is surrounded by a specific striated muscular mass named genital striated muscles. These muscles–the external urethral sphincter, the urethralis muscles, the bulbospongiosus (including its portion termed levator ani) and ischiocavernosus muscles and the external anal sphincter (Fig. 1, Panel 1)–conform a sophisticated group of striated muscles that are recruited during intromission and ejaculation responses (Fig. 1, Panel 2), for relatively brief periods at high activation levels (Holmes et al., 1991; McKenna et al., 1991; Ishihara et al., 1997; Carro-Juárez and Rodríguez-Manzo, 2000). One of the most conspicuous features of this group of muscles is that it exhibits a predominance of IIb fibres and possesses only alpha motoneuron innervation, lacking of gamma motoneurones and muscle spindles (Ishihara et al., 1997). Several studies have shown that the pudendal nerve is providing the innervation of all genital muscles (McKenna and Nadelhaft, 1986; Kontani and Shiraoya, 2002; Pacheco et al., 1997; Carro-Juárez, 2005).

2.2.3. *Spinal innervation of genital structures*

Electrophysiological and anatomical studies indicate that there is an extensive system of neurones in the thoracic, lumbar and sacral spinal segments that receive reproductive organ inputs mainly from the pudendal nerve. Studies investigating the features of single interneurones in the dorsal and intermediate zone of lumbosacral segments have shown that L6–S1 spinal segments have sensory fields on the penis that are significantly larger than the receptive fields for single primary afferents, thus demonstrating a central convergence of penile sensory inputs (Hubscher, 2006; for review). Penile interneurones have bilateral receptive fields and their responses suggest a monosynaptic input from the ipsilateral and contralateral penis dorsal nerve fibres. Afferent fibres in the dorsal penile nerve produce crossed and uncrossed reflex facilitation of pudendal responses (Hubscher, 2006; for review).

Pudendal motoneurones that provide somatic motor outputs to genital striated muscles are located in the lumbosacral spinal cord, in a single spinal nucleus known in humans as Onuf's nucleus (Sato et al., 1978; Schroder, 1980). In the rat, this nucleus has two different portions formed by the dorsomedial and the dorsolateral groups of motoneurones (McKenna and Nadelhaft, 1986). Pudendal motoneurones are organised in different clusters in the medial ventral area of the spinal cord region that encompasses L6 to S1 segments. As compared with other motoneuron spinal nuclei, such as the soleus and extensor nuclei, pudendal nerve motoneurones exhibit prominent contralaterally projecting dendritic arborizations (Rose and Collins, 1985). It has been suggested that this crossing dendrites arrangement of pudendal motoneurones may be a substrate for their synchronised activation during penile reflexes (Rose and Collins, 1985). In addition, it has been observed that these crossing dendrites are located in close proximity to contralateral and ipsilateral pudendal motoneuron somas and dendrites. The existence of sub-threshold, short latency and excitatory interactions between ipsilateral and contralateral dorsomedial motoneurones has been shown (Collins et al., 1991). This type of communication between pudendal motoneurones can be considered as the substrate for the coordinated contraction of genital muscles seen during ejaculation.

3. Evidence for the existence of a spinal pattern generator for ejaculation

3.1. *Behavioural studies*

The first evidence suggesting the existence of a central pattern generator for ejaculation came from the analysis of spontaneous ejaculation in the 60′s. In man and other mammals, seminal ejaculation occurs most frequently as a result of genital stimulation derived from coitus or masturbation. Occasionally, ejaculation takes place spontaneously, in the apparent absence of external stimulation, and it is properly seen as a response to endogenous stimuli ordinarily occurring during sleep. This type of ejaculation have also been observed during sleep in domestic cats and hamsters and, particularly, in rats. Experimental data show that spontaneous ejaculation is not due to genital sensory stimulation, since animals prevented from reaching their genitals show them regular daily (Orbach, 1961; Orbach et al., 1967; Beach 1975). In rats, testosterone maintains spontaneous ejaculation in a similar fashion to coital ejaculation (Orbach et al., 1967). Interestingly, coital ejaculation during mating tests temporarily inhibits the spontaneous ejaculation and this inhibition is more pronounced after 5 successive coital ejaculations than after one (Beach, 1975). It has been shown that cortical, hippocampal and hypothalamic lesions do not influence the expression of spontaneous ejaculation; neither does spinal transection at a high lumbar level (Orbach et al., 1967). These data pointed out the possibility that the neural commands involved in the control of spontaneous ejaculation were located at the spinal level. In addition, they put forward that the cumulative effect of coital ejaculation upon spontaneous ejaculation is similar to the effect of repeated coital ejaculation triggering sexual

exhaustion, where the inhibitory effect of recurrent ejaculation is also cumulative.

3.2. *Ex-copula reflex test model studies*

Another indication that a spinal generator controls ejaculatory mechanisms was provided by Bacq (1931). He transected the spinal cord of male guinea pigs between T12 and L1 and reported that rhythmic movements occurred in the anogenital region, that the penis became longer, thicker and protruded completing penile erection, and that this genital activity was accompanied by the discharge of semen. This pattern of reflexive sexual responses in spinal animals bears some striking resemblances to those seen in spinally-intact animals. Detailed studies by Hart (1968) described sexual reflexes evoked by genital stimulation in spinal male rats maintained in a supine position on a laboratory table. These males were lightly restrained on their backs and the prepuce was held retracted so the penis extended well beyond it. Under these circumstances clusters of penile responses occurred which were identified as erections, short flips and long flips followed by ejaculation. Further studies by Sachs and Garinello (1979) provided the first quantitative evidence that the rhythm of these reflex clusters was the same in spinal and intact male rats, thus concluding that the reflexes were paced by spinal neural mechanisms, whose timing was independent of suprasegmental influence. These reflex clusters could be evoked in animals with neurectomy of the dorsal penile nerves and with spinal cord transection (Sachs and Garinello, 1980). The existence of a hypothetical spinal pacemaker regulating penile sexual reflexes was also inferred from the occurrence of the phasic expression of penile reflex clusters without the application of phasic genital stimulation. These results suggested not only that the final ejaculatory mechanism was dependent of the release of a spinal inhibition of more rostral origin, but that the sequential pattern of successive pre-ejaculatory sensory inputs might be programmed at a spinal level. In support of this view was Beach's proposal that the temporary post-ejaculatory refractory period was a reflection of a transitory reduction of the excitability of the spinal mechanisms and their additive effects (Beach, 1967).

3.3. *The coital reflex model studies*

An animal model based on the ex-copula reflex test proposed by Hart (1968) was presented by McKenna et al. (1991) using urethane anaesthetised and acutely spinally-transected rats. The anaesthesia allowed invasive manipulations and neurophysiological recordings. In this model, the mechanical distension of the urethra by the injection of saline solution (a stimulus mimicking the deposition of seminal secretions into the urethra), while briefly occluding the penile meatus, elicits a reflexive response featured by clonic contractions of the perineal muscles, rhythmic autonomic firing, phasic penile erections and penile movements and expulsion of the urethral contents in male rats. According to McKenna et al. (1991), the eliciting stimulus for this pelvic complex response was the stimulation of the urethra. Interestingly, it could also be elicited in female rats. The response was named urethrogenital reflex, a term reflecting the eliciting stimulus site and the responding organs. Although this model was proposed for the study of sexual reflexes in general, it is considered as the neural concomitant of ejaculation (McKenna et al., 1991; Carro-Juárez and Rodríguez-Manzo, 2000; Carro-Juárez et al., 2003).

By using this model, the notion of the existence of a spinal pattern generator controlling ejaculation gained some support. Thus, McKenna et al. were able to record genital rhythmic activity in the hypogastric and cavernosus nerves in animals with neuromuscular and autonomic blockade, but with an intact pudendal sensory nerve. They suggested that this reflex response could be the product of a spinal pattern generator analogous to other fictive locomotion generators (McKenna et al., 1991). Yet, the presence of the pudendal sensory nerves in that study did not preclude the activation of a sequence of spinal reflexes.

There are several theoretical principles that allow considering a given rhythmic response to be under the control of a CPG. These principles are: 1) the motor pattern recorded must represent the pattern that would have produced the rhythmic behaviour under study in an intact animal; 2) the motor response has to be evoked in the absence of higher neural structure influence; 3) in the absence of sensory information the activation of a CPG must still be able to originate the rhythmic behaviour under study; 4) reflexive and spontaneously expressed rhythmic motor patterns ought to exhibit a rhythm in its expression as a result of the activation of the CPG; and 5) individual rhythmic motor patterns should be elicited or modulated pharmacologically (Delcomyn, 1985).

Based on some of the principles above listed and by using the urethrogenital model as a model system, the definitive physiological evidence of the involvement of a spinal pattern generator in the control of ejaculation was presented by Carro-Juárez and Rodríguez-Manzo in (2000). These authors evidenced the existence of an intraspinal intrinsic rhythm of expression of ejaculation by showing that the genital motor pattern of ejaculation appeared spontaneously, at 3-min intervals, after the selective deafferentation of genitalia in male spinal rats (Fig. 4). The motor pattern registered in deafferentated animals consisted of the rhythmic activation of all genital muscles surrounding the male genital tract (Fig. 1, Panel 2), accompanied by penile movements, penile erections and the potent expulsion of seminal secretions. Electromyographic recordings from the rhythmic ejaculatory motor pattern revealed a stereotyped striated muscle response (Fig. 1, Panel 2), featured by an ejaculatory rhythmic motor train always in parallel with penile and ejaculatory actions (Carro-Juárez and Rodríguez-Manzo, 2000). These rhythmic contractions of the genital muscles and its motor consequences proved to be similar to those registered in copulating male rats (Figs. 3 and 4) during ejaculation (Holmes et al., 1991), as well as to those recorded in spinally-intact anaesthetised (Carro-Juárez et al., 2003) and spinally-transected anaesthetised, non-deafferentated male rats (McKenna et al., 1991; Marson and McKenna, 1990, 1994; Marson et al., 1992,). Carro-Juárez and colleagues demonstrated that the rhythmic motor pattern of ejaculation, exhibiting similar features, was

obtained after pharmacological manipulations (Carro-Juárez et al., 2003; Carro-Juárez and Rodríguez-Manzo, 2001, 2003, 2005a,b, 2006a,b).

4. Neural control of the spinal generator for ejaculation

4.1. *Afferent control*

An approach to the understanding of the functioning of a central pattern generator is to stimulate afferents that activate neural circuits (Gordon and Whelan, 2007). This technique is useful, since it relies on the endogenous release of neurotransmitters and neuromodulators to excite neural circuits. The temporal course of pattern generator motor outputs is determined to a great extent by the intrinsic properties of the generator neurones (Arshavsky, 2003). However, peripheral sensory feedback is required for the generation of a normal motor behaviour pattern (Roberts and Perrins, 1995). There is no question that sensory feedback during the performance of a motor behaviour act plays an important role in stabilizing that behaviour. Under normal conditions, the spinal generator for ejaculation must integrate command signals from the brain with sensory feedback from the genital region and produce rhythmic outputs to the motor neurones responsible for ejaculation. The activity in the neuronal networks underlying movement and other rhythmic behaviours can start or stop in response to specific sensory stimuli. The role played by afferent activity in rhythmic motor patterns is to help shaping the pattern, to control phase-transitions and to reinforce ongoing activity (Pearson et al., 2003). Afferent input is so elementary that it can be seen as an essential element of the

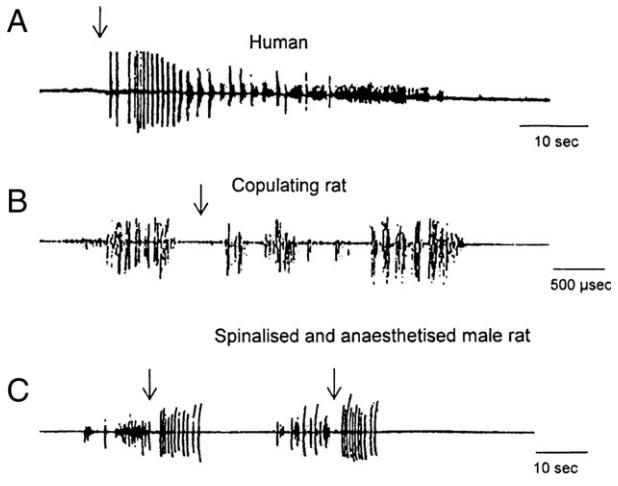

Fig. 3 – Electromyographic activity of the bulbospongiosus muscle during ejaculation in the man (A), in copulating (B) and in spinalised and anaesthetised male rats (C). Notice that the similarities among the three responses including rhythmicity in the motor trains and its after-discharge component are preserved between species and experimental conditions. Modified from: Gerstenberg et al., 1993; Holmes et al., 1991; McKenna et al., 1991). Arrows indicate the initiation of ejaculation.

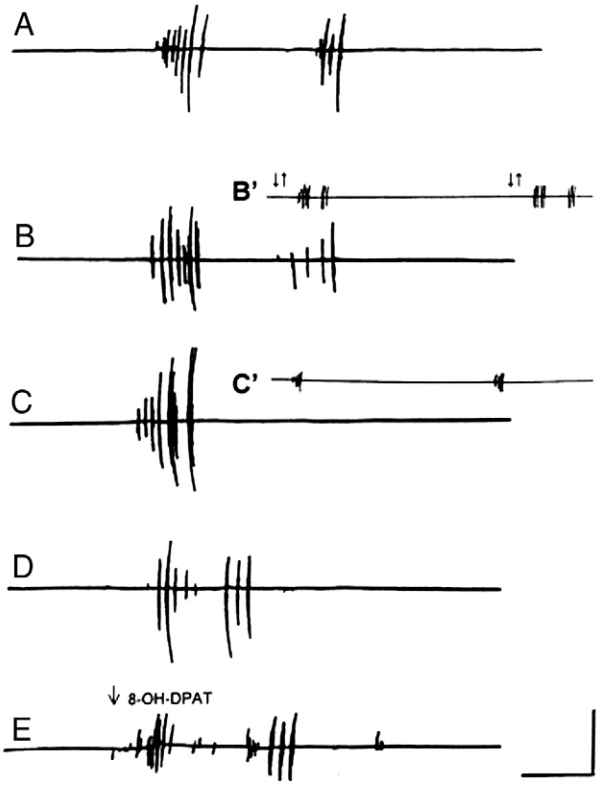

Fig. 4 – The basic principles to demonstrate that ejaculation is under the control of a spinal pattern generator include: the expression of ejaculation in spinally-intact and anaesthetised animals (A), the activation of ejaculation after spinal cord transection (B), the spontaneous activation of ejaculatory trains in deafferentated animals (C), after repeated genital sensory elicitation at different intervals (D) and after its pharmacological activation (E). From Carro-Juárez et al., 2003; with permission.

definition of a central pattern generator (Pearson et al., 2003). A spinal pattern generator for ejaculation should make the connection between the sensory afferents from the genitalia and the autonomic and somatic neurones of the circuit. This hypothesis proved to be true and it was established, by using the coital reflex model that, once released from its higher control by spinal cord transection, the spinal generator for ejaculation can be activated by sensory signals from the penis, the scrotal skin or the urethra, three of the main sensory structures involved in ejaculation (Carro-Juárez and Rodríguez-Manzo, 2006a,b). Mechanical stimulation of all these genital structures elicited the stereotyped ejaculatory motor pattern whose electromyographic response consists of a first motor ejaculatory train followed by an after-discharge component. By contrast, in deafferentated rats the spontaneous ejaculatory electromyographic patterns lack the after-discharge component. Thus, sensory stimulation activates the ejaculatory motor pattern with this noticeable difference with the spontaneous ejaculatory pattern (Carro-Juárez and Rodríguez-Manzo 2000). Consequently, sensory inputs modulate the activity of the spinal pattern generator facilitating the expression of the complete ejaculatory motor rhythmic

sequence. Besides, overlapped sensory stimulation applied to an *in progress* ejaculatory motor response fully stops the expression of the rhythmic pattern, evidencing a direct effect of the superimposed mechanical stimulation upon the ongoing rhythmic bursting activity. Thus, a direct effect of afferent inputs on the spinal circuits controlling ejaculation and a short-lasting nature of the inhibitory process, that develops after recurrent inflow was evidenced (Carro-Juárez and Rodríguez-Manzo, 2005a,b). Repeated elicitation of the ejaculatory motor pattern by genital sensory means modifies the activity of the ejaculation generator inducing a long-lasting inhibitory state (Carro-Juárez and Rodríguez-Manzo, 2000). A mean number of 7 sensory-elicited ejaculatory motor patterns are registered prior to its inhibition (Carro-Juárez and Rodríguez-Manzo, 2006a,b). It has been suggested that inhibition of the spinal ejaculation generator is a process that occurs at a spinal level when ejaculation is repeatedly evoked through the activation of a positive sensory feedback mechanism (Carro-Juárez and Rodríguez-Manzo, 2000, 2001, 2003, 2005a). Thus, rhythmogenic activity of the ejaculation generator is highly susceptible to genital inputs which pace pattern generation by exerting a modulatory influence.

4.2. *Neurochemical modulation*

The modulatory systems–intrinsic and extrinsic–of central pattern generators are essential to its operation (Grillner, 2003). It has been demonstrated that the 5-HT system is an integral part of the CPG networks that are located into the spinal cord, which fine-tunes neuronal properties (Grillner, 2003). 5-HT seems to promote a regular motor pattern in all vertebrates studied, though in some species this modulation depends mainly on descending projections (Grillner, 2003). Dopamine has a complementary action to that of 5-HT. The GABA system in the spinal cord is also active during network activity and it can therefore be also considered as an integral part of the locomotor system (Grillner, 2003). Thus, intrinsic modulation is turned on whenever the generator is active. Both intrinsic and extrinsic modulatory systems can be activated by descending brainstem fibres to adapt the motor networks to different external demands (Grillner, 2003). Whether the activity of the spinal generator for ejaculation is activated by extrinsic and/or intrinsic mechanisms is a topic that deserves experimental studies. Even so, the general plan of the neurochemical control of the spinal generator for ejaculation can be offered. It has been demonstrated that 5-HT released by descending pathways from the brainstem exerts an inhibitory control upon sexual reflexes including ejaculation (Marson and McKenna, 1990; Marson et al., 1992, 1994; De Jong et al., 2006, Gravitt and Marson, 2007). However, elevation of synaptic 5-HT and dopamine by blockade of re-uptake mechanisms with *p*-chloramphetamine facilitates the activity of the spinal generator for ejaculation (Stafford et al., 2006a,b). Besides, activation of 5-HT1A receptors facilitates ejaculation (Ahlenius and Larsson, 1997; Carro-Juárez et al., 2003) and reverses the inhibition of ejaculation in spinal male rats induced by repeated genital sensory stimulation (Carro-Juárez and Rodríguez-Manzo, 2001). In addition, activation of lumbosacral 5-HT2C receptors induces bursts of rhythmic activity in sympathetic nerves to the vas deferens in spinal male rats. This is a response associated with the contraction of the vas deferens and with ejaculation that includes the contraction of genital muscles (Stafford et al., 2006a,b). Thus, the existence of a descending 5-HT excitatory mechanism, in addition to the described inhibitory one, has been postulated (Stafford et al., 2006a,b). The data showing that after spinal cord transection an intraspinal intrinsic rhythm of expression of the ejaculatory motor pattern is uncovered suggest that the facilitatory actions of 5-HT are intrinsic in nature. In line with this notion, it has been shown that systemic administration of the 5-HT1A receptor agonist 8-OH-DPAT in spinal animals turns on an intraspinal rhythm of ejaculatory activity inducing the expression of complete sequences of the rhythmic ejaculatory pattern (Carro-Juárez et al., 2003).

Dopaminergic pathways are also involved in the control of the ejaculatory motor pattern. Thus, it has been demonstrated that activation of D2-like receptors mediates the expulsive phase of ejaculation (Clement et al., 2006a,b). Ejaculation is also induced by the i.c.v. injection of a preferential D3 dopaminergic receptor agonist (Clement et al., 2007). The increase in extracellular dopamine by *p*-chloramphetamine also induces an ejaculatory-like genital sequence in anaesthetised rats (Stafford et al., 2006a,b) similar to that obtained with the systemic administration of dopamine (Carro-Juárez and Rodríguez-Manzo, unpublished observations). It has been suggested that these effects are exerted at the spinal generator for ejaculation. Activation of D1- and D2-like dopamine receptors by apomorphine induces ejaculation-like responses in spinal cord transected animals (Stafford and Coote, 2006a,b) and a similar effect is obtained when injecting selective D2/D3 receptor agonists (Stafford and Coote, 2006a,b). These studies demonstrate a facilitatory role of the dopaminergic spinal system in the control of the central generator for ejaculation.

The possible participation of the noradrenergic central system in the modulation of the spinal generator for ejaculation has also been evaluated by examining the effect of α-adrenoceptor agonists and antagonists upon the activity of the ejaculation generator in the rat's disinhibited spinal cord (Carro-Juárez and Rodríguez-Manzo, 2003, 2006a). Thus, it has been shown that ejaculatory rhythmic patterns, accompanied by the expulsion of urethral contents and phasic penile movements, can be elicited by the i.v. injection of methoxamine or yohimbine (Carro-Juárez and Rodríguez-Manzo, 2006a,b). As a result, it could be demonstrated that an increased noradrenergic tone, either by blockade of pre-synaptic α2-adrenoceptors or by stimulation of post-synaptic α1-adrenoceptors, results in the activation of the ejaculation generator (Carro-Juárez and Rodríguez-Manzo, 2006a,b). After demonstrating a role for the noradrenergic system in the functioning of the generator for ejaculation, the stimulatory effects of yohimbine and methoxamine were tested in the model of inhibited ejaculation due to repeated genital mechanical stimulation. Systemic administration of either yohimbine (Carro-Juárez and Rodríguez-Manzo, 2003) or methoxamine (unpublished data) reverses the inhibition of ejaculation thus, supporting the notion of a facilitatory role of the noradrenergic system on ejaculation in spinal male rats (Carro-Juárez and Rodríguez-Manzo, 2003). Reversal of ejaculation inhibition by methoxamine or yohimbine can be

prevented by the pre-treatment with clonidine or prazosin, respectively (Carro-Juárez and Rodríguez-Manzo, 2003). Thus, these studies provide the evidence that the spinal generator for ejaculation might be importantly influenced by the noradrenergic system which exerts a facilitatory control on the expression of the genital motor pattern of ejaculation.

The participation of the cholinergic spinal system in the control of the genital pattern of ejaculation has also been shown. Thus, administration of muscarin produces a facilitation of the expression of the ejaculatory motor pattern (Gil et al., 2000; Vargas et al., 2004), whereas the administration of the muscarinic antagonist homatropine blocked it, suggesting that the ejaculation generator may be activated through muscarinic receptors (Gil et al., 2000). The cholinergic modulation of the ejaculatory motor pattern is probably mediated by the M2, M3 and M4 muscarinic receptor subtypes (Gómez et al., 2005).

A potential role for peptides in the modulation of the ejaculation generator has also been evidenced. Thus, it has been demonstrated that in adult as well as in neonatal male rats, the systemic administration of oxytocin commences the rhythmic genital pattern associated with ejaculation (Carro-Juárez and Rodríguez-Manzo, 2005a,b). The ejaculation activating effects of oxytocin can be blocked by pre-treatment with hexamethonium, a selective oxytocinergic antagonist (Carro-Juárez et al., 2006). A different set of experiments evaluated the hypothesis that spinal opioids participated in the control of ejaculation by exerting an inhibitory influence upon the spinal generator for ejaculation. Thus, it was shown that morphine, a μ opioid receptor ligand, exerts an inhibitory effect on the genital motor pattern of ejaculation and that the pre-treatment with the non-selective opioid receptor antagonist naloxone blocked the inhibitory actions of morphine on ejaculatory sequences. These data support the notion that spinal opioids modulate the activity of the spinal generator for ejaculation by exerting an inhibitory influence (Carro-Juárez and Rodríguez-Manzo, submitted for publication).

5. Identification of the spinal generator for ejaculation

5.1. *Anatomical evidence in males*

Although a biological CPG is actually composed by many highly interconnected neurones, it is still useful to consider the CPG as a holistic entity (or a few entities) instead of thousands of separated parts (Vogesltein et al., 2007). Identifying neurones and connections within spinal networks is challenging because cells that comprise the locomotor network form part of a heterogeneous mixture of interneurones within the ventral spinal cord. Activity-labelling studies and physiological evidence show that locomotor-related neurones are concentrated in the ventral cord (lamina VII, VIII and X) suggesting that all the critical elements of the locomotor circuit in mammals are located there (Kihen, 2006, for review). The rhythmogenic capacity is also located in the ventral cord and, at least in rodents, into the lower thoracic spinal cord, with rostral segments having a greater capacity to generate a rhythm than the caudal ones (Kihen, 2006 for review). The organisational principle suggests that instead of having one rhythm-generating core localised in the upper lumbar cord, mammalian CPGs are composed of multiple distributed rhythm-generating core networks/modules (Kihen, 2006). It has been hypothesised that an integral part of the spinal generator for ejaculation is a group of interneurones, located into the lumbosacral spinal cord, which converts sensory signals into motor and autonomic outputs (McKenna, 1999). Thus, recent studies have permitted the identification of a population of interneurones in the central gray of lumbar segments L3–L4 that could play a pivotal role in the control of ejaculation (Truitt and Coolen, 2002). This population consists of cells located in lamina X and the medial portion of lamina VII of these lumbar segments. They contain galanin, cholecystokinin and enkephalin (Fig. 5) and send projections to a nucleus located within the posterior intralaminar thalamus; the parvocellular subparafascicular thalamic nucleus (Truitt and Coolen, 2002; Coolen et al., 2004). Based on their location and on its thalamic projections, these particular cells are referred to as lumbar spinothalamic cells (LST). Studies using c-fos as a marker of neural activation, have demonstrated the involvement of these cells in male sexual behaviour. It was shown that LST cells expressed Fos with ejaculation, but not following other components of sexual behaviour such as intromissions or mounts. Further studies showed that lesions to LST cells did not affect the display of mounts and intromissions, but completely disrupted the display of ejaculatory behaviour. Examination of female partners revealed the consistent absence of seminal plugs (Truitt and Coolen, 2002). The LST cells have connections to sensory inputs as well as to autonomic and somatic centres (Coolen et al., 2004). These neurones also have close connections to motoneurones innervating the bulbospongiosus muscles (Xu et al., 2005). Moreover, a convergence of retrograde labelling from the prostate and from the bulbospongiosus muscles on the same lumbar LST neurones has been demonstrated (Xu et al., 2006). Likewise, it has been shown that the prostate and bulbospongiosus muscles receive dual innervation from L3–L4 and from L6–S1 spinal segments, though only the L3–L4 spinal cells significantly express galanin and neurokinin 1. Thus, within the lumbosacral spinal cord the lamina X of the L3–L4 segment is, from an anatomical perspective, specialised in the coordination of L1–L2 sympathetic centres and L6–S1 parasympathetic and motor centres that innervate the prostate and bulbospongiosus muscles (Xu et al., 2006). All in all, it is probable that the lumbosacral spinal cord contains one major ejaculation-generating core located in the ventral portion of lamina X in the upper lumbar cord, directed towards multiple core pattern generation networks of the lumbosacral segment, whose operation mode is still unknown.

It could be hypothesised that at least two core pattern generation networks are sequentially activated by genitosensory stimulation during ejaculation: one of them could be related to the autonomic component of ejaculation, involved in the production and voiding of seminal secretions, and the other would be in charge of the somatic component of ejaculation, involved in the control of muscular contractions seen during ejection. In support of this hypothesis is the fact that the fictive ejaculatory response, observed in spinal transected male rats after the activation of sensory pathways

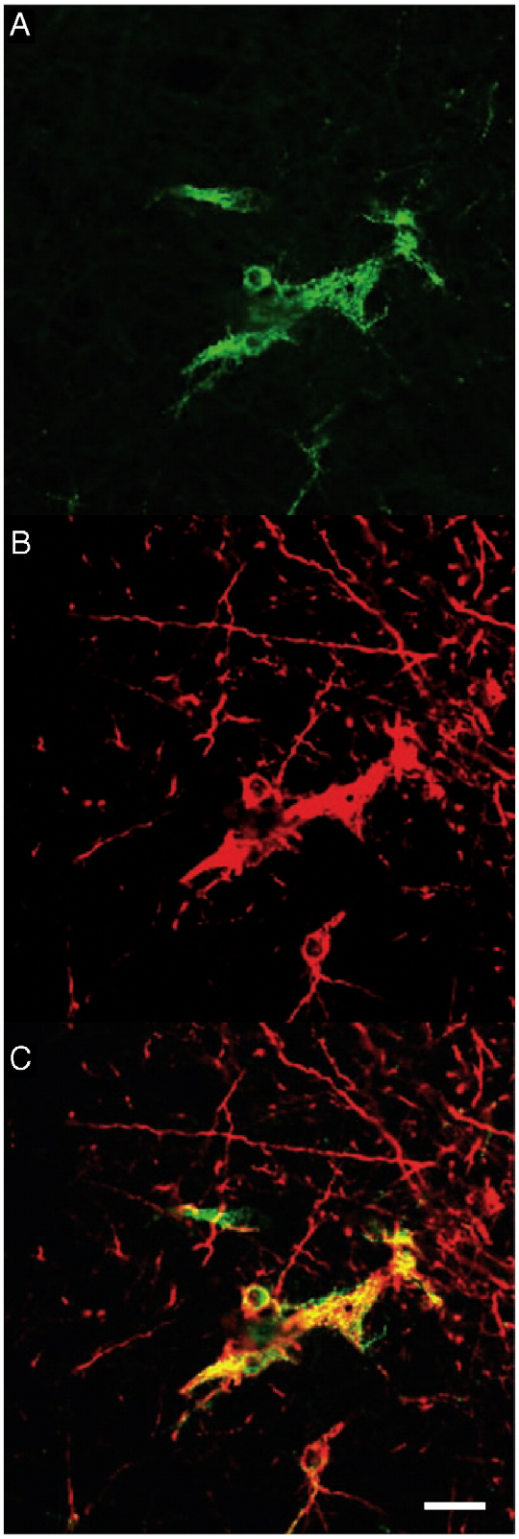

Fig. 5 – Fluorescent images illustrating the coexpression of galanin (A) and NK-1R B) in LSt neurones (C) Overlay of (A) and (B). Scale bar, 50 μm. From Truitt and Coolen, 2002; with permission.

mediating ejaculation, includes both autonomic and somatic components that, even though turned on simultaneously during ejaculation could obey different spinal mechanisms. Interestingly, Marson and Gravitt (2004) described the activation of neurones immunoreactive to galanin, choline acetyl transferase or serotonin after the elicitation of the urethrogenital reflex (UG). Spinal circuits involved in the UG reflex include neurones relaying afferent information from the pudendal sensory nerve to the dorsal horn and medial cord of L5–S1. Interneurones specifically activated with the UG reflex were identified in the medial, intermediate and lateral gray, as well as a small proportion of parasympathetic and sympathetic preganglionic neurones in the intermediolateral cell column of L5–S1 and medial gray of T13–S1, respectively (Marson and Gravitt, 2004).

5.2. *Studies in neonates*

A criterion to establish that a given rhythmic motor response is under the control of a CPG is to provide evidence for its existence at early developmental stages. It has been proposed that the pattern-generating neural circuitry shaping a specific CPG is present early in development, but remains silent undergoing a progressive maturation until its full expression (Pearson et al., 2003). By using the urethrogenital reflex model, it was demonstrated that ejaculatory rhythmic motor patterns are present in neonatal male rats. A complete ejaculatory motor sequence can be elicited in male rats after the application of an ejaculation-like releasing stimulus since postnatal day 2 (Carro-Juárez and Rodríguez-Manzo, 2005a,b). Similar ejaculatory motor patterns can be obtained in neonatal rats after the application of a pharmacological stimulus. The ejaculatory motor activity of neonates is accompanied by ejaculatory movements that propel urethral contents, but lack of penile erection and movements (Carro-Juárez and Rodríguez-Manzo, 2005a,b). The motor trains of ejaculation in neonates from postnatal day 7 to 21 are facilitated in the sense that they show an augmented number of muscular discharges (Fig. 6). The ejaculatory trains undergo a progressive maturation until postnatal day 28, when they exhibit an adult-like ejaculatory motor pattern (Fig. 6). These data strongly suggest that the establishment o f the intrinsic inhibitory spinal mechanisms controlling ejaculation takes place during postnatal development. Besides, data showing that male rats are able to express ejaculation, but not penile erection responses at early developmental stages reveal a differential organisation and maturation of the spinal circuits controlling penile erection and ejaculation and reinforce the notion that the rhythmic motor pattern of ejaculation is an innate motor pattern (Carro-Juárez and Rodríguez-Manzo, 2005a,b).

5.3. *Studies in females*

It has been proposed that the neural organisation of male and female sexual functions is similar (McKenna, 2000). Though genital sexual reflexes differ between males and females it can be observed that both sexes possess the anatomical substrate for ejaculation. Thus, several studies have shown the presence of both orgasmic and ejaculatory motor patterns in human and non-human female primates, as well as in female rats, that are very similar to the orgasmic and ejaculatory patterns of their conspecific males (Emery and Sachs, 1975; Marson

et al., 2003). Female ejaculatory and orgasmic patterns exhibit parallelisms with those of males that include the presence of genital fluids and the occurrence of rhythmic contraction of genital muscles (Kratochvil, 1994; Emery and Sachs, 1975; Shehata, 1980). In addition, there is evidence suggesting that female ejaculation appears to be followed by a refractory period, just as in males (Emery and Sachs, 1975). Hence, both female and male ejaculatory responses share the facilitatory and inhibitory phenomena associated to ejaculation. In a previous study, the presence of the ejaculatory motor pattern was evidenced in the prostatic region of the female urethra. This ejaculatory-like response consists of a strong rhythmic motor pattern registered in the urethralis muscle in response to sensory stimulation of genital structures such as the urethra, the vagina and the clitoris (Carro-Juárez and Rodríguez-Manzo, 2005a,b). The motor components of the female rat ejaculatory motor pattern are indistinguishable from those registered in male rats. It could be demonstrated that this female ejaculatory motor pattern is controlled by a spinal pattern generator and that the spinal circuits in charge of that ejaculatory-like response can be modulated by sensory and pharmacological means (Carro-Juárez and Rodríguez-Manzo, 2006a,b) in a similar fashion to that observed in males, thus providing support for the notion of the involvement of a spinal generator in the control of female ejaculation.

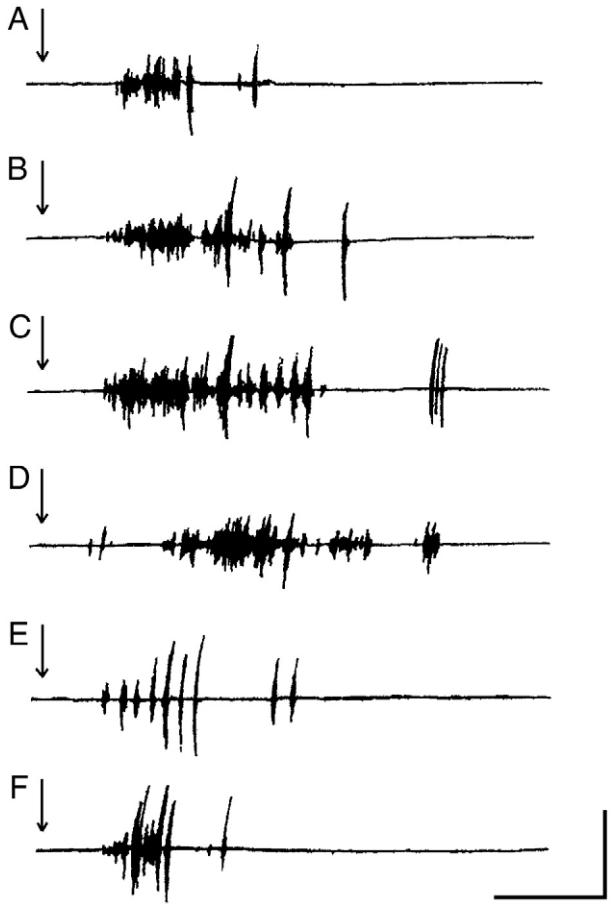

Fig. 6 – Postnatal developmental expression of the rhythmic motor pattern of ejaculation in spinal male rats. Note the similarities among the fictive ejaculatory motor sequences of PO2 (A), PO7 (B), PO14 (C), PO21 (D), PO28 (E) and adult rats (F) registered in the bulbospongiosus muscles. Arrows indicate stimulus off. From Carro-Juárez and Rodríguez-Manzo, 2005a; with permission.

6. Conclusions and perspectives

The physiology of ejaculation has become recently a central matter of investigation. The studies described herein highlight one of the most interesting findings on ejaculatory function: the existence of a spinal pattern generator controlling this sexual response. In spite of the progress in the understanding of the functioning of the spinal generator for ejaculation many questions remain to be solved. Thus, for instance, it would be important to establish not only the precise location of the spinal generator within the spinal cord as well as its participation in the control of the two components of ejaculation, but also to analyse the contribution of each individual core of such circuit in modulating the pacemaker activity that drives simultaneously seminal emission and striated muscle contraction. In this respect, it is important to highlight that the genital model offers, as a model system of ejaculation, a particularly attractive experimental approach that has provided important insights into the operation of the ejaculation generator and the participation of spinal neurotransmitter systems in the modulation of its inhibitory and excitatory mechanisms (Table 1). Besides, it would be interesting to analyse the participation of the spinothalamic cells in mediating the mechanisms of emission and/or ejection in both sexes. Particularly, it would be important to establish their participation in the ejaculatory motor pattern.

Finally, we propose that an altered activity of the spinal generator for ejaculation could underlie some pathological conditions responsible for ejaculation-related sexual dysfunctions, where an imbalance between inhibition/facilitation prevails. For example, in premature ejaculation redundant activity of the spinal generator could produce the non expected early ejaculatory response. By contrast, an enhanced inhibition of the ejaculatory generator could underlie retarded ejaculation.

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Table 1 – Role played by different neurotransmitter systems in the functioning of the spinal generator for ejaculation (SGE) in rats

Neurotransmitter system	Compound	Effects on male rat SGE functioning	References
Serotonergic	5-HT	• Inhibits the expression of ejaculation in spinal rats, i.e. inhibits SGE activity	Marson and McKenna, 1990, 1994; Marson et al., 1992; Stafford et al., 2006a,b
	5-HT1A receptor agonist 8-OH-DPAT	• Activates the SGE • Reverses SGE inhibition elicited by repeated genital sensory stimulation	Carro-Juárez and Rodríguez-Manzo, 2001; Carro-Juárez et al., 2003; Stafford et al., 2006b
	5-HT1A receptor antagonist WAY-100635	• Activates the SGE • Blocks the 8-OH-DPAT-induced activation of the SGE and the reversal of the repeated genital stimulation-induced SGE inhibition	Carro-Juárez and Rodríguez-Manzo, 2001; Stafford et al., 2006a
	5-HT2C receptor agonist Ro600175	• Activates the SGE	Stafford et al., 2006b
	5-HT2B/C antagonist SB206553	• Blocks pharmacological activation of SGE with PCA	Stafford et al., 2006b
Noradrenergic	α1-receptor agonist methoxamine	• Activates the SGE	Carro-Juárez and Rodríguez-Manzo, 2006a
	α1-receptor antagonist prazosin	• Inhibits the activity of the SGE	Carro-Juárez and Rodríguez-Manzo, 2003, 2006a
	α2-receptor agonist clonidine	• Inhibits SGE activity • Blocks the yohimbine-induced SGE activation	Carro-Juárez and Rodríguez-Manzo, 2003, 2006a
	α2-receptor antagonist yohimbine	• Activates SGE functioning • Reverses the sensory-induced inhibition of the SGE	Carro-Juárez and Rodríguez-Manzo, 2003, 2006a
Dopaminergic	Dopamine	• Activates the SGE	Watcho and Carro-Juárez, submitted for publication
	D1/2-receptor agonist apomorphine	• Activates the SGE in female and male rats	Stafford and Coote, 2006a
	D2/3 receptor agonist piribedil	• Activates SGE functioning in male and female rats	Stafford and Coote, 2006a
	D2 receptor antagonist remoxipride	• Inhibits the apomorphine-induced activation of the SGE in female and male rats	Stafford and Coote, 2006a
	D3 receptor antagonist nafadotride	• Inhibits the apomorphine-induced activation the SGE in female and male rats	Stafford and Coote, 2006a
Cholinergic	Cholinergic agonist muscarine	• Facilitates activity of the SGE	Vargas et al., 2004; Gómez et al., 2005; Gil et al., 2000
	Muscarine receptor antagonist homatropine	• Inhibits SGE activity	Gil et al., 2000
	M3 and M4 receptor antagonists 4-DAMP and tropicamide	• Block the muscarine-induced SGE activation	Gómez et al., 2005
	M2 receptor antagonists methoctramine and AFDX	• Reverses the muscarine-induced facilitation of the SGE	Gómez et al., 2005
Nitrergic	NO releaser sodium nitroprusside	• Activates the SGE in female rats	Carro-Juárez and Rodríguez-Manzo, 2006a,b
Peptidergic systems			
Oxytocinergic	Oxytocin	• Activates the SGE in neonate and adult male rats	Carro-Juárez and Rodríguez-Manzo, 2005a,b
	Oxytocin receptor antagonist hexamethonium	• Blocks the oxytocin-induced activation of the SGE	Carro-Juárez et al., 2006
Opioidergic	μ receptor agonist morphine	• Inhibits the activity of the SGE	Carro-Juárez and Rodríguez-Manzo, submitted for publication
	μ and δ receptor antagonist naloxone	• Facilitates the activity of the SGE • Prevents morphine-induced inhibition of the SGE	Carro-Juárez and Rodríguez-Manzo, submitted for publication

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