SEXUAL RESPONSES IN THE HUMAN SPINAL CORD

by

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Abstract

Altered sexual function is one of the most devastating consequences of spinal cord trauma (SCT). Despite this fact, current knowledge of the neural circuitry regulating sexual response in the spinal cord (SC) in healthy humans is remarkably incomplete. In order to better understand the changes that occur to sexual responses following SCT, we must elucidate the neural transmission of sexual function in healthy humans. Functional magnetic resonance imaging (fMRI) techniques to map neuronal function have been adapted for the SC and can now reveal this neural circuitry. We mapped, with spinal fMRI, neuronal activity in the lower thoracic, lumbar and sacral SC in healthy men (n = 10) and women (n = 9) that occurs in response to intermittent audiovisual stimulation (AVS), intermittent genital self-stimulation (GSS) and the combination of the former and latter, applied continuously and simultaneously until orgasm (AVGSS). MR images revealed predominantly increased signal intensity changes (∆S+) in the autonomic preganglionic nuclei of the lower thoracic, lumbar and sacral SC in women and mostly decreased signal intensity changes (∆S-) in comparable regions in men. In functional MR images, ∆S+ are related to increased neuronal input while ∆S- are associated with diminished neuronal input to a particular region. Linear regression analyses uncovered a greater number of inverse correlations between SC ∆S and scores of sexual function in women than in men indicating greater descending modulation of SC circuits regulating sexual responses in women than in men. Taken together, our results demonstrate that spinal fMRI is an effective and sensitive technique that can reveal signal intensity changes in the lower thoracic, lumbar and sacral SC associated with AVS, GSS and AVGSS in healthy men and women.
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<td>Autonomic ganglion</td>
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<td>AVS</td>
<td>Audiovisual stimulation</td>
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<td>AVGSS</td>
<td>Audiovisual and genital self-stimulation</td>
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<td>BNST</td>
<td>Bed nucleus of the Stria terminalis</td>
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<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>DCN</td>
<td>Dorsal commissural nucleus</td>
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<td>DGC</td>
<td>Dorsal grey commissure</td>
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<td>DGM</td>
<td>Dorsal grey matter</td>
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<tr>
<td>DH</td>
<td>Dorsal horn</td>
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<td>DM</td>
<td>Dorsomedial nucleus of the thalamus</td>
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<td>DMN</td>
<td>Dorsal motor nucleus</td>
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<tr>
<td>DNP</td>
<td>Dorsal nerve of the penis</td>
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<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<td>EPI</td>
<td>Echo-planar imaging</td>
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<tr>
<td>FLAIR</td>
<td>Fluid-attenuated inversion recovery</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<tr>
<td>FSFI</td>
<td>Female sexual function index</td>
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<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
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<tr>
<td>GRE</td>
<td>Gradient-echo</td>
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<tr>
<td>GSS</td>
<td>Genital self-stimulation</td>
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<tr>
<td>HASTE</td>
<td>Half Fourier single-shot fast spin-echo</td>
</tr>
<tr>
<td>IIEF</td>
<td>International index of erectile function</td>
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<tr>
<td>IML</td>
<td>Intermediolateral cell column</td>
</tr>
<tr>
<td>LC</td>
<td>Locus ceruleus</td>
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<tr>
<td>LMN</td>
<td>Lower motor neuron</td>
</tr>
<tr>
<td>LSt</td>
<td>Lumbar spinothalamic cells</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MD</td>
<td>Mediodorsal nucleus of the thalamus</td>
</tr>
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<td>MEA</td>
<td>Medial amygdaloid nucleus</td>
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<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
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<td>MPOA</td>
<td>Medial preoptic area</td>
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<td>MR</td>
<td>Magnetic resonance</td>
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<tr>
<td>MRF</td>
<td>Medullary reticular formation</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NCTS</td>
<td>Nucleus cuneatus</td>
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<td>NPGi</td>
<td>Nucleus paragigantocellularis</td>
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<tr>
<td>NTS</td>
<td>Nucleus of the solitary tract</td>
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<tr>
<td>ON</td>
<td>Onuf’s nucleus</td>
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<tr>
<td>PAG</td>
<td>Periaqueductal grey</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>PMSA</td>
<td>Perceived mental sexual arousal</td>
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<tr>
<td>PSA</td>
<td>Perceived sexual arousal</td>
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<tr>
<td>PPSA</td>
<td>Perceived physical sexual arousal</td>
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<tr>
<td>PVN</td>
<td>Paraventricular nucleus of the hypothalamus</td>
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<tr>
<td>rCBF</td>
<td>Regional cerebral blood flow</td>
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<tr>
<td>RESPITE</td>
<td>Retrospective spinal cord motion time-course estimates</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
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<tr>
<td>SC</td>
<td>Spinal cord</td>
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<td>SE</td>
<td>Spin echo</td>
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<td>SEEP</td>
<td>Signal enhancement by extravascular water protons</td>
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<td>SNS</td>
<td>Sympathetic nervous system</td>
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<td>SPFp</td>
<td>Subparafascicular thalamic nucleus</td>
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<td>SPN</td>
<td>Sacral parasympathetic nucleus</td>
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<td>STN</td>
<td>Substantia nigra</td>
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<tr>
<td>TE</td>
<td>Echo time</td>
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<td>TR</td>
<td>Repetition time</td>
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<td>UMN</td>
<td>Upper motor neuron</td>
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<td>VGM</td>
<td>Ventral grey matter</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>VH</td>
<td>Ventral horn</td>
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<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
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<tr>
<td>ΔS+</td>
<td>Increased signal intensity changes</td>
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<tr>
<td>ΔS-</td>
<td>Decreased signal intensity changes</td>
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Chapter 1

Introduction

“A major obstacle to understanding our own sexuality is realizing we are prisoners of past societal attitudes towards sex”. (Bullough, 1976)

Sexual function is an exceptionally important aspect of human life. The subject of sexuality is inherently appealing to men and women alike because it is intrinsically pleasurable and instrumental to reproduction. The venerable thinker and physician, Sigmund Freud, recognized the sexual drive as a powerful psychological and biological force of humankind (Masters, Johnson, and Kolodny 1988). Freud saw human sexuality as the motivating force behind all of human actions as well as the primary trigger of most neuroses (Masters et al. 1988). Thus, in the study of human sexuality we are essentially learning about the very essence of human nature (Masters et al. 1988).

Alfred Kinsey (1894 – 1956), a zoologist at Indiana University, was a pioneer in the study of human sexuality (Masters et al. 1988). Along with his colleagues, Wardell Pomeroy, Clyde Martin, and Paul Gebhard, he published two important volumes: Sexual Behavior in the Human Male (Kinsey 1948) and its sequel, Sexual Behavior in the Human Female (Kinsey 1953). These extensive reports described the sexual experiences of 12,000 individuals from across all walks of life. Unfortunately, Kinsey’s reports were met with shock and dismay from critics and the general public, who attacked it on moral and methodological grounds (Masters et al. 1988). Nevertheless, Kinsey’s groundbreaking research was the force that propelled the start of the sexual revolution that took place shortly (Masters et al. 1988). Negative attitudes about sex were replaced with openness to sexual experience and its expression outside of marriage. Female sexuality in particular, increasingly became regarded as a source of pleasure instead of duty, as in earlier times. The advent of the sexual revolution made sex a legitimate topic of discussion,
naturally giving rise to public curiosity. People with sexual dysfunction were more likely to seek the help of trained professionals. However, the lack of objective knowledge of human sexual responses was clear, making it difficult for clinicians to effectively treat sexual disorders in their frantic patients (Masters et al. 1988).

Shortly after Kinsey’s publication, *Sexual Behavior in the Human Female*, William H. Masters and Virginia E. Johnson, a physician and a behavioral scientist at Washington University in St. Louis, decided to take matters into their own hands. Skeptical of using animal data to understand human sexual responses, they embarked on a quest to investigate human sexuality in their own laboratory, using objective measures (Masters et al. 1988). Masters and Johnson viewed humans as unique creatures due to the interplay of psychological, sociological, anatomical and physiological elements in human sexual responses (Masters et al. 1988). Furthermore, Masters and Johnson realized that in order to effectively treat sexual abnormalities, it is first essential to understand the normal anatomy and physiology of human sexual function (Masters et al. 1988). Thus, Masters and Johnson decided to investigate sexual responses in a large population of healthy men and women. Conclusions drawn from their data in healthy individuals would ultimately be utilized in assessing and diagnosing sexual dysfunction. In only a decade, Masters and Johnson had observed more than 10,000 incidents of sexual interactions in 382 women and 312 men (Masters et al. 1988) publishing the controversial (for the time) volume, *Human Sexual Response* (Masters and Johnson 1966). In this volume, Masters and Johnson first described the sexual response cycle comprised of 4 distinct stages: *excitement, plateau, orgasm and resolution*. Since then, sex researchers throughout the world have utilized variations of the human sexual response cycle based on the original model proposed by Masters and Johnson in 1966.

The central nervous system (CNS) mechanisms regulating sexual responses in humans are not well understood. Because the control of sexual responses is organized at the level of the
CNS, it is essential to consider when assessing, diagnosing and treating sexual dysfunction in humans. However, there are many problems associated with studying the central mechanisms in humans in vivo. Direct approaches to studying the brain and spinal cord physiology are quite invasive, necessitating the implementation of animal models of human sexual function. In addition, there are few animal models of female sexual function and those that exist are focused primarily on the hormonal control of sexual behavior in rodents (McKenna 2000). It is known that vasocongestion of female and male genitalia (penis and clitoris) as well as orgasmic responses that occur concomitantly with ejaculation (Truitt and Coolen 2002; Allard, Truitt, McKenna and Coolen 2005) in men and in women (Wimpissinger, Stifter, Grin and Stackl 2007; Kratochvil 1994; Levin and Riley 2007) are organized at the level of the spinal cord and brainstem (McKenna and Marson 1997). Advances in functional magnetic resonance imaging (fMRI) techniques (Logothetis, Pauls, Augath, Trinath and Oeltermann 2001; Menon et al. 1992; Ogawa et al. 1992) and specifically their modification for imaging the spinal cord (Stroman, 2005; Stroman, Tomanek, Krause, Frankenstein and Malisza 2002a; Figley and Stroman 2008), spinal fMRI, have permitted the direct study of sexual responses in the human spinal cord of men and women. Spinal fMRI investigations of sexual responses in healthy men and women will enable researchers to determine what changes occur to the SC mechanisms regulating sexual responses following injury and facilitate more accurate and complete assessments of SC function post-trauma, thus facilitating rehabilitation of sexual responses in individuals with SCT.

1.1 Human sexual arousal

In women, increases in heart-rate, blood pressure, rate of respiration, pupil dilation, nipple erection and muscle tension accompany sexual arousal (Levin and Riley 2007) and all reside under sympathetic control (Blumenfeld 2002). Sexual arousal can occur in response to psychogenic and/or reflexogenic cues (Forsythe and Horsewell 2006) producing genital vasocongestion and clitoral tumescence in women which is physiologically equivalent to penile
erection in men (Levin and Riley 2007). The sympathetic nervous system (SNS) plays a significant role in psychogenic sexual arousal at the level of the TL pathway (T11-L2) in women (Forsythe and Horsewell 2006) and studies of healthy women have shown that pharmacologically increasing sympathetic activity or inducing anxiety results in significant increases in genital vasocongestion (Sipski 2002). Parasympathetic mechanisms of sexual arousal are facilitatory in women (Giraldi et al. 2004) and parasympathetic preganglionic neurons in the SPN send projections via pelvic efferents to the vagina and clitoris (Giraldi et al. 2004). The pudendal nerves in turn, supply the perineum, urethra and clitoris with sensory innervation (Giraldi et al. 2004).

Although the thoracolumbar SC is usually considered to be anti-erectile (Giuliano, Rampine, Bernabe, and Rousseau 1995), sympathetic neurons of the DCN may be responsible for the tone of smooth muscle fibers while IML neurons mediate vascular tone of the erectile tissues (Rampin, Bernabe, and Guiliano 1997). In men, penile erection occurs in response to parasympathetic activity that causes relaxation of the corpus cavernosum and corpus spongiosum, smooth muscles of the penile arteries (Rampin et al. 1997). The dorsal nerve of the penis (DNP) transmits sensory input from the penis to the SC (Kitchell, Gilanpour, and Johnson 1982). In the SC, the DNP projects to the dorsal horn (DH), SPN and DGC of the lumbosacral SC of male rats (Rampin et al. 1997). Penile erection can be induced by a variety of stimuli under different circumstances. Erections can be psychogenic – elicited by erotic pictures, movies, fantasies, pleasant odors or non-genital touch. Erections can also be reflexive such as those elicited by masturbation (Rampin et al. 1997). However, in healthy humans with an intact SC, the divide between psychogenic and reflexogenic erections is less clear because one system necessarily feeds into the other for normal sexual responses to occur. Furthermore, it is likely that many people would implement psychogenic components such as fantasy during masturbation. The SPN in the sacral SC is the chief spinal nucleus mediating erectile responses (Rampin et al. 1997).
Finally, supraspinal structures in the brainstem and cortex modulate reflexive penile erection (Rampin et al. 1997).

1.2 Male orgasm

Male ejaculation is comprised of two separate events. The first, emission, is controlled by the thoracolumbar (T11-L2) pathway while expulsion is mediated by the sacral (S2-S4) pathway (Giuliano and Clement 2005). It is clear that the thoracolumbar (TL) and sacral pathways are necessary for orgasm in humans. Ejaculation in men is accompanied by pleasurable orgasmic sensations (Giuliano and Clement 2005). Indeed, ejaculation is tightly coupled with reward in male rats (Pfaus, Kippin, and Centeno 2001). Recently, Truitt & Coolen isolated the region in the spinal cord of male rats that controls ejaculatory responses. The spinal ejaculation generator (Marberger 1974), located in the lower lumbar spinal cord in rats (L3-L4), controls the two components of ejaculation, emission and expulsion, by integrating sympathetic, parasympathetic and motor outputs (Coolen, Allard, Truitt and McKenna 2004). The spinal ejaculation generator is under the control of various supraspinal regions of the brainstem and hypothalamus in rodents (Coolen et al. 2004). Exactly how the sensory perception of orgasm occurs is currently not understood, but it is proposed that following ejaculation, sensory information travels to the brain and gives rise to the pleasurable feelings we call orgasm (Coolen et al. 2004).

Essentially, ejaculation is a reflex response. This has been substantiated by the fact that men with complete spinal cord injury above the entry of all the known genitospinal nerves are able to ejaculate through vibrostimulation of the penis (Sonksen and Ohl 2002). Similarly, animals retain the ability to ejaculate following complete lesion of the spinal cord (McKenna, Chung, and McVary 1991). Taken together, these data support the existence of the spinal ejaculation generator in the lumbar spinal cord of animals and humans since in the former as in the latter ejaculation persists in spite of complete transection of the spinal cord above the level of entry of all the known genitospinal nerves.
1.3 Female orgasm

Sipski and colleagues postulate that orgasm in women is the function of a sacral reflex arc that is facilitated or inhibited by higher brain regions and is associated with pleasurable sensations (Sipski, Alexander, and Rosen 1995). Indeed, Burns and colleagues have shown that women with complete SCT above T9 are able to experience orgasms comparable in sensation, level of heart rate and blood pressure to those of healthy controls (Burns, Rivas, and Ditunno 2001), suggesting that the integrity of the sacral reflex arc underlies orgasmic potential. In addition, women with complete LMN trauma at S2-S5 are substantially less likely to experience orgasm (Sipski 2001).

The controversy between vaginally and clitorally-derived orgasms can be resolved based on the findings from a recent study. Foldes & Buisson used sonography to visualize the movements of the clitoris and its anatomical relationship with the anterior wall of the vagina (the alleged location of the controversial G-spot) during voluntary perineal contraction and vaginal penetration without sexual stimulation (Foldes and Buisson 2009). After detailed examination and dynamic analysis of the movement of the clitoris during vaginal penetration in 5 healthy, 34 year old women, the authors concluded that the pleasurable region known as the “G-spot” may be a product of the contact between the internal clitoris and the anterior vaginal wall. This contact is enhanced during erotic stimulation by reflex contractions of the bulbocavernosus and ischiocavernosus striated muscles of the pelvis and increased blood flow to the region (Foldes and Buisson 2009). Hence, the distinct sensitivity attributed to the G-spot may in fact be the result of friction between the densely innervated root of the clitoris and the anterior vaginal wall (Wimpissinger et al. 2007).

Reports of female ejaculation at orgasm have prompted investigations of the female prostate. Formerly known as Skene’s paraurethral glands (Skene 1880), this mysterious and highly variable organ has been implicated in the forceful expulsion of prostate-like fluid from the
female urethra at the moment of orgasm (Wimpissinger et al. 2007). Indeed, women report stronger and more pleasurable orgasms that are accompanied by ejaculation than those that are not (Davidson, Darling, and Conway-Welch 1989). MRI of the female prostate in seven women confirmed the suspicion that this organ is highly variable from one woman to another (Wimpissinger, Tscherney, and Stackl 2009) which can explain why some women experience ejaculation at the time of orgasm while others do not.

1.4 Spinal Cord Control of Sexual Responses

Sexual responses are coordinated by neural circuits in the spinal cord (McKenna 2001). Interneurons underlie this neural circuitry, effectively establishing connections between sympathetic, parasympathetic and somatic divisions of the nervous system that control sexual responses (McKenna 2001) (Figure 1). Sexual responses can be of two types: psychogenic and reflexogenic. Psychogenic sexual responses occur as a result of descending modulation from higher brain regions to the spinal cord interneurons. Reflexogenic sexual responses, on the other hand, result from sensory stimuli activating spinal interneurons (McKenna, 2001). The reproduction system in males and females is innervated by two neural pathways (Levin and Riley, 2007; Weiss 1972). The first pathway synapses in the spinal cord at the thoracolumbar level while the second pathway enters the spinal cord at the sacral level (Courtois, Macdougall, and Sachs 1993). Sexual responses that occur in response to fantasy, visual, auditory and olfactory cues activate the thoracolumbar (TL) pathway (Weiss 1972; McKenna 2001).
Figure 1 Autonomic Nervous System

Visceral afferents conveying sympathetic and parasympathetic input from smooth muscle, cardiac muscle and glands to the hypothalamus first synapse in the dorsal horn of the SC before ascending in the lateral column to the hypothalamus. Descending information from the hypothalamus is conveyed to the lateral horn of the SC. Autonomic motor neurons in the lateral horn of the SC synapse with the autonomic ganglion (AG) which in turn provides autonomic innervation to the peripheral organs.

The TL originates in the thoracolumbar SC segments T11-L2 (Courtois et al. 1993). Preganglionic sympathetic neurons at T11-L2 synapse with postganglionic neurons of the hypogastric nerves (Weiss et al. 1972). The sacral pathway, on the other hand, ascends from the genital organs (in response to touch, pressure, and vibration) via the dorsal nerve of the penis and
clitoris and enters the spinal cord at the 2nd sacral to the 5th sacral segments as pelvic nerve afferents (McKenna and Marson 1997; Courtois et al. 1993). At this level, the pelvic nerve afferents synapse with the preganglionic parasympathetic fibers. Pelvic and pudendal nerve efferents produce genital vasocongestion in women (Levin and Riley 2007) and promote vasodilation and penile rigidity in men (Lavoisier, Proulx, Courtois, and Durand 1988). Courtois et al found that spinal transection of the sacral pathway eliminated penile responses to genital stimulation (Courtois et al. 1993). However, when stimulation was applied centrally to the medial preoptic area (MPOA) in the animals with lesions to the sacral pathway, 85% demonstrated penile responses (Courtois et al. 1993). These data supports the role of the TL pathway in mediating erectile responses in males. Therefore, erectile function is mediated by the combination of sympathetic and parasympathetic mechanisms (Giuliano and Rampin 2004).

The intermediolateral cell column (IML) and the dorsal commissural nucleus (DCN) of the thoracolumbar spinal cord (T13-L2) in rats (Hancock and Peveto 1979; Nadelhaft and McKenna 1987) and T11-L3 in humans, contain sympathetic preganglionic cell bodies that innervate the visceral organs involved in ejaculation (Coolen et al. 2004). This innervation occurs primarily via the hypogastric nerves (Nadelhaft and McKenna 1987). The sacral parasympathetic nucleus (SPN) is found in the intermediolateral column of the sacral spinal cord (L6-S2) in rats and S2-S5 in humans, contains preganglionic parasympathetic cell bodies innervating visceral organs via pelvic nerve efferents (McKenna and Marson 1997).

The dorsal gray commissure (DGC) of the L6-S1 SC segments in male rats (Rampin et al. 1997) and S2-S5 SC segments in humans (Adel and Ronald 1998) integrates afferent information from the pelvic somatic organs and visceral structures conveyed by the pudendal and pelvic genitospinal nerves (McKenna and Marson 1997). Extensive peptidergic connections exist between the SPN and DGC suggesting strong interconnection between these sacral SC nuclei (Rampin, Bernabe, and Giuliano 1997). There is a direct projection from the rat DGC to
Barrington’s nucleus in the pons (Ding et al. 1997). In addition, DGC neurons express numerous γ-Aminobutyric acid (GABA) and glycine receptors that underlie inhibitory synaptic transmission in the SC (Xu 1999) and modulation of autonomic function (Bereiter and Gann 1989).

Motoneurons in Onuf’s nucleus, located at the S2-S4 spinal segments in humans, mediate penile tumescence and control the forceful expulsion of semen from the male urethra at ejaculation (Forger and Breedlove 1986). Pudendal efferents leave Onuf’s nucleus to innervate striated perineal muscles in males and females. However, the magnitude of innervation and the size of the muscles are significantly greater in males than in females. Indeed, Onuf’s nucleus is known to be a sexually-dimorphic spinal nucleus in rats, dogs and humans (Forger and Breedlove 1986; McKenna and Marson 1997) and the influence of early androgen has been implicated as one of the factors contributing to this sexual dimorphism (Forger and Breedlove 1986). While the functional significance of the ON in males is clear, its role in mediating sexual responses in females is unknown (Levin and Riley 2007) (Figure 2).

**Figure 2 Spinal Cord Regions Mediating Sexual Function**

Proposed regions of the spinal cord that control sexual responses in men and women, based on work in rats. Cross sections of the thoracolumbar and sacral spinal cord are shown in radiological orientation. Lines indicate pathways while black circles represent the location of spinal nuclei. Figure was created by Natalie Kozyrev and Oleg Portnoy. Adapted from McKenna, K.E. (2001). Neural circuitry involved in sexual function. The journal of spinal cord medicine, 24(3), 148:154.
1.5 Brainstem Control of Sexual Responses

Brainstem regions provide descending modulation of spinal cord reflexes (McKenna 2000). Afferent information from the clitoris and visceral structures is transmitted to the brainstem by the spinothalamic and spinoreticular pathways (McKenna 2000) (Figure 3).

Figure 3 Spinothalamic Pathway Conveys Sexual Information

Sexual information enters the spinal cord in the dorsal horn and crosses the midline before ascending in the anterolateral spinal column to various thalamic nuclei. Thalamic neurons project to the somatosensory cortex of the postcentral gyrus, insula and other cortical regions. Spinothalamic fibers also synapse in the reticular formation of the caudal medulla.
Fast myelinated fibers travel in the anterolateral columns of the spinothalamic pathway, terminating in the posterolateral nucleus of the thalamus (Campbell 1976). From here, fibers are conveyed to the medial thalamus (Campbell 1976). Spinoreticular fibers are slower than spinothalamic fibers and ascend in the contralateral lateral spinal columns subsequently terminating in the reticular formation of the brainstem (McKenna 2000). In addition, mechanosensation from the genital organs is conveyed to the somatosensory cortex via the dorsal column-medial lemniscus pathway (Nolte 2009). Komisaruk et al also proposed a role for the nucleus of the solitary tract in the medulla oblongata in mediating orgasmic responses in women with complete SCT above the level of entry of the known genitospinal nerves, via the Vagus nerves (Komisaruk et al. 2004).

Genital afferents from the penis and clitoris are conveyed to the nucleus paragigantocellularis (NPGi) which in turn project to pelvic efferents and interneurons in the lumbosacral spinal cord (McKenna and Marson 1997). Lesioning NPGi abolishes tonic inhibition of orgasmic-like responses in rats (Marson and McKenna 1990). The NPGi contains serotonergic neurons that are inhibitory to spinal sexual reflexes (Marson and McKenna 1992). Apart from its role in mediation of sexual responses, the NPGi is involved in the cardiovascular (Brown and Guyenet 1984) and pain modulation (Azami, Wright and Roberts). The NPGi also projects to the locus ceruleus (LC) (Ston-Jones, Ennis, Pieribone, Nickell, and Shipley 1986) but the functional significance of this projection for sexual responses is unknown. The LC contains norepinephrine fibers that project to the pudendal motoneurons in the lumbosacral SC and may control orgasmic responses (Dean and Lue 2005; Schroder and Skagerberg 1985).

The raphe nuclei in the pons represent another brainstem site that has serotonergic projections to the lumbosacral spinal cord (Holstege, Kuypers and Boer 1979). Neurons in Barrington’s nucleus in the parabrachial pons also project to the lumbosacral SC (Holstege et al.
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1979). Barrington’s nucleus is known to mediate micturition (Kuru 1965), defecation and parturition (Fukuda and Fukai 1986; Fukuda and Fukai 1988) but its role in mediation of sexual responses is currently unclear (McKenna 2000).

The periaqueductal gray (PAG) of the midbrain is an important mediator of sexual responses in humans and animals (McKenna 2000). Indeed, neurons of the PAG are labeled during sexual behavior (Rose 1990) and after viral injections into the uterus and clitoris (Marson 1995). Experiments on ejaculatory behavior in rats utilizing the urethrogenital reflex (Marson, and McKenna 1992) have shown that the PAG is integral to triggering expulsion during ejaculation in rats (Marson 2004). Immunocytochemical techniques have revealed extensive populations of androgen and estrogen receptors in the PAG, mostly localized to its caudal two-thirds (Murphy, Shupnik, and Hoffman 1999). The PAG also has numerous synaptic connections with the NPGI, raphe nuclei, Barrington’s nucleus, LC and parts of the hypothalamus that modulate sexual behavior (McKenna 2000). In fact, recent anatomical studies have shown that the PAG acts as a relay centre between the NPGI and the medial preoptic area (MPOA) of the hypothalamus (Coolen, Peters, and Veening 1998).

Not surprisingly, Holstege and colleagues have found the strongest activation during ejaculation in humans in the ventral tegmental zone (VTA), a region involved in reward, and the subparafascicular nucleus (SPFp) (Holstege et al. 2003) which has previously been shown to be important for ejaculation in rats (Coolen et al. 2004). In the ventral midbrain, the substantia nigra (STN) mediates the rate of copulation and the frequency of ejaculation in rats (Salamone 1992) such that bilateral lesions of the STN result in a decline of these functions.

1.6 The hypothalamus

The hypothalamus plays a pivotal role in sexual behavior and reproduction (McKenna 2000; McKenna 2001). Although the medial preoptic area (MPOA) of the hypothalamus facilitates male sexual behavior in rodents, reptiles, birds and amphibians (Hull, Muschamp, and
Sato 2004), neuroimaging studies have failed to find activation of the MPOA during sexual responses in men suggesting that the MPOA may not be involved in human sexual function. In male rodents, the MPOA promotes mate selection and does not directly control sexual performance or motivation (McKenna 2001; McKenna 2000). Sexual dimorphism of the MPOA may be related to differences in the organizational and activational effects of gonadal steroids between males and females (Commins, D., & Yah, P. 1984). The paraventricular nucleus (PVN) of the hypothalamus, which has direct connections to the MPOA and NPGi (Bancila et al. 1999), secretes oxytocin, a neurohormone involved in pair-bonding, lactation, parturition and parental behavior (Nelson 2005), from the posterior pituitary into the blood in males and females during sexual arousal and orgasm (McKenna 2000; McKenna 2001). Neurons of the PVN project to motoneurons (McKenna and Nadelhaft 1986) and parasympathetic preganglionic neurons (Saper, Loewy, Swanson and Cowan 1976) in the lumbosacral spinal cord in rats.

1.7 The Thalamus

Classically, the thalamus, comprised of relay nuclei, has been considered to be a gateway for the transmission of sexual information from the SC to the brain (Temel, Visser-Vandewall, Ackermans, and Beuls 2004). However, there is substantial evidence for the thalamus as an integrator and mediator of penile erection (Temel et al. 2004) and ejaculation (Coolen, Veening, Wells, and Shipley 2003). There are two specific nuclei in the thalamus that appear to facilitate sexual responses. The first is the medial dorsal (MD) nucleus, which when electrically stimulated in squirrel monkeys, elicits erectile responses (Maclean, Denniston, and Dua 1963). The second is the SPFp thalamic nucleus. Neurons of the SPFp are labeled with C-fos protein, an immediate-early gene expressed at neuronal activation (Temel, Helmy, Pinnock, and Herbert 2003), following ejaculation (Coolen, Peters, and Veening 1997; Coolen et al. 2003). It is likely that sexual sensations from the genital organs are conveyed via the anterolateral spinothalamic pathway to its relay nucleus, the SPF, and from there to regions of the brain (Guyton and Hall
1996). Taken together, this information suggests that the MD and SPFp nuclei of the thalamus are not simply relay nuclei but centers that integrate and process incoming sexual signals from the SC.

1.8 Cerebral Cortex

Anatomical retrograde and anterograde tracing studies have revealed that lumbar spinothalamic neurons (LSt) cells in the lumbar spinal cord send projections to the SPF which in turn projects to the MPOA, bed nucleus of the stria terminalis (BNST), and medial amygdaloid nucleus (MEA) in the forebrain (Coolen et al. 2003). This highlights the importance of the SPF in conveying signals specifically related to ejaculation from the lumbar SC to the brain. In terms of male ejaculation, an entire network of brain regions has been recently revealed. Fos protein expression has identified activation of the BNST, the posterodorsal MEA and the SPFp to be distinctively associated with ejaculation (Coolen et al. 1998; Hamson and Watson 2004).

The striatum, comprised of the globus pallidus, putamen and nucleus accumbens (Nolte 2009), is crucial in mediating motivation and reward (Spinella 2007). In particular, the core region of the nucleus accumbens underlies reward (Kelley 1999) and copulation in the rat results in increased C-fos activation in this region (Robertson et al. 1991). Indeed, Dopamine, an excitatory neurotransmitter mediating reward, is abundant in the core region of the nucleus accumbens (Nelson 2005; Nolte 2009) and lesions of the nucleus accumbens result in anhedonia and a loss of interest in sexual activity in men (Goldenberg, Schuri, Gromminger and Arnold 1999) and female rodents (Rivas and Mir 1990; Rivas and Mir 1991).

The prefrontal systems play a vital role in mediating human sexual responses (Spinella, 2007). Through their interconnections with the cortex, striatum and thalamus, the prefrontal systems regulate human sexual behavior (Spinella 2007). Indeed, lesions of the anterior cingulate gyrus, found in the medial prefrontal cortex, decrease mounting, intromissions and ejaculation in male rats (Yamanouchi and Arai 1992; Agmo and Villalpando 1995). The anterior cingulate
seems to regulate initiation of sexual behavior as opposed to its maintenance (Agmo and Villapando 1995). In addition, several neuroimaging studies revealed increased cerebral blood flow in the anterior cingulate in males and females during sexual arousal (Redoute et al. 2000; Arnow et al. 2002; Karama et al. 2002).

The orbitofrontal cortex, located in the ventromedial part of the prefrontal cortex (Spinella 2007), is also important in reward (Spinella 2007). The orbitofrontal cortex is activated in response to pleasurable touch (Francis et al. 1999) and eating chocolate (Small, Zatorre, Dagher, Evans, and Jones-Gotman 2001) and deactivated at aversion that results from continuous consumption of chocolate (Small, Zatorre, Dagher, Evans, and Jones-Gotman 2001). Moreover, humans with orbitofrontal cortex lesions exhibit inappropriate sexual behavior such as masturbation in public (Miller, Cummings, McIntyre, Ebers, and Grode 1986). The dorsolateral prefrontal cortex is involved in generation of erotic fantasy (Spinella 2007) and individuals with lesions to this region have difficult conjuring erotic imagery (Crowe and Ponsford 1999). Finally, increases in cerebral blood flow are greatest in the right prefrontal cortex, compared to the left prefrontal cortex or other cortical regions, during human sexual arousal and orgasm in men (Tiihonen et al. 1994).

1.9 Altered Sexual Function after Spinal Cord Trauma

Spinal cord trauma (SCT) frequently results in devastating changes to sexual function in men and women (Sipski and Arenas 2007; Sipski, Alexander, and Rosen 2006). There are two types of injuries to the spinal cord that have severe implications for sexual responses in humans. The first type is upper motor neuron (UMN) trauma that transects the primary efferent neuron which normally descends from the brain to synapse with neurons found in the sacral spinal cord (Sipski et al. 2007) and corresponds to injuries above the 11th thoracic (T11) spinal segment. The second type is termed lower motor neuron (LMN) trauma which transects the cell body of the neuron in the sacral spinal cord (Sipski et al. 2007) and corresponds to injuries below T11. Sexual
arousal in men and women can be classified as either mentally-evoked (psychogenic) or physically-evoked (reflexogenic). Bors and Comarr observed that 93% of men with complete UMN trauma are able to have reflexogenic but not psychogenic erections (Bors and Comarr 1960). Likewise, Courtois and colleagues found that reflexogenic stimulation works best for men with UMN injuries (Courtois et al. 1999). Similarly in women, perception of light touch and pinprick in the T11-L2 dermatomes is highly predictive of women’s capacity to experience psychogenic genital lubrication (Sipski, Alexander, and Rosen 1997) and women with no sensation in the T11-L2 dermatomes are unable to achieve psychogenic lubrication (Sipski et al. 1997).

Geiger and colleagues found that of men with complete LMN lesions encompassing the sacral spinal cord, 26% continued to have psychogenic erections while none were able to have reflexogenic erections (Geiger 1980). In line with this, Courtois et al demonstrated that in men with LMN trauma, psychogenic stimulation is most effective in producing erectile responses (Courtois et al. 1999). In those with incomplete injuries, Courtois and colleagues demonstrated that up to 80% of men with LMN trauma retain psychogenic erectile responses and 100% of men with UMN lesions retain reflexogenic erectile responses (Courtois et al. 1999), suggesting that erectile function is organized at the level of the lumbar SC by central generators (Levin and Riley 2007). Similarly, genital stimulation is effective in producing vaginal lubrication in all women except those with complete spinal cord trauma at the level of the sacral reflex pathway (S2-S4). Indicating that reflex lubrication in women is mediated by a sacral reflex arc (Sipski 2001).

It is known that up to 80% of men with lower motor neuron (UMN) lesions can experience psychogenic erection while almost 100% of men with upper motor neuron (LMN) lesions can experience reflexogenic erections (Courtois, Goulet, Charvier, and Leriche 1999). In a recent study of 81 men with SC lesions ranging from C2 to S5 and complete as well as incomplete SCT, as assessed by the American Spinal Injury Association (ASIA) exam, up to 20%
of men with upper motor neuron (UMN) lesions spanning from C2 to T2 achieved ejaculation during combined masturbation and erotic film (natural stimulation). Up to 60% of men with lesions between T11 and L2 and up to 90% of men with lesions below L3 experienced ejaculation through ‘natural’ stimulation (Courtois et al. 2008). On the other hand, men with lesions between T7 and T10 experienced ejaculation only through penile vibratory stimulation or administration of medodrine, a drug that targets the sympathetic nervous system and lowers the threshold for emission, thereby facilitating ejaculation (Courtois et al. 2008). Although the success rate for ejaculation is quite high (95-100%) (Biering-Sorensen and Sonksen 2001), the percentage of men that reach orgasm is between 41-65% (Sipski et al. 2006; Anderson, Borisoff, Johnson, Stiens, and Elliott 2007; Dahlberg, Alaranta, Krautainen, and Kotia 2007) indicating that ejaculation and orgasm are distinct events that may not always coincide, especially after SCT. This finding supports the effectiveness of penile vibratory stimulation in eliciting ejaculation in men with intact thoracolumbar (T11-L2) and sacral (S2-S5) SC segments.

In women, up to 25% with LMN lesions can experience psychogenic lubrication but none report reflexogenic lubrication highlighting the importance of the sacral SC in reflex lubrication in women (Sipski et al. 2001). Laboratory research in 25 women revealed that 52% of women with complete UMN trauma at or above T6 reached orgasm by genital self-stimulation while women with LMN trauma including the sacral segments (S2-S5) were significantly less likely to reach orgasm (Sipski 2001).

1.10 MRI Basics

The hydrogen nucleus, or proton, which is abundant in the human body, underlies the concept of magnetic resonance imaging (MRI). The spin of the proton generates a magnetic field. The strength and direction of the magnetic field is known as the magnetic moment. The proton’s spin also generates angular momentum. Every MRI scanner emits a static magnetic field expressed in units of Tesla. While the static magnetic field of the earth is only 0.00005 Tesla,
typical MRI scanners are between 1.5 and 3 Tesla with some scanners boasting field strengths as high as 9 Tesla. In the absence of a strong magnetic field generated by an MRI scanner, the spins are randomly oriented. However, in the presence of a strong magnetic field, the spins align their axes parallel or antiparallel to the magnetic field of the MRI scanner and engage in precession in the direction of this magnetic field, akin to a spinning top (Huettel, Song, and McCarthy 2004). In a stronger magnetic field, more energy is required to switch from a parallel (low-energy) to an antiparallel (high-energy) state. Therefore, as the strength of the magnetic field increases, so does the number of spins in the orientation parallel to the magnetic field. Because the parallel orientation of the spins is more stable and requires less energy, more spins will always assume the parallel than the antiparallel state.

MR techniques measure the total magnetization of all the spins in a particular volume. The total or net magnetization is comprised of a longitudinal and a transverse component. The transverse component of the spins is cancelled out due to the large number of spins in a given volume and the strength of the net magnetization would depend on the number of spins in the parallel orientation. The stronger the external magnetic field applied, the more spins will assume the parallel orientation and the stronger the signal that can be derived. This is why it is advantageous to use MRI scanners with strong magnetic fields of 3 Tesla or even higher (Huettel et al. 2004).

In order to measure the net magnetization, or signal of the spins in an external magnetic field, the spins must first be ‘excited’. Excitation of spins is achieved by radiofrequency (RF) coils in MR scanners that generate another magnetic field for the spins to precess around. This allows us to rotate the spins into new orientations. Magnetic field gradients are applied in order to alter the precessional frequency of the spins as a function of their position. The Larmor frequency is the frequency of the spins’ precession within an external magnetic field. The MR signal is derived from the net transverse magnetization of all the voxels in the excited sample. In order to
excite spins in a single slice, the radiofrequency (RF) pulse must correspond to only the frequencies of the spins in that slice. Because the frequency of the RF pulse is specific to that of the spins in the slice, all the other spins from other slices are effectively ignored. The RF pulse affects only the spins in specific positions with a particular range of frequencies that correspond to it. In a typical MRI experiment, the area being imaged (e.g. the brain) is placed in the centre of the scanner where the external magnetic field is uniformly strong. When we introduce an excitation pulse to the uniform magnetic field, this perturbs the spins away from equilibrium. Subsequently, in the period of time when the spins are perturbed, MR signal can be measured. However, the MR signal decays over time through a process known as relaxation (Huettel et al. 2004).

There are two types of relaxation, longitudinal and transverse relaxation. Following the excitation pulse, the spins begin to lose energy that was absorbed during the excitation. Longitudinal relaxation, T1, refers to the time it takes for excited spins that are antiparallel to the magnetic field to switch to the lower energy (parallel state), thereby losing energy to their surroundings. Hence T1 is the recovery of the longitudinal component of the relaxation over time. Transverse relaxation, T2, occurs because of accumulated phase differences and spin-spin interactions. Spin-spin interactions refers to the phenomenon that when the spins are excited at the same time, their individual effects on each other produce instability, with some spins precessing at a faster and some at a slower rate. In addition, there are external influences on signal decay due to transverse relaxation referred to as T2*. T2* is the time constant that describes the signal decays in the transverse component due to the combined influences of spin-spin interactions and external field inhomogeneities. External field inhomogeneities are caused by variations in MR signal strength from one location to another in a given sample. Therefore, the spins precess at different frequencies in different spatial locations resulting in the loss of
coherence of the MR signal. Among the types of relaxation, T1 is the longest time constant, followed by T2 and finally T2* (Huettel et al. 2004).

Two important elements regulate the time of MR image acquisition. The first element, repetition time (TR) refers to the time interval from one excitation pulse to the next. The second element, echo time (TE) refers to the span of time between excitation and data acquisition. Furthermore, there are two primary types of pulse sequences used in MRI. The first type is gradient-echo (GRE) imaging that uses spatial gradients to generate the MR signal changes measured during data acquisition. Spatial gradients are applied to change the strength of the magnetic field over space. The second type is spin-echo (SE) imaging that uses a second 180 degree electromagnetic pulse, or refocusing pulse, to generate the MR signal changes that can be measured during data acquisition (Huettel et al. 2004).

In order to obtain spatial information from the MR signal, an RF pulse is applied to flip the net magnetization away from equilibrium (parallel orientation). When the RF pulse is turned off, the transverse magnetization begins to decay according to time T2*, T2 or T1. The decay of the transverse magnetization provides contrast between different tissues that have different rates of decay (e.g. CSF versus white matter). As the transverse magnetization precesses around the Larmour frequency, it generates a time-varying magnetic field. The transverse magnetization is measured by detector coils positioned around the anatomical region of interest (e.g. SC). Raw MR signal data is acquired in K-Space, a notational scheme that is used to describe MR data. An excitation pulse is applied to select a single slice within the total imaging volume. A linear magnetic field gradient is applied in the x direction in order to determine the spatial location of magnetic moments. This phenomenon is termed frequency encoding because we are obtaining information about the amount of signal present at each frequency of the MR signal. Phase encoding is achieved by applying another gradient in the y direction that serves to alter the phase of the MR signal relative to its position. Finally, a two-dimensional inverse Fourier transform is
applied to reconstruct the raw frequency data acquired in K-space into an image. While the centre of K-space determines image contrast, the edges of K-space are responsible for image resolution. Data is sampled in K-space as spatial frequencies and increased sampling frequency yields a larger image ((Huettel et al. 2004).

1.11 MR Contrasts

1.11.1 Proton Density Contrast

Proton density contrast in MR is dependent on the concentration of protons in voxels. In order to maximize proton density contrast, one must utilize pulse sequences that minimize T1 and T2 contrasts. A long TR helps to minimize T1 contrast while a short TE effectively minimizes T2 contrast. Thus, proton density pulse sequences generally have a long TR and a short TE. The long TR ensures that tissues are almost completely recovered after excitation while a short TE decreases decay of the MR signal prior to image acquisition. In general, the TR of proton density weighted pulse sequences should be bigger than the T1 values while TE should be smaller than the T2 values of the tissues being imaged. Proton density weighted images are lightest in regions with the greatest concentration of protons, such as CSF and darkest in regions with the least concentration of protons such as air spaces (Huettel et al. 2004).

1.11.2 T1 Contrast

T1 contrast in MR is commonly utilized for anatomical imaging. T1 contrast, as its name implies, is dependent on the T1 values of the tissues being imaged. Pulse sequences that utilize a T1 contrast have an intermediate TR. T1 values for grey matter and white matter are quite similar. Hence, a long TR would result in similar levels of recovery of longitudinal magnetization for both tissues while a short TR would not allow adequate recovery of longitudinal magnetization and thus no MR signal would be recorded. Conversely, an intermediate TR permits recovery of longitudinal magnetization for both tissues, but because the T1 values for grey matter are greater than for white matter, grey matter would appear darker in a T1-weighted image than white matter.
resulting in good contrast between the two types of tissues. CSF, which has a much greater T1 value compared to white matter and grey matter, would be darkest in T1 weighted images because it would take longest for the longitudinal magnetization to recover (Huettel et al. 2004).

1.11.3 T2 Contrast

MR pulse sequences utilizing T2 contrast utilize an intermediate TE in order to take advantage of transverse magnetization to obtain signal intensity changes. A very long TE would result in almost a complete loss of signal while a very short TE would result in not enough signal decay. In both cases, there will be no T2 contrast because with a long TE all transverse magnetization decay and with a short TE, not enough transverse magnetization will decay. In addition, T2 contrast requires a long TR for longitudinal magnetization to recover completely and effectively minimize T1 contrast. T2 contrast is brightest in fluid filled regions such as the ventricles and darkest in regions with the least fluid such as bone and lipid. Only spin-echo methods can be used to create T2-weighted images because spin-echo unlike gradient echo methods, allow spin-spin relaxation and are not dependent on field inhomogeneities (Huettel et al. 2004).

1.11.4 T2* Contrast

T2* contrast in MR is dependent on spin-spin interactions and magnetic field inhomogeneities of tissues. Blood oxygenation level dependent (BOLD) contrast for functional magnetic resonance imaging (fMRI) utilizes T2* contrast as it is sensitive to the concentration of deoxygenated hemoglobin present in the blood. Gradient echo sequences must be used to generate T2*-weighted images because spin-echo sequences diminish the effects of field inhomogeneities by introducing refocusing pulses. Similarly to proton-density-weighted images, T2* weighted images require a long TR in order to minimize T1 weighting. However, unlike proton-density-weighted images, T2* weighted images necessitate an intermediate TE in order to
ensure sensitivity to magnetic field inhomogeneities which is important for BOLD contrast (Huettel et al. 2004).

1.12 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) is a noninvasive neuroimaging technique that utilizes a strong magnetic field to elucidate the function of the human nervous system by acquiring time-series of images while the subject engages in a particular task. In general, periods of stimulation or ‘task’ are interleaved with periods of baseline. The observed fMRI signal intensity changes, related to changes in neuronal activity, represent the difference between the stimulation and baseline conditions. Thus, the design of the baseline condition should take into account all the variables present in the stimulation condition, except for the variable of interest being studied. For example, if we are interested in investigating human responses to anxiety-provoking visual stimuli, the baseline condition should comprise neutral visual stimuli such that the variable of viewing visual stimuli is constant across the stimulation and baseline conditions. In addition, it is important to repeat periods of stimulation and baseline several times to improve reliability in detecting signal intensity changes that arise in response to changes in neuronal activity. Spin-echo and gradient-echo are the two main pulse sequences used for fMRI. Spin-echo imaging introduces a second 180 degree electromagnetic pulse, also known as a refocusing pulse, in order to reverse the loss of phase coherence that occurs after excitation and is used to generate T2 and proton-density weighted images. On the other hand, gradient-echo pulse sequences introduce spatial gradients which in turn produce the MR signal changes. Gradient-echo pulse sequences are useful to obtain T2* weighted images because they are sensitive to inhomogeneities in the magnetic field (Huettel et al. 2004).

FMRI experimental design is crucial to obtaining reliable signal intensity changes that reflect changes in neuronal activity in response to a particular stimulus or task. Signal intensity changes obtained in fMRI substantially lag the timing of neuronal activity. Therefore, we are
actually measuring the correlation between fMRI signal intensity changes and neuronal activity. When designing an fMRI experiment, it is important to consider the temporal onset of fMRI signal intensity signal changes. Ideally, each stimulation condition should be longer than the time it takes for signal intensity changes to occur, so that it can be reliably detected. Another factor is the baseline condition which inevitably includes the effects of random noise, blood and CSF flow, cardiac motion and perhaps some bulk motion. The main goal of an fMRI study design is to reveal the psychological or physiological aspect being investigated while minimizing confounds including but not limited to random noise, blood and CSF flow and cardiac motion.

There are two types of fMRI study design. The first, event-related fMRI study design, demonstrates signal intensity changes in response to each application of the stimulus. Conversely, the block design demonstrates the average signal intensity changes in response to a repeated stimulus. Typically, the signal intensity changes obtained using the block design, are higher than those obtained using the event-related study design. The decision to use a block design versus an event-related design is determined by what is being investigated. One must keep in mind that the BOLD signal intensity changes peak 4-6 seconds after stimulus onset and take approximately 10-12 seconds after stimulation to return to baseline (Huettel et al. 2004).

While MRI is useful in obtaining structural images of the brain and spinal cord, functional images require the use of functional magnetic resonance imaging techniques. Early attempts to map brain function have begun with positron emission tomography (PET), a neuroimaging technique that involves the injection of radioactive tracers in order to detect changes in the brain associated with blood flow and glucose metabolism during a cognitive or motor task. However, there are several important disadvantages to using PET for functional neuroimaging. First, PET is a rather invasive technique as it involves injections of radioactive tracers. Second, PET is quite slow (60 – 120 seconds) at image acquisition while some functional MR imaging sequences such as echo-planar imaging (EPI) can acquire an entire image following...
one excitation pulse (approximately 20 slices per second) (132). Fast acquisition of CNS function is advantageous because it more closely matches the rate of physiological processes as they occur. Thirdly, images acquired with PET have much lower signal to noise ratio and a poorer spatial resolution (4-5 mm) (Cherry and Phelps 2002), compared to other techniques such as fMRI (1-2 mm) (Logothetis 2008), making it difficult to localize to specific neuronal regions.

Other neuroimaging techniques such as electroencephalography (EEG) and magnetoencephalography (MEG) can be used to measure the electrical and magnetic potential of the brain respectively, by attaching electrodes to the surface of the scalp. However, these techniques have poor spatial resolution (25 mm and 5 mm for EEG and MEG respectively) (Lounasmaa, Hamalainen, Hari, and Salmelin 1996; Dale and Halgren 2001) and therefore are impractical when attempting to localize a given pattern of activity to a specific brain region. In order to do this, the electrodes would have to be inserted directly into the brain and the activity of neuronal networks of different brain regions can thus be recorded. While such single-unit recordings allow for precise localization of function, they are quite invasive and therefore rarely used in humans (Huettel et al. 2004).

**1.13 FMRI contrast mechanisms**

**1.13.1 Blood Oxygenation Level-dependent (BOLD) Contrast**

The field of functional neuroimaging is still quite new. In fact, the first MRI studies to map human brain function are less than two decades old (Ogawa et al. 1992; Bandettini, Wong, Hinks, Tikofsky and Hyde 1992). In order to detect neuronal function, MRI methods most commonly rely on blood oxygenation level-dependent (BOLD) contrast. The idea behind BOLD is that a change in neuronal activity is closely accompanied by a change in blood oxygenation, cerebral blood flow and volume (Ogawa et al. 1993). Pauling and Coryell were the first to demonstrate the paramagnetic properties of hemoglobin and that the level of oxygen in the blood impacts its magnetic properties (Pauling and Coryell 1936). While oxygenated hemoglobin is
diamagnetic and is weakly repulsed from a magnetic field, deoxygenated hemoglobin is paramagnetic and has a significant magnetic moment. Deoxygenated blood has magnetic susceptibility and when placed in a magnetic field, produces spin dephasing and subsequently a decay of transverse magnetization at the time constant $T_{2^*}$. Since oxygenated hemoglobin has very little influence on the surrounding magnetic field, oxygenated blood would result in a stronger MR signal than its deoxygenated counterpart. Ogawa and colleagues demonstrated precisely this idea by showing that the $T_{2^*}$ effect of blood is diminished when it is deoxygenated (Ogawa, Lee, Nayak and Glynn 1990; Huettel et al. 2004).

In another pioneering experiment, Ogawa and colleagues showed that manipulating blood oxygenation in rodents’ brains alters the visibility of $T_{2^*}$-weighted images of blood vessels. When the rodents breathed 100% oxygen, there was little noise in the resulting images. In contrast, when the rodents breathed normal air (20% oxygen), the resulting images showed significant decrease in the signal to noise ratio and deteriorated even further when oxygen content was reduced to 0%. Ogawa and colleagues concluded that the change in signal intensity in the images was the result of increased amounts of deoxyhemoglobin in the blood with decreasing levels of oxygen consumption (Ogawa et al. 1990). Since oxygen metabolism is required for neuronal activity, Ogawa and colleagues speculated that increases in neuronal activity would be accompanied by an increase in oxygen consumption and subsequently a decrease in oxygenated hemoglobin (Huettel et al. 2004).

Changes in blood flow form a second mechanism for the BOLD effect. With increased blood flow coupled to an unchanging metabolic demand, the amount of oxygenated blood would increase over time and with it, the MR signal. The resulting contrast between oxygenated and deoxygenated hemoglobin in the blood allowed researchers to indirectly measure changes in neuronal activity. At present, there is a general consensus that as neuronal activity increases, so does metabolic demand for oxygen resulting in increased oxygenated hemoglobin and therefore
increased MR signal. Although it has been shown that the BOLD signal is associated with neuronal input (Logothetis, Pauls, Augath, Trinath and Oelterman 2001), the precise relationship between glucose metabolism and blood flow is still not clear. Therefore, caution must be exercised in interpretations of experimental findings from studies utilizing the BOLD contrast for fMRI (Huettel et al. 2004).

1.13.2 Limitations of BOLD

While BOLD is the most commonly used contrast in neuroimaging studies, it presents important disadvantages. BOLD is affected by differences in magnetic susceptibilities between different tissues including lipids, bone, cartilage and air. In fact, functional MR imaging studies utilizing the BOLD contrast are particularly susceptible to signal loss and subsequent distortion in their images, especially in the temporal and frontal lobes of the brain, the brainstem and spinal cord where such tissue interfaces are abundant (Cusack et al. 2005). Thus, proton-density-weighted spin-echo imaging which is relatively insensitive to magnetic susceptibilities between tissues, is the ideal contrast to counteract the negative effects of BOLD in imaging regions of the brain, brainstem and spinal cord (Stroman, Krause, Malisza, Frankenstein and Tomanek 2002).

The majority of fMRI studies to date have been performed in the brain as opposed to the SC. These studies utilized mainly gradient-echo sequences sensitive to magnetic field inhomogeneities. While gradient-echo sequences work well in detecting the BOLD response in the brain, they contribute to poor image quality and signal loss in the SC due to differences in magnetic field susceptibilities between CSF, bone and lipids that are found in close proximity to the SC (Stroman 2005). Therefore, it is practically impossible to obtain good contrast-to-noise ratio and image quality in the SC by utilizing gradient-echo imaging parameters designed to detect the BOLD contrast.

One relatively successful BOLD fMRI study of the brainstem was performed recently by Mainero and colleagues (Mainero, Zhang, Kumar, Rosen and Sorensen 2007) who mapped
changes in the trigeminal nucleus and supraspinal brainstem nuclei following heat/capsacin-induced primary and secondary mechanical allodynia in the trigeminal system in humans. Functional MRI was performed on a 3 Tesla MR scanner. In order to eliminate the effects of pulsatile brainstem motion, fMRI acquisition was synchronized to a given phase of each participant’s cardiac cycle. Another advantage of this study is that sagittal slices, as opposed to transverse or coronal slices of the brainstem were acquired, helping to eliminate partial volume effects while maximizing the coverage of the brainstem. Furthermore, although the image quality was adequate, it could have been improved by acquiring proton-density weighted images.

Another fMRI study of the brainstem by Zambreanu and colleagues attempted to map regions of the brain and brainstem during central sensitization in humans (Zambreanu, Wise, Brooks, Iannetti and Tracey 2005). Notably, the authors only ventured as far caudally as the rostral pons, although central sensitization is known to begin in the SC. The authors state that they used specific anatomical landmarks to localize brainstem nuclei. However, with decreasing image quality, it would be hard to find such landmarks not to mention localize specific brainstem nuclei, particularly when using gradient echo-planar imaging (EPI) sequence which is quite sensitive to magnetic field inhomogeneities. Slices were acquired axially with a thickness of 6 mm increasing susceptibility to partial-volume effects when attempting to localize activity to a specific, miniscule brainstem nucleus. Partial volume effects refer to the presence of signal contributions from two or more distinct functional regions within a single voxel (Huettel et al. 2004).

Hawley and colleagues investigated the effect of sound on fMRI activation in the human auditory brainstem nuclei (Hawley, Melcher, and Fullerton 2005). Contiguous sagittal images were acquired on a 3 Tesla scanner using gradient-echo BOLD sequence. Cardiac-gating was implemented in order to reduce image artifacts due to the movement of the cervical spinal cord and brainstem with each heart-beat. However, fluctuations in the heart-rate of each subject
resulted in TR variability and subsequent variations in signal strength from one image to another, contributing to T1 effects (Hawley et al. 2005).

Komisaruk et al were one of the first to attempt to functionally localize brainstem and cervical spinal cord nuclei in humans (Komisaruk et al. 2002). Using gradient-echo echo-planar sequences to detect the BOLD effect, they acquired coronal images of the brainstem and cervical spinal cord on a 1.5 Tesla MR scanner. Although they were able to localize specific nuclei during tasks designed specifically to target these nuclei, for example the nucleus of the solitary tract was activated by tasking a sweet-sour-bitter-salty mixture, they admit that the fMRI BOLD signal does not provide an exact representation of the anatomical location of the brainstem nuclei but only approximate localization.

Govers and colleagues imaged the cervical spinal cord during finger tapping using BOLD fMRI (Govers et al. 2007). The authors acquired contiguous transverse slices from the 5th cervical spinal segment to the 2nd thoracic spinal segment. They used a single-shot interleaved EPI-sequence to acquire the T2*-weighted images. The quality of the images acquired was quite poor and it was difficult to distinguish the corresponding anatomical regions of active voxels in the data. Govers and colleagues explained the difficulties with this study to be associated with limited spatial resolution, periodic movement of the spinal cord with each heart-beat and hemodynamic washout (Govers et al. 2007).

In summary, each of the aforementioned studies demonstrates that it is difficult to obtain high-quality fMRI data of the cervical spinal cord and brainstem using gradient-echo methods sensitive to the BOLD effect. Stroman et al (2002) demonstrated that there is a non-BOLD contribution to signal intensity changes (ΔS/S) observed in the spinal cord. They measured the signal intensity changes in spinal fMRI at very short echo times and showed that the observed signal intensity changes have a value of up to 3.3 % in the SC at a TE of 11 ms. BOLD fMRI is sensitive to T2* weighted images that require an intermediate TE. Therefore, ΔS/S obtained at a
very short TE are not T2* weighted and do not represent the BOLD effect. Stroman and colleagues proposed that this signal contribution is due to local increases in proton density at sites of neuronal activity. Thereby a second contrast mechanism emerged called Signal Enhancement by Extravascular Water Protons (SEEP) contrast (Stroman et al. 2002b).

### 1.13.3 Signal Enhancement by Extravascular Water Protons (SEEP) Contrast

The SEEP mechanism is derived from the shift of water to sites of neuronal activity resulting in cell swelling accompanied by subsequent increases in proton density, resulting in an increased MR signal (Stroman et al. 2002b; Stroman, Tomanek, Krause, Frankenstein, and Malisza 2003; Stroman, Kornelsen, Lawrence, and Malisza 2005; Stroman, Lee, Pitchers, and Andrew 2008b). Active neurons release potassium ions (K+) into the extracellular space. Subsequently, astrocytes accumulate the excess K+ with water following intracellularly. The uptake of water results in astrocyte swelling and is the basis of the SEEP mechanism (Andrew and MacVicar 1994; Fujita et al. 1997; Andrew, Labron, Boehnke, Carnduff and Kirov 2007; Stroman et al. 2008b). Support for the SEEP theory is derived from studies utilizing positron emission tomography (PET) in brain imaging that have shown that increased water flow accompanies increased blood flow to regions of neural activity (Ohta et al. 1996; Fujita et al. 1997). In line with this, Buxton and Frank have shown that neuronal activation results in a 30% increase in the rate of blood flow through the capillaries resulting in the diffusion of water through the walls of the capillaries (Buxton and Frank 1997). Further support for the SEEP theory emerged from a recent study demonstrating the presence of MRI signal, associated with neuronal activity, in living rat brain slices (Stroman et al. 2008b). There was no blood present in the slices of rat brains that were imaged, signifying that the presence of blood is not required for detecting fMRI signal in living tissues. Furthermore, the signal intensity markedly and reversibly increased during neuronal depolarization associated with increased extracellular potassium (Stroman et al. 2008b). Curiously, the SEEP mechanism is about three times greater in the spinal cord as in the
brain (Stroman, Krause, Frankenstein, Malisza, and Tomanek 2001). This apparent difference can be explained by the fact that the blood-brain barrier is much more selectively permeable than the barrier between the spinal cord and the blood, permitting fewer substances (including water) to enter (Prockop, Naidu, Binard, and Ransohoff 1995).

Stroman and colleagues have compared the regions of signal intensity changes visualized with SEEP and BOLD contrasts in duplicate experiments in healthy volunteers (Stroman et al. 2005). They observed regions of SEEP activity to be immediately adjacent to areas of BOLD activity with insignificant overlap between the two regions. In addition, two distinct response functions were delineated for BOLD and SEEP respectively. Furthermore, the peak SEEP response occurs 1 second after the peak BOLD response but decays slower and lacks a post-stimulus undershoot (Stroman et al. 2005). Results from this important study provide additional support for the existence of the second contrast mechanism, SEEP, which is sensitive to local increases in proton density at sites of neuronal activity.

The SEEP response can be distinguished from the BOLD response in that it does not depend on the strength of the magnetic field (Stroman 2005). Indeed, spin-echo, proton-density-weighted protocols at a short echo time (21-23 ms) designed to detect SEEP, demonstrated consistent signal intensity changes at around 2% during sensory stimulation of the hand at 3, 1.5 and 0.2 tesla (Stroman 2005). Conversely, the BOLD effect depends on the strength of the magnetic field (Gati, Menon, Ugurbil, and Rutt 1997) and thus would be insignificant at 0.2 tesla (Stroman 2005). Another distinguishing characteristic between BOLD and SEEP is that the BOLD effect depends on the effects of T2* and requires gradient-echo protocols with a long TR, in order to minimize the effects of T1, and an intermediate TE in order to detect local field inhomogeneities. Conversely, SEEP can be detected with spin-echo protocols with a long TR, in order to minimize the effects of T1, and a short TE, in order to minimize the effects of T2. The refocusing pulse in spin-echo methods minimize sensitivity to magnetic field inhomogeneities.
and improve image quality. Thus, SEEP is detected by acquiring proton-density-weighted image data while BOLD is detected by acquiring T2*-weighted image data (Stroman 2005). Finally, while BOLD signal intensity changes are known to occur near veins, venules and capillaries, SEEP signal intensity changes occur proximal to capillaries, arteries and arterioles (Stroman et al. 2003).

1.14 Spin-echo versus gradient-echo spinal fMRI

Functional MRI of the spinal cord (spinal fMRI) is valuable for assessing functional changes associated with neuroplasticity following spinal cord injury (Kornelsen, 2007). In fact, spinal fMRI can detect neuronal function caudal to the site of injury in individuals with trauma to the spinal cord (Kornelsen and Stroman 2007). Unquestionably, the primary advantage of spinal fMRI is that it is a noninvasive method that can be used to study spinal cord function in patients and healthy individuals. Spinal fMRI utilizes contributions from BOLD (Stroman et al. 2002b) and SEEP (Stroman et al. 2005) contrasts to indirectly detect neuronal activity in response to motor (Kornelsen and Stroman 2007) and sensory (Stroman et al. 2004; Lawrence, Stroman, and Kollias 2008) stimulation in the gray matter of the spinal cord (Kornelsen and Stroman 2007).

The advent of spinal fMRI presents significant benefits for clinicians assessing spinal cord injuries and devising treatment options for patients in order to facilitate recovery of function (Kornelsen and Stroman 2007). Another advantage of spinal fMRI is that it can reveal neuronal activity in the spinal cord even if sensation in the corresponding dermatome is lacking, in contrast to conventional clinical assessments available today (Kornelsen and Stroman 2007). Furthermore, spinal fMRI can be used to compare neuronal activity maps between individuals with SCT and healthy age and sex-matched control participants in order to elucidate the changes that result following SCT. Conveniently, spinal fMRI can be performed on any standard MR scanner and
the resulting images can be displayed in three-dimensional (3D) volume (Kornelsen and Stroman 2007).

Several groups have conducted spinal fMRI studies. Li and colleagues report on the spinal effects of acupuncture stimulation assessed by proton density-weighted fMRI at 0.2 Tesla (Li, Ng, Wong, Luk, and Yang 2005). Using SEEP contrast, Li and colleagues demonstrated consistent proton-density changes in regions of motor and sensory activity in the C6/C7 segments of the 8 out of 11 volunteers studied, at sites consistent with spinal cord neuroanatomy (Li et al. 2005). This study demonstrated that SEEP spinal fMRI can reliably assess neuronal activity in the cervical spinal cord during stimulation of upper limb acupoints.

Moffitt and colleague used spin-echo spinal fMRI to image the human lumbar SC (Moffitt, Dale, Duerk, and Grill 2005). Experiments were performed on a 1.5 Tesla MR scanner with half-Fourier single shot spin-echo (HASTE) sequence as well as with its modified version – fluid-attenuated inversion recovery (FLAIR)-HASTE. Unfortunately, their efforts to visualize neuronal activity in the SC did not succeed and actually served to increase the variability and artifacts in the functional MR images. Moreover, Moffitt and colleagues did not observe consistent areas of activation in the SC with either method (Moffitt et al. 2005).

Ng and colleagues conducted proton-density-weighted spinal fMRI in the cervical spinal cord during sensorimotor stimulation at a 0.02 Tesla (Ng et al. 2006). They reported that over 70% of the participants demonstrated an average signal intensity change of 4.06% in the ventral and dorsal horns of cervical SC during sensorimotor stimulation. Thus, results from this study provide further support for proton-density-weighted spin-echo spinal fMRI as a reliable and useful technique that can be used to detect sensory and motor neuronal activity in the SC (Ng et al. 2006).

Govers et al conducted a spinal fMRI study to determine whether a reliable fMRI signal can be detected and spatially localized to specific neuronanatomical sites in the cervical SC
during fngertapping, fMRI signal intensity changes in the cervical SC were detected with gradient-echo echo-planar sequence on a 1.5 tesla scanner. T2*-weighted, transverse slices spanned from the 5th cervical (C5) to the 1st thoracic (T1) spinal segments. SC responses were observed bilaterally in most subjects during stimulation. However, there was a great deal of variability in the responses across subjects. For example, motor activity at C8, in response to fngertapping, was observed in only half of all the participants. Furthermore, the functional images had a great deal of distortion and artifacts such that functional activity had to be superimposed on anatomical reference images of the same regions. The original echo-planar images were so distorted that it was very difficult to see the anatomy. The authors also admitted that most of the BOLD signal was detected in the vicinity of major draining veins and not the grey matter of the SC. Because gradient-echo sequences are sensitive to magnetic field inhomogeneities, the resultant T2* images of the cervical SC had local susceptibility artifacts due to magnetic field inhomogeneities. Although motion correction was applied, regions of activity near tissue interfaces between bone and CSF likely contributed to regions of false activity (Govers et al. 2007).

Stracke et al investigated if functional activity with SC somatosensory stimulation in nine healthy volunteers can be visualized with BOLD fMRI. However, similarly to Govers et al, results were inconsistent across subjects and the functional echo-planar images revealed substantial distortions and susceptibility artifacts. In addition, the image artifacts hindered delineation of the SC, especially in the cervical segments. Due to considerable distortion in the echo-planar images, it was impossible to match the statistical results with the anatomical reference images and also very difficult to determine spatial localization of activity in the SC (Stracke, Pettersson, Schoth, Moller-Hartmann, and Krings 2005).

Backes et al tested the feasibility of BOLD to image the cervical SC by use of median nerve stimulation and fst clenching. Specifically, they tested whether stimulation of the median
nerve could evoke an fMRI response in the cervical SC at 1.5 Tesla. The fMRI responses to median nerve stimulation and fist clenching were compared. Similarly to Govers et al (2007) and Stracke et al (2005), the quality of the T2* weighted images was poor due to the sensitivity of gradient-echo sequences to magnetic field inhomogeneities compounded by the effects of SC motion and CSF flow (Backes, Mess, and Wilmink 2001).

Komisaruk et al conducted spinal fMRI on a 1.5 tesla system to visualize the location of cranial and spinal nerve nuclei. Localization of nuclei was achieved by cross-correlation analysis of BOLD signal intensity changes during particular motor and sensory procedures based on the known functions of specific nuclei. They detected regions of activity in several nuclei in response to different sensory and motor stimuli. For example, the nucleus abducens was activated in response to left-right movement of the eyes, while the hypoglossal nucleus became activated in response to pushing the tongue against the hard palate. This study demonstrated that cranial nerve and spinal nuclei can be visualized with fMRI in humans in response to sensory and motor stimuli (Komisaruk et al. 2002).

Stroman and colleagues have used spinal fMRI to noninvasively map neuronal function in the healthy and injured human SC in response to graded thermal stimuli. The method involved single-shot fast spin-echo sequence with a long TR and a short TE = 34 ms. Flow compensation gradients were applied in the rostral-caudal direction and spatial saturation pulses were applied to eliminate signal from anterior to the spine. All the individuals with SCT had injuries between the 6th cervical (C6) and the 8th thoracic (T8) segments. Activity in the lumbar SC, below the level of injury, was detected in all individuals with SCT. Furthermore, patterns of activity observed in people with SCT differed from those observed in healthy individuals. Signal intensity changes detected in response to different temperature stimuli were comparable to those found in healthy participants indicating the presence of neuronal activity below the level of the SC lesion. This study represents the first noninvasive demonstration of activity in the human SC below the level
of injury. The advantage of spinal fMRI compared to standard assessments such as the ASIA exam is that it can provide insight into SC function objectively without reliance upon subjective reports of sensation. Furthermore, spinal fMRI can be used to enhance current assessments following SCT (Stroman et al. 2002a).

Maieron et al recently performed spinal fMRI using BOLD contrast in the cervical SC in 13 right-handed volunteers during a finger-to-thumb opposition task. Although signal intensity changes observed during the task were consistently greater than those observed during rest, the poor resolution of the images of the SC made it impossible to distinguish between different neuronatomical regions within the SC (Maieron et al 2007).

Agosta et al used proton-density-weighted spinal fMRI to scan the cervical spinal cord in 12 healthy volunteers during a tactile stimulation of the palm of the right hand (Agosta et al. 2009). Activity was detected predominantly on the right side and the highest frequencies of fMRI activity were observed in the C6 and C7 SC segments. Furthermore, the task-related mean signal change of all activated voxels of the cord was 3.2%. The authors concluded that spinal fMRI is a reliable technique for obtaining physiological information on the activity of human spinal cord circuitry of tactile stimulation (Agosta et al. 2009).

Recently, Lawrence and colleagues used proton-density-weighted spinal fMRI to investigate regions of the cervical and lumbar spinal cord that are activated in response to vibration stimulation of different dermatomes in seven healthy humans (Lawrence, Stroman, and Kollias 2008). The main findings of the study were that the segmental distribution of activity visualized with fMRI was in line with the known SC neuroanatomy and that the rostrocaudal distribution of activity in the SC matched the dermatome that had been stimulated. The authors concluded that spinal fMRI can reveal a dermatome-dependent pattern of SC activity during vibratory stimulation (Lawrence et al. 2008).
Review of spinal fMRI literature indicates that acquiring SC images with spin-echo, proton-density-weighted sequences, as compared to gradient-echo T2*-weighted sequences, increases the quality of the image data and reduces distortions associated with magnetic field inhomogeneities. It is clear that studies utilizing spin-echo fMRI with a short TE were able to acquire better quality images that revealed more reliable signal intensity changes at sites of neuronal activity. An overwhelming majority of spin-echo spinal fMRI studies demonstrated consistent signal intensity changes across subjects whereas gradient-echo fMRI studies failed to do so. Thus, spin-echo spinal fMRI with a short echo time is an optimal noninvasive method that can be used to reveal consistent signal intensity changes at sites of neuronal activity in the human SC (Stroman 2005).

1.15 Challenges of Spinal fMRI

Apart from its numerous advantages, like any technique, spinal fMRI presents with its share of challenges. Because the SC is situated inside the protective sheath of the vertebral column, differences in magnetic susceptibilities between bone, cartilage and tissues, previously discussed, result in the formation of gradients which in turn reduce the MR signal and generate artifacts in functional MR images of the SC. This challenge has been successfully resolved, for the most part, by acquiring proton-density spin-echo weighted images that are less sensitive to differences in magnetic susceptibilities between different tissues.

Another problem has to do with the diminutive cross-section of the SC spanning an average of 16 × 10 mm in the cervical enlargement compared to its significant length averaging 45 cm. Such dimensions make it difficult to obtain high resolution images of the SC along with a high signal-to-noise ratio. In order to improve spatial resolution and increase-signal-to-noise ratio, one must ascertain an optimal balance of slice orientation, thickness and image resolution (Stroman 2005). Stroman and colleagues have shown that by acquiring contiguous sagittal slices with a minimum thickness irrespective of the signal-to-noise ratio, reliable images of the spinal
cord can be successfully acquired in the sagittal orientation (Stroman, Kornelsen, and Lawrence 2005). In order to increase signal-to-noise ratio, smoothing of reformatted 3D data should be performed in the rostral-caudal direction in relation to the spinal cord (Stroman et al. 2005).

A third challenge in spinal fMRI is associated with motion of the spinal cord. While it is acceptable for the spinal cord to move slightly and still reliably detect neuronal activity, excessive motion, particularly in the rostral-caudal direction, can create image artifacts that may erroneously enhance the signal intensity in a given region. The sources contributing to spinal cord motion is the flow of the cerebrospinal fluid (CSF), heart-beat and movement of the lungs during respiration. In addition, there is the inevitable presence of slight physiological motion during fMRI experiments as it is quite difficult for volunteers to stay still for prolonged periods of time. However, the biggest challenge appears to be cardiac-related motion in spinal fMRI experiments (Stroman 2005). SC motion varies throughout the cord’s length with maximum velocity appearing in the cervical SC and virtually no motion present in the lumbosacral SC (Feinberg and Mark 1987; Figley, Yau, and Stroman 2008). Moreover, motion in the anterior/posterior direction appears to be more problematic than motion in the right/left direction, which is negligible (Figley and Stroman 2007). Recently, Figley and Stroman (2009) developed and validated a novel method to compensate for cervical SC motion, by using retrospective spinal cord motion time-course estimates (RESPITE) to model the components of motion-related signal changes in fMRI data (Figley and Stroman 2009). The development of RESPITE allowed motion in the cervical SC, arising as a result of CSF flow and heart-rate, to be accounted for in the functional dataset, using a general linear model (GLM) approach, thereby greatly diminishing the impact of cord motion on the magnitude and location of fMRI signal arising in response to neuronal activity. The GLM approach is a class of statistical tests that assume that the experimental data are comprised of a linear combination of basis set functions that describe the model paradigm, as well as confounding variables including noise and cardiac-related motion, among others. The linear
sum of these basis functions describes the time course for each voxel in the dataset (Huettel et al. 2004).

### 1.16 Neuroimaging Studies of Sexual Function

The advent of powerful and sensitive fMRI techniques has made it possible to study brain regions involved in sexual function. Park and colleagues conducted the first BOLD fMRI experiment to elucidate regions of the brain involved in sexual arousal in healthy women during visually-evoked erotic stimulation (Park et al. 2001). They found increased signal intensity changes in regions of the brain implicated in reward including the thalamus, caudate nucleus and globus pallidus during viewing of erotic compared to non-erotic film. Several brain regions implicated in sexual function became activated in response to erotic but not the non-erotic visual stimuli. However, Park and colleagues observed no significant differences between the women’s perceived sexual arousal (PSA) ratings of the erotic versus the non-erotic film (Park et al. 2001) suggesting that the women may not have found the erotic films presented to be sexually arousing.

Hamann and colleagues utilized BOLD fMRI to examine men’s and women’s responses to visual sexual stimuli (Hamann, Herman, Nolan, and Wallen 2004), discovering that men have greater activation in the amygdala than women in response to identical erotic film excerpts. Interestingly, this difference was sustained despite the women’s greater PSA ratings (Hamann et al. 2004). Moreover, the sex differences were largest in the limbic areas of the brain and were more pronounced in the left than the right amygdala. There were also many similarities apparent between men and women in fMRI activity of various brain regions, particularly the striatum (Hamann et al. 2004) which is closely associated with pleasure and reward.

Karama and colleagues investigated regional brain activation in males and females during the viewing of erotic film excerpts (Karama et al. 2002). Results from 20 men and 20 women revealed increased BOLD signal in the anterior cingulate cortex, amygdala, ventral striatum and parts of the cortex (Karama et al. 2002). Furthermore, men showed greater activation of the
thalamus and hypothalamus than women in response to viewing of erotic film. In addition, only men demonstrated a positive correlation between the levels of PSA and hypothalamic activity (Karama et al. 2002), reflecting the higher level of sexual arousal experienced by men compared to women in response to processing of visual erotic cues (Karama et al. 2002).

Safron and colleagues investigated the neural substrates of sexual arousal in homosexual and heterosexual men using event-related BOLD fMRI (Safron et al. 2007). They found that men exhibit much higher levels of genital and subjective arousal to sexual stimuli depicting the preferred sex compared to the nonpreferred sex. In whole brain analysis, Safron and colleagues found increased activation of a large number of regions in response to the ‘preferred sex’ including the posterior cingulate, precuneus, left superior parietal lobule, left globus pallidus, thalamus, left putamen, left insula, left claustrum, hypothalamus, nucleus accumbens, and left cerebellum among others (Safron et al. 2007). Notably, most of the activity was localized to the left hemisphere suggesting that the left hemisphere plays a greater role in sexual arousal than the right, in men. The authors also noted that a greater number of brain regions became activated in response to the preferred sexual stimuli (Safron et al. 2007).

An intriguing report by Walter and colleagues recently demonstrated the neural differentiation between emotional and sexual components of human sexual arousal (Walter et al. 2008). The authors defined emotional components as general feelings of happiness, joy, anger or fear and the sexual components as feelings that reflect specific sexual intensity. They showed that activity in the ventral striatum and hypothalamus was specifically related to the sexual intensity of the stimulus, while activity in the anterior cingulate cortex was linked to an interaction between emotional factors and sexual intensity. Activity in the dorsomedial prefrontal cortex, mediodorsal thalamus and amygdala were related only to the emotional component of sexual arousal. Moreover, Walter and colleagues found no differences between males and females in the
magnitude or regions of activation, suggesting that the neural substrates governing sexual arousal are very similar between the two sexes (Walter et al. 2008).

Another report by the same group describes high resolution fMRI of subcortical regions during visual erotic stimulation at 7 Tesla (Walter, Stadler, Tempelmann, Speck and Northoff 2008). While erotic and non-erotic pictures were presented in an event-related design, high resolution EPI images with an in-plane resolution of $1.4 \times 1.4 \text{ mm}^2$, were acquired in only 13.6 minutes. Activity was observed in the anterior caudate and mediodorsal thalamus. The authors concluded that fMRI at high fields represents an optimal tool for studying the functional neuroanatomy of subcortical brain regions. Best of all, the high resolution images did not require additional spatial smoothing because of the high signal-to-noise ratio.

Arnow and colleagues investigated brain activation and sexual arousal in healthy heterosexual males using fMRI (Arnow et al. 2002). Consistent with previous neuroimaging studies (Redoute et al. 2000), they observed strong increased activations, associated with penile turgidity, in the left caudate, putamen and claustrum (Arnow et al. 2002). At the onset of erection and during sustained erection, Arnow and his team found activation in the rostral anterior cingulate, hypothalamus and secondary somatosensory cortex. However, during sustained erection but not at its onset, they observed increased activity in the insula (Arnow et al. 2002). Comparisons between the sustained erection and the onset of erection conditions yielded significant increased activation in the secondary somatosensory cortex and insula albeit not in the anterior cingulate. There was also a decrease in hypothalamic activation during sustained erection, consistent with the theory proposed by Georgiadis and Holstege attributing the role of the hypothalamus as a mediator of erectile onset as opposed to sustained erectile responses in men (Georgiadis and Holstege 2005).

Georgiadis and Holstege also investigated brain activation in men during sexual stimulation of the penis using PET (Georgiadis and Holstege 2005). The researchers utilized PET
to measure regional cerebral blood flow (rCBF) in the men attributing the choice of this technique to its greater resilience to movement compared to fMRI (Georgiadis and Holstege 2005). However, PET has poor temporal and average spatial resolution (Cherry and Phelps 2002) compared to fMRI not to mention it is quite invasive, particularly in studies of human sexual function. Georgiadis and colleagues observed increased rCBF in the right hemisphere, including the posterior insula and secondary somatosensory cortex while decreased rCBF was observed in the right amygdala (Georgiadis and Holstege 2005). The researchers ascribed the lack of increased rCBF in the thalamus, hypothalamus and primary somatosensory cortex to their involvement in the initiation of sexual arousal as opposed to sexual performance (Georgiadis and Holstege 2005).

Holstege et al also used PET to measure rCBF increases during ejaculation compared to sexual stimulation in heterosexual men (Holstege et al. 2003). They observed increased rCBF in the ventral tegmental area, a region of the brain rich in dopamine cell bodies that is associated with pleasure and reward (McBride, Murphy and Ikemoto 1999). Additional rCBF was detected in the midbrain lateral central tegmental field, zona incerta and SPF nucleus of the thalamus, a region associated with the ejaculation generator in the lumbar spinal cord in rats (Coolen, Veening, Wells, and Shipley 2003). In contrast, decreased rCBF was observed in the amygdala, a region of the brain implicated in fear responses (Rauch, Shin, and Wright 2003), suggesting that fear must be ‘turned-off’ during orgasm. Furthermore, powerful rCBF was observed in the cerebellum during ejaculation. This finding is consistent previous studies that implicated the cerebellum in mediating emotionally-pleasurable behaviors including sexual arousal (Redoute et al. 2000), drug addiction (Sell et al. 1999) and even financial incentive (Martin-Solch et al. 2001). Activity in the cerebellum is not likely to be related to motion artifacts in this study because the participants did not engage in self-stimulation. Instead, penile stimulation was
performed by each individual’s partner. Interestingly, rCBF was predominantly localized to the right side, suggesting that the right hemisphere plays an important role in ejaculation in men.

Recently, Georgiadis et al used PET to identify regions of the brain involved in clitorally-induced orgasm in healthy women (Georgiadis et al. 2006). In line with their hypotheses, decreased rCBF was observed in the neocortex during female orgasm. It seems that the neocortex ‘shuts down’ at the moment of orgasm, allocating synaptic input to regions of the basal ganglia and midbrain involved in motivation and reward instead (Georgiadis et al. 2006). In fact, PSA scores obtained from participants during the study, positively correlated with rCBF in the ventral midbrain and right caudate nucleus, regions of the brain rich in dopamine receptors and associated with feelings of pleasure (Georgiadis et al. 2006). Consistent with previous findings in men during ejaculation (Holstege et al. 2003), strong increased rCBF was observed in the cerebellum, specifically in the deep cerebellar nuclei, during clitoral stimulation culminating in orgasm in women.

It is known that women diagnosed with complete SCT above T10 and hence above the level of entry of the hypogastric, pelvic and pudendal sensory nerves, still perceive and experience orgasms in response to vaginal and cervical sexual stimulation (Sipski et al. 1995; Komisaruk, Gerdes, and Whipple 1997). Komisaruk and his group proposed that in people with a high SCT (e.g. T10), the Vagus nerves transmits sensory sensation from the genitals to the nucleus of the solitary tract in the medulla oblongata in the brainstem, effectively bypassing the SC lesion (Komisaruk et al. 2004). Using BOLD fMRI, they investigated brain activation during vaginocervical self-stimulation and orgasm in women with complete SCT (Komisaruk et al. 2004). Indeed, results revealed that women demonstrated activity in the inferior region of the nucleus of the solitary tract, in addition to other brain regions, at orgasm triggered by cervical self-stimulation (Komisaruk et al. 2004). Komisaruk and colleagues concluded that the Vagus nerves provide a spinal cord bypass pathway for the transmission of vaginal/cervical sensation to
the brain in women with SCT above the level of the known genitospinal nerves (Komisaruk et al. 2004). However, there are several limitations to this study. First, the women were scanned with gradient-echo echo-planar sequence. This sequence, when applied in the brainstem and cervical spinal cord regions, is sensitive to magnetic field inhomogeneities making it difficult to obtain high-quality fMRI data because gradient-echo methods are highly sensitive to the BOLD effect (Stroman 2005). Moreover, it is known that the highest quality images of the spinal cord and brainstem can be obtained with proton-density weighted spin-echo imaging parameters because it’s the least sensitive to spatial inhomogeneities in the static magnetic field between various tissues commonly found around the spinal cord, including CSF, bone and air spaces of the lungs (Stroman 2005). Secondly, the thickness of the brain slices was set at 4 mm which is quite thick if the intention is to localize activity in the nucleus of the solitary tract in the medulla oblongata, which is quite small. Thirdly, there were only 5 women imaged in this experiment, and only 4 of them had complete SCT as verified by the ASIA exam (Komisaruk et al. 2004) limiting the impact and reliability of the findings. Finally, there were no control subjects scanned to compare their responses and neuronal activity patterns to those of the group with SCT.

Although previous fMRI studies have revealed several important regions of the brain mediating sexual responses in men and women, no experiments thus far have been performed to investigate the SC mechanisms governing sexual function in humans. SC nuclei integrate autonomic, somatic and central NS mechanism and trigger the desired response (e.g. ejaculation) to sexual stimulation. Thus, it is essential to have a clear understanding of the neural circuitry of the human SC mediating sexual function in order to recognize how sexual responses occur in healthy humans.
1.17 Research Proposal

1.17.1 Background and Rationale

A large number of men and women experience sexual dysfunction following SCT. Depending on the level of SC injury, the capacity for penile erection and vaginal lubrication is preserved to some extent, either through psychogenic or reflexogenic sexual stimulation. However, a large number of victims of SCT lose the ability to reach orgasm post-trauma and this in turn, negatively impacts their relationships, quality of life, satisfaction, and in fertility in men. Given such drastic consequences to sexual function following SCT, it is imperative to understand the neural circuitry of sexual responses in healthy individuals in order to determine what changes occur to this neural circuitry post-trauma.

Despite the desperate need for information about the SC control of sexual responses in humans, there have been no fMRI studies performed to date on the SC patterns of neuronal activity during sexual arousal and orgasm in humans. Commonly utilized gradient-echo BOLD fMRI sequences are unsuitable for functional imaging of the SC as these are sensitive to differences in magnetic susceptibilities between different tissue types that are abundant in close proximity to the SC. Further complicating matters is the pulsatile movement of the cervical SC with each heart-beat, creating artifacts in the resultant MR images of the adjacent brainstem. Fortunately, the advent of proton-density-weighted spinal fMRI methods has finally made it possible to investigate sexual responses in the human spinal cord and brainstem.

Studies of individuals with SCT indicate that the ability to perceive sensation in the T11-L2 dermatomes is closely associated with preserved ability to experience psychogenic penile erection (Sipski et al. 2007) and vaginal lubrication (Sipski et al. 2001). On the other hand, an intact sacral spinal cord is highly predictive of the capacity to experience orgasm in men (Sipski and Alexander et al. 2006) and women (Sipski et al. 2001) with SCT, suggesting that orgasm is a reflex response involving the autonomic nervous system.
In this project, we aim to reveal, by means of spinal fMRI, the neural circuitry of sexual responses in the lower thoracic, lumbar and sacral spinal cord during sexual responses in healthy men and women. We investigated the neural correlates of sexual responses in the lower thoracic, lumbar and sacral spinal cord. The study comprised three experimental conditions. In the first condition, we mapped the neural correlates of audiovisually-evoked sexual responses while participants engage in intermittent viewing of erotic film excerpts and we termed this intermittent audiovisual stimulation (AVS). In the second condition, we aimed to reveal the neural correlates of genitally-evoked sexual responses while participants engage in intermittent genital self-stimulation (penile for men and clitoral for women) and this was termed intermittent genital self-stimulation (GSS). Finally, in the third condition, we explored the neural effects of simultaneous audiovisual and genital self-stimulation culminating in orgasm and this was termed continuous audiovisual and genital self-stimulation (AVGSS). Throughout AVGSS, participants engaged in viewing of erotic film excerpts and genital self-stimulation, continuously and simultaneously, until orgasm was reached.

The purpose of our experiment was threefold. First, we aimed to investigate whether spinal fMRI can reveal reliable signal intensity changes, indicative of neuronal activity in the grey matter of the human SC during sexual responses. Our second goal was to determine which regions of the SC are activated in response to sexual stimulation and whether these regions are in line with previous work describing neuroanatomical locations involved in sexual function in humans and animals. Lastly, we were interested in investigating individual and gender differences in sexual responses in humans. We divided the experiment into three parts. The first part, audiovisual stimulation (AVS) was aimed at targeting neuronal circuitry involved in psychogenic sexual responses to viewing erotica. The second part of the study, genital self-stimulation (GSS), was meant to trigger parts of the nervous system involved in reflexive sexual responses in men and women. It is important to note that in healthy humans with an intact SC, unlike in individuals
with complete SCT, it is difficult to separate the psychogenic and reflexogenic components of sexual responses, as one pathway necessarily feeds into the other. Finally, the third part of the experiment, continuous audiovisual and genital self-stimulation (AVGSS), was designed to reveal the neural correlates of combined psychogenic and reflexogenic stimulation culminating in orgasm.

1.17.2 Hypothesis

We hypothesized that spin-echo proton-density-weighted spinal fMRI, which has previously been shown to reveal consistent and reliable signal intensity changes in response to sensory (Lawrence et al. 2008) and motor (Kornelsen and Stroman 2007) stimuli, can reveal consistent signal intensity changes in the human thoracic, lumbar and sacral SC in response to sexual stimuli in humans. Since the SC is an evolutionary old structure, we expected that the patterns of signal intensity changes in the human SC activated in response to sexual cues will closely resemble the neuroanatomical regions previously observed in animal experiments. We also hypothesized that there may be gender differences, associated with sexual dimorphism, in the human SC. Indeed, several neuroimaging studies report on sexual dimorphic structures in the brain. For example, it has recently been demonstrated that the amygdala shows a greater degree of activation in men compared to women during visual sexual stimulation, even when women’s perceived sexual arousal ratings are higher (Hamann et al. 2004). Similarly, Karama et al have observed greater activation of the caudate nucleus, thalamus and hypothalamus in men than in women in response to viewing erotic films (Karama et al. 2002). Furthermore, male rats, dogs, and humans possess a greater number of motoneurons in the ON of the lumbosacral SC than female conspecies (Forger and Breedlove 1986; Schroder 1980). Thus, we postulated that sexual dimorphism may also exist in other regions of the human SC.
1.17.3 Objective

To map the signal intensity changes in the lower thoracic, lumbar and sacral SC during AVS, GSS and AVGSS in 10 healthy men and 9 healthy women (Chapter 2).
Chapter 2

Neuroanatomical Correlates of Sexual Responses in the Human Spinal Cord

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(This manuscript is in preparation for submission).

2.1 Introduction

Until the relatively recent development of functional magnetic resonance imaging (fMRI) (Menon et al. 1992; Ogawa et al. 1992) and its specific adaptation for use in the spinal cord (SC) (spinal fMRI) (Stroman 2005; Agosta et al. 2008; Kornelsen and Stroman 2007) non-invasive in vivo studies of the healthy human SC were not possible. Therefore, to obtain information about SC responses, researchers have had to rely on animal experiments, postmortem assessments, or clinical studies of the effects of trauma. While these studies have been essential to our understanding, our knowledge of normal human SC function is based on very little information from neurologically-intact healthy humans (Hochman 2007; Fields, Heinricher, and Mason; Bandler and Shipley 1994; Gebhart 2004). Not only are there significant differences between humans and research animals in descending modulation of responses related to emotional and cognitive processes, but neuroplasticity can impact the neural pathways that are recruited post-
injury. Thus, the direct study of neurologically intact humans via spinal fMRI provides more accurate information about the neural correlates of human sexual responses.

It is known that even after complete SC injuries some people retain aspects of sexual function. In fact, 55% of women with SC injuries spanning the cervical to the lumbar levels are able to self-stimulate to orgasm, in comparison to only 17% with complete disruption of the sacral (S2-S5) reflex arc (Sipski et al. 2001). How this occurs is the subject of some debate (Sipski et al. 2001; Komisaruk and Whipple 2005; Alexander 2008). Nevertheless, the question remains as to precisely how sexual function is altered by SCT, and why some individuals maintain the ability to achieve orgasm, while others are not as fortunate.

In humans, sympathetic components of sexual responses are controlled by the thoracolumbar segments, T11 to L2, whereas parasympathetic components are mediated by the sacral segments, S2 to S5 (Sipski et al. 2001; Sipski et al. 2007). The sympathetic component involves mentally-evoked (psychogenic) penile erection in men, and vaginal vasocongestion in women and is mediated by preganglionic neurons in the dorsal commissural nucleus (DCN) and the intermediolateral cell column (IML) (Giuliano and Rampin 2000; Giuliano, Rampin and Allard 2002). Conversely, parasympathetic preganglionic neurons in the sacral parasympathetic nucleus (SPN) mediate physically-evoked (reflexogenic) erection, clitoral engorgement, vaginal vasocongestion and lubrication in humans (Blumenfeld 2002). Furthermore, the SPN, dorsal gray commissure (DGC) and dorsal horn (DH) of the sacral SC receive afferent input from the penis and clitoris (Veronneau-Longueville et al. 1999; Marson and Murphy 2006) and an intact sacral SC appears to be required for reflexogenic sexual responses to occur (Steers 2000; Giuliano et al. 2002).

Overall, the human sexual response is mediated by the integrated functions of the sympathetic, parasympathetic and somatic components (Blumenfeld 2002; Levin and Riley 2007; Neuhuber 1982; Schroder 1980). The latter group is found in Onuf’s nucleus (ON) which controls muscle
and sphincter contraction during penile erection and ejaculation in men (Blumenfeld 2002). However, the role of ON in women’s sexual function is currently not understood.

This study is the first demonstration of neuronal function associated with autonomic and sexual responses in the healthy human SC as detected with spinal fMRI. Furthermore, we show, for the first time, that psychogenic and reflexogenic components of sexual responses are integrated in neurologically-intact humans and reveal the presence of sexually-dimorphic circuits that provide insight into gender and individual differences in human sexual function.

2.2 Methods

2.2.1 Participants

Healthy volunteers (10 men, 10 women) were recruited from the local community, and participated only after giving informed consent. The study was approved by the Queen’s University Health Sciences Research Ethics Board. Out of these volunteers, one woman withdrew prior to the fMRI experiments, and of those remaining there were 8 heterosexual males, 2 homosexual males, 8 heterosexual females, and 1 homosexual female (mean age = 21.8 ± 3.4 years). The results from all participants were included because we anticipated individual variability regardless of sexual orientation. Nine of the male and eight of the female participants completed a medical/sexual history checklist and a short questionnaire of sexual function. Males completed the International Index of Erectile Function (IIEF) (Rosen et al. 1997) and females completed the Female Sexual Function Index (FSFI) (Rosen et al. 2000). The participants had no history of sexual dysfunction, central nervous system injury or disease and all underwent screening prior to testing to ensure that they generally find erotica sexually arousing and are comfortable with genital self-stimulation.
2.2.2 Study Design

Functional magnetic resonance imaging studies were carried out in the Queen’s University MRI facility, with a 3 Tesla Siemens Magnetom Trio MRI system. Each participant was provided with head-phones for two-way communication and for the audio portions of the stimuli, and was positioned lying supine on the bed of the MRI system, to enter the bore of the MRI magnet head-first. Padding was put in place to elevate the knees and raise the arms/elbows. This provided comfort and served to reduce any task-related motion of the spine. A wide elastic band was placed across the participant’s midsection (attached on each side to the bed) to further decrease subject motion. After this initial positioning, the participant was carefully aligned to ensure that their spine was as straight as possible, and to center the initial imaging volume at the bottom of the xiphoid process to be approximately at the T12 vertebra. Each participant lay on top of the MRI phased-array spine coil, which was integrated into the bed. A mirror was positioned in front of the participant’s eyes, supported by a clear plastic frame, so that he/she could comfortably view a rear-projection screen positioned outside of the MRI system. An optical sensor was attached to the second digit of the non-stimulating hand throughout the study to record the peripheral pulse. Subsequently, the bed was advanced into the MRI system to position the T12 vertebra at roughly the center of the magnet.

Initial localizer images were acquired in 3 planes, and were used for subsequent positioning of image slices. A higher-resolution set of coronal images were acquired, followed by a set of 9 sagittal slices, in order to verify the positioning for the subsequent fMRI studies.

2.2.3 Functional Imaging Protocol

Functional MRI in the SC was carried out using the signal changes arising from altered tissue water content at sites of neuronal activity (Schummers, Yu, and Sur 2008; Stroman et al. 2008b; Stroman 2005). “Signal enhancement by extravascular water protons” (SEEP) contrast, is distinct from the more widely used “blood oxygenation-level dependent” (BOLD) contrast for
fMRI. This deviation from conventional brain fMRI methods is necessary because of the magnetic field distortions caused in the SC by its proximity to the bone and cartilage in the spine and to the air/tissue interfaces in the lungs. The functional image data are acquired by means of proton-density-weighted, spin-echo imaging. For this study we used a half-Fourier single-shot fast spin-echo (HASTE) sequence (Stroman et al. 2008a) to image the lower thoracic, lumbar, and sacral SC with relatively high resolution (1.5 mm × 1.5 mm × 2 mm), in 9 contiguous 2 mm thick sagittal slices. These spanned a 280 mm x 140 mm field of view (head/foot × anterior/posterior) centered at the 12th thoracic (T12) vertebral body and encompassed the 7th thoracic (T7) to the 4th lumbar (L4) vertebral bodies. The echo time (TE) was 38 ms and the repetition time (TR) was 1000 ms per slice. A spatial saturation pulse was applied to eliminate signal from regions anterior to the spine, and flow compensation gradients were applied in the head/foot direction to reduce image artifacts produced by flow of cerebrospinal fluid (CSF). With these acquisition parameters, the 3D volume spanning the lower thoracic, lumbar, and sacral SC was imaged every 9 seconds, to describe the signal intensity response time-series with each stimulus paradigm.

Following the fMRI scans, high resolution (1 mm x 1 mm x 2 mm) anatomical scans were also acquired with T1-weighting, with an inversion-recovery turbo spin-echo pulse sequence, with a minimum echo time (12 ms), repetition time (TR) of 2 seconds and two averages.

2.2.4 Stimulation Paradigms

Each volunteer participated in three sequential parts of the study. Due to the sensitive nature of the subject, participants were given maximal privacy during the experiment. All cameras were turned off, lights in the room were dimmed and lights inside the bore of the magnet were turned off. Sounds emanating from the bore of the magnet were minimized by reducing the volume, except during AVGSS because it was necessary for each participant to verbally report the occurrence of orgasm. Audiovisual stimulation (AVS) was designed to detect SC activity in
response to viewing erotic films. The films, depicting heterosexual couples engaged in sex play, vaginal sexual intercourse, and oral sex were presented in two 5 minute blocks, separated by a baseline condition (blank screen) for 3 minutes, and the stimulation blocks were also preceded and followed by two baseline conditions of 1.5 minutes each. This entire part lasted a total of 16 minutes. The selected films were chosen by the investigators and were all very highly rated by independent viewers. The homosexual participants were shown the same erotic films as the heterosexual participants, in order to maintain consistency of the stimuli.

The second part of the study comprised genital self-stimulation (GSS) and was designed to target the neuronal activity associated with penile and clitoral self-stimulation in men and women respectively. Participants were instructed by means of written instructions projected onto a rear-projection screen, indicating whether to rest, or to perform self-stimulation. Interleaved between 1.5 minute initial and final baseline conditions, two equal blocks of 1.5 minutes of stimulation were separated by a 1.5 minute block of rest. This part of the study lasted a total of 7.5 minutes. Stimulation was manual for males and was applied with an MRI-compatible vibrating stimulator for females. Because the type of genital stimulation differed between men and women, it may have resulted in slightly different patterns of signal intensity changes depending on the neuronatomical pathways activated.

Audiovisual and genital self-stimulation (AVGSS) involved continuous viewing of an erotic film along with continuous genital self-stimulation, and was designed to investigate the combined effects of AVS and GSS, culminating in orgasm. Images were acquired during an initial 1.5 minute baseline period (blank screen, no stimulation), followed by a continuous epoch of erotic film presentation combined with genital self-stimulation, until orgasm ensued. Following the stimulation block, or a maximum of 20 minutes, regardless of whether the participant reached orgasm or not, the erotic film presentation was aborted, and the participant was instructed to rest while images were acquired for an additional 3 minutes of baseline. This part of the study lasted a maximum of 24.5 minutes (Figure 4)
2.2.5 Data Analysis

Data were analyzed using custom-made software written in MatLab (The MathWorks Inc. Natick, MA) by means of a general linear model (GLM) using the RESPITE basis set (Stroman, Figley et al. 2008; Figley et al. 2008; Stroman 2006). The RESPITE basis set is designed to estimate a number of physiological noise regressors in spinal fMRI data. These physiological noise regressors are derived from the cardiac traces recorder from each participant during the study, as well as the principal components of SC motion. Incorporating RESPITE into the GLM
allows to model functional signal intensity changes associated with the stimulus paradigm as well as signal fluctuations associated with motion of the SC and CSF flow, in order to distinguish between them. Including RESPITE in the GLM analysis has been shown to reduce the occurrence of type 1 (false-positive) and type two (false-negative) errors, thus improving the sensitivity (15-20%) and specificity (5-6%) for detecting signal intensity changes associated with neuronal function (Figley and Stroman 2009). The output of the analysis included the magnitudes of the signal changes (beta values) corresponding to each function in the basis set, and a test for the significance of the value of $\beta_1$, which corresponds to the stimulus paradigm. Throughout AVGSS, participants were reaching orgasm at different times. Therefore, the time course for AVGSS differed across participants. This difference in the time course was accounted for because we performed individual analysis of the data first before combining the time courses for each part of the study across each group of participants. The combined group data represents the average time course of all the participant time courses, for each part of the study. The analysis results were spatially normalized to enable voxel-by-voxel comparisons between individuals (Stroman et al. 2008a). Group analyses were carried out separately for males and females, by means of random effects analysis, based on the mean and standard error of the mean of the $\beta_1$ values in each voxel across the group of participants (McGonigle et al. 2000). The random effects analysis permits inference about the larger population from which the participants are sampled. Each individual within the group of subjects is considered to be one of several potential participants that may have taken part in the experiment. Furthermore, the random effects analysis assumes that experimental manipulations or stimuli can elicit different responses in every subject, accounting for inter-subject variability (Huettel et al. 2004). For example, some participants may demonstrate greater SEEP signal intensity changes than other participants in response to the same experimental stimuli.

Group responses were taken to be significant at a T-value greater than 2.5 or less than -2.5 ($p < 0.04$). Group results were also analyzed on a voxel-by-voxel basis by testing for
correlations between β1 values and subjective ratings. These included the ratings of perceived physical sexual arousal (PPSA) and perceived mental sexual arousal (PMSA) obtained after each of the three experimental conditions, the total scores on the IIEF and the FSFI, and sub-sections of these questionnaires including the domains of Sexual Desire, Orgasmic Function, Sexual Arousal, and Erectile Function. The IIEF and FSFI assessed sexual functioning in the past four weeks in males and females respectively, while PPSA and PMSA scores obtained after each experimental condition examined sexual responses of each individual at the time of the study. Therefore, the latter scores are more directly related to signal changes in the SC throughout the experiment, while the former scores are taken as an indication of general sexual functioning.

2.2.6 Statistical Analyses

Two separate statistical analyses were performed on the PPSA and PMSA scores. First we compared the PPSA and PMSA means between men and women for each part of the study using a two-tailed, unpaired Student’s t-test (p < 0.05). Then we compared the PPSA and PMSA means for each experimental condition, separately within each gender group using a two-tailed paired Student’s t-test (p < 0.05).

2.3 Results

Here we report the fMRI results from 19 healthy volunteers, including 10 men and 9 women that were studied. It is important to note that the neuroanatomical regions of interest revealed by fMRI analysis and discussed below, are based on visual comparisons with anatomical texts and atlases (Adel and Ronald 1998; Blumenfeld 2002; Nolte 2009). Therefore, the regions described herein are approximations of the known location of neuroanatomical structures and are not exact.

2.3.1 FMRI signal intensity changes

Spinal fMRI studies spanning the lower thoracic, lumbar and sacral regions of the SC demonstrated consistent patterns of activity in response to AVS, GSS and AVGSS in each of the
19 healthy volunteers that were studied. Positive as well as negative signal changes were observed upon stimulation (hereafter referred to as positive or negative “activity”), and are expected to reflect increased or decreased neuronal input, respectively (Logothetis et al. 2001; Stroman et al. 2005 (Figures 5-7).

Figure 5 Spinal Cord Responses to AVS
Activity during stimulation in the lower thoracic, lumbar and sacral spinal cord throughout AVS in 10 men and 9 women. Each transverse segment of the SC is 18 mm in the horizontal plane and 3 cm in the vertical plane. Arrows indicate region of interest activity in the spinal cord. IML – intermediolateral cell column. DCN – dorsal commissural nucleus. DH – dorsal horn. VH – ventral horn. SPN – sacral parasympathetic nucleus.

**Figure 6 Spinal Cord Responses to GSS**

Activity during stimulation in the lower thoracic, lumbar and sacral spinal cord in 10 men and 9 women throughout GSS. Each transverse segment of the SC is 18 mm in the horizontal plane and 3 cm in the vertical plane. Arrows indicate regions of interest in transverse sections of the spinal cord. IML – intermediolateral cell column. VH – ventral horn. DCN – dorsal commissural nucleus. DH – dorsal horn. SPN – sacral parasympathetic nucleus.
Figure 7 Spinal Cord Responses to AVGSS

Activity during stimulation in the lower thoracic, lumbar and sacral SC throughout AVGSS in 10 men and 9 women. Cross sections of the SC are shown in radiological orientation. Each transverse segment of the SC is 18 mm in the horizontal plane and 3 cm in the vertical plane. Arrows indicate regions of interest in the transverse sections of the spinal cord. IML – intermediolateral cell column. VH – ventral horn. DCN – dorsal commissural nucleus. DGC – dorsal grey commissure. SPN – sacral parasympathetic nucleus.
2.3.1.1 Audiovisual stimulation (AVS)

In the lower thoracic SC, women demonstrated increased activity in the IML and DCN while men revealed decreased activity in the IML. In the mid-lumbar SC, men showed increased activity in the VH and women showed increased activity in the IML and DCN. In the sacral SC, men demonstrated increased activity in the SPN. Similarly, women revealed increased activity in the SPN and DH (Figure 5, Tables 1, 2a and 3a).

2.3.1.2 Genital self-stimulation (GSS)

In the lower thoracic SC, men demonstrated increased activity in the IML while women revealed decreased activity in the VH. Throughout the mid-lumbar SC, men showed decreased activity in the DCN. In contrast, women demonstrated very powerful increased activity in the IML, and decreased activity in the VH. In the sacral SC, men showed decreased activity in the SPN and DH while in women there was an absence of activity altogether (Figure 6). However, because AVS always preceded GSS, it is likely that sexual responses to AVS could have contributed to the signal intensity changes observed during GSS.

2.3.1.3 Audiovisual and genital self-stimulation culminating in orgasm (AVGSS)

In the lower thoracic SC, men demonstrated decreased activity in the IML while women revealed increased activity in the IML and decreased activity in the VH. Throughout the mid-lumbar SC, men demonstrated decreased activity in the DCN while women showed increased activity in the IML and decreased activity in the VH. Finally, in the sacral SC, men demonstrated decreased activity in the DGC while women showed increased activity in the SPN (Figure 7).
2.4 Neural correlates of sexual responses

In order to supplement our group fMRI data, we investigated subject-by-subject correlations between fMRI activity and measures of sexual function based on questionnaires and PSA ratings. Thus, the group fMRI data demonstrates consistent features of responses across people while correlations between fMRI activity and measures of sexual function provide insight into individual differences in sexual responses in the SC. Using custom-made programs written in MatLab, we uncovered all the voxels in our fMRI t-value maps that were correlated with different scores of sexual function, including the total scores on the IIEF and FSFI, their individual domains, and PPSA and PMSA ratings. Out of the labeled voxels, only regions of signal intensity changes in the SC that demonstrated consistent areas of activity across several segments were chosen.

2.4.1 Part 1: Neural correlates of audiovisually-evoked sexual stimulation

In the first part of the study we investigated correlations between SC activity and measures of sexual function during audiovisual stimulation. The details of these results are presented in Tables 1, 2b and 3b.

2.4.1.1 Thoracolumbar spinal cord

Male and female participants demonstrated similar patterns of spinal cord activity in response to AVS. In women, activity observed in the left IML of the lower thoracic segments was positively correlated with physical sexual arousal ($r = 0.97$). Similarly in men, activity in the left IML and DCN of the upper lumbar spinal segments was positively correlated with physical sexual arousal ($r = 0.76$) (Figure 8a-8e).
Figure 8 Correlations between Scores and fMRI Activity during AVS

Male (n=10) and female (n=9) responses to intermittent erotic film. Transverse spinal segments show activity (highlighted in yellow) in the left IML in a) females and b) in the DCN and left IML in males. Physical sexual arousal is positively correlated with fMRI activity in c)* females (r = 0.97) and in e)* males (r = 0.76). d) The levels of spinal cord activity in males and females are color-coded and presented in sagittal view. Transverse spinal segments show activity (highlighted in yellow) e) bilaterally in the VH, left IML and left DH in females and f) bilaterally in the VH and DCN in males. Mental sexual arousal is positively correlated with fMRI activity in g) females* (r = 0.83) and h) in males* (r = 0.95). * Blue circles = heterosexuals, green circles = homosexuals, red circles = participants that did not reach orgasm during AVGSS

These findings are consistent with studies that found the presence of excitatory neurotransmitter glutamate in neurons of the IML in the thoracolumbar cord (Swanson and
McKellar 1979; Morrison, Callaway, Milner, and Reis 1989). Furthermore, there are dense excitatory, oxytocinergic projections from the paraventricular nucleus of the hypothalamus (PVN), which is known to regulate sexual function, to neurons in the IML within the T13-L2 segments of the thoracolumbar spinal cord in rats (Shafton, Ryan and Badoer 1998). In addition, both sexes displayed activity in the IML and neighboring regions throughout the mid-lumbar spinal segments that was positively correlated with mental sexual arousal in males (r = 0.95) and in females (r = 0.83) (Figure 8f-8i).

In contrast, activity in the VH, DH and DCN of the lower thoracic segments was negatively correlated with the erectile function domain (EF) of the International Index of Erectile Function (IIEF) in men (r = -0.90) (Table 2a) and with the sexual arousal domain of the Female Sexual Function Index (FSFI) in women (r = -0.86) (Table 3a).

<table>
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<th><strong>a) Female Responses</strong></th>
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<tr>
<td><strong>Mode of Stimulation</strong></td>
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<tr>
<td>1) Intermittent audiovisual stimulation (AVS)</td>
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<td>2) Intermittent genital self-stimulation (GSS)</td>
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<td>3) Continuous audiovisual and genital self-stimulation (AVGSS)</td>
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<th><strong>b) Male Responses</strong></th>
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<tr>
<td><strong>Mode of Stimulation</strong></td>
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<tr>
<td>1) Intermittent audiovisual stimulation (AVS)</td>
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<td>2) Intermittent genital self-stimulation (GSS)</td>
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<td>3) Continuous audiovisual and genital self-stimulation (AVGSS)</td>
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**Table 1 Spinal Cord Activity during AVS, GSS and AVGSS**

Positive and negative activity in the spinal cord in response to 1) intermittent audiovisual stimulation (AVS), 2) intermittent self-stimulation (GSS) and 3) continuous audiovisual and self–stimulation (AVGSS) in men and women.
Table 2 Correlations between Scores and fMRI Activity

Table 3 Correlations between Scores and fMRI Activity in Women


This finding is in line with previous reports that visceral afferents of the hypogastric nerve, which convey noxious inputs from the internal genitalia including the vagina, cervix and uterus, terminate on the DCN of the thoracolumbar segments (Thor, Morgan, Nadelhaft, Houston and de
Groat 1989) and act to inhibit sexual responses in female rats (McKenna 2002). Similarly in male rats, the inhibitory neurotransmitters glycine and GABA are colocalized to the DCN of the thoracolumbar cord (Popratiloff, Valtschanoff, Rustioni, and Weinberg 1996).

2.4.1.2 Lumbosacral spinal cord

Due to the psychogenic nature of the visual stimuli, it was remarkable to observe considerable activity in the lower lumbar and sacral regions, which are typically associated with reflexogenic responses (Giuliano and Rampin 2000; Giuliano et al. 2002; Steers 2000; Sipski et al. 2007). In females, activity in the DGC, VH and DH of the lower lumbar/upper sacral spinal segments was negatively correlated with the sexual desire domain of the FSFI ($r = -0.92$) (Table 3a) while in males, activity in the DGC, ON and SPN of the upper sacral segments was positively correlated with the sexual desire domain of the IIEF ($r = 0.91$) (Table 2a). Although the DGC is known to suppress sexual responses when receiving noxious input from the internal genitalia (McKenna 2002), studies also suggest that some DGC neurons in the sacral segments facilitate sexual responses (Giuliano and Clement 2005). Similarly, the SPN receives dense afferent parasympathetic input from the penis/clitoris via the pelvic nerve (McKenna and Marson 1997) and contains neurons that stain positively for dopamine and nitric oxide, neurotransmitters that promote sexual responses (Marson and Murphy 2006; Sasek, Seybold and Elde 1984). This suggests that neurons in the SPN have an excitatory role in mediating sexual responses and is substantiated by our finding that activity in the SPN in males was positively correlated with sexual desire. Previous work also demonstrated extensive functional networks between the DGC and the SPN in the lumbosacral segments of the SC (Sasek et al. 1984). Perhaps following input from the SPN, the DGC facilitates sexual responses in males and females.

In line with the known neuroanatomy and neurophysiology (Hart 1972; McKenna and Marson 1997; Tang, Rampin, Giuliano, and Ugolini 1999) activity observed in the ON was positively correlated with sexual desire in males. Situated in the ventral horn (VH) throughout the S2-S4
spinal segments in humans, ON contains pudendal motoneurons that innervate pelvic floor musculature, urethral and anal sphincters in male rats (Schroder 1980), and is important in regulating erectile and ejaculatory functions in male dogs (Hart 1972). These pudendal efferent fibers are also known to innervate penile musculature including the ischiocavernosus and bulbospongiosus muscles, thereby enhancing erections and controlling contraction of the urethral sphincter during emission (Schroder 1980).

2.4.2 Part 2: Neural correlates of genital self-stimulation

In the second part of the study we tested correlations between activity in the SC during genital self-stimulation and measures of sexual function. The details of these results are presented in Tables 1, 2b and 3b.

2.4.2.1 Thoracolumbar spinal cord

Although the stimulus in this part of the experiment was designed to be reflexogenic, and was expected to involve predominantly the sacral spinal cord, we also observed consistent activity in the lower thoracic and upper lumbar cord regions, corresponding to psychogenic components of sexual responses (Sipski et al. 2007; Giuliano et al. 2002). Thus, both the viewing of erotic films in Part 1 and genital self-stimulation in Part 2 appear to involve psychogenic and reflexive components. In response to intermittent self-stimulation, there were many gender similarities regarding the spatial distribution of SC activity. Tables 2b and 3b illustrate that activity in the sympathetic nuclei of the thoracolumbar cord, DCN and IML, was positively correlated with the total sexual function score of the FSFI in women \( r = 0.78 \) and of the IIEF in men \( r = 0.80 \) (Figure 9a-9e).
Figure 9 Correlations between Scores and fMRI Activity during GSS

Transverse spinal segments reveal activity (highlighted in yellow) a) in the right VH and right IML in females and b) in the DCN and left DH in males. The total score of sexual function is positively correlated with fMRI activity in c) females* (n = 8, r = 0.78) and in e) males* (n = 9, r = 0.80). d) The levels of spinal cord activity in males and females are color-coded and presented in sagittal view. Transverse spinal segments show activity (highlighted in yellow) in f) the right IML and the right VH in females and g) in the DCN in males. Physical sexual arousal and fMRI activity are negatively correlated in h) females* (n = 9, r = -0.95) and positively correlated in i) males* (n = 10, r = 0.86). * Blue circles = heterosexuals, green circles = homosexuals, red circles = participants that did not reach orgasm during AVGSS.
Similarly, in the upper lumbar spinal segments, activity in the DCN in males and in the IML in females was positively correlated with the sexual desire domain of the IIEF (r = 0.83) (Table 2b) and the FSFI (r = 0.83) (Table 3b).

Conversely, while in males activity in the IML and DCN of the upper lumbar spinal segments was positively correlated with physical sexual arousal (r = 0.86), similar activations across identical spinal segments in females were negatively correlated with physical sexual arousal (r = -0.95) (Figure 9f – 9i). These findings are consistent with labeling studies in female rats, which found that the thoracolumbar IML can sometimes impede sexual responses (Neuhuber 1982). Therefore, in human females similarly to females of other species, sympathetic neurons of the thoracolumbar spinal cord likely function primarily to regulate sexual responses, actively restricting them or passively permitting them to occur.

2.4.2.2 Lumbosacral spinal cord

We did not observe similarities between males and females in the lumbosacral SC throughout intermittent self-stimulation. Here, males displayed activity in the DH of the upper sacral spinal segments that was negatively correlated with mental sexual arousal (r = -0.76) (Table 2b) while females demonstrated activity in the VH and IML of the upper lumbar segments that positively correlated with mental sexual arousal (r = 0.86) (Table 3b). This suggests that males who experience heightened sexual arousal during self-stimulation have decreased input to the DH of the sacral spinal segments while in females, increased sexual arousal during stimulation is accompanied by increased descending and sympathetic input to the thoracolumbar cord.

2.4.3 Part 3: Neural correlates of continuous AVGSS

During the third and final part of the study we investigated the effects of simultaneous AVS and GSS culminating in orgasm (See Methods). The details of these results are presented in Tables 1, 2c and 3c.
Here, activity in the lower lumbar segments was positively correlated with the sexual desire domain of the IIEF in males ($r = 0.98$) and of the FSFI in females ($r = 0.90$) indicating that the lower lumbar SC is important in mediating sexual responses leading up to orgasm in humans (Tables 2c, 3c and Figure 10a-10e).

![Figure 10 Correlations between Scores and fMRI Activity during AVGSS](image)

**Figure 10 Correlations between Scores and fMRI Activity during AVGSS**

Transverse spinal segments show activity (highlighted in yellow) in the (a) medial dorsal gray (MDG) in females and (b) in the medial ventral gray (MVG), left DH and right VH in males. Sexual desire is positively correlated with fMRI activity in c) females* ($n = 8$, $r = 0.90$) and in e) males* ($n = 9$, $r = 0.98$). d) The spinal cord levels of activity in males and females are color-coded and presented in sagittal view. Transverse spinal segments show activity (highlighted in yellow) in f) the left DH and left IML in females and g) in the left DH and left SPN in males. h) Mental sexual arousal is negatively correlated with fMRI activity in females* ($n = 9$, $r = -0.94$). i) The total score on the IIEF is negatively correlated with fMRI activity in males* ($n = 9$, $r = -0.90$). * Blue circles = heterosexuals, green circles = homosexuals, red circles = participants that did not reach orgasm during AVGSS.
While females showed lateral and DH activity in the mid-lumbar spinal segments that negatively correlated with mental sexual arousal \((r = 0.94)\), no similar pattern was observed in males. Likewise, while males revealed sacral cord activity that was negatively correlated with the total sexual function score of the IIEF \((r = 0.90)\) (Figure 10f-10i) no similar patterns were observed in females. Notably, female but not male group responses showed increased thoracolumbar and sacral cord activity during continuous sexual stimulation leading up to orgasm (Table 1a-1b) indicating that in general, these structures are involved in female but not male sexual responses leading up to orgasm. This sexual dimorphism can be explained by the presence of spinothalamic neurons in the lower lumbar SC that are integral to the spinal ejaculation generator in male rats (Coolen et al. 2004). Perhaps in men, similarly to male rats, the spinal ejaculation generator in the lower lumbar (but not in the lower thoracic or sacral) SC controls sexual responses culminating in orgasm. However, it is currently unclear whether such a mechanism also exists in females.

Finally, there were no significant correlations between fMRI activity extracted from salient regions of the spinal cord in part 3 and physical sexual arousal in both sexes. In addition, throughout part 3, fMRI activity was not significantly correlated with the erectile function domain of the IIEF and mental sexual arousal in males.

### 2.5 Perceived Sexual Arousal

Women reported significantly greater PMSA than men in response to GSS \((t = 2.15, p = 0.045)\). In addition, women also reported greater PPSA than men in response to GSS. However, this difference did not quite reach statistical significance \((t = 2.05, p = 0.056)\). Men and women did not differ significantly on scores of PPSA and PMSA in response to AVS or AVGSS suggesting that the level of mental and physical sexual arousal experienced by men and women were analogous during those parts of the experiment. Therefore, differences in neural activity
during AVS and AVGSS cannot be attributed to differences between men and women in PPSA and PMSA. Moreover, there were significant differences between PSA ratings corresponding to each of the three parts of the study within each gender group. These results are presented in detail in table 4a-b.

2.5.1 Men

In men, reported PPSA scores were significantly higher in response to AVGSS than AVS ($t = 5.30, p = 0.0005$) and GSS ($t = 5.30, p = 0.0005$). Similarly, reported PMSA scores were significantly higher in response to AVGSS than AVS ($t = 4.43, p = 0.0016$) and GSS ($t = 5.5, p = 0.0004$). However, there was no significant difference between PPSA or PMSA scores in response to AVS compared to GSS. These results are presented in detail in table 4c-d.

2.5.2 Women

In women, PPSA scores were significantly higher during AVGSS compared to AVS ($t = 6.32, p = 0.0002$). Similarly, PMSA scores were significantly higher during AVGSS than AVS ($t = 3.82, p = 0.005$) and GSS ($t = 2.79, p = 0.02$). PPSA scores were significantly higher during GSS than AVS ($t = 4.89, p = 0.001$) but PMSA scores did not differ significantly from GSS to AVS in women. These results are presented in detail in table 4e-f.

2.6 Reported Orgasms

Nine men and seven women reached orgasm in response to AVGSS. The participants reported orgasm as soon as it occurred by stating “done” or “finished” into the microphone positioned inside the bore of the magnet. All the homosexual participants reached orgasm during the experiment. Anecdotally, all the individuals who did not reach orgasm happened to participate in our study during the time of final exams. Perhaps this is an example of when anxiety and stress, associated with the sympathetic nervous system, can negatively impact on sexual SC circuits and inhibit orgasm in men and women. Generally, participants who did not reach orgasm reported lower ratings of PPSA and PMSA and also tended to score lower on the IIEF and FSFI.
This tendency may indicate that sexual function remains stable in the course of 4 weeks. However, because the number of individuals that did not reach orgasm following AVGSS is quite small (n = 3), it is difficult to make any conclusive statements. The man who did not reach orgasm stated that the erotic films presented during the study were not sexually arousing. Moreover, he demonstrated strong increased activity in the lumbar SC and highly rated PMSA in response to AVS.

One of the women who did not reach orgasm attributed her inability to orgasm to issues with comfort and privacy during the experiment. She revealed average signal intensity changes and sometimes no change in signal at all during stimulation. This woman did not report experiencing pain during sexual intercourse and consistently reported average PPSA and PMSA ratings during AVS, comparable to other women that had reached orgasm. Conversely, the second woman that did not reach orgasm during AVGSS consistently reported lower ratings of PPSA and PMSA and also demonstrated lower scores on the FSFI. Moreover, this woman reported frequently experiencing pain during sexual intercourse and consistently demonstrated low signal intensity changes (in positive correlations between fMRI activity and scores) and high signal intensity changes (in negative correlations between fMRI activity and scores).

We included individuals that did not orgasm in the group analysis of AVGSS because we were interested in elucidating individual differences in sexual responses. Furthermore, we reasoned that it would be interesting to observe what regions of the SC are associated with decreased sexual responses in humans. As well, the main focus of our investigation was combined audiovisual and genital sexual stimulation, while orgasm was secondary for our purposes. By including the three participants who did not orgasm in the AVGSS group analyses, we are by no means equating the summation effect obtained from the combined application of AVS and GSS, with the effect of orgasm on the neural circuitry. In fact, the summation effect and the effect of orgasm may involve physiologically different phenomena and may recruit different neuroanatomical pathways. Nevertheless, although the AVGSS condition is not specific to
orgasm, the AVGSS activity maps certainly include orgasm in the great majority of people studied.

Table 4  PPSA and PMSA in Men and Women

Comparison of A) perceived physical sexual arousal (PPSA) and B) perceived mental sexual arousal between men and women across the three experimental conditions. C) Comparisons of PPSA in men during AVS, GSS and AVGSS. D) Comparisons of PMSA in men during AVS, GSS and AVGSS. E) Comparisons of PPSA in women during AVS, GSS and AVGSS. F) Comparisons of PMSA in women during AVS, GSS and AVGSS. 1 Corrected for multiple comparisons. * Statistically significant. ** Very statistically significant.

2.7 Discussion

We have described the areas of neuronal activity in the SC that mediate human sexual responses elicited by AVS, GSS and AVGSS culminating in orgasm. The primary finding of this study is that neuronal activity associated with sexual responses in the healthy human SC can be mapped, for the first time, with spinal fMRI. In addition, to the best of our knowledge, this is the
first time that autonomic responses have been successfully mapped in humans with fMRI, as indicated by the extensive activity detected in the lateral horn of the thoracolumbar and sacral SC. Importantly, we also detected DH and VH activity across all three parts of the study. The DH of the SC receives ascending sensory input from the periphery while the VH mediates descending motor input from the cerebrum (Blumenfeld 2002; Nolte 2009). This signifies that the sum of sensory, motor, somatic, and autonomic inputs are integral to sexual responses in the human SC. Furthermore, the observed activity in response to the sexual stimuli did not selectively involve only areas ascribed to psychogenic (thoracolumbar) or reflexogenic (sacral) functions. Therefore, we conclude that psychogenic and reflexogenic components of the sexual response may not be separable, but are necessarily integrated in able-bodied men and women. However, there is a possibility of order effects since GSS was always preceded by AVS. Thus, it is probable that the effects of viewing erotic film clips during AVS contributed to the SC activity observed in response to GSS.

To the best of our knowledge, we are the first to demonstrate the specifics of SC of sexual responses in healthy humans. It is noteworthy that the majority of activity in the SC was found in sympathetic and parasympathetic preganglionic nuclei, indicating that the autonomic nervous system plays an important role in the regulation of sexual responses in humans. Furthermore, the great majority of activity observed in the SC in women was positive while men revealed predominantly negative activity in the same SC segments (Figure 5 - 7). It is interesting that as the experiment progressed closer to orgasm, women revealed less descending input as indicated by decreased activity in the VH. A similar pattern emerged in men such that the greatest increased activity was observed during AVS and subsequently declined until all activity observed in the SC was decreased. Certain sites in the brainstem are notorious for their role in the inhibition of SC circuitry involved in sexual function (Marson and McKenna 1992). Hence, decreased input to the SC in men may indicate decreased inhibition from regions of the brainstem.
Various regions of the SC involved in sexual function in humans revealed signal intensity changes that were powerfully correlated (negatively in some cases) with subjective scores of mental and physical sexual arousal, and with validated measures of sexual function (IIEF and FSFI), at the time of each study (Tables 2 and 3). Although fMRI activity maps do not differentiate between excitatory or inhibitory input to a particular region, we can infer whether the input to a specific region facilitated or blocked sexual responses based on whether activity in a given region was positively or negatively correlated with a specific measure of sexual function.

In men as compared to women, a greater number of SC regions salient to sexual responses, revealed activity that was positively correlated with measures of sexual function (Tables 2 and 3) although men and women reported analogous levels of PSA except during GSS when women reported significantly greater PSA than men (Table 4a – 4b). This may in part be due to the presence of sexually dimorphic structures/pathways that facilitate sexual responses in males but block them in females. A number of sexually-dimorphic spinal structures have been described which comprise the spinal ejaculation generator (Truitt and Coolen 2002; Coolen et al. 2004). Moreover, these neurons are localized to the DGC, ON and SPN in the lower lumbar and sacral SC and directly mediate erection and ejaculation in male rats (Sakamoto et al. 2008). However, in female rats, these nuclei are less well understood and thought to be vestigial (Sakamoto et al. 2008). Our results suggest that the sexual dimorphism in rats can be extended to humans. Another possible explanation for a greater number of positive correlations between activity in the SC and scores of sexual function in men as compared to women is that men demonstrated much more decreased input to the SC than women. Indeed, women revealed mostly increased activity in the SC and also a number of negative correlations between this activity and scores of sexual function indicating that increased input to the SC in women suppresses sexual responses.

However, there is also evidence of facilitation of spinal sexual responses in females. Recent studies have found that climax-like responses in female rats are controlled by the pudendal motor nerve in response to stimulation of pudendal afferents, which innervate the SC at the L5-S1
segments and terminate in the DGC, SPN and medial dorsal horn (Cai, Alexander, and Marson 2008; Wiedey, Alexander, and Marson 2008). In line with these studies, we observed increased activity in the medial dorsal horn of the lower lumbar spinal segments in females during part 3, which was positively correlated with the sexual desire domain of the FSFI \((r = 0.90)\) (Table 3c, Figures 10a and 10c). In addition, females but not males showed increased activity in the right SPN and ON throughout continuous audiovisual and self-stimulation culminating in orgasm (Table 1). The sacral cord activity in women throughout this part is notable as it may be related to the theory that the neurological occurrence of orgasm is a reflex response involving the sacral spinal cord (Sipski et al. 2001). Taken together, these findings suggest that the ON, SPN and DGC in humans, as in other animals, contain populations of sexually-dimorphic neurons in the sacral SC.

A similar pattern of sexual-dimorphism emerged in the thoracolumbar SC such that activity in the DCN and IML was predominantly positively correlated with measures of sexual function in males but primarily negatively correlated with measures of sexual function in females. This finding is consistent with those of previous psychophysiological studies which have revealed a strong correlation between male sexual arousal and penile circumference, but a poor correlation between female psychogenic sexual arousal and increases in vaginal blood flow (Chivers and Bailey 2005). Perhaps, similarly to the SPN and DGC, the IML and DCN in the thoracolumbar SC also contain sexually-dimorphic populations of neurons that are early androgen-dependent thereby enhancing sexual responses in men but not in women. However, this idea requires further investigation.

**2.8 Conclusions**

We have advanced the understanding of the neural circuitry of human sexual responses in five important ways. First, spinal fMRI is a technique which permits, for the first time, observations of the SC regions that mediate sexual responses in humans. Secondly, using spinal fMRI, we are the
first to map autonomic nervous system function in humans as evidenced by the extensive activity
detected in the lateral horn of the thoracolumbar and sacral spinal cord. Thirdly, our results reveal
the presence of sexual dimorphism in several spinal structures including the IML, DCN, SPN,
DGC and ON and this is in line with previous work in animals. Fourthly, we have shown that the
sympathetic and parasympathetic components of sexual responses are integrated and cannot be
functionally separated. Finally, not only can we see differences between men and women but we
can see individual differences in sexual responses as well.

Future research needs to examine the functional connectivity between spinal, brainstem and
higher brain structures mediating human sexual responses. A clearer understanding of these
functional networks will enhance the knowledge of normal sexual function and reveal the
changes that occur, in individuals, following SC trauma or disease.
Chapter 3: General Discussion

3.1 Main Findings

The primary finding of this research is that spin-echo proton-density-weighted spinal fMRI can reveal activity in the human thoracic, lumbar and sacral SC in response to sexual stimuli. To the best of our knowledge, this is the first time that autonomic activity has been detected in the human SC with fMRI as is evident by the large number of activations observed in the lateral horn of the thoracolumbar and sacral SC in men and women. This finding indicates that spinal fMRI is a sensitive and reliable technique that can be used to visualize autonomic inputs to the SC, in addition to sensory and motor inputs which has been shown previously. Moreover, the observed activity in the SC in response to sexual stimulation in humans is in accord with neuronanatomical locations previously described in rodents suggesting that SC circuits mediating sexual responses are very similar across species.

Throughout AVS, men and women revealed activity in the preganglionic sympathetic (IML and DCN) and parasympathetic (SPN) nuclei in the SC. Activity was also observed in the VH and DH corresponding to descending and ascending neuronal input respectively. We have shown that activity in the SC spanning the TL – mid-lumbar segments is strongly, positively correlated with PPSA and PMSA in men and women. Notably, signal intensity changes in the SC were predominantly found in the lower thoracic and lumbar SC in men and women during AVS, supporting the role of the TL pathway in mediating psychogenic sexual responses. Throughout each experimental condition and particularly during AVS, women revealed a greater number of inverse correlations between activity in the lower thoracic, lumbar and sacral SC and measures of sexual function than men. In addition, women demonstrated predominantly increased activity in the autonomic preganglionic SC nuclei while men revealed mostly decreased activity in these regions, associated with decreased descending modulation. Inverse correlations observed between activity in the autonomic preganglionic SC nuclei and measures of sexual function in men and
women are likely due to NPGi-mediated descending inhibition of spinal sexual reflexes. Descending projections from the NPGi are serotonergic (McKenna and Marson 1997) and a large body of evidence implicates the neurotransmitter serotonin in the regulation of sexual responses in humans, via inhibitory mechanisms (Marson, List, and McKenna 1992; Giuliano and Clement 2005). Serotonin is also abundant in the thoracolumbar and lumbosacral SC (Giuliano and Clement 2006), and is mainly inhibitory to sexual responses in male (Hull, Muschamp, and Sato 2004) and female rats (Marson, cai, and Makhanova 2003). Similarly, selective serotonin reuptake inhibitors are known to delay ejaculation in men (Waldinger and Olivier 2004; Waldinger, Zwinderman, and Olivier 2004).

Throughout GSS, men and women demonstrated increased activity in the thoracolumbar IML. Men demonstrated decreased activity in the lumbar DCN, sacral SPN and DH, indicative of decreased input to these regions while women revealed decreased activity in the thoracic and lumbar VH, specifically associated with decreased descending input. In spite of its role in reflexogenic sexual responses, there was very little activity in the sacral SC in women during GSS, perhaps due to order effects. AVS always preceded GSS in our experiments and thus AVS-related activity may have contributed to the signal intensity changes observed during GSS.

Throughout AVGSS, women demonstrated consistently decreased activity in the VH, associated with diminished descending modulation, and increased activity in the thoracolumbar and lower lumbar IML, related to increased autonomic input. Furthermore, women demonstrated increased activity in the sacral SPN, in the absence of activity in the VH, supporting the theory that the sacral reflex arc is involved in sexual responses culminating in orgasm in women (Sipski et al. 2001). Indeed, the sacral reflex arc plays a key role in orgasm in women with SCT (Sipski et al. 2001; Sipski and Arenas 2006). In addition, it has been shown that stimulation of the sensory branch of the pudendal nerve or urethral distention in anesthetized, spinally transected female rats can elicit vaginal vasodilation and climax-like responses (Cai et al. 2008). Pudendal sensory nerve stimulation results in significant increase in c-fos positive cells in the ipsilateral
dorsal horn and bilaterally in the medial, lateral and intermediate gray of L5-S1 (Wiedey et al. 2008), and this pattern of activation is similar to that observed in men and women during AVGSS. Conversely, bilateral lesions of the pudendal nerve, which innervates Onuf’s nucleus in the ventral horn in humans (Levin and Riley 2007), abolish climax-like responses in spinally-transected female rats (Cai et al. 2008), indicating that in the absence of descending modulation, orgasm becomes a reflex response that is mediated by the sacral SC. In an intriguing report, Meloy (2007) describes a serendipitous discovery which he termed “neurally augmented sexual function”. Meloy discovered that by positioning electrodes approximately at the level of the cauda equina, in close proximity to the sacral SC and pelvic and pudendal nerves, can elicit pleasurable sensations, akin to orgasm, in adult women suffering from inorgasmia (Meloy 2007). This discovery provides further support to the importance of the sacral SC, situated in close contact with the cauda equina and the genitospinal nerves, in mediating orgasmic responses in women.

Similarly, decreased activity in the lower lumbar DCN in men supports the prospect of a spinal cord ejaculation generator. The NPGi sends direct inhibitory projections to the lower lumbar DCN (L3-L5) (Marson and McKenna 1992) which forms part of the spinal cord ejaculation generator in male rats (Truitt and Coolen 2002). Decreased activity in the lower lumbar DCN in men indicates decreased descending modulation of SC circuits associated with sexual responses culminating in ejaculation and orgasm. Furthermore, decreased activity in the sacral DGC in the absence of activity in the VH supports the idea that the sacral reflex arc is associated with sexual responses culminating in orgasm in men, as in women. Indeed, spinal afferents that mediate ejaculation travel in the pudendal nerve and dorsal nerve of the penis terminating in the DGC and DH of the lumbosacral SC in male rats and rhesus monkeys (McKenna and Nadelhaft 1986; Ueyama, Arakawa, and Mizuno 1987; Ropolo, Nadelhaft, and de Groat 1985). Furthermore, men with complete SCT that afflicts the sacral SC are significantly
less likely to reach orgasm than men with an intact sacral SC (Sipski et al. 2006) and the same is true for women with SCT (Sipski et al. 2001).

We observed activity in the lower lumbar SC in men and women during AVGSS that was powerfully positively correlated with the sexual desire domain of the IIEF and FSFI. This suggests that the lower lumbar SC in men and women facilitates AVGSS. Indeed, a cluster of spinothalamic neurons (LSt cells) located between the 3rd and 4th lumbar segments comprise the spinal ejaculation generator in male rats (Truitt and Coolen 2002; Coolen et al. 2004). The LSt cells are activated specifically following ejaculation but not other components of the sexual response and their lesion completely abolishes ejaculation (Coolen et al. 2004). Moreover, LSt cells project to the SPFp in male rats (Coolen et al. 2004). In turn, the SPFp conveys ejaculation-specific information and may also be involved in relaying the pleasurable sensations associated with orgasm to the brain (Coolen et al. 2004).

3.2 Individual Variability in Sexual Responses

While fMRI activity maps can reveal what neural regions are associated with AVS, GSS and AVGSS, they cannot differentiate between excitatory and inhibitory inputs. This drawback makes it difficult to interpret fMRI data. In order to elucidate the nature of the SC signal intensity changes, we tested for correlations between fMRI activity and perceived sexual arousal ratings obtained from participants after each experimental condition. Positive significant correlations indicated that increased input to a particular area is associated with increased sexual arousal. On the other hand, a negative correlation suggested that increased input to a given neuronal region is associated with lower ratings of sexual arousal. Thus, we were able to determine whether neuronal input to a particular region facilitates or blocks sexual responses. We observed many inverse correlations between activity in the SC and scores of sexual function indicating that much descending input to the spinal cord is regulatory to sexual responses. Not only was this regression analysis useful in distinguishing neuronal inputs that block from those that facilitate sexual
responses in humans, but it gave us an opportunity, for the first time, to tap into the individual differences in sexual functioning at the level of the lower thoracic, lumbar and sacral SC in humans. The advantage of this type of analysis is that while it explores the relationship between neuronal input and scores of sexual functioning at the group level, it also preserves each person’s individual activity and corresponding sexual function score. Consequently, it can be used as a powerful clinical tool to determine each individual’s sexual function ‘profile’ that takes into account not only self-reports but also the patterns of neuronal activity associated with them.

We utilized the block design to reveal the magnitude of signal intensity changes in response to each stimulation paradigm. The block design proved to be very suitable for our purposes because it correlated quite well with perceived physical and mental sexual arousal and scores of sexual function. We conclude that the block design is well-suited for detecting neuronal activity in the SC associated with sexual responses. While it is true that the sexual response is not comprised of on/off events, our block design allowed for sufficient time in the stimulation periods for the sexual response to ‘turn on’ and enough time in the baseline for it to ‘turn off’.

3.3 Limitations

One important limitation is that the experiment comprised individuals with different sexual orientations and handedness, potentially confounding variables. Another limitation of this work is associated with reliability of spinal fMRI. While the reliability of spinal fMRI is very high for group data, the reliability of individual fMRI experiments is reduced by the effects of Type 1 and Type 2 errors (Stroman 2006). Type 1 error is defined as rejecting the null hypothesis when it is actually true (Huettel et al. 2004) and in fMRI occurs when voxels that do not represent real signal intensity changes, are activated (false-positive results). FMRI activity that represents a Type 1 error is commonly found outside of the spinal cord in the CSF and can easily be distinguished from real activity found inside the spinal cord (Stroman 2006). Cardiac and CSF-related motion of the SC contributes to activity found outside of the cervical and upper thoracic
SC (Stroman 2005). However, recent evidence indicates that motion of the lower thoracic, lumbar and sacral spinal cord is negligible (Figley et al. 2008). Thus, activity observed outside of the SC in chapter 2 is likely due to slight arm, hand and body movements of the participants during self-stimulation and orgasm. We attempted to decrease movement as much as possible by ensuring participants were very comfortable prior to scanning. We raised the arms and legs with padded cushions for extra support. We also attached a flexible elastic band over the midsection of each participant which served more as a reminder not to move rather than as a restraint. The vibratory stimulator also helped to reduce movement during the experiment by eliminating the need for rhythmic movements of the hand in women during GSS. However, the hand-held vibrating stimulator was designed such that each women could manipulate the angle and intensity of self-stimulation applied to the genital area. Thus, both men and women used their hands to some extent in order to perform self-stimulation. Any activity arising in the SC as a function of hand or arm movement would not be detected in our images as the nucleus cuneatus, representing the arm region of the medial lemniscus pathway (Nolte, 2009), is located in the caudal medulla. In addition, during data analysis, bulk motion correction was applied to each SC in the time-series data by aligning subsequent images of the SC with the initial image (Stroman et al. 2008a). Motion correction was applied in the sagittal orientation in order to ascertain that different slices of the SC, acquired at various times in the series, are not pooled together. Custom-made software written in MatLab (The Mathworks Inc., Natick, MA) was used for motion correction and entailed manually drawing a reference line along the anterior edge of the SC in a midline slice extending from the cauda equina to the T7 vertebral body. Next, two position reference points were marked, one at the conus medullaris and the other at the T8/T9 vertebral disc. Furthermore, spatial smoothing was applied in the rostrocaudal direction in order to prevent partial volume effects (Stroman et al. 2008a).

In order to further decrease the occurrence of Type 1 errors, it is advantageous to recruit a greater number of subjects per group. Indeed, consistent fMRI activity across many subjects
retains fewer erroneously activated voxels and more closely represents real neuronal activity. To further reduce Type 1 errors, a rigorous statistical threshold ($p < 0.0001$) was applied to the individual analyses of fMRI activity in the spinal cord. Hence, the fMRI maps show only statistically significant activity. Type 2 error is defined as accepting the null hypothesis when it is actually false (Huettel et al. 2004) and in fMRI occurs when voxels that represent real signal intensity changes are not activated (false-negative results) (Stroman 2006). It has been shown that including the peripheral pulse traces in the GLM analysis reduces type 2 errors and considerably improves sensitivity and reliability of the fMRI data (Stroman 2006). Furthermore, the RESPITE basis set incorporated into the GLM analysis, effectively models physiological noise associated with SC motion, CSF flow and heart-beat. Thus, signal intensity changes associated with the stimulus paradigm may be distinguished from physiological noise. Including RESPITE in the GLM analysis has decreased instances of type 1 as well as type 2 errors (Figley and Stroman 2009).

The different modes of self-stimulation utilized by men and women in our experiments represent another important limitation. While women used an MRI-compatible vibratory stimulator for clitoral self-stimulation, men self-stimulated manually during GSS and AVGSS. Thus, it is possible that some of the resultant differences in fMRI activity between men and women may be due to the different modes of self-stimulation between the sexes. Vibration stimulation is an optimal method to induce orgasm in individuals with SCT (Sipski et al. 2005) but not in healthy men. Because it is our intention to compare data collected from individuals with SCT and healthy control volunteers, the women in our experiments used the vibrating stimulator. Conversely, healthy men who used the vibrating stimulator in a pilot study found it to be an ineffective means of self-stimulation and opted for manual self-stimulation instead.

Another limitation of our work is associated with the possibility of order effects in our fMRI data. Specifically, the three experimental conditions that comprised our studies were not randomized, possibly contributing to order effects. Viewing erotic film clips first may have
contributed to the activity observed during genital self-stimulation. In addition, our choice of baseline condition was not ideal because we cannot rule out the effects of emotional/affective components associated with viewing erotica. Previous fMRI experiments of sexual function have used sports clips (Ferretti et al. 2005) and short videos of therapeutic massage (Hamann et al. 2004) as a baseline condition in order to account for the effects of watching people interacting in a non-sexual manner. Because we used a blank screen as a baseline condition, we cannot be certain that the observed activity in the spinal cord and brainstem is specifically related to sexually-salient stimuli. Thus, the observed activity in the spinal cord and brainstem probably corresponds to the sexual stimuli as well as to watching human beings interact.

Lastly, our study design could be improved by separating the event of orgasm from the AVGSS condition. This would enable us to more precisely distinguish which regions of the spinal cord are activated specifically during orgasm, during AVGSS, and which SC regions respond during both conditions. Moreover, including an ‘imitation’ condition in which participants mimicked the rhythmic hand and body movements associated with GSS and orgasm, similarly to the procedure adopted by Georgiadis et al. (2006), would enable us to control for the effects of motion during GSS and AVGSS.

3.4 Future Directions

Future studies should use spinal fMRI to investigate sexual responses in the SC in men and women with SCT. Our results from healthy men and women can be used as a control in such studies. Comparing the patterns of neuronal activity associated with sexual responses in healthy individuals and people with SCT will advance our understanding of how sexual function is altered by trauma. In turn, this will enable practitioners and clinicians to devise more effective treatments for sexual dysfunction following SCT or disease.
Chapter 4

4.1 Summary and Conclusions

This project represents the first demonstration of neuronal activity in the human SC in response to sexual stimulation. Utilizing spinal fMRI, we have demonstrated the regions of the SC that are involved in AVS, GSS and AVGSS in healthy men and women. Thus, spinal fMRI is a sensitive and reliable technique that can reveal neuronal activity associated with sexual responses in humans.

Chapter 2 describes the first non-invasive demonstration of autonomic nervous system activity in the human spinal cord during sexual responses. Our results indicate that the autonomic nervous system is closely associated with spinal sexual responses in humans. Indeed, most of the signal intensity changes observed in response to AVS, GSS and AVGSS were localized to sympathetic and parasympathetic preganglionic nuclei in the thoracolumbar and sacral SC. Furthermore, we observed interesting gender differences in the SC whereby men revealed predominantly decreased activity in the SC while women showed mostly increased activity in analogous SC regions. In addition, women demonstrated more inverse correlations between SC activity and scores of sexual function than men. We propose that there is greater descending modulation of spinal sexual responses in women than in men, particularly during AVS. This descending modulation is likely initiated by serotonergic projections from the NPGi to sympathetic preganglionic neurons in the thoracolumbar SC in women. On the other hand, decreased input observed in the SC in men suggests decreased descending modulation of spinal sexual responses and is corroborated by the fact that most correlations between SC activity and scores of sexual function were positive in men.
The ability to conduct effective, non-invasive research of the human SC during sexual responses opens up an expanse of opportunities for researchers to utilize spinal fMRI in continuing to study sexual function and dysfunction in humans. Due to the difficulty of examining neuronal activity in the human SC, this area of research has been an unchartered domain – until now. We believe that spinal fMRI can provide greater insight into the regions of the SC that mediate sexual responses in humans and will enable researchers to make important discoveries that will benefit the sexual future of humankind.
References


Li, G., Ng, M. C., Wong, K. K., Luk, K. D., & Yang, E. S. (2005). Spinal effects of acupuncture stimulation assessed by proton density-weighted functional magnetic resonance imaging at 0.2 T. *Magnetic Resonance Imaging*, 23, 995-999.


Appendix A Information/Consent Form

TITLE OF PROJECT:
The Development of Spinal fMRI to Assess Sexual Sensations after Spinal Cord Injury

BACKGROUND INFORMATION: (Overview of study)
You are being invited to participate in a research study directed by Patrick Stroman at Queen’s University in collaboration with Dr Marca Sipski from the University of Alabama at Birmingham. The research involves magnetic resonance imaging (MRI) of healthy volunteers between the age of 18 and 50. The purpose of this study is to develop and improve new methods for using functional magnetic resonance imaging (fMRI) to map where the spinal cord functions to relay information between the brain and the body with particular tasks or sensations, which will later be applied to assess spinal cord injury and to monitor effects of treatment or therapy. The current stage of the research is to determine the function in the brainstem that is related to sexual stimulation as a result of viewing an erotic movie, and that is related to self-stimulation to orgasm. The results obtained from healthy volunteers will provide the baseline for the next stage of studies in spinal cord injured people. Participation in the study involves one visit to the Queen’s fMRI Facility in the lower level of the Cancer Research Institute, and will take about 2 hours.

AIM
The aim of this study is to develop the procedures to map function in the brainstem and brain by means of fMRI during sexual stimulation in able-bodied individuals.

DETAILS OF THE STUDY

1. Description of visits, and tests to be performed as part of the study
If you agree to participate, your brain will be imaged while you are lying in a magnetic resonance imaging (MRI) scanner in the Queen’s fMRI Facility, and your heart beat will be monitored using entirely non-invasive methods. The entire session may last up to 2 hours including getting ready for the study and positioned in the magnet etc. This study involves a single visit to the lower level of the Cancer Research Institute for imaging.
a) You will begin by filling out a checklist and questionnaire to make sure you are eligible to participate in MRI experiments. This will be completed first, and will take about 5 minutes. If you are pregnant or are trying to conceive you will not be eligible. If there is any uncertainty regarding whether or not you are pregnant and you want to participate in the study, a pregnancy test must be done prior to the experiment.

b) You will then be asked to complete a brief history report, including questions related to sexual functioning, in order to determine that you are free from medical, psychiatric or neurologic impairment which could impact upon the study findings.

c) Prior to the scan, the vibrating stimulator that you may use for self-stimulation during the study will be shown to you, and you will be able to turn it on to test it. This is an MRI-compatible device that only works in the magnetic field of the MRI system.

d) Please try to wear clothing containing no metal, or to bring a change of clothing. Metal in zippers, snaps, and the wire and metal clasps in some bras can interfere with the imaging. Many shoes contain metal as well. You will be asked to remove or change out of any clothes that contain metal that will be near the area being imaged, and you will be asked to remove your shoes.

e) You will be asked to wear earplugs to protect your ears from the noise of the magnet, and you will also be provided with head-phones for communication and for audio presentation.

f) You will be asked to lie on your back on the well-padded bed of the magnet. Pillows will be placed under your legs for comfort and a blanket will be placed over your legs if you wish.

g) Your heart-beat will be monitored with a small device that is clipped onto one finger, and uses light to sense your blood flow. Your breathing will be monitored with a belt containing a flexible air-filled tube that will be placed around the lower portion of your chest.

h) Your head will rest in a support that is fitted with a mirror so that you can see a rear-projection screen outside of the head end of the magnet where visual presentations will be projected.

i) Once you are comfortably positioned, you and the bed will then slide into a 2 meter long tube (the magnet) until your lumbar spinal cord is at the center. This will place your head
approximately 2 feet from the opening at one end of the magnet, and your feet sticking out the other end.

j) You will need to keep still at all times while the images are taken, and to not change position between sets of images. To help you, we will make you as comfortable as possible and we will pack soft foam around your head if needed.

k) The MR system has a two-way intercom for communication. During the imaging to map the function in your brain the MRI system is quite loud, and the intercom will be turned off. However, you will also be given a squeeze ball that you can squeeze if you want to talk to the operator.

l) All functional MRI studies require periods of rest interleaved with periods of sensation or activity so that we can detect the differences in the spinal cord that show where there was activity. There will be three parts to the experiments that we will do. After we do a few quick images to check your position, the first part of the study will involve continuous MR imaging of your brain, while you watch a non-erotic film for several minutes, followed by an erotic film for several minutes, and then the film will be switched back to the non-erotic one, and the cycle will be repeated. This will continue for a total of 16 minutes. At the end you will be asked via the intercom to provide a rating of your mental level of arousal, and another rating of your genital level of arousal, on a scale from 1 to 10. The second part of the study will start with an initial 2 minute baseline period, while you rest and take no action, and then you will be prompted via instructions on the rear-projection screen to stimulate yourself with the vibrating stimulator for 1 minute, followed by 1 minute of rest/recovery when you rest again, and then the cycle will repeat for a total of 10 minutes. At the end of the session, you will again be asked via intercom to provide a rating of your level of mental and genital arousal. During the third and final part of the experiment, images of the brain will again be acquired continuously. After an initial two minute baseline period you will be prompted to self-stimulate until you reach orgasm, up to a maximum of 30 minutes. You will be asked to indicate by squeezing the squeeze-ball when you are about to experience orgasm, and then imaging will be continued for another 3 minutes of baseline afterward.

m) At the end of the study (approximately 1.5 hours) you will be removed from the MRI system.
2. MRI Procedures

The MRI scanning procedure is very much like other medical imaging used in hospitals, but you will not be exposed to x-rays. This MRI machine uses a strong magnet and radio waves to make images of the interior of your body, based primarily on the water content. You will not feel the magnetic field or the radio waves. The MRI being used in this study is a 3 Tesla MRI that is twice that used for most clinical imaging, although 3 tesla systems are becoming more common in hospitals. The levels of magnetism and radio waves used in the MRI have not been shown to cause harmful effects. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic objects must be removed from your person before you approach the scanner. If you have a cardiac pacemaker or a metallic clip in your body (e.g., an aneurysm clip in your brain or an I.U.D.) you should not participate in any MRI study. In addition, credit cards and other cards with magnetic strips should also be removed as these will be damaged. (These items will be kept safe for you).

You will be in audio contact with the operator except during imaging, and you will have a squeeze-ball to signal the operator at any time. You may ask the operator to stop the experiment at any time. While you are in the MRI system we will not be able to see or hear you, you will have privacy. You should ask to stop the experiment if you feel tired, claustrophobic, or uncomfortable.

3. What are the risks of participating in the study?

There are no known risks involved with magnetic resonance imaging. However, the MR scanner uses a very strong magnet that will attract metal. Therefore ALL metallic or magnetic objects must be removed from your person before you approach the scanner.

4. What are the benefits of participating in the study?

You will not get a personal medical benefit from participating in this study but your participation will help us to improve functional imaging of the brain.

5. Exclusion criteria

Do to the very high magnetic field you should not be a subject in any MRI experiment if any of the following apply to you:

a) have a history of head or eye injury involving metal fragments.
b) have ever worked in a metal shop

c) have some type of implanted electrical device (such as a cardiac pacemaker or neurostimulator)

d) have implanted metal objects as a result of surgery such as artificial joints, aneurysm clips, metal staples

e) have severe heart disease (including susceptibility to arrhythmias) or any other serious illness

f) have non-removable jewelry (body piercing)

g) are, or may be, pregnant

h) are left-handed

i) are homosexual or bisexual

j) have never viewed pornographic films before

k) have never engaged in masturbation before

l) have a history of brain or spinal cord injury

m) have a history of psychiatric disorder (e.g. major depression)

n) are currently taking medication to treat anxiety or depression or any other condition

o) have never engaged in sexual intercourse with a partner

p) have had any surgery performed to the genitals (except male circumcision)

q) experience pain during sexual intercourse

6. Confidentiality

The findings of this study will be reported in scientific journals but your name will remain confidential. Data from your images will be stored on a secure computer system and identified only with the date and a subject code. Only the researchers directly related to this study will have access to the data files and the subject codes. You will not be identified in any publication or reports.

Although this is not a diagnostic scan and any images obtained are for research purposes only, it is possible that the MR scan may disclose an unknown abnormality. In this event, a medical imaging specialist will be asked to review the images and we would send a report to your physician. The researchers directly involved with this procedure do not have the credentials to diagnose medical conditions.
7. **Voluntary nature of study/Freedom to withdraw or participate**

Your participation in this study is voluntary. You may withdraw from this study at any time before, during or after, and your withdrawal will not affect your future medical care, academic standing, or career.

8. **Withdrawal of subject by principal investigator**

The study Director may decide to withdraw you from this study if:
1) You do not meet the criteria in the Magnetic Resonance Screening Form.
2) You are unable to perform the tasks requested.

9. **Liability**

"In the event that you are injured as a result of taking study medication or of the study procedures, medical care will be provided to you until resolution of the medical problem.

By signing this consent form, you do not waive your legal rights nor release the investigator(s) and sponsors from their legal and professional responsibilities."

10. **Compensation**

You will receive $50 to cover your costs for parking, transportation to Queen’s, etc, for participating in this study.
SUBJECT STATEMENT AND SIGNATURE SECTION:

I have read and understand the consent form for the study entitled: The Development of Spinal fMRI to Assess Sexual Sensations After Spinal Cord Injury. I have had the purposes, procedures and technical language of this study explained to me. I have been given sufficient time to consider the above information and to seek advice if I chose to do so. I have had the opportunity to ask questions which have been answered to my satisfaction. I have named Dr. ________________ at ________________ as the physician to be contacted for follow-up purposes. I am voluntarily signing this form. I will receive a copy of this consent form for my information. If at any time I have further questions, problems or adverse events, I can contact

Dr. Patrick Stroman (P.I.)

Centre for Neuroscience Studies
Queen's University
Kingston, Ontario
K7L 2V7
Phone: (613) 533-3245
Fax: (613) 533-6840

If I have questions regarding my rights as a research subject I can contact
Dr. Albert Clark, Chair, Research Ethics Board at Queen’s Univ. (1613)533-6081

By signing this consent form, I am indicating that I agree to participate in this study.

_______________________  ______________________
Signature of Volunteer          Date

_______________________  ______________________
Signature of Witness           Date

STATEMENT OF INVESTIGATOR:
I, or one of my colleagues, have carefully explained to the subject the nature of the above research study. I certify that, to the best of my knowledge, the subject understands clearly the nature of the study and demands, benefits, and risks involved to participants in this study.

_________________________________________  _______________________
Signature of Principal Investigator  Date
Participant Consent Form

Project title: The Development of Spinal fMRI to Assess Sexual Sensations After Spinal Cord Injury

I have read the Letter of Information, have had the nature of the study explained to me, and I agree to participate. All questions have been answered to my satisfaction.

Subject Name (please print): ____________________________________________

Signature: __________________________________ Date: ________________

________________________

Individual responsible for obtaining consent:

Signature: __________________________________ Date: ________________

________________________

Investigator:
Signature: ______________________________ Date: ______________
Appendix B MR Safety Checklist

Centre for Neuroscience Studies

**MAGNETIC RESONANCE (MR) IMAGING SAFETY CHECKLIST FOR RESEARCH SUBJECTS**

This MR system has a very strong magnetic field of 3 Tesla. It may be hazardous to individuals wearing the magnet room if they have certain metallic or electronic objects. Therefore, all individuals are required to fill out this form BEFORE entering the magnet room. Be advised, the magnet is ALWAYS ON. This questionnaire must be completed accurately to ensure safety. An answer of "Yes" in a category may not necessarily exclude you from entry into the MRI or its vicinity.

Name: ____________________________ Age: ________ Weight: ________ Height: ________

**Please Circle:**

- Have you had prior surgery or an operation of any kind? Yes No
- Have you had an injury to the eye involving a metallic object (e.g., metallic suture, foreign body)? Yes No
- Have you ever been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.)? Yes No
- Are you pregnant or suspect you are pregnant? Yes No
- Do you have any history of claustrophobia, panic attacks, or seizures? Yes No
- Do you have any history of heart disease (e.g., arrhythmias, heart attack, etc.)? Yes No

**WARNING:** Certain implants, devices, or objects may be hazardous to you in the MR environment or the magnet room. DO NOT ENTER the MR environment or the magnet room if you have any questions or doubts regarding an implant, device, or object.

Please indicate if you have any of the following:

- Yes No Aneurysm clips(s)
- Yes No Cardiac pacemaker
- Yes No Intracranial cardiovascular stent(s) (ICD)
- Yes No Electronic implant or device
- Yes No Magnetically activated implant or device
- Yes No Any type of prosthesis or implant
- Yes No Any external or internal metallic object (e.g., dentures, permanent retainer, IUD, metal plate)
- Yes No Artificial or prosthetic limb
- Yes No Any metallic fragments or foreign body
- Yes No Medication patch (Nicotine, Nitroglycerine)
- Yes No Tissue expander (e.g., breast)
- Yes No Body piercing
- Yes No Other implants

**IMPORTANT INSTRUCTIONS:** Remove all metallic objects before entering the MR environment or the magnet room. This includes hearing aids, dentures, or other metallic objects. Any metallic objects are especially prohibited in the magnet room and MRI environment.

I certify that the above information is correct to the best of my knowledge. I have read and understand the entire contents of this form and have had the opportunity to ask questions regarding the information on this form.

Person Completing Form:

Print Name: ____________________________ Signature: ____________________________ Date: __________

Form Reviewed By:

Print Name: ____________________________ Signature: ____________________________ Date: __________ Position: ____________________________

For research study volunteers (to be completed at the end of the study):

Total time spent in magnet (minutes) ____________________________

Time entered by (name): ____________________________
Brain Research of Sexual Function

Healthy Volunteers are Needed for fMRI studies at Queen’s University
(Studies starting January 2009)

If you are:
♦ Heterosexual
♦ Right-handed
♦ Without history of neurological or psychiatric conditions (e.g. Major depression)
♦ Currently NOT taking centrally-acting drugs (i.e. anti-anxiety or anti-depressant medication)

We need you for studies of brain physiology using functional magnetic resonance imaging (fMRI) to map neuronal function. Participating in the study requires one visit to the Queen’s MRI Facility, and takes about 2 hours. The studies are completely non-invasive and confidential. A small honorarium ($50) will be provided to cover your time and expenses (parking etc).

For more information and/or to be sent an information package for either of these studies, please contact:
Janet Mirtle-Stroman, Recruitment Coordinator, by e-mail at stroman_fMRI@sympatico.ca