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Role of Descending Monoamines in the Spinal Control of Micturition in Cat

by

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Submitted in partial fulfilment of the requirements

for the degree of Doctor of Philosophy

at

Dalhousie University,

Halifax, Nova Scotia

December, 1994

ISBN 0-612-05194-3

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Abstract

Monoamines, such as noradrenaline (NA) and serotonin (5HT), are contained in descending pathways to the spinal cord. These compounds modulate several functions, such as antinociception and locomotion, at a spinal level. There is evidence to suggest that NA is the spinal transmitter in pathways forming the descending limb of the spino-bulbo-spinal reflex underlying micturition. 5HT has been implicated as an inhibitory modulator of micturition. We proposed to examine the physiological role of NA and 5HT in the spinal control of micturition. A conscious preparation for monitoring voiding was developed. Monoaminergic transmission was modulated on an acute and chronic basis, using intrathecal administration of selective receptor ligands and neurotoxins, respectively.

Blockade of noradrenergic transmission did not alter bladder function, suggesting that NA is not the spinal mediator of micturition. Acute blockade of serotonergic transmission decreased bladder capacity while stimulation of serotonergic receptors increased capacity, suggesting that 5HT, at a spinal level, can inhibit micturition. A modulatory action of serotonergic receptors on the ascending limb of micturition was investigated in an acute, anesthetized preparation. 5HT1D/2A/2C and 5HT3 receptors were implicated in the inhibition of ascending activity, which was consistent with the effects of serotonergic ligands in the conscious preparation..

The possibility that 5HT, as a spinal inhibitory modulator of bladder function, could diminish detrusor hyperreflexia after spinal cord injury was examined in a chronic, spinally-transected preparation. However, cats initially prepared for these experiments did not develop reflex bladder activity. Further experiments suggested that cutaneous afferent input from the perineum can influence the emergence of reflex bladder activity. 5HT was tested in cats with reflex bladder activity after spinal transection and produced opposite effects to those seen in spinally-intact cats. However, 2-methyl-5HT, a 5HT3 agonist, increased the volume at which micturition occurred. These results suggest that some, but not all, aspects of serotonergic pharmacology are altered in the portion of the spinal cord isolated by transection.

Table of Abbreviations

5,7-dHT	5,7-dihydroxytryptamine
6-OHDA	6-hydroxydopamine
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
DβH	dopamine-β-hydroxylase
CFU	colony forming units
G	gauge
HD	hourly diuresis
hr	hour
Hz	hertz
i.m.	intramuscular
i.p.	intraperitoneal
i.t.	intrathecal
i.v.	intravenous
kg	kilogram
mg	milligram
ml	millilitre
mm	millimetre
mmol	millimole
MOhm	megaohm
ms	millisecond
nmol	nanomole
P_C	pressure of contraction (cmH20)
SEM	standard error of the mean
T_V	duration of voiding (seconds)
μmol	micromole
V_R	residual volume (ml)
V_T	volume threshold (ml)

Acknowledgements

I would like to take this opportunity to thank the people who provided such wonderful support during the duration of my thesis.

First, I would like to express my profound appreciation to Dr. Dale Paley and in turn to Dr. Sylvia Craig and the staff of the Animal Care Centre. Their helpful ideas, professional approach and obvious care for the animals made these experiments considerably easier.

Second, to Leslie Ingraham, Dr. Huan-Ji Du, Gary Bialik and Dr. Jerzy Gajewski, a sincere thanks for your technical expertise and your precious weekend time for emptying cat bladders. Thank you to Cindee Leopold for the immunohistochemical work and Kay Murphy for coaching in tissue slicing.

Third, to Drs. Jana Sawynok and Tom White, thank you for your scientific insight and good comraderie.

On a personal level, I would like to thank my husband, Bob, and good friends Leslie, Sandy, Shelley, Helen and Judy and the Department of Pharmacology en masse for helping me maintain a sense of humour through it all.

Thank you to Rick Hansen Man in Motion Foundation and the Medical Research Council of Canada for providing financial support for both research in bladder function and for the studentships I received.

And last, and most importantly, to my supervisor, Dr. John Downie. Thank you so much for your patience and understanding. Your scientific approach and willingness to examine new ideas have provided me with a solid foundation for my future endeavours.

I. Introduction

A. Anatomy of the Urinary Tract

Urine is produced in the kidneys and flows through the ureters into the bladder where it is stored until vesical evacuation occurs (Fig. 1). The body of the bladder is a meshwork of three interdigitated muscles: the outer and inner layers run longitudinally, while the middle layer is circular. The bladder muscle is specialized in the trigonal area - defined as "the triangular area of the posterior bladder wall which lies between the ureteric orifices and the inferior urethral meatus" (Dixon and Gosling, 1987). The bladder and urethra are joined at the bladder neck. In the male, an internal urethral sphincter, composed of smooth muscle cells, forms a complete circle around the bladder neck. In the female, the internal urethral sphincter is not well-defined. The distal end of the urethra is encircled by striated muscle forming the external rhabdosphincter (Fig. 1).

B. Peripheral Innervation of the Lower Urinary Tract

Afferent information from the bladder and urethra reaches the spinal cord via the pelvic, hypogastric and pudendal nerves. In cat, afferent fibres originate in S1-S3 (pelvic), T12-L5 (hypogastric) and L7-S3 (pudendal) dorsal root ganglion cells (Morrison, 1987, Baron et al., 1985). Afferent activity subserving the micturition reflex is carried in the pelvic nerve. Firing in pelvic afferents commences within the physiological range of pressures (Floyd and Lawrenson, 1979), increases during

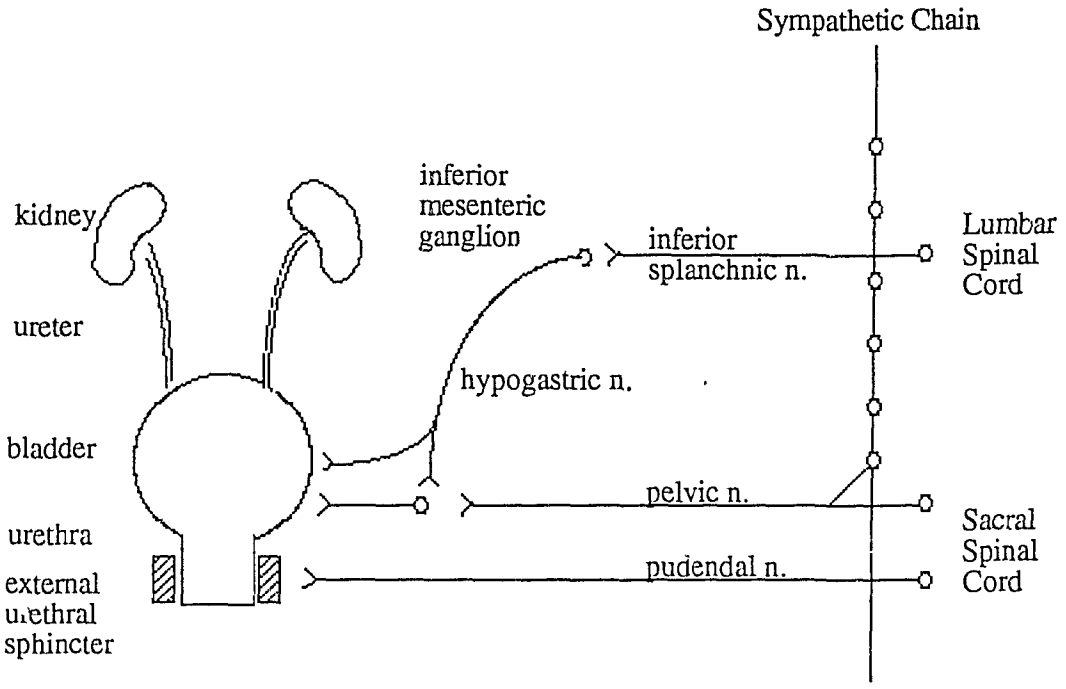


Figure 1: Anatomy and Peripheral Innervation of the Lower Urinary Tract

bladder filling and plateaus at high pressures, which has been attributed to adaptation of nerve endings (Janig and Morrison, 1986). Hypogastric afferent neurons respond over the same range of bladder tension as those travelling in the pelvic nerve but there are substantially fewer mechanoreceptive afferents in the hypogastric nerve than in the pelvic nerve and the hypogastric nerve alone cannot mediate micturition as the pelvic nerve is essential for bladder reflexes (Morrison, 1987). Afferents in the pudendal nerves innervate the urethral mucosa, the perigenital skin and the pelvic floor musculature (Dixon and Gosling, 1987).

The lower urinary tract is innervated by all divisions of the nervous system. The neuronal organization can be described in terms of the two functions of the bladder: storage and evacuation.

(i) Storage - Urinary continence is promoted by bladder relaxation and urethral constriction (Fig. 2). Stimulation of the hypogastric nerves, part of the sympathetic innervation to the bladder, inhibits spontaneous or evoked bladder contractions. Sympathetic preganglionic neurons are located in the intermediolateral cell column of L2-L5 (de Groat and Lalley, 1972) and project to the bladder in the hypogastric nerves and sympathetic chains (Downie et al, 1984, Kuo et al., 1984). Sympathetic inhibition of bladder activity can be blocked by β-adrenoceptor antagonists (de Groat and Saum, 1972), presumably acting on receptors located primarily in the body and dome of the bladder (Awad et al., 1974). It has been proposed that sympathetic inhibition of pelvic ganglion transmission through α-adrenoceptors could reduce the parasympathetic outflow to the bladder and thereby inhibit bladder activity (de Groat

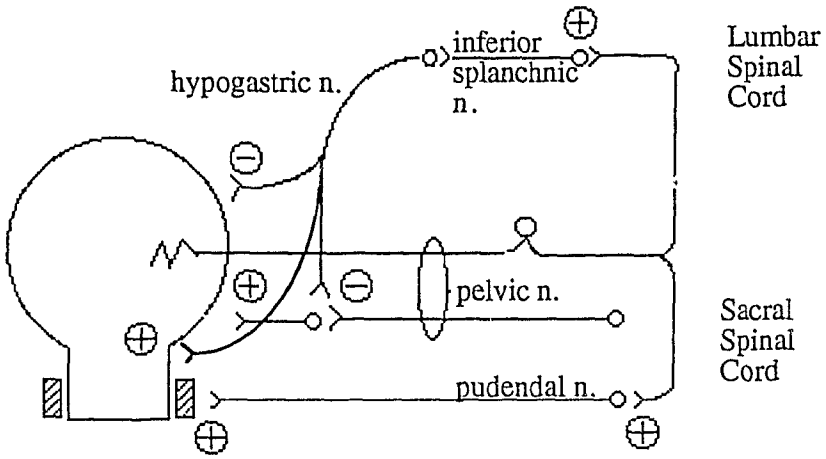


Figure 2: Spinal Reflex Mechanisms Controlling Continence

and Saum, 1972, de Groat and Theobald, 1976). However, during bladder filling at physiological rates, pelvic efferent nerve activity is either absent or can be attributed to sympathetic nerve fibres (Satchell and Vaughan, 1989). This observation argues against a physiological role for inhibition of pelvic ganglionic transmission as ganglionic filtering should require pelvic efferent activity during bladder filling. It is unlikely that ganglionic filtering occurs under extreme conditions as it is only demonstrated at low frequencies of stimulation (de Groat and Saum, 1972). Thus, the physiological role for ganglionic filtering has not been determined.

Urethral closure is also important for the maintenance of continence and both the sympathetic and somatic divisions of the nervous system mediate this action. Hypogastric nerve stimulation and administration of noradrenaline, a neurotransmitter in post-ganglionic sympathetic neurons, produce urethral constriction (Awad and Downie, 1977) by α-adrenoceptor activation. In cat, the striated muscle of the external urethral sphincter is innervated by the pudendal nerve arising from Onuf's nucleus in the ventral horn of S1 and S2 spinal segments (Ueyama et al., 1984). Contraction of the sphincter is mediated by the release of acetylcholine and subsequent stimulation of nicotinic cholinergic receptors.

(ii) Evacuation - Parasympathetic preganglionic neurons, as shown by retrograde tracing studies using HRP in cat, are located in a lateral band of cells in lamina VII of S1-S3 spinal segments (de Groat et al., 1981). Parasympathetic neurons travel in

the pelvic nerves and provide excitatory input to the bladder via bladder postganglionic neurons in the pelvic plexus. Bladder contraction is mediated by muscarinic receptors in the bladder wall (de Groat, 1975). In some species, there is also a non-cholinergic, non-adrenergic component to bladder contraction which may be mediated by ATP (Burnstock et al., 1972, Dean and Downie, 1978, Brading and Mostwin, 1989, Luheshi and Zar, 1990).

C. Central Reflexes Controlling Bladder Function

During filling at physiological rates, the bladder is under sympathetic inhibition (Maggi et al., 1985) which mediates relaxation of the bladder muscle. The lumbar sympathetic outflow to the bladder is excited by pelvic afferent activity via an intersegmental spinal reflex (de Groat and Lalley, 1972). EMG activity in the urethral sphincter increases during bladder filling (Fowler and Fowler, 1987) as bladder afferents, also via a spinal reflex, activate the pudendal nerve outflow. Bladder relaxation and sphincter constriction promote continence and as these reflexes are spinally-organized, continence mechanisms can proceed without conscious intervention.

When micturition occurs, the excitatory somatic outflow to the external sphincter and sympathetic inhibition of the bladder are depressed whereas the parasympathetic outflow to the bladder is activated (de Groat, 1975). The resultant

bladder contraction coupled with sphincter relaxation produces efficient bladder emptying.

Micturition is dependent on a spino-bulbo-spinal reflex arc. Ascending information from the abdominal viscera travels in the dorsal and lateral columns of the spinal cord. Dorsal column pathways carry sensory fibre projections from the pelvic and pudendal nerve which transmit information concerning touch and pressure in the urethra (Nathan and Smith, 1951). The ascending limb of micturition is thought to be located in the white matter of the lateral columns in the spinal cord. Studies examining human beings with bilateral lesions of the spino-thalamic tract who have lost the normal sensations indicating the need to void have implied that the ascending pathway for micturition is located superficially along the equatorial plane of the lateral columns (Nathan and Smith, 1951). In cats, this pathway is located more dorsally in the superficial layer of the dorsolateral funiculus (Barrington, 1933, Kuru, 1965, de Groat et al., 1981). This ascending path has been suggested to be a sacrobulbar projection to the paraalar, juxtasolitary, and lateral nuclei of the brainstem (Kuru, 1965). The juxtasolitary nucleus in turn projects to the pontine micturition centre (PMC, Kuru, 1965). The PMC was first identified by Barrington (1925) who employed electrolytic lesions to show that bilateral destruction of an area "just ventral to the internal edge of the superior cerebellar peduncle... is followed by a permanent inability to empty the urinary bladder". Bladder contractions can be elicited by electrical (Kuru, 1965, de Groat, 1975, Holstege et al., 1986, Lumb and

Morrison, 1987 and Shefchyk, 1989) and chemical (Lumb and Morrison, 1987, Sugaya, 1987, Mallory et al., 1991) stimulation in the dorsal pontine tegmentum but a precise location of the pontine micturition centre has not been produced. Electrical stimulation of the medial portion of the dorsolateral pontine tegmentum (the M-region, Holstege et al., 1986) produces coordinated bladder contraction and sphincter relaxation. Injections of ^{3}H-leucine into the M-region labels fibres in the sacral intermediolateral cell nuclei (Holstege et al., 1986) that contain parasympathetic preganglionic neurons (Nadelhaft et al., 1980). A pathway from the PMC descends mainly ipsilaterally in the superficial dorsolateral funiculus of the spinal cord (Loewy et al., 1979), but terminates bilaterally in the intermediolateral regions of the sacral spinal cord. There are no terminations in the thoracic or lumbar spinal cord and as such this projection is specific to the sacral cord.

The dorsolateral pontine tegmentum provides other descending inputs to the sacral spinal cord. Lateral to the M-region is another region that, when electrically stimulated, causes sphincter contraction (Holstege et al., 1986). This area (the L-region) has projections to Onuf's nucleus, the origin of motoneurons innervating the external urethral sphincter (Holstege et al., 1986). Electrical simulation in a "ribbon-like band" (Griffiths et al., 1990) between the M and L regions also produces sphincter activity. This has been suggested to indicate a connecting pathway between these regions, but the function of this pathway has not been determined.

Complete bladder emptying may require a positive feedback system (Lindström et al., 1984). The requirement for a feedback system can be demonstrated by lowering

bladder pressure during bladder contraction. This reduction in bladder pressure results in the elimination of efferent activity in the pelvic nerve (Fall et al., 1990), presumably by decreasing pelvic afferent input. As well, large amplitude, long duration micturition contractions elicited by electrical stimulation of the PMC can be eliminated by deafferentation of lumbosacral dorsal roots, which would remove pelvic input (Kruse et al., 1991). However, deafferentation of lumbosacral dorsal roots has also been reported not to alter PMC-evoked contractions (Shefchyk, 1989). Kruse et al. (1991) suggested that prolonged activation of the descending micturition pathway in Shefchyk's experiments (1989) may have been responsible for the lack of effect of deafferentation. A reflex, with afferent and efferent components contained in the pelvic nerve, may underly this feedback system. This reflex is evoked by running fluid through the urethra (Barrington, 1925), which would occur during micturition, and results in bladder contraction.

A spinal circuit may also be important for micturition. Recordings of activity in ascending axons in the thoracic cord elicited by pelvic nerve stimulation have shown that these neurons are not only responsive to bladder input but are also excited by stimulation of other nerves such as the pudendal nerve (McMahon and Morrison, 1982a,b). This would suggest that the spino-bulbo-spinal reflex that underlies micturition could be activated by afferent input from areas other than the bladder. The magnitude of pelvic nerve activity evoked by stimulation of cutaneous nerves could be facilitated by elevating intravesical pressure (McMahon and Morrison, 1982c). This was interpreted to suggest that there was a bladder-specific pathway that could modulate the excitability of parasympathetic preganglionic neurons by a spinal reflex pathway (McMahon and

Morrison, 1982c). Thus, although the spino-bulbo-spinal reflex can be initiated by afferent input from other nerves, such as the pudendal, micturition will only occur when pelvic nerve afferent input has elevated the excitability of the preganglionic neurons to a level where they will fire.

The presence of this spinal circuit has been a matter of debate and two models of the central organization of micturition have been proposed (Fig. 3). The first states that micturition is mediated solely via the previously described spino-bulbo-spinal reflex. This model invokes a bladder-specific afferent pathway to the pontine micturition centre and descending pathways from the pons mediating bladder contraction and sphincter relaxation (herein termed the de Groat model, de Groat, 1975). The second model also requires a spino-bulbo-spinal reflex but the ascending pathway is not bladder-specific. A sacral spinal circuit is required for this model (herein named the McMahon and Morrison model, McMahon and Morrison 1982b,c and McMahon, 1986). This sacral circuit would determine the responsiveness of parasympathetic preganglionic neurons to excitatory inputs from descending pathways originating in supraspinal centres. These models will be used to discuss the results obtained in these experiments.

D. Monoaminergic Innervation of the Spinal Cord

The spinal cord is not merely a conduit for pathways originating in supraspinal centres to access autonomic and somatic outflow to peripheral organs. It is an important integrative centre for autonomic and somatic control. Autonomic functions such as maintenance of urinary continence are subserved by intersegmental spinal reflexes without

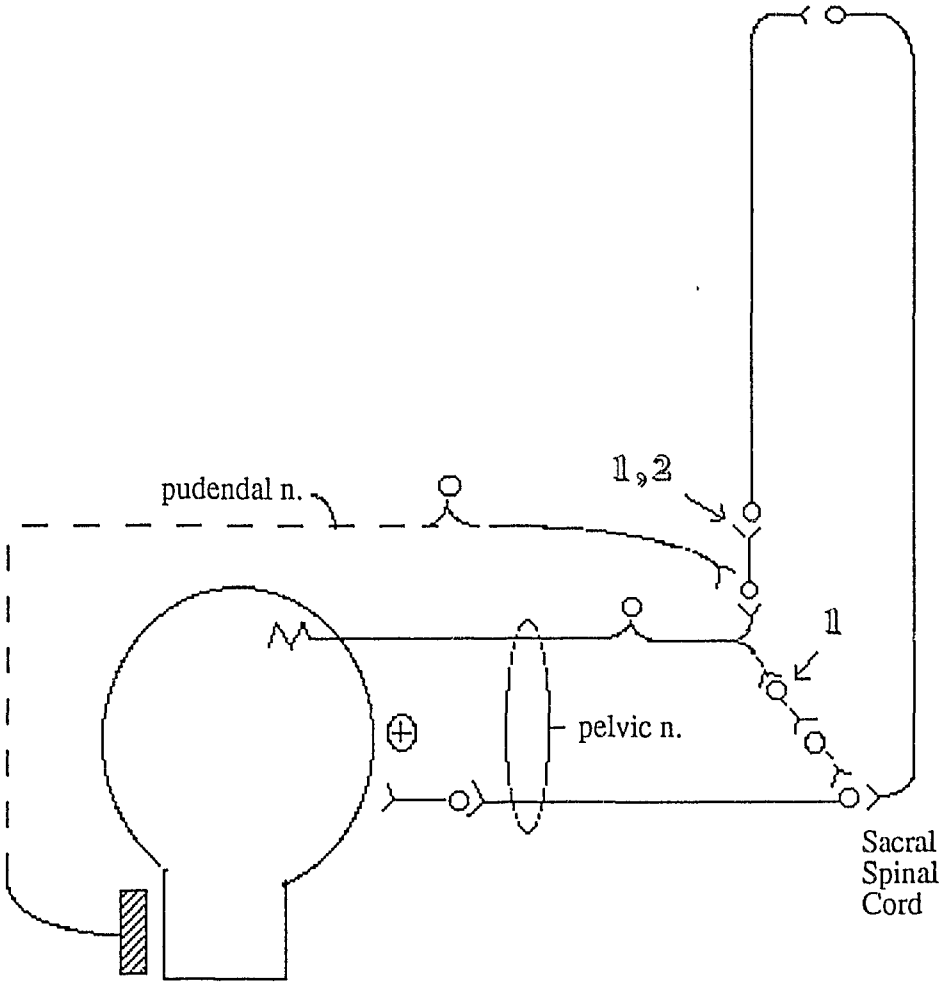


Figure 3: Two Models of the Central Organization of the Micturition Reflex. Solid and dashed lines denote McMahon & Morrison model (1) while solid lines alone demonstrate deGroat model (2). Numbers indicate site of modulation for respective models.

need for supraspinal input. Supraspinal centres can control spinal cord function through the use of descending pathways. Descending monoaminergic pathways, containing dopamine, noradrenaline (NA) or serotonin (5HT), provide a rich innervation to the spinal cord and can control a variety of functions at the spinal level.

Monoamines, such as NA and 5HT, are contained in descending projections to the spinal cord. The cells of origin of noradrenergic terminals are located in the locus coeruleus, subcoeruleus, medial and lateral parabrachial and Kolliker-Fuse nuclei as well as in an area adjacent to the superior olivary nucleus (corresponding to the A5-A7 pontine cellular groups, Westlund et al., 1980, 1983). In the cat, the Kolliker-Fuse nucleus is the principal source of pontine catecholaminergic cells projecting to the lumbar spinal cord (Stevens et al., 1982).

Noradrenergic terminals, identified by immunocytochemical staining for dopamine-β-hydroxylase (DβH), are concentrated in the marginal layer of the dorsal horn, in the ventral horn among motoneurons and in the autonomic lateral cell columns of the thoracic and sacral spinal cord (Westlund et al., 1983). In the superficial layers of the dorsal horn, NA-containing fibres and varicosities, identified using antibodies recognizing NA, rarely form classical synapses with their targets (Rajaofetra et al., 1992b). Classical synapses, however, are common in the motoneuron areas of the ventral horn and the intermediolateral cell column.

Adrenoceptors have been identified in the spinal cord using autoradiography. Tritiated prazosin, an α_1-adrenoceptor ligand, binds to receptors in the ventral horn and deeper laminae of the dorsal horn whereas tritiated rauwolscine labels α_2-adrenoceptors

throughout the grey matter of the lumbar cord (Dashwood et al., 1985) but concentrated in laminae I/II and the intermediomedial zone. Tritiated paraaminoclonidine (a ligand of α_2-adrenoceptors) was localized to the substantia gelatinosa and intermediolateral cell column (Unnerstall et al., 1984). These experiments have demonstrated that both α_1- and α_2-adrenoceptors are present in the spinal cord. However, their respective laminar distribution is different, with α_1-adrenoceptors in the ventral horn and deeper laminae of the dorsal horn and α_2-adrenoceptors in the superficial layers of the dorsal horn and in the intermediate zone.

Serotonergic projections from the brain to the spinal cord have been classified into three subsystems: dorsal, intermediate and ventral (Skagerberg and Björklund, 1985). The dorsal pathway originates in the nucleus raphe magnus, nucleus reticularis gigantocellularis and nucleus paragigantocellularis. This pathway travels unilaterally in the dorsolateral funiculus (Bullitt and Light, 1989) in both myelinated and unmyelinated fibres (Westlund et al., 1992), contains both 5HT and other compounds and terminates in the dorsal horn and intermediate zone (Skagerberg and Björklund, 1985) but in the dorsal horn, classical synapses are rare (Marlier et al., 1991).

The intermediate pathway originates in the nucleus raphe obscurus, nucleus raphe pallidus and the arcuate cell group (Skagerberg and Björklund, 1985) and projects to the intermediate grey in the thoracolumbar and upper sacral levels of the spinal cord. 5HT is colocalized with somatostatin, substance P, thyrotropin-releasing hormone and met-enkephalin (Chiba and Masuko, 1989). The ventral pathway descends in the ventral cord and innervates the ventral horn (Skagerberg and Björklund, 1985). In the ventral spinal

cord 5HT is colocalized with thyrotropin-releasing hormone and substance P (Ulfhake et al., 1987). There is also a small spinal projection of serotonergic neurons originating in the locus coeruleus (Lai and Barnes, 1985). In many animals, there is also an intrinsic serotonergic innervation of the spinal cord (Lamotte et al., 1982, DiTirro et al., 1983, Rajaofetra et al., 1992a). The cell bodies are located around the central canal and innervate Onuf's nucleus (Rajaofetra et al., 1992a). However, in cat, the serotonergic innervation of Onuf's nucleus is exclusively supraspinal in origin as spinal transection eliminates all serotonergic terminals in the ventral horn (Ramirez-Leon et al., 1994).

The investigation and classification of 5HT receptors is a continuing process. 5HT receptor subtypes can be separated into at least three, but up to seven classes (reviewed in Hoyer et al., 1994). Three classes that have been extensively studied are 5HT1, 5HT2 and 5HT3. The 5HT1 class has been further subdivided into 5HT1A,B,D,E and F and the 5HT2 class into 5HT2A/2C. 5HT receptor subtypes demonstrate a differential distribution within the spinal cord. Autoradiography using [^{125}I]iodo-zacopride, a 5HT3 antagonist (Laporte et al., 1992), and *in situ* hybridization for 5HT3 receptor mRNA (Tecott et al., 1993), demonstrated labelling restricted to the superficial layers of the dorsal horn of the rat spinal cord. A large population of these sites are on the central projections of dorsal root ganglion neurons (Hamon et al., 1989, Kidd et al., 1993). Autoradiography using tritiated 5HT in cat demonstrated 5HT1 binding sites throughout the grey matter with concentrations in Laminae II, III and the thoracic intermediolateral cell column (Pubols et al., 1992). Tritiated ketanserin, a 5HT2

antagonist, labelled receptors in the thoracic sympathetic area and the ventral horn (Marlier et al., 1991b).

E. Monoaminergic Influences on Spinal Function

Monoamines, such as NA and 5HT, modulate a variety of functions at a spinal level including nociception (reviewed in Basbaum and Fields, 1984, Fields et al., 1991), blood pressure, sexual reflexes and locomotion (Jacobs and Fornal, 1993). Both NA and 5HT are antinociceptive at a spinal level. Intrathecal administration of NA (Reddy et al., 1980) and 5HT (Yaksh and Wilson, 1979) elevated nociceptive thresholds as measured by hot plate and tail flick tests. The mechanism of action for suppression of nociceptive transmission for NA is likely mediated through α_2-adrenoceptors. NA decreases nociceptive responses in dorsal horn neurons (Belcher et al., 1978), an action which can be blocked by yohimbine, an α_2-adrenoceptor antagonist (Davies and Quinlan, 1985). NA also reduces non-nociceptive transmission from group II afferents to first order interneurons (Bras et al., 1989) in the intermediate and ventral spinal cord via α_2-adrenoceptor activation (Bras et al., 1990). Intrathecal 5HT decreases blood pressure in rats (Solomon and Gebhart, 1988), probably by activation of 5HT1A receptors (Ramage and Fozard, 1987, Ramage et al., 1988, Shepheard et al., 1990). Stimulation of spinal serotonergic receptors facilitates seminal emission and suppresses penile erection (Mas et al., 1985). MCPP, a non-selective serotonergic agonist (Hoyer, 1988) can facilitate sacral preganglionic input to the penis (which would produce erection) but this action is thought to be due to inhibition of serotonergic input to preganglionic neurons (Steers and

de Groat, 1989). In spinally-injured patients, cyproheptadine, a 5HT2A/2C antagonist (Hoyer, 1988) and clonidine, an α_2-agonist, reduce limb spasticity (Fung et al., 1993). Cyproheptadine reduces spasticity by blocking excitation of motoneurons by 5HT, which may be mediated by 5HT2A/2C receptors (Yamazaki et al., 1992).

Both NA and 5HT appear to be important in the spinal control of micturition. Clonidine can inhibit urethral constriction (Nordling et al., 1979). Clonidine, at a site within the spinal cord, depresses a vesicosympathetic (Krier et al., 1979) and a vesicosomatic reflex (Downie and Bialik, 1988) which mediate urethral closure. Clonidine, a centrally-acting antihypertensive agent, is an α_2-adrenoceptor agonist. However, the action of clonidine in the control of blood pressure is attributable to the imidazole structure of clonidine, not its capacity to bind to α_2-adrenoceptors (Bousquet et al., 1984) whereas clonidine's action on lower urinary tract function has been demonstrated to be α_2-adrenoceptors activation (Downie et al., 1991).

Noradrenaline has been suggested to be the compound contained in descending pathways originating in the pontine micturition centre that mediates micturition at a spinal level in cat. Bladder contractions elicited by bladder filling or electrical stimulation of the locus coeruleus were blocked by intrathecal administration of phentolamine, a non-selective α-adrenoceptor antagonist, and prazosin, an α_1-adrenoceptor antagonist (Yoshimura et al., 1988). As well, 6-hydroxydopamine, a noradrenergic neurotoxin, eliminated bladder contractions in anesthetized cats (Yoshimura et al., 1990a). However, in conscious rats, intrathecal administration of phentolamine did not affect bladder contractions (Durant and Yaksh, 1988) but the dose of drug used in the rat experiments

was considerably lower than for the cat (30μg vs 1mg) and the discrepancy may have been due to a species difference. In summary, at a spinal level, noradrenaline may inhibit urethral closure through α_2-adrenoceptors and elicit micturition in anesthetized cats via α_1--adrenoceptors.

5HT is emerging as an inhibitory modulator of micturition at a spinal level. Stimulation of the raphe nuclei inhibited recordings of the micturition reflex potential, which was partially reversed by LSD, a non-selective serotonergic antagonist (McMahon and Spillane, 1982, Morrison and Spillane, 1982). Application of 5HT to bladder preganglionic neurons in the sacral spinal cord depressed evoked neural activity in these cells (de Groat and Ryall, 1972). MCPP, a serotonergic agonist (Steers and de Groat, 1989), and 5HTP, the precursor of 5HT (de Groat et al., 1982) depressed rhythmic bladder activity. All of these experiments were performed in anesthetized animals and have demonstrated that 5HT is inhibitory to bladder function.

F. Effect of Spinal Transection on Urinary Tract Function

Following spinal cord injury, urine retention occurs as the spino-bulbo-spinal reflex which mediates micturition is interrupted by transection of the spinal cord. Bladder afferent input to the sacral spinal cord, lumbar sympathetic outflow and motor innervation to the urethral sphincter are still intact, allowing sphincter reflexes to be retained. Loss of bladder contractions coupled with maintenance of sphincter tone results in urine retention. Urine retention can produce autonomic dysreflexia manifested as headache, sweating and blurred vision. Autonomic dysreflexia occurs when spinal cord regions

controlling blood pressure are isolated from normal modulatory influences by spinal transection (for review see Shea et al., 1973). This allows afferent information from the distended bladder to elevate blood pressure below the spinal lesion via a reflex mechanism. Baroreceptors detect this increase in blood pressure and in response mediate slowing of the heart rate and dilatation of the skin vasculature.

The spinal circuitry controlling bladder function is altered following spinal cord injury. In cat, distension-evoked bladder contractions emerge 1-2 weeks following spinalization but the afferent path subserving these contractions is mediated by C-fibres rather than the Aδ-fibres in spinally-intact cats (de Groat et al., 1981). These contractions produce high intravesical pressures but are usually not sustained (Morrison, 1987). The coordination of sphincter relaxation with bladder contraction is lost as this is mediated via a supraspinal pathway and now the bladder contracts against a constricted urethra (Blaivas et al., 1981, Galeano et al., 1986). This phenomenon, termed bladder-sphincter dyssynergia, is often exhibited by paraplegic patients. Bladder-sphincter dyssynergia can result in high intravesical pressures which puts renal function at risk (Gerridzen et al.. 1992). Bladder emptying is incomplete due to unsustained bladder contractions occurring against a closed outlet. Residual urine in the bladder provides an environment for bacterial growth and bladder infections can be difficult to treat in spinally-injured persons.

The response of the bladder to cutaneous input is also altered by spinal injury. In spinally-intact subjects, tactile stimulation of the perineal region inhibits bladder activity. However, after spinal cord injury, perineal stimulation causes bladder contraction (Kuru, 1965, de Groat et al., 1981, Thor, 1985). There is a similar perineal-bladder reflex in

neonatal kittens which the mother cat employs to empty her offsprings' bladders (de Groat et al., 1975). However, this reflex is lost as the kitten is weaned (Thor, 1985).

II. Research Proposal

A. Rationale

The two functions performed by the urinary bladder are urine storage and evacuation. Storage can occur without supraspinal intervention but urine evacuation requires supraspinal pathways. Little is known about the supraspinal control of the sacral spinal circuitry involved in micturition. Other systems, such as antinociception and locomotion, are modulated by descending monoaminergic pathways originating in the brainstem. Previous research has implicated both NA and 5HT in the spinal control of micturition. However, most of these experiments have been performed in anesthetized animals and have not looked at voiding behaviour. The physiological role of descending monoamines in the spinal control of micturition, therefore, has not been established.

NA has been suggested to be the spinal neurotransmitter mediating bladder contraction. It has also been suggested that NA, at a spinal site, can inhibit sphincter contraction. The contribution of NA to micturition evoked in awake animals will be examined in the following experiments. The **first hypothesis** that will be tested is that spinal noradrenaline is required for micturition in the conscious, spinally-intact cat.

Earlier studies have implicated 5HT as a spinal inhibitor of micturition, but again the physiological role of 5HT in the control of micturition is unknown. The **second hypothesis** to be tested in this thesis is that 5HT inhibits micturition at a spinal level in the conscious spinally-intact and spinally-transected cat. If either hypothesis is true, then a potential mechanism of action for the relevant compound(s) will be investigated.

Spinal cord injury results in severe disturbances to bladder function. The mechanisms underlying bladder dysfunction after spinal cord injury have not been determined. Several theories have been proposed to explain these changes. The simplest theory is that elimination of descending pathways allows spinal mechanisms to be revealed which subserve the distorted bladder function in the spinally-transected cat. Descending monoaminergic pathways originate in supraspinal centres and thus are lost after spinal cord injury. The information provided by these experiments would be very useful clinically because if NA is determined to be the spinal mediator of micturition and can inhibit sphincter function, replacement of NA following spinal cord injury may produce a coordinated voiding pattern.

An inhibitory modulator of micturition would also be very useful clinically. Hyperactive bladder activity due to spinal transection could be depressed between catheterizations to minimize bladder contractions thereby protecting the kidneys against pressure-induced damage.

If either hypothesis is true, then the usefulness of the relevant compound(s) will be tested in spinally-transected cats. During the course of experiments in spinally-transected cats, it was observed that cats with bladders drained via an implanted catheter did not develop distension-evoked bladder contractions. The **third hypothesis** to be tested is that removal of descending pathways by spinal transection is an insufficient stimulus for the emergence of distension-evoked bladder contractions after spinal injuyr.

B. Aims and Summary of Experimental Preparations

The goal of these experiments was to test the hypotheses that, at a spinal level, NA mediates micturition and 5HT inhibits bladder function under physiological conditions. Many experiments to-date have examined bladder function in anesthetized animals. However, anesthesia does affect bladder function (Rudy et al., 1991). Thus, to avoid this complicating factor, a conscious preparation was developed wherein urodynamic parameters were examined during surreptitious bladder filling. Conflicting data have been produced in rat and cat for NA (Durant and Yaksh, 1988 and Yoshimura, 1988). Cats were chosen for the present experiments as in dogs and rats voiding is linked with episodic decreases in external urethral sphincter (EUS) activity. However, in humans (de Groat, 1990) and cats, EUS activity is quietened for the duration of voiding. Cats are also an easy animal to handle for testing of bladder function parameters and are an appropriate size for the surgeries performed in the present experiments. Cats were implanted with cannulae in the dorsal surface of the bladder muscle. The cannulae were tunnelled subcutaneously to exit at the head. This preparation allowed bladder filling with concomitant pressure monitoring (cystometrogram) and unobstructed voiding from which various bladder function parameters could be obtained. An intrathecal cannula was positioned at L7-S1 for administration of compounds to the sacral spinal cord. Noradrenergic and serotonergic transmission was altered on an acute and chronic basis through the intrathecal administration of receptor ligands and neurotoxins, respectively.

The effects of alteration of noradrenergic or serotonergic transmission were determined by comparing bladder function parameters before and after intrathecal injection.

In light of results obtained in conscious spinally-intact cats, the mechanism of action of 5HT was investigated. These experiments were performed in anesthetized cats as axonal recording *in situ* was required. The pelvic afferent supply from the bladder was stimulated and ascending activity was recorded in the lower thoracic spinal cord. In addition, input from the pudendal nerve onto ascending activity was monitored and the pelvic-pudendal (viscerosomatic) and pudendal-pudendal (somato-somatic) reflexes were examined. The effects of intrathecally-administered compounds on ascending activity and spinal reflexes were determined.

In conscious, spinally-intact cats, 5HT inhibited micturition. Cats used in the conscious, spinally-intact experiments were to be used for examination of the effects of 5HT after spinal transection and underwent lower thoracic spinal cord transection. However, bladder hyperactivity did not develop in these cats. Two factors, bladder infection and perineal stimulation, were examined to determine if there was any correlation between emergence of reflex bladder activity and the presence of bladder infection or perineal stimulation. If either of these factors were linked with the emergence of distension-evoked contractions, it could be concluded that removal of descending pathways was an insufficient stimulus for the production of bladder hyperactivity after spinal transection. In the course of these studies, cats demonstrating reflex bladder activity were produced and tested with serotonergic ligands.

III. Materials and Methods

All experiments were performed with the approval of the University Committee for Laboratory Animals. Experiments on chronic cats were performed under a standard operating procedure developed using guidelines established by the Canadian Council on Animal Care and approved by the University Committee for Laboratory Animals.

A. Behavioural Studies in Conscious, Spinally-Intact Cats

1. Acute Manipulation of Spinal Monoaminergic Transmission

(i) Surgical Preparation - After a conditioning period of three weeks, adult male cats (3.5-7.0 kg) underwent two aseptic surgical procedures. In the first, under halothane anesthesia, cats were placed in the ear bars of a stereotaxic apparatus with the body supported by a box such that the spinal column was straight. A midline skin incision over the skull was made and the neck muscles were dissected along the midline to reach the cisterna magna. The head was bent forwards and the dura and arachnoid membranes over the cisterna magna were removed using the bent tip of an 18G 1.5 inch needle. A Teflon cannula (24G, dead volume=0.08 ml) was inserted via the cisterna magna into the intrathecal (i.t.) space and advanced to the level of the lumbosacral cord. The length of cannula was estimated for each cat based on the length from the occipital protuberance to the L6 vertebral process. The cannula end was fitted with an L-shaped joiner (Plastics

One, Roanoke, Virginia) with a screw cap and secured to the neck muscles. The skull skin incision was closed over the cannula and the cat recovered for a minimum period of 1 week. In the second surgery (1-3 weeks later), 2 silastic cannulae (20G) were implanted in the dorsal surface of the bladder. Each silastic cannula was fitted with 2 buttons, 2 mm apart, placed 1 cm from the distal end of the cannula. A purse-string suture was made in the bladder muscle, the cannula inserted and the muscle closed around the cannula between the two buttons. These buttons ensured the cannula would neither advance nor retract. The cannula end was fenestrated with 3-4 holes. The bladder cannulae exited the abdominal musculature to the right of the incision. The muscular layer was closed and the cannulae were tunnelled subcutaneously, using stainless steel trochars, to exit at the head incision. The bladder and i.t. lines were led to joiners with screw caps. A headcap was formed by drilling 3-5 holes in the skull and screwing in self-tapping screws. The joiners were tied to the screws, excess portions of scalp were cut away and dental acrylic was applied to secure the joiners to the screws.

(ii) Bladder Testing - After a minimum recovery period of 1 week, bladder function was tested. Unanesthetized cats were placed in 3'deep x 2'wide x 3'high cages provided with a litter box. One of the bladder cannulae was connected to an infusion pump through a fluid swivel (Harvard Apparatus, South Natick, Maine). The bladder was infused at a physiological rate (Klevmark, 1974) of 10 hourly diuresis units (1 hourly diuresis unit=1.1 ml/kg/h) with sterile saline from a 60 ml syringe using an infusion pump (Sage, model 385) with an adjustable infusion rate. Bladder pressure was monitored on a side arm of the filling line with a pressure transducer (Micron Instruments, LA). The pressure

transducer was connected to a two channel Gould chart recorder, providing a hard copy of the pressure trace. The Gould chart recorder was connected to a computer equipped with MacLab software to store the trace. Cystometrograms (CMGs) were performed (bladder pressure monitored during bladder filling). The following parameters of micturition were measured: volume threshold for micturition (V_T), maximum amplitude of pressure during contraction (P_C), and voiding time (T_V) (Fig. 4). The bladder was emptied following micturition to determine post-void residual volume (V_R). Each experiment consisted of: two baseline CMGs, one CMG following vehicle injection and one after injection of the compound of interest. Only one dose of one compound was administered per session. Testing sessions were separated by at least one day except for one cat in which the duration of methysergide action was monitored.

(iii) Drug Studies - Compounds were administered via the i.t. cannula in a volume of 0.1 ml and flushed with 0.15 ml of sterile saline. CMGs were started 10 min after injection of the agent. The following drugs were injected: methysergide maleate (Sandoz), prazosin hydrochloride (Sigma Chemical Company), phentolamine mesylate (Ciba), 5HT hydrochloride (Research Biochemicals International (RBI)), and zatosetron maleate (Lilly Research Laboratories) (Appendix "B"). Methysergide, phentolamine, 5HT and zatosetron were dissolved in sterile saline (0.9%). Prazosin was dissolved in 10% dimethyl sulfoxide (DMSO), 20% distilled water and 70% saline.

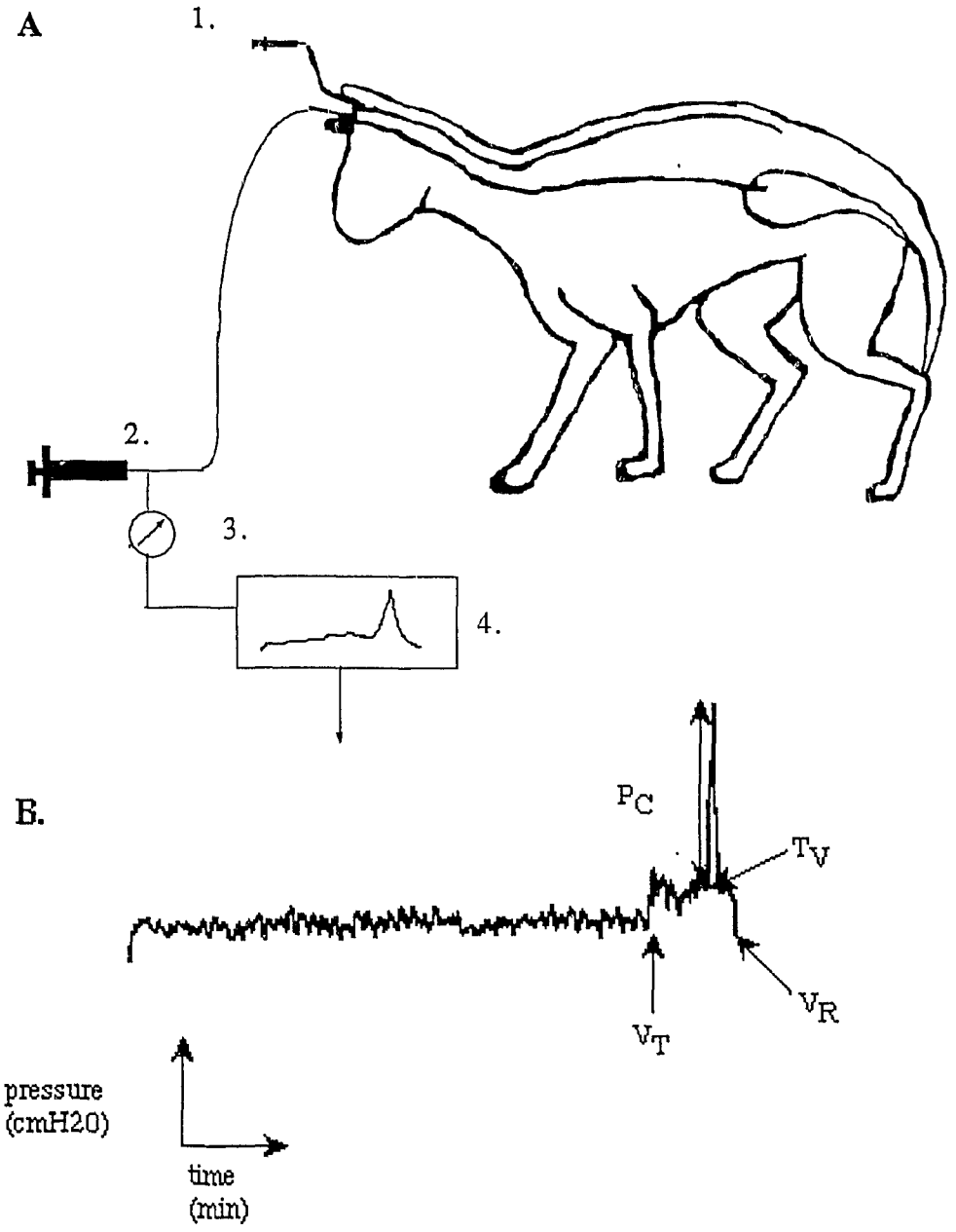


Figure 4: Cystometrograms in Conscious Cats.
A)Diagrammatic representation of experimental preparation 1. Injection into intrathecal line. 2. Infusion of sterile saline into bladder at 10HD and 3. Pressure monitor on side arm of infusion line 4. Output of pressure transducer on chart recorder. B) Representative cystometrogram showing bladder function parameters. Abbreviations are explained in text.

2. Chronic Interruption of Spinal Serotonergic and Noradrenergic Transmission

Cats used in Section 1 for testing of methysergide, phentolamine or prazosin were used for the toxin studies (Fig. 5). This was a blinded study and therefore toxins and uptake blockers were administered by another person in the laboratory while bladder function testing was done by the author.

(i) Neurotoxin Administration - The neurotoxins 6-hydroxydopamine (6-OHDA) hydrobromide and 5,7-dihydroxytryptamine (5,7-dHT) creatinine sulfate (both from Sigma Chemical Company) were used to deplete noradrenergic and serotonergic nerve terminals, respectively. Neurotoxins were administered via the indwelling intrathecal line (Howe et al., 1987) in an effort to localize the lesion to the lumbosacral cord. The toxic action of these compounds is dependent upon uptake into the nerve terminal. Consequently, injections of neurotoxins were preceded by uptake blocker administration to protect the alternate neurochemical population of neurons. Imipramine, a noradrenergic uptake blocker, was administered i.p. 1 hr prior to 5,7-dHT to protect noradrenergic nerve terminals. This was later changed to desipramine as desipramine is a more selective uptake blocker (Richelson and Pfenning, 1984). Fluoxetine, a serotonergic-uptake blocker, was injected i.p. 3 hr prior to 6-OHDA in order to prevent uptake of the neurotoxin into serotonergic neurons. Toxin administration followed the protocol outlined in Fig. 6a,b which underwent some modifications and the actual toxins and uptake blockers administered will be described in the results section.

(ii) Bladder Testing and Infection - Bladder function was tested 4-26 days post-toxin administration. Delayed testing dates were due to the presence of urinary tract infection.

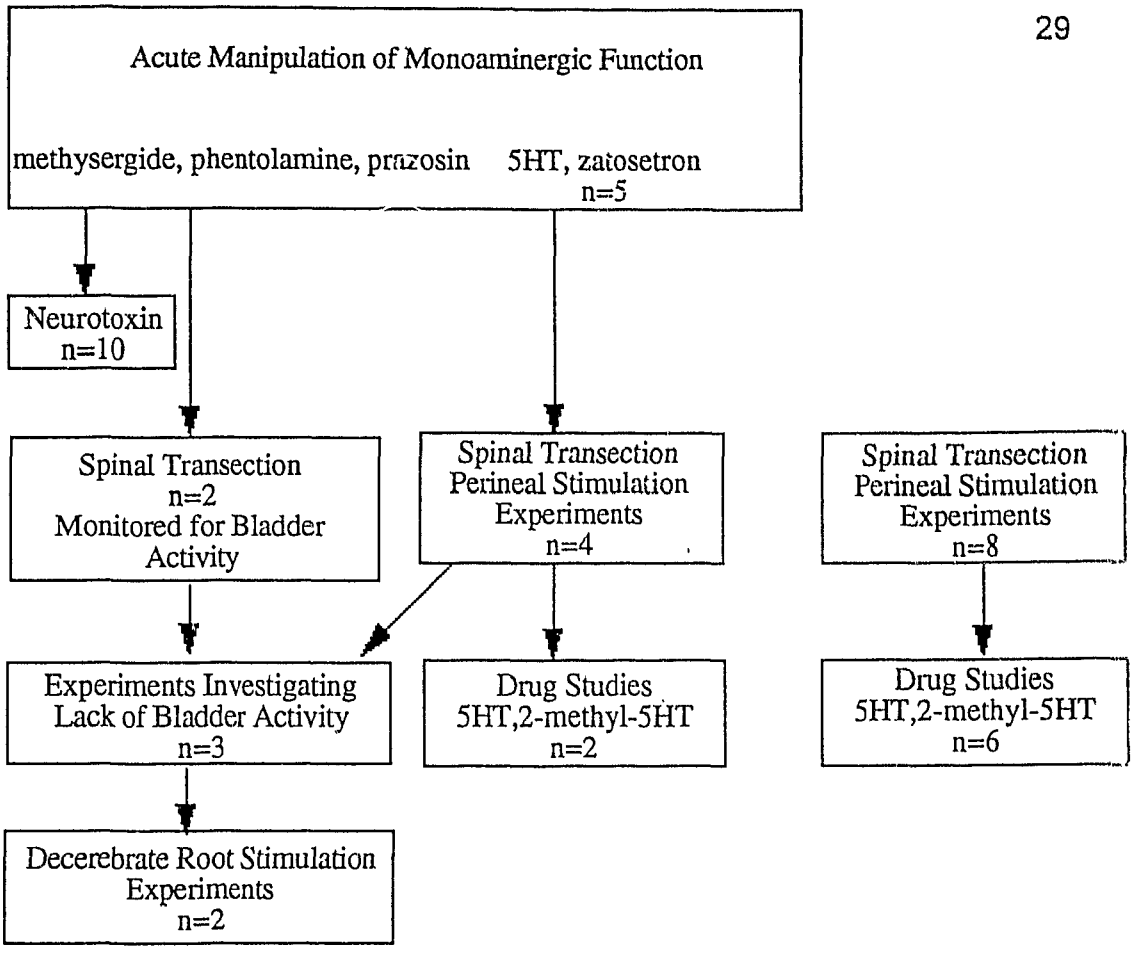


Figure 5: Animal Usage for Conscious Cat Experiments

A. Daily Protocol

1. Night Before-Cat OFF FEED
2. Day Of- sedation (Rompun {xylazine} 1.1-2.2 mg/kg i.m.)

↓

uptake blocker, i.p.
1hr - imipramine, desipramine
3h - fluoxetine

↓

toxin i.t. in 0.5 ml 0.1% ascorbic acid in saline
i.t. flush with 0.1ml saline

↓

observe for 1 hr

B. Dosing Protocol

Low Dose Toxin 1
500ug 6-OHDA, 600ug 5,7-dHT

↓ *

High Dose Toxin 1
1000ug 6-OHDA, 1200ug 5,7-dHT

↓ *

High Dose Toxin 2

↓

Acute Experiment and Harvest of Spinal Cord Tissue for Immunohistochemistry

*If there is an effect of any toxin proceed directly to acue experiment.
Effect=VR>25%VT OR 50%VT>VT(toxin) or VT(toxin)>150%VT

Figure 6: Protocols for Neurotoxin Administration A) Daily and B) Sequence of neurotoxins

Urinary tract infection was determined by culture and sensitivity testing of samples obtained from the indwelling bladder line. If infection was present (> 10,000 CFU, colony forming units) then appropriate antibiotic therapy was administered. Testing of intrathecal compounds was done only on cats without urinary tract infection. If bladder function was affected by toxin administration (defined as bladder residual volume > 25% of bladder capacity or > 50% change in volume threshold) then the cat was used for an acute experiment to test the effects of intrathecal antagonists under anesthesia and the spinal cord was taken for immunohistochemistry.

(iii) Immunohistochemistry - Immunohistochemistry was performed in Dr. Alan Fine's laboratory, Department of Physiology and Biophysics, Dalhousie University. Serotonergic neurons were identified using an antibody directed against serotonin. Noradrenergic neurons were located using an antibody raised against dopamine-β-hydroxylase. This enzyme converts dopamine into noradrenaline and as such is a marker for noradrenergic nerve terminals. Details of tissue processing are presented in Appendix "A".

B. Experiments on Anesthetized Cats

1. Ascending Activity

(i) Surgical Preparation - Anesthesia was induced in adult male cats (2.8-3.6 kg, n=77) with ketamine (25 mg/kg i.m.). The trachea was cannulated and a mixture of 2-3% halothane was administered in N_2O/O_2 in a ratio of 1:1 for a total flow rate of 2 L/min. The right jugular or femoral vein was cannulated for chloralose administration (50 mg/kg

i.v.) and for fluid supplementation (lactated Ringers, 15 ml/kg/hr). Halothane anesthesia was decreased after chloralose administration based on blood pressure readings and was discontinued after completion of the surgical preparation. The cats were artificially respired using 100% O_2. The right carotid artery was cannulated for blood pressure recording. Temperature was measured through a probe placed in the abdomen. Blood pressure, temperature and pCO_2 were monitored and kept within physiological ranges. A representation of the experimental preparation is outlined in Fig. 7.

A laparotomy was performed through a midline abdominal incision. The urethra was cannulated 2-3 cm below the bladder neck. Pelvic nerves were dissected bilaterally by taking 1 branch per side, separating the branch from the surrounding fascia in two 4 mm sections, 3 mm apart. Silver 'plate' electrodes were secured to the urethra, placed under the 2 dissected sections and bent back over the nerve. The dissected areas were then insulated from surrounding tissue with Plastibase (Squibb Canada Inc., Montreal), a thick, gelatinous, non-conducting material. The abdomen was closed in two layers, muscle and skin. Laminectomies were performed at T11-T12 and L4 for microelectrode recording and for intrathecal cannula implantation respectively. After making a small opening in the dura at L4, an intrathecal cannula (polyethylene tubing 10G) was inserted and threaded to the lumbosacral junction. In some experiments, cervical laminectomies were performed for administration of local anesthetic. A paravertebral incision at the level of the ischial tuberosities was made to reach the left pudendal nerve. The left sensory and urethral motor branches were dissected and cut distally. The central cut ends

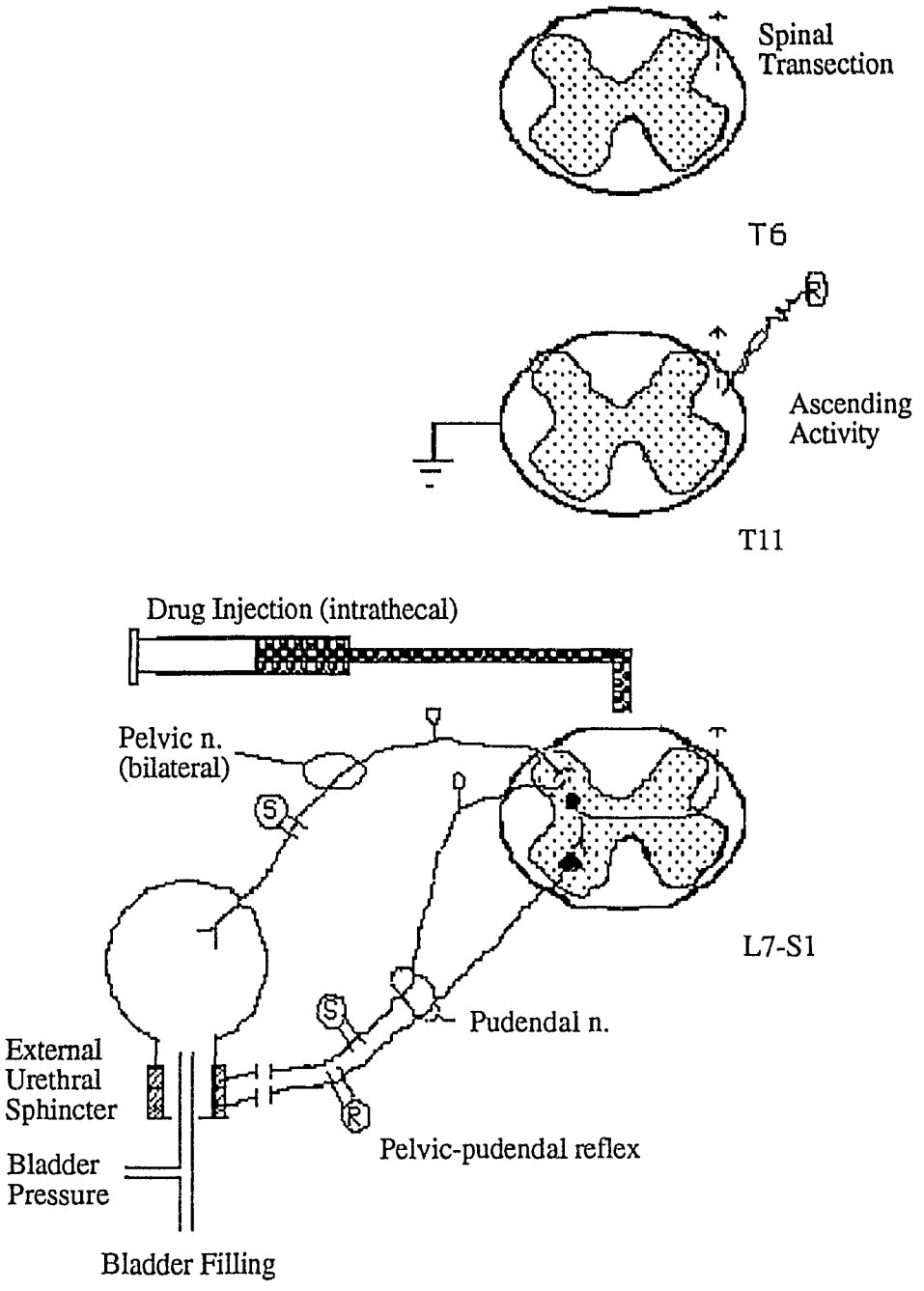


Figure 7: Diagram of Anesthetized Preparation. S=stimulating electrode.R=recording electrode

of the nerves were placed on bipolar platinum-iridium electrodes in a mineral oil pool. Bilateral pneumothoraxes were performed to reduce chest excursion.

(ii) Nerve Stimulation and Recording - Spinally-Intact and Acutely-Spinalized Cats - The pelvic nerve was stimulated using a train of 2-3 pulses of 0.5 ms width, 3 ms apart and at 0.2 Hz. The intensity used was 5-10 times the threshold for eliciting a pelvic-pudendal reflex (2.5-9 V). The lateral funiculus of either side of T11-T12 spinal segments was explored with a 10 MOhm tungsten microelectrode. Contralateral pudendal nerve stimulation of a single pulse of 0.1 ms width at 0.2 Hz was used as a search stimulus. Single unit responses were differentially amplified, stored on videotape after A/D conversion and pulse-code modulation with a Vetter Digital 3000A, led to a window discriminator and analyzed on-line by a computer equipped with MacLab Histogram software. Twenty successive responses to nerve stimulation were taken to form post-stimulus time histograms. Response was defined as the sum of all twenty discharges. If spontaneous activity was present, an average activity/bin was calculated, multiplied by response duration, and subtracted from the total spike number. The measure of treatment effect was the number of spikes after treatment expressed as a percentage of pretreatment control. Location of units was determined by depth shown by micropositioner and by electrolytic lesion after recording.

(iii) Acute Spinalization - Two experiments were performed on acutely-spinalized cats. Spinalization was carried out at T5-T6 after cooling of the spinal cord with iced saline for 10 min.

(iv) Drug Studies - 5HT hydrochloride (RBI), methysergide maleate (Sandoz), 2-methyl-5HT maleate (RBI), zatosetron maleate (Lilly Research Laboratories), 8-OH-DPAT hydrobromide (RBI), NAN-190 hydrobromide (RBI) were dissolved in 0.9% saline just prior to injection.

2. Viscerosomatic and Somato-somatic Reflexes

Spinally-intact Cats - The pelvic-pudendal (viscerosomatic) and pudendal-pudendal (somato-somatic) reflexes were monitored concomitantly with ascending activity. Compound action potentials were recorded in response to either pelvic or pudendal nerve stimulation as used in the ascending experiments. Responses were differentially amplified and stored on videotape after A/D conversion. Experiments were analyzed on a computer with a MacLab Scope program by playing the tape back after the experiments were completed. This aspect of the experiments could not be analyzed on-line as the computer was occupied analyzing ascending activity. Twenty successive action potentials were averaged and the area under the averaged compound action potential calculated. Treatment effect was expressed as post-treatment value calculated as a percentage of pre-treatment control.

C. Behavioural Studies in Conscious, Spinally-Transected Cats

(i) Surgical Preparation - Six of the cats in these studies were used to examine the effects of 5HT and zatosetron on bladder function and thus the method for surgical preparation is similar to that in the section for Behavioural Studies in Conscious Spinally-Intact Cats in Section A with the following exceptions. Only one 20G Silastic

cannula was implanted in the dorsal surface of the bladder. A second 20G Silastic cannula was fitted with a balloon-end fitting made from the finger tip of a latex glove. This second cannula was implanted in the peritoneal space and exited the abdominal musculature through a hole punctured in the muscle to the right side of the abdominal incision. This second cannula was used to monitor intra-abdominal pressure. Spinal cord transection at T11-T12 was performed in an aseptic surgery 8-16 weeks after bladder line implantation.

The second group of male cats (n=6) was prepared as for section A but did not receive an intrathecal cannula and was not used for intrathecal testing of drugs prior to transection. After a minimum recovery period of a week, the cats were spinally transected as for the first group.

(ii) Bladder Drainage - Following spinal cord transection, bladder drainage was accomplished twice daily by one of three methods: by clean intermittent urethral catheterization (n=4) or via the indwelling bladder cannula with (n=4), or without (n=4) the addition of perineal stimulation. Perineal stimulation attempted to mimic that caused by urethral catheterization. Thus, extension of the erect penis was done and continued for the duration of bladder emptying as would be done for urethral catheterization. Urine samples were obtained through the implanted cannula or by cystocentesis and sent to a local hospital for bacterial culture and sensitivity testing before and after transection and when multistick or sediment exam suggested infection. If urinary tract infection was present (defined as $>10{,}000$ CFUs/ml), appropriate antibiotic therapy was instituted based on sensitivity testing.

(iii) Bladder Testing - Bladder function was assessed using the same testing protocol employed for the spinally-intact cats except for one cat who was tested using an indwelling urethral catheter as the bladder line had become clogged. At post-mortem the line was found to be plugged with crystalline material. Bladder function testing was carried out only on cats without bladder infection. Bladder function was tested before and after spinal cord transection in i.t.-implanted cats and after transection in non-implanted cats.

(iv) Investigations in Areflexic Cats - Naloxone (750 nmol) was injected intrathecally in three cats. Cold saline was instilled into the bladders of two cats that failed to demonstrate reflex bladder activity after spinal transection.

(v) Drug Studies in Reflexic Cats - Intrathecal application of 5HT or 2-methyl-5HT was accomplished by one of two methods: (1) through a chronically-implanted intrathecal line or (2) by lumbar puncture with a spinal needle (20G) inserted into the epidural space at the L7 vertebral level and advanced until spinal fluid was observed in the needle hub. Lumbar puncture was performed by a qualified anesthetist, Dr. G. Doak. The dead volume of the needle was calculated to be 0.05 ml. 0.15 ml was injected and the needle removed.

(vi) Statistics - Paired t-tests ($P<0.05$) were used to compare bladder parameters before and after spinal transection.

D. Acute Root Stimulation Experiments on Chronic Spinally-Transected Cats

Two out of three cats without distension-evoked bladder contractions underwent an acute experiment to determine if a sensory or motor deficiency was responsible for the lack of reflex activity.

(i) Surgical Preparation - Ketamine (25 mg/kg i.m.) was used for induction and was followed by halothane (2.5%) in O_2 and N_2O. The trachea, left carotid artery and right femoral vein were cannulated for artificial ventilation, blood pressure recording, and fluid and drug administration respectively. An intercollicular decerebration was performed and halothane was discontinued. Lactated Ringer's solution (15 ml/kg/hr) was used for fluid supplementation.

(ii) Bladder Testing - The bladder was distended with 20 ml of warm saline and bladder pressure in response to S1-S3 sacral root stimulation with bipolar electrodes was monitored on a side arm of the fluid filling line. Whole roots were stimulated with a Medtronic Inc. 3623 stimulator to determine the root giving the largest bladder pressure response. Root stimulation values of 5 V, 500 ms pulses at 25 Hz gave reliable pressure responses in both experiments. The root giving the largest contraction was then divided intradurally into dorsal and ventral portions. The ventral root was stimulated to ensure that the motor input to the bladder was intact and the dorsal root was stimulated to determine if the sacral spinal circuitry was capable of producing bladder contraction.

IV Results

A. Behavioural Studies in Conscious, Spinally-Intact Cats

1. Acute Manipulation of Spinal Monoaminergic Transmission

The reproducibility of V_T between trials was examined and found to be consistent in repeated trials (Fig. 8A) and was not affected by vehicle (saline). Zatosetron (10 nmol) decreased V_T in 3 cats to 65% of control (range 57%-71%, Fig. 8A,B, Table 1). Methysergide dose-dependently reduced V_T (n=5, Fig. 9A). There was considerable inter-animal variability in the susceptibility of V_T to inhibition by methysergide (Fig. 9A). In 4/5 cats, a dose was attained beyond which methysergide was less effective. At doses exceeding this level, considerable hind-limb motor impairment was observed. The highest dose of methysergide used was 60 nmol as larger doses routinely resulted in allodynia manifested as sensitivity to light brushing of the fur along the abdomen and hindquarters. Using the maximum inhibition of V_T, methysergide decreased V_T to 33% of control (range 18%-50%, Table 1). Methysergide's duration of action was monitored in one cat for 4 h. At 4 h post-injection, V_T was still depressed. On the following day, the cat was retested and V_T had returned to control levels. None of the other bladder function parameters were consistently altered (Fig. 9B). 5HT (2350 nmol) was administered to 5 cats. V_T was increased to an average of 201% (range 143%-300%, Fig. 10, Table 1). Other CMG parameters were not consistently affected (Fig. 10B). There did not appear to be any other behavioural effects caused by intrathecal administration of 5HT.

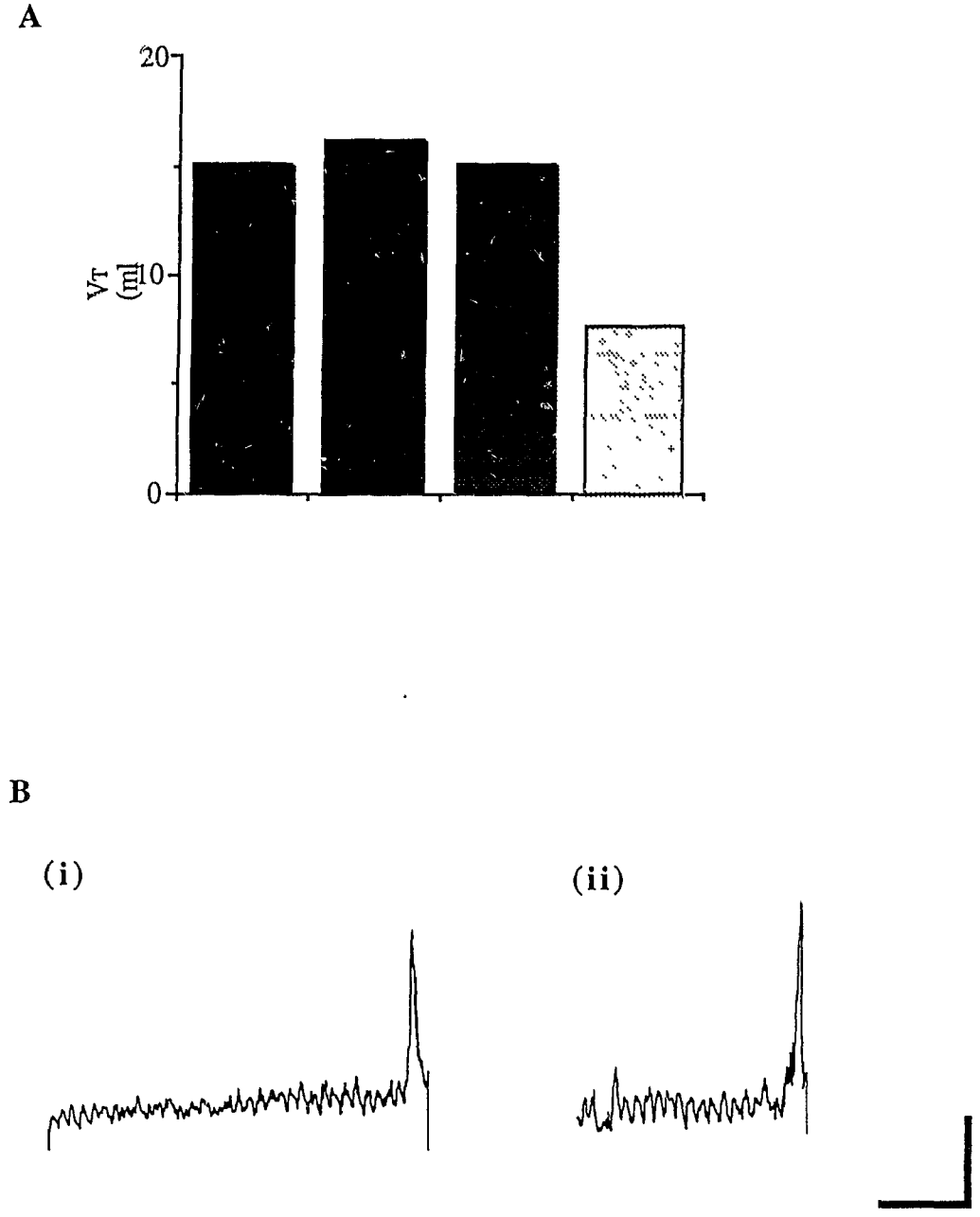
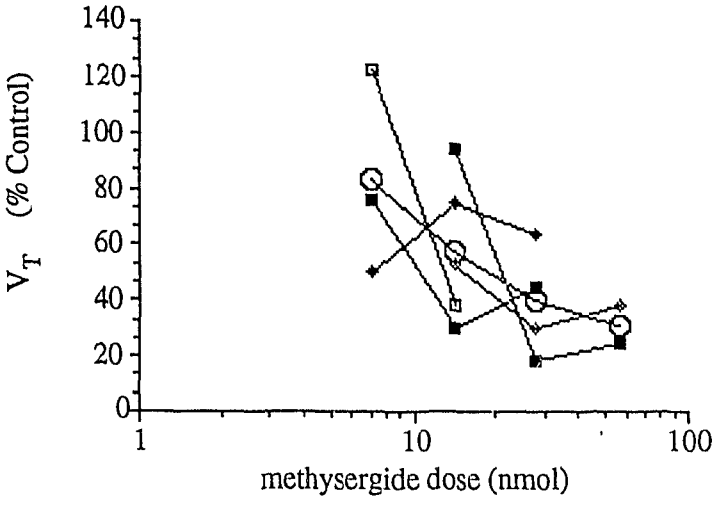


Figure 8: Effect of Zatosetron on Bladder Function in Conscious Cats A) VT for two baseline CMGs (solid bars), after i.t. injection of saline (dark stippled bar) and after i.t. injection of zatosetron (10 nmol, light stippled bar) and B) Representative CMG from one animal. (i) control and (ii) after zatosetron, 10nmol. Horizontal bar=9 min.Vertical bar=20 cmH20.

A



B.

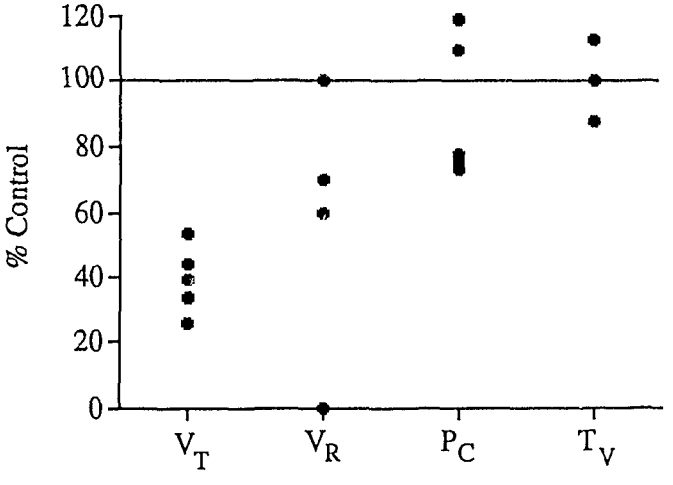


Figure 9: Effect of Methysergide on Bladder Function in Conscious, Spinally-Intact Cats. A) on V_T. Small symbols represent individual animals. Large circle represents average. B) on all functional parameters.

Compound	Dose (nmol)	n	Action	V_T (Average, % Control)	Range
5HT	2350	5	5HT agonist	201	143-300
methysergide	7-60	5	5HT1D/2A/2C antagonist	33	18-50
zatosetron	10	3	5HT3 antagonist	65	57-71
prazosin	25	4	α1-antagonist	85	71-100
phentolamine	890	4	α-antagonist	55	37-68

Table 1: Effect of Serotonergic and Noradrenergic Ligands on Volume Threshold (VT) in Conscious, Spinally-Intact Cats.

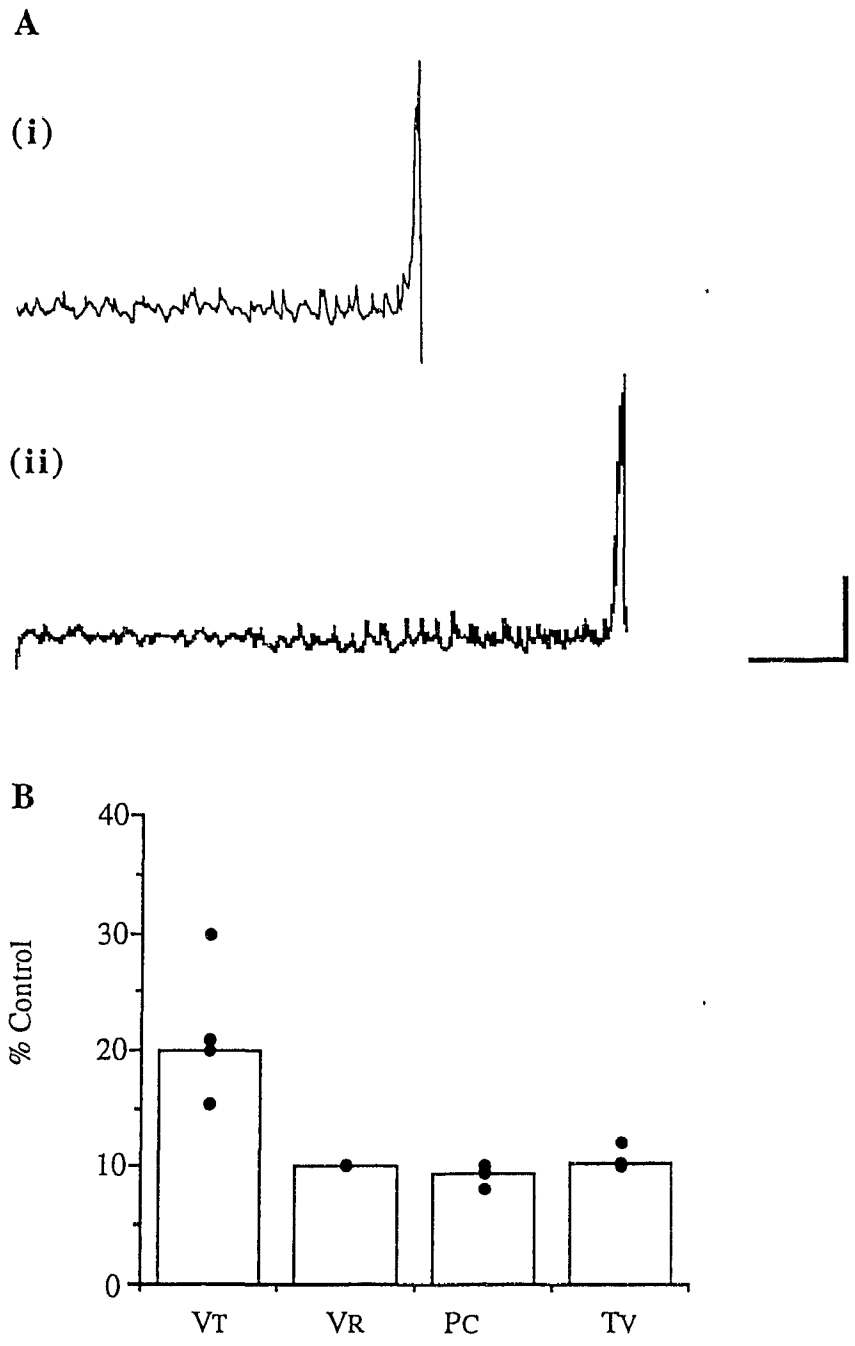


Figure 10: Effect of 5HT on Bladder Function in Conscious Spinally-Intact Cats A) Representative CMG from one animal (i) control (ii) after 5HT 2350 nmol. Horizontal bar=9 min, Vertical bar=20 cmH20. B) on all bladder parameters measured (n=5). Bar represents average and filled circles represent individual animals.

Prazosin was tested at 25 μmol on four cats and 50 μmol in one cat. No consistent effects were seen on any parameter, V_T included (Fig. 11A). Motor impairment and allodynia were not observed. Phentolamine (890 nmol) was tested on 4 cats. This was the maximum dose used as larger doses resulted in allodynia. V_T was decreased on average to 55% of control. The other CMG parameters were not consistently affected (Fig. 11B).

2. Chronic Interruption of Spinal Serotonergic and Noradrenergic Transmission

Bladder function testing and immunohistochemical analysis were done without knowledge of the identity of the neurotoxin or uptake blocker injected. The following results were correlated after the bladder testing and immunohistochemistry had been done.

(i) Bladder Infections - 8 out of 12 cats developed 1 or more bladder infections during their stay in the Animal Care centre. *Pseudomonas cepacia* and *aeruginosa* were responsible for 66% of all infections. *Escherichia coli*, *Enterococcus* and *Staphylococcus* were the causative bacteria in the remaining infections.

(ii) Uptake Blocker Controls - Control injections were performed for imipramine and fluoxetine. Imipramine was injected i.p. 1 hr and fluoxetine 3 hr prior to intrathecal administration of 0.5 ml of 0.1% ascorbic acid in 0.9% sterile saline. Imipramine (administered twice to the same cat), a noradrenergic uptake blocker, did not affect bladder function measured one week after injection. Intraperitoneal administration of fluoxetine, however, did increase V_T (n=2) to 210% and 274% of control 6 and 7 days after administration (Fig. 12).

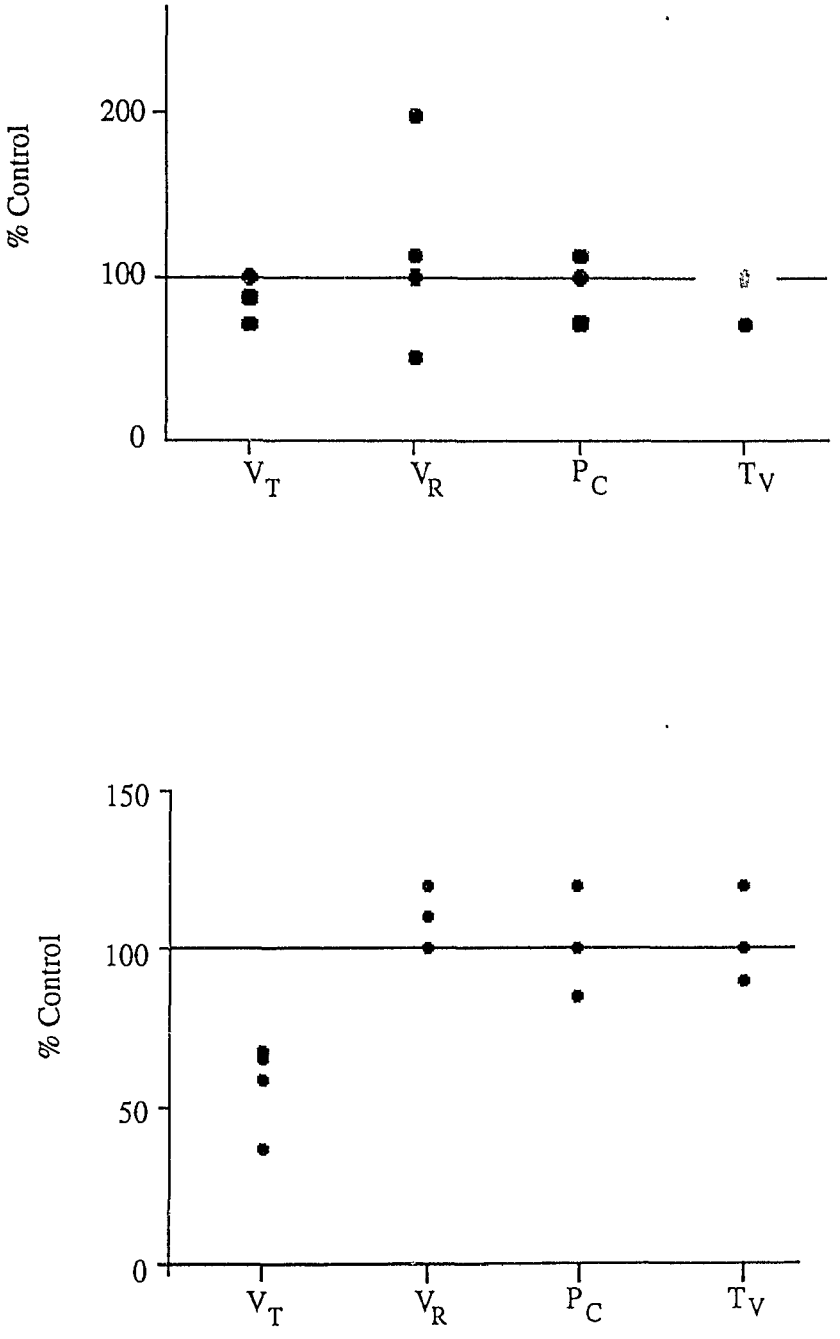


Figure 11: Effect of Adrenoceptor Antagonists on Bladder Function Parameters in Conscious, Spinally-Intact Cats A) Prazosin (25 μmol, n=4) and B) Phentolamine (890 nmol, n=4).

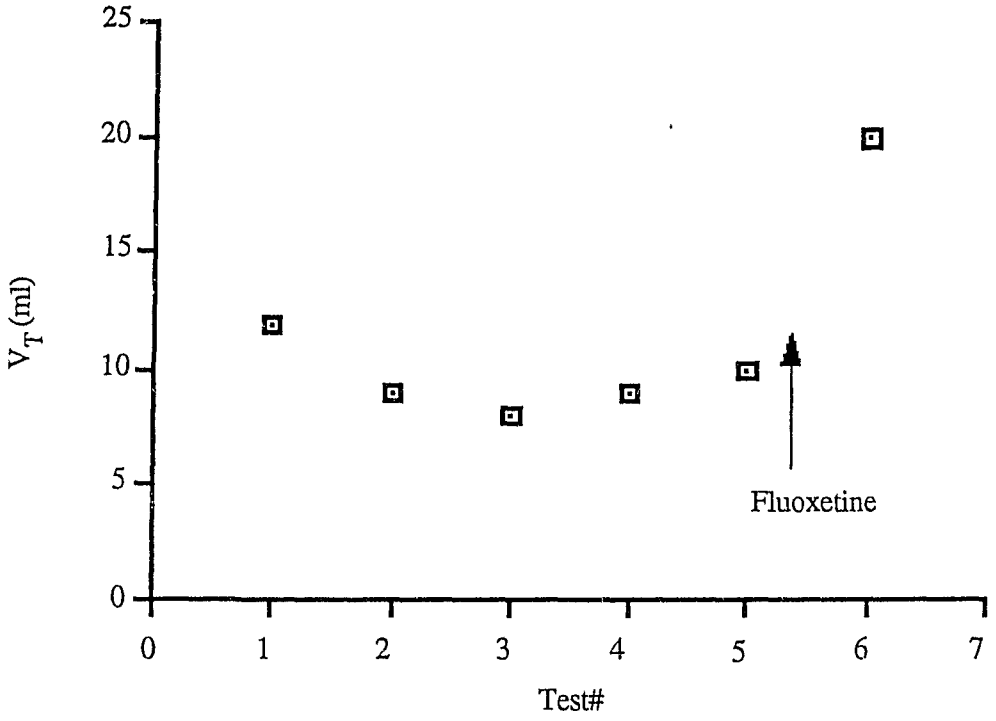


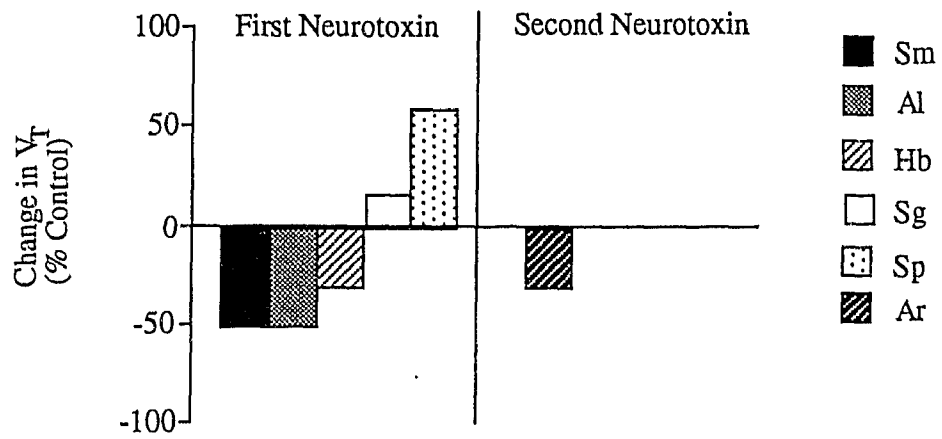
Figure 12: Effect of Fluoxetine (7.5mg/kg i.p.) on V_T in One Conscious Cat.

(iii) Neurotoxins - (a) 5,7-dHT - Five cats received 5,7-dHT as the first neurotoxin. In one cat the low dose (1.5 mmol) produced a decrease in V_T whereas in a second cat, the low dose was ineffective but the high dose (3 mmol) decreased V_T. As the low dose was not always effective, a single high dose was administered to 2 cats. One cat was unaffected but the second showed an increased V_T (Fig. 13).

(b) 6-OHDA - 4 cats were administered 6-OHDA as the first neurotoxin. In 1 cat, a low dose (2 mmol) 6-OHDA increased V_T. In 1 cat, the low dose was ineffective but a second dose increased V_T. As the low dose was not always effective the third cat received a single, high dose (4 mmol) and demonstrated an increased V_T. The fourth cat was unaffected even by the largest dose and went on to receive the high dose of the second neurotoxin (5,7-dHT, 3 mmol). As fluoxetine caused a large increase in V_T by itself in 2 cats, a correction factor had to be instituted. The 2 cats who received fluoxetine alone demonstrated large increases in V_T although their basal V_T levels varied considerably. Thus a percentage correction factor representing an average of the 2 values was instituted. When this correction factor was applied, 4 of the 5 cats were unaffected by 6-OHDA while the fifth demonstrated a large decrease in V_T (Fig. 13).

(c) 6-OHDA and 5,7-dHT - 2 cats were administered both neurotoxins as they were unaffected by the first neurotoxin. The cat initially injected with 6-OHDA received 5,7-dHT 20 days later and showed a decreased V_T (Fig. 13). The second cat injected initially with 5,7-dHT had 6-OHDA injected 9 days later and demonstrated a slight increase in V_T (Fig. 13).

A



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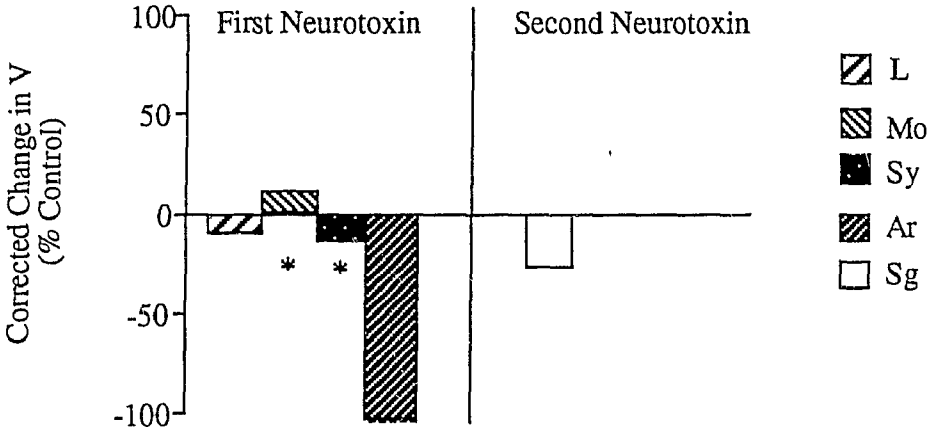


Figure 13: Effect of the Neurotoxins A)5,7-dHT and B)6-OHDA on V_T in Conscious Cats. Bars represent change in V_T in individual animals expressed as a percentage of their respective control volumes. Asterisk denotes low dose of fluoxetine.

(iv) Immunohistochemistry - (a) 5,7-dHT - In 2 cats, 1 receiving the low dose (1.5 mmol) and the other the high dose (3 mmol) of 5,7-dHT, no depletion of 5HT or DβH was observed. In 2 cats receiving the high dose of 5,7-dHT, both 5HT and DβH were completely absent from the sacral spinal cord (Table 2).

(b) 6-OHDA - The cat receiving two doses of 6-OHDA had no depletion of either 5HT or DβH. The cat administered one high dose of 6-OHDA (4 mmol) was depleted of both DβH and 5HT immunoreactivity. The cat receiving the low dose of 6-OHDA (2 mmol) was depleted of DβH but not 5HT.

(c) 5,7-dHT and 6-OHDA - The 2 cats receiving both neurotoxins were depleted of 5HT in the lumbosacral cord. The cat who received 6-OHDA first was not depleted of DβH (Table 2).

B. Experiments on Anesthetized Cats

1. Ascending Activity

(i) Location and Latency of Units - Figure 14 demonstrates the location of recorded units with respect to stimulating sites from Fedirchuk and Shefchyk, and recording sites from McMahon and Morrison. Most units were found between 1-1500 μm depth (Fig. 15A) and had latencies from the peripheral stimulus of 11-50 ms (Fig. 15B) The latencies and depths of the units did not correlate (Fig. 15C). The 2 units examined in spinal cats with 2-methyl-5HT were found at depths of 1360 and 1150 microns. Twenty successive

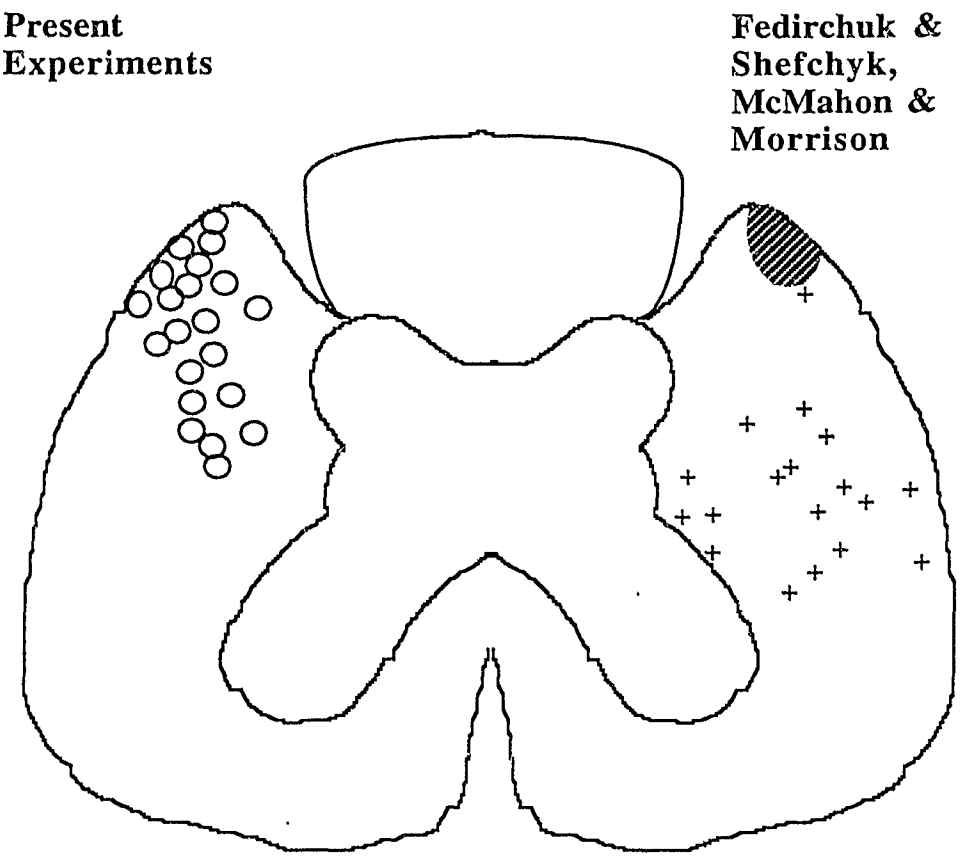
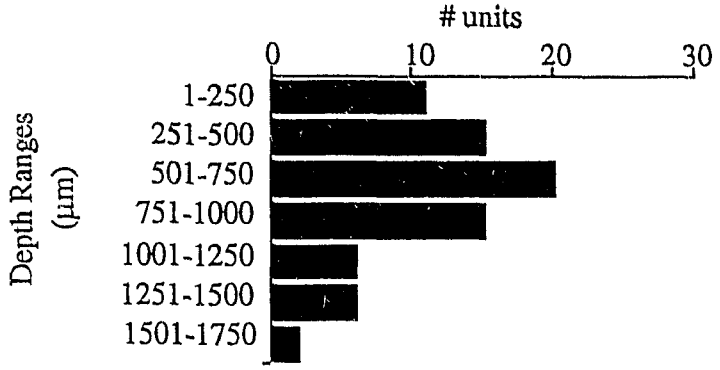


Figure 14: Location of Ascending Units Recorded or Stimulated in Anesthetized Cats. Left side (open circles) denotes units recorded from in present experiments. Hatched area on right represents area stimulated in Fedirchuk and Shefchyk experiments. Crosses denote location of units recorded in McMahon and Morrison experiments.

A



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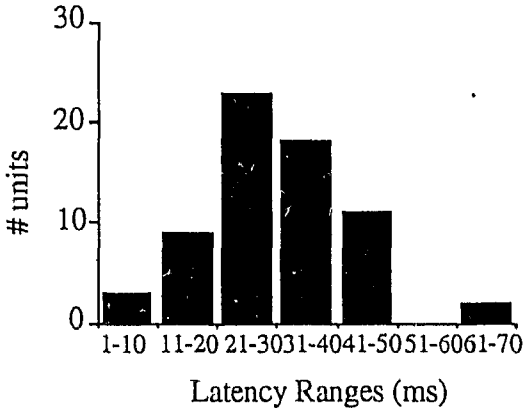

C

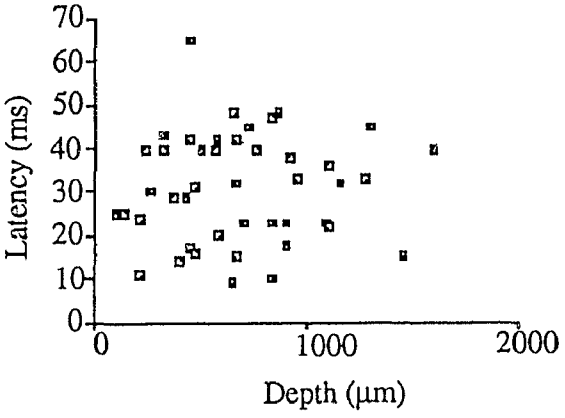

Figure 15: Depth and Latency Distributions of Ascending Units Recorded in Anesthetized Cats. A) Distribution of depths recorded in present experiments. B) Distribution of latencies C) Lack of correlation between depth and latency of ascending units.

Table 2: Doses, Immunohistochemistry and Behavioural Effects of Neurotoxins in Conscious Cats

Cat	Toxin	Dose (nmol)	Uptake Blocker	DβH †	5HT †	V_T* (% Control)
Sm	5,7-dHT	1.5 3	Imipramine	(-)	(-)	50
Al	5,7-dHT	1.5	Imipramine	(+)	(+)	51
Hb	5,7-dHT	3	Imipramine	(+)	(+)	67
Sp	5,7-dHT	3	Desipramine	(-)	(-)	155
Sg	5,7-dHT 6-OHDA	3 4	Imipramine Fluoxetine	(-)	(-)	118 75
Sy	6-OHDA	2 2	Fluoxetine Fluoxetine	(+)	(+)	 82
Mo	6-OHDA	2	Fluoxetine	(-)	(+)	114
L	6-OHDA	4	Fluoxetine	(-)	(-)	86
Ar	6-OHDA 5,7-dHT	4 3	Fluoxetine Desipramine	(+)	(-)	0 66
Fr	-	-	Fluoxetine (15mg/kg)	(+)	(+)	210
Mar	-	-	Fluoxetine (15mg/kg)	(+)	(+)	274

†DβH,5HT- (+)=normal immunoreactivity, (-)=depletion in sacral spinal cord
*6-OHDA values are after correction for fluoxetine's effect

unit responses to pelvic nerve stimulation were monitored. Pelvic nerve stimulation did not always produce unit activation in all twenty trials. The spike number varied from 0-9. On-going activity was present in 43 of 77 neurons. Units could be activated by both left and right pelvic nerve stimulation as well as by pudendal nerve stimulation (Fig. 16). Pudendal nerve stimulation produced an earlier and larger response of statistically similar duration compared to pelvic nerve stimulation. In two acutely-spinalized cats, two units with latencies of 20 and 25 ms were recorded.

(ii) Drug Effects - 5HT (0.3-6 mmol i.t.) was tested in 7 cats. Ascending activity was depressed in 3 cats and unaffected in 4 cats (Fig. 17). When ascending activity was affected, it was dramatically decreased (Fig. 17). 8-OH-DPAT (60-150 nmol) caused increases (n=2) and decreases (n=3) in ascending activity (Fig. 17). NAN-190 (4-520 nmol) decreased ascending activity in four cats but was ineffective in the fifth. Methysergide (60 nmol, n=5) increased ascending activity, in all experiments in which it was tested, to an average of 165% of control (Fig. 17, 18). Unit latencies were not significantly altered. 2-Methyl-5HT (800 nmol, n=4) decreased ascending activity to 52% of control (Fig. 17, 19). Zatosetron partially reversed the action of 2-methyl-5HT (n=4, Fig. 20). Zatosetron alone (10 nmol, n=4) increased activity to a mean of 198% (Fig. 17, 21). Latencies of units were not significantly altered. In spinal cats, 2-methyl-5HT (800 nmol, n=2) depressed ascending activity to 65% and 75% of control (Fig. 17) but did not alter the latencies.

2. Viscerosomatic and Somato-somatic Reflexes

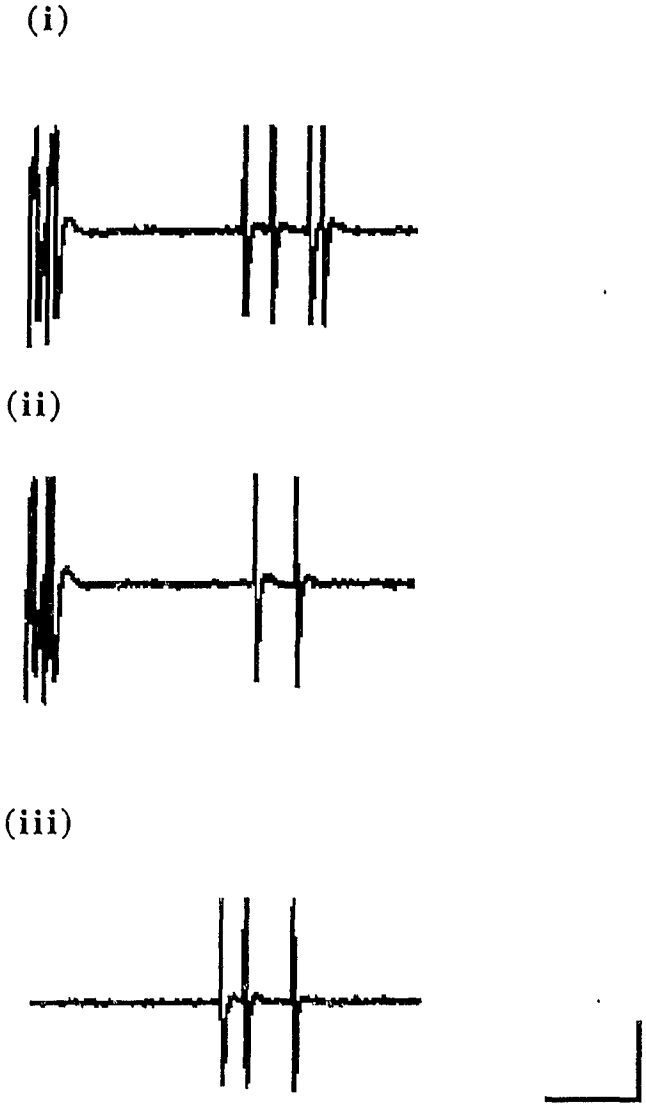

Figure 16: Afferent Input onto Ascending Units Recorded in Anesthetized Cats. Unit response to (i)ipsilateral (ii)contra-lateral pelvic and (iii) pudendal nerve stimulation. Horizontal bar=10ms, Vertical bar=300μV

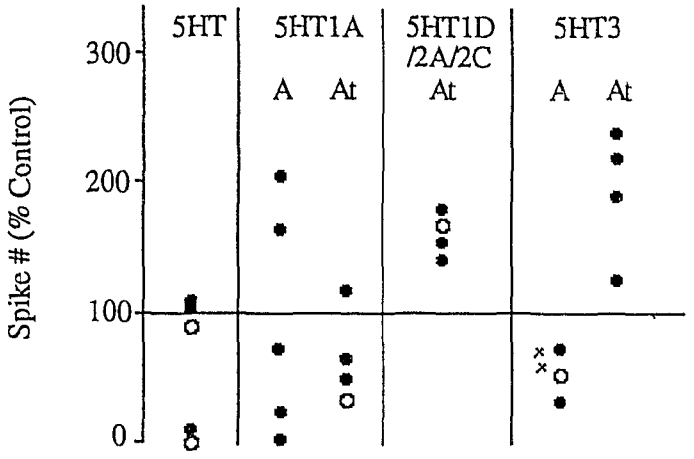

Figure 17: Effect of Serotonergic Compounds on Ascending Activity Recorded in Anesthetized Cats. Responses were evoked by pelvic nerve stimulation and are expressed as a percentage of control. Filled circles represent individual experiments, empty circles represent the values for two experiments. Crosses denote experiments in acutely-spinalized cats. Circles above the horizontal line indicate increased activity. A=agonist, At=antagonist. 5HT1A A=8-OH-DPAT, At=NAN-190, 5HT1D/2A/2C At=methysergide, 5HT3 A=2-methyl-5HT, At=zatosetron.

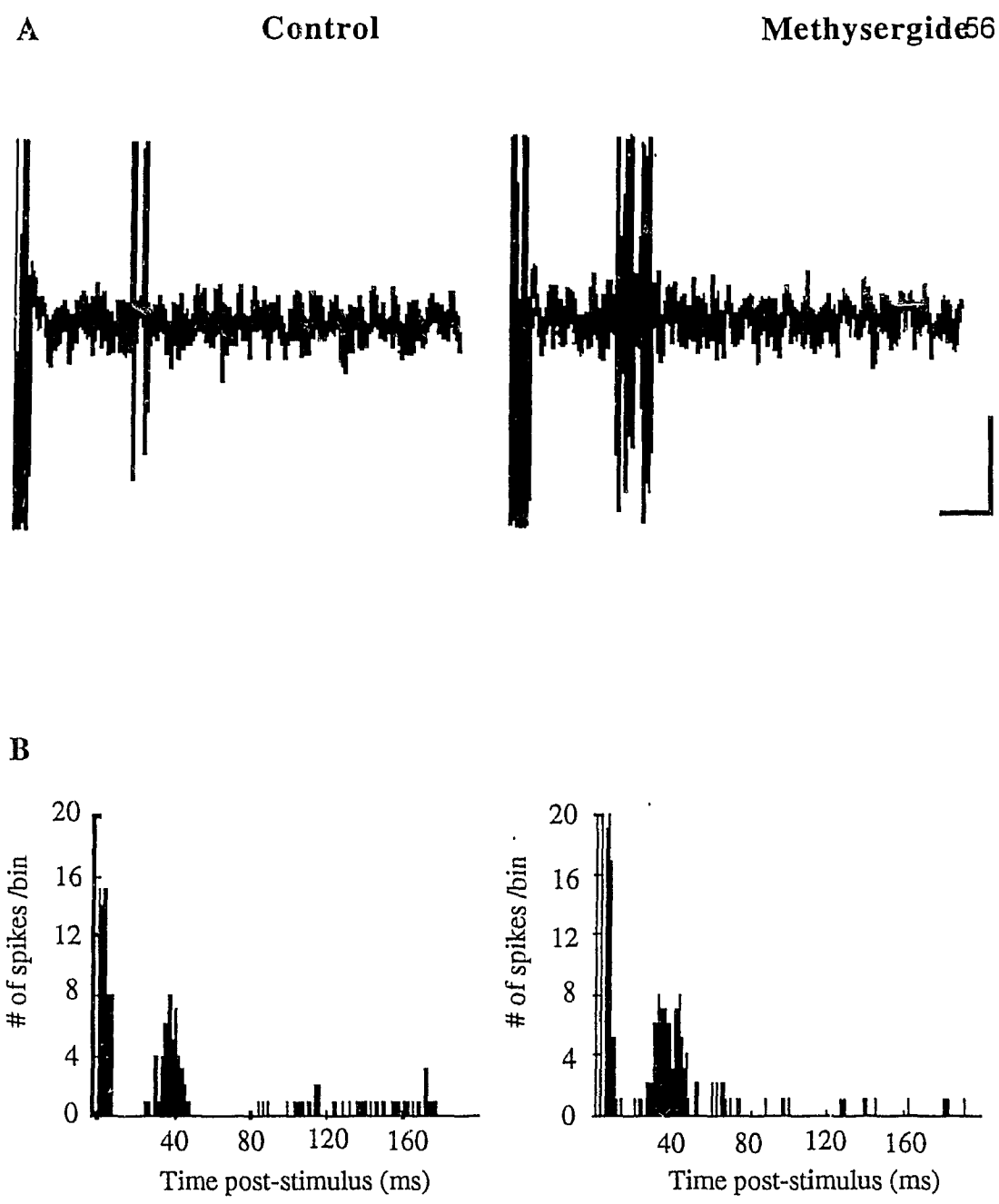

Figure 18: Effect of Methysergide (60 nmol i.t.) on Ascending Activity Recorded in One Anesthetized Cat. Responses were evoked by pelvic nerve stimulation. A) Raw traces B) post-stimulus time histograms. Horizontal bar=10ms. Vertical bar=50μV

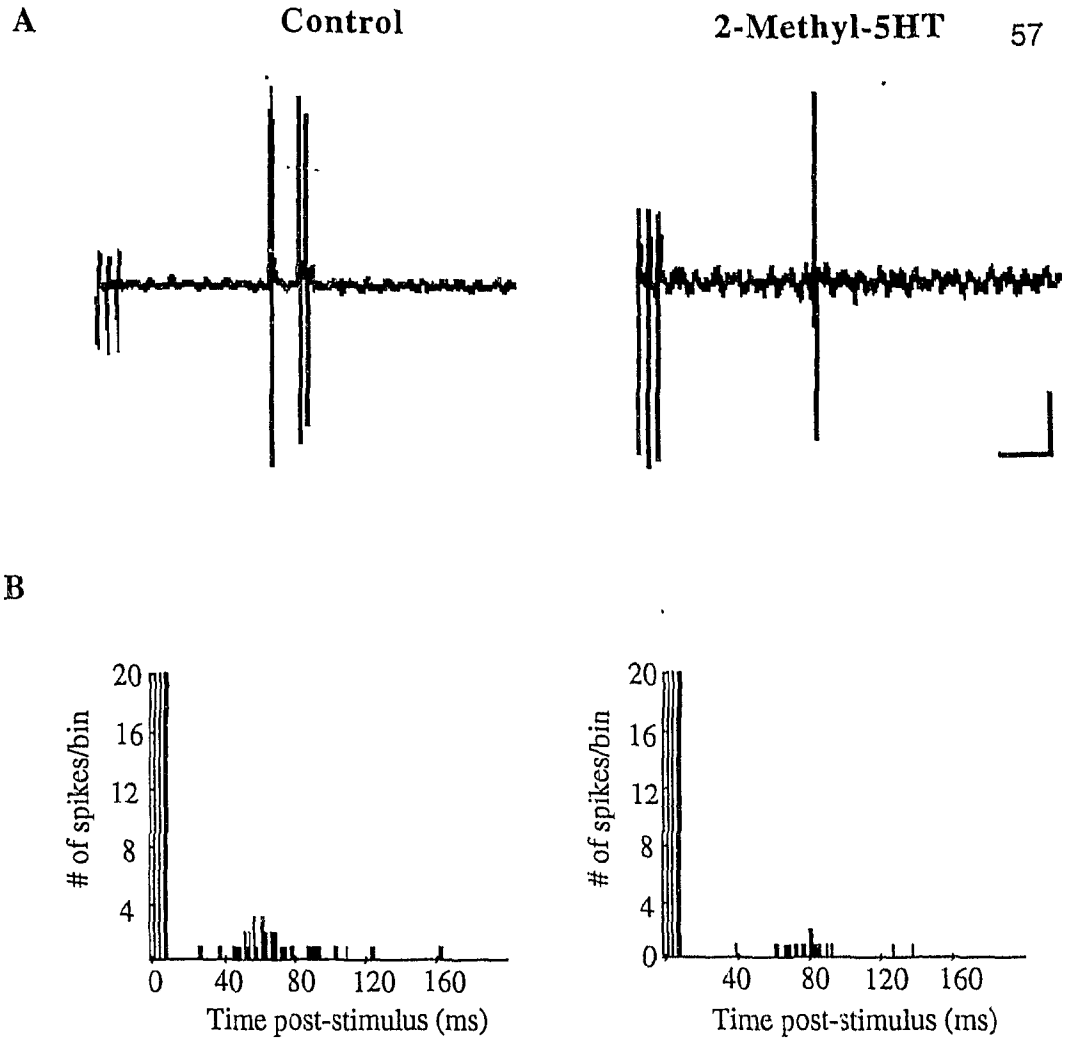

Figure 19: Effect of 2-Methyl-5HT (800 nmol) on Ascending Activity Recorded in One Anesthetized Cat. Response evoked by pelvic nerve stimulation. A) Raw traces B) post-stimulus time histograms. Horizontal bar=10ms Vertical bar=200 μV in A, 100 μV in B.

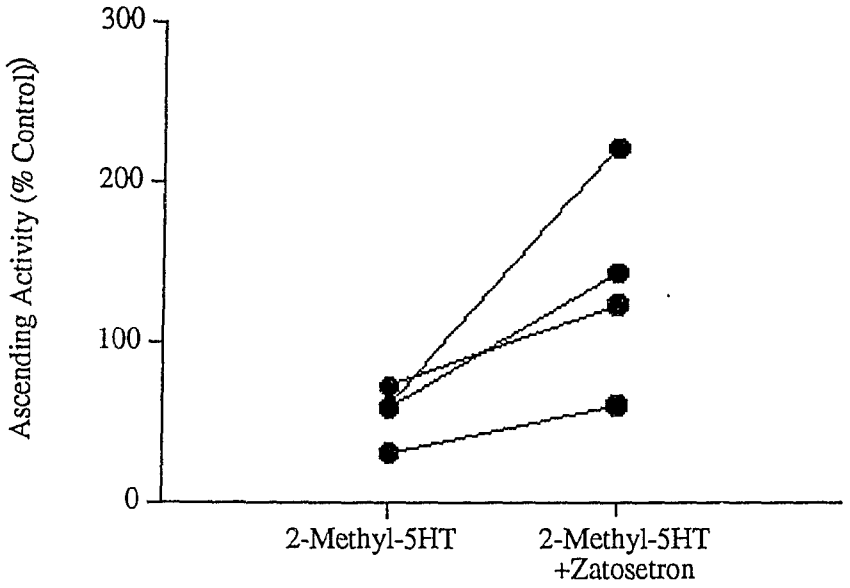

Figure 20: Effect of 2-Methyl-5HT (800 nmol, n=4) on Ascending Activity and Reversal with Zatosetron (10 nmol) as Recorded in Anesthetized Cats. Responses were evoked by pelvic nerve stimulation.

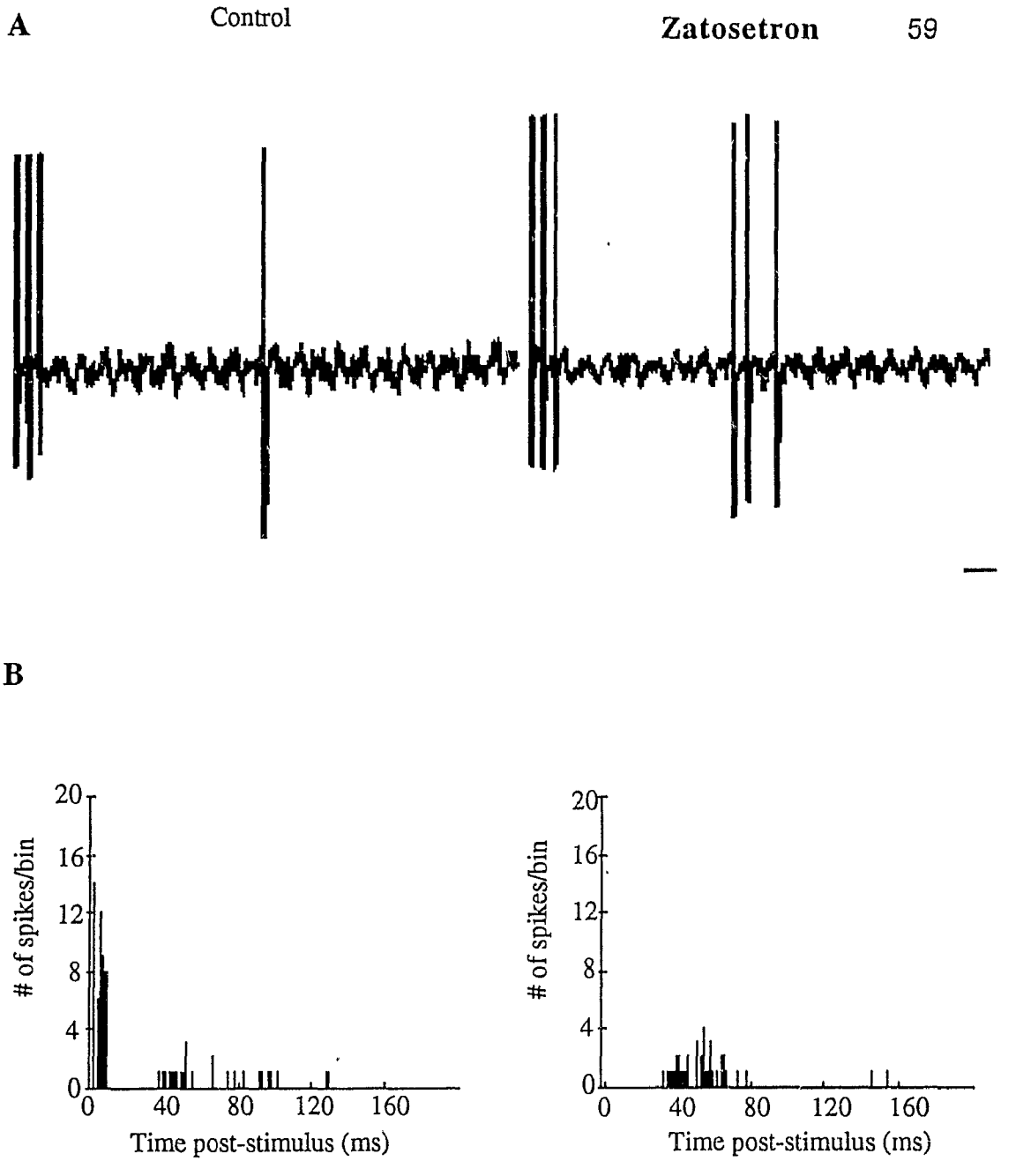

Figure 21: Effect of Zatosetron (10nmol) on Ascending Activity in an Anesthetized Cat. Responses evoked by pelvic nerve stimulation. A) Raw traces B) Post-stimulus time histograms. Horizontal bar=10 ms. Vertical bar=10 μV.

Spinally-Intact Cats - Methysergide, a non-selective 5HT1D/2A/2C antagonist, (60 nmol, n=5) decreased the pelvic-pudendal reflex to 65% of control (Fig. 22). 2-Methyl-5HT, a 5HT3 agonist, (800 nmol, n=4) increased the pelvic-pudendal reflex to 173% of control (SEM 92%, Fig. 22) which was reversed by zatosetron (Fig. 23). Zatosetron, a 5HT3 antagonist, (10 nmol, n=4) decreased pelvic-pudendal reflex to 23% of control (SEM=12%, Fig. 22).

C. Behavioural Studies in Conscious Spinally-Transected Cats

(i) Bladder Infection - The infection status prior to first bladder testing is shown in Table 3. Of the headcap-drained group, 1/3 was infected, while 1/5 of the headcap plus perineal stimulation group was infected. Half (2/4) of the catheterized group had developed infections prior to the first testing session. Infectious organisms cultured included *Pseudomonas aeruginosa, Pseudomonas cepacae* and *Enterobacter cloacae*.

(ii) Reallocation in Bladder Care Groups - In three cases, circumstances dictated a change in bladder drainage method. After one week, one headcap-managed cat's bladder line clogged and consequently the cat was subsequently managed by urethral catheterization. To balance the groups, a catheterized cat was switched to the headcap group after 1 week. In the last case, a cat designated to be managed by headcap drainage-only was receiving perineal stimulation from the other cats in the colony licking his perineum. We therefore began managing him by headcap drainage plus perineal stimulation. The respective methods and durations of bladder drainage for each cat are shown in Table 3.

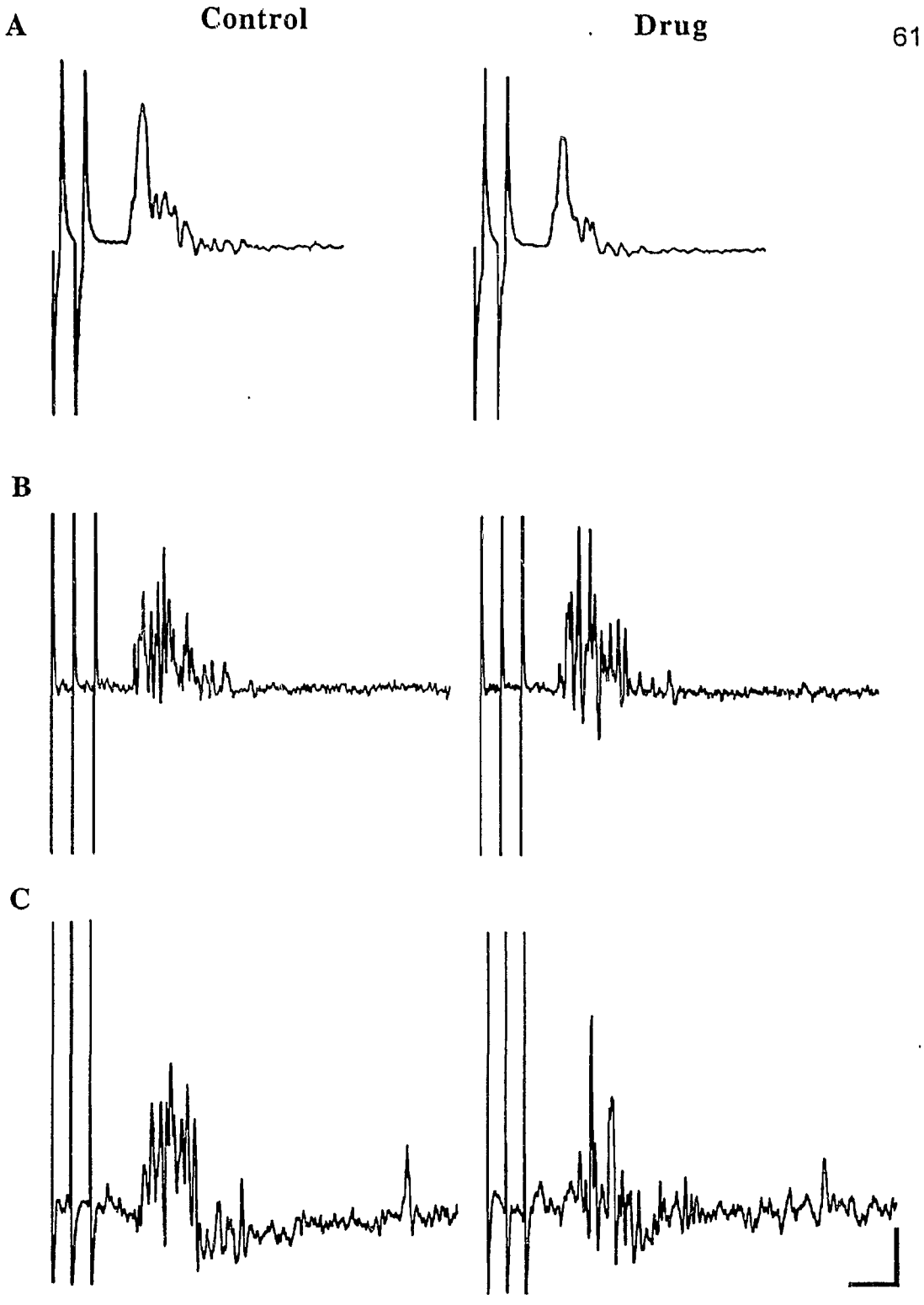

Figure 22: Effect of Serotonergic Compounds on the Pelvic-Pudendal Reflex Recorded in Anesthetized Cats. A) methysergide (60 nmol) B) 2-methyl-5HT (800 nmol) and C) zatosetron (10 nmol). Horizontal bar= 5 ms. Vertical bar=100 μV for A,B and 50 μV for C.

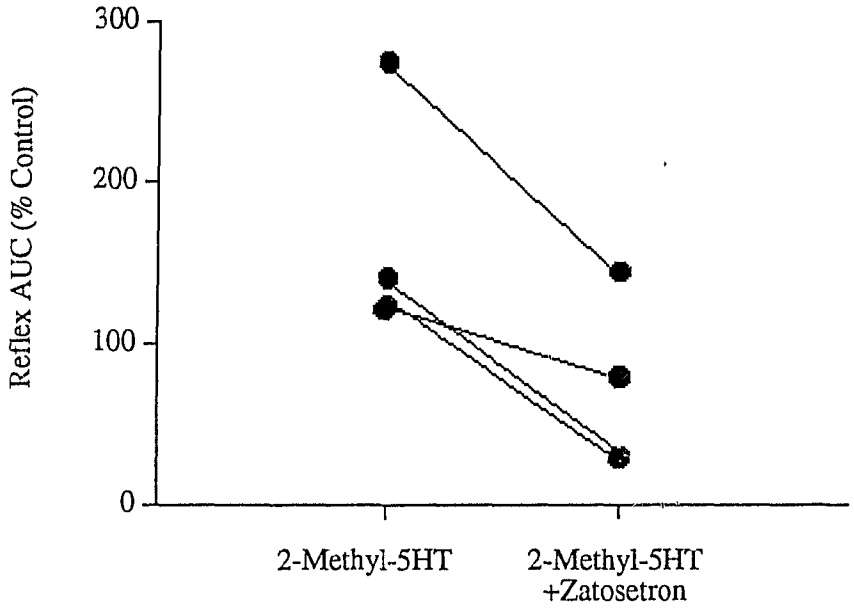

Figure 23: Effect of 2-Methyl-5HT (800 nmol,n=4) on the Pelvic-Pudendal Reflex and Reversal by Zatosetron (10 nmol) as Recorded in Anesthetized Cats.

Table 3: Bladder Management, Bladder Function and Infection Status. *VT=volume threshold for first contraction greater than 15 cmH20. †UTI=Urinary Tract Infection. (+)=presence of bladder infection before testing date, not at time of testing.+ N/A=not applicable as contractions were absent.§Numbers in parentheses indicate V_T prior to spinal transection.¥ initially from other cats.

		First Test			Last Test		
Cat Number	Drainage Method	Test Time (wks)	V_T*(ml)	UTI†	Test Time (wks)	VT (ml)	# of UTIs
1	Headcap[16wks+2wk catheter]	3.0	N/A+ (7) §	(-)	18	N/A	1
2	Headcap[16wks+2wk catheter]	3.0	N/A (35)	(-)	18	N/A	1
3	Headcap	3.0	N/A (15)	(+)	15	N/A	1
4	Catheterization	2.0	16 (14)	(-)	13	25	2
5	Catheterization [1wk headcap, 11.8wk catheter]	4.0	6 (15)	(+)	12.8	17	3
6	Catheterization [1wk catheter, 10wk headcap]	2.0	18 (20)	(-)	11	15	1
7	Catheterization	3.0	7	(+)	6.6	5	2
8	Headcap Plus Perineal Stimulation	2.2	6	(-)	5	29	0
9	Headcap Plus Perineal Stimulation	3.2	15	(-)	4.3	10	0
10	Headcap Plus Perineal Stimulation¥	1.2	25	(-)	8.2	23	0
11	Headcap Plus Perineal Stimulation	2.5	25	(-)	7	23	2
12	Headcap Plus Perineal Stimulation [5.1wks with, 3.9 without]	1.2	27	(-)	9	8	0

(iii) Bladder Activity - Reflex bladder activity after spinal cord transection differed from normal activity in duration of contraction. The duration of contraction in spinal cats is 1-2 sec while in intact cats (Fig. 24A,B), the duration is approximately 20 s. This difference was statistically significant ($P<0.05$). The P_C did not differ statistically nor did V_T. These contractions generated similar intravesical pressures to those in the intact cat but were not sustained. Bladder activity evoked by tactile perineal stimulation was distinctly different from that evoked by bladder distension (Fig. 24C). These pressure responses are of longer duration and lower amplitude than those evoked by bladder filling.

Two cats drained through the headcap for 16 weeks did not demonstrate bladder activity although their bladders were filled beyond their pre-spinalization volume thresholds for micturition. In order to determine if urethral catheterization could aid in the emergence of distension-evoked bladder contractions, they received intermittent catheterization for two weeks at 16 weeks post-spinalization, but did not develop bladder filling-evoked contractions (Fig. 25A).

(iv) Investigations in Areflexic Cats - The ability of the bladder to contract in response to other stimuli known to cause bladder contractions (such as i.t. administration of naloxone and intravesical instillation of cold saline) in spinal cord-transected cats was tested. Naloxone (150-750 nmol i.t.) administration did not reveal bladder contractions in response to bladder filling (Fig. 25B). Cold saline (about 10°C) infused into the bladder did not produce contractions (Fig. 25C).

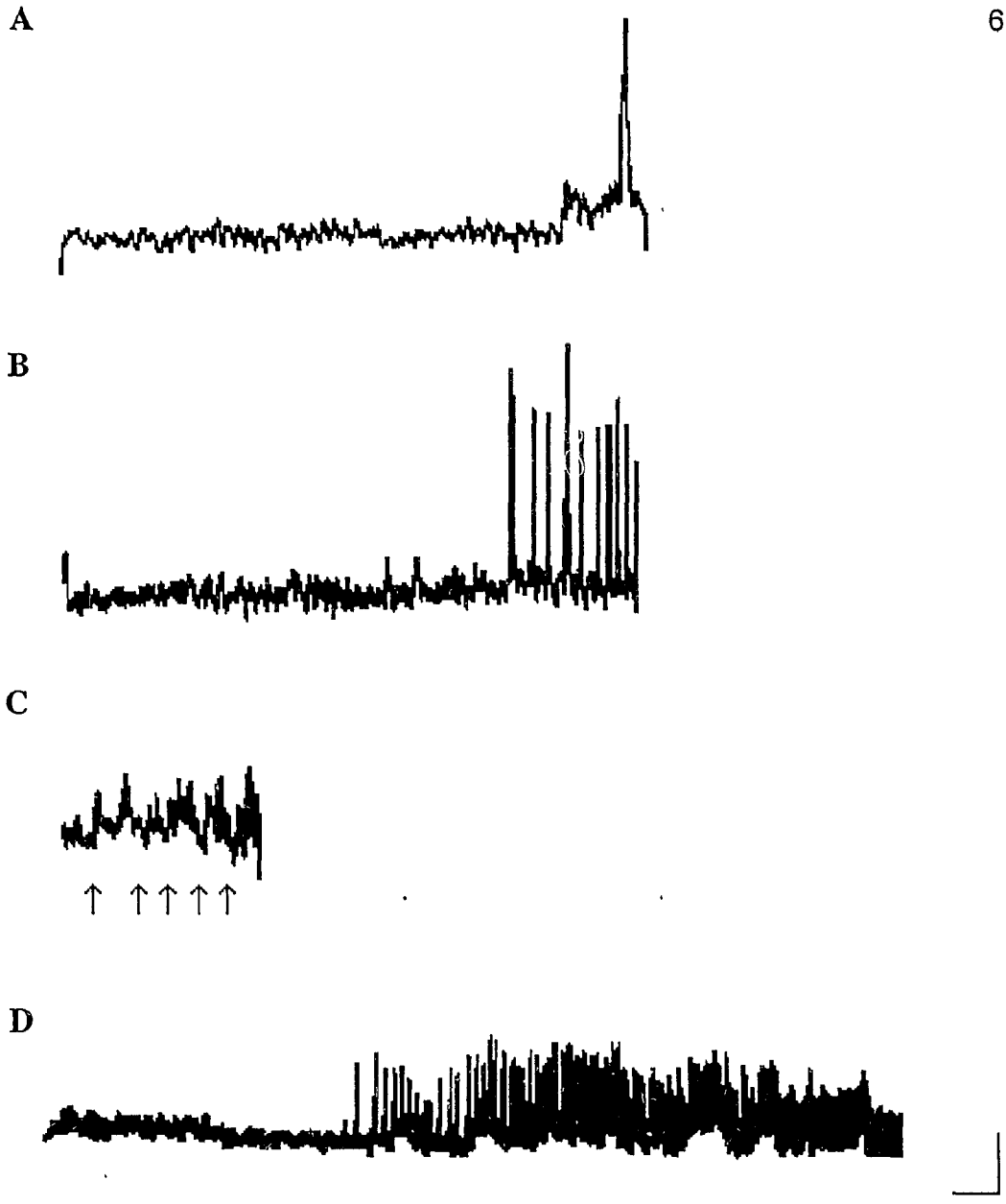

Figure 24: Bladder Pressure Recordings in One Conscious Cat (Cat#6, Table 1) demonstrating responses to: A) bladder filling with sterile saline before spinal transection; B) bladder filling 2 weeks after spinal transection at T11/12; C) touching (arrows) the perineal area 2 weeks after spinal transection; D)bladder filling 11 weeks after spinal transection. Horizontal bar=2 min, vertical bar= 20 cmH20.

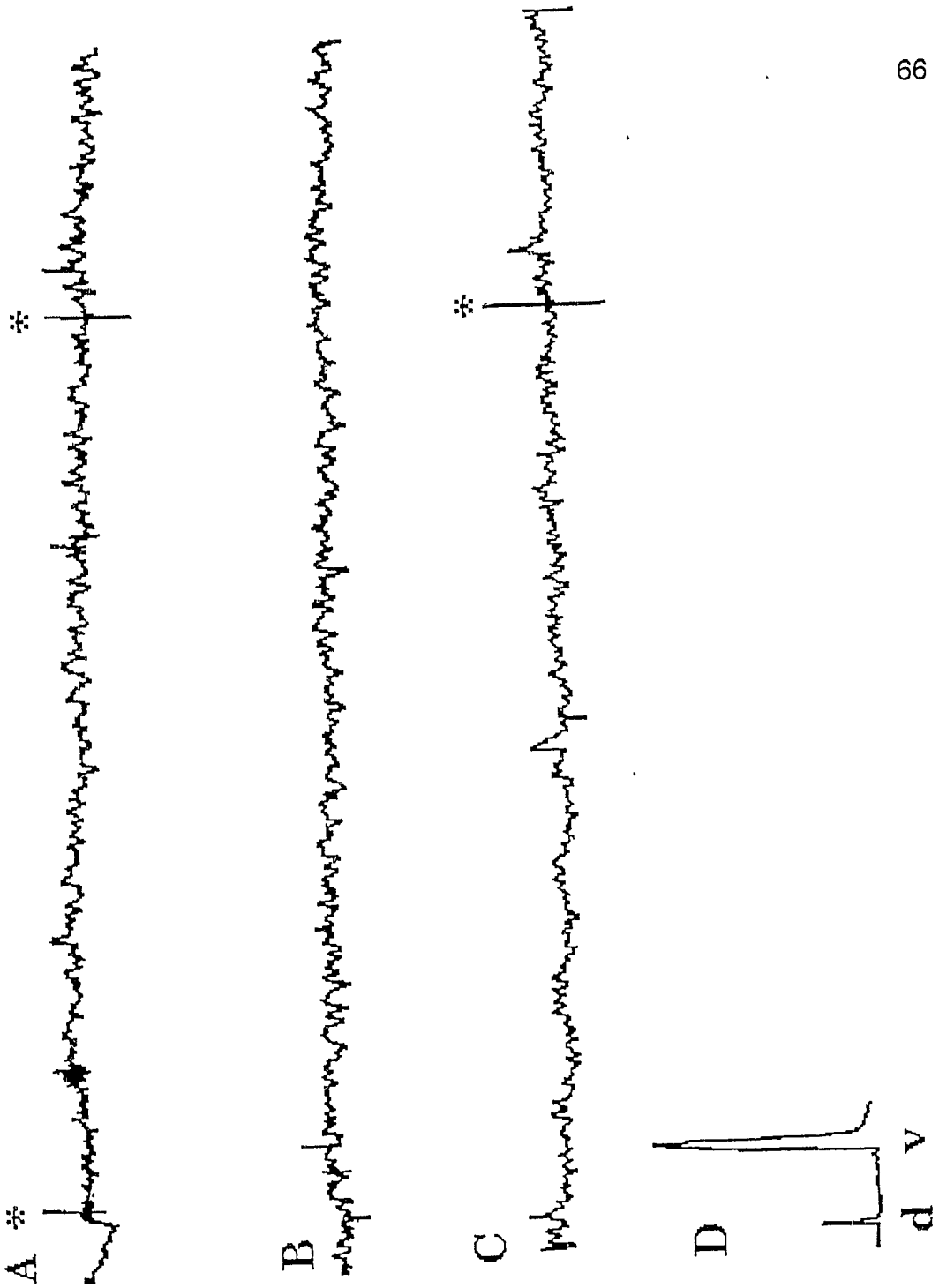

Figure 25

Figure 25: Bladder Pressure Recordings from One Cat (Cat #1, Table 3), during the period in which its bladder was drained through the headcap only, demonstrating lack of response to: vesical distension (panels A-C) with sterile saline (A), with sterile saline after i.t. naloxone (750 nmol) (B), and with cold saline (C). Sacral root stimulation (D) at a bladder volume of 20 ml (d=dorsal, v=ventral) elicits contractions. Asterisks denote movement of external infusion line (artifact). A-C were done in conscious cats whereas D was done following decerebration. Horizontal bar=5 min for A,B and C, 2 min for D. Vertical bar=10 cm H_20 for A,B and C and 20 cm H_20 for D.

(v) Withdrawal of Perineal Stimulation - After the establishment of bladder activity perineal/urethral stimulation was withdrawn in two cats. In one, the bladder was drained via the headcap for 10 weeks after 1 week of intermittent catheterization. In the other, no perineal stimulation was provided for 3.9 weeks after 5.1 weeks in which it was provided. Both cats demonstrated distension-evoked bladder activity during the stimulation-free period (Fig. 24D) although the height of contractions appeared diminished.

The presence of bladder activity correlated well with the application of perineal stimulation either directly or indirectly via urethral catheterization (Fig. 24, Table 3) as all cats with perineal stimulation developed bladder activity while all those without did not.

(vi) Drug Studies - 5HT (470 nmol) was tested in four cats after spinal transection. Three cats had bladders drained via urethral catheterization and one cat was drained via headcap with addition of perineal stimulation. In 3 of 4 cats, 5HT reduced the volume at which bladder contractions occurred to an average of 59% (range 54%-67%, Fig. 26). These cats were all drained by urethral catheterization. The fourth cat was unaffected by 5HT (106% of control) and was drained by head-cap with perineal stimulation. Two cats received 5HT through the indwelling intrathecal cannula and two by lumbar puncture. Both cats who received 5HT via lumbar puncture demonstrated spasticity of the hind limbs. Intrathecal administration of 5HT did not appear to cause hind limb extension. The cat not affected by 5HT received the compound by lumbar puncture but demonstrated hind limb spasticity. No other side effects of 5HT were seen.

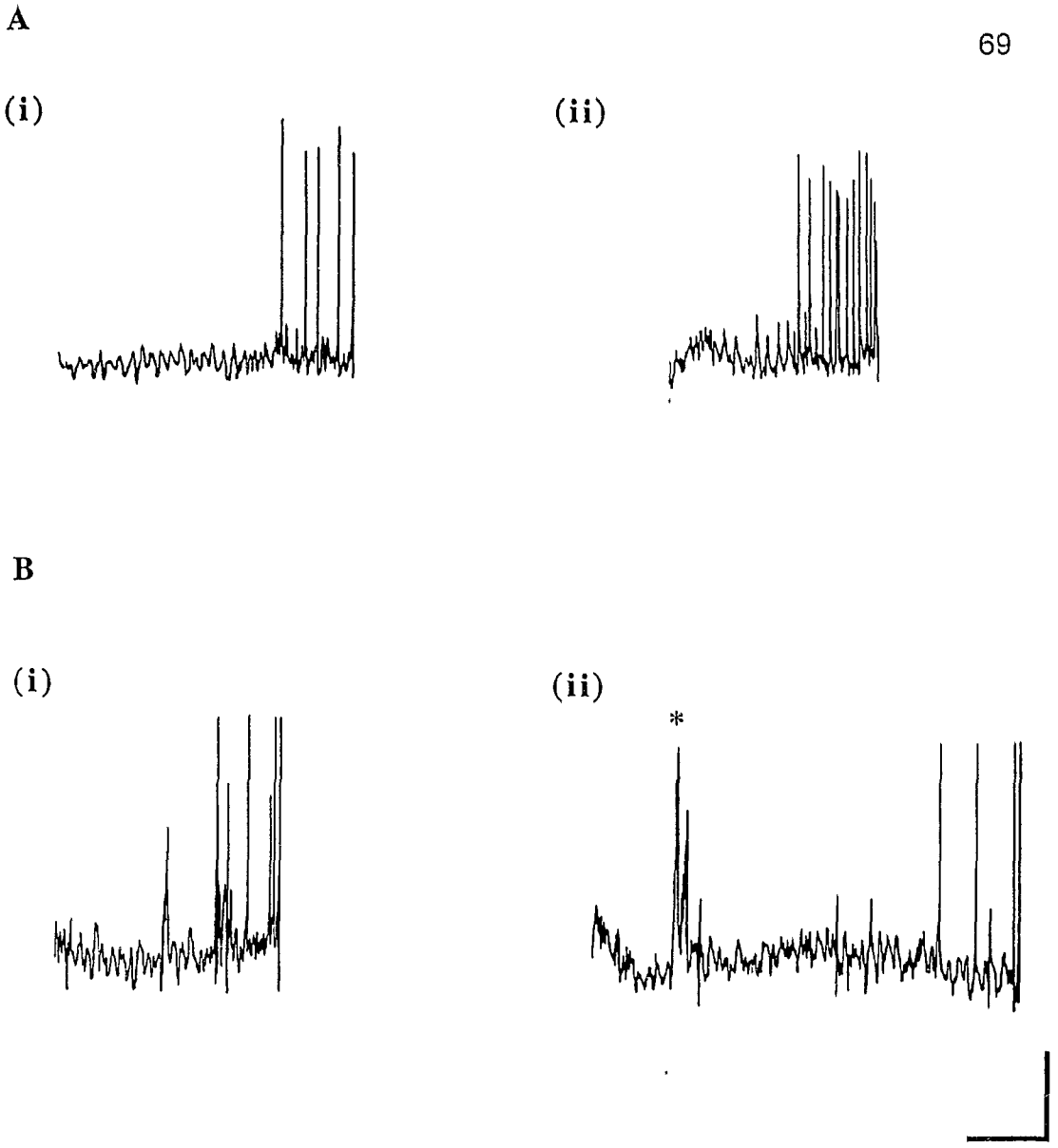

Figure 26: Distension-Evoked Bladder Contractions in the Conscious, Spinally-Transected Cat after Intrathecal Administration of A) (i) saline (0.25ml) (ii) serotonin (470 nmol) B) (i) saline (0.25ml), (ii) 2-methyl-5HT (800 nmol). Asterisk denotes defecation. Horizontal bar=9 min.

2-Methyl-5HT was tested in four cats and the volume at which micturition contractions occurred increased to an average of 141% in all cats tested (range 122%-160%, Fig. 26). Two cats were injected with 2-methyl-5HT by lumbar puncture and the other two via the indwelling cannula. In cats administered 2-methyl-5HT by lumbar puncture, the hind limbs became spastic. One cat receiving 2-methyl-5HT by lumbar puncture defecated shortly after drug injection. Bladder drainage was accomplished by urethral catheterization in two cats and by headcap-drainage with perineal stimulation in two cats.

D. Acute Root Stimulation Experiments in Chronic Spinally-Transected Cats

The two cats without bladder reflex activity that underwent sacral root stimulation both demonstrated bladder pressure responses to S2 dorsal and ventral root stimulation (Fig. 25D). The stimulation parameters were optimum at 5 V, 500 ms pulses at 25 Hz.

V. Discussion

Descending pathways originating in the brainstem can modulate a variety of functions at a spinal level. In an effort to understand the spinal modulation of micturition by two monoamines contained in descending pathways projecting to the sacral spinal cord, two hypotheses were tested. The first hypothesis is that noradrenaline is a spinal mediator of micturition in conscious cats. The second hypothesis is that 5HT is an inhibitory modulator of micturition under physiological conditions. The third hypothesis is that removal of descending pathways alone is inadequate for the emergence of reflex bladder activity following spinal transection. In the following sections, the experiments performed to examine these hypotheses will be discussed. Factors contributing to the emergence of distension-evoked bladder contractions will also be examined.

A. Behavioural Studies in Conscious, Spinally-Intact Cats

1. Acute Manipulation of Spinal Noradrenergic and Serotonergic Transmission

The experiments in conscious, spinally-intact cats lead to the rejection of the first hypothesis that NA is the spinal mediator of micturition as intrathecal administration of α-adrenoceptors did not abolish micturition. However, evidence has been provided to support the second hypothesis that 5HT plays an inhibitory role in micturition as serotonergic antagonists decreased V_T and 5HT increased V_T. Further, 5HT1D/2A/2C and 5HT3 receptor subtypes may be involved in inhibition of bladder activity by 5HT.

Further examination of receptor subtypes was not attempted due to the inavailability of very selective subtype antagonists and the large time requirement for the conscious cat experiments.

In the experiments in conscious, spinally-intact cats, the contribution of spinal NA and 5HT to micturition under physiological conditions was tested by examining the effects of intrathecal administration of noradrenergic antagonists and serotonergic ligands on distension-evoked bladder contractions in conscious cats. Doses of phentolamine, a non-selective α-adrenoceptor antagonist, and methysergide, a 5HT1D/2A/2C antagonist, were limited by the presence of allodynia ($>$890 nmol for phentolamine, $>$60 nmol for methysergide). As administration of receptor antagonists for NA and 5HT in these experiments caused allodynia, descending noradrenergic and serotonergic pathways may be tonically active in depressing transmission of nociceptive information in these cats. This is consistent with the observation that both NA and 5HT are analgesic at a spinal level (Archer et al., 1986). However, bladder effects can be separated from allodynia by using lower antagonist doses. Either higher antagonist doses are required to reach the site of action for hyperaesthesia than for bladder function or bladder function is more susceptible to interruption of serotonergic modulation.

Intrathecal prazosin, an α_1-adrenoceptor antagonist, did not noticeably alter bladder function parameters. The lack of effect for prazosin is not due to a deficiency in the experimental preparation as cats tested with prazosin were also tested with phentolamine, which did demonstrate an effect. This lack of effect conflicts with the reported inhibition of bladder contractions evoked by bladder distensions or electrical

stimulation of the locus coeruleus by similar doses of intrathecal prazosin (Yoshimura et al., 1988) in chloralose-anesthetized cats. The conclusion drawn from these experiments was that stimulation of the pontine micturition centre, located in the locus coeruleus, produced activity in descending noradrenergic pathways which resulted in excitation of the sacral parasympathetic outflow to the bladder. However, prazosin does not inhibit locus coeruleus stimulation-evoked excitation of parasympathetic preganglionic neurons in chloralose-anesthetized cats (Yoshimura et al., 1990b). We cannot provide an explanation for these apparently conflicting results. However, the latter results are in accordance with our observation that prazosin was ineffective in blocking micturition. The difference in susceptibility of bladder distension-evoked bladder contractions to prazosin in our experiments versus those of Yoshimura may be due to confounding effects of chloralose anesthesia in Yoshimura's experiments. Anesthesia alters the response of the lower urinary tract to drugs. MK-801, a non-competitive NMDA antagonist, inhibits bladder activity in urethane-anesthetized rats (Maggi et al., 1990, Yoshiyama et al., 1990) whereas in awake rats MK-801 either has no effect (Yoshiyama et al., 1990) or facilitates the micturition reflex (Vera and Nadelhaft, 1991). Injection of the amino acid L-glutamate into the pontine micturition centre produced bladder excitation in decerebrate cats but both excitatory and inhibitory effects in chloralose-anesthetized cats (Mallory et al., 1991) were observed. Responses to serotonergic ligands are also altered by anesthesia. In vivo voltammetry studies have shown that, in anesthetized rats, a 5HT1B agonist produces a decrease in 5-hydroxyindole levels (Puig et al., 1993). Thus, anesthesia alters the response of the urinary tract to pharmacological manipulation and the

actions of serotonergic ligands in other systems. At present this explanation is only conjecture and the impact of the state of consciousness should be investigated further.

Intrathecal phentolamine, a non-specific α-adrenoceptor antagonist, reduced V_T in the present studies, suggesting an inhibitory role for spinal α-adrenoceptors in the control of micturition. In rat, a low dose (27 nmol) of intrathecal phentolamine had no effect on micturition (Durant et al., 1988). However, this dose was considerably less than the one used in this experiment (890 nmol) and it may have been too low to be effective. These conflicting results may be explained by the presence of anesthesia as for prazosin. Phentolamine, at simi8lar doses (890 nmol) to those used in this study, inhibited bladder contractions in cats (Yoshimura et al., 1988). These conflicting results may be explained by the presence of anesthesia as for prazosin. In the present experiments. phentolamine's reduction of volume threshold is unlikely to be mediated by α_1- adrenoceptors as prazosin did not affect micturition. Phentolamine also blocks α_2-adrenoceptors and 5HT2A/2C (Hoyer, 1985) receptors. In urethane-anesthetized rats, intravenous administration of clonidine, an α_2-adrenoceptor antagonist, inhibited distension-evoked bladder contractions (Maggi et al., 1985), which was opposite to our observations. Supraspinal sites are responsible for this action as intracerebrally-administered clonidine also depressed bladder activity (Maggi et al., 1985). Intrathecal administration of α_2-adrenoceptor antagonists to conscious rats did not effect distension-evoked bladder activity (Durant et al., 1988). Therefore, α_2-adrenoceptors are unlikely to mediate phentolamine's reduction in V_T. Phentolamine's effect in the present experiments was similar to that of methysergide, a

non-specific 5HT antagonist, as described below. A decrease in V_T by phentolamine may be attributable to antagonism of 5HT 2A or 2C receptors.

Methysergide caused a long-lasting (>4 h) depression of V_T. There was a large inter-animal variability in this response. This may be attributable to the i.t. cannula position relative to the antagonist's site of action. The ability of methysergide, a 5HT1D/2A/2C receptor antagonist, to decrease V_T implies that endogenous 5HT increases V_T through activation of one or more of these receptors. 5HT3 receptors may also be involved in endogenous serotonergic inhibition of V_T as zatosetron, a 5HT3 antagonist, also decreased V_T. Exogenous 5HT increased V_T, confirming that V_T can be inhibited at a spinal level by 5HT. 5HT may be inhibitory to micturition at a spinal level through an action on 5HT1D/2A/C and 5HT3 receptors.

An inhibitory action of 5HT is consistent with the observations that stimulation of the raphe nuclei (source of spinal 5HT terminals) inhibited recordings of the micturition reflex potential (McMahon and Spillane, 1982) and activation of post-synaptic serotonergic receptors with high doses of 5MeODMT decreased spontaneous bladder contractions (Thor et al., 1990). However, administration of a 5HT1A agonist (8-OH-DPAT) elicited contractions in filled bladders of urethane-anesthetized rats (Lecci et al., 1992). It is not unusual for serotonergic receptor subtypes to mediate actions in opposite directions in the central nervous system. 5HT2, 5HT1B and 5HT3 receptor activation produces antinociception (Solomon and Gebhart, 1988, El-Yassir et al., 1988, Alhaider et al., 1991) whereas 5HT1A receptors are pronociceptive (Zemlan, 1983, Murphy and Zemlan, 1990). It appears that in nociception and micturition, similar classes of

serotonergic receptors mediate inhibitory actions (5HT2/5HT3) versus excitatory actions (5HT1A). Thus, our results substantiate an inhibitory role for 5HT in the spinal control of micturition.

In the present experiments, only the volume threshold of micturition was consistently affected. Alterations in the patterns of micturition by compounds acting in the sacral spinal cord can be predicted from both de Groat (de Groat, 1975) and McMahon and Morrison (McMahon and Morrison 1982b,c) models of bladder function. In both models, alterations of ascending activity to the pontine micturition centre would alter the volume at which micturition occurs but would not affect other micturition parameters. An inhibitory action of a compound on transmission from the descending pathway to the parasympathetic nucleus would be observed as a decrease in the bladder contraction pressure as fewer neurons would be excited and the parasympathetic outflow to the bladder would be reduced. The McMahon and Morrison model provides a third site of action as a compound could inhibit the spinal reflex pathway to reduce the excitability of bladder preganglionic neurons. Bladder parasympathetic preganglionic neurons would not be sufficiently depolarized to be fired by descending pathways mediating micturition. Such an action would alter volume threshold of micturition but should not affect pressure or duration of contraction. As V_T was the only parameter affected by alteration of serotonergic transmission the possible sites of action are:(1) on the ascending path and (2) on a spinal circuit controlling the excitability of parasympathetic preganglionic neurons. An action on a spinal circuit is very difficult to examine directly as the circuit at present is hypothetical and thus the neuronal pathway

has not been identified which precludes recording from neurons within the pathway. However, an action on the ascending path could be examined by recording from neurons projecting to the pontine micturition centre. This was attempted in the experiments in anesthetized cats.

2. Chronic Interruption of Spinal Monoaminergic Transmission

Another method of determining the spinal involvement of NA and 5HT in micturition is to eliminate specific descending pathways using selective neurotoxins. In order to restrict the site of the chemical lesion, the neurotoxins were applied intrathecally. As the neurotoxic effects of these compounds are dependent upon uptake into nerve terminals, pretreatment with uptake blockers was employed to protect other chemical populations of neurons against destruction. It is possible that the uptake blockers could affect micturition and thus control injections of the uptake blockers, imipramine and fluoxetine, were tested by injecting the blockers prior to intrathecal injection of 0.1% ascorbic acid in saline (the vehicle used to dissolve the neurotoxins). Desipramine was not tested as the switch from imipramine to desipramine was made later in the experiment and the number of cats did not permit another set of vehicle injections. Imipramine, a noradrenergic uptake antagonist, did not affect bladder function parameters or protect against noradrenergic neuron depletion, based on DβH immunoreactivity, in one cat. Acute administration of imi8pramine can increase functional bladder capacity in conscious cats (Shaffer et al., 1979). It is not surprising that imipramine was without effect in our experiments as bladder function was tested one week after imipramine administration.

Imipramine has a half-life of 18 hours (Goodman and Gilman, 1990) and therefore would not be present in effective amounts one week after injection. Fluoxetine, a serotonergic uptake blocker, did not prevent depletion of serotonergic terminals by 6-OHDA in 3 cats but increased V_T (n=2) one week after injection. Fluoxetine has a long half-life ($t_{1/2}$=1-3 days) and forms an active metabolite (norfluoxetine) with a long half life (7-15 days, Lemberger et al., 1985). Thus, blockade of 5HT uptake could still be possible almost 1 week after injection due to the presence of the active metabolite. However, the number of cats tested was small and therefore this conclusion is at best tentative.

Both cats demonstrated a large increase in V_T and it was felt that a correction factor should be instituted. Affter correction for the increase in V_T by fluoxetine, no change in V_T due to 6-OHDA was observed in 4/5 cats and 1 cat demonstrated a large decrease in volume threshold. Other experiments depleting NA with 6-OHDA have yielded conflicting results. In rats, intravenous administration of 6-OHDA reduces V_T (Maggi et al., 1989). Removal of peripheral noradrenergic terminals may contribute to this effect as sympathetic neurons are reported to decrease, through α-adrenoceptors, parasympathetic transmission to the bladder (de Groat and Saum, 1972, de Groat and Theobald, 1975). Elimination of ganglionic inhibition by intravenous administration of 6-OHDA could increase parasympathetic outflow, resulting in a decreased V_T. However, the physiological role of ganglionic inhibition is questionable (Satchell and Vaughan, 1989) and thus a central mechanism of action cannot be ruled out. Intrathecal injection of 6-OHDA in rats produces small increases in V_T (Durant and Yaksh, 1988) whereas injection of 6-OHDA into the locus coeruleus greatly reduces distension-evoked bladder

contractions (Yoshimura et al., 1990a) in chloralose-anesthetized cats. The results from the locus coeruleus experiments suggested that the locus coeruleus contained the PMC and that descending noradrenergic pathways mediated micturition in the spinal cord (Yoshimura et al., 1990a). However, a descending pathway has been identified that projects solely to the sacral spinal cord (Loewy et al., 1979) and as such was suggested to be the anatomical substrate for the descending limb of the micturition reflex. This projection is left intact when noradrenergic neurons are eliminated with 6-OHDA (Loewy et al., 1979) and it was concluded that the descending path for micturition was not noradrenergic. The locus coeruleus provides a rich noradrenergic innervation to supraspinal sites including the hypothalamus, cortex and various brainstem nuclei (reviewed in Moore and Bloom, 1979). Injection of cholera toxin subunit B has demonstrated that Barrington's point (the PMC) receives input from several supraspinal structures including nuclei in the hypothalamus and brainstem and from cortical structures (Valentino et al., 1994). Thus it is possible that lesions of ascending noradrenergic pathways interfered with supraspinal sites important for micturition and produced the results seen in Yoshimura's experiments (1990a). Micturition was not abolished by intrathecal toxin administration in our and Durant and Yaksh's experiments. The lack of effect of 6-OHDA is in accordance with our observations that intrathecal administration of prazosin did not alter bladder function and the suggestion that phentolamine's action may be due to serotonergic receptor blockade.

5,7-dHT produced a decrease in V_T in 3/5 cats. This did not correlate with elimination of 5HT immunoreactivity as one cat exhibiting a decrease in volume threshold

was not depleted of 5HT and a cat with altered bladder function was depleted. It may be that 5HT levels may have been decreased but that the immunohistochemical methods weren't sensitive enough to detect this change. We are unable to draw any conclusions about 5,7-dHT administration. Conflicting results have been obtained using removal of 5HT neurons by neurotoxin administration. In rats 5,7-dHT produced hyperactive bladder activity in 50% of animals (Suzuki et al., 1990) whereas i.t. administration of 5,6-dHT, a serotonergic neurotoxin, produces small increases in bladder capacity (Durant and Yaksh, 1988).

The conclusions that can be drawn from these experiments are (1) spinal NA is not an absolute requirement for micturition as removal of NA terminals did not abolish micturition and (2) tentatively that 5HT may be inhibitory to micturition as blockade of 5HT uptake produced an elevation in V_T. The site of action of fluoxetine is likely to be central as peripherally, serotonergic receptors cause bladder contraction, not inhibition (Chen, 1990).

B. Experiments on Anesthetized Cats

From the experiments in conscious cats, NA does not appear to mediate micturition at a spinal level. Further investigation of NA involvement in spinal control of micturition was not attempted in favour of further examination of the mechanism of action of 5HT. The drugs that were initially examined were the ones used in the conscious preparation. This was done to determine if any correlations between the effects on ascending activity and the observations in the conscious cat. Other serotonergic ligands

were also examined to determine which compounds would be suitable for testing in the conscious cat.

1. Ascending Activity

In earlier experiments in this thesis, activation of 5HT receptors increased volume threshold while blockade decreased V_T. None of the other bladder function parameters were consistently affected by alteration of serotonergic transmission. Changes in V_T could be a consequence of serotonergic inhibition of the ascending limb of the micturition reflex. V_T is thought to be determined in the pons where afferent information from the bladder, upon reaching a threshold intensity, activates the parasympathetic outflow to the bladder (Kruse et al., 1991). 5HT, by decreasing the sensory information ascending the spinal cord, would allow the bladder to fill to a larger volume before the pontine micturition centre was activated and micturition occurred.

(i) Afferent Path of Micturition - The afferent pathway subserving micturition has not been identified. Barrington (1933) reported that ascending fibres transmitting sensation of bladder fullness travelled in the posterior superficial layer of the lateral column in the lumbar cord. Stimulation of the superficial dorsolateral funiculus in the thoracic spinal cord elicits coordinated micturition, presumably due to activation of the ascending limb of the spino-bulbo-spinal reflex (Fedirchuk and Shefchyk, 1991). The spinocervical tract travels in the dorsolateral funiculus but transmission in this pathway is not affected by bladder filling (Cervero and Iggo, 1978). de Groat et al. (1981) and Kuru (1965) identified ascending tracts originating from neurons just dorsal to the sacral

parasympathetic neurons innervating the bladder. The path was partially crossed in cats and was located in the dorsolateral funiculus at lumbar levels but shifted ventrally as the tracts ascended the spinal cord (Kuru, 1965). Spinal neurons activated by pelvic nerve stimulation and projecting out of the spinal cord in pathways similar to that described by Kuru (1965) have been examined electrophysiologically (McMahon and Morrison, 1982a). The latency of the evoked response of these units was approximately 20 ms and a large population of units responded to pudendal nerve stimulation. In our experiments, ascending activity could be recorded in acutely-spinalized cats, suggesting that the units were ascending axons, not descending. The recording sites were located in the dorsolateral funiculus both superficially and approaching the intermedial zone of the lateral funiculus. These neurons were responsive to both pelvic and pudendal nerve stimulation and had latencies similar to that described by McMahon and Morrison (1982a). Thus the units recorded in these experiments possess functional and anatomical properties attributed to the ascending path subserving micturition. The present experiments explored the effects of spinal administration of serotonergic compounds on axonal activity in the thoracic cord elicited by pelvic nerve stimulation. From these experiments, inferences could be drawn about the modulation of the afferent path subserving micturition at a spinal level by 5HT.

(ii) Drug Actions - Blockade of spinal 5HT1D/2A/2C receptors by intrathecal methysergide increased ascending activity, suggesting that endogenous 5HT inhibits ascending information at the spinal level through activation of these receptors. Endogenous 5HT could inhibit bladder activity at a spinal level in the dorsal horn via

5HT1D/2A/2C receptors by decreasing ascending activity to the pons. Further dissociation of receptor subtypes was not attempted as highly selective, receptor subtype ligands are not available. The time required to dissect these receptor subtypes was thought to be better spent examining other receptors implicated in the spinal modulation of micturition in the conscious cat.

Intrathecally-administered 2-methyl-5HT, a 5HT3 agonist, depressed ascending activity which was antagonized by intrathecal administration of the 5HT3 receptor antagonist, zatosetron, suggesting that activation of 5HT3 receptors can inhibit ascending activity. Moreover, zatosetron alone increased pelvic nerve-evoked ascending activity, indicating that endogenous 5HT inhibits ascending activity through 5HT3 receptor activation. 5HT3 receptors are believed to be ligand-gated, cation-selective channels and are associated with membrane depolarization and neuronal excitation (for review see Peters et al., 1992). In this respect, there are at least two ways in which descending 5HT axons can inhibit spinal transmission of inputs from bladder afferents through a presumably excitatory ligand-gated channel. 5HT may depolarize primary afferent terminals and in turn result in presynaptic inhibition of the secondary or projecting neurons. This mechanism is strongly supported by recent findings that 5HT3 receptors are located presynaptically on small diameter primary afferent fibres terminating in the superficial layers of the dorsal horn (Kidd et al., 1993; Hamon et al., 1989). Alternatively, some 5HT3 binding sites remain after dorsal root rhizotomy (LaPorte et al., 1991) which may be located on intrinsic dorsal horn neurons. Endogenous 5HT could activate, via postsynaptic 5HT3 receptors, dorsal horn interneurons containing

inhibitory neurotransmitters, such as GABA, that in turn depress activity of supraspinally-projecting neurons, as suggested by Basbaum and Fields (1984) and Fields et al. (1991). Behaviourally-measured analgesia and activity of dorsal horn neurons projecting to the brain can be inhibited by 2-methyl-5HT, a 5HT3 agonist which can be blocked not only by 5HT3 antagonists, but by GABAergic antagonists as well (Alhaider et al., 1991). Stimulation of the nucleus raphe magnus, a source of serotonergic terminals in the spinal cord, results in inhibitory post-synaptic potentials (IPSPs) in primate spinothalamic tract cells. These IPSPs can be blocked by hyperpolarization or intracellular chloride application (Giesler et al., 1981). GABA receptors are linked to chloride channels, thus GABA may be involved in descending inhibition of spinal activity by raphe stimulation. Either by activation of intrinsic inhibitory interneurons or by presynaptic depolarization, an excitatory action of 5HT3 receptors could result in an inhibition of bladder input processing at the sacral spinal level.

It has been hypothesized that descending serotonergic pathways inhibit the micturition reflex but that 5HT1A receptors could provide a negative feedback on the descending pathways, thereby causing excitation of the micturition reflex (Lecci et al., 1992). Opposing actions of serotonergic compounds have been attributed to autoreceptor-mediated depression of serotonergic function in the regulation of sexual function (Steers and de Croat, 1989). Penile erection is depressed by serotonergic ligands (Mas et al., 1985). Excitatory actions of serotonergic compounds on penile erection is thought to be due to depression of serotonergic function (Steers and de Groat, 1989). It could be expected that 5HT1A receptor activation would increase ascending activity if 5HT1A

receptors can depress a presumably inhibitory serotonergic pathway onto ascending cells. However, in the present experiments, spinal administration of 8-OH-DPAT, a 5HT1A agonist, did not reliably affect ascending activity whereas blockade of 5HT1A receptors by NAN-190 decreased ascending activity in 4 of 5 cats. Thus, endogenous 5HT may act on 5HT1A receptors to increase ascending activity. If the 5HT1A receptors are close to saturation by endogenous 5HT then further activation of 5HT1A receptors by an exogenous ligand may produce inconsistent effects.

We propose that inhibition of the micturition reflex may be due to the depression of afferent information mediated by 5HT1D/2A/2C and 5HT3 receptors. 5HT1A receptors may also participate in this modulation although its exact function remains to be determined. The action of methysergide and zatosetron on ascending activity provides an explanation for the observation in our earlier experiments that, in awake cats, spinal administration of similar doses of methysergide or zatosetron decreased the volume threshold for micturition but not the other bladder function parameters. Blockade of serotonergic inhibition by methysergide or zatosetron would increase ascending activity to the pons, which in turn would decrease the volume threshold. An effect on the ascending path of micturition would be consistent with the modulation of other systems by 5HT. In the dorsal horn of the spinal cord, classical synaptic connections of serotonergic terminals are rare (Marlier et al., 1991a). This suggests that serotonin has access to a variety of targets. Pain reflexes (Hammond and Yaksh, 1984) and locomotion (Jacobs and Fornal, 1993, Schotland and Grillner, 1993) are modulated by 5HT at a spinal level. The antinociceptive actions of 5HT may be subserved by inhibition of sensory processing as

5HT reduces excitation of dorsal horn neurones evoked by noxious and non-noxious stimulation in anesthetized cats (Belcher et al., 1978, El-Yassir et al., 1988). 5HT depresses transmission from Group II muscle afferents in the cat (Bras et al., 1989) and in the spinal control of motor function, 5HT has been suggested both to inhibit sensory information processing and to facilitate motor output (Jacobs and Fornal, 1993). Thus 5HT may inhibit sensory processing in a variety of systems. However, in our experiments we only observed allodynia at high antagonist doses, suggesting that nociceptive and micturition processing can be separated. As well, hindlimb spasticity evoked by 5HT was seen only in spinal animals where receptor supersensitivity could occur. It may be possible, therefore, that 5HT could affect sensory processing in several systems but could do it selectively.

There are two other mechanisms by which 5HT could alter volume threshold. The first is based on the model of bladder function proposed by McMahon and Morrison. In this model, a spinal reflex controls the excitability of the parasympathetic preganglionic neurons. Increasing levels of afferent information from the bladder would activate the spinal circuit thereby making the preganglionic neurons more susceptible to activation by descending pathways from the pontine micturition centre. In effect, a spinal 'gate' would be present on the efferent side of the spino-bulbo-spinal reflex where the action of descending pathways is determined by a spinal reflex controlling the excitability of preganglionic neurons. 5HT, by inhibiting the spinal reflex, would decrease the excitability of the preganglionic neurons such that the descending pathways would require elevated activity in order to excite the parasympathetic outflow to the bladder.

The second possibility is that 5HT produces an excitation of the sympathetic outflow to the bladder. Activation of the sympathetic outflow to the bladder allows the bladder to accommodate larger volumes due to direct inhibition of the bladder musculature via β-adrenoceptors and perhaps indirectly via inhibition of transmission in the peripheral ganglia relaying the parasympathetic excitatory input to the bladder. Hypogastric-denervated rats show decreased bladder capacity when compared to sympathetically-intact cats (Morikawa et al., 1990) and volume threshold for micturition is significantly decreased by sympathectomy in cats (Flood et al., 1988). However, there are conflicting data concerning the actions of 5HT on sympathetic nerve activity. Both excitatory and inhibitory actions on sympathetic nerve activity by 5HT have been described (Yusof and Coote, 1988). Application of 5HT onto sympathetic preganglionic neurons causes excitation (de Groat and Ryall, 1976). Stimulation of the nucleus raphe pallidus and obscurus, the source of the serotonergic innervation of the intermediolateral cell column (Skagerberg and Björklund, 1985), inhibits sympathetic preganglionic neurons which can be blocked by LSD, a serotonergic antagonist (Gilbey et al., 1981). However, in the same experiments, methysergide, cinanserin and cyproheptadine depressed sympathetic discharge in the absence of brain stimulation. It was suggested that under basal conditions, endogenous 5HT activates sympathetic efferent activity while 5HT released by raphe stimulation can inhibit the same activity (Gilbey et al., 1981). The physiological role of 5HT in the control of the sympathetic innervation of the bladder has not been determined.

In summation, endogenous 5HT may modulate ascending activity through the activation of 5HT1D/2A/2C receptors which may explain the effect of volume threshold for micturition observed in the conscious cat experiments. Selective drugs will be required for dissection of different 5HT receptors. The results with exogenous 5HT were inconsistent. Activation of multiple subtypes of receptors could underly the variable effects of 5HT. However, in conscious cats 5HT increased V_T. As serotonergic ligands have been shown to have different effects in awake and anesthetized animals, variable results with 5HT may be associated with the use of chloralose anesthesia. It would be prudent in further experiments to observe modulation of ascending activity by 5HT and other ligands in decerebrate cats. As well, we are unsure of the exact target of the ascending axons that we were recording from and therefore we may have been recording from different populations of ascending axons which may respond differently to 5HT administration. Definition of the afferent input to the recorded cells is very difficult. Recording from axons in the thoracic cord requires a very stable preparation, even a small change in blood pressure can cause the unit to be lost. Distension of the bladder can alter blood pressure in anesthetized cats which makes observation of responsiveness of individual units to the natural stimulus for micturition (bladder filling) extremely difficult. Another possibility would be to record directly from the pontine micturition centre. However, recording from Barrington's point would be very difficult as it receives input from a variety of spinal and supraspinal sources (Valentino et al., 1994) whereas reliable recordings can be obtained from axons recorded in the thoracic spinal cord.

2. Viscero-somatic Reflex

In this thesis, the electrical response evoked on the pudendal nerve by stimulation of the pelvic nerve will be called the pelvic-pudendal reflex. Although no muscle activity was actually monitored in the present experiments, this electrical response is thought to be an electrophysiological correlate of the reflex and the terminology aids in the description of the experiments. In the experiments monitoring ascending activity in anesthetized cats, the viscero-somatic and somatic reflexes were initially recorded to determine the stimulation parameters capable of activating the nerve and to ensure that the stimulating electrodes continued working throughout the experiment. However, it became clear that the reflexes were also being modulated by intrathecal administration of serotonergic compounds. Methysergide, a 5HT1D/2A/2C antagonist, decreased the amplitude of the pelvic-pudendal reflex as did zatosetron, a 5HT3 antagonist. These results suggest that endogenous 5HT, through 5HT1D/2A/2C and 5HT3 receptors, facilitates the pelvic-pudendal reflex. 2-Methyl-5HT, a 5HT3 agonist, increased the pelvic-pudendal reflex. Zatosetron, a 5HT3 antagonist, reversed the increase in the reflex caused by 2-methyl-5HT. These data imply that 5HT3 receptor activation can facilitate the pelvic-pudendal reflex.

To date, the results of our experiments have substantiated an inhibitory role for 5HT in the spinal control of micturition. However, the actions of 5HT compounds on the spinal reflexes suggest that 5HT is excitatory at a spinal level. 5HT has been suggested to have opposite effects on spinal versus supraspinal actions and will be discussed below in the section on drug studies in conscious, spinally-transected cats.

C. Behavioural Experiments in Conscious, Spinally-Transected Cats

1. Emergence of Distension-Evoked Bladder Contractions

Following the observation that 5HT was inhibitory at a spinal level to micturition in conscious, spinally-intact cats, an investigation of the possible inhibitory action on bladder hyperactivity following spinal cord injury was attempted. The cats examined in these experiments had been used in the intrathecal drug studies and as such possessed a cannula implanted in the dome of the bladder. After spinal transection, bladders were drained using the implanted cannula as this was a faster and less stressful method and was expected to decrease the incidence of urinary tract infection. However, cats with spinal cord transection whose bladders were drained through the implanted cannula did not develop reflex bladder contractions in response to bladder distension over a period of 16 weeks. Neither did they develop urinary tract infection. These findings were contrary to experiments done previously in this laboratory on cats with urinary drainage accomplished with intermittent catheterization (Gajewski, J.B., unpublished observations, Rudy, 1988).

In addition to the lack of distension-evoked contractions, the bladder function in these cats differed in other aspects from spinal cats examined in previous studies. Naloxone was ineffective at producing bladder contractions in the three cats managed by headcap-drainage and without distension-evoked bladder activity. It has been demonstrated (Thor et al., 1983) that naloxone can produce bladder contractions 2-10 weeks after lower thoracic spinal transection in cats managed with manual expression and perineal stimulation. This finding has been interpreted as representing tonic enkephalinergic inhibition of the bladder filling reflex after spinal cord injury. Bladder

contractions can be elicited by the instillation of cold saline into the bladder in anesthetized, spinally-intact cats (Fall et al., 1990). This bladder cooling reflex has been used as a diagnostic tool following spinal injury (Bors and Blinn, 1957) as this reflex cannot be demonstrated in conscious humans. The cooling reflex was not present in the two spinally-transected cats tested. C-fibres are thought to form the afferent path for the cooling reflex (Fall et al., 1990). C-fibres are also thought to mediate distension-evoked bladder contractions in spinal cats (de Groat et al., 1990). As the cats did not respond to cold saline, C-fibre afferents may not form functional connections in these cats. This would imply that the sacral circuitry of these cats differs from that of cats in which distension-evoked bladder contractions have been described. As our cats without distension-evoked bladder contractions did not respond to intrathecal naloxone or instillation of cold saline into the bladder, there may be pharmacological and functional differences between animals with distension-evoked contractions versus those without. Nevertheless, bladder contractions could be elicited by either sacral ventral or dorsal root stimulation in two cats without reflex bladder activity. This finding implies that the appropriate spinal circuitry and motor innervation to the bladder were available. The disparity between electrical sacral dorsal root stimulation and bladder distension as adequate stimuli for evoking bladder reflex activity may arise in different ways. First, electrical stimulation of the dorsal roots may provide an afferent stimulus of different character than does bladder filling and as such may be able to activate spinal circuitry that the natural stimulus (bladder filling) cannot access. Devor et al. (1977) have suggested that dorsal horn cells receive two types of synaptic input: one effective in driving the cell

in response to natural stimulation and the other relatively ineffective at activating the cell unless there is a synchronous volley of synaptic input evoked by electrical stimulation of convergent axons. An alternative explanation is that dorsal root stimulation may activate afferents in addition to those from the bladder, (e.g. from the perineum) which could cause bladder contraction (Thor, 1985). In any case, bladder areflexia following spinal cord injury is not attributable to destruction of either the efferent path to the bladder or of sacral spinal circuitry.

Distension-evoked reflex bladder contractions appeared in the range of 1 to 8 weeks following spinal cord transection in previous studies employing intermittent catheterization (Rudy, 1988; J.B. Gajewski, personal communication) or manual expression with perineal stimulation (Thor, 1985, Galeano et al., 1986a,b, Roy et al., 1992) for bladder drainage. However, in our experiments, distension-evoked bladder contractions did not appear even up to 16 weeks after spinal cord transection in cats drained by indwelling cannulae. Evidently, the method of bladder drainage affects the emergence of distension-evoked bladder contractions.

Bladder drainage through an indwelling cannula decreased the incidence of bladder infections, which is frequently a problem both with manual expression (Roy et al., 1992) and intermittent catheterization (J. B. Gajewski, personal communication). In this study, infection did not correlate with the emergence of bladder activity as some cats who had activity were never infected while some cats who developed infections that were cleared subsequently did not have activity. Thus, bladder infection is not required for emergence of bladder contractions.

To the best of our knowledge, previous studies involving chronic spinal cord transection have used animals that were managed with direct or indirect (via urethral catheterization) perineal stimulation. In fact, perineal stimulation is recommended when emptying the bladder in chronic spinal animals (Roy et al., 1992). In the present experiments, spinal cord transected cats drained through the indwelling bladder cannula without perineal stimulation did not develop distension-evoked bladder activity. However, the addition of perineal stimulation to similarly-prepared cats resulted in the emergence of distension-evoked bladder contractions after spinal transection. All of the catheterized cats also developed bladder contractions and they would have received perineal stimulation during the catheterization process. The present data imply that perineal stimulation may be required for distension-evoked bladder contractions to appear after spinal cord injury.

The fact that perineal stimulation was associated with the development of distension-evoked bladder contractions may be a consequence of the convergence of the sacral spinal circuitry for the perineum and the bladder. Spinal interneurons and projection neurons related to bladder function receive input from both cutaneous and vesical sources (McMahon and Morrison, 1982a,b). Similarly, spinothalamic tract neurons and neurons near the central canal in the sacral spinal cord receive both visceral and somatic input (Milne et al., 1981, Honda, 1985). Thus, there is a close association between perineal and bladder input. Perineal circuitry is closely associated with bladder function as demonstrated by the importance of perineal stimulation in kittens for vesical emptying. Light tactile stimulation to the perineum, provided by the mother cat, elicits

micturition. Prior to weaning, the kitten does not demonstrate distension-evoked bladder contractions and if tactile stimulation is withdrawn, the bladder becomes overdistended and the kitten may die. This perineal-bladder excitatory reflex changes after weaning such that perineal stimulation inhibits bladder activity (de Groat and Ryall, 1969). However, perineal excitation of bladder activity is not abolished but rather suppressed as in spinally-intact cats the reflex can be elicited when descending serotonergic systems are inhibited (Thor et al., 1990). After chronic spinalization (which would also eliminate spinal serotonergic terminals) the excitatory perineal-bladder reflex re-emerges (Thor et al., 1986). The close relationship between perineal and bladder circuitry may provide the framework that allows the "priming" of spinal pathways that mediate distension-evoked bladder contractions and that all cats receiving perineal stimulation either directly or indirectly via urethral catheterization demonstrated distension-evoked bladder contractions leads us to suggest that perineal stimulation is a requirement for the emergence of distension-evoked bladder contractions after spinal transection.

There may be a limited time in which perineal stimulation can alter sacral spinal circuitry after spinal injury so that distension-evoked bladder contractions can emerge. Delay of introduction of perineal stimulation for 1 week did not appear to affect the appearance of bladder activity. However, in 2 of 3 cats drained by headcap and monitored for 16 weeks, 2 weeks of urethral catheterization, sufficient for cats having just received spinal cord transection to develop activity, was ineffective at producing contraction. This suggests that following spinal injury, there is a time window in which perineal stimulation must be introduced if distension-evoked contractions are to emerge.

According to the present data this time is greater than 1 week but less than 16 weeks. A more precise definition of this time period must await further studies.

We have observed a phenomenon wherein peripheral afferent input can produce a long-term change in bladder reflex excitability. The underlying basis of bladder hyperactivity after spinal cord injury has been likened to that subserving bladder hyperactivity following partial urethral ligation (de Groat et al., 1990). In experiments in conscious rats, partial urethral ligation caused an increase in voiding frequency, apparently due to the emergence of a short latency, spinal reflex (Steers and de Groat, 1988). A short latency, C-fibre afferent mediated spinal reflex also underlies bladder hyperactivity after suprasacral spinal cord transection (de Groat). In our experiments, cutaneous afferent input from the perineum results in the emergence of a spinal reflex allowing the bladder to contract in response to bladder afferent input.

The mechanisms by which distension-evoked bladder contractions emerge after spinal cord injury have not been defined. Elimination of bulbospinal inhibitory pathways, strengthening of existing synapses or formation of new synaptic connections due to axonal sprouting, change in synthesis, release or action of neurotransmitters and alterations in afferent input from peripheral organs have all been suggested as possible mechanisms for the change in micturition in paraplegic animals (de Groat et al., 1990). We would suggest that although elimination of descending pathways may be an initiating stimulus for alterations in bladder function following spinal cord injury, it is not solely responsible. This is evidenced by the lack of distension-evoked bladder contractions in the cats drained

through the indwelling bladder cannula without perineal stimulation. They lost all sacral projections of descending pathways but they did not develop reflex bladder activity.

There is evidence that new synaptic connections are formed after spinal cord injury. The underlying basis of bladder hyperactivity after spinal cord injury has been likened to that subserving bladder hyperactivity following partial urethral ligation (de Groat et al., 1990). In experiments in conscious rats, partial urethral ligation caused an increase in voiding frequency, apparently due to the emergence of a short latency, spinal reflex (Steers and de Groat, 1988). A short latency, C-fibre afferent-mediated spinal reflex also underlies bladder hyperactivity after suprasacral spinal cord transection (de Groat et al., 1990). This is behavioural evidence that new synaptic connections are formed. There is also anatomical data to show that synaptic connections may be changed after spinal cord transection. Pudendal nerve afferents demonstrate an expanded terminal distribution in the dorsal horn after spinal cord injury (Thor et al., 1986). Primary afferent sprouting has been suggested to underly the recovery of function after hemisection (Helgren and Goldberger, 1993). Onuf's nucleus undergoes extensive synaptic reorganization following spinal cord injury (Beattie et al., 1993). It also appears that some synapses may be eliminated as the sacral parasympathetic nucleus shows a reduction in the number of synaptic contacts (Beattie et al., 1993) after spinal cord injury. Thus, there is evidence that synaptic connections are altered following spinal transection.

There is evidence also for changes in synthesis, release or action of neurotransmitters. Leucine-enkephalin containing axons and varicosities are increased in the dorsal horn and intermediate gray matter and in cell bodies in spinal animals (Thor et

al., 1986). This was interepreted as an increase in synthesis as leucine enkephalin was not detected in cells in spinally-intact animals unless colchicine, a compound that interferes with axonal transport is administered (Thor et al., 1986). VIP, (vasoactive intestinal peptide, a transmitter contained in primary afferents from the bladder (reviewed in Maggi and Meli, 1986)), inhibited bladder contractions in the spinally-intact cat but elicited bladder contractions in spinally-transected cats (de Groat et al., 1990). Thus, synthesis of transmitters and action of transmitters are altered following spinal cord injury.

Alterations in afferent input from peripheral organs is a mechanism that is substantiated by the present experiments. In our experiments, cutaneous afferent input from the perineum contributed to the emergence of distension-evoked bladder contractions following spinal transection. Cutaneous afferent input can cause relatively long term changes in spinal cord function. Hyperalgesia due to tissue injury can be observed in areas remote from the site of injury (Coderre et al., 1993 for review). This hyperalgesia is associated with spinal cord hyperexcitability. A network of intraspinal neurons, activated by tissue injury, facilitates other spinal interneurons' activity, allowing hyperalgesia to spread to uninjured body regions. In the present situation, perhaps perineal stimulation activates spinal interneurons which increase the responsiveness of interneurons associated with bladder contraction. Due to the heightened excitability of bladder interneurons, bladder afferent activity can now activate the efferent output to the bladder, resulting in bladder contraction.

These various mechanisms may not be mutually exclusive but rather dependent on or a consequence of another mechanism. A hypothetical chain of events could consist of elimination of descending pathways to the spinal cord removing synaptic onnections in the sacral cord, allowing new synaptic connections to form. The formation of these connections may be influenced by afferent input from peripheral organs. If the plastic changes occur in a time window after spinal cord injury, this may explain the restricted time period in which perineal stimulation can influence the emergence of reflex bladder activity. These connections may have a different pharmacology which may explain the changes in neurotransmitter action. This may be very important in the treatment of bladder dysfunction following spinal cord injury and may be an explanation for the observations in the following experiments in chronic spinal cats.

2. Drug Studies in Conscious Spinally-Transected Cats

Spinal cord injury interrupts the spino-bulbo-spinal reflex that subserves micturition and thus distension-evoked bladder contractions are eliminated. After a recovery period of 1-8 weeks, bladder contractions in response to bladder filling emerge (de Groat et al., 1981). These can be strong contractions but are not sustained and do not empty the bladder. These contractions expose the kidneys to high pressures and can result in renal damage (Gerridzen et al., 1992). A compound that inhibited these contractions between bladder drainage sessions could protect the kidneys from pressure-induced damage. The experiments in spinally-intact cats demonstrated that 5HT inhibits bladder contractions at a spinal level. The results with methysergide

(5HT1D/2A/2C antagonist) and zatosetron (5HT3 antagonist) implicated 5HT1D/2A/2C and/or 5HT3 receptors in this action. 5HT and 2-methyl-5HT were tested in conscious spinally-transected cats to determine if serotonergic inhibition of distension-evoked bladder was maintained following spinal transection.

After spinalization, 5HT reduced V_T in 3 of 4 cats. The unaffected cat received 5HT via lumbar puncture but the method of 5HT injection was not responsible for the ineffectiveness of 5HT. Both cats receiving drug by lumbar puncture demonstrated motor effects. Motoneuron activity is facilitated by serotonin (White and Neuman, 1983) through 5HT2A/2C receptors (Yamazaki et al., 1992). Motor spasticity due to incomplete spinal injury can be reduced by administration of a serotonergic antagonist, cyproheptadine (Wainberg et al., 1986, 1990), implying that 5HT promotes muscle activity. As the cat with the unaffected volume threshold demonstrated motor effects, 5HT was probably reaching the ventral horn of the spinal cord. The cat with the unaltered V_T had its bladder drained by headcap with the addition of perineal stimulation while the other cats received urethral catheterization. It is possible that the pharmacology of the spinal pathways mediating bladder contractions after spinal cord injury differs between animals drained by headcap with the addition of perineal stimulation and animals drained by urethral catheterization, but this explanation is only conjecture.

Intrathecal administration of 5HT in 3 of 4 cats resulted in an effect opposite to that demonstrated in spinally-intact cats. As mentioned above, other compounds, such as VIP, demonstrate a similar reversal in action after spinal cord transection. One possible mechanism is that removal of descending pathways allows a spinal mechanism of action to

be unveiled which is suppressed in the spinally-intact animal. This hypothesis has been advanced for pain transmission wherein two populations of dorsal horn nociceptive units have been proposed: one mediating spinal nociceptive reflexes and facilitated by 5HT, the other projecting to the brain and inhibited by 5HT (Zemlan et al., 1983). In our experiments in anesthetized cats, ascending activity elicited by pelvic nerve stimulation was increased by compounds that block serotonergic receptors while spinal reflexes were decreased. Removal of the ascending pathway by spinal transection may allow an excitatory effect on the spinal reflex mediating distension-evoked bladder contractions to be observed. Thus, administration of 5HT in a spinal animal could cause a decrease in V_T by exciting the spinal reflex. An alternative explanation is that anatomical changes in sacral cord circuitry may be reflected in changes in pharmacology. Our earlier experiments demonstrated that perineal stimulation was required for emergence of distension-evoked bladder contractions. This implies that removal of descending pathways is insufficient for bladder reflex activity to appear. An alteration in the sacral spinal circuitry must therefore occur after spinal cord transection. After spinal cord injury, sacral spinal cord circuitry undergoes anatomical changes.

Stimulation of spinal 5HT3 receptors by intrathecal administration of 2-methyl-5HT increased V_T. This is consistent with the results obtained with zatosetron in spinally-intact cats. Spinal cord injury, therefore, does not alter the action of 5HT3 receptors on bladder function. The opposite effects of 5HT and 2-methyl-5HT indicate that 5HT3 receptors are not responsible for the action of 5HT after spinal cord injury. Earlier in the discussion, two mechanisms were suggested for 5HT3 modulation of

bladder activity: primary afferent depolarization and/or activation of GABAergic interneurons. After spinal transection, the first mechanism may be eliminated as the afferent path for distension-evoked bladder contractions changes from Aδ- to C-fibres (de Groat et al., 1981). However, an action on GABAergic interneurons would be maintained. This may allow 5HT3 receptors to maintain an inhibitory action after spinal transection.

In summary, the action of 5HT is reversed by spinal cord injury whereas 2-methyl-5HT remains inhibitory to bladder function. 2-Methyl-5HT requires further investigation to determine its usefulness in prevention of high bladder pressures after spinal cord injury.

VI. Conclusions

The following conclusions can be drawn from the present experiments:

1. Noradrenaline is not an essential spinal mediator of micturition as intrathecal administration of adrenoceptor antagonists did not abolish micturition. Therefore, the first hypothesis can be rejected. Whether noradrenaline has a more subtle modulatory role in micturition has not been proven.

2. 5HT plays an inhibitory role in the spinal control of micturition as intrathecal administration of 5HT to conscious cats increased the volume at which micturition occurred

3.. Furthermore, 5HT1D/2A/2C and 5HT3 receptors may mediate the suppression of bladder activity by 5HT as blockade of these receptors decreased bladder capacity in the conscious cat.

4. The mechanism of action for 5HT may be through a reduction in the activity of the ascending limb for micturition. Although 5HT produced inconsistent effects on ascending activity, use of other serotonergic agonists and antagonists suggests that there is an endogenous serotonergic inhibition of ascending activity which may be responsible for 5HT's effects on bladder function.

5. Some aspects of 5HT pharmacology are altered in the portion of the spinal cord isolated from supraspinal centres by transection of the cord as 5HT produced opposite

effects in spinally-intact and spinally-transected cats. However, 5HT3 receptors retained their inhibitory function.

6. Spinal transection does not inevitably lead to detrusor hyperreflexia and that elimination of descending pathways is an inadequate stimulus for the development of reflex bladder contractions as cats who did not receive perineal stimulation did not develop reflex bladder activity. Moreover, peripheral input in the form of perineal stimulation may be very important in the emergence of bladder contractions after spinal cord injury.

VII. Implications of Thesis Experiments

The experiments contained in this thesis have demonstrated that NA is not the spinal mediator of micturition. Thus it is necessary, before making any conclusions about the role of a substance in the control of micturition, to test the relevant compounds in a conscious animal. Second, the results show that 5HT is inhibitory to bladder function at a spinal level in spinally-intact cats. Therefore, manipulation of the serotonergic system should be investigated in cases of bladder hyperreflexia such as those caused by bladder inflammation. If 5HT can inhibit bladder hyperactivity, its mechanism of action would be very important. Does it act directly or through an intermediate such as GABA or adenosine? As well, dissociation of the receptors responsible for the actions of 5HT would allow subtype-selective ligands to be selectively chosen for a therapeutic purpose, thereby minimizing side effects.

Third, the results showing the importance of cutaneous input for the emergence of distension-evoked contractions after spinal cord injury provide the basis for further experiments which could lead to control of the emergence of bladder activity following spinal injury. As well, the ability of 5HT3 receptors to inhibit reflex bladder activity following spinal injury requires further investigation to ascertain the possibility of pharmacological control of bladder hyperreflexia. A drug controlling bladder hyperreflexia would be very useful as high intravesical pressures that lead to renal failure could be avoided. As this drug would have a central mechanism of action, voiding could still be produced by electrical stimulation of the ventral roots.

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IX. Appendices

Appendix "A"

1. Prepare sections
2. Rinse 1x 10min with .01M PBS*
3. Rinse 1x 30min with 0.3% H_2O_2*
4. Rinse 1x 10min with 0.01M PBS*
5. Block 30 min with 10% Normal Serum PBS* (type of serum depends on host species of secondary antibody)
6. Place in Primary antibody 48hrs @ 4 C*
   Eugene Tech anti-Tyrosine Hydroxylase TE 101 (rabbit) 1:2000
7. Rinse 3x 10min with 0.01M PBS*
8. Place in secondary antibody 1hr*
   Biotinylated goat anti-Rabbit 1:500
9. Rinse 3x 10min with 0.01M PBS*
10. Add ABC solution*
    Vectastain ABC Kit PK 4000 from Vector Laboratories
11. Rinse 3x 10min with 0.01M PBS*
12. Add DAB solution for 5min* 1ml/well
13. Add Glucose oxidase to DAB - 100ul/1ml DAB (up to 45 min)
14. Rinse 3x 10min with 0.01M PBS*
15. Mount sections on gel coated slides and air dry overnight.
16. Run slides through an alocohol dehydration steps (70% x 2, 95% x 2, 100% x 2 at 10 minutes each)
17. 2 x 10 minutes each in xylene
18. Coverslip and air dry

* with agitation

Solutions

0.01M PBS
NaCl - 8.76g/L
KCl - 0.2 g/L
Triton X 100 - 2ml/L
PO4 Buffer - 50ml/L of 0.2M

Solution for Primary and Secondary Antibodies and ABC
1% Normal Serum in 0.01M PBS (serum depends on host species of secondary antibody)

ABC Solution
Add 4ul "A"/ml of 1% Normal Serum PBS
Add 4ul "B"/ml
Mix gently and let sit at least 15min before using

DAB Solution

50mg Diaminobenzidine (Sigma D 5637)
200mg D-glucose
40mg Ammonium Chloride (Sigma A 4514)
100ml 0.1M Phosphate Buffer
Mix and filter before using.

Glucose Oxidase
1mg glucose oxidase/16.6ml H20 (Sigma G 6125)

Appendix "B" - Table of Ligands Used in the Present Studies

Compound	Dose	Site of Action
5HT	0.3-6mmol	5HT agonist
Methysergide	7-60nmol	5HT1D/2A/2C antagonist
2-Methyl-5HT	800nmol	5HT3 agonist
Zatosetron	10nmol	5HT3 antagonist
8-OH-DPAT	60-150nmol	5HT1A agonist
NAN-190	4-520nmol	5HT1A antagonist
prazosin	25,50μmol	α1-antagonist
phentolamine	890nmol	α-antagonist

Appendix "C"- Abstracts and Papers from Ph.D. thesis experiments

1. Espey, M.J., J.W. Downie and A. Fine Effect of 5-HT receptor and adrenoceptor antagonists on micturition in conscious cats. Eur. J. Pharmacol. 221:167-170, 199?.

2. Espey, M.J.and J.W. Downie. Effect of serotonergic compounds on bladder function in the awake cat before and after spinal transection. Eur. J. Pharmacol. in preparation.

3. Espey, M.J., J.W. Downie and J.B. Gajewski. Perineal stimulation and the emergence of distension-evoked bladder contraction in spinal cats. Am. J. of Physiol. submitted

4. Espey, M.J., H.-J. Du and J.W. Downie. Serotonergic modulation of pelvic nerve-evoked ascending activity in anesthetized cats. J. Neurosci. In preparation

5. Espey, M.J., H.-J. Du and J.W. Downie. Effect of serotonergic compounds on a viscerosomatic reflex. In preparation.