Modulation of Cutaneous and Urethral Afferent Transmission During Micturition in the Decerebrate Cat

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Robert R. Buss

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Abstract

Urinary continence is assisted by the activation of pudendal and perineal afferents that activate excitatory interneuronal pathways to striated urethral sphincter (EUS) motoneurones. During micturition, these excitatory pathways are inhibited by several mechanisms including postsynaptic inhibition of EUS motoneurones and presynaptic inhibition of primary afferent transmission. Attenuation of the excitatory pathways contributes to relaxation of the EUS thus promoting efficient bladder emptying during voiding.

This study examined the role of primary afferent depolarization (PAD), a phenomenon closely associated with presynaptic inhibition, of cutaneous and urethral afferent transmission as one mechanism contributing to reduced activity in excitatory pathways to EUS motoneurones during micturition in the decerebrate cat. PAD was inferred by measuring changes in the electrical excitability of individual afferents within the dorsal horn of the spinal cord. PAD was observed in 21 of 30 cutaneous afferents (perineal and hindlimb) terminating in the rostral sacral segments of the spinal cord during micturition evoked by bladder distension or electrical stimulation of the pontine micturition centre. Cutaneous afferents terminating in the lower lumbar spinal cord (5 of 5) did not received PAD during micturition.

It is suggested that the PAD observed in sacral cutaneous afferents may be mediated by a previously described population of sacral PAD interneurones that were shown to give PAD to primary afferents entering the sacral spinal cord. Activation of these PAD interneurones by the central micturition circuitry would suppress transmission from all cutaneous afferents terminating in the sacral spinal cord whether or not they have excitatory actions to EUS motoneurones.

The effects of electrical stimulation of urethral afferents in the sensory pudendal nerve were

examined in isolation of cutaneous (*i.e.*, dorsal penile) pudendal afferents. Excitability measurements showed that urethral afferents gave PAD to and received PAD from perineal and hindlimb cutaneous afferents. Stimulation of muscle afferents at group II but not group I strength was effective at producing PAD in urethral afferents. Stimulation of urSPud afferents evoked excitatory postsynaptic potentials in 7 of 7 EUS motoneurones. Intracellular recordings from two EUS motoneurones revealed that urethral-evoked excitatory postsynaptic potentials were depressed during micturition when there was no activity in the EUS ENG. During micturition, excitability measurements showed that 6 of 16 urethral afferents examined received PAD during the period of EUS electroneurogram (ENG) suppression that was followed by PAH when activity returned in the EUS ENG at the termination of micturition. Three of 16 urethral afferents received PAD, 2 of 16 PAH, and 5 of 16 no excitability change during micturition. PAD or PAH was also observed in 6 urethral or cutaneous afferents during bladder filling, prior to micturition.

The biphasic PAD-PAH of urethral afferents observed during micturition is hypothesized to be mediated by interneurones directly accessed by the central micturition circuitry. The close correlation of urethral PAD with EUS ENG inactivity suggests that the central micturition circuitry accesses in parallel: PAD interneurones with connections to urethral afferents, the inhibitory interneurones responsible for postsynaptic inhibition of EUS motoneurones and perhaps other interneurones impinging on the motoneurones.

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List of Abbreviations

μA	microamp
μm	micrometre
AC	alternating current
CCF	caudal cutaneous femoral
CCS	caudal cutaneous sural
CPG	central pattern generator
cutSPud	cutaneous sensory pudendal
DC	direct current
DRP	dorsal root potential
EAS	striated external anal sphincter
ENG	electroneurogram
EPSP	excitatory postsynaptic potential
EUS	striated external urethral sphincter
GABA	γ-amino-butyric acid
GAD	γ-amino-*n*-butyric acid decarboxylase
GS	gastrocnemius soleus
h	hour
HRP	horseradish peroxidase
Hz	hertz
IVP	bladder pressure

IML	intermedio-lateral region of the grey matter
kg	kilogram
L	lumbar
LCS	lateral cutaneous sural
M	molar
MΩ	megaohm
mg	milligram
min	minute
mL	millilitre
mmHg	millimetre mercury
ms	millisecond
m s^{-1}	metres per second
mV	millivolt
n	number
N wave	negative wave
P wave	positive wave
PAD	primary afferent depolarization
PAH	primary afferent hyperpolarization
PBST	posterior biceps semitendinosus
PMC	pontine micturition centre
s	second
SFP	superficial perineal

SMAB	semimembranosus anterior biceps
T	threshold
Tib	posterior tibial
urSPud	urethral sensory pudendal
$\bar{x}$	mean
°C	degrees Celsius

Introduction

An Introduction to the Mechanisms of Continence and Micturition

The function of the urinary bladder is to collect and store urine until it can be evacuated at an appropriate time and place. Urine storage is maintained by constriction of bladder neck and proximal urethral smooth muscle and by contraction of the striated urethral sphincter. The actions of these muscles prevent the escape of urine through the urethra. Urine storage is further facilitated by stretching of the bladder body smooth muscle which accommodates the increasing volume of urine during bladder filling and minimizes bladder pressure increases. Mechanisms such as these, that promote the storage of urine, are known as continence mechanisms.

Urine evacuation, or micturition, requires a suppression of the continence mechanisms. The smooth muscle of the bladder neck and proximal urethra and the striated muscle of the external urethral sphincter (EUS) must relax to allow urine to flow through the urethra. Relaxation of the bladder outlet must be coordinated with a contraction of the bladder body smooth muscle sufficient to expel a stream of urine.

Mechanical actions of the smooth and striated muscle subserving continence and micturition are controlled by the autonomic (sympathetic and parasympathetic) and somatic nervous systems. During continence, stretch sensitive receptors located in the bladder wall (Iggo, 1955) convey information (*via* the pelvic nerve) concerning the state of bladder fullness to the spinal cord and supraspinal centers. Bladder wall stretch receptors are increasingly activated as the bladder fills. Several continence-promoting spinal neural pathways are

reflexly activated by bladder wall stretch. These reflex pathways include an adrenergic sympathetic (hypogastric nerve) mediated direct relaxation of bladder body smooth muscle and a modulation of cholinergic transmission at the bladder parasympathetic ganglion, which functions to reduce excitatory input to the bladder. Together, these actions prevent unwanted contractions of the bladder body smooth muscle. Continence is further maintained by a hypogastric nerve mediated excitation of proximal urethral and bladder neck smooth muscle. Activation of an excitatory pathway to the motoneurones controlling the striated urethral sphincter and pelvic floor muscles also aids in preventing urine leakage.

The contribution of the EUS to the maintenance of continence is unique because this muscle is under voluntary control. The EUS encircles the urethra from the prostate gland to the root of the penis in males while in females, the sphincter forms an additional encirclement around the vagina (Martin, Fletcher & Bradley, 1974a). Innervation of the EUS is provided by the pudendal nerve (Martin, Fletcher & Bradley, 1974b). Voluntary contraction of the EUS can be used to delay or terminate micturition. The descending pathway subserving the voluntary control of micturition has been attributed to direct cortical actions to EUS motoneurones (Fukuda & Koga, 1992). However, findings of Shefchyk, Espey & Buss (unpublished observations) suggest the cortical motor actions are mediated indirectly through the medullary reticular formation (Mackel, 1979), as electrical stimulation of the medullary pyramidal tract did not produce monosynaptic excitatory postsynaptic potentials (EPSPs) in EUS motoneurones.

In addition to activation by descending motor pathways and bladder wall stretch receptor activated pathways, the EUS is also excited by changes in intra-abdominal pressure

(Koga & Fukuda, 1990), tactile stimulation of genital and perineal skin, and flow of urine through the urethra (Barrington, 1914). Reflex activation of the EUS is critical for preventing the accidental escape of urine during behaviours such as retching or vomiting (Koga & Fudkuda, 1990), and stimulation of genital and perineal skin may evoke reflex activation of the EUS and help prevent urine escape during movements such as walking. The EUS clearly plays a very important role in maintaining continence.

As mentioned earlier, a signal for initiating micturition is provided by bladder wall stretch receptors (Iggo, 1955) which trigger a central micturition circuitry when the bladder has filled to capacity. At this stage the neural pathways responsible for maintaining continence must be modulated by some mechanism to allow efficient bladder emptying. Voluntary contraction of the EUS can temporarily delay micturition, however, micturition will eventually proceed once higher bladder volumes are reached.

Once activated, the central micturition circuitry excites parasympathetic bladder preganglionic neurones which send an excitatory signal *via* the pelvic nerve and the peripheral ganglia, to bladder body smooth muscle and the bladder body contracts. The sympathetic reflex pathways contributing to constriction of the bladder neck, proximal urethra, and EUS are inhibited and pathways which contribute to effective bladder emptying are released. For example, during micturition, flow through the urethra does not initiate closure of the EUS but instead facilitates contraction of the bladder and suppression of EUS activity. The neural pathways activated by cutaneous stimulation in the genital region which reflexly contract the EUS between voids are also inhibited.

Reflex Pathways to the External Urethral Sphincter

The pudendal nerve carries afferent fibres activated by cutaneous stimulation to the genital region. Studies have shown that the receptive field of the common pudendal nerve includes the labia, vulva, clitoris and one to two centimeters of skin surrounding the vaginal orifice (Cueva-Rolon, Muñoz-Martínez, Delgado-Lezama & Raya, 1994) in females, and the skin of the glans penis (Cooper, 1972) in males. In addition, flow through the urethra appears to activate small lamellate receptors located in the superficial urethral mucosa which produce a sustained discharge in afferent fibres carried by the pudendal nerve (Todd, 1964; Talaat, 1937, in dogs). The afferent fibres carried in the pudendal nerve activate strong excitatory pathways to EUS motoneurones. Fedirchuk, Hochman & Shefchyk (1992b) found that 45 of 47 EUS motoneurones examined received EPSPs when the pudendal nerve was stimulated. The strength of electrical stimulation used in their study would have activated (Hunt & McIntyre, 1960) low threshold cutaneous afferents (tactile afferents) innervating the genital region.

Superficial perineal (SFP) nerve afferents also convey cutaneous sensation. The receptive field has not been well documented in cats, but has been shown to extend from the base of the tail to medial surface of the thigh and scrotum in male dogs (Spurgeon & Kitchell, 1982; Spurgeon & Reddy, 1986). Superficial perineal nerve afferents have a weaker excitatory action on EUS motoneurones than pudendal afferents; 24 of 37 EUS motoneurones received EPSPs from SFP nerve stimulation (Fedirchuk *et al.*, 1992b). The effectiveness of the SFP nerve in exciting the striated urethral sphincter is not unexpected because of the proximity of the SFP receptive field to pudendal nerve afferents. However,

the SFP nerve appears to carry only cutaneous information and does not carry afferents activated by flow through the urethra.

Cutaneous nerves with receptive fields distant to the genital region generally have much weaker excitatory connections onto EUS motoneurones. Lateral (LCS) and caudal cutaneous sural (CCS) and caudal cutaneous femoral (CCF) afferents were largely ineffective (22 of 26) in producing EPSPs in EUS motoneurones (Fedirchuk *et al.*, 1992b). The receptive fields of these afferents included the skin of the thigh, lower limb and foot (Korber & Brown, 1982; Ekholm, 1967). Based upon available evidence, it appears that cutaneous stimulation in the genital and perineal region activates a reflex contraction of the EUS. Cutaneous stimulation of this area caused by activities such as walking, jumping, and especially copulation, undoubtedly contributes to continence by activation of the EUS.

The interneurones involved in the reflex pathways from pudendal, perineal and hindlimb cutaneous afferents are largely unidentified in the cat. Latency measurements (Fedirchuk *et al.*, 1992b) reveal a polysynaptic excitatory pathway with a strong possibility that a disynaptic pathway exists. The first order interneurones in this pathway have been revealed by anatomical and physiological methods. The pudendal nerve afferents terminate primarily in the first two sacral segments of the cat spinal cord with a small distribution in the last lumbar and lower sacral and coccygeal segments (Ueyama, Mizuno, Nomura, Konishi, Itoh & Arakawa, 1984; Fedirchuk, Song, Downie & Shefchyk, 1992a). Field potential recording (Fedirchuk *et al.*, 1992a) and horseradish peroxidase (HRP) anterograde labeling studies (Ueyama *et al.*, 1984) have revealed the dorso-medial grey matter in the first two sacral segments as the principal site of termination. However, this HRP labeling

(Ueyama *et al.*, 1984) also revealed a termination in the intermedio-lateral (IML) region of the grey matter, the site of bladder preganglionic parasympathetic neurones. This region may contain the first-order interneurones activated by urethral nerve (urSPud) stimulation, the small branch of the pudendal nerve containing afferents activated by flow through the urethra. Although fields evoked by the sensory pudendal nerve (containing the urSPud branch) were investigated by Fedirchuk *et al.* (1992a), the focus was on dorsal horn cutaneous fields, and for this reason the urSPud component may have been ignored. Bladder afferents also terminate in this area of the spinal cord (Sugaya & Mori, 1988), and this close proximity supports the possibility that the same first-order interneurones are activated by both bladder and urethral afferents. Further support for this possibility comes from the observation of Buss & Shefchyk (1997b) and Shefchyk & Buss (1997) that electrical stimulation of the urethral nerve can access micturition networks similar to those activated by bladder afferents.

Modulation of Excitatory Reflex Pathways to the External Urethral Sphincter During Micturition

During micturition, the excitatory reflex pathways to EUS motoneurones are inhibited. The primary site of inhibition of this reflex is the EUS motoneurone. Fedirchuk & Shefchyk (1993) showed that the EUS motoneurone membrane potential becomes hyperpolarized and there is an associated increase in somatic input conductance during micturition. This hyperpolarization could be reversed by ejection of chloride into the EUS motoneurone suggesting the inhibition was mediated by a chloride conductance. γ-amino-

butyric acid (GABA) and glycine were suggested as likely candidates mediating the hyperpolarization because they are common inhibitory neurotransmitters in the spinal cord. Glycine-mediated inhibition of EUS motoneurones likely predominates during micturition as Espey, Buss, Nance, Sawchuk, Carr & Shefchyk (1996) showed that activity in the EUS electroneurogram (ENG) was not decreased during micturition when the glycine antagonist strychnine was administered intravenously. Immunohistochemical methods revealed glycine receptors on the membrane of EUS motoneurones which supported the conclusion that the action of the strychnine was at least in part at the sphincter motoneurone.

Fedirchuk, Downie & Shefchyk (1994) have provided evidence that inhibition of the excitatory cutaneous reflex pathway also occurs at a premotoneuronal level. They found that pudendal and perineal EPSPs recorded in EUS motoneurones were reduced in amplitude during micturition. However, the conductance changes occurring in EUS motoneurones during micturition could also have changed the EPSP sizes. To address this possibility, pudendal, perineal and hindlimb cutaneous evoked EPSPs were recorded in hindlimb motoneurones that did not undergo a conductance change during micturition. EPSPs recorded in these motoneurones were also suppressed during micturition. These findings were strong evidence supporting the existence of a premotoneuronal mechanism. It was proposed that interneurones located in polysynaptic pathways were inhibited and/or that the primary afferents were subjected to presynaptic inhibition during micturition.

The widespread depression of pudendal, perineal and hindlimb cutaneous pathways suggested a nonspecific mode of inhibition. A global inhibition of cutaneous transmission would manifest itself as nonspecific inhibition. For this reason presynaptic inhibition of

cutaneous afferent transmission was first investigated. Angel, Fyda, McCrea & Shefchyk (1994) showed that approximately half the pudendal afferents studied received primary afferent depolarization (PAD), a phenomenon that has been closely correlated with presynaptic inhibition, during micturition. This finding demonstrated that PAD pathways were activated during micturition and the suggestion was made that transmission from perineal and hindlimb afferents might also be inhibited by a PAD mechanism during micturition.

The History of Primary Afferent Depolarization

The phenomenon that would eventually be labeled PAD was described by several investigators in the 1930s. Several approaches were used to reveal the then undescribed depolarization of primary afferents. Not until the end of the 1930s was it realized that the same phenomenon was being investigated.

Gasser & Graham (1933) and Hughes & Gasser (1934) showed a long lasting inhibitory phenomena activated by stimulation of a dorsal root. Stimulation of a dorsal root produced three distinct waveforms as recorded from the dorsal surface of the spinal cord. The three waveforms recorded were: 1) a triphasic spike corresponding to the incoming afferent volley, 2) a negative wave, called the N wave, which represented the activation of interneurones (average latency, 10.2 ms), and 3) a long lasting positive wave (P wave) peaking 20 ms after the spike and lasting 80 to 100 ms. Of relevance to PAD was that the N wave evoked by a second volley to the dorsal root was reduced in amplitude for as long as the P wave, produced by the first volley, existed. That is, the nerve cells producing the P

wave were inhibiting the excitation of interneurones within the spinal cord. Further evidence for an inhibitory action of the P wave was provided by the demonstration that muscle force developed during a flexor reflex was reduced for as long as the P wave produced by a preceding volley lasted. The P wave is still used as a measure of PAD (Quevedo, Equibar, Jimenez, Schmidt & Rudomin, 1993) and is attributed to activity of PAD interneurones and the associated depolarization of primary afferent fibres.

At a similar time, Matthews (1934), Barron & Matthews (1934), and Barron & Matthews (1935a) reported that stimulation of a dorsal root could produce centrifugal discharges in an adjacent dorsal root. This centrifugal activity was attributed to fibres coming from a peripheral receptor and branching into collaterals within the spinal cord which sent axons back to the periphery in the same and adjacent roots (Barron & Matthews, 1935b). No sign of action currents were observed in muscle by the antidromic discharges; thus they suggested the fibres went to receptors in the periphery.

In addition to the dorsal root discharges observed upon stimulation of a dorsal root, Barron & Matthews (1938a) also observed a long lasting depolarization (*i.e.*, 120 ms in duration). This depolarization was recorded as a potential difference between one pole of an electrode placed on the dorsal root where it enters the cord and the other lead, placed on the distal cut end of the dorsal root. The electrotonic spread of depolarization of the central terminals of the dorsal root fibre was thus measured. The depolarization of the dorsal root, called the dorsal root potential (DRP), was evoked by stimulation of an adjacent or contralateral root, or a root up to six segments away.

Barron & Matthews (1935a) suggested the depolarization arose from changes in

external ion concentrations following impulse activity in that fibre and neighboring fibres. An electrotonus, originating in the grey matter, spread along the afferent collaterals and into the dorsal roots. The depolarization of primary afferents was hypothesized to prevent the impulse from reaching interneurones and motoneurones. The time course of inhibition following an afferent volley was similar to the duration of the DRPs observed and they suggested the DRP was producing part of the inhibition.

Toennies (1938) observed that stimulation of the saphenous nerve could evoke a reflex discharge originating in the spinal cord and traveling distally through the ipsilateral and contralateral saphenous nerve. The reflex was made larger when appropriately timed conditioning volleys were given to the saphenous, tibialis or peroneus nerves. Toennies (1938) believed it was the same phenomenon described by Matthews (1934) and Barron & Matthews (1935a) because the fibres of the saphenous nerve are nearly exclusively of dorsal root origin (Heinbecker, O'Leary & Bishop, 1933). Toennies (1938) showed the delay to the reflex volley was too slow to have been from recurrent collateral afferent fibres as suggested by Barron & Matthews (1935a). Along with the observation that dorsal root reflexes were facilitated, Toennies correctly concluded that fibres of dorsal root origin made synaptic connections with interneurones within the spinal cord and that these interneurones were responsible for reflex discharge observed in fibres of the dorsal root.

Toennies (1939) showed that a tap applied to the skin over the tibia could evoke a reflex discharge in the saphenous nerve which was reduced when it was preceded by a dorsal root discharge. This study was critical because it showed that dorsal root discharges could inhibit incoming afferent information elicited by natural stimulation.

Barron & Matthews (1938b) amalgamated Toennies' dorsal root discharge and the DRP by making the observation that dorsal root discharges were most often observed when evoked by a nearly synchronous centripetal volley that produced an abruptly rising DRP. Less synchronous volleys, produced by pressure to the foot, seldom produced a dorsal root reflex. Such stimulation would not produce an abruptly rising DRP. They concluded that dorsal root reflexes occur when the depolarizations are rapid enough in onset to elevate the membrane potential to action potential threshold. Collectively, these studies provided a reasonable hypothesis that primary afferent fibres could depolarize adjacent primary afferent fibres through interneuronal reflex pathways located within the spinal cord, and that the depolarization of these afferents could reduce transmission to motor reflex pathways.

Eccles and colleagues (reviewed in Eccles, 1964) did extensive studies on dorsal root potentials and reflexes and named the phenomenon primary afferent depolarization. This name was given because of the depolarization observed in the terminals of primary afferent fibres. They found the time course of presynaptic inhibition closely followed the duration of depolarization of afferent terminals. The best evidence came from the recording of Ia monosynaptic EPSPs which were maximally depressed when the depolarization was greatest. The depression lasted for as long as primary afferents were depolarized.

Afferent terminal depolarization was measured by intracellular recording from primary afferent terminals, recording of dorsal root potentials, and by measuring increases in the excitability of the membrane to electrical stimulation. This last technique was developed by Wall (1958) and is based upon the premise that when afferent fibres undergo a depolarization, their membrane potential is brought closer to action potential threshold and

less current is required to produce an action potential in the fibre. Eccles hypothesized the depolarization of the afferent terminal was directly responsible for the presynaptic inhibition. The depolarization would reduce the size of the afferent spike and in turn reduce the amount of transmitter liberated from the terminal. The depolarization itself was believed to be mediated by a neurotransmitter (postulated to be GABA) which increased the permeability of the primary afferent to chloride. An active chloride pump was hypothesized to maintain a positive chloride gradient within the terminals of primary afferents. Opening chloride channels would allow an outward depolarizing flow of chloride through the membrane.

The EPSP depression observed during presynaptic inhibition does not exclude the possibility that postsynaptic mechanisms contributed to the depression. The absence of hyperpolarization in the postsynaptic cell is often taken as evidence for an absence of postsynaptic inhibition. However, inhibition taking place on dendrites distant from the soma will not necessarily spread to the soma and be observed as a hyperpolarization. Such a possibility was suggested by Frank (1959) and named remote inhibition.

Kellerth (1968) showed a homonymous gastrocnemius soleus monosynaptic EPSP reduced by a conditioning volley in the hamstrings nerve. There was no hyperpolarization of the gastrocnemius soleus motoneurone corresponding to the EPSP reduction, which was consistent with a presynaptic mechanism. However, a postsynaptic action was revealed by showing that the frequency of action potential firing induced by intracellular current injections was reduced during hamstring conditioning stimuli. The decrease in firing frequency revealed that the existence of a postsynaptic effect, hypothesized to be acting on the remote portions of the dendrites, was indeed present. Kellerth (1968) stated that a pure

case of presynaptic inhibition was never observed. However, McCrea, Shefchyk & Carlen (1990) reported a 70% reduction in Ia EPSPs without a change in the shape of the EPSP. As a change in EPSP shape is generally accepted as evidence for a postsynaptic action, the Ia afferent EPSPs were unlikely to have undergone postsynaptic inhibition.

GABA is the Neurotransmitter Depolarizing Primary Afferents

Levy (1977) reviewed the PAD literature and concluded that the available evidence provided strong support for GABA as the transmitter mediating PAD. The most compelling facts are: 1) γ-amino-*n*-butyric acid decarboxylase (GAD), the rate-limiting enzyme for GABA synthesis, was found in the presynaptic terminal at axo-axonic synapses on primary afferents, 2) GABA depolarized primary afferents, 3) the GABA receptor antagonists bicuculline, picrotoxin and penicillin antagonized GABA-evoked depolarizations, 4) depletion of GABA by inhibition of GAD reduced PAD, and 5) inhibition of GABA transaminase, an enzyme mediating the degradation of GABA, enhanced PAD.

More recently, it has been shown that GABA can activate two distinct classes of receptors; $GABA_A$ and $GABA_B$. Both receptor classes have pre- and postsynaptic actions in the mammalian central nervous system (for review see, Misgeld, Bijak & Jarolimek, 1995). $GABA_B$ receptors are activated by baclofen and antagonized by OH-saclofen and CGP 55845A, all of which make them pharmacologically distinguishable from $GABA_A$ receptors. $GABA_A$ receptors are activated by muscimol and antagonized by bicuculline and picrotoxin. At the presynaptic terminal, baclofen has been shown to reduce transmitter release by increasing potassium conductances, decreasing calcium conductances, and by a mechanism

independent of potassium or calcium conductances. These actions are mediated through a G protein pathway which in some instances may include an additional second messenger system.

Jiménez, Rudomin & Enriquez (1991) showed that intravenous administration of baclofen reduced the size of the monosynaptic EPSP, the ventral root potential, and the extracellular field potential recorded from the motor nucleus. The action was attributed to a presynaptic mechanism because baclofen administration did not change the membrane potential of motoneurones or the time course of the monosynaptic EPSP. However, these experiments cannot rule out the possibility that baclofen facilitated a tonic PAD pathway. A later study (Quevedo, Eguibar, Jiménez & Rudomin, 1992) revealed that baclofen can abolish PAD evoked by segmental stimulation and concluded that baclofen can inhibit the segmental PAD pathway. They showed that transmission from the last-order interneurone to primary afferents is inhibited by baclofen, as was revealed by an abolishment of PAD evoked by intraspinal stimulation of last order interneurones.

The possibility exists that different sets of interneurones are responsible for the activation of $GABA_A$ and $GABA_B$ receptors located on primary afferents. Such a possibility would increase the effectiveness of the central nervous system in controlling transmission from primary afferents in the spatial and temporal domains (Rudomin, 1994). If so, situations may exist where there is strong suppression of afferent transmission, perhaps as revealed by a reduction in monosynaptic field potentials, and no observable PAD in the immediate vicinity. Alternatively, common interneurones may activate both $GABA_A$ and $GABA_B$ receptors and the two modes of inhibition may function to reduce synaptic transmission at

the same sites. Additionally, depolarizing action of the $GABA_A$-mediated chloride channel opening may additionally facilitate the conductances activated by the $GABA_B$ receptor activation. Such a process would be conceptually similar to the prolonged calcium current, mediated by a depressed potassium current, described in Aplysia; for review see Klein, Shapiro & Kandel (1980).

GABAergic Synapses onto Primary Afferent Fibres

The existence of GABAergic synapses presynaptic to primary afferent fibres was an essential requirement of the hypothesis of Eccles (1964). Maxwell, Christie, Short & Brown (1990) have used electron microscopy to shown GABAergic terminals presynaptic to both *en passant* and terminal boutons of Ia afferent collaterals that had been identified and filled with HRP. The finding of axo-axonic synapses on Ia afferents was critical because Eccles (1964) and later investigators have used the reduction of the monosynaptic Ia EPSP as an indication of presynaptic inhibition produced by PAD.

Current PAD Studies in the Mammalian Spinal Cord

Besides the mechanism of action, Eccles and colleagues were interested in the interneuronal pathways subserving PAD in primary afferents. Several interneuronal PAD pathways were revealed by observing segmental sources of PAD (reviewed in Schmidt, 1971). More recent investigations have concentrated on characterizing the interneurones involved in these PAD pathways. The dedicated work of Rudomin and colleagues has characterized several PAD pathways to Ib muscle afferents (reviewed in Rudomin, 1990) and

Jankowska & Riddell (1994; 1995) have started to investigate the interneurones subserving PAD of group II muscle afferents.

How PAD mechanisms are used during behaviours is another area of PAD study. During fictive locomotion, flexor and extensor groups I and II muscle (Gossard, Cabelguen & Rossignol, 1991) and cutaneous (Gossard, Cabelguen & Rossignol, 1989) afferents have been shown to undergo a prominent depolarization during the extension phase of the step cycle. A less prominent depolarization is sometimes observed during the flexion phase of the step cycle. The observed depolarizations presumably regulate the flow of afferent information to the locomotor central pattern generator (CPG) during phases of the step cycle. Evidence supporting an inhibitory action of the observed afferent PAD was recently provided by Perreault, Jimenez, Shefchyk & McCrea (1994) who showed that short latency muscle group I and II and cutaneous field potentials were depressed during fictive locomotion.

Goals, Aims and Hypothesis of this Thesis

The goal of this thesis was to determine whether cutaneous afferents terminating in the sacral spinal cord receive PAD during micturition. PAD of cutaneous afferents would contribute to the suppression of perineal and hindlimb cutaneous afferent reflexes during micturition and facilitate EUS relaxation and efficient bladder emptying. This was a likely possibility as Angel *et al.* (1994) had shown that pudendal afferents receive PAD during micturition.

One issue addressed was why would the reflex pathway from hindlimb muscle afferents need to be suppressed during micturition. Hindlimb cutaneous afferents were shown

to have only a weak excitatory action on EUS motoneurones, yet these reflex pathways were suppressed during micturition (Fedirchuk *et al.*, 1994). We hypothesized that the micturition central circuitry utilized a preexisting segmental PAD circuitry, shared perhaps with other behaviours such as locomotion, to suppress the cutaneous excitatory reflex pathways to sacral motoneurones. The sacral segmental PAD circuitry had access to cutaneous afferents terminating in the sacral spinal cord and its activation by the micturition central circuitry would result in reduced transmission of all cutaneous afferents terminating in the sacral spinal cord.

Another issue is whether synaptic transmission from flow activated urethral afferents (urSPud) was modulated differently from pudendal afferents conveying cutaneous sensation from skin in the genital region (cutSPud). Our initial hypothesis predicted that urSPud afferents would receive primary afferent hyperpolarization (PAH), a removal of tonic PAD (Mendell & Wall, 1964), during micturition; a suggestion proposed in the study of Angel *et al.* (1994) to account for PAH observed in some fibres carried in the pudendal nerve (urethral and cutaneous branches not separated). However, the later finding that urSPud stimulation evoked strong excitatory reflex actions in EUS motoneurones and the EUS ENG lead to an alternative hypothesis. We hypothesized urSPud afferent branches contributing to strong excitatory reflex actions to EUS motoneurones would receive PAD during micturition. However, urSPud afferent branches that activated reflex pathways which facilitate bladder contraction (Barrington, 1914) would not receive PAD. It was hypothesized urethral afferents traveling in the pudendal nerve would receive a more prominent PAD during micturition than cutaneous afferents because a micturition specific PAD pathway, distinct from the

segmental PAD pathway giving PAD to sacral cutaneous afferents, was accessing urethral afferents.

Methods

General Surgery

Data shown were collected during experiments performed on16 cats, 11 male and 5 female (2.2-4.5 kg). The surgery was performed under Halothane (1 to 3%) in a mixture of nitrous oxide and oxygen. The adequacy of anaesthesia was verified by testing withdrawal reflexes and monitoring blood pressure. The femoral artery and vein were cannulated for blood pressure recording and fluid and drug administration respectively and a tracheostomy was performed. Bladder pressure was measured *via* a catheter inserted and tied into the ventral surface of the bladder.

The following nerves were routinely cut and dissected on the left side: branch of the pudendal nerve conveying cutaneous sensory information from skin located in the genital region (cutSPud), branch of the pudendal nerve conveying information from flow activated receptors located in the urethral mucosa (urSPud), superficial perineal (SFP), caudal cutaneous femoral (CCF), caudal cutaneous sural (CCS), lateral cutaneous sural (LCS), posterior tibial (mixed muscle and cutaneous innervation of the plantar surface of the foot, Tib), posterior biceps and semitendinosus (PBST), anterior biceps and semimembranosus (SMAB), medial and lateral gastrocnemius and soleus (GS), external urethral sphincter motor branch of the pudendal nerve (EUS), and external anal sphincter motor branch of the pudendal nerve (EAS). In some experiments the ligaments joining the left femur to the ilium and ischium were cut so that the mineral oil pool was easier to form and the femoral and obturator nerves cut to reduce afferent input to the spinal cord. Other nerves branching off

the sciatic nerve were cut and ligated.

An L7 to L4 laminectomy was performed and the cat was then moved to a rigid recording frame. Skin from the back and hindlimbs were formed into a continuous pool containing mineral oil. Core and pool temperatures were maintained between 37 and 38 °C using radiant heat. Bipolar chlorided silver hook electrodes were used to record from and/or stimulate the peripheral nerves. Electroneurograms (ENGs) were filtered (high pass cutoff 300 Hz; low pass 3000 Hz) and amplified 1000 to 10000 times. The dura over the exposed spinal cord was cut and reflected laterally and patches of pia matter removed to permit insertion of the microelectrode. A precollicular-postmammillary decerebration was performed (the cerebral cortices and the tissue rostral to the transection removed) and the anaesthetic discontinued. Dextran was administered to replace blood loss and a 5% glucose and bicarbonate solution was infused at a rate of 5 mL h^{-1} throughout the experiment. The animals were then paralyzed with gallamine triethiodide (5 mg every 45 minutes) and artificially ventilated; bilateral pneumothoraxes were made to minimize thoracic movements. Mean blood pressure was always kept above 80-100 mmHg (dextran was administered to elevate blood pressure) and end-tidal CO_2 levels were maintained between 3 and 4%.

Micturition

Room temperature saline solution was infused through the bladder catheter at 2 to 3 mL min^{-1} to initiate distension-evoked reflex micturition; inter-cat volume thresholds for micturition ranged between 11 and 40 mL. Alternatively, electrical stimulation of the pontine

micturition centre (PMC) (Shefchyk, 1989; Fedirchuk & Shefchyk, 1991) with 40 to 500 μA, 0.2 to 0.5 ms square wave pulses at 20-50 Hz through monopolar tungsten stimulating electrodes was used to initiate micturition. Bladders were filled to approximately 50% of their distension volume threshold prior to electrical stimulation of the PMC. Excitability changes were recorded in 42 fibres (8 animals) during distension-evoked micturition and in 34 fibres (11 animals) during PMC-evoked micturition; note that 12 fibres were observed during both distension- and PMC -evoked micturition. All data reported are from voids where EUS ENG activity was suppressed during micturition and the bladder contraction was sufficiently strong to expel a stream of urine.

Recording Location

Glass microelectrodes filled with 2 M sodium citrate (tip diameters 2.0 to 2.4 μm; resistance around 2 MΩ) were used for field potential recording and intra-spinal stimulation. Peripheral nerve stimulation was used to locate the largest short-latency field potentials in the dorsal horn, presumably reflecting the site of termination of a large number of afferent fibres.

Recordings were made in the sacral and lumbar spinal cord. In the sacral spinal cord, recordings were made from the dorsal surface of the sacral spinal cord using a monopolar chlorided silver ball electrode to locate a site where the group II component of a PBST volley was the largest; this area overlaid the EUS motor nucleus and was the site of the sacral relay interneurones (Jankowska & Riddell, 1993; 1994) which are the first interneurones in sacral PAD pathways (Jankowska & Riddell, 1995). The electrode was inserted medial to the dorsal

root entry zone and lateral to the midline with the electrode tip angled 0 to 10° lateral. The lumbar recording site extended from the L6-L7 border to the middle of L7.

Excitability Testing

Once a field was located within the spinal cord, the microelectrode was connected to a constant current simulator which could deliver 0.5 ms square wave current pulses ranging from 0.2 to 10 μA. Pulses were delivered at a rate between 2 and 7.5 Hz. The stimulus strength was manually adjusted until antidromically activated units could be observed in a particular peripheral nerve ENG. A time and amplitude window discriminator was adjusted to isolate a single unit activated by the intra-spinal stimulation. The unit was not studied if increases or decreases in intra-spinal stimulus current changed the amplitude of the unit. A computer program was then used to adjust the amount of current ejected through the electrode, in steps of 0.2 to 0.6 μA, so that the unit would fire about 50% of the time (see Madrid, Alvarado, Dutton & Rudomin, 1979; Angel *et al.*, 1994). The unit triggering within the discriminator window was continuously monitored during recording to ensure the absence of false triggering. A decrease in the stimulation current required to maintain firing of the unit about 50% of the time was interpreted as an increase in fibre excitability and the presence of primary afferent depolarization. The current necessary to maintain unit firing 50% of the time often fluctuated prior to and during bladder filling. Baseline current was determined by visual inspection of the current trace. When both excitability increases and decreases occurred in a unit, both measurements were taken from the same baseline. Routinely, baseline current measurements were taken in the pre-void

period, however, baseline was taken during the post-void period when gradual current changes during bladder filling occurred. Current changes of ≥5% were the criteria for an excitability change.

Stimulus Parameters and Conduction Velocity Calculations

The strengths of peripheral nerve stimulation were expressed in multiples of the threshold current (T) which produced the first detectable volley at the cord dorsum electrode following constant current stimulation with 0.2 ms duration single pulses. Conduction times of the afferents were calculated from the onset of the intraspinal stimulus to the unit firing recorded in the peripheral nerve. The conduction velocities reported in this study will be underestimated, as the minimum distance from the intra-spinal site to the peripheral recording electrodes was measured without compensation for action potential activation time or slower conduction through intra-spinal afferent branches.

Segmental Paradigm

Segmental sources of PAD were tested by conditioning stimulation of cutaneous or muscle nerves (trial frequency 2.0 to 3.3 Hz). Cutaneous nerves were conditioned with a train of three stimuli at 300 Hz with a condition-test interval of 20 ms measured from the last conditioning shock to the test stimulus. Muscle nerve conditioning stimulation was composed of a train of five stimuli at 300 Hz with a condition test interval of 25 ms.

Blood Pressure Changes

Blood pressure increases were sometimes observed during distension- and PMC-evoked voiding. Caution was taken to ensure the excitability changes observed were not an artifact associated with movements and changes in recording conditions caused by changes in blood pressure. If the excitability change in a fibre closely followed blood pressure fluctuations the data were not used in the analysis. In some instances, a rapid injection of dextran was used to raise blood pressure to determine whether a blood pressure change altered intraspinal stimulating currents.

Intracellular Recordings

EUS and EAS motoneurones were antidromically identified by stimulation of the EUS or EAS nerve. Potassium acetate-filled (2 M, 1.6 to 1.8 μm) glass microelectrodes were used for intracellular recording. Amplification was through a Dagan 8700 Cell Explorer; DC traces were digitized at 5000 Hz and AC traces at 10000 Hz.

Statistics

Use of the term significant indicates statistical significance at the 95% confidence interval. T-tests, or the Mann-Whitney Rank Sum Test (where applicable) were used for comparison of means. The Fisher Exact Test was used to compare the occurrence of excitability changes observed among the various species of afferents studied.

Results

Fibre excitability changes were assessed in 64 afferent fibres during micturition evoked by bladder distension or stimulation of the PMC. Fifty-six units were recorded in the dorsal horn of the first and second segments of the sacral spinal cord, and eight from the seventh lumbar spinal segment. In the sacral cord, the rostral-caudal location of the microelectrode was at the site where the largest group II PBST cord dorsum potential could be recorded. This area corresponds with the rostral extent of the EUS motor nucleus located in the ventral horn (Jankowska & Riddell, 1993). Figure 1 shows the depths of the stimulating electrode from the dorsal surface of the spinal cord and the conduction velocities of the fibres sampled in the sacral segments in this study.

Part A: Cutaneous Afferents

Excitability Changes Observed in Sacral Cutaneous Afferents During Micturition

Excitability increases were observed during micturition in 21 of 30 cutaneous afferents terminating in the first or second sacral segments. Ten units were examined during distension-evoked micturition, 11 during PMC-evoked micturition, and one during both distension- and PMC-evoked micturition. The average increase in excitability of cutSPud, SFP, CCF, CCS and LCS fibres during micturition is presented in Table 1. Representative examples of excitability increases during distension- and PMC-evoked micturition are shown in Figures 2 and 3. In Figure 2A the current required to maintain firing in 50% of the trials

was 4.82 μA (horizontal line i) prior to micturition and gradually decreased to a minimum current of 4.06 μA (horizontal line ii) at the end of the bladder contraction (arrow iii). The current required to maintain firing in 50% of the trials was reduced by 0.76 μA or (15.8 %) of the prevoid value, *i.e.*, fibre excitability increased 15.8%. In this fibre the excitability change commenced at the onset of the bladder contraction (arrow iv) and returned to baseline around 20 seconds after the bladder contraction had ended (arrow v).

In 14 of 21 fibres the excitability increases observed during micturition were observed during the period of bladder contraction and sphincter relaxation and regularly persisted after activity returned in the EUS ENG (see Figures 2A & B, 3B-D). However, in 4 of 21 units, fibre excitability returned to baseline in the midpoint of the bladder contraction (not shown), and in 3 of 21 units the excitability increase did not occur until the middle of the bladder pressure increase (see Figure 3A). The 3 of 21 fibres in which late onset excitability increases occurred were observed only during distension-evoked voids. As illustrated in Figure 2B, during PMC-evoked voids, the excitability increase always began at the onset of the void and were more distinct. The effect of brainstem stimulation alone (*i.e.*, in the absence of micturition) was not systematically investigated in this study. Panels C and D in Figure 2 show similarity in the time course of the excitability change in one cutaneous afferent (SFP) recorded during PMC (C) and distension-evoked (D) voiding. Although short episodes of excitability decreases (arrows) appear in Figure 2C and D, these events appeared sporadically prior to and after micturition (not shown) and were not directly attributed to micturition.

Table 2 summarizes the species of cutaneous afferent fibres that were examined

during micturition. There was no significant difference in the occurrence of excitability changes during micturition among the different cutaneous afferents. This finding suggests there was no relationship between the location of cutaneous nerve receptive field and the occurrence of excitability changes during micturition.

Excitability Changes Observed in Lumbar Cutaneous Afferents During Micturition

Recordings were made from 8 units located in the seventh lumbar segment of the spinal cord. Observations from these units are presented separately because of their location outside the sacral region specialized for mediating micturition behaviours. Excitability changes were uninterpretable in three units, absent in four units (1 LCS, 2 CCS and 1 CCF) and in one unit (LCS) there was a small decrease in excitability (5.4%) during the period of bladder contraction as shown in Figure 4. This unit was observed in an animal in which other units recorded in first sacral segment (CCF and urSPud) displayed an increase in excitability of 10.3% and 7.1% (respectively) during micturition.

Excitability Increases Evoked by Segmental Cutaneous and Muscle Nerve Stimulation

Excitability changes evoked by 5T stimulation of peripheral nerves were examined in 19 units in the absence of micturition; these 19 units were also examined during micturition. Nerves stimulated included the cutaneous nerves cutSPud, urSPud, SFP, CCF, CCS and LCS; the muscle nerves PBST, SMAB and GS; and the mixed nerve Tib. Seventeen fibres exhibited excitability increases from stimulation of at least one nerve. The two fibres which did not display an excitability change were tested with stimulation of 6 to

8 segmental sources. These two fibres were observed in two animals in which afferent stimulation increased the excitability of other afferent fibres. Neither fibre was subject to an excitability change during micturition.

Part B: Urethral Afferents

This is the first study in which the excitability and effects of electrical stimulation of urethral (urSPud) afferents in the sensory pudendal nerve were examined separately from perineal cutaneous afferents (*i.e.*, cutSPud afferents). The EUS ENG records illustrated in Figure 5 show that urSPud 2T stimulation evoked a large reflex whereas 2T stimulation of the cutSPud nerve did not. 5T cutSPud stimulation evoked a small reflex in the EUS ENG at a longer latency than that evoked by urSPud stimulation (16.1 ms vs 8.9 ms). In the other preparation tested, 2T urSPud stimulation evoked a large EUS reflex which diminished in amplitude with repeated 1 Hz stimulation; 2T cutSPud stimulation evoked a smaller reflex that did not decrement.

Examination of EPSPs recorded in a sample of 7 EUS motoneurones revealed that urSPud afferents produced shorter latency EPSPs (central latency, $\bar{x} = 3.9 \pm 0.38$ ms, range = 3.4-4.5 ms) in EUS motoneurones than did cutSPud afferents (central latency, $\bar{x} = 5.0 \pm 0.93$ ms, range = 3.5-6.2 ms). The amplitude of urSPud (4T) and cutSPud (5T) evoked EPSPs were compared in four EUS motoneurones; see Figure 6 for a representative example. In the four EUS motoneurones, urSPud stimulation produced larger EPSPs ($\bar{x} = 2.0 \pm 0.28$ mV) than did cutSPud stimulation ($\bar{x} = 1.42 \pm 1.34$ mV). In EAS motoneurones, 2T

stimulation of urSPud- and cutSPud-evoked EPSPs with similar mean latencies; 3.5 ● 0.62 ms, range = 3.0-4.5 ms, n = 8 and 3.4 ● 0.38 ms, range = 2.6-3.8 ms, n = 7 respectively. A comparison of cutSPud- and urSPud-evoked EPSPs within the same EAS motoneurones is not possible because such data was not collected, however, a comparison of 4-5T evoked EPSPs from different cells revealed that cutSPud afferent stimulation evokes larger EPSPs (2.17 ● 1.51 mV, n = 5) than urSPud afferent stimulation (0.83 ± 0.04 mV, n = 3). UrSPud-evoked EPSPs (1.6-5T) were larger in EUS (2.51 ● 1.19 mV, n = 3) than in EAS (1.36 ± 0.55 mV, n = 7) motoneurones. In contrast, cutSPud (3.5-5T)-evoked EPSPs were larger in EAS (2.01 ± 1.41 mV, n = 6) than in EUS (0.72 ± 0.20 mV, n = 4) motoneurones.

Stimulation of Segmental Sources Can Produce Excitability Increases in Urethral Afferents

The same cutaneous and group II muscle afferents previously shown to evoke excitability increases in sensory pudendal (cutSPud and urSPud recorded together) afferents (Angel *et al.*, 1994) produce excitability changes in urSPud afferents. The conduction velocities ($\bar{x}$ = 35.9 ± 13.4 m s^{-1}, range = 13-62 m s^{-1}) of urSPud afferents examined in the present study were not significantly different from the conduction velocities of cutSPud ($\bar{x}$ = 35.2 ± 10.4 m s^{-1}, range = 23-62 m s^{-1}) afferents and were similar to the conduction velocities of common pudendal afferents ($\bar{x}$ = 41 m s^{-1}, range = 18-64 m s^{-1}) reported by Angel *et al.* (1994).

An example of cutaneous and muscle afferent evoked excitability changes in an urSPud afferent is shown in Figure 7. Stimulation of muscle afferents at group I strength (Figure 7B) failed to produce excitability increases in urSPud afferents whereas stimulation

at group II strength (5T PBST) did. Excitability changes were observed in this fibre during micturition. Stimulation of the urSPud nerve at supra-threshold strengths produced excitability increases in perineal (cutSPud and SFP) and hindlimb cutaneous (CCF, CCS and LCS) afferents (data not shown).

Excitability Changes in Urethral Afferents During Micturition

The excitability of sixteen urethral units was examined during distension and/or PMC-evoked micturition. Five of the sixteen urSPud fibres showed no change in excitability during micturition; these units were observed in animals in which excitability changes were documented in other urethral or cutaneous afferents during micturition. All five units displayed an increase in excitability with stimulation of one or more cutaneous or muscle nerves (*i.e.*, cutSPud, SFP, CCF, CCS, LCS, Tib, PBST, SMAB or GS).

The most frequently observed (6 of 11 fibres) pattern of excitability change observed during micturition was biphasic in nature. First there was an increase in excitability (ranging from 5.4% to 13.6%) at the onset of the bladder contraction that lasted to the midpoint of the bladder contraction. During this period there was reduced EUS ENG activity. This was followed by a decrease in excitability (ranging from 5.0% to 23.3%) during the last half of the bladder contraction when EUS ENG activity returned. The excitability decrease was closely associated with the return of activity in the EUS ENG. When the biphasic excitability change occurring in the six fibres was averaged, the excitability increase was 9.1% and the excitability decrease was 11.8%. This characteristic biphasic increase-decrease in excitability is shown in Figure 8 and was observed in 6 units (3 cats) during distension- or PMC-evoked

voids.

Two of the 6 fibres exhibiting the biphasic increase-decrease in excitability were examined during both distension- and PMC evoked voids. While both fibres underwent the characteristic biphasic excitability change during PMC-evoked voiding, one of the fibres underwent a distinctly different pattern of excitability change during distension-evoked voiding. In this unit (not shown), a gradual decrease in excitability was observed during bladder filling which was removed (an excitability increase of 7.0% using the prevoid current level as baseline) at the onset of the bladder contraction during which time there was little EUS ENG activity. An additional small decrease in excitability occurred during the post-void period of enhanced activity in the EUS ENG but did not approach the level of fibre excitability observed immediately prior to the void. It appeared that in addition to the biphasic excitability change, this unit had an excitability decrease during bladder filling.

Five urSPud fibres did not exhibit a biphasic excitability increase-decrease during micturition. An excitability increase (7.1%) in one fibre, and an excitability decrease (6.1%) in another fibre were observed at the onset of the bladder contraction and did not persist for longer than the bladder contraction. Two other afferents were observed in the preparation where gradual excitability increases or decreases were observed during bladder filling. These fibres underwent a decrease in excitability during bladder filling that recovered at the onset of the bladder contraction; the recovery observed in the fibres was a 10.0% and 10.7% increase in excitability when the prevoid current level was used as baseline. The remaining fibre displayed an excitability decrease (46.6%) during the last half of the bladder contraction during the same period when excitability decreases were observed in the six fibres exhibiting

the biphasic increase-decrease in excitability.

In one animal, 6 units displayed a gradual increase (3 units) or decrease (3 units) in excitability during bladder filling. An example of an excitability increase in a urSPud afferent during bladder filling is shown in Figure 9. Though the pattern of excitability change shown in Figure 9 is not identical to the characteristic biphasic increase-decrease in excitability shown in Figure 8, the excitability increase-decrease of this fibre during micturition was classified as biphasic. This is because the excitability of the fibre increased during the period of EUS ENG suppression and the excitability decreased when activity returned in the EUS ENG.

In total, 8 urSPud afferents displayed a decrease in excitability during micturition and two additional fibres displayed a decrease in excitability during bladder filling. However, only in one of these urSPud fibres did the excitability change begin during the onset of the bladder contraction. In the other 7 units, excitability decreases were observed at the end of the bladder contraction when activity returned in the EUS ENG and presumably (a neuromuscular blocker, gallamine triethiodide, prevented contraction of the EUS muscle) when the EUS muscle would contract and terminate micturition. These 7 units included the 6 units displaying biphasic excitability changes during micturition and one unit which did not display a biphasic excitability change but did exhibit a decrease in excitability during the same period the biphasic units displayed an excitability decrease.

Some Cutaneous Afferents Receive the Same Pattern of Excitability Changes During Micturition as Urethral Afferents

During micturition, the pattern of excitability changes in urSPud afferents was distinctly different from that seen in the majority of perineal or hindlimb cutaneous afferents studied. Increases in excitability, but no excitability decreases, were observed in 19 of the 21 perineal and hindlimb cutaneous afferents studied. This contrasts with urSPud afferents where excitability decreases were observed in 8 of 11 fibres observed to undergo an excitability change during micturition. However, two of the 21 cutaneous afferents did display an excitability change similar to urSPud afferents. Both fibres displayed an increase in fibre excitability during the period of EUS ENG suppression and the value of this excitability change is included in Table 1. These exceptions were a SFP and a cutSPud afferent observed in two preparations; both were subject to a similar biphasic excitability change as a urethral afferent recorded in the same experiment. This is shown in Figure 10 for the SFP afferent recorded 475 μm away in the same track as the urSPud afferent. The amplitude of the excitability change observed in the urSPud and SFP fibre is normalized and the two stimulus current traces are superimposed, showing a similar pattern and time course of excitability change in both fibres (Figure 10C). In a second preparation, a cutSPud and a urSPud afferent, shown in Figure 11, underwent an identical pattern of excitability change during micturition. These two units were recorded in the same track, a distance of 48 μm apart. The other 19 cutaneous afferents did not display excitability changes similar to those observed in urSPud afferents.

Evidence Suggesting a Modulatory Action of the Excitatory Changes Observed in Sacral Afferents During Micturition

Evidence from intracellular recording from two EUS motoneurones shows that urSPud-evoked EPSPs can be suppressed during micturition. In the two EUS motoneurones, EPSPs were suppressed by 84% and 88% during the onset of the bladder contraction; some evoked EPSPs were completely suppressed during the first half of the bladder contraction. The EPSPs increased in amplitude from the midpoint of the bladder contraction prior to the return of activity in the EUS ENG. An example of such a pattern of changes in EPSP sizes is shown in Figure 12.

Male and Female Animals and Differences in the Incidence of Excitability Changes Observed During Micturition

There was a significantly higher incidence (19 of 20) of excitability changes noted during micturition in female cats, while in male animals, only half the fibres observed (13 of 26) displayed a change in excitability during micturition (see Table 2). The conduction velocities and intraspinal recording depths of the fibres sampled in male and female cats were compared to determine whether sampling biases could be responsible for the higher incidence of excitability changes during micturition in female cats. The conduction velocities and intraspinal recording depths of the fibres sampled in male and female cats were not significantly different, nor was there a significant relationship between conduction velocity or recording depth and the occurrence of excitability changes during micturition in male and/or female animals. The variation in excitability of fibres observed during

micturition was not related to experiment chronology, differences in nerves dissected, or the weight of the animal. However, there was a significant relation between the peak void bladder pressure and the occurrence of excitability changes during micturition. Fibres recorded in the sacral spinal cord that exhibited excitability changes during micturition occurred during voids where peak bladder pressures were lower ($\bar{x} = 35.3 \pm 9.5$ mmHg, range = 22-64 mmHg, n = 32) in comparison to voids where excitability changes were not observed and peak void bladder pressures were higher ($\bar{x} = 40.9 \pm 5.4$ mmHg, range = 31-49 mmHg, n = 14). There was also a significant difference between peak void bladder pressures in male and female cats. The maximum bladder pressure reached during voiding was between 22 and 40 mmHg ($\bar{x} = 29.1 \pm 4.3$ mmHg, n = 20) in females and from 31 to 64 mmHg ($\bar{x} = 42.5 \pm 7.3$ mmHg, n = 26) in males. The findings do not imply that excitability changes only occur during voids with low peak bladder pressures, as excitability changes were observed during voids characterized by a wide range of bladder pressures in both sexes.

Discussion

The findings presented in this thesis clearly demonstrate that both cutaneous (perineal and hindlimb) and urethral afferents undergo excitability changes during micturition. The distinct onset and termination of the excitability changes, and especially the biphasic excitability changes observed in urethral afferents, reduce the possibility that the changes observed were due to extracellular potassium accumulation which would depolarize the afferent membranes in a nonspecific manner. Although not demonstrated, PAD of cutaneous and urethral afferents is believed to inhibit afferent transmission to first order spinal interneurones and reduce activation of excitatory pathways to EUS motoneurones. Suppression of these excitatory reflex pathways would contribute to EUS relaxation and facilitate efficient bladder emptying during voiding.

PAD of Cutaneous Afferents During Micturition

This study examined excitability changes in cutaneous afferents during micturition to test the hypothesis that the central micturition circuitry activates PAD interneurones which in turn modulate cutaneous afferent transmission during voiding. Previous findings have shown that pudendal- and perineal-evoked EPSPs in both EUS and sacral hindlimb motoneurones were attenuated during micturition (Fedirchuk *et al.*, 1994) and that sensory pudendal afferents received PAD during micturition (Angel *et al.*, 1994). At the time, it was suggested PAD was one mechanism responsible for the suppression of hindlimb cutaneous reflexes.

The present study provides direct evidence that the central micturition circuitry evokes PAD in all sources of sacral cutaneous afferents (Figure 2 & 3). However, not all sources of cutaneous afferents examined in this study (Table 2) evoke excitatory reflexes to EUS motoneurones. Why the micturition circuitry would need to inhibit transmission from afferents whose activation does not excite sphincter motoneurones is not immediately obvious. It is difficult to imagine the evolution of a specialized population of micturition PAD interneurones that would establish connections with all cutaneous afferents entering the sacral spinal cord, in order to inhibit the sub-population that mediate excitation of EUS motoneurones. A more tenable possibility is that the micturition circuitry is accessing a pre-existing population of PAD interneurones which is organized for modulating cutaneous transmission in the sacral spinal cord.

Recently, a population of sacral PAD interneurones has been described (Jankowska & Riddell, 1995; Riddell, Jankowska & Huber, 1995) which may evoke PAD in sacral group II muscle afferents (Figure 13) and sacral cutaneous afferents (Buss & Shefchyk, 1997). Since the first order interneurones described by Jankowska & Riddell (1994) may be the first relay in reflex pathways to EUS and EAS motoneurones (Fedirchuk *et al.*, 1992b), activation of the sacral PAD interneurones described by Jankowska & Riddell (1995) and Riddell *et al.* (1995) could be used to control the activation of excitatory reflex pathways to EUS motoneurones. It is suggested that this population of sacral PAD interneurones is activated by the central micturition circuitry (Figure 14) and is the reason PAD was observed in all classes of cutaneous afferents that terminated in the sacral spinal cord. The sacral PAD interneurones distinguished by Jankowska & Riddell (1995) and Riddell *et al.* (1995) most

likely have actions on the units investigated in this study because cutaneous afferents receiving PAD during micturition also received PAD upon stimulation of segmental cutaneous or muscle nerves described by Riddell *et al.* (1995). In addition, the variability of the timing of PAD in the different cutaneous afferents is consistent with an indirect activation of PAD interneurones during micturition. The absence of PAD in lumbar cutaneous afferents during micturition is consistent with a restricted action of the central micturition circuitry through the sacral PAD interneurones described by Jankowska & Riddell (1995) and Riddell *et al.* (1995). Why a small PAH was observed in one lumbar LCS unit is not readily apparent. The central micturition circuitry may directly inhibit PAD interneurones in the lumbar spinal cord, or alternatively, the PAH observed could have been indirectly mediated by the central micturition circuitry through suppressed sacral excitability (due to micturition mediated PAD) and associated diminished excitatory drive to the lumbar spinal cord.

The sacral PAD interneurones speculated to be activated by the central micturition circuitry may also be accessed by the locomotor CPG as Perrault *et al.* (1994) have shown that sacral cutaneous and group II muscle afferent field potentials are reduced in amplitude during fictive locomotion. The field potential reduction was attributed to primary afferent depolarization and an associated reduction of synaptic transmission to first order interneurones. Both cutaneous and group II muscle afferent field potentials were reduced the greatest during the extensor phase of the step cycle which may reflect the presence of shared PAD interneurones acting on both cutaneous and group II muscle afferents. These findings, though consistent with the locomotor circuitry utilizing the sacral PAD interneurones to

inhibit cutaneous and group II muscle afferent transmission during locomotion, are not the only mechanism by which cutaneous and group II muscle afferent transmission may be suppressed.

If the central micturition circuitry is accessing the same population of sacral PAD interneurones as the locomotor CPG, group II afferents should also receive PAD during micturition. Group II muscle afferent excitability changes during micturition has not yet been examined but group II muscle afferent field potentials could easily be examined to provide further evidence for the utilization of the sacral PAD interneurones described by Jankowska & Riddell, 1995 and Riddell *et al.*, 1995, during micturition.

PMC and urSPud Stimulation as Tools to Study the Central Micturition Circuitry

Previous findings revealed that stimulation of the PMC can produce micturition similar to that evoked by bladder distension (Shefchyk, 1989; Fedirchuk & Shefchyk, 1991). PMC stimulation was shown to evoke a bladder contraction and hyperpolarize EUS motoneurones *via* a chloride conductance (Fedirchuk & Shefchyk, 1993). The present study found that like distension-evoked voiding, PMC-evoked micturition produced PAD in cutaneous and urethral afferents. This is further evidence that descending PMC pathways and bladder distension activates similar central micturition circuitries. The more rapid onset of PAD, and the presumably more synchronous activation of PAD interneurones, observed during PMC-evoked voiding may facilitate the finding of micturition related PAD interneurones in future studies.

Recent findings of Shefchyk & Buss (1997) and Buss & Shefchyk (1997b) have

shown that electrical stimulation of urethral afferents can also evoke micturition, thus providing another tool for studying the central micturition circuitry. UrSPud stimulation has already been used to provide evidence that the central micturition circuitry is located in the spinal cord (Buss & Shefchyk, 1997b) as originally hypothesized by Denny-Brown & Robertson (1933). Present knowledge is also consistent with the proposed activation (for review; DeGroat, Booth & Yoshimura, 1993) of the central micturition circuitry through a spino-bulbo-spinal loop. The ascending arm of the loop conveys information from stretch sensitive bladder afferents to a control centre in the pons. The descending pathway of the spino-bulbo-spinal loop accesses parasympathetic bladder preganglionic neurones producing a bladder contraction and activating inhibitory pathways to the EUS. The original evidence for this loop organization comes from the fact that spinal transection prevented coordinated micturition (for review; DeGroat, 1975). Buss & Shefchyk (1997b), however, demonstrated that urSPud stimulation in acute spinal cats suppresses EUS ENG activity and evokes a small bladder contraction. Furthermore, spinal intact animals which did not micturate when the bladder was distended responded to urethral nerve stimulation with coordinated micturition including a bladder contraction sufficient to expel a stream of urine (Buss & Shefchyk, 1997b; Shefchyk & Buss, 1997). It was hypothesized that urethral afferents access the same spinal micturition circuitry to coordinate bladder and sphincter function as bladder afferents. Activation of the central micturition circuitry by urethral stimulation may permit the study of the spinal micturition circuitry in the absence of descending influences. It is thus an appropriate model system for the development of treatments for conditions in which descending pathways are damaged due to lesions or disease.

Urethral-Evoked Excitation of EUS Motoneurones

The central latencies of urSPud-evoked EPSPs in EUS motoneurones were investigated to provide insight on the interneuronal pathways from urSPud afferents to EUS motoneurones. The central latencies of urSPud-evoked PSPs in EUS motoneurones ($\bar{x}$ = 3.9, range = 3.4-4.5 ms) were similar to the latencies reported for common sensory pudendal afferent effects ($\bar{x}$ = 3.8, range = 2.0-5.0 ms; see Fedirchuk *et al.*, 1992). However, the central latencies of urSPud evoked EPSPs in EAS motoneurones were longer in this study ($\bar{x}$ = 3.5, range = 3.0-4.5 ms) than those observed by Fedirchuk *et al.* (1992) for common pudendal afferents ($\bar{x}$ = 2.6, range = 1.5-4.5 ms). It is likely that the shortest pudendal to EUS motoneurone latencies reported in Fedirchuk *et al.* (1992) were evoked by urSPud afferents contained in the common sensory pudendal nerve, the longer latency actions were likely due to a mixed action of urSPud and cutSPud afferents. The latencies of urSPud evoked EPSPs reported in this study, all longer than 3.0 ms, suggest that are mediated by polysynaptic pathways.

Sources of PAD to Urethral Afferents

The cutaneous and muscle afferent sources evoking PAD in urSPud afferents were not extensively examined in this study because Angel *et al.* (1994) examined sources of PAD to common sensory pudendal afferents (urSPud together with cutSPud). UrSPud afferents examined in this study received PAD (though not every urSPud afferent received PAD from every nerve) upon stimulation of the same cutaneous sources reported by Angel *et al.* (1994) and the predominance of group II but not group I muscle afferents in evoking PAD was also

observed. Although the occurrence and/or strength of PAD (not examined) evoked in urSPud afferents may be different from common sensory pudendal afferents, this study reveals that urSPud afferents receive PAD from segmental cutaneous and group II muscle afferents, likely through the same sacral PAD interneurone pathways which act on other sacral cutaneous afferents.

PAD and PAH of Urethral Afferents During Micturition

In an earlier work, Angel *et al.* (1994) hypothesized that urSPud afferents would receive PAH during micturition because flow through the urethra augments bladder reflexes. In the present study, urethral afferents were shown to activate strong excitatory reflexes to EUS motoneurones (Figure 5 & 6) so it appeared that Angel's hypothesis would not be supported since PAD, not PAH, would be expected to occur during micturition in afferents which evoked reflex excitation of the EUS. In the present study, urethral afferents were found to receive PAD (19%), PAH (12%), a biphasic PAD-PAH (38%; Figure 8 & 9), or no change in excitability(31%) during micturition. The present finding that stimulation of urethral afferents evoked strong excitation of EUS motoneurones leads to the possibility that urethral afferent branches which activate excitatory reflex pathways to EUS motoneurones are the ones that receive PAD during micturition. The PAD of these medial terminating afferents during the period of sphincter suppression would contribute to depression of transmission from urethral afferents activated by flow through the urethra during micturition. Inhibition of this reflex pathway (Figure 15 and 16) would be essential for sphincter relaxation and coordinated micturition. Urethral afferents received a PAD linked closely to

the period of EUS suppression and a PAH at the termination of micturition corresponding to when activity returned in the EUS ENG. The PAH observed near the termination of the void could contribute to sphincter closure and the increased ENG activity accompanying the end of the void.

The observation of PAH in urethral afferents implies that some PAD interneurones synapsing onto urethral afferents are tonically active in the decerebrate cat. Tonic activity of PAD mechanisms is interesting because it allows the gain of reflex pathways to increase or decrease at the first synapse in the central nervous system, before there is convergence of input onto spinal interneurones. Thus, this study has provided evidence for the use of PAD mechanisms to tonically control synaptic transmission, to exert gradual changes in the potency of synaptic transmission (observations of PAD and PAH during bladder filling; Figure 9) and for phasic modulation of reflex pathways, as demonstrated during the expulsion phase of micturition.

The biphasic pattern of PAD-PAH observed in 6 of 16 urethral afferents during micturition differed from the PAD of cutaneous afferents that was not closely correlated with the period of EUS suppression. The distinct pattern of PAD-PAH observed in urethral afferents suggests that the central micturition circuitry activates a sub-population of PAD interneurones that specifically target urethral afferents (Figure 15) and are not utilized by other behaviours such as locomotion as previously discussed in relation to hindlimb cutaneous afferents. However, a SFP and cutSPud afferent were found to undergo a time-course of PAD-PAH identical to two urSPud afferents examined in the same experiment during micturition. The SFP and cutSPud fibres were recorded in the same electrode tract in

very close proximity to the urSPud afferent with the identical time course of PAD-PAH. CutSPud and urSPud afferents likely received PAD from the same PAD interneurones as the urSPud afferents recorded in the same tracks. The close proximity of the cutSPud and SFP afferents to urSPud afferents is consistent with the possibility that all were converging on the same first order interneurones in an excitatory reflex pathway to EUS motoneurones. Although there is no direct evidence for such convergence, it is possible because first order sacral interneurones receive convergence from many cutaneous sources (Jankowska & Riddell, 1994). Thus, it is hypothesized that urSPud afferents, as well as a smaller number of cutaneous afferents, evoke strong excitatory reflexes to EUS motoneurones through a common interneuronal reflex pathway. During micturition, the central micturition circuitry specifically targets these urSPud and cutSPud afferents (see Figure 16, right side). The other cutaneous afferents (and possibly group II muscle afferents) entering the sacral spinal cord also receive PAD during micturition, but this PAD is indirectly mediated (see Figure 16, left side) by a population of sacral segmental PAD interneurones (Jankowska & Riddell, 1993; 1994) which may be utilized by other behaviours, including locomotion.

Only one of 16 urSPud fibres received PAH during a bladder contraction, a period when flow through the urethra could contribute to secondary bladder reflexes. A potential explanation for this lack of PAH during a bladder contraction is that the recording sites in this study may not have sampled the more caudal and lateral terminating branches of urethral afferents that end near bladder preganglionics or the interneurones that facilitate bladder reflexes (De Groat, Vizzard, Araki & Roppolo, 1996). It is now hypothesized that urSPud fibres terminating in this region will display PAH during the onset of the bladder contraction.

Feedback from urSPud fibres traveling in the lateral region of the sacral spinal cord and terminating near bladder preganglionic interneurones will receive PAH, and these urSPud afferent branches are the ones mediating the urethra to bladder reflex described by Barrington (1914).

Considering their prominent role in micturition, it was surprising that so few urSPud fibres underwent excitability changes during micturition. A possible reason is that these fibres, or fibre branches, did not activate interneurones which were part of excitatory reflex pathway to EUS motoneurones. These fibres could have activated reflex pathways facilitating the bladder contraction, ascending pathways relaying the sensation of urine flow to supraspinal targets, or other pathways, supraspinal or spinal, not part of the excitatory pathway to the EUS.

Urethral afferents examined in this study could not be activated by urine flow as the ipsilateral urethral nerve was transected in order to record unit activity evoked by intraspinal stimulation. Thus, excitability changes observed were mediated by the central micturition circuitry and/or contralateral segmental systems (for example, contralateral urSPud afferents or pelvic afferent fibres) during micturition. Since Angel *et al.* (1994) have previously shown that excitability changes occur in the absence of flow through the urethra (proximal urethra ligated), the excitability changes observed in this study are likely attributed to the action of the central micturition circuitry. Additionally, the PAD of perineal, hindlimb cutaneous and urethral afferents could itself decrease transmission from these afferents to first order interneurones, some of which activate sacral PAD interneurones.

Preliminary evidence has revealed that the time course and pattern of urSPud EPSP

modulation during micturition (Figure 12) can closely follow the time course and pattern of excitability changes observed in urethral afferents during micturition. Two explanations for this finding exist. First, the PAD and PAH of the urSPud afferents may be the cause of the attenuation of the urSPud EPSP during the period of sphincter suppression and the EPSP potentiation when activity returns in the EUS ENG at the end of the bladder contraction. An alternative explanation is that the central micturition circuitry activated, in parallel, last order PAD interneurones and interneurones that inhibit excitatory interneurones or EUS motoneurones directly; such an organization is presented in Figure 15. Recent recordings of urethral evoked EPSPs in EAS motoneurones (Buss and Shefchyk, unpublished observations) have revealed that urethral evoked EPSPs observed in EUS motoneurones do not undergo the biphasic attenuation and potentiation during micturition. This finding provides additional evidence for a private micturition specific reflex pathway from urethral afferents to EUS motoneurones.

PAD interneurones may also synapse directly onto EUS motoneurones and inhibit them postsynapticaly. Although this study did not provide evidence for such an action, pre- and postsynaptic actions by PAD interneurones has been reported by Rudomin, Jimenez, Quevedo & Solodikin (1990).

Occurrence of PAD in Male and Female Cats

In this study, excitability changes were observed in 95% of the fibres examined in female cats but only 50% of the fibres examined in female cats during micturition. Excitability changes observed occurred during voids for which peak bladder pressures were

lower ($\bar{x}$ = 35.3 mmHg) compared to peak bladder pressures ($\bar{x}$ = 40.9 mmHg) reached during voids for which no excitability changes were observed. In addition, male cats voided with higher peak bladder pressures ($\bar{x}$ = 42.5 mmHg) than female ($\bar{x}$ = 29.1 mmHg) cats revealing that gender differences or peak void bladder pressures were equally likely to be the cause of the different occurrences of excitability changes observed in male and female cats. The reason why excitability changes were less frequently observed during high bladder pressure voids is unknown, however, it could be because bladder afferents also activate PAD pathways to cutaneous and urSPud afferents and at higher bladder pressures, when stretch receptive bladder afferents are maximally activated, their contribution to fibre excitability may mask the excitability changes evoked by the central micturition circuitry.

Future Directions

The existence of a central micturition circuitry located in the spinal cord, originally suggested by Denny-Brown & Robertson (1933) is reproposed. A spinal central micturition circuitry is hypothesized because of the finding that repetitive urSPud stimulation suppresses EUS ENG activity which is coordinated with a contraction of the bladder (Buss & Shefchyk, 1997b; Shefchyk & Buss, 1997). Part of the central micturition circuitry is proposed to exist in the IML region of the sacral spinal cord. The IML region contains many of the neurones involved in micturition including interneurones presynaptic to bladder preganglionic neurones, bladder preganglionic neurones, the termination site of stretch receptive bladder afferents, and perhaps urethral afferents and descending PMC pathways. Anatomical tracing techniques and urSPud-evoked fields could be used to determine whether urSPud afferents

preferentially terminated in the IML region.

It is expected that urSPud- and PMC-evoked field potentials can be found in the IML region. At present, urSPud or PMC fields have not been described but this is likely because previous studies have sampled the more rostral and medial regions of the dorsal horn (Fedirchuk *et al.*, 1992a), not including the IML region. Future studies need to record field potentials in the IML region to determine if PMC, urSPud and bladder afferent field potentials occur in the same area. If overlapping fields are observed, interneurones in this area will need to be investigated for convergent input from urethral and bladder afferents and descending PMC pathways. The finding of convergent input onto sacral IML interneurones would provide substantial evidence that these cells are an important component of the central micturition circuitry. If these hypotheses are substantiated, knowledge of the pharmacology and receptor types on these cells could be used to develop targeted pharmaceutical treatments for lower urinary tract dysfunction.

Although the evidence presented in this thesis suggests that the sacral PAD interneurones described by Jankowska & Riddell (1995) and Riddell *et al.* (1995) are used by the central micturition circuitry to inhibit cutaneous transmission during micturition, one further test of this hypothesis should be completed. As presented in the discussion, group II muscle afferents should also receive PAD during micturition if the same sacral segmental PAD interneurones are involved. A simple test for this would be to record group II muscle afferent (for example, PBST or GS) field potentials in S1 during micturition. Suppression of short latency group II field potentials would suggest a reduction of presynaptic transmission, most likely due to PAD.

Concluding Remarks

The central micturition circuitry located in the lumbo-sacral spinal cord accesses two populations of PAD interneurones, one that is used by multiple behaviours and likely overlaps to a considerable extent with the sacral segmental PAD circuitry and another more restricted to micturition (Figure 16). Interneurones in the central micturition circuitry likely access several downstream interneurones in parallel, including last order PAD interneurones, glycinergic or GABAergic inhibitory interneurones synapsing onto EUS motoneurones, excitatory interneurones in reflex pathways to EUS motoneurones, and bladder preganglionic neurones (Figure 15). A single population of interneurones may be activated during micturition and drive the sacral circuitries utilized during micturition.

Figure 1: Conduction velocities and recording depths of urethral and cutaneous afferents studied in the sacral spinal cord. Each symbol represents one of the urSPud, cutSPud, SFP, CCF, CCS or LCS fibres examined during micturition. Filled circles represent fibres that underwent an excitability change during micturition and, unfilled circles represent those that did not. Three additional fibres were not plotted as their conduction and/or recording depths were not recorded. One fibre had a conduction velocity of 44 m s^{-1} and two were recorded at depths of 516 and 1304 μm.

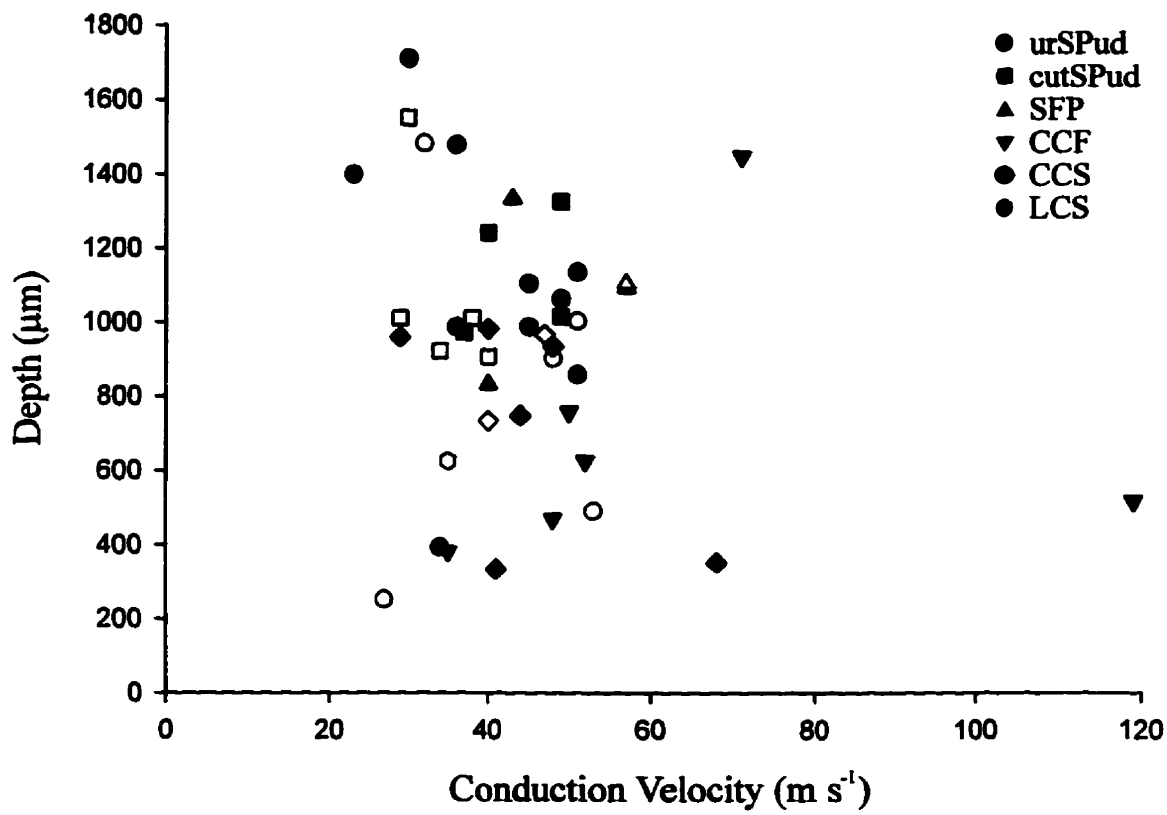
1800
1600
1400
1200
1000
800
600
400
200
0
Depth (µm)
0
20
40
60
80
100
120
Conduction Velocity (m s-1)
urSPud
cutSPud
SFP
CCF
CCS
LCS

Figure 1

Figure 2: Excitability changes in representative cutSPud and SFP afferent fibres recorded in the sacral cord during distension- and PMC-evoked micturition. Excitability increases are shown for three fibres observed during voiding. The depth and conduction velocity of the fibre is given above the traces. The upper trace is the intraspinal current value, the middle trace is the bladder pressure (IVP), and the lower trace is the EUS ENG. During micturition, the excitability of the fibre shown in A increased 15.8%; see results for a description of measurements, lines i and ii, and arrows iii to v. The excitability of the fibre shown in B increased an average of 26.4% for the three voids shown. In C, the excitability of the fibre increased 9.5% during micturition. The averaged fibre excitability increases during the two voids shown in D was 17.6%. C and D are records taken from the same fibre and show the similarity of excitability changes during distension- and PMC-evoked voids. The arrows directed at the current trace in C and D point to excitability decreases which were sporadically observed in the fibre throughout the recording period and are not related to micturition. Grey horizontal lines show periods of PMC stimulation in B and D.

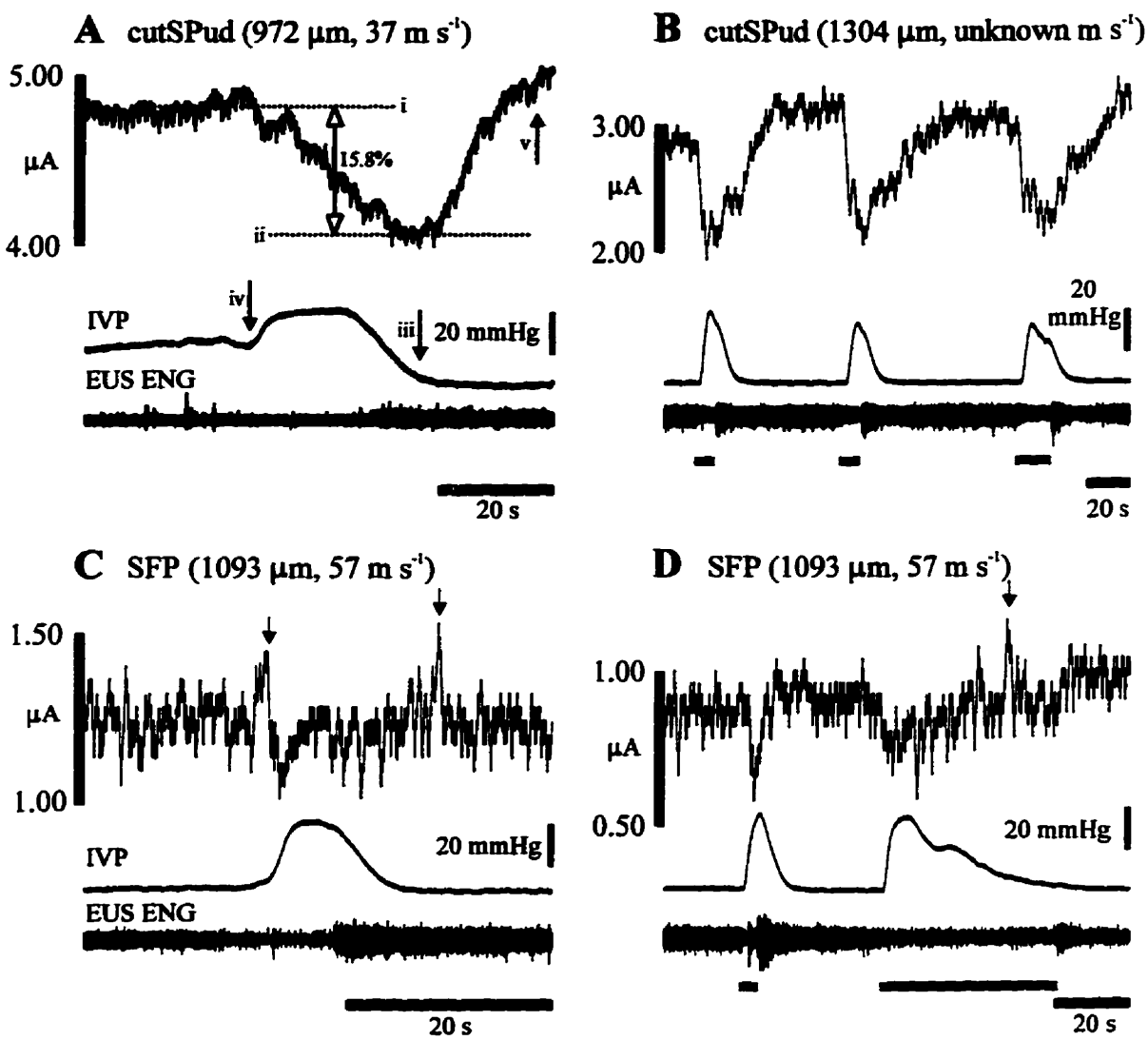
A cutSPud (972 µm, 37 m s-1)
5.00
µA
4.00
i
ii
15.8%
v
IVP
iv
iii
20 mmHg
EUS ENG
20 s
B cutSPud (1304 µm, unknown m s-1)
3.00
µA
2.00
20
mmHg
20 s
C SFP (1093 µm, 57 m s-1)
1.50
µA
1.00
IVP
20 mmHg
EUS ENG
20 s
D SFP (1093 µm, 57 m s-1)
1.00
µA
0.50
20 mmHg
20 s

Figure 2

Figure 3: Excitability changes in representative CCF and CCS afferent fibres recorded in the sacral cord during distension- and PMC-evoked micturition. Organization is the same as in Figure 2. Excitability changes for the fibres shown in A, B, C, and D increased 10.3%, 7.0%, 7.3% and 7.4%, respectively, during micturition. Grey horizontal lines show periods of PMC stimulation in B and D.

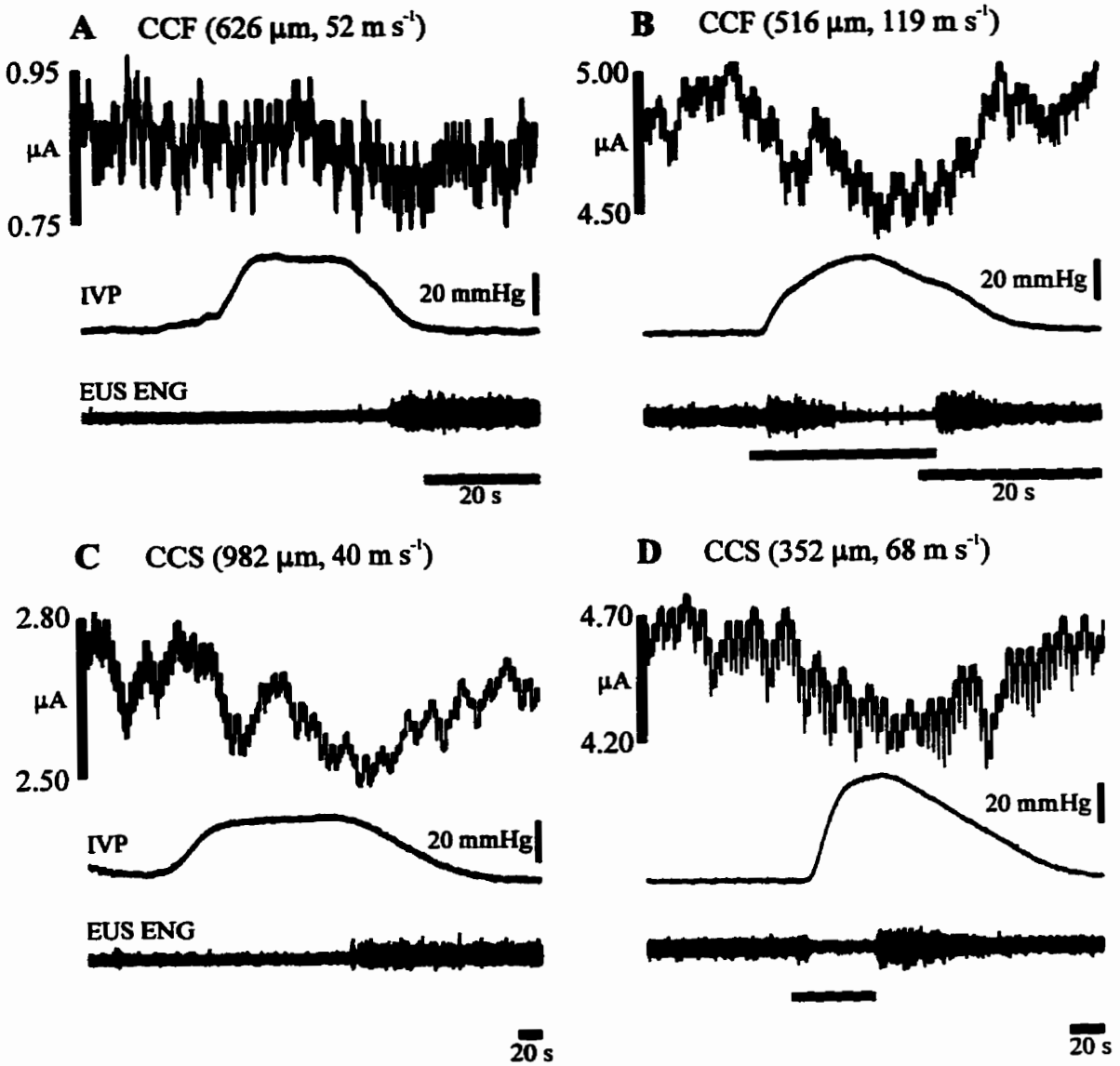
A CCF (626 µm, 52 m s-1)
0.95
µA
0.75
IVP
20 mmHg
EUS ENG
20 s
B CCF (516 µm, 119 m s-1)
5.00
µA
4.50
20 mmHg
20 s
C CCS (982 µm, 40 m s-1)
2.80
µA
2.50
IVP
20 mmHg
EUS ENG
20 s
D CCS (352 µm, 68 m s-1)
4.70
µA
4.20
20 mmHg
20 s

Figure 3

Figure 4: Excitability decrease in a lumbar LCS afferent observed during a distension-evoked void. The excitability of this lumbar LCS fibre decreased 5.4% during micturition.

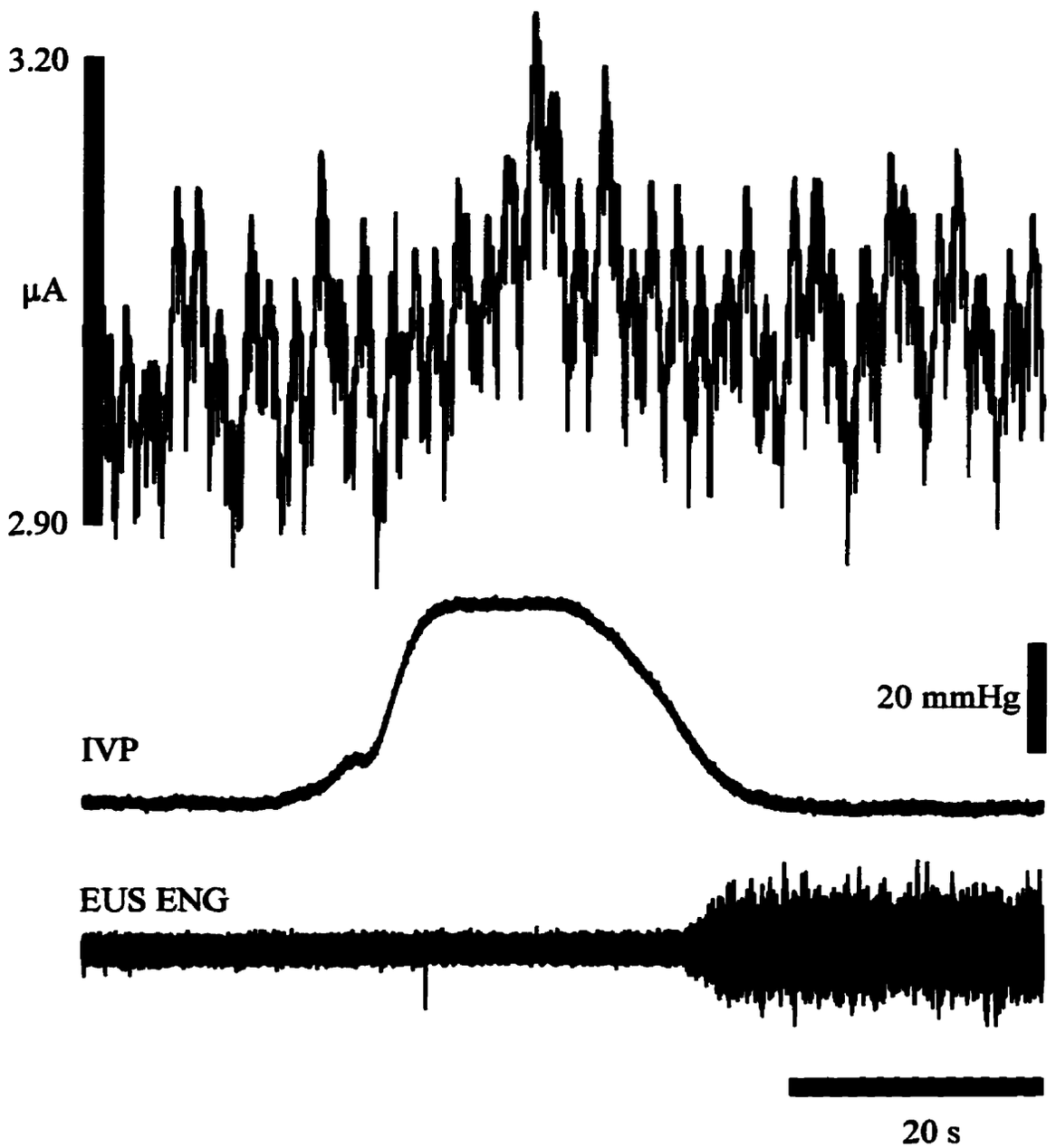

Figure 4

Figure 5: CutSPud- and urSPud-evoked activity in the EUS nerve: A: electrical stimulation to urSPud at 2T. B: electrical stimulation to cutSPud at 2T. C: electrical stimulation to cutSPud at 5T. Grey vertical bars represent the application of the single 0.5 ms square wave stimulation pulses delivered at 1Hz. All traces are raw records and each set of 3 records was taken in succession.

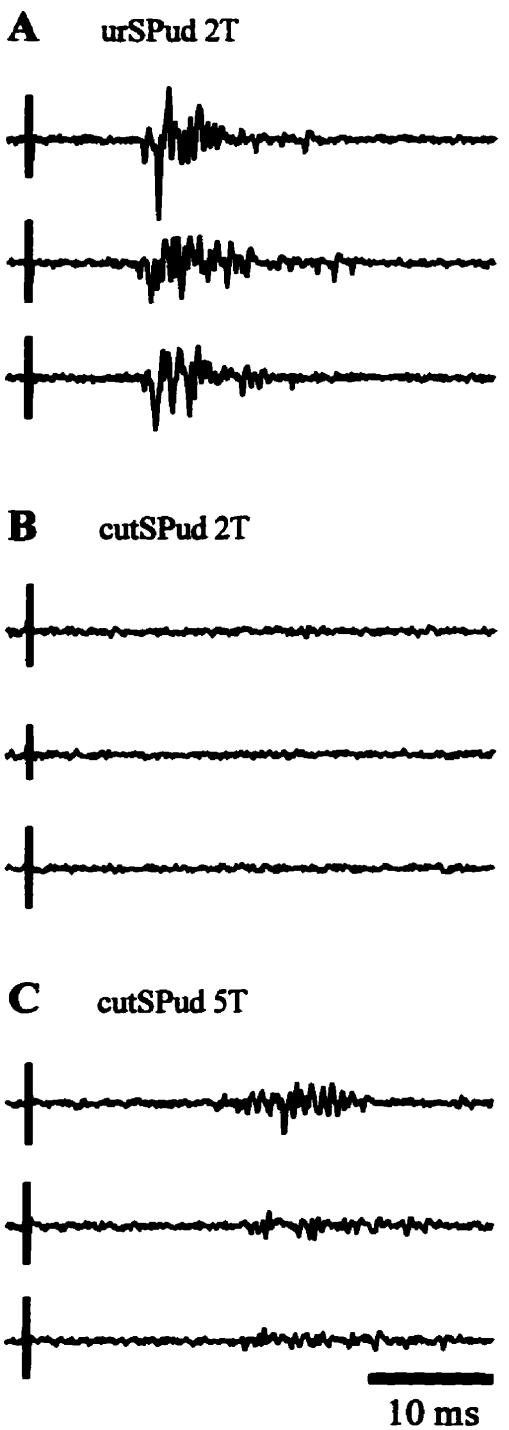

Figure 5

Figure 6: An EUS motoneurone receives larger excitation from urSPud than cutSPud afferent stimulation: Upper traces are the superimposed intracellular records from an EUS motoneurone (spike height 80 mV, V_M = -63 mV), lower traces are the S1 cord dorsum record. Averages of 30 sweeps were made during stimulation of urSPud (solid line) and cutSPud (dashed line). Calibration pulse at start of intracellular records is 2 mV and 2 ms. Grey vertical bars represent the application of the 0.5 ms square wave stimulator pulse at a rate of 3.3 Hz.

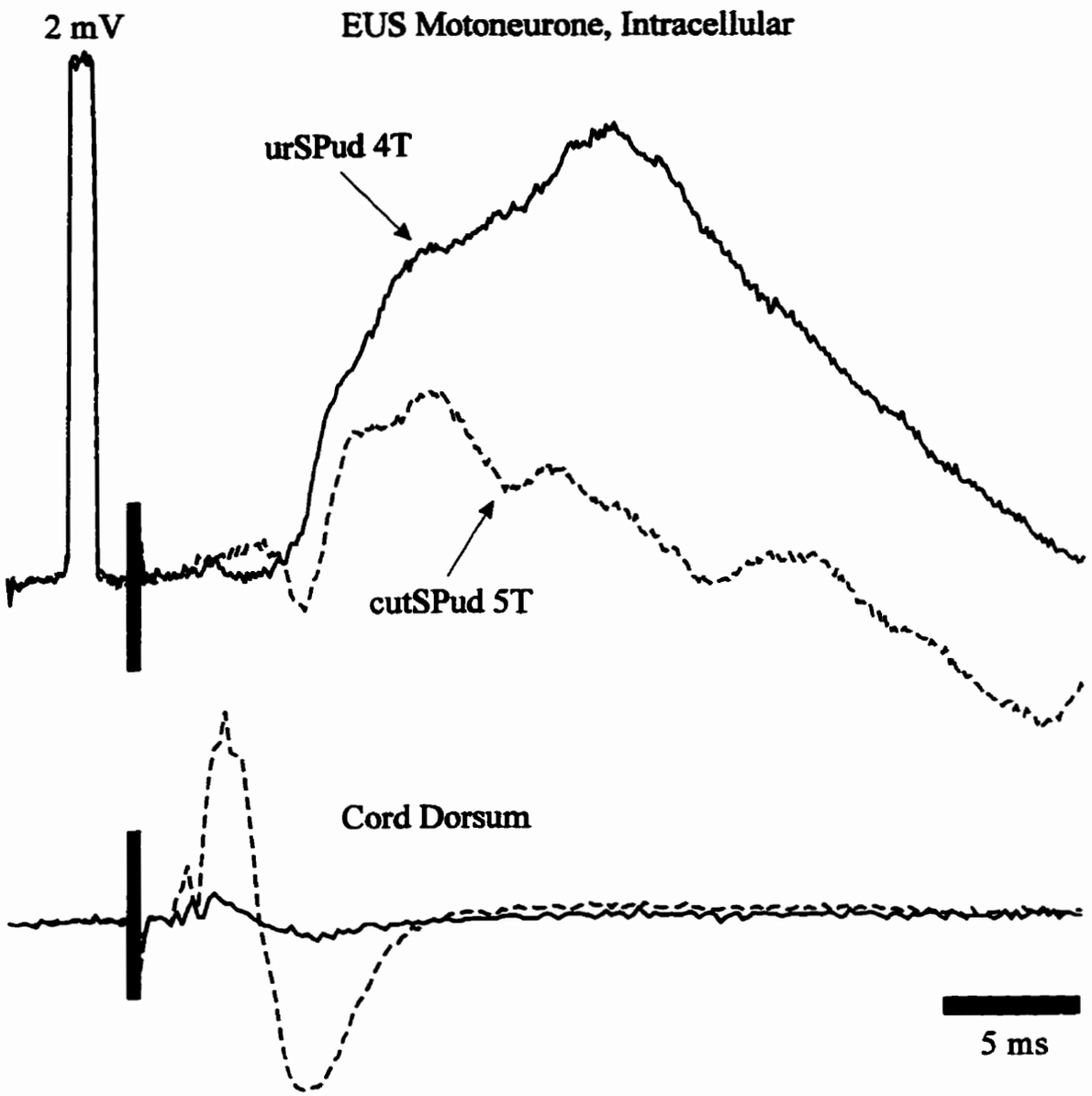
2 mV
EUS Motoneurone, Intracellular
urSPud 4T
cutSPud 5T
Cord Dorsum
5 ms

Figure 6

Figure 7: Excitability increase in an urSPud afferent fibre evoked by cutaneous and muscle nerve stimulation. In A, the urSPud afferent (conduction velocity of 45 m s^{-1}, depth 988 μm) displayed an increase in excitability during 2T stimulation of cutaneous nerves whereas 5T but not 2T muscle afferent stimulation (B) was effective. Percent changes in fibre excitability are shown, for each period of segmental nerve stimulation, beneath the current traces. Grey horizontal bars show periods of segmental nerve conditioning stimuli.

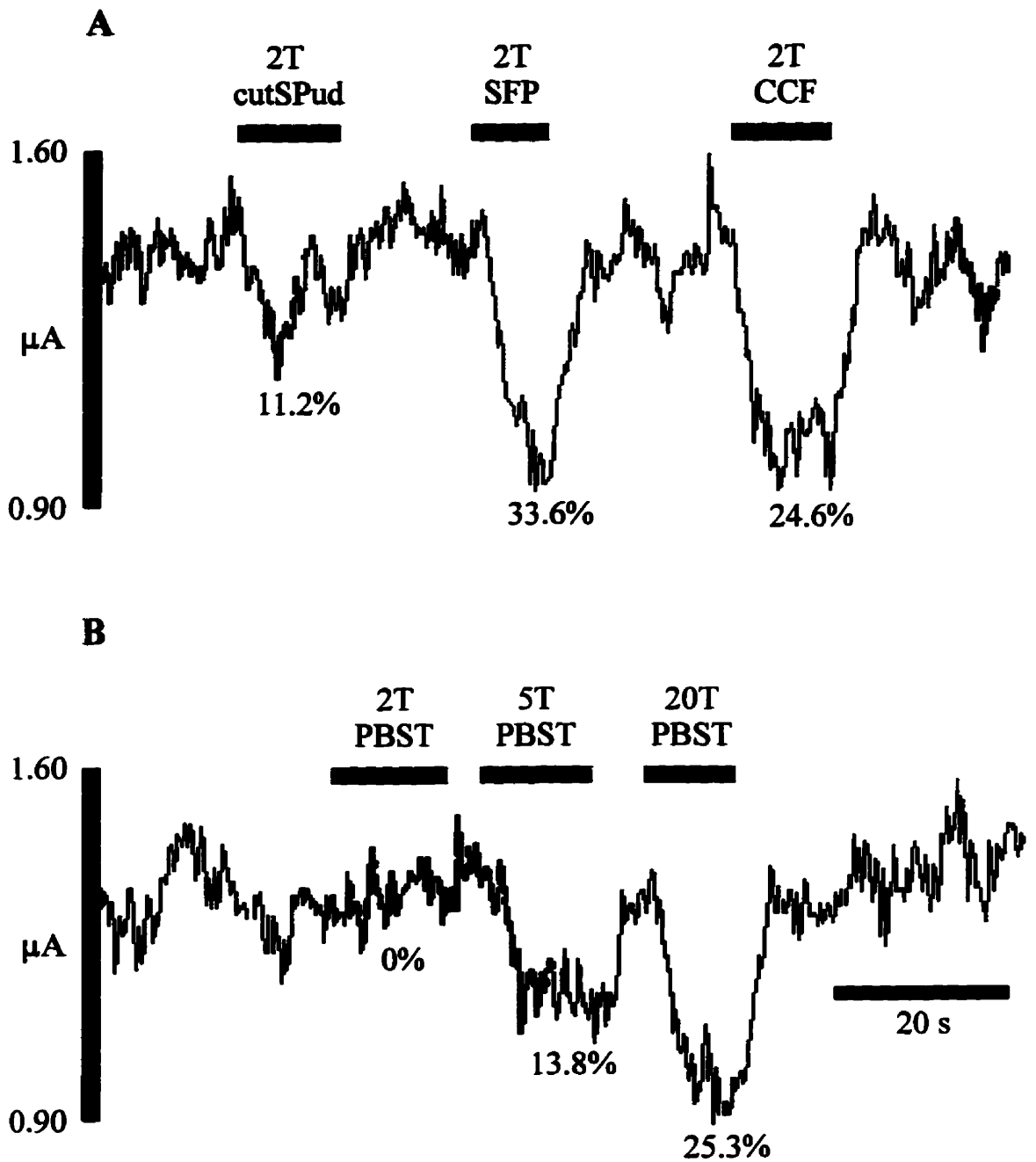
A
2T
cutSPud
2T
SFP
2T
CCF
1.60
µA
0.90
11.2%
33.6%
24.6%
B
2T
PBST
5T
PBST
20T
PBST
1.60
µA
0.90
0%
13.8%
25.3%
20 s

Figure 7

Figure 8: Characteristic biphasic increase-decrease in excitability observed in an urSPud afferent during PMC-evoked micturition. The excitability increased 8.3% from the prevoid current levels and then decreased 11.6% from prevoid current levels when activity returned in the EUS ENG. The conduction velocity of this urSPud fibre was 36 m s^{-1} (recording depth = 988 μm). The grey horizontal bar shows the period of PMC stimulation.

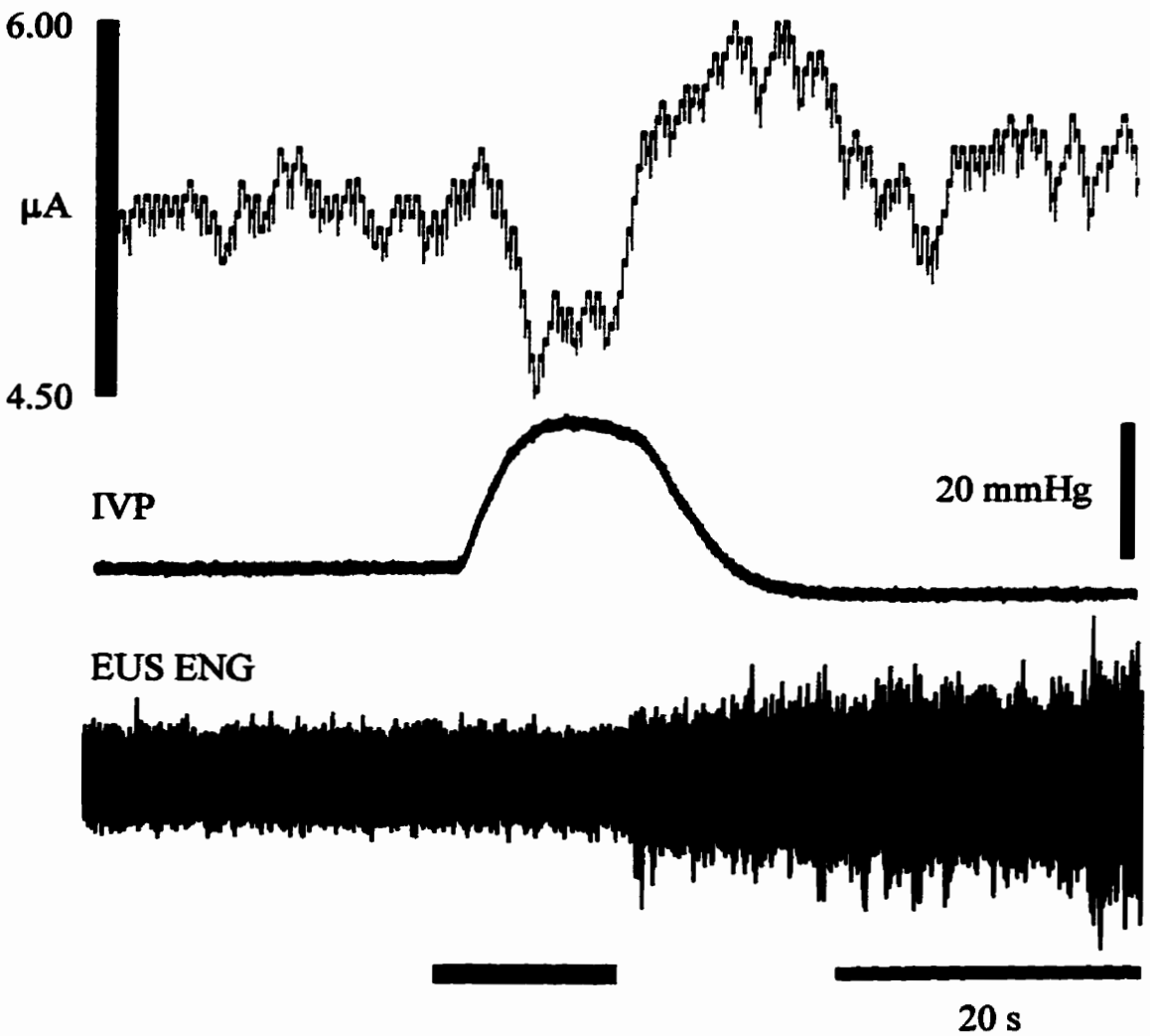

Figure 8

Figure 9: Biphasic excitability change in an urSPud fibre during bladder filling and distension-evoked voiding: Activity in the EUS ENG gradually decreased during bladder filling and a parallel increase in excitability of the urSPud fibre is observed. In this fibre it was difficult to determine what the prevoid current level was so the excitability increase (9.3%) was measured from the postvoid current level. During the bladder contraction, the excitability of the fibre decreases. The fibre was recorded at a depth of 1064 μm and had a conduction velocity of 49 m s^{-1}.

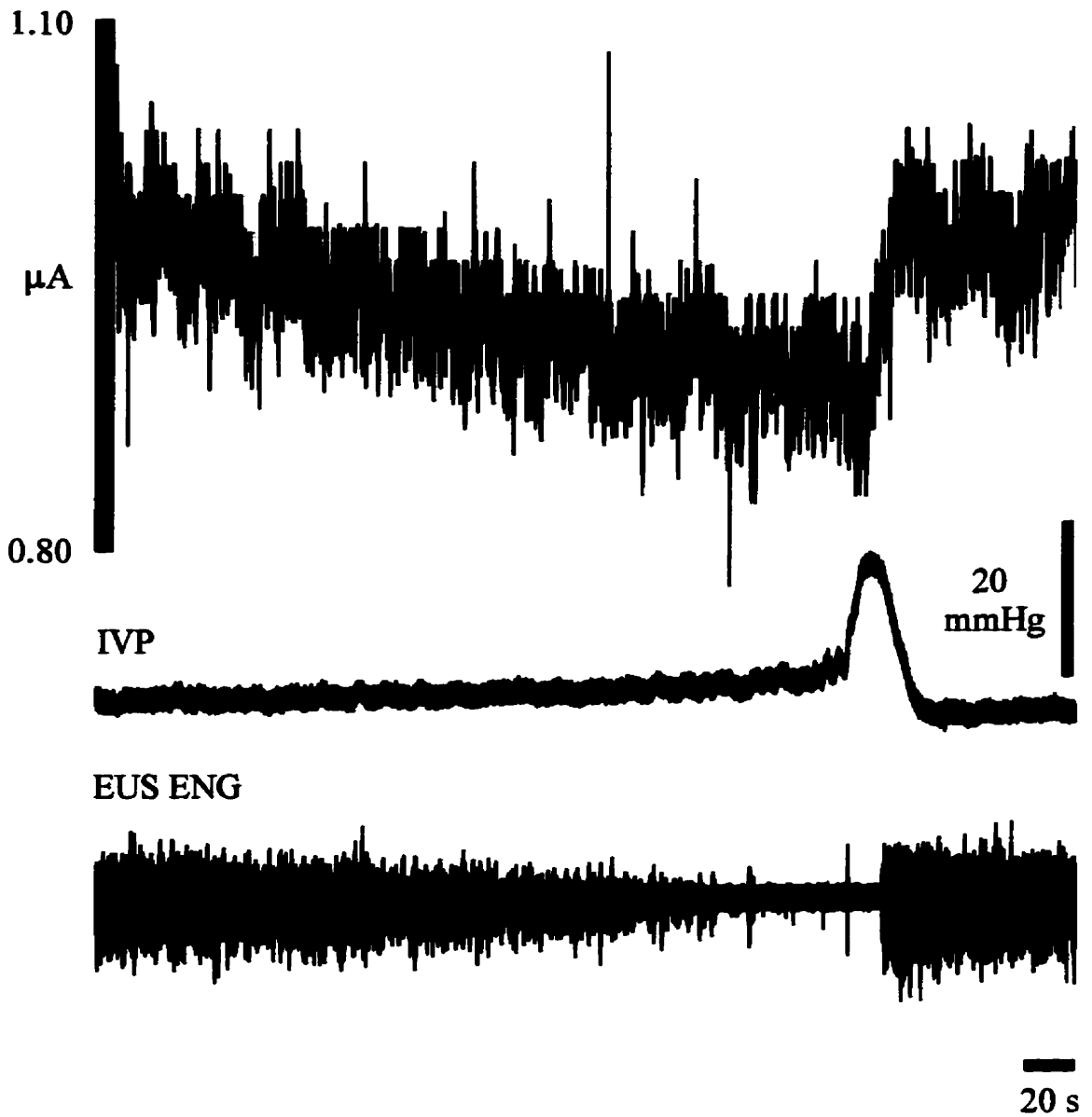

Figure 9

Figure 10: Similar patterns of excitability changes in a SFP and urSPud fibre during micturition. A SFP (A) and urSPud (B) fibre were recorded 475 μm apart in the same track in the sacral spinal cord. The excitability traces are amplitude normalized and overlaid in C, which reveals a similar pattern of excitability change in both fibres.

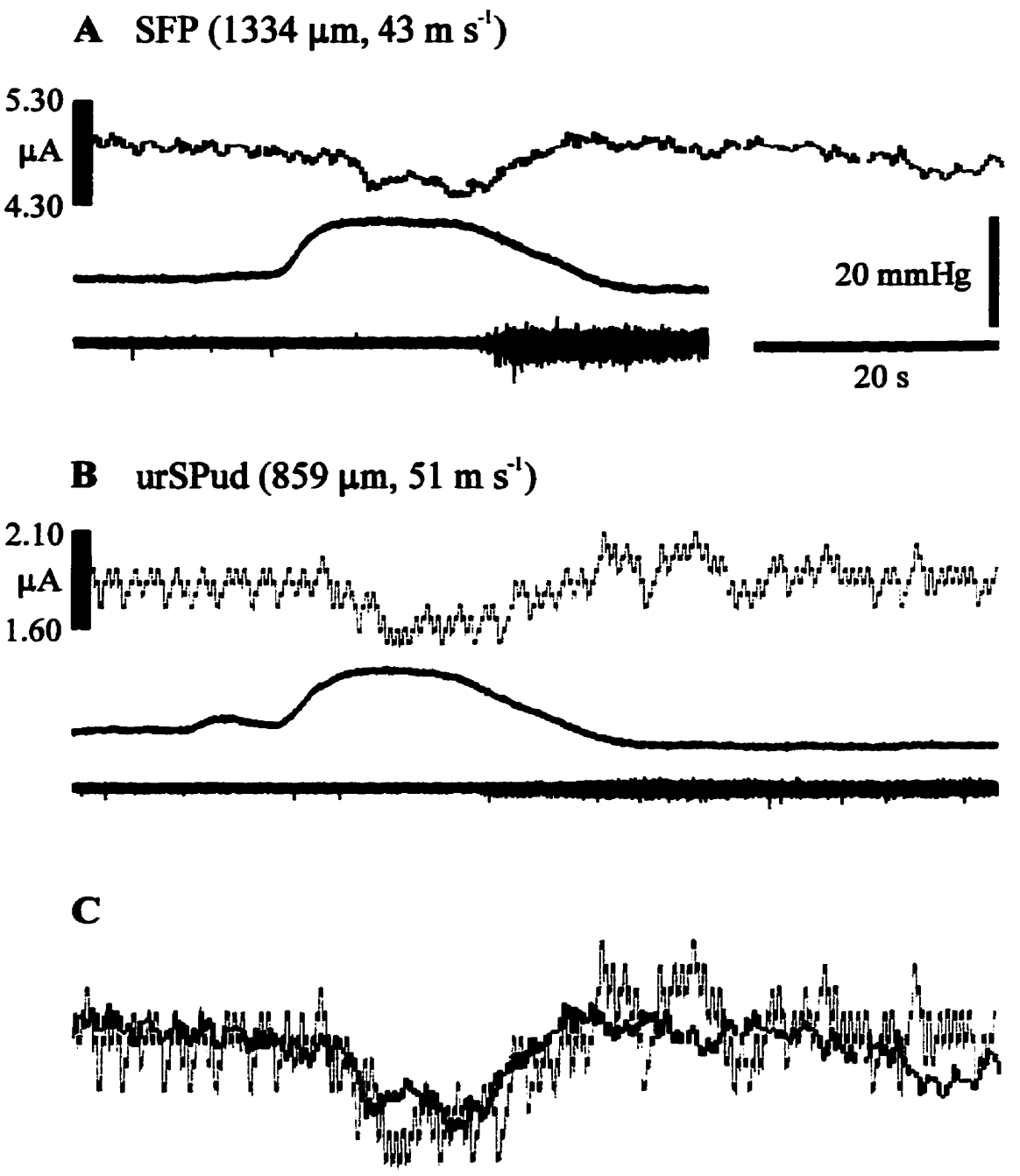
A SFP (1334 µm, 43 m s-1)
5.30
µA
4.30
20 mmHg
20 s
B urSPud (859 µm, 51 m s-1)
2.10
µA
1.60
C

Figure 10

Figure 11: Similar excitability changes in a cutSPud and urSPud fibre during distension-evoked micturition. The cutSPud (A) and urSPud (B) fibres were recorded 48 μm apart in the same track in the sacral spinal cord. In C, the excitability traces are amplitude normalized and overlaid as in Figure 10.

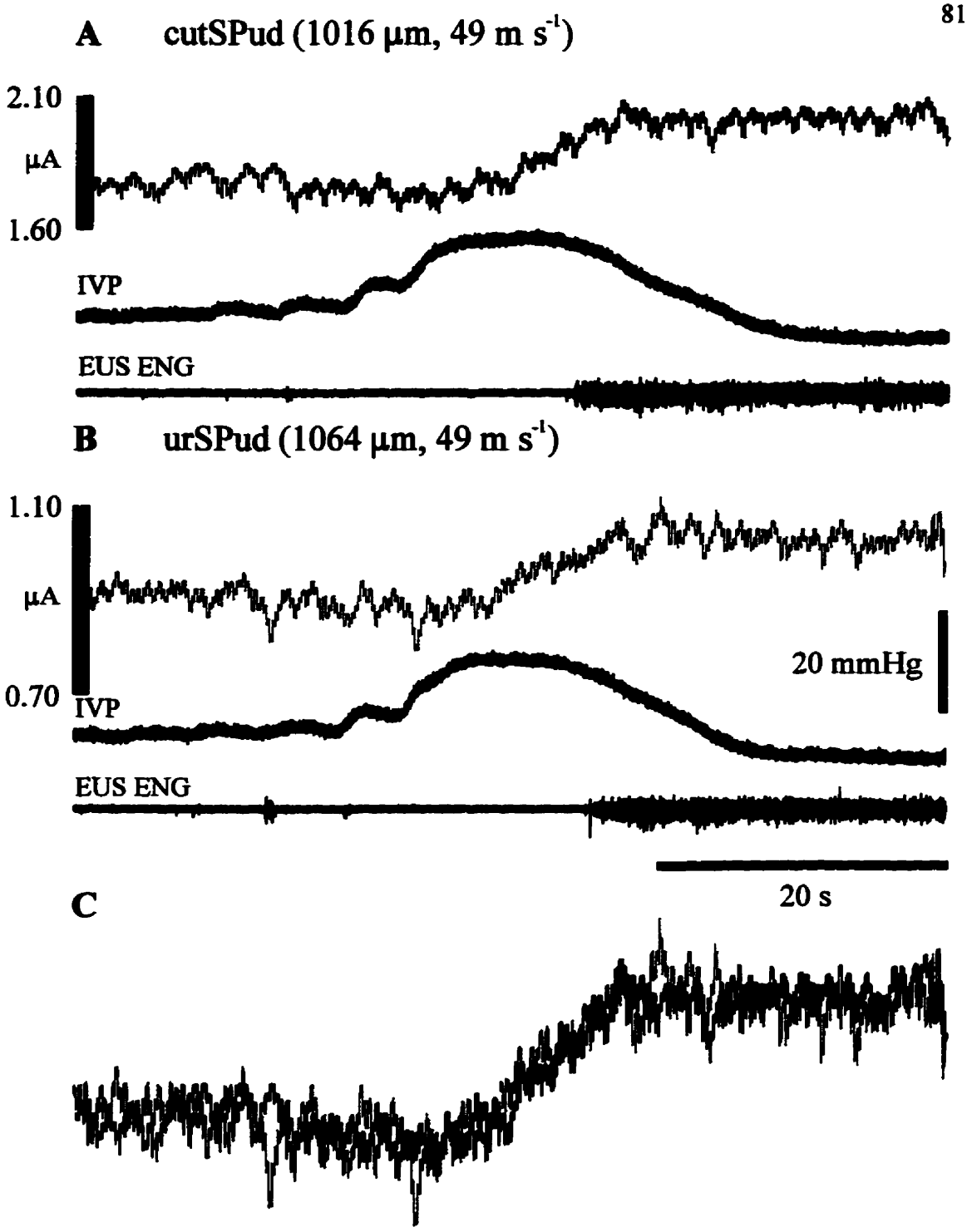
A cutSPud (1016 µm, 49 m s-1)
2.10
µA
1.60
IVP
EUS ENG
B urSPud (1064 µm, 49 m s-1)
1.10
µA
0.70
IVP
20 mmHg
EUS ENG
20 s
C

Figure 11

Figure 12: Attenuation and potentiation of urSPud-evoked EPSPs during distension-evoked micturition. Traces from top to bottom show the bladder pressure, the EUS nerve ENG, membrane potential of the EUS motoneurone (note that spikes are truncated), stimulus markers and EPSPs evoked by every fifth urSPud stimulus. The urSPud nerve was stimulated at 1.6T. EPSPs were attenuated at the onset of the bladder contraction and EUS hyperpolarization, and are nearly completely suppressed for much of the void.

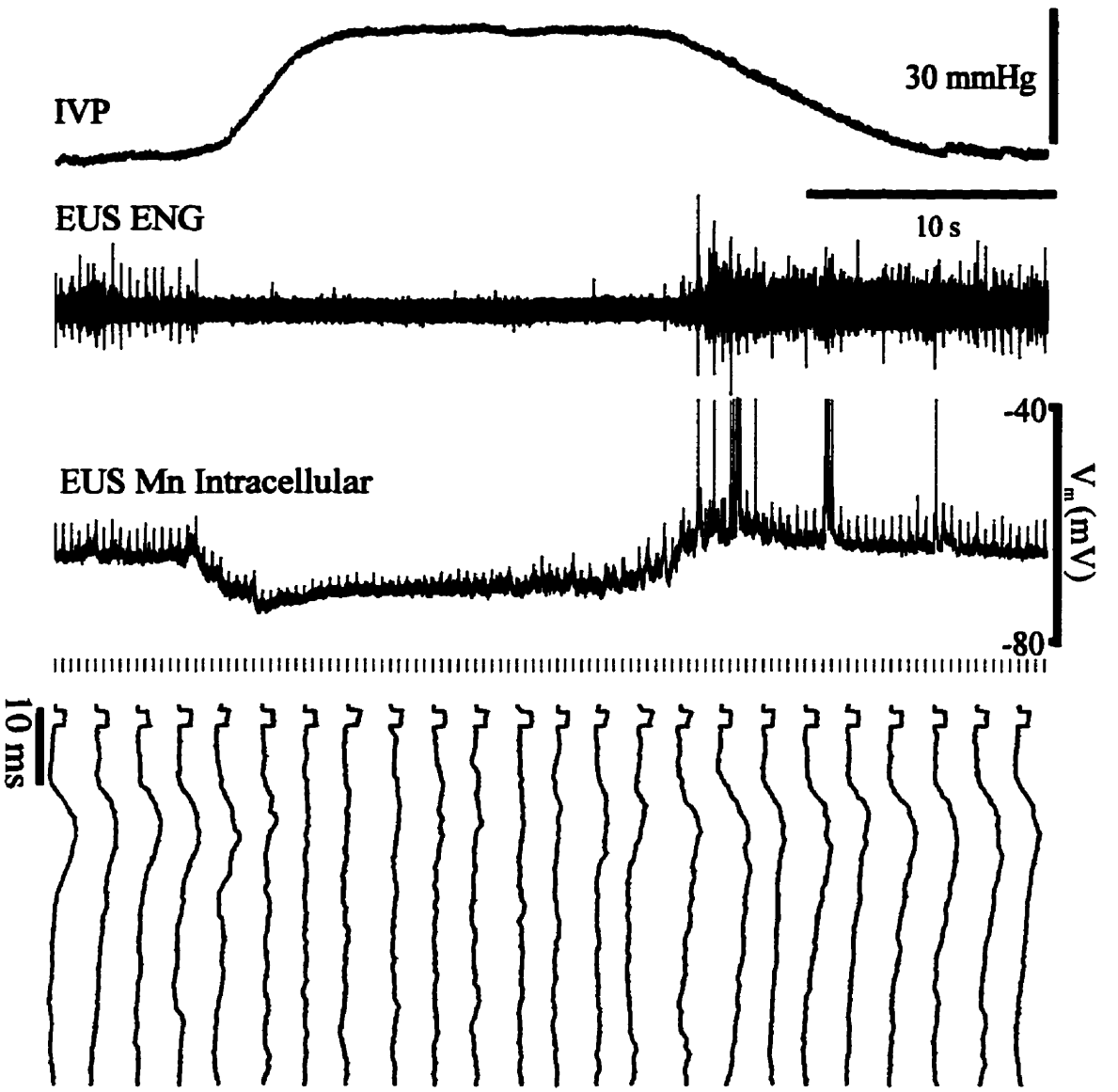

Figure 12

Figure 13: Schematic representation of interneuronal relays from group II muscle and cutaneous afferents, described by Jankowska & Riddell (1994), terminating in the sacral spinal cord. Group II and cutaneous afferents synapse onto common sacral interneurones (1). These first order sacral interneurones activate a number of interneuronal pathways including polysynaptic pathways to sacral motoneurones (2), disynaptic negative feedback pathways to sacral interneurones (3), and disynaptic (4) and polysynaptic (5) PAD pathways to group II muscle afferents terminating in the sacral spinal cord. Unfilled circles represent excitatory interneurones, black circles inhibitory interneurones and grey circles PAD interneurones. The PAD interneurone shown represents the connections of one or more populations of PAD interneurones and their axo-axonic synapses (6) onto cutaneous and group II muscle afferents.

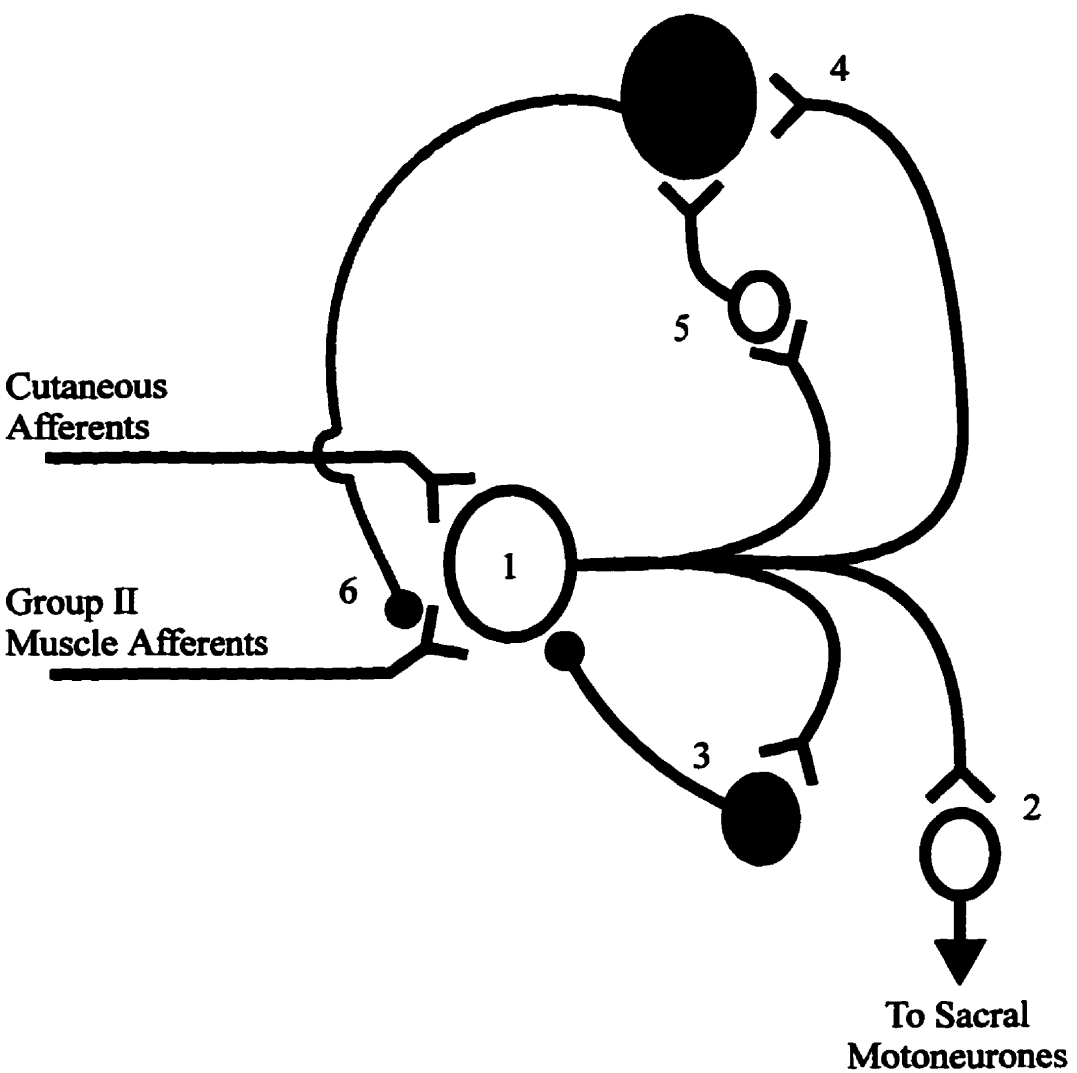
4
5
Cutaneous
Afferents
1
6
Group II
Muscle Afferents
3
2
To Sacral
Motoneurones

Figure 13

Figure 14: Schematic showing the suggested utilization of the sacral PAD interneurones by the central micturition circuitry. The central micturition circuitry is shown accessing PAD interneurones and interneurones presynaptic to these PAD interneurones (shown in grey circle). Activation of these PAD interneurones would inhibit cutaneous and group II muscle afferent transmission, thus contributing to suppression of reflex pathways to EUS motoneurones during micturition.

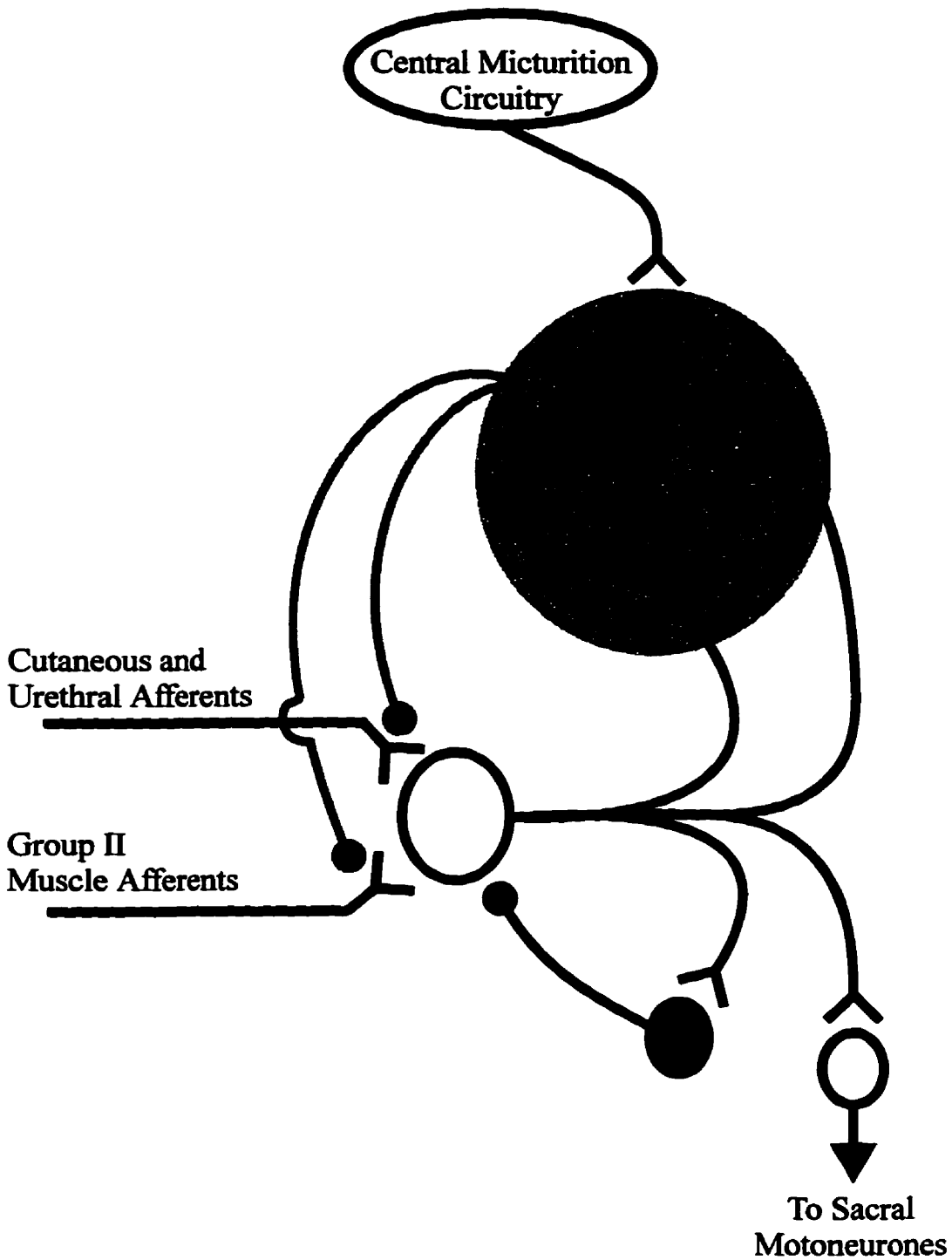
Central Micturition Circuitry
Cutaneous and Urethral Afferents
Group II Muscle Afferents
To Sacral Motoneurones

Figure 14

Figure 15: Schematic of hypothesized and known interneuronal pathways contributing to suppression of excitatory pathways to EUS motoneurones and activation of pathways to bladder preganglionic neurones during micturition. The central micturition circuitry is shown to synapse onto spinal interneurones (1) which have connections to PAD interneurones (2), bladder preganglionic neurones (3), and interneurones (4) exerting postsynaptic inhibition onto interneurones in polysynaptic excitatory pathways to EUS motoneurones, or interneurones (5) with direct inhibitory inputs to EUS motoneurones. The grey PAD interneurones (2) modulate transmission from urethral afferents as well as a portion of cutaneous afferents that synapse onto sacral interneurones (6) in an excitatory pathway to EUS motoneurones. We suggest that these PAD interneurones may additionally produce postsynaptic inhibition (7) in EUS motoneurones. Note that branches of urethral afferents synapsing onto the sacral interneurones (6) are accessed by the PAD interneurones (2) but branches (8) traveling to bladder preganglionics (3) are not. However, this is not meant to imply that other populations of PAD interneurones do not access branches of urethral afferents going to bladder preganglionic neurones. During micturition, activation of the interneuronal population (1) would excite bladder preganglionic neurones and lead to contraction of the bladder. Urine forced through the urethra would excite urethral afferents (8) which contribute to excitatory bladder reflexes during micturition. The selective PAD of urethral afferent branches activating excitatory pathways to EUS motoneurones prevents the reflex excitation of EUS motoneurones observed during continence. Postsynaptic inhibition of EUS motoneurones and interneurones exciting EUS motoneurones also contributes to EUS sphincter relaxation.

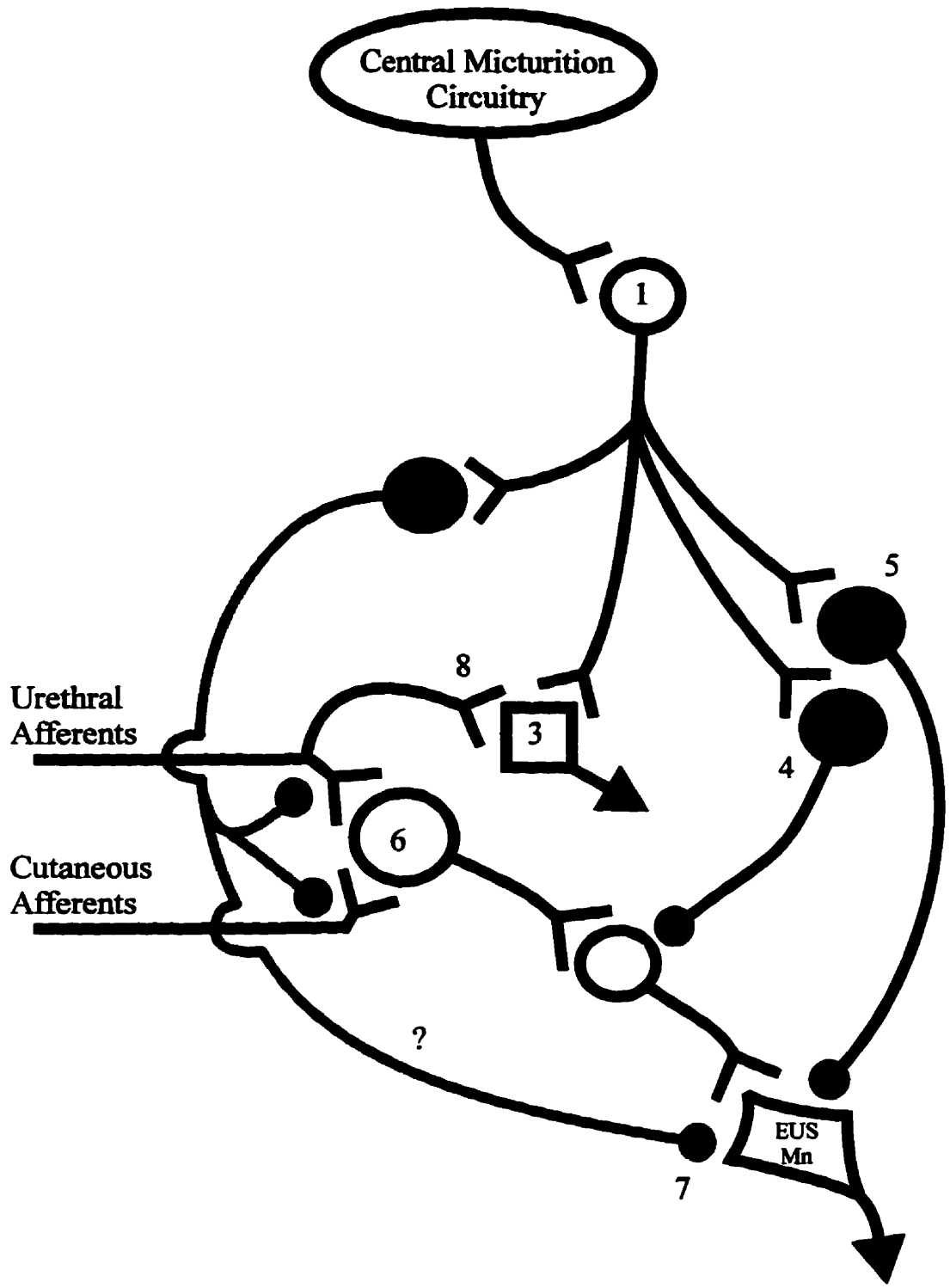
Central Micturition Circuitry
1
5
8
Urethral Afferents
3
4
6
Cutaneous Afferents
?
EUS Mn
7

Figure 15

Figure 16: A simplified schematic representation of the two hypothesized PAD interneurone populations (1 & 2) and their control of urethral and cutaneous afferent transmission during micturition. (1) represents the sacral segmental PAD interneurones presented in Figure 14 and (2) the micturition PAD interneurones presented in Figure 15. It is hypothesized that the sacral segmental PAD interneurones (1) modulate transmission of cutaneous and group II muscle afferents which activate excitatory pathways to sacral motoneurones, including a weaker link with EUS motoneurones. The micturition PAD interneurones (2) are more specific in targeting urethral and cutaneous afferents that activate interneurones with strong excitatory connections to EUS motoneurones. Locomotor circuitries may also utilize the sacral PAD interneurones during locomotion.

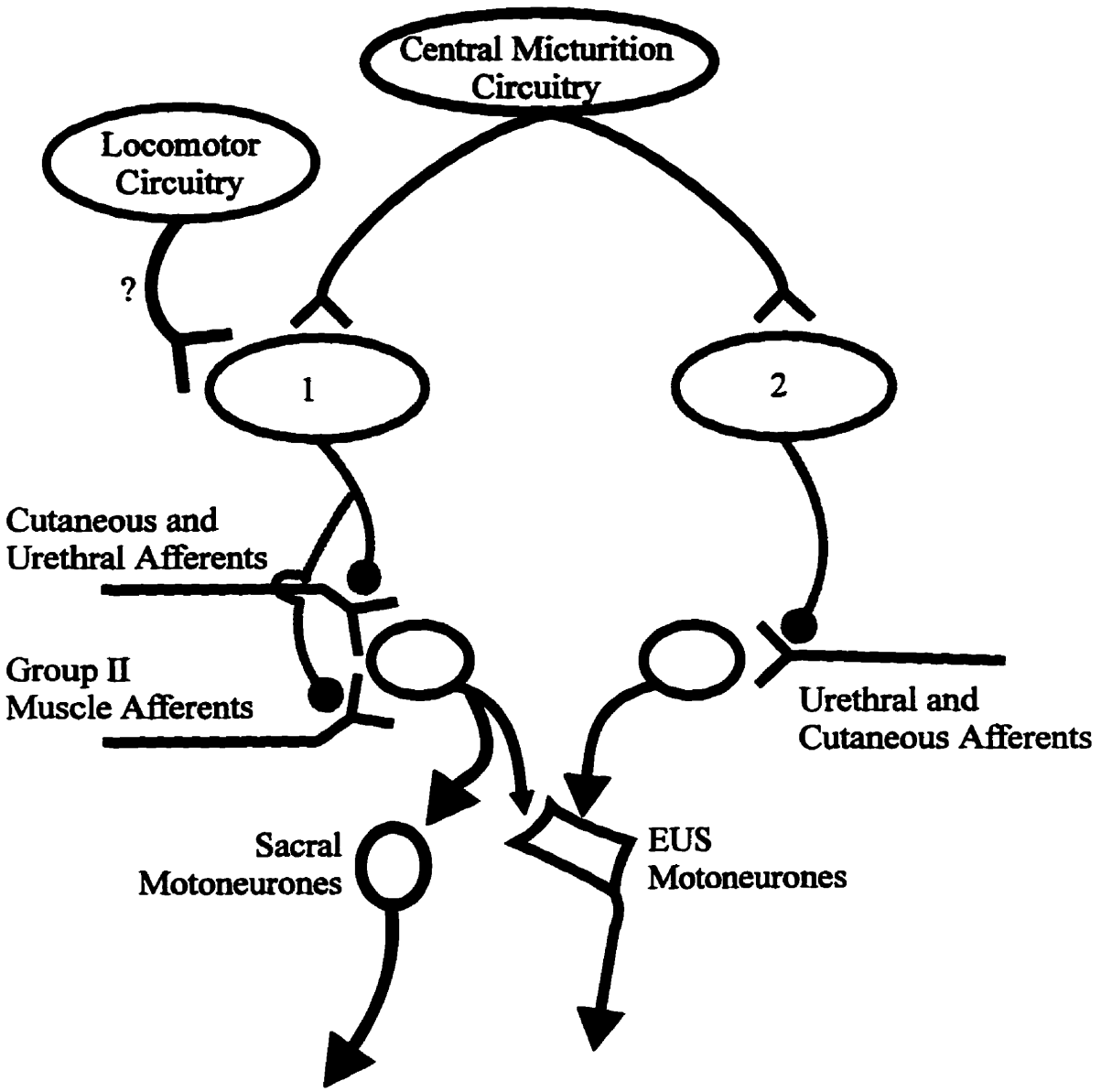
Central Micturition Circuitry
Locomotor Circuitry
?
1
2
Cutaneous and Urethral Afferents
Group II Muscle Afferents
Urethral and Cutaneous Afferents
Sacral Motoneurones
EUS Motoneurones

Figure 16

Table 1: Excitability increases in cutaneous fibres examined in the sacral spinal cord during micturition.

Cutaneous Fibre	Increase in Fibre Excitability During Micturition		
cutSPud	$\bar{x}$ = 12.9%	range = 5-26.4%	n = 5
SFP	$\bar{x}$ = 10.7%	range = 7.7-14.9%	n = 3
CCF	$\bar{x}$ = 13.5%	range = 8.5-30%	n = 6
CCS	$\bar{x}$ = 6.2%	range = 5-7.6%	n = 6
LCS	9.4%		n = 1

Table 2: Afferents examined in the sacral spinal cord during micturition.

Fibre	Excitability Change		No Excitability Change		Uninterpretable Excitability Change	
	Male	Female	Male	Female	Male	Female
urSPud	3	8	4	1		
cutSPud	2	3	5			
SFP	1	2	1			
CCF	2	4				1
CCS	4	2	2		6	
LCS	1		1		3	
Total	13	19	13	1	9	1

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