TITLE:

Sacro-lumbar Intersegmental Spinal Reflex in Autonomic Pathways Mediating Female Sexual Function

AUTHORS:

[1,3]Shi Yong Yuan, PhD, [1,3]Ian L Gibbins, PhD, [2,3]Vladimir P Zagorodnyuk, PhD, [1,3]Judy L Morris, PhD

INSTITUTION:

[1]Anatomy and Histology, [2]Human Physiology, and [3]Centre for Neuroscience, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.

CONFLICT OF INTEREST:

None

ACKNOWLEDGEMENTS:

Supported by grants from the National Health and Medical Research Council of Australia (Project Grant 375100, Principal Research Fellowship 160083 to J. Morris). We thank Patricia Vilimas for excellent technical support throughout the experiments, and Yvette DeGraaf and Dr Jennifer Clarke for providing culture medium and assistance with confocal microscopy.

INTRODUCTION

Sexual activity in females is associated with increased pelvic blood flow, local mucous secretion, and rhythmic contractions of the vagina and uterus[1,2,3,4] that promote subsequent fertilization and implantation[5]. These responses are produced by autonomic pathways with final motor neurons in the paracervical (anterior pelvic) ganglia,[6-9] nearly half of which mediate vasodilation.[8-12]

In males and females, pelvic vasodilation is often considered to be controlled mainly by sacral parasympathetic pathways.[13-18] Nevertheless, in several species, including humans, lumbar sympathetic pathways contribute to increased pelvic blood flow and sexual arousal.[10,13,17-21] Indeed, many pelvic vasodilator neurons in the paracervical ganglia receive their dominant synaptic input from sympathetic preganglionic neuroins in mid-lumbar (L3) spinal cord; these neurons often receive convergent preganglionic inputs from sacral spinal cord.[7,8]

The segmental organization of the splanchnic, hypogastric, pelvic and pudendal nerves is well conserved across species. However there are some small differences. For example, spinal outflows to the lumbar splanchnic nerves are at T11-L2 levels in humans, T12-L3 in dogs, mid to upper lumbar levels in cats and rats[18] and mid lumbar levels in guinea pigs.[7,8] Preganglionic axons project from the ventral roots via the sympathetic chain ganglia to the lumbar splanchnic nerves. They then project through the inferior mesenteric ganglia (IMG) and along hypogastric nerves to enter pelvic plexus where they make synapses with pelvic neurons, including those in the paracervical ganglia.[18]

Sensory nerves from the reproductive tract enter the lumbo-sacral spinal cord via the pudendal, pelvic and hypogastric nerves: the pudendal nerve carries fibres from the external genitalia, the pelvic nerves from vagina, cervix and caudal uterus while the hypogastric nerve carries sensory information from cervix and uterus.[15-17,22-26] Sensory information transmitted by pudendal and pelvic sensory nerves facilitates arousal and conception, while information from lumbar sensory nerves is involved more in pregnancy, parturition and nociception.[16,17,23,25,26] Sensory inputs to lumbar spinal cord potentially could stimulate sympathetic preganglionic neurons,[22] including those in vasodilator pathways.[25,27] Indeed, stimulation of pudendal sensory fibres activates spinal reflexes that trigger perineal and vaginal contractions with associated increases in vaginal blood flow.[17,28-30] Therefore, we would expect the lumbar spinal outflow to paracervical ganglia to be activated by stimulation of pudendal sensory nerves, either via descending pathways after relay of sensory information to the brainstem,[30] or via intra-spinal circuits. To date, however, there has been no direct evidence for such a circuit. If present, this circuit would add another level of subtlety in the control of the pelvic viscera that would need to be considered in any interpretation and treatment of dysfunction in the urogenital tract.

AIM

This study aims to investigate the activation of lumbar preganglionic neurons projecting caudally along the hypogastric nerve to paracervical ganglia in response to stimulation of ascending or descending spinal pathways. Specifically, we set out to determine whether the mid-lumbar preganglionic outflow was activated by an intersegmental spinal reflex initiated from pudendal sensory fibres. We used a novel preparation from female guinea pigs, a species for which there are considerable functional and anatomical data on the organisation of

pelvic autonomic pathways.

METHODS

Immature female guinea-pigs (6-8 weeks old; 200–240 g body weight; Hartley-IMVS) were anaesthetised with 50% urethane (up to 1.8g/kg i.p.) as approved by the institutional animal welfare committee according to national guidelines. Guinea-pigs were used in this study as the neurochemistry, synaptic organization and function of their pelvic vasodilator neurons are well known, comparable to what has been found in other species including humans.[12,16] Furthermore, the pathways and functions of other autonomic and sensory pathways supplying the abdominal and pelvic viscera in female guinea-pigs are also well known, further facilitating the design and interpretation of these experiments.[7-9,11,31]

Electrophysiology

Anaesthetised animals were opened with a mid-ventral line incision, the aorta was cannulated at T13-L1 spinal level or just proximal to the iliac bifurcation and perfused at 4 ml min^{-1} with preheated (37ºC) HEPES-buffered physiological salt solution (composition in mM: 146 NaCl, 4.7 KCl, 0.6 $MgSO_4$, 1.6 $NaHCO_3$, 0.13 NaH_2PO_4, 2.5 $CaCl_2$, 7.8 glucose, 20 HEPES, 1.25% Ficoll, adjusted to pH 7.4 with NaOH and bubbled with 100% O_2). During the experiment the preparations were perfused only via the aorta. The initial 200 ml of perfusate contained 1000 IU heparin and 0.15 mM sodium nitrite to prevent clotting and to dilate blood vessels. To maximise aortic perfusion of the spinal cord, arteries supplying abdominal and pelvic organs were ligated and transected.

Descending spinal-hypogastric pathways

A segment of perfused spinal cord from T12 to L6 was transferred to a dissecting dish containing oxygenated HEPES-buffered physiological salt solution, changed every 15 minutes during the dissection. In guinea pigs, the hypogastric nerves originate mainly from L2-L4 lumbar splanchnic nerves, traverse the IMG, and project caudally to the pelvic region.[7,8] The caudal ends of left and right hypogastric nerves at the level of the iliac bifurcation before entering paracervical ganglia were isolated for extracellular recording. The preparation was pinned ventral side uppermost in a recording chamber and equilibrated for at least 30 minutes at 37ºC. Compound action potentials were recorded from right and left hypogastric nerves with extracellular suction electrodes (200-500 μm inner tip diameter; **Figure 1A**). Electrical stimulation of T12-T13 spinal cord (0.3ms, 50V, single pulse or repeated pulses with an interval of 400 ms) was delivered by a pair of parallel stainless entomology pins (0.2 mm in diameter, 1.5 mm apart, insulated by epoxy resin to within 5 mm of the tips), inserted into both lateral funiculi of the spinal cord, and connected to a Grass S8800 stimulator. Control stimuli were applied twice at an interval of 2 minutes. Stimulation was repeated either after transecting the lumbar splanchnic nerves (LSN) bilaterally at L3 or after perfusing the preparation with physiological solution containing 0.6 μM tetrodotoxin (TTX) for 10 minutes.

Pudendal-spinal-pelvic pathways

A perfused segment of spinal cord from L2 to the caudal end of the vertebrae was isolated and transferred to a recording chamber as above. In guinea pigs, pudendal nerves and pelvic nerves originate mainly from S1-S3 and S2-S4 respectively.[32,33] After exposure of pudendal and pelvic nerves, extracellular electrophysiological recordings were carried out with suction electrodes on the central ends of pelvic nerves. Electrical pulses were delivered by a suction

stimulating electrode (inner tip diameter: 100-300 μm) on the ipsilateral pudendal nerve branch projecting to the female reproductive region to test for activation of pudendal-spinal-pelvic pathways (**Figure 1B)**. The peripheral end of pudendal nerve was sectioned to eliminate the possible effect of spinal efferent activation within the pudendal nerve during the electrical stimulation.

Pudendal-spinal-hypogastric pathways

A longer segment of spinal cord from T13 to the cauda equinae was prepared as above for extracellular recording. In one group of experiments, the distal end of the left or right hypogastric nerve before it entered the pelvic ganglia was isolated for extracellular recording. In a second group, the right L3 splanchnic nerve was exposed, transected and recordings were made with a suction electrode from the distal end of the proximal portion that maintained its connection to the spinal cord. In both groups, electrical pulses (0.3ms, 50V, 1, 5 and 10 pulses at 200 Hz) were delivered by a suction stimulating electrode attached to the pudendal nerve ipsilateral to the recording electrode to test for activation of pudendal-spinal–hypogastric pathways (**Figure 1C**). In some experiments, the selective $GABA_A$ receptor antagonist, bicuculline (10 μM), was added to the perfusate for 20 min after a set of control stimuli before repeating the same stimulation protocol.

Confirmation of L3 preganglionic neurons in the hypogastric nerve

To test the preganglionic nature of the L3 spinal output to hypogastric nerve and paracervical ganglia, two groups of experiments were carried out in an isolated segment of perfused spinal cord from T12 to the cauda equinae, prepared as above. A suction stimulating electrode was attached to either L3 splanchnic or pudendal nerves on one side of the preparation (**Figure**

1D). A suction recording electrode was attached to the caudal ends of both left and right hypogastric nerves. After control stimulation, a selective nicotinic receptor blocker, hexamethonium (200 μM) was added to the perfusate and recording bath for 15-30 minutes before repeating the stimulation procedure. TTX (1 μM) was subsequently added in the experiments with pudendal nerve activation and its effect was tested after 15 minutes. Bicuculline (10 μM) was present throughout a subset of experiments in this group.

Extracellular compound action potentials and electrical stimuli were recorded with PowerLab/4SP hardware (AD Instruments, Sydney, Australia) connected to an iMac computer using Chart version 5.5.4 at 1000 samples/second. The maximal amplitude of the response to stimulation was measured.

Morphology

Detection of spinal cord infarction with TTC staining

To confirm viability of the perfused spinal cord preparations, 2,3,5-triphenyl-2*H*-tetrazolium hydrochloride (TTC) staining was used. Transverse sections (1 mm thick) were taken from perfused spinal cord preparations after dissection for 2 hours and electrophysiological recording for up to 5 hours and were incubated in 3% TTC for 10 minutes at 37ºC in the dark. In four animals, TTC staining of perfused isolated thoracic spinal cord was deep pink colour indicating viable tissue[34,35] during the first 2 hours of tissue dissection, and after continuous perfusion of lumbar cord for 5 hours. Spinal cord kept isolated at the room temperature for 5 hours without perfusion was white, indicating extensive tissue infarction.[34,35]

Neuronal tracing procedure for detection of sensory pathways from pudendal nerves

Neuronal tracing in organ culture was performed by coating two glass beads (150–200 μM in diameter; Sigma Chemical Co., St. Louis, MO) with DiI (1,18-didodecyl-3,3,38,38-tetramethyl indocarbocyanine perchlorate; Molecular Probes, Eugene, OR).[31] The beads were placed on a small branch of the right pudendal nerve at S3, attached to an isolated spinal cord preparation from T12 to cauda equinae. The tissue was covered with sterile culture medium (DME/F12, Sigma Chemical Co.) supplemented with 10% heat inactivated bovine serum, penicillin 100 IU/ml, streptomycin 100 μg/ml, amphotericin B 2.5 μg/ml, gentamycin 20 μg/ml (Cytosystems, Castle Hill, N.S.W., Australia), and adjusted to pH 7.4. The preparation was placed in a humidified incubator (37°C, equilibrated with 5% CO2 in air) for 3 days and gently agitated. DiI-coated beads were left in place during the culture, and the medium was changed daily. Cultured tissue was fixed for 48 hours at 4°C with 0.5% picric acid and 2% formaldehyde in 0.1 M phosphate buffer, pH 7.0. Segments of spinal cord with attached DRG from L1 to S3 were cut in serial transverse sections 50 μm thick on a cryostat (Leica 1800; Reichert Jung). Sections were mounted on glass slides in buffered glycerol (70% glycerol in 0.5 M sodium carbonate buffer, pH 8.6) to locate DiI labeled neurons under an AX70 epifluorescence microscope (Olympus Optical, Tokyo, Japan).

Immunohistochemistry

DiI labeled DRG sections mounted on glass slides were incubated for 48 hours with a mixture of well-characterised antisera against calcitonin gene-related peptide (CGRP) raised in goat (1:1000; Arnel Products 1780, NY, USA) and substance P (SP) raised in rat (1:600; Chemicon International MAB356, Temecula, CA, USA) at room temperature in a humid chamber, washed, and incubated for 2 hours with species-specific secondary antibodies [indodicarbocyanine (Cy5)-conjugated donkey anti-sheep IgG (Jackson ImmunoResearch

Laboratories, West Grove, PA, USA) which recognises the goat anti-CGRP antibody, and fluorescein isothiocyanate (FITC)-conjugated donkey anti-rat IgG (Jackson). Tissues were remounted in buffered glycerol (pH 8.6) and viewed under an Olympus AX70 epifluorescence microscope with highly-selective filter blocks (Chroma 41008 for Cy5 and Chroma 31001NB for FITC, Chroma Technology, Bellows Falls, VT) or a Leica TCS SP5 confocal microscope (Leica Microsystems P/L, Mannheim, Germany).

Single, double or triple labeled neurons (DiI alone; DiI and CGRP; DiI and SP; DiI, CGRP and SP) were counted using an AX70 epifluorescence microscope. Digital images of labeled neurons were captured by a Hamamatsu Digital Camera (C4742-95, Japan) mounted on an AX70 microscope running AnalySIS (version 5.0, Olympus Soft Imaging Systems) or a Leica TCS SP5 confocal microscope (LASAF software; Leica Microsystems P/L, Mannheim, Germany).

Data Analysis

Effects of drugs on compound action potentials were determined by measuring the maximum amplitude of individual responses at each recording site after subtracting baseline noise, defined as high-frequency fluctuations of 10-30 μV in the baseline. Mean response amplitudes were determined from 4 to 7 animals. Traces were filtered using a band pass from 2000 Hz (high cut-off) to 10 Hz (low cut-off) before the measurement.

Data were expressed as mean ± standard error from at least four experiments. Differences were analysed by analysis of variance (ANOVA) using SPSS 16.0 for Macintosh (SPSS, Chicago, IL, USA) with $P < 0.05$ taken as statistically significant.

Drugs used

TTX (Alomone Laboratories, Jerusalem) was stored in aqueous solution at 10 mM. The $GABA_A$ receptor antagonist, bicuculline methiodide (Sigma-Aldrich), was dissolved in phosphate buffered saline at a concentration of 10 mM and used at a final concentration of 10 µM. Hexamethonium hydrochloride (Sigma-Aldrich) was dissolved in phosphate buffered saline and used at a final concentration of 200 µM.

RESULTS

Responses in hypogastric nerve to activation of descending spinal pathways

Electrical stimulation of T12-T13 spinal cord (1 or 5 pulses) evoked multi-phasic compound action potentials recorded at the caudal ends of the hypogastric nerves (HN). These responses were abolished by bilaterally transecting lumbar splanchnic nerves (LSN) at L3 (**Figure 2A** and **2C**) or by adding TTX (0.6 μM) to the perfusate (n = 6).

Responses in pelvic nerve to stimulation of pudendal nerve

Electrical stimulation (1 or 5 pulses) of the pudendal nerve evoked compound action potentials recorded in the ipsilateral pelvic nerve 30-50 ms after the stimulation artifact. All responses in pelvic nerves were blocked by transecting the pudendal nerves ipsilaterally confirming that these responses required intact spinal circuitry and were not an electrical artifact due to current spread (n = 6) (**Figures 2B** and **2D**).

Responses in hypogastric or lumbar splanchnic nerves to stimulation of pudendal nerve

Electrical stimulation of the pudendal nerves (1, 5, or 10 pulses) evoked compound action potentials recorded in the caudal ends of the hypogastric nerves that were abolished by transecting the splanchnic nerves bilaterally at L3 or the ipsilateral pudendal nerves. Similar responses were recorded at the central ends of ipsilateral splanchnic nerves at L3. These mono- or multi-phasic responses occurred after a 30-50 ms delay. The amplitude of responses recorded in hypogastric nerves increased significantly with increasing number of stimulating pulses from 44 ± 3 μV (single pulse) to 64 ± 8 μV (5 pulses) and 77 ± 11 μV (10 pulses) (n = 7, ANOVA, $p < 0.001$). A similar increase in response amplitude was observed in the L3

splanchnic nerve as the number of stimulating pulses was increased (53 ± 6 μV, single pulse; 66 ± 8 μV, 5 pulses; 81 ± 7 μV, 10 pulses) (n = 7, $p < 0.001$).

The selective $GABA_A$ receptor antagonist, bicuculline (10 μM for 20 minutes) significantly increased the amplitude of evoked responses at both recording sites compared with controls The responses in both nerves were enhanced to a similar degree by bicuculline (**Figure 3** and **4**).

Effect of nicotinic blockade on hypogastric nerve responses to stimulation of pudendal or lumbar splanchnic nerves

Electrical stimulation of either L3 splanchnic or pudendal nerves evoked compound action potentials in the caudal ends of the hypogastric nerve. The response amplitude increased with increasing number of stimulating pulses at splanchnic nerve [(n = 4): 51 ± 4 μV (single pulse), 102 ± 19 μV (5 pulses), 160 ± 38 μV (10 pulses)]. Pudendal nerve stimulation with 10 pulses evoked much smaller responses [(n = 4): 120 ± 19 μV (10 pulses)] compared with splanchnic nerve stimulation, in spite of presence of bicuculline to maximize the response. The response amplitudes at both activation sites were not significantly affected by the nicotinic receptor blocker, hexamethonium (200 μM) [splanchnic nerve, n = 4: 49 ± 3 μV (single pulse), 100 ± 18 μV (5 pulses), 163 ± 38 μV (10 pulses); pudendal nerve, n = 4: 115± 21 μV (10 pulses)]. The responses to pudendal nerve activation were blocked by addition of TTX (0.6 μM) (**Figure 5B**). The combined effects of hexamethonium and TTX indicate that these responses were due predominantly to preganglionic fibres projecting from the L3 spinal output to hypogastric nerve and paracervical ganglia.

Neuronal tracing sensory pathways and immunohistochemical labeling

DiI beads placed on an exposed branch of pudendal nerves for three days labeled sensory neuronal axons and cell bodies in the DRG at S3 level. In two preparations from different animals, DiI labeled 99 and 122 neurons, respectively, in S3 DRG. DiI labeled cells were not found in the spinal cord, nor in DRGs at lumbar and other sacral levels. Labeling for immunoreactivity to SP and CGRP revealed that there were four groups of DiI labeled neurons in the S3 DRG (DiI alone: n = 34 ± 4 (30% of total DiI labelled cells, A1 = 30, A2 = 38); DiI and CGRP: n = 16 ± 1 (14%, A1 = 15, A2 = 16); DiI and SP: n = 19 ± 2 (17%, A1 = 17, A2 = 20); DiI, CGRP and SP: n = 43 ± 6 neurons (39%, A1 = 37, A2 = 48); A1 and A2 for animal 1 and 2) (**Figure 6**).

DISCUSSION

This study provides clear evidence for the existence of multiple spinal pathways likely to be involved in autonomic control of the female reproductive organs. Most notably, we have shown that stimulation of sensory fibres in the pudendal nerve activates spinal pathways ascending from sacral to mid-lumbar levels where they excite sympathetic preganglionic outflows including those projecting to paracervical ganglia that innervate the pelvic viscera and associated vasculature.

Spinal descending pathways to the hypogastric nerve

The hypogastric nerve provides a major route for lumbar sympathetic outflow to the pelvic viscera, including the female reproductive tract and its vasculature. Indeed, many neurons in the paracervical ganglia receive convergent preganglionic inputs from the hypogastric and pelvic nerves.[7-9,18,22] Previous studies, mostly in males, have indicated that the lumbar outflow receives both excitatory and inhibitory descending spinal input.[18,36] Our data indicate that bulk electrical stimulation of descending spinal tracts activates these pathways with dominant excitatory input to preganglionic neurons projecting out the hypogastric nerve. Our lesioning experiments show that most of this outflow reaches the hypogastric nerve via the L3 splanchnic nerve.

Local spinal pathways from pudendal to pelvic nerves

As predicted from observations in male[13,18] and female rats,[15-17] pudendal nerve stimulation activated spinal outputs via the pelvic nerve in the isolated spinal cord preparation of female guinea-pigs. This pathway provides sacral parasympathetic preganglionic input to paracervical ganglion cells, and seems to be very robust, since single pulses delivered to the

pudendal nerve reliably generated compound action potentials in the pelvic nerve, presumably mediated via local circuits in sacral spinal cord. Comparing the effects of electrical activation on two neuronal pathways (eg. spinal-splanchnic-hypogastric descending pathway and pudendal-spinal-pelvic pathways) much more robust effects were found in the former. This could mean that the descending central inputs to these spinal circuits can drive a greater level of output than can local sensory inputs derived from the pudendal nerve.

Ascending spinal pathways from pudendal to hypogastric nerves

A key observation from our study is that pudendal nerve stimulation activated sympathetic preganglionic outflows from mid-lumbar spinal levels in addition to sacral parasympathetic pathways projecting via the pelvic nerves. Thus there is an ascending intersegmental spinal circuit that transmits sensory information from the pudendal nerve to the lumbar spinal cord. This ascending spinal pathway activated hypogastric preganglionic neurons at the same levels as were activated by descending spinal stimulation (ie. L3). Although sensory projections from deep areas of the genital tract reach the spinal cord via the hypogastric and pelvic nerves, the pudendal nerve mostly transmits sensory input from the lower urogenital tract, especially the external genitalia.[15-17,22-26]

The amplitude of the output of the ascending pathway was related to the strength of stimulation of the pudendal nerve, suggesting that the circuit is capable of generating graded motor responses to varying levels of pudendal nerve activation. The magnitude of the response in the hypogastric nerve was enhanced by blockade of spinal $GABA_A$ receptors with bicuculline, implying significant tonic or collateral inhibitory regulation of the circuit within the spinal cord.

Taken together the data indicate that pudendal nerve stimulation activates local and ascending spinal pathways to generate sacral parasympathetic and lumbar sympathetic outflows, respectively (**Figure 7**). Indeed, preganglionic inputs to vasodilator neurons projecting from paracervical ganglia to the pelvic vasculature arise primarily from mid-lumbar spinal levels in guinea-pigs.[7,8] However, *in vivo* studies based on nerve lesions in anaesthetized rats concluded that hypogastric pathways to reproductive organs did not contribute significantly to increased blood flow in response to pudendal sensory nerve activation.[14,17] Perhaps the GABA inhibitory modulation of the ascending intersegmental reflex demonstrated in this study masks the lumbar outflow in some conditions. Nevertheless, there is evidence that sympathetic pathways may contribute to sexual arousal in able-bodied women or after spinal cord injury.[20,37]

The lumbar and sacral sensory pathways presumably play separate roles in response to stimulation from different parts of the reproductive tract or at different stages of sexual activity in females. The neurochemical diversity of sacral sensory neurons labeled from the pudendal nerve implies that both mechanoceptive neurons (large diameter, lacking neuropeptides) and polymodal or nociceptive neurons (small diameter, expressing neuropeptides) can contribute to these reflexes[38].

If comparable pathways exist in humans, we predict there would be at least four ways of activating autonomic pathways to the reproductive tract: descending central excitation of lumbar or sacral output pathways, and reflex spinal excitation of the same outputs after stimulation of pudendal nerve afferents. The functional differences between these pathways

are unclear, as are the consequences of an ascending inhibitory spinal pathway. Furthermore, although we have focussed on the regulation of the reproductive tract, comparable pathways are likely associated with other pelvic viscera that are innervated by the pudendal nerve, most notably the distal bowel and lower urinary tract. This diversity of pathways regulating the pelvic viscera has been largely unrecognised, yet it raises the possibility of multiple therapeutic targets for women with abnormal autonomic regulation of reproductive function.

CONCLUSIONS

Our data show an ascending intersegmental spinal pathway from the pudendal nerve to mid-lumbar preganglionic outputs projecting to paracervical ganglia, that is under inhibitory spinal modulation, in addition to the local spinal pathway from the pudendal nerve to preganglionic outputs at sacral levels. Both pathways may mediate spinal reflex activation of pelvic vasodilation in sexual function, augmenting descending excitation from supra-spinal levels.

REFERENCES

1 Giuliano F, Rampin O, Allard J. Neurophysiology and pharmacology of female genital sexual response. J Sex Marital Ther, suppl 2002;1:101-121.

2 Kim SW, Jeong SJ, Munarriz R, Kim NN, Goldstein I, Traish AM. An *in vivo* rat model to investigate female vaginal arousal response. J Urol 2004;171:1357- 1361.

3 Gruenwald I, Lowenstein L, Gartman I, Vardi Y. Physiological changes in female genital sensation during sexual stimulation. J Sex Med 2007;4(2):390-394.

4 Traish AM, Botchevar E, Kim NN. Biochemical factors modulating female genital sexual arousal physiology. J Sex Med. 2010;7:2925-2936.

5 Hammarström M. Autonomic nervous control of cervical secretion in the guinea-pig. Acta Physiol Scand 1989;135:367-371.

6 Keast JR. Unusual autonomic ganglia: connections, chemistry and plasticity of pelvic ganglia. Int Rev Cytol 1999;193:1-69.

7 Jobling P, Gibbins IL, Morris JL. Functional organization of vasodilator neurons in pelvic ganglia of female guinea pigs: comparison with uterine motor neurons. J Comp Neurol 2003;459(3):223-241.

8 Morris JL, Gibbins IL, Jobling P. Post-stimulus potentiation of transmission in pelvic ganglia enhances sympathetic dilatation of guinea-pig uterine artery *in vitro*. J Physiol 2005;566:189-203.

9 Morris JL, Gibbins IL. Neuronal colocalization of peptides, catecholamines and catecholamine-synthesizing enzymes in guinea pig paracervical ganglia. J Neurosci 1987;7:3117-3130.

10 Fahrenkrug J, Ottesen B. Nervous release of vasoactive intestinal polypeptide from the feline uterus: pharmacological characterisation. J Physiol 1982;331:451-460.

11 Morris JL. Co-transmission from autonomic vasodilator neurons supplying the guinea-pig uterine artery. J Autonom Nerv Syst 1993;42:11-21.

12 Hoyle CH, Stones RW, Robson T, Whitley K, Burnstock G. Innervation of vasculature of the human vagina by NOS and neuropeptide-containing nerves. J Anat 1996;188:633-644.

13 Dail WG, Manzanares K, Moll MA, Minorsky N. The hypogastric nerve innervates a population of penile neurons in the pelvic plexus. Neuroscience 1985;16:1041-1046.

14 Sato Y, Hotta H, Nakayama H, Suzuki H. Sympathetic and parasympathetic regulation of the uterine blood flow and contraction in the rat. J Autonom Nerv Syst 1996;59:151-158.

15 Papka RE, Traurig H. Autonomic efferent and visceral sensory innervation of the female reproductive system: special reference to neurochemical markers in nerves and ganglionic connections. In: Maggi CA, ed. Nervous Control of the Urogenital System. Luxembourg, Harwood, 1993, p 423-466.

16 Traurig H, Papka RE. Autonomic efferent and visceral sensory innervation of the female reproductive system: special reference to functional roles of nerves in reproductive organs. In: Maggi CA, ed. Nervous Control of the Urogenital System. Luxembourg, Harwood, 1993, p 103-141.

17 Cai RS, Sipski Alexander M, Marson L. Activation of somatosensory afferents elicit changes in vaginal blood flow and the urethrogenital reflex via autonomic efferents. J Urol 2008;180:1167-1172.

18 de Groat WC, Booth AM. Neural control of penile erection. In: Maggi CA, ed. Nervous Control of the Urogenital system. Luxembourg, Harwood, 1993, p 467-524.

19 Chapelle PA, Durand J, Lacert P. Penile erection following complete spinal cord injury in man. Br J Urol 1980;52:216-219.

20 Sipski ML, Rosen RC, Alexander CJ, Gomez-Marin O. Sexual responsiveness in women with spinal cord injuries: differential effects of anxiety-eliciting stimulation. Arch Sex Behav 2004;33:295-302.

21 Salonia A, Giraldi A, Chivers ML, Georgiadis JR, Levin R, Maravilla KR, McCarthy MM. Physiology of Sex Med. 2010;7: 2637-2660.

22 Jänig W, McLachlan EM. Organization of lumbar spinal outflow to distal colon and pelvic organs. Physiol Rev 1987;67:1332-1404.

23 Berkley KJ, Robbins A, Sato Y. Functional difference between afferent fibers in the hypogastric and pelvic nerves innervation female reproductive organs in the rat. J Neurophysiol 1993;69(2):533-544.

24 McLachlan EM. The components of the hypogastric nerve in male and female guinea-pigs. J Auton Nerv Syst 1985;13:327-342.

25 Jänig W, Koltzenburg M. Pain arising from the urogenital tract. In: Nervous Control of the Urogenital System. Ed. Maggi CA, Luxembourg, Harwood, 1993;p 525-578

26 Wiedey J, Alexander MS, Marson L. Spinal neurons activated in response to pudendal or pelvic nerve stimulation in female rats. Brain Res 2008;1197:106-114.

27 Traub RJ, Murphy A. Colonic inflammation induces fos expression in the thoracolumbar spinal cord increasing activity in the spinoparabrachial pathway. Pain 2002;95:93-102.

28 McKenna KE, Chung SK, McVary KT. A model for the study of sexual function in anaesthetised male and female rats. Am J Physiol 1991;261:R1276-1285.

29 Giuliano F, Allard J, Compagnie S, Alexandre L, Droupy S, Bernabe J. Vaginal physiological changes in a model of sexual arousal in anaesthetised rats. Am J Physiol Regul Integr Comp Physiol 2001;281:R140-R149.

30 Marson L, Cai R, Makhanova N. Identification of spinal neurons involved in the urethrogenital reflex in the female rat. J Comp Neurol 2003;462:355-370.

31 Yuan SY, Costa M. Brookes SJH. Neuronal control of the gastric sling muscle of the guinea pig. J Comp Neurol 1999;412:669-680.

32 Kanno Y. Gross and light microscopic observations of the pudendal nerve of the guinea pig. Hokkaido Igaku Zasshi 1976;51(5):383-407.

33 Costa M, Furness JB. Observations on the anatomy and amine histochemistry of the nerves and ganglia which supply the pelvic viscera and on the associated chromaffin tissue in the guinea-pig. Z Anat Entwicklungsgesch 1973;140:85-108.

34 Anderson MF, Sims NR. Mitochodrial respiratory function and cell death in focal cerebral ischemia. J Neurochem 1999;73:1189-1199.

35 Bederson JB, Pitts LH, Germano SM, Nishimura MC, Davis RL, Bartkowski HM. Evaluation of 2,3,5-trophenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. Stroke 1986;17:1304-1308.

36 de Groat WC, Booth AM, Yoshimura N. Neurophysiology of micturition and its modification in animal models of human disease. In: Maggi CA, ed. Nervous Control of the Urogenital System. Luxembourg, Harwood, 1993, p 227-290.

37 Meston CM, Gorzalka BB. The effects of sympathetic activation on physiological and subjective sexual arousal in women. Behav Res Ther 1995;33:651-664.

38 Papka RE. Sensory ganglia. In: Squire L, ed. Encyclopedia of Neuroscience, San Diego, Academic Press, 2009, p 657-668.

FIGURE LEGENDS

Figure 1: Diagram of the preparation and experimental arrangement for electrical stimulation and recording to test for a descending lumbar spinal-hypogastric pathway from supra-spinal levels (A), a pudendal-spinal-pelvic pathway (B), a pudendal-spinal-hypogastric pathway (C) and the preganglionic nature of L3 spinal output to hypogastric nerve (D) in female guinea-pigs. SG: sympathetic chain ganglia. IMG: inferior mesenteric ganglion. S: sacral spinal cord. L: lumbar spinal cord.

Figure 2: Electrical stimulation of T12-T13 spinal cord (A) or pudendal nerves (B) (0.3 ms, 50 V, single pulse) evoked compound action potentials in the hypogastric nerve (Aa) or pelvic nerves (Ba). All responses occur with a delay of 30-50 ms after the stimulation artifact. Evoked responses were reproducible and abolished by transecting the lumbar splanchnic nerves at L3 (Ab) or the pudendal nerves at S3 ipsilateral to the stimulation site (Bb) (n=6). Ac and Bc represent corresponding electrical stimulation time-points. Vertical calibration bars: 500 μV in A and 400 μV in B; Horizontal bars: 100 ms. C: group data (n=6) for experiments in A. D: group data (n=6) for experiments in B. White bars: controls; black bars: after transection.

Figure 3: Compound action potentials (arrows) recorded extracellularly from splanchnic (A) and hypogastric (B) nerves following stimulation of pudendal nerves with 1, 5 or 10 pulses. Selective $GABA_A$ receptor antagonist, bicuculline, significantly increased the amplitude of evoked responses at both recording sites when compared with controls (n=7). C: Electrical stimulation time-points. Vertical calibration bar: 200 μV for all responses; Horizontal bar: 50 ms for 1 and 5 pulses, 100 ms for 10 pulses.

Figure 4: Maximum amplitudes of compound action potentials (mean ± S.E) recorded from hypogastric and splanchnic nerves during electrical stimulation of the pudendal nerve. Multiple pulses evoke responses with significantly larger amplitude responses (ANOVA, effect of pulse number, $p<0.001$). Bicuculline (10μM) in the perfusate for 20 minutes increased amplitudes of evoked responses significantly in both pathways (ANOVA, effect of bicuculline, $p < 0.001$, $n = 7$). The magnitude of the increase was similar in each pathway (no significant interaction between bicuculline and pathway). White bars: control; Filled bars: after addition of bicuculline.

Figure 5: Effect of nicotinic receptor antagonist, hexamethonium (200 μM) on responses to electrical activation of lumbar splanchnic-hypogastric (A) and pudendal-spinal-hypogastric (B) pathways. Compound action potentials (arrows) were recorded extracellularly at distal ends of hypogastric nerves during electrical activation of splanchnic nerves at L3 (A) or pudendal nerves (B) respectively with different pulses (0.3 ms, 50 V, 200 Hz) in the presence (B) or absence (A) of $GABA_A$ receptor antagonist, bicuculline (10 μM) (Left: recording traces; Right: group data, $n = 4$ for both A and B). After application of hexamethonium for 15-30 minutes the responses showed no significant change, indicating that there were no nicotinic synapses in the pathway (A and B). Further addition of tetrodotoxin (1 μM) blocked the responses to pudendal nerve activation (B). White bars: control; black bars: after hexamethonium. Calibration bars: horizontal 50 ms, vertical 200 μV for A and 100 for B. EST: electrical stimulation time-points.

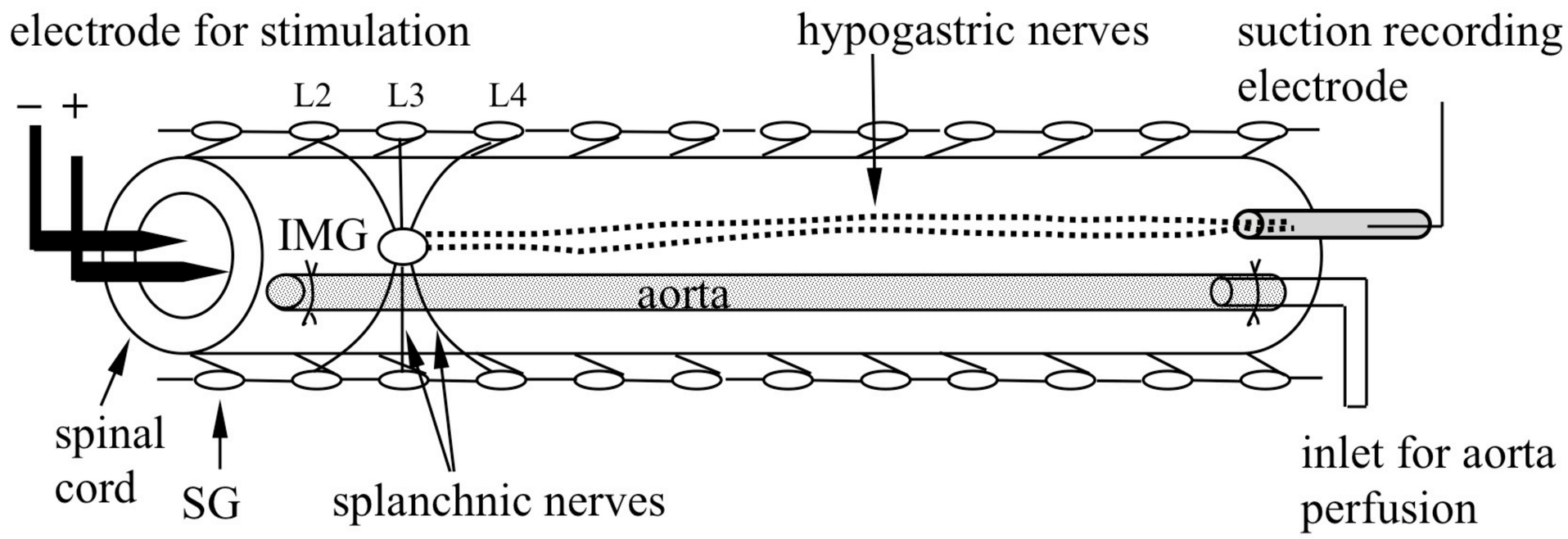
Figure 6: Confocal images of DiI labeled DRG sensory neurons at S3 with double immunohistochemical labeling for SP and CGRP. There are four classes of labeled neurons:

DiI alone (red arrows); DiI+CGRP (white arrows); DiI+SP (green arrows); DII+CGRP+SP (yellow arrows). Each image is the projection of 24 optical sections at an interval of 2 µm. Calibration bar: 75 µm.

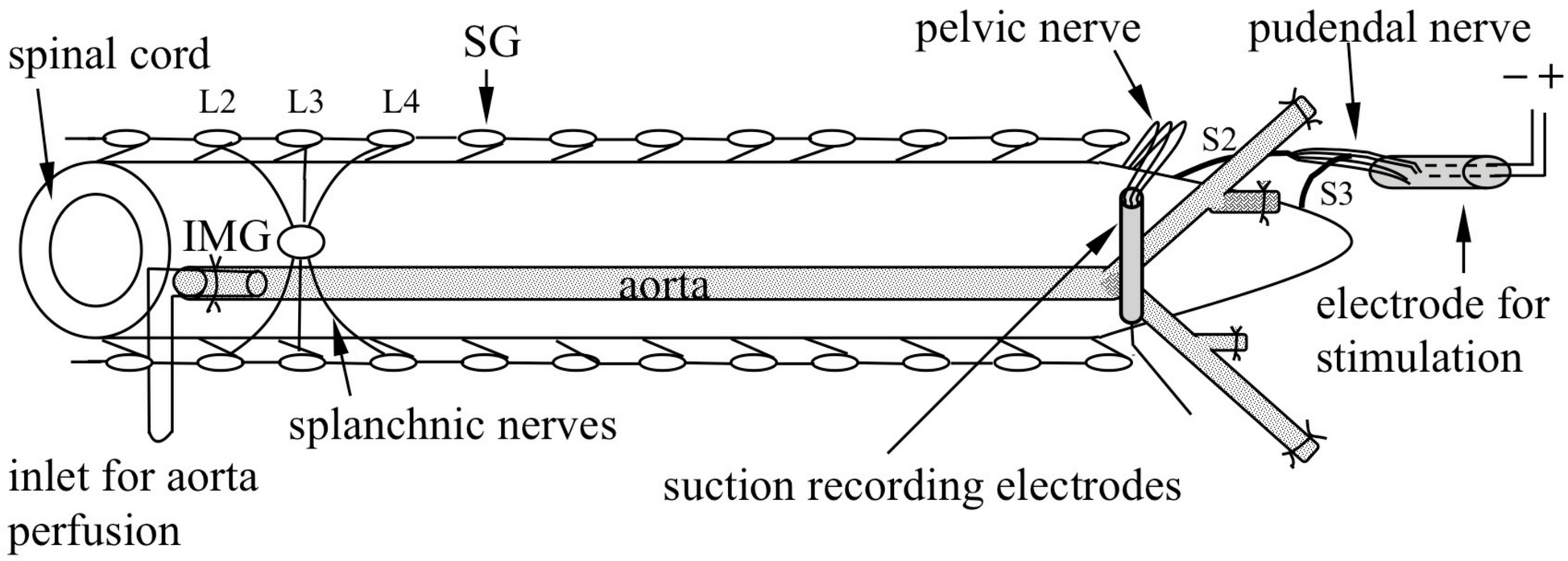
Figure 7: Summary of spinal pathways projecting to the paracervical ganglia (PG) of female guinea-pigs examined in this study. Descending spinal and ascending pudendal-lumbar spinal pathways activate sympathetic preganglionic neurons projecting via L3 lumbar splanchnic nerves (LSN) and hypogastric nerves (HN) to the paracervical ganglia. Sacral spinal output to the paracervical ganglia is via parasympathetic preganglionic neurons projecting out the pelvic nerve (PN, thick black arrows). IMG, inferior mesenteric ganglion.

Figure 1.

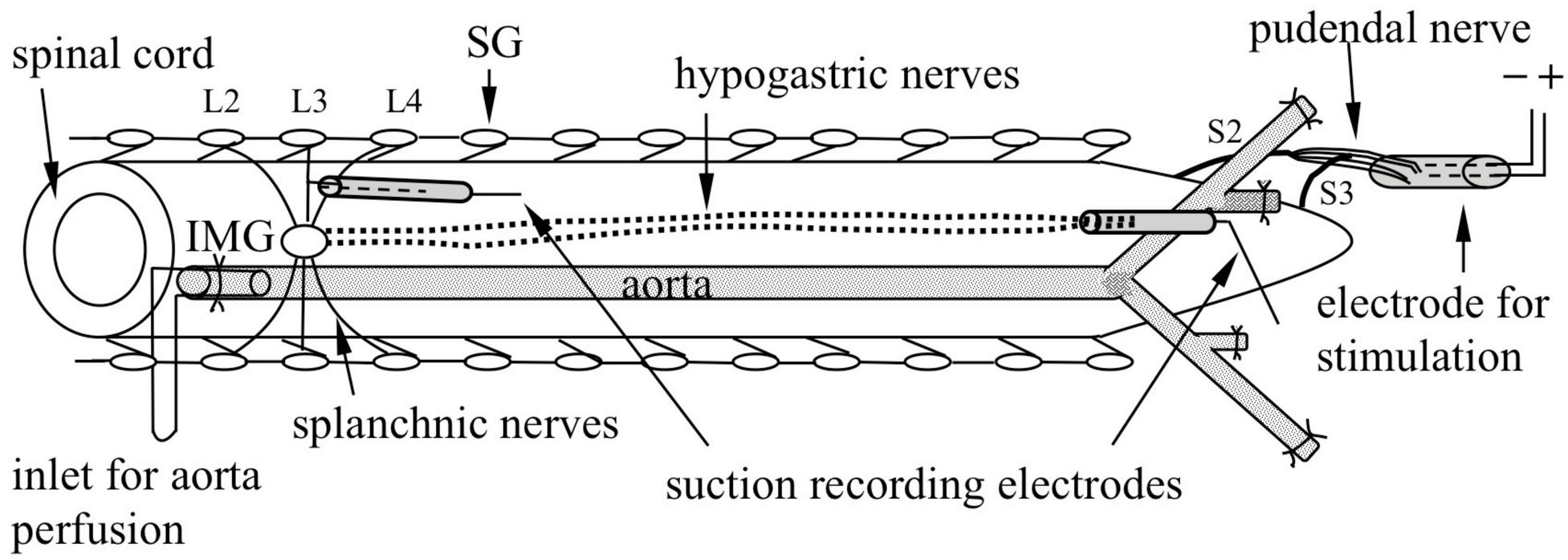
A.



B.



C.



D.

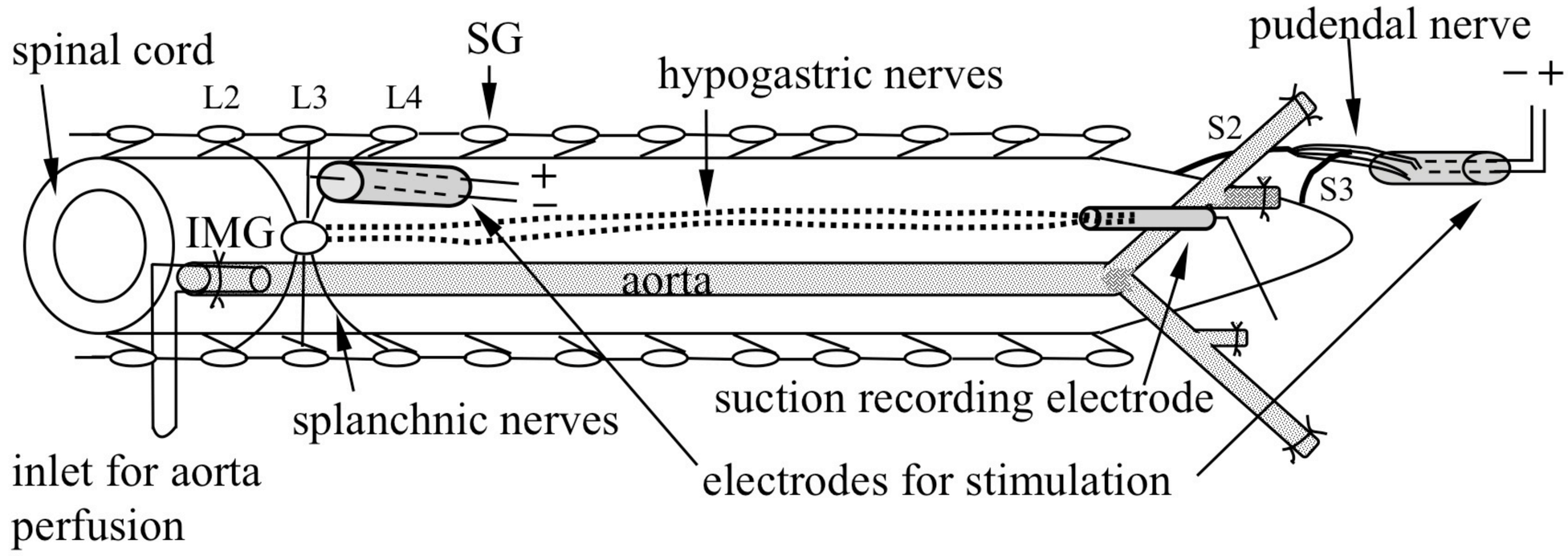
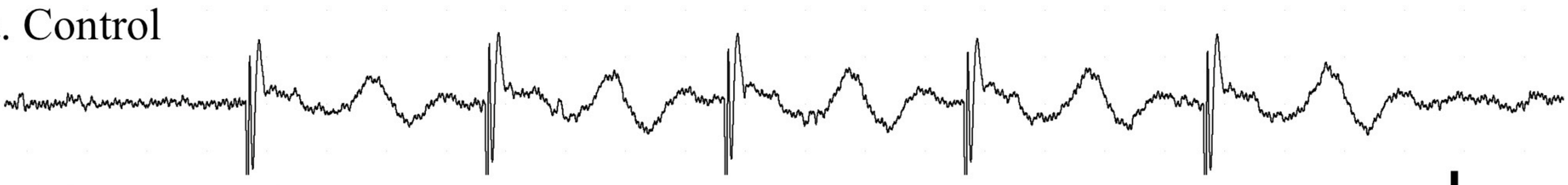


Figure 2.

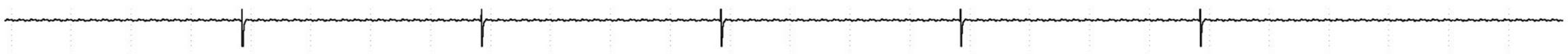
A.

a. Control



b. After transection

c. Electrical stimulation



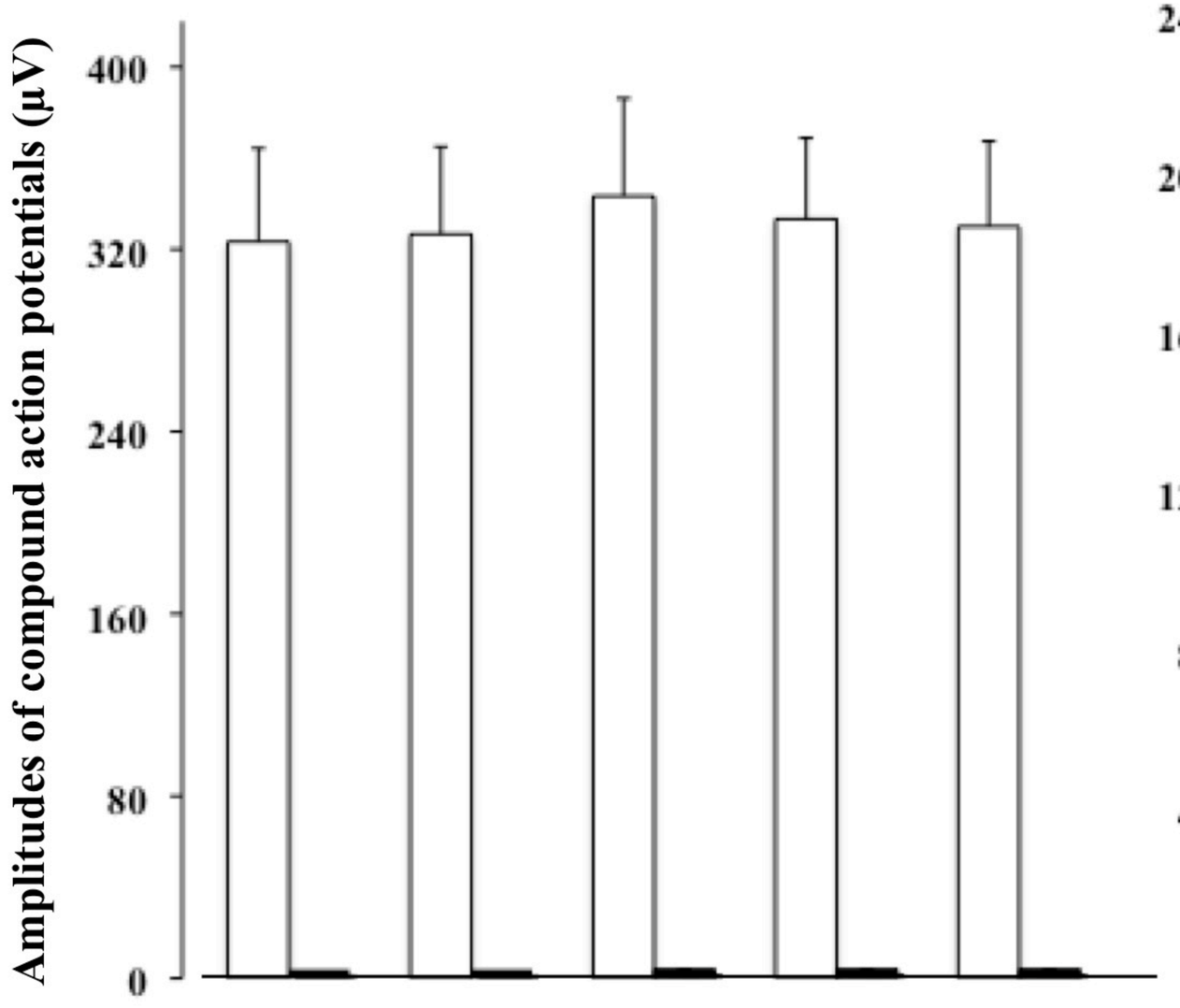
B.

a. Control

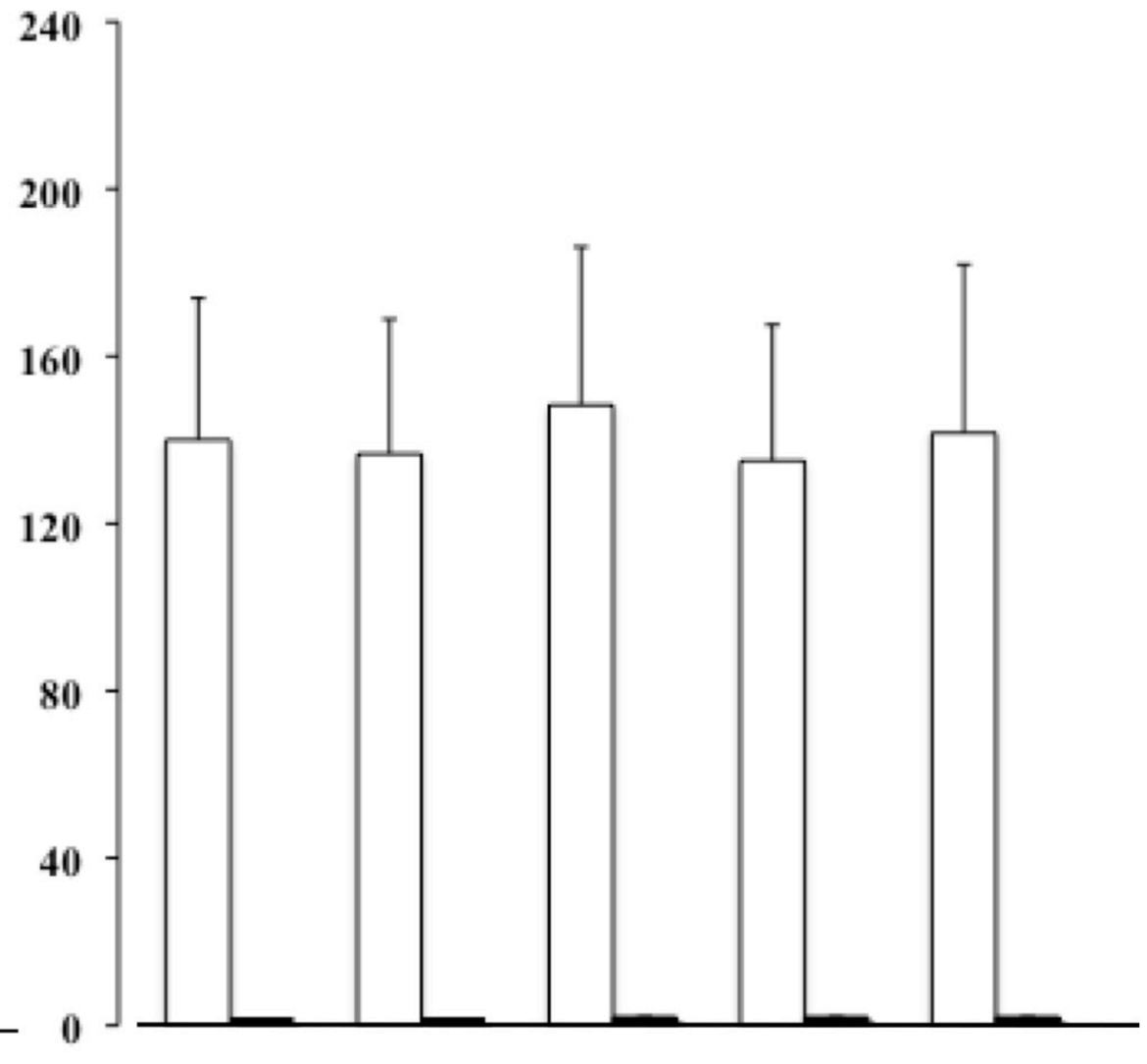
b. After transection

c. Electrical stimulation

C.

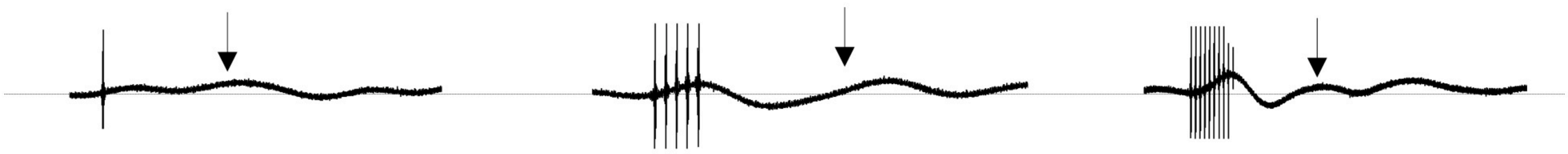


D.

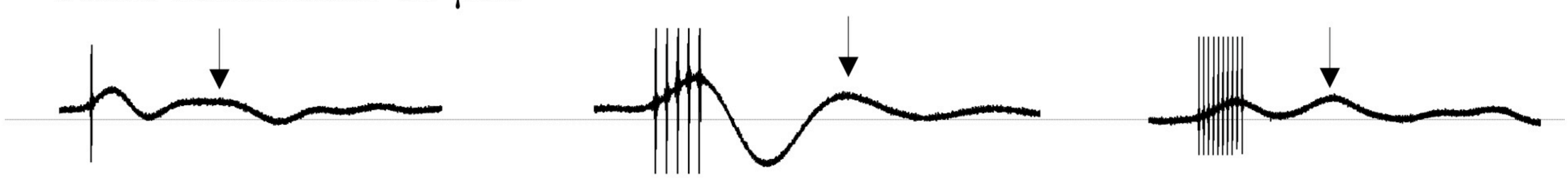


A. Splanchnic nerves

Control



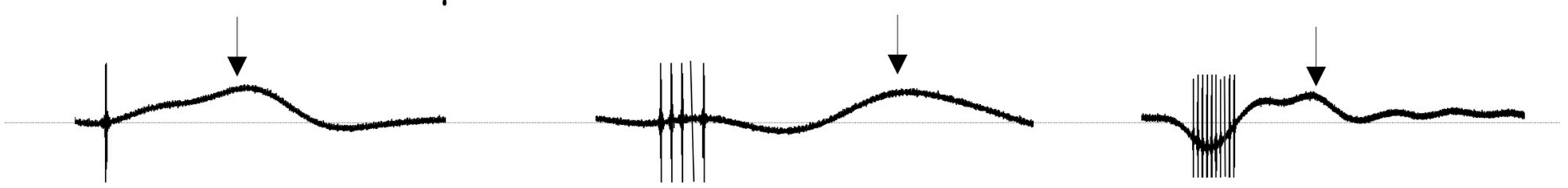
After bicuculline 10 μM



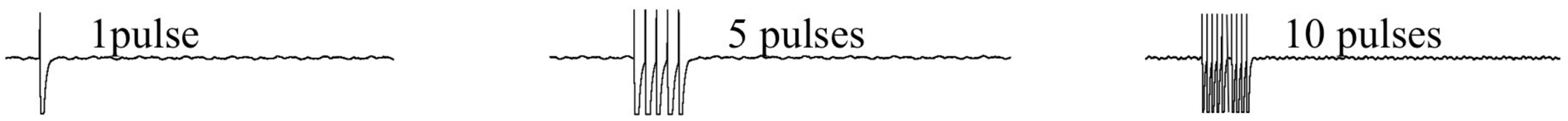
B. Hypogastric nerves

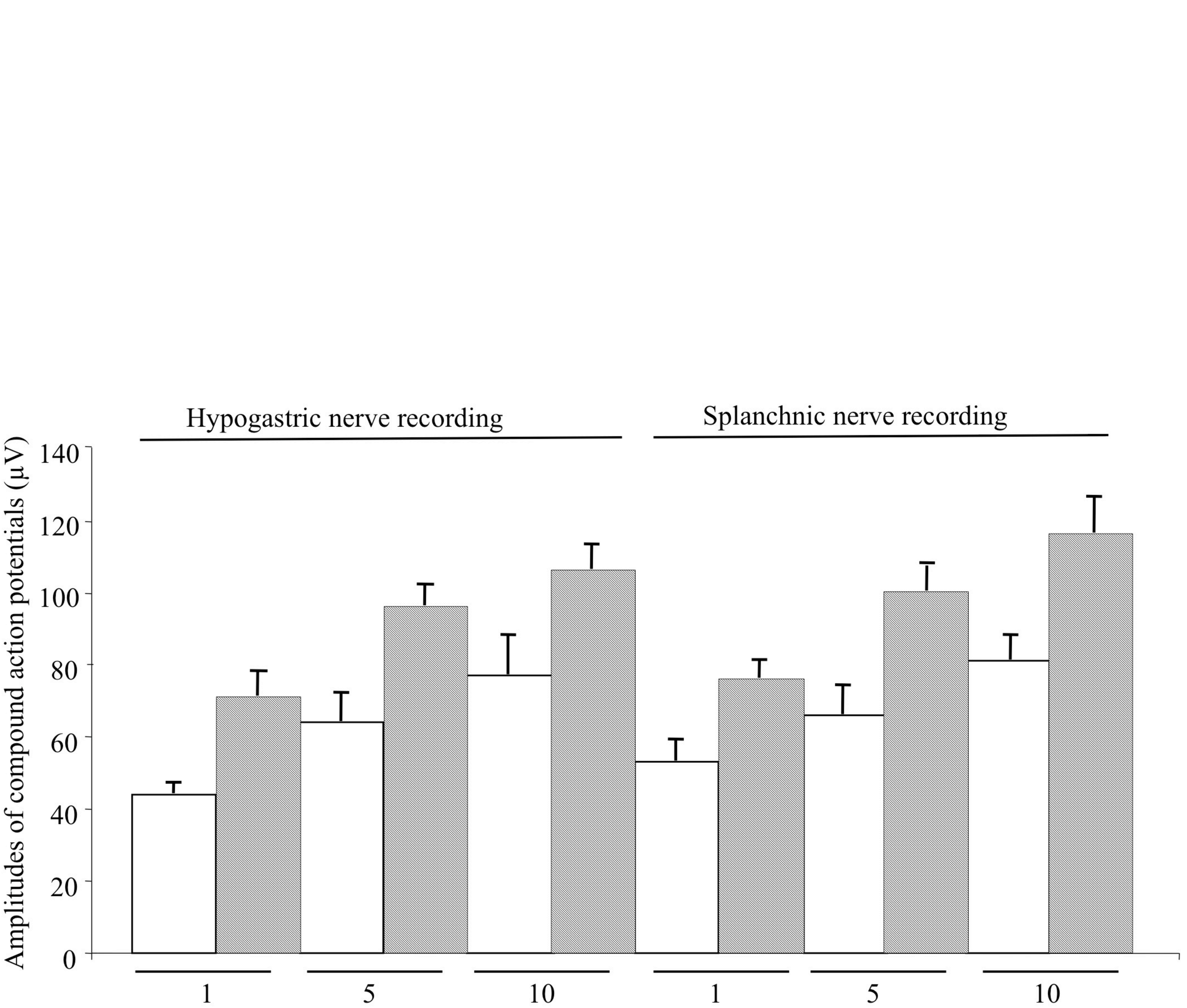
Control

After bicuculline 10 μM

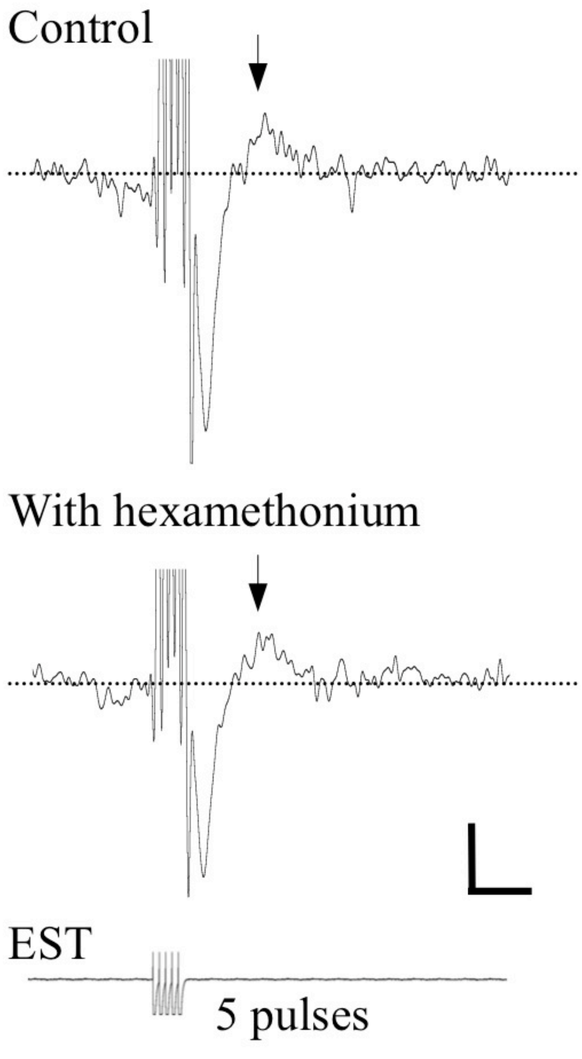


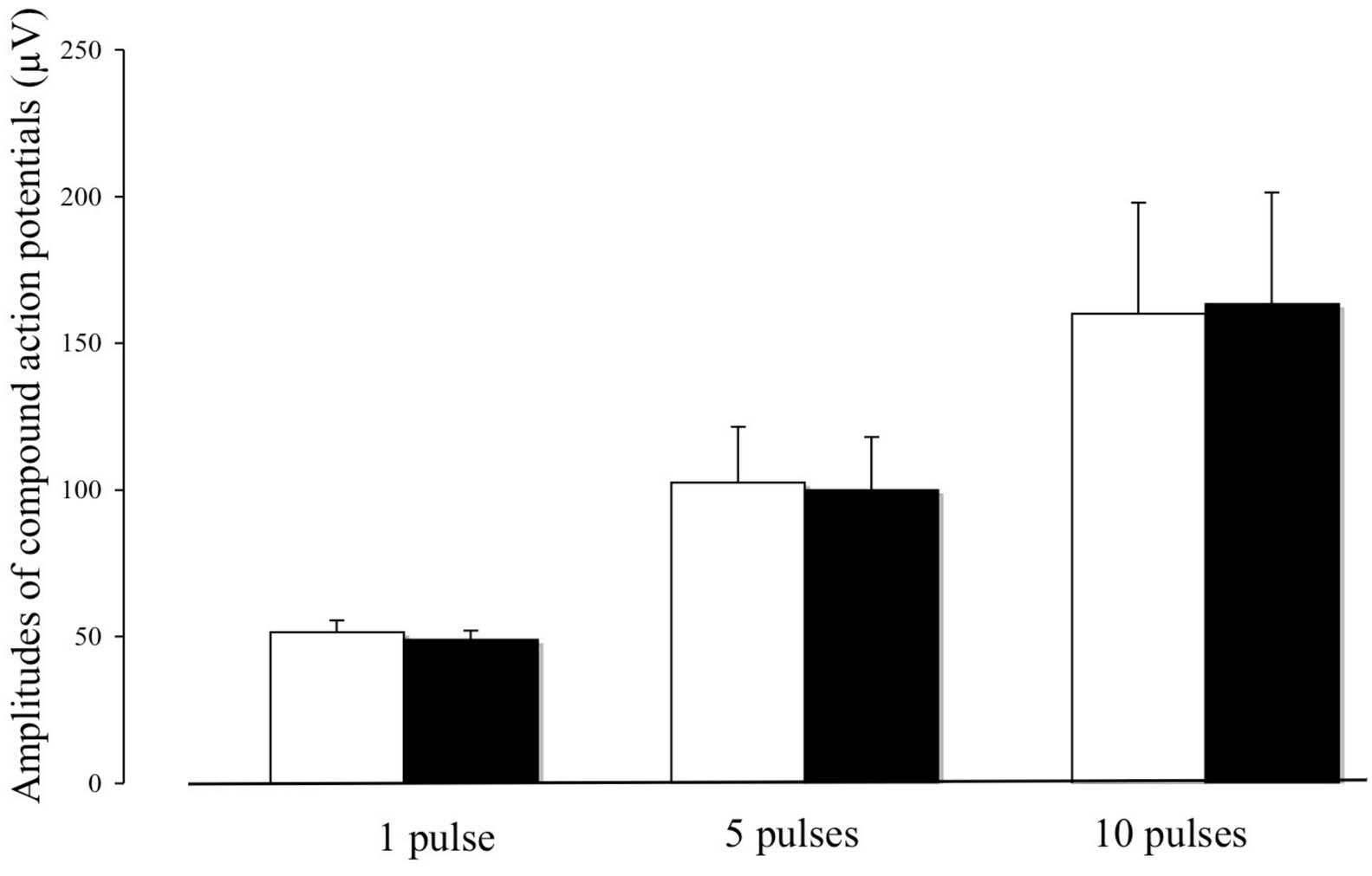
C. Electrical stimulation



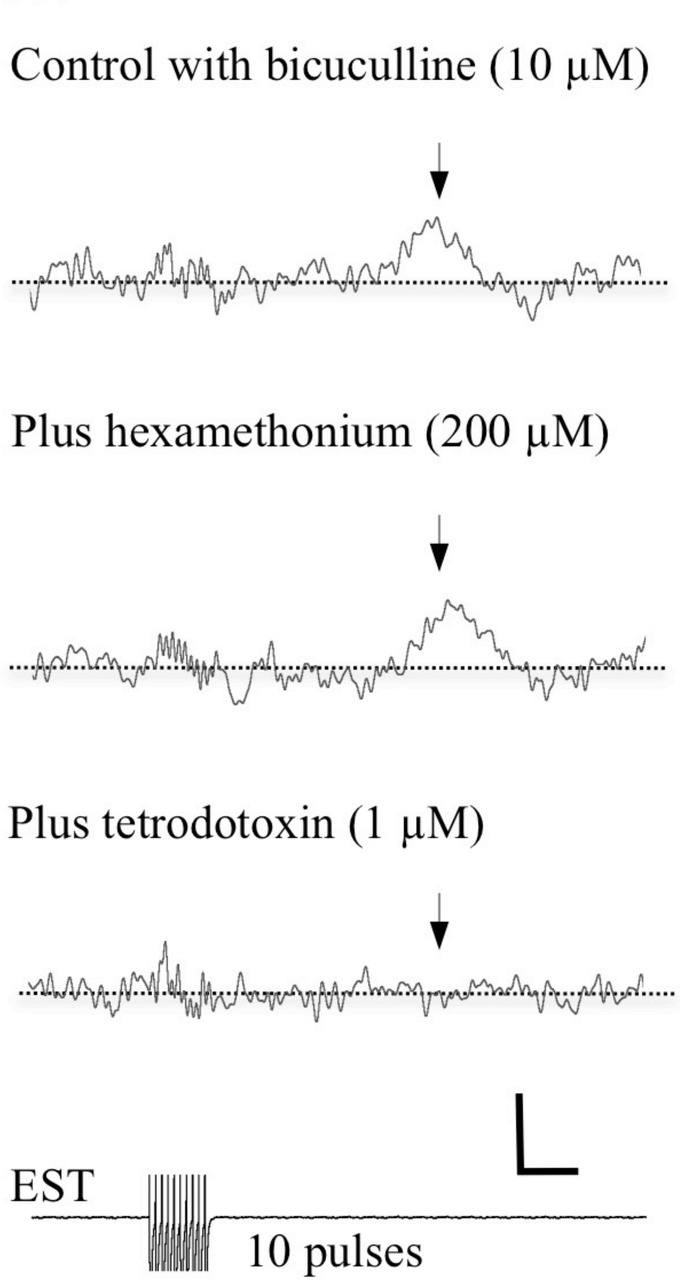

Hypogastric nerve recording
Splanchnic nerve recording
Amplitudes of compound action potentials (μV)
140
120
100
80
60
40
20
0
1
5
10
1
5
10
Number of stimulating pulses

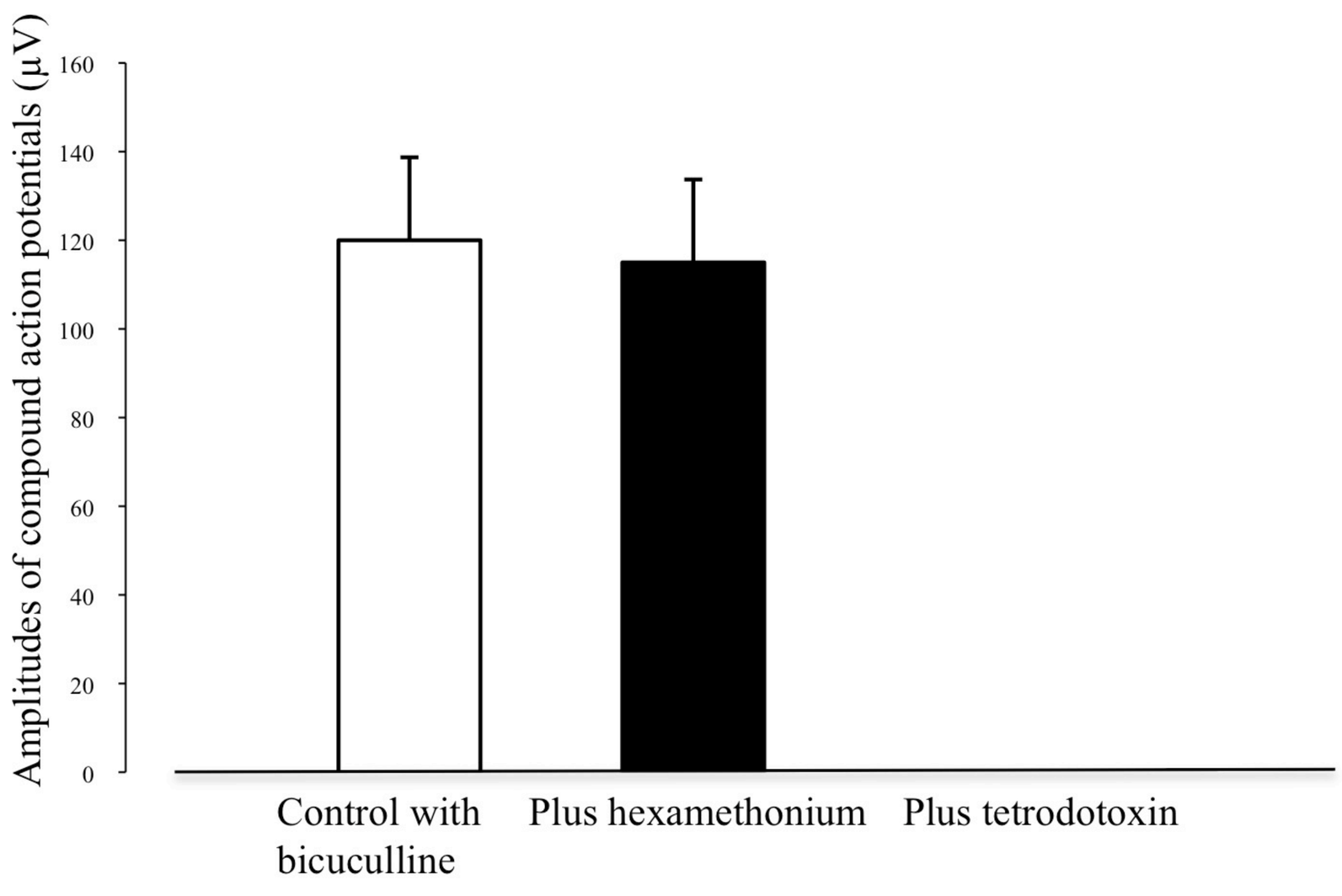
A.

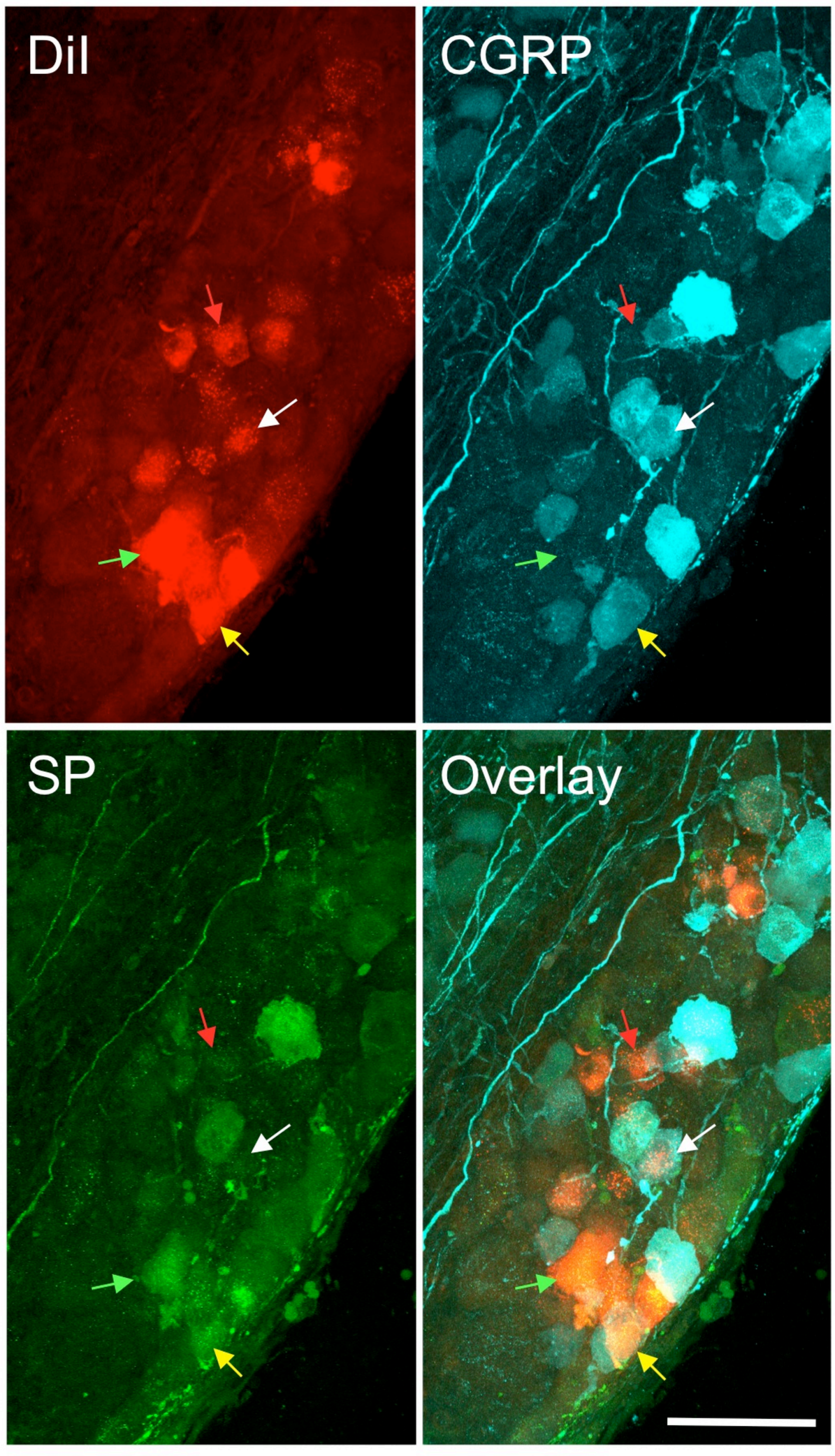

Control
With hexamethonium
EST
5 pulses

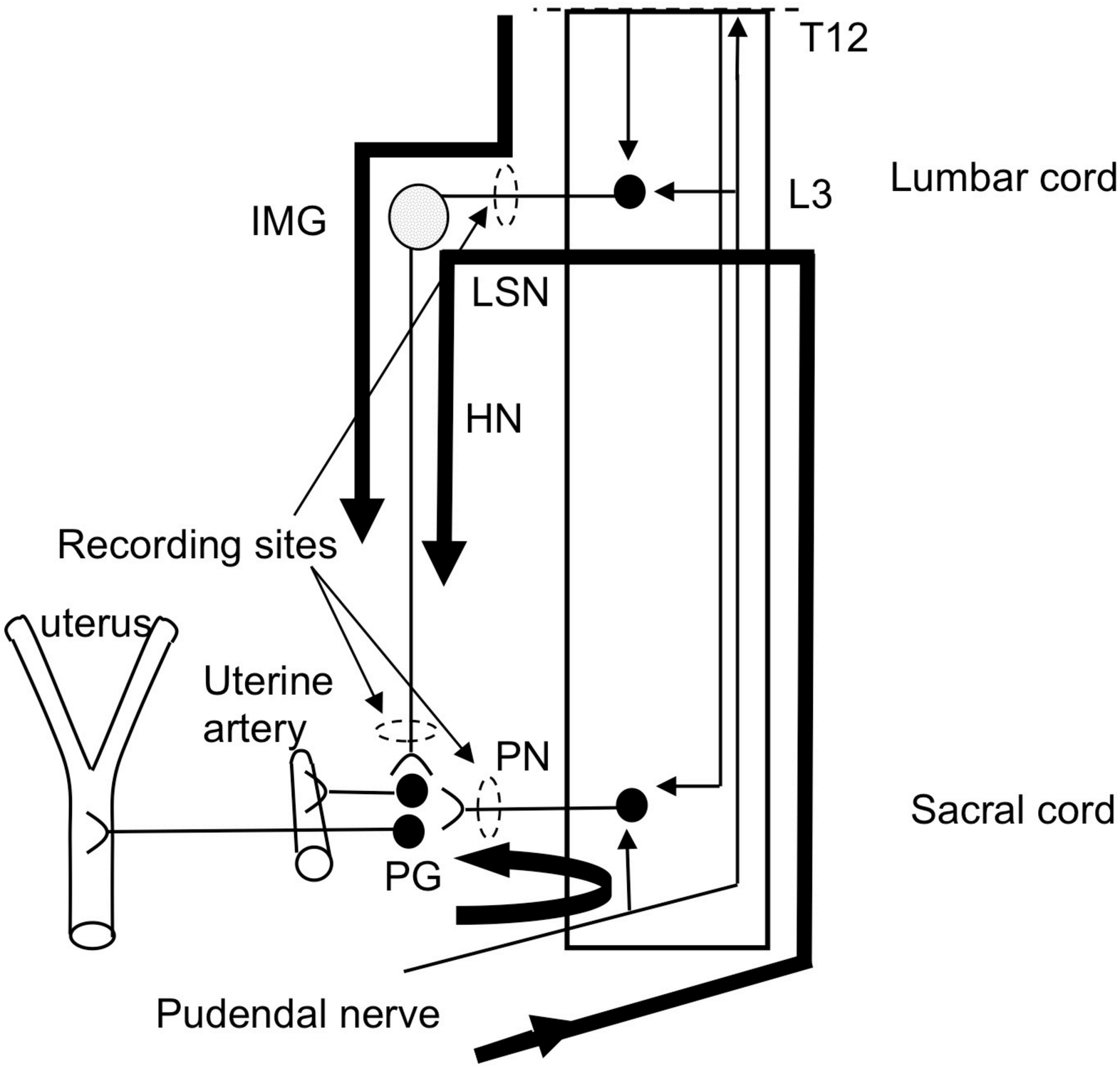

Amplitudes of compound action potentials (μV)
250
200
150
100
50
0
1 pulse
5 pulses
10 pulses

B.


Control with bicuculline (10 μM)
Plus hexamethonium (200 μM)
Plus tetrodotoxin (1 μM)
EST
10 pulses


Amplitudes of compound action potentials (μV)
160
140
120
100
80
60
40
20
0
Control with bicuculline
Plus hexamethonium
Plus tetrodotoxin


DiI
CGRP
SP
Overlay


T12
L3
Lumbar cord
IMG
LSN
HN
Recording sites
uterus
Uterine
artery
PN
Sacral cord
PG
Pudendal nerve