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Mid-lumbar (L3) epidural stimulation effects on bladder and external urethral sphincter in non-injured and chronically transected urethane-anesthetized rats V.2

Daniel Medina Aguinaga[1], Robert Hoey[1], Natasha L. Wilkins[1], Beatrice Ugiliweneza[2,3,4], Jason Fell[1], Susan J. Harkema[2,3], Charles H. Hubscher[1,3]

[1]Department of Anatomical Sciences and Neurobiology, University of Louisville School of Medicine;
[2]Department of Neurological Surgery, University of Louisville School of Medicine, Louisville, KY, USA;
[3]Kentucky Spinal Cord Injury Research Center, University of Louisville, Louisville, KY, USA;
[4]Department of Health Management and Systems Science, School of Public Health and Information Science, University of Louisville, Louisville, KY, USA

SPARC
Tech. support email: **info@neuinfo.org**

Daniel Medina Aguinaga

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We use this protocol and it's working

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ABSTRACT

Current experiments are utilizing epidural stimulation, and the measurement of numerous systems, after either sham transection or full T9 transection. Transected Wistar rats (male and female) are tested after 6 weeks of recovery. Initial mapping experiments have targeted the L3 segment of the spinal cord for epidural stimulation. The testing paradigm subjects this segment of the spinal cord to combinations of frequency (5, 10, 30, 45, 60 Hz) and intensity (50, 75, 100, 150, 300µA) to determine the most effective stimulus parameters for external urethral sphincter modulation. Endpoints include: rectal and distal colon pressure activity, external urethral sphincter electromyography (EUS EMG), external anal sphincter electromyography (EAS EMG), bladder pressure changes, voiding and drop patterns, volume of voided fluid, and muscle movement thresholds. By recording these responses, and the changes due to stimulation, these experiments will provide insight into the best stimulus parameters to influence bladder activity after spinal cord injury.

Before starting

Prior to terminal testing procedures, animals are implanted with: a jugular catheter, a tracheal cannula, a bladder catheter, bilateral fine wire electrodes in the External Urethral Sphincter, and a modified Medtronic 5-6-5 array electrode.

MATERIALS

	A	B	C	D
	ITEM	Company	catalog number	website link
	ketamine	Dechra Vet Products	VINV-CIII-0016	https://northamerica.covetrus.com/Product?sku=080524
	xylazine	Covetrus	1XYL006	https://northamerica.covetrus.com/Product?sku=080896
	4–0 suture	Ethicon	1629H	https://northamerica.covetrus.com/Product?sku=034049

	A	B	C	D
	wound clips (9 mm autoclip)	MikRon Precision	ACS- CS	https://www.braintreesci.com/surgical-tools-supplies/wound-closure/appliers-removers-clips/9mm-autoclip/
	Meloxicam	Covetrus	6451603845	https://northamerica.covetrus.com/Product?sku=049756
	Gentafuse, gentamicin	Covetrus	VINB-0069-1300	https://northamerica.covetrus.com/Product?sku=006913
	penicilin G	Bimeda	1PRO304	https://northamerica.covetrus.com/Product?sku=069322
	Isoflurane	Covetrus	11695067772	https://northamerica.covetrus.com/Product?sku=029405
	urethane	Thermo Scientific Chemicals	AC325542500	https://www.fishersci.com/shop/products/urethane-97-thermo-scientific/AC325542500#?keyword=urethane
	water-heated pad	Gaymar	TP-700	https://www.braintreesci.com/temperature-measurement-control/heat-therapy-pumps-pads/gaymar-heating-cooling-t-pump-back-in-stock/
	PE-60 tubing	BD Intramedic‰ PE Tubing	BD 427416	https://www.fishersci.com/shop/products/intramedic-pe-tubing-16/1417012C?keyword=true
	electrodes (0.002" diameter, stainless steel)	A-M Systems	790500	https://www.a-msystems.com/p-809-pfa-coated-stainless-steel-wire.aspx
	multi-electrode epidural stimulation array (Specify 5–6–5)	(Medtronic, Minneapolis, MN)	multi-electrode epidural stimulation array (Specify 5–6–5)	https://www.medtronic.com/us-en/healthcare-professionals/services/innovation-lab.html

	A	B	C	D
	infussion pump	Braintree Scientific, Braintree, MA, USA	BS-300	https://www.braintreesci.com/syringe-surgical-pumps/programmable-syringe-pumps/just-infusion-syringe-pump/
	BLPR2 Transducer	World Precision Instruments [WPI, LLC]; Sarasota, FL, USA	503067	https://www.wpiinc.com/503067-blpr2-transducer-without-cable.html
	differential amplifier	A-M Systems, Sequim, WA, USA	Model 3000 AC/DC Differential Amplifier	https://www.a-msystems.com/p-254-model-3000-acdc-differential-amplifier.aspx
	balance	Research Products International Corp (Ohaus)	SPX123	https://www.fishersci.com/shop/products/ohaus-scout-spx-spx123/502114168#?keyword=ohaus%20scout%20spx123
	USB device interface cable (RS232)	StarTech	ICUSB232PROC	https://www.staples.com/StarTech-USB-C-to-Serial-Adapter-with-COM-Port-Retention-USB-C-to-DB9-Cable-or-RS232-Cable-ICUSB232PROC/product_IM12DG846
	software for balance	Research Products International Corp (Ohaus)	Serial Port Data Collection, SPDC, Ohaus, V2.01	https://us.ohaus.com/en-us/support/software-and-drivers
	FORT100 weight transducer	WPI, LLC	FORT100	https://www.wpiinc.com/var-2858-large-fort-force-transducer.html
	Transbridge 4 M amplifier	WPI LLC	SYS-TBM4M	https://www.wpiinc.com/sys-tbm4m-4-channel-transducer-amplifier.html
	Software to record data	Cambridge Electronic Device	Spike 2, V8.21	https://ced.co.uk/products/spkovin
	CED Mircro3 1401 unit	Cambridge Electronic Device	Micro3 1401 unit	https://ced.co.uk/products/mic4in
	4-channel differential AC amplifier	AM-Systems, model 1700	Model 1700 Differential AC Amplifier	https://www.a-msystems.com/p-202-model-1700-differential-ac-amplifier.aspx
	Pressure probes for anorectal manometry Millar SPR-524	AD Instruments	SPR-524	https://www.adinstruments.com/products/pressure-catheters

Spinal cord transections

1 Under aseptic conditions, the body temperature was maintained within the range of 36–37°C via a warm water recirculator.

2 All animals were anesthetized with a mix of ketamine/xylazine (80/10 mg/kg, intraperitoneally).

3 A dorsal longitudinal incision was made to expose the T7 vertebra, and a laminectomy was made to expose the T8 spinal cord. The dura mater was lateralized and the spinal cord was transected using surgical scissors. Gelfoam was placed in the lesion cavity as hemostatic.

4 The muscular layer and the skin were closed with wound clips (Mikrotek, 9 mm autoclip), and the animals were allowed to recover from anesthesia.

Post-surgical care

5 Pain medication (Meloxicam,1/day for 3 days intramuscular) and antibiotics (Gentafuse, gentamicin, 1/day for 3 days; Penject, penicillin G, 1/day for 3 days, subcutaneous) were applied to all subjects.

6 After surgery, the rat's urinary bladder was manually emptied 3/day until voiding reflexively.

Terminal mapping - Anesthesia and airway care

7 Animals were initially anesthetized with Isoflurane (3l/min 5% for induction, and 2% for maintenance) and placed in supine on a surgical table conditioned with a water-heated pad (Gaymar) to maintain body temperature.

8 A sagittal mid-line incision was made in the neck to expose the jugular vein and the trachea.

9 A PE-60 (Intramedic, Clay Adams) jugular catheter was inserted into the vessel and secured with silk suture to urethane infusion.

10 A polyethylene tubing was placed into the trachea to ensure airway opening.

11 Anesthesia was switched from isoflurane to 50% urethane solution (1.2 g/kg), reducing the isoflurane percentage and slowly infusing the urethane, maintaining continuous surgical depth of anesthesia.

12 The skin was closed with a silk suture securing the tracheal and jugular catheters with the same suture.

Terminal mapping - Electromyography and cystometrogram s...

13 The urinary bladder was exposed by a midline abdominal incision and a PE-60 tubing with a heat-flared end) inserted into the vesical lumen through a dome incision and secured with a collar of silk suture.

14 The external urethral sphincter (EUS) was exposed and implanted bilaterally with two thin wires (A-M Systems, 0.002" diameter, stainless steel). A third electrode (A-M Systems, 0.003" diameter,

stainless steel) was inserted into the abdominal wall as a reference.

15 The bladder catheter and electrodes were tunneled and exteriorized through the skin on the back of the neck.

16 The muscular abdominal wall and the skin were closed with a silk suture.

Terminal mapping - Epidural electrode implant

17 The spinal cord was exposed by a midline abdominal incision and quadruple laminectomy. A modified multi-electrode epidural stimulation array (Specify 5–6–5, Medtronic, Minneapolis, MN) modified for use as one electrode row was placed on the epidural surface, one over L3-L2 segment.

18 The muscular layer and the skin were closed with a silk suture and the wire of the electrode was secured with the same suture.

Terminal mapping - Mapping procedure

19 The rat is placed on their ventrum throughout testing. The hindlimbs are taped down to the platform as the electrical stimulation can cause motor movements that may move the animal out of position. The tail is held upright and out of line of sight by a movable arm anchored to the table by a magnet.

20 Bilateral fine wire electrodes are implanted into the external anal sphincter (EAS), using 27g needles. EAS electrodes are implanted at an oblique angle so as to travel from midline to lateral aspect of the sphincter.

21 SPR-524 pressure sensors (AD instruments) are inserted into the rectum (2 cm from anal verge) and the distal colon (10 cm from the anal verge) and secured to the base of the tail using tape. These probes have their own control unit which is then fed into our data acquisition unit.

22 A perfusion pump is connected to the urinary bladder catheter hub and set to deliver saline at a rate of 0.25 ml per minute. The pump syringe has a pressure sensor attached so that pressures in the bladder can be detected during filling. A 60 ml syringe is used to ensure enough saline for the entire testing procedure.

23 All wire electrodes (ground wire, bilateral EUS, and EAS) are connected to wires fitted with copper duck bill clip connectors. The end of the electrode wire is borne and clipped with the connectors. Only strip as much insulation as necessary for a good hold by the clip, as any extra wire with cause noise in the signal. Electrode wires are amplified (A-M Systems, 4 channel, differential amplifier) and then sent to the data acquisition unit.

24 A balance (OhausScout) is placed underneath the surgical platform to collect voided material and to relay that information to our acquisition computer via RS-232 connector and a Serial Port Data Collect (SPDC) software.

25 The animal, perfusion pump, and table are grounded to the electrophysiology cabinet containing the stimulator and associated electrical components.

26 Data acquisition unit is a CED 1401 micro 3 system. The software used is Spike 2 version 8.

27 Spike 2 is opened and a configuration file is loaded that contains the setup for all of the channels being recorded (EUS, EAS, 2 cm probe, 10 cm probe, leaks, stim marker, keyboard input).

28 Electrical stimulation equipment is connected to the Medtronic interface which controls the electrode implant. A grass stimulator (S88) with a current isolation unit provides the electrical stimulation.

29 Once the animal has all necessary components set up, the acquisition software starts recording. Shortly afterward, the perfusion pump is turned on and bladder pressure begins to rise.

30 The animal is allowed to have several fill-void cycles until there is a consistent time in between voids.

31 Five baseline periods of activity are collected. A timer is used to ensure 2 minute baseline period measurements.

32 After the baseline periods, the stimulation is turned on for either: 2 minutes (if fill-void cycle is shorter than 2 minutes or there is a dripping pattern) or until one void occurs (with a longer than 2-minute interval).

33 Stimulation parameters are changed after each presentation.

- Frequency parameter: 5, 10, 30, 45, 60 Hz.
- Intensity parameter: 50, 75, 100, 150, 300, 500 μA.

The frequency-intensity pairings are presented in an increasing fashion.

- If frequency is varied first, the stimulations would proceed: 5 Hz 50 μA, 10 Hz 50 μA, etc. through all frequency-intensity pairings.
- If intensity is varied first, the stimulations would proceed: 5 Hz 50 μA, 5 Hz 75 μA, etc.

Each stimulation is followed by an off period of 2 minutes to allow for any residual energy to dissipate and the system to return to baseline.

34 During this period, data is collected on bowel function (rectal and distal colon), urethral sphincter activity (EUS EMG), external anal sphincter activity (EAS EMG), bladder pressure, when urine is expelled via marker button, exact electrical stimulation markers, volume of urine voided, and any notes made via keyboard input.