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GENERAL INFORMATION
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1. Title of Dataset: Nerve transfer for restoration of lower motor neuron-lesioned bladder, urethral and anal sphincter function. Part 4: Effectiveness of the motor reinnervation

2. Author Information:

Principal Investigator Contact Information
Name: Mary F Barbe, PhD
Institution: Aging + Cardiovascular Discovery Center at the Lewis Katz School of Medicine, Temple University
Address: 3500 North Broad Street,
Philadelphia, Pennsylvania, United States of America
Email: mary.barbe@temple.edu
ORCID: 0000-0002-5235-9803

3. Date of data collection (single date, range, approximate date): 2016-2023

4. Geographic location of data collection: < Philadelphia, Pennsylvania, United States of America >

5. Information about funding sources or sponsorship that supported the collection of the data:
Research reported in this publication was supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number R01NS070267 to Dr. Mary F. Barbe.

SHARING/ACCESS INFORMATION
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1. Licenses/restrictions placed on the data, or limitations of reuse: Creative Commons BY license

2. Recommended citation for the data:

In Press. Barbe, MF et al. Nerve transfer for restoration of lower motor neuron-lesioned bladder, urethral and anal sphincter function. Part 4: Effectiveness of the motor reinnervation American Journal of Physiology- Regulatory, Integrative, and Comparative Physiology.

3. Citation for and links to publications that cite or use the data:

4. Links to other publicly accessible locations of the data:

5. Links/relationships to ancillary or related data sets:

6. Was data derived from another source? No
   If yes, list source(s):

DATA & FILE OVERVIEW

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1. File list (filenames, directory structure (for zipped files) and brief description of all data files, add additional entries as necessary):

   A. Filename: Data for Tiwari et al 2024_AJP_03-21-2024.xlsx

 Short description:
An excel file is provided that includes the raw data for analyses for Figures 1-8. Each tab includes the data from each different figure.

B. Filename: Supplemental Videos for Tiwari et al 2024_AJP_03-21-2024.docx

Short description:
Representative images and videos showing recovery of squat-and-void postures at the Final testing point for Tiwari et al 2024_AJP. Representative images and videos showing recovery of squat-and-void postures at the Final testing point. Slide 1) Images of a squat-and-void posture in the home cage of one ObNT-ScNT Reinn animal before decentralization (PreSurgery) and a video collected at 14 months after onset of the experiment (1 year after decentralization and 4 months after reinnervation surgery). Slide 2) Images of a squat-and-void posture in the home cage of a second ObNT-ScNT Reinn animal before decentralization (PreSurgery) and a video collected at 14 months after onset of the experiment (1 year after decentralization and 4 months after reinnervation surgery). Slide 3) Videos of a third ObNT-ScNT Reinn animal at 6, 7 and 9 months after the reinnervation surgery. These videos were collected during awake bladder filling sessions (i.e., awake urodynamics).

2. Relationship between files, if important for context:

N/A

3. Additional related data collected that was not included in the current data package:

N/A

4. Are there multiple versions of the dataset? No

METHODOLOGICAL INFORMATION

Animals

Prior to onset, the protocol for this study was submitted to and approved by the Temple University Institutional Animal Care and Use Committee in accordance with the guidelines of the National Institute of Health for the Care and Use Laboratory Animals, United States Department of Agriculture, and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Upon approval, this study was then under the oversight of the Temple University Institutional Animal Care and Use Committee and in accordance with the guidelines of the National Institute of Health for the Care and Use Laboratory Animals, United States Department of Agriculture, and AAALAC.
Thirty female mixed-breed mongrel hound dogs were included in this study that were 6-8 months of age and 20-25 kg body weight at experimental onset, from Covance Research Products, Inc., Lancaster County, PA, USA, or Marshall BioResources, North Rose, NY, USA. Animals were housed in an AAALAC-accredited central animal facility and provided free access to food and water and maintained in a 12:12 hour light-dark cycle in 22-24ºC housing rooms. They were housed in large, connected pens, typically with 1 or 2 cage companions. Standard environmental enrichment and/or exercise were performed during group/single housing as per protocol.

Original design and necessary modifications of the project plan

The original design and necessary modifications of the project plan are as described in part 1 of this series (26). The current study is part 4 of a series reports and results from 30 animals with three main groups (Fig. 1A). The ObNT-ScNT Reinn group consisted of 8 dogs that were in the study a total of 22 months (22 ± 0.4 mo, mean ± SEM, Fig. 1B). At study onset, these ObNT-ScNT Reinn animals functionally decentralized by bilaterally transecting the dorsal roots of L7, all spinal roots caudal to L7, and the hypogastric nerves, followed by a 9-13 mo recovery period (10.4 ± 0.7 mo, Fig. 1C), then reinnervation by transfer of the obturator nerve to the vesical branch of the pelvic nerve, as well as a branch of the sciatic nerve to the pudendal nerve, that was then followed by an additional 8-12 mo recovery (11.9 ± 0.4 mo, Fig. 1C). The Decentralized group consisted of 4 animals that underwent similar decentralization followed by an 11-21 mo recovery (18 ± 2.5 mo, Fig. 1B), but no reinnervation surgeries. Controls consists of 7 sham-operated and 11 unoperated animals (18 total; Fig. 1A).
There is some overlap with the pilot study (22) and part 2 (25) of this series. Specifically, we included squat-and-void posture data from 3 ObNT-ScNT Reinn animals, 1 Decentralized animal, and 8 Control animals (5 sham-operated and 3 sham-unoperated) from the small pilot study, and *in vivo* electrophysiology data for peripheral nerve-evoked bladder, urethra and anal sphincter contractility from 3 ObNT-ScNT Reinn animals from the pilot study (22). We also included the *in vivo* peripheral nerve-evoked bladder contractility graph from Part 2 of this series (25) for comparison purposes to new additional data.

Unique to this manuscript are: 1) data from 18 additional animals, 2) report of defecation postures; 3) segmental spinal root/cord-evoked bladder, urethra and anal sphincter contractility data from all animals from L2-S3 to more closely match reports that spinal cord input to the obturator nerve is nearer to L3-L6 (20, 27-29); 4) peripheral nerve-evoked urethra and anal sphincter contractility data from the Decentralized and Control animals, and 5 additional ObNT-ScNT Reinn animals; 5) retrograde dye labeling data in the spinal cord ventral horn segments from L2-S3 after dye injections into the bladder and urethra sphincter; 6) Rexed laminar location of these labeled neurons in the spinal cord; and 7) correlations between these various outcomes. Because of the inclusion of the urethral and anal sphincter data, we renamed the reinnervated group to ObNT-ScNT Reinn (different from prior studies in which we focused on the obturator nerve transfer to the pelvic nerve results, and thus named the reinnervated group as “ObNT-Reinn”).

Decentralization of the bladder and nerve transfer surgeries

Surgical decentralization and nerve transfer procedures were as previously described (22, 25, 26). Briefly, dogs were sedated with propofol (6 mg/kg, i.v.) for endotracheal intubation, and then anesthetized using isoflurane (2-4% maximum alveolar concentration) with oxygen. Double balloon catheters were placed in the urethra and bladder (30). All animals except for unoperated controls underwent laminectomy of L6-S3 vertebrae. L7 and S1-3 ventral roots were identified electrophysiologically, as previously described (17, 18). Decentralized and ObNT-ScNT Reinn animals underwent decentralization from pelvic end organs by bilateral extradural transection of dorsal roots of L7, bilateral extradural transection of dorsal and ventral roots of S1-3, and bilateral transection of hypogastric nerves within the abdomen. Dorsal root ganglia (L7-S3) were also excised in these animals, except for the 4 animals from the pilot study (3 from the ObNT-ScNT Reinn group and 1 from the Decentralized group) (22). Sham-operated controls underwent lumbosacral laminectomy, nerve root identification using electrical stimulation without root transection, and abdominal laparotomy for identification of pelvic vesical nerve branches and hypogastric nerves.

For the nerve transfer surgeries, at 8-12 months after decentralization, 8 of the decentralized animals were re-anesthetized and catheterized with balloon catheters, as described above. For reinnervation of the bladder detrusor muscle, obturator nerves were accessed abdominally, divided longitudinally using a micro-scalpel; approximately 25% of the fascicles were transected, transferred, and sutured end-to-end to the transected vesical branch of the pelvic nerve, bilaterally, using described methods for identification of pelvic nerve branches (20), obturator nerve division (22), and end-on-end anastomosis . For reinnervation of external urethral and anal sphincters, a redundant branch of the sciatic nerve was identified in the posterior mid-thigh and then transferred cranially to branches of the pudendal nerve that induced urethral and anal sphincter contractions with intraoperative electrical stimulation (22) (the pudendal nerve and its branches were identified within Alcock’s canal (31)). Axoguard nerve connectors (Axogen Corp, Alachua FL, USA) were used to maintain transferred nerve coaptation and to reinforce the coaptation site which was covered in Tisseel fibrin sealant (Baxter, Deerfield, IL, USA) (26).

Postoperative care

Postoperative care procedures were as described previously (22, 26). Urine samples were collected, and urinalysis results were previously reported in Part 1 of this series (26).

Observation of squat-and-void and defecation postural behaviors

Squat-and-void postures and defecation postures were tracked pre and post decentralization, and post-reinnervation by recording them for 24 hours at monthly interval in 5 of the sham operated control, all 4 Decentralized, and all 8 ObNT-ScNT Reinn animals. Postures/day are reported for 3 time points: presurgery, post decentralization (recorded during the month immediately prior to the nerve transfer surgery in the reinnervated group and half-way to euthanasia for other two groups), and final (a recording was made within the month prior to terminal surgery). These videos were assessed by observers not aware of the animals' treatment allocation.

Retrograde dye injections

Dogs were injected with retrograde labeling dyes three weeks prior to euthanasia, as previously described (30) using a telescope (Hopkins II 30" Telescope, 2.9 mm x 30 cm and Tele Pack x LED, TP 100, Karlz Storz, Tuttlingen, Germany). Briefly, animals were sedated and anesthetized, and the bladder was cystoscoped. Dogs received injections of Fluoro-Gold (FG; 4-5% w/v in 0.9% saline solution, Fluorochrome, LLC, Denver, CO, USA) into the detrusor muscle at four different sites lateral to each ureteral orifice, and True Blue (TB; 2% w/v in 74% dimethyl sulfoxide, Life Technologies Corporation, Grand Island, NY, USA) into four different sites of the urethral sphincter. All dogs except 3 of the 11 unoperated control received these dye injections. All dogs were allowed to recover from anesthesia before being returned to their home cages.

In vivo functional electrical stimulation at 10 months after reinnervation surgery, or 18 months post-decentralization

An average 10-month reinnervation recovery time was chosen based on data from the pilot study showing functional recovery of squat-and-void postures between 4-6 months after obturator nerve transfer in 3 ObNT-ScNT Reinn animals (32). For this, prior to euthanasia, the animals were anesthetized and catheterized, as described above. Bladder, external urethral sphincter, rectal, and anal sphincter pressures were monitored throughout the surgeries, as described , as were vital signs. Three successive filling cystometrograms were obtained to determine bladder capacity, as previously described (33). Detrusor pressure was calculated by subtracting rectal pressure (as an estimate of intra-abdominal pressure) from bladder pressure (also termed intravesical pressure). After laminectomy, L1-S3 spinal ventral roots or spinal cord segments were systematically stimulated (0.5-10 mAmp, 20-Hz, and a pulse duration of 0.2 msec in a train of 4-7 second duration) , bilaterally, using hand-held monopolar or bipolar electrodes while recording the induced bladder, urethra or anal sphincter pressures. Evoked maximum detrusor pressure (MDP), maximum urethral sphincter pressure (MUSP), maximum anal sphincter pressure (MASP) data was recorded at 10 Hz sampling rate and is reported as an average for each stimulated root/spinal cord segment, as well as after binning the data in L2-6 and L7-S3 groupings, since in dogs, L7-S3 roots are the primary segments innervating the

bladder (30), and the obturator nerve primarily originates from L3-L6 spinal cord segments in a variable range as described earlier. We included L2 data in the binning data due to past findings from the lab that this segment also induces bladder contraction (32). Additionally, right and left transferred nerves (obturator-to-pelvic and sciatic-to-pudendal) were identified and stimulated in ObNT-ScNT Reinn animals, as were right and left intact pelvic and pudendal nerves in Control and Decentralized animals (0.5-10 mAmp, 20-Hz, and a pulse duration of 0.2 msec in a train of 4-7 second duration). Changes in pressures were continuously recorded with external pressure transducers interfaced with the PowerLab® multichannel data acquisition system and LabChart® software (ADInstruments, Colorado Springs, CO, USA). The strength of evoked contractions was derived from differences between the resting baseline pressure and the peak pressure obtained during continuous stimulation. The resulting MDP, MUSP, MASP are reported in cmH_2O.

Euthanasia and tissue collection

After the above electrophysiological testing, all animals were euthanized with Euthasol (Virbac Corporation, Fort Worth, TX, USA; 1 ml for each 4.5 kg of body weight, i.v.). Thereafter, the spinal cord was collected from all animals from lumbar (L) level 2 caudally to the sacral (S) level 3, in a segmental manner. Each segment was fixed by immersion in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 2 days. They were then equilibrated in 10% and then 30% sucrose in phosphate buffer for two days each, before being frozen in OCT compound (Optimal Cutting Temperature Compound, Sciagen, Fisher-Scientific, Hempstead, NH, USA) for future cryosectioning. Samples were cryosectioned into 14 µm cross sections, with every fifth section mounted onto charged slides (Fisher Plus, Thermo Fisher Scientific Inc., Waltham, MA, USA). All sections were dried onto the slides overnight, washed in phosphate-buffered saline (PBS) and coverslipped with 80% glycerol in PBS as a mounting medium. Sections were quantitatively evaluated using fluorescence microscopes for the presence of retrogradely labeled cell bodies.

Quantitative analysis of retrogradely labeled neuronal cell bodies

Three sections of each spinal cord segment, per dog and per vertebral level, from L2 to S3, were analyzed quantitatively and bilaterally for the number of Fluoro-Gold labeled and True Blue retrogradely labeled neuronal cell bodies per area of the ventral horn assayed, using previously described methods (30, 34). Briefly, regions of interest were landmarked and circumscribed at 40 x magnification, using a 4X objective, on live microscopic images (see Fig. 6 in Ruggieri et al, 2011). All fields of the intermediate and ventral horns were sampled systemically at 400 x magnification (using a 40 x objective) in three non-adjacent sections per tissue. To avoid bias in estimating the number of neurons, only retrogradely labeled perikarya with a clearly visible nucleus were counted. The sum of labeled cells counted per retrograde dye was divided by the size of ventral horn region of the spinal cord segment assayed, to provide an estimate of the number of cells per mm^2 per segment. Additionally, the Rexed laminar location of the retrogradely labeled cells was recorded onto the cord maps, in order to provide an estimate of the number of cells per mm^2 per lamina and per segment (see Rexed lamina divisions used in Fig. 5 in Gomez et al, 2015 (20)).

Statistical analyses

Statistical analyses were performed using GraphPad Prism 10, as was the graphing (GraphPad Software, La Jolla, CA, USA) for all but the retrograde dye labeling data, which were analyzed using SPSS (IBM SPSS Statistics, Armonk, NY, USA). The level of significance was set at $p < 0.05$ for all analyses and adjusted p values are reported for the post hoc analysis results. All data are presented as means and 95% confidence intervals (CI). The statistical inter-group comparisons were pre-defined before the data were collected.

Mixed-effects regression models were used for the analyses of repeated-measures of behavioral postures and spinal roots/cord evoked contractions, followed by multiple comparison post hoc tests, with results confirmed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Behavior postures (squat-and-void and defecations postures) were analyzed using two factors: *surgical group* and *time point* (pre-surgery, post-decentralization, and final), followed by Tukey-Kramer's multiple comparison post hoc tests. Analyses of spinal roots/cord evoked MDP, MUSP, and MASP contractions were performed for individual segmental levels (L1-S3), or with the root/spinal cord segments combined (L2-6 versus L7-S3), using two factors: *surgical group* and *segment(s)*, followed by Fisher's LSD multiple comparison post hoc tests. For data examining MDP, MUSP, and MASP responses after peripheral nerve stimulation, Kruskal–Wallis ANOVAs were used followed by Dunn's multiple comparison post hoc tests. Control group statistics for peripheral nerve stimulation included only 5 sham-operated control animals since peripheral nerve stimulation was not performed in the other control animals. Analyses of the numbers of retrogradely labeled neurons in spinal cord ventral horns were first performed for each dye using repeated-measures mixed-effects models with two factors: *surgical group* and individual *segment* (from L1 to S3). Next, this same data was reanalyzed similarly after combining the data into L2-6 vs L7-S3 segmental groupings. Additionally, this data was reanalyzed using a repeated-measures mixed-effects model and three factors: *surgical group, segmental group* (L2-6 vs L7-S3), and *lamina location* of the labeled neurons (VII, VIII and IX). These were followed by Tukey-Kramer multiple comparison post hoc tests. Because this set of analyses did not test a prespecified statistical null hypothesis, the result are exploratory, therefore the calculated *p*-values are interpreted as descriptive, not hypothesis testing . For succinctness sake, the statistical findings from the mixed-effects models are listed in Appendix Table 1 and post hoc multiple comparison results are depicted in the figures.

Correlations between outcomes was also performed (Pearson's r correlations) to inform if the outcomes were linked and if the responsiveness of one outcome was linked to responsiveness to another outcome. For this, electrical stimulation responses of L2-L6 were averaged before use in the correlation assay, as were L7-S3 spinal root responses. Similarly, retrograde dyes result for L2-L6 ventral horn segments were averaged, as were L7-S3 dye results.

3. Software- or Instrument-specific information needed to interpret the data, including software and hardware version numbers:

Indicated above, in Retrograde dye injections, In vivo functional electrical stimulation, and Statistical analyses

4. Standards and calibration information, if appropriate:

Indicated above, in In vivo functional electrical stimulation

5. Environmental/experimental conditions:

Indicated above, in Animals, Quantitative analysis of retrogradely labeled neuronal cell bodies, Decentralization of the bladder and nerve transfer surgeries, Observation of squat-and-void and defecation postural behaviors and In vivo functional electrical stimulation

6. Describe any quality-assurance procedures performed on the data:

Indicated above, in Animals, Quantitative analysis of retrogradely labeled neuronal cell bodies, Decentralization of the bladder and nerve transfer surgeries, Observation of squat-and-void and defecation postural behaviors, In vivo functional electrical stimulation, and Statistical analyses

7. People involved with sample collection, processing, analysis and/or submission:

Ekta Tiwari, Danielle S. Porreca, Alan S. Braverman, Lewis Holt-Bright, Nagat A. Frara, Justin M. Brown[5] Benjamin R. Johnston, Stanley F. Bazarek[8] Brendan A Hilliard, Michael Mazzei, Michel A. Pontari, Daohai Yu, Michael R. Ruggieri, Sr, and Mary F. Barbe

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DATA-SPECIFIC INFORMATION FOR: [FILENAME] (Repeated as necessary)

Data for Tiwari et al 2024_AJP_03-21-2024.xlsx
Supplemental Videos for Tiwari et al 2024_AJP_03-21-2024.docx
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1. Number of variables:
Provided in methodological section.

2. Number of cases/rows:
Provided in methodological section.

3. Variable List, defining any abbreviations, units of measure, codes or symbols used:
Provided in methodological section.

4. Missing data codes:
Code/symbol Definition
Code/symbol Definition

5. Specialized formats or other abbreviations used:

DATA-SPECIFIC INFORMATION FOR CODE: [FILENAME] (Repeated as necessary)

1. Language used:

2. Software necessary for editing and running:

3. Purpose of code:

4. Overview of operation of code (inputs, outputs, etc.):

DATA-SPECIFIC INFORMATION FOR VISUALIZATION: [FILENAME] (Repeated as necessary)

1. Kinds of visualization: Graphs and videos

2. Program used to generate visualization: Graphpad Prism version 9.4.1 (GraphPad Software, La Jolla, CA) and Microsoft office Powerpoint

3. Data file(s) used to generate visualization: Data for Tiwari et al 2024_AJP_03-21-2024.xlsx
Supplemental Videos for Tiwari et al 2024_AJP_03-21-2024.docx

4. Describe the process used to generate the visualization: Data for individual animal bladder for each treatment from different dog groups were put into the prism graphing program. Videos were captured with a video camera.