Molecular characterization of the spinal circuitries controlling lower urinary tract function

Andrea M. Sartori[1,2], Natalie Klymko[1], Richard Lee[1], Mark L. Zeidel[1,2], Anne M.J. Verstegen[1,2]

[1]Department of Medicine, Beth Israel Deaconess Medical Center, Boston, USA,
[2]Harvard Medical School, Boston, USA

HARVARD MEDICAL SCHOOL
TEACHING HOSPITAL

Beth Israel Lahey Health
Beth Israel Deaconess Medical Center

INTRODUCTION

- Lower urinary tract function (LUTF) is a complex physiological process involving the coordination of the somatic and autonomic nervous system.
- Efficient bladder storage and voiding rely on the precise interaction of various brain and spinal nuclei.
- Despite its clinical significance, the specific spinal cord neurons contributing to LUTF remain poorly characterized, presenting a barrier to targeted therapeutic interventions.
- This study aims to molecularly define and characterize the specific neuronal subpopulations in the spinal cord that are involved in LUTF.

METHODS

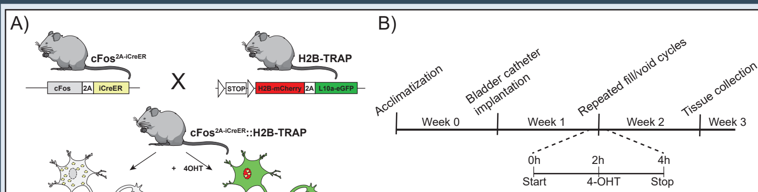

Fig. 1) Experimental design. A transgenic mouse line expressing a Cre-recombinase from the cFos promoter (TRAP2) was crossed to a reporter strain carrying Cre-dependent nuclear and cytosolic tags (H2B-Trap). Adult mice were implanted with a catheter in the dome of the bladder, and the open end connected to an infusion harness. After a one-week recovery period, the animals were acclimatized to the urodynamic setup. On the experimental day, saline was infused into the bladder of awake, freely behaving mice at 25 µl/min for 2 hours. This was followed by an intraperitoneal injection of 4-Hydroxytamoxifen (4-OHT, 50mg/kg) and the infusion continued for 2 more hours. One week after the experiment, the animals were sacrificed and brain and spinal cord tissues were collected.

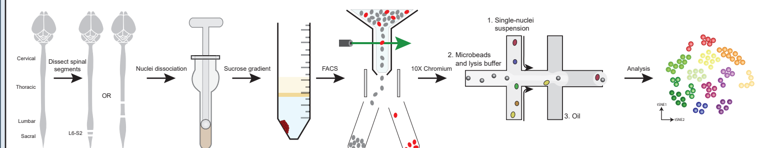

Fig. 2) Experimental procedure for single-nuclei RNA-seq. Pieces of lower thoracic and lumbosacral spinal cord were cut and separately dounce-homogenized to release the nuclei. Single-nuclei suspensions were cleaned of myelin and debris by using a density gradient with centrifugation. mCherry-positive nuclei found in these single-nuclei suspensions were then isolated using fluorescence-activated nuclei sorting, followed by library preparation and sequencing using the 10x Chromium platform.

TRAP2 mouse allows for enrichment of neurons likely involved in lower urinary tract function

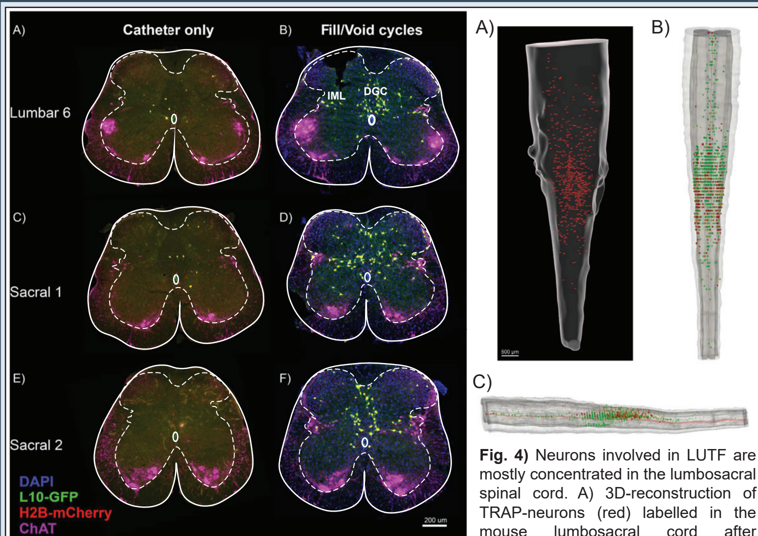

Fig. 3) Inducing continuous fill/void cycles (B, D, F) led to significant labeling at the lumbosacral spinal cord levels compared to animals implanted with a catheter that did not undergo fill/void cycles (A, C, E). Elevated cell densities were seen in the dorsal gray commissure (DGC) of lumbosacral segments L5 to S1 and in the intermediolateral cell column (IML) of L6 to S2. Cholinergic neurons were identified with immunofluorescence against choline acetyltransferase (pink).

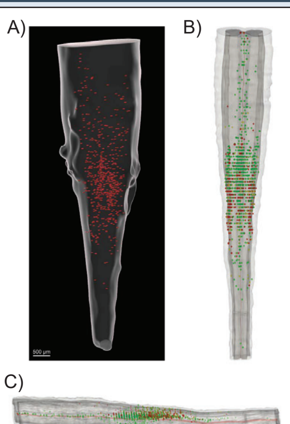

Fig. 4) Neurons involved in LUTF are mostly concentrated in the lumbosacral spinal cord. A) 3D-reconstruction of TRAP-neurons (red) labelled in the mouse lumbosacral cord after stimulating the lower urinary tract by continuous fill-void cycles. B-C) Top and lateral view of the reconstructed labelled neurons in the rat spinal cord following Pseudorabies virus (PRV) injections into either the external urethral sphincter (PRV-152, green) or bladder (PRV-614, red).

Single-nucleus transcriptomic profiling of the lumbosacral spinal cord identifies multiple neuronal subpopulations

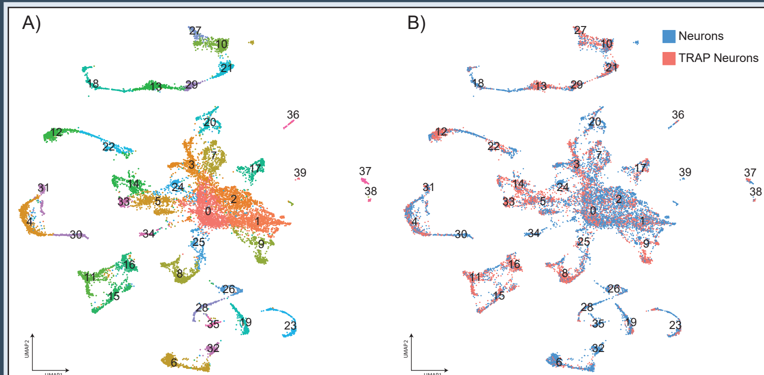

Fig. 5) Uniform manifold approximation and projection (UMAP) plot of neurons identified from lumbosacral spinal cord tissue samples using single-nucleus RNA-seq with the 10X Chromium platform. A) Unsupervised clustering of 15,288 nuclei revealed 40 main subpopulations. B) UMAP plot from A) grouped by sample origin. Neurons (NeuN+) obtained from the L6-S2 spinal levels are shown in blue, while neurons that activated during repeated fill-void cycles (TRAP+) are depicted in red.

Neuronal subpopulations from the lumbosacral spinal cord can be characterized based on their molecular profiles

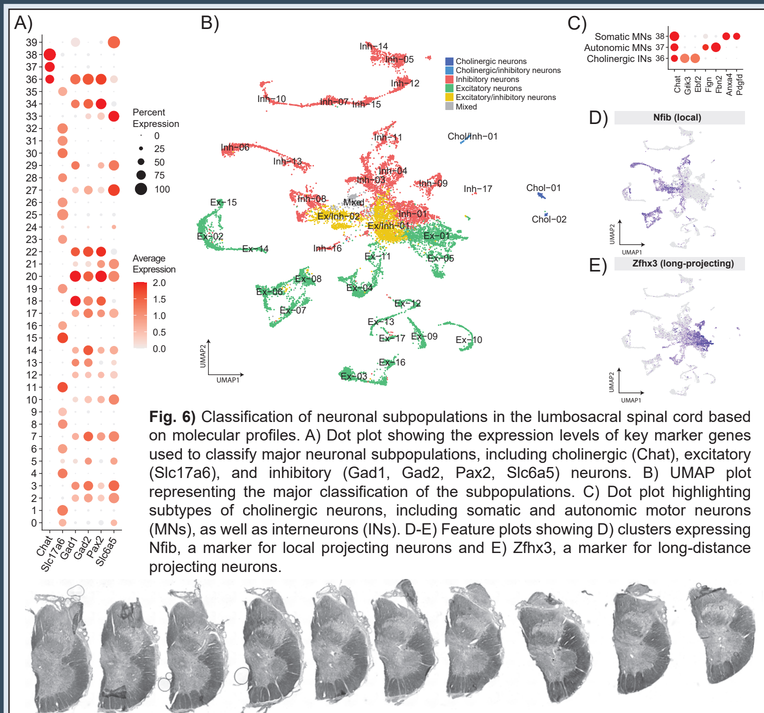

Fig. 6) Classification of neuronal subpopulations in the lumbosacral spinal cord based on molecular profiles. A) Dot plot showing the expression levels of key marker genes used to classify major neuronal subpopulations, including cholinergic (Chat), excitatory (Slc17a6), and inhibitory (Gad1, Gad2, Pax2, Slc6a5) neurons. B) UMAP plot representing the major classification of the subpopulations. C) Dot plot highlighting subtypes of cholinergic neurons, including somatic and autonomic motor neurons (MNs), as well as interneurons (INs). D-E) Feature plots showing D) clusters expressing Nfib, a marker for local projecting neurons and E) Zfhx3, a marker for long-distance projecting neurons.

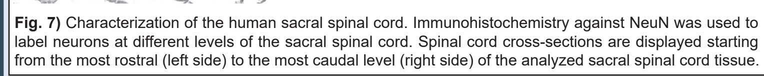

Fig. 7) Characterization of the human sacral spinal cord. Immunohistochemistry against NeuN was used to label neurons at different levels of the sacral spinal cord. Spinal cord cross-sections are displayed starting from the most rostral (left side) to the most caudal level (right side) of the analyzed sacral spinal cord tissue.

CONCLUSIONS & OUTLOOK

- We have successfully used the TRAP2::H2B-Trap transgenic mouse to enrich for neurons activated by a specific behavior (LUTF).
- The molecular profiles of subpopulations identified via single-nuclei RNA-seq can be used to investigate the functional role of these spinal neurons in lower urinary tract control.
- Spatial validation of clusters is necessary to pinpoint the populations of interest.
- These findings will significantly contribute to our understanding of LUTF neurobiology in mice and lay the foundation for comparative investigations in humans, with implications for the development of targeted therapies aimed at addressing lower urinary tract dysfunction.

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Correspondence: Andrea M. Sartori; asartori@bidmc.harvard.edu