

Single cell transcriptome profiling to define cell types in brain nuclei controlling bladder function

Anne M.J. Verstegen¹, Natalie Klymko¹, Harini Srinivasan², Deepti Pant², Linus Tsai², Mark L. Zeidel¹

¹Division of Nephrology, Department of Medicine, Harvard Medical School, Boston, MA 02215, USA

²Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, Harvard Medical School, Boston, MA 02215, USA.

Introduction/ background:

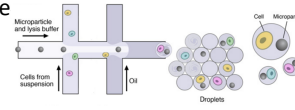
The pontine micturition center (PMC) is involved in the supraspinal control of bladder function. For micturition to occur, an excitatory signal from PMC has to reach preganglionic bladder motoneurons. The glutamatergic PMC neuron population includes multiple, yet to be identified subgroups that express additional specific marker genes, and some of these subpopulations may provide the descending control of detrusor and sphincter function.

Goals:

- Identify all neuron subtypes in PMC
- Map the functional connections of specific bladder controlling neurons using anterograde and retrograde tracing,
- Characterize the functional contribution to micturition of those neurons that innervate bladder motoneurons in the spinal cord.

Methods:

1) Single nuclei RNA-sequencing involves tissue dissection and preparation of a suspension of single nuclei. Individual nuclei are then encapsulated in droplets together with microparticles (beads), using a custom-designed microfluidic device



Following droplet formation, each nucleus is lysed within the droplet and mRNAs released hybridize to primers on the bead surface. mRNAs are reverse-transcribed into cDNAs and amplified for high-throughput sequencing.

2) Anatomical tract tracing:

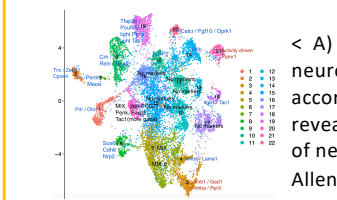
(Forward/) in anterograde direction: tracing axonal projections from PMC neurons and (backward/) in retrograde direction: neurons in 'upstream' locations are labeled (after axon terminals have taken up the label and transported this back up to the cell body).

3) Histological and anatomical study of newly identified neuron subpopulations includes -Validation of existing transgenic mouse Cre-lines, -Assessing if neurons project axons to the sacral spinal cord, and if there is no mouse line available then instead of tracing anterograde axonal projections; -Determining the extent of colocalization of spinally projecting neurons with mRNA expression in PMC.

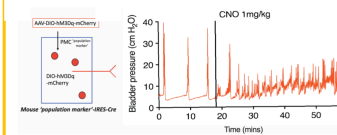
4) Chemogenetic neuron stimulation:

Binding of GPCR-ligand Clozapine-n-Oxide (CNO) to stereotactically injected Designer Receptors (Activated specifically by Designer Drugs), stimulates specific neuron subpopulations in a Cre-dependent manner. Here we use the excitatory version of DREADDs (Gq) which activates intracellular signaling pathways.

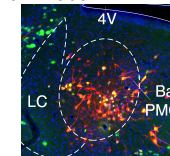
(Techniques 2-4 applied to each identified subpopulation). **Results:**



< A) Drop-Seq with nuclei (DroNc-Seq) revealed separate clusters of neurons in and near PMC: UMAP plot of 6423 neurons annotated according to known gene expression. The unbiased algorithm reveals 6 clusters of excitatory PMC neurons and four more clusters of neurons in nearby regions based on gene expression data in the Allen Brain Atlas.

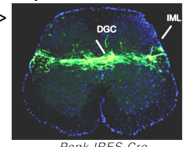
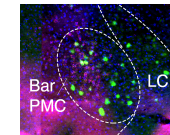


(up) C) DREADDs activation of 'Crh' subpopulation: Bilateral stereotactic injection of Cre-dependent (excitatory) Gq in combination with urodynamics readout shows that CNO induced stimulation of this subpopulation increases void frequency and increases baseline bladder pressure with lower amplitude contractions.



< B) 'Tac1' subpopulation marker gene expression near PMC: AAV-DIO-mCherry expression colocalizes with Tac1-GFP reporter labeled neurons.

D) Axonal projections of 'Penk' neurons to sacral spinal cord: Cre-specific anterograde label injected in the PMC reveals projections to two spinal cord regions with bladder motoneurons, or inhibitory interneurons that connect to EUS motoneurons >



E) In-situ Hybridization for 'Oprk1' mRNA (^ magenta) upon rgCre in reporter mouse: Retrograde label injected in the spinal cord labels spinally projecting neurons in the brain. ISH for this marker gene mRNA shows expression in retrogradely labeled neurons.

Conclusions and future directions:

We have identified 10 subpopulations within the pool of glutamatergic neurons near the mouse PMC. For each of these we assessed if marker expression was restricted to PMC or not-specific, if neurons sent axonal projections to the spinal cord (for innervation of bladder and EUS motoneurons), and whether activation had an effect on micturition behavior. Five subpopulations remain that passed the above 'criteria'. All findings and single cell transcriptomics data will be shared as **Resource** to the community.

Support: NIH/NIDDK, P20 DK119798 (Zeidel - PI). References: Macosko et al., 2015 (*Cell*) - Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets; Campbell et al., 2017 (*Nat Neurosci*) - A molecular census of arcuate hypothalamus and median eminence cell types; Bakken et al., 2018 (*Plos*) - Single-nucleus and single-cell transcriptomes compared in matched cortical cell types