Robustness between FOXP transcription factors maintains striatal function

UT Southwestern Medical Center

Newaz Ahmed, Nitin Khandelwal, Ashley Anderson, Karthigayini Sivaprakasam, Jay Gibson, and Genevieve Konopka

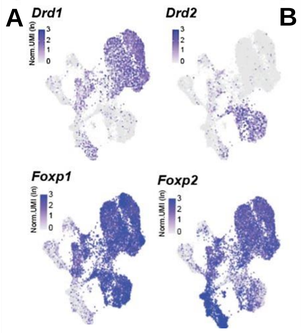
Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX

Neuroscience 2022

Abstract

Variants in the transcription factors FOXP1 and FOXP2 are significantly associated with autism spectrum disorder (ASD) and expressive language impairments. Both genes are expressed in spiny projection neurons (SPNs) in the striatum, where they may work together to regulate gene expression. Knockout of Foxp1 from the dopamine 2 receptor (D2) SPNs in mice results in significant behavioral, morphological, and physiological impairments, while there are fewer changes in all of these domains upon deletion of Foxp1 from the dopamine 1 receptor (D1) SPNs. This difference may be due to the differential expression of Foxp1 and Foxp2 in the SPNs; Foxp1 is highly expressed in both D1 and D2 SPNs whereas Foxp2 is more highly expressed in the D1 SPNs relative to D2 SPNs. Therefore, we hypothesize that Foxp1 and Foxp2 have compensatory functions in the D1 SPNs. Utilizing mice that have a Drd1 specific knockout of Foxp1, Foxp2, or both genes, we find that loss of both genes results in impaired motor learning, hypoactivity, and social behavior as well as increased firing of the D1 SPNs. Differential gene expression analysis from single nuclei RNA-sequencing implicates genes involved in ASD risk, maintenance of electrophysiological properties, and neuronal development and function. These data support the hypothesis that Foxp1 and Foxp2 functionally compensate for each other in D1 striatal neurons.

Background and Introduction

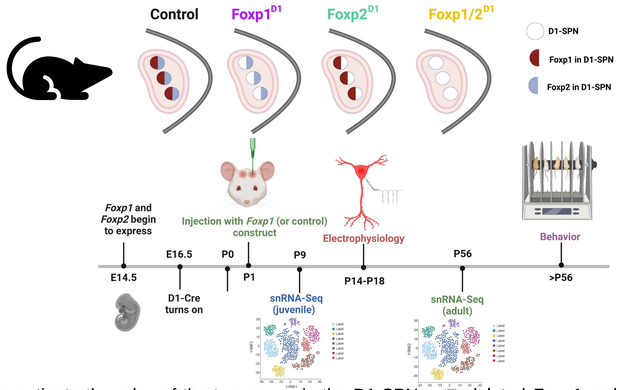


B

	D1 cKO of *Foxp1* (*Foxp2* expressed in all cells)	D2 cKO of *Foxp1* (*Foxp2* only expressed in sub-populations)
SPN Specification	No Change	Decreased
Striatal Projections	No Change	Impaired
Motor Learning	No Change	Impaired
Locomotive Activity	No Change	Hyperactive
Social Behavior	Decreased	No Change
SPN Excitability	Hyperactive	Hyperactive (due to impaired K_{IR} and K_{Leak} function)

A) Feature plots generated from single cell RNA-Seq shows that *Foxp1* is expressed in both D1- and D2-SPNs whereas *Foxp2* expression is limited to the D1-SPNs[1]. **B)** Table summarizing the morphological, behavioral, and electrophysiological findings from previous *Foxp1*-cKO studies[1,2].

Experimental Design



To investigate the roles of the two genes in the D1-SPNs, we deleted *Foxp1* and/or *Foxp2* from the D1 SPNs and then performed: a battery of behavioral tests, single-nuclei RNA-Sequencing (snRNA-Seq) of striatal tissue, or electrophysiological experiments. An AAV-mediated injection of *Foxp1* was also done in a subset of mice.

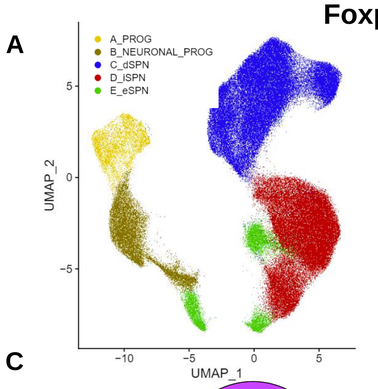
Acknowledgments

This study was supported by funding from NIH/NIMH R01MH102603 (G. Konopka), NIH/NIMH R01MH126481 (G. Konopka), Simons Foundation for Autism Research 573689 (G. Konopka & J. Gibson), T32-HL 139438 NHLBI Institutional training Grant (N. Ahmed), and F31-NS117030-01 NIH Training Grant (N. Ahmed).

References

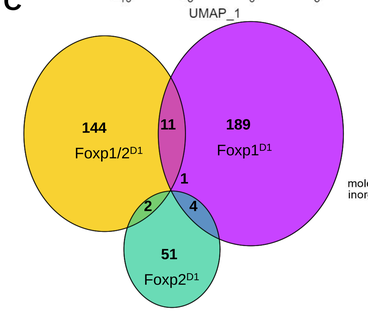
1. Anderson, A., et al. (2020). Cell Reports: **30**: 3051-3066
2. Khandelwal, N., et al (2021). Molecular Psychiatry: **26**: 1761-1774
3. Saunders, A., et al. (2018). Cell: **174**(4): 1015-1030

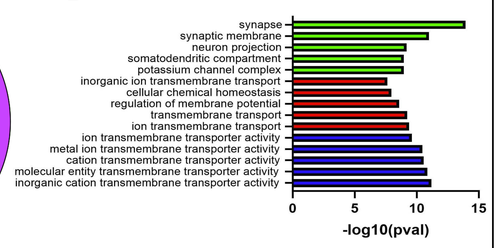
snRNA-seq of juvenile striatum with Drd1 deletion of Foxp1/Foxp2



B

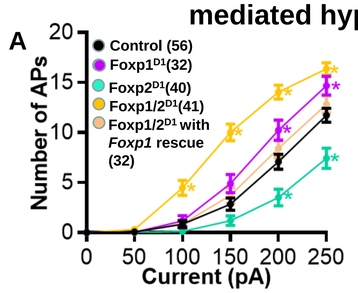
Genotype	# nuclei	# SPNs	# dSPNs
Control (n=3)	46,307	33,331	15,510
Foxp1^{D1} (n=3)	48,801	28,132	12,743
Foxp2^{D1} (n=3)	51,529	28,418	10,964
Foxp1/2^{D1} (n=3)	45,745	28,422	12,907

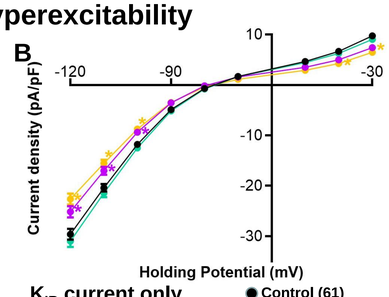


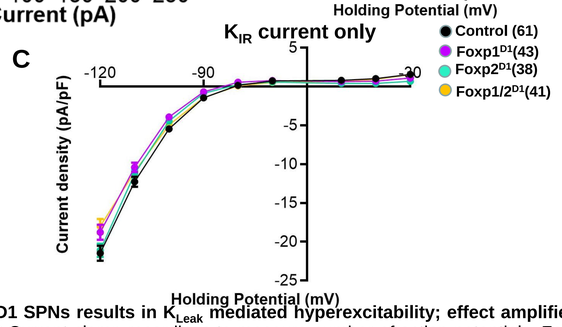


Striatal snRNA-seq data from juvenile Drd1cKO of Foxp1 and/or Foxp2. Using the 10X Genomics Chromium platform, we targeted 10,000 nuclei/genotype from mice at postnatal day 9. We used Cell Ranger and then Cell Bender to demultiplex, align, count, and aggregate runs filter out nuclei with >10,000 UMI, >1.5% mitochondrial content, or high ambient RNA. Clusters were annotated using previously published adult mouse brain single-cell RNA-seq data[3]. **A)** UMAP clustering after removal of non-neuronal cell-types. **B)** Table showing number of total nuclei and SPNs included in the analysis. **C)** Scaled Venn diagram with the number of overlapping and unique differentially expressed genes per genotype per MAST-GLM. **D)** Bar plot showing Gene Ontology terms enriched in Foxp1/2^{D1} D1-SPN DEGs.

Loss of both *Foxp1* and *Foxp2* results in K_{Leak} mediated hyperexcitability

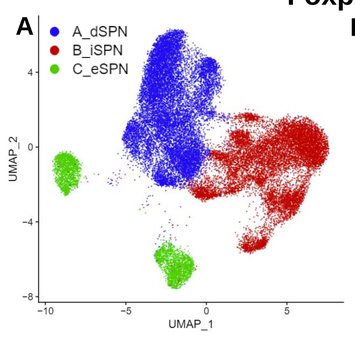






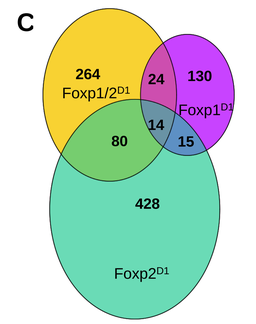
Loss of *Foxp1* from the D1 SPNs results in K_{Leak} mediated hyperexcitability; effect amplified by further loss of *Foxp2*. **A)** Current clamp recordings to measure number of action potentials. Foxp1^{D1} D1-SPNs exhibit hyperexcitability and Foxp1/2^{D1} have even greater hyperexcitability. Foxp2^{D1} have hypoexcitabiliy. Foxp1/2^{D1} mice injected with AAV-mediated *Foxp1* construct had normal excitability. **B)** Current-Voltage plots generated in absence of cesium. Foxp1^{D1} and Foxp1/2^{D1} mice show differences from controls. **C)** Current-voltage plot showing impact of K_{IR} channels. Lack of significant differences upon subtracting the two implicates K_{Leak} channels as being causal. * p <0.05. Two-way ANOVA with Holm-Šidák's post-hoc analysis.

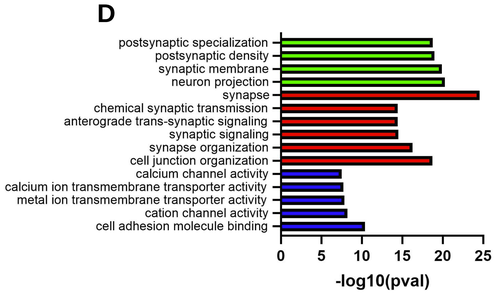
snRNA-seq of adult striatum with Drd1 deletion of Foxp1/Foxp2



B

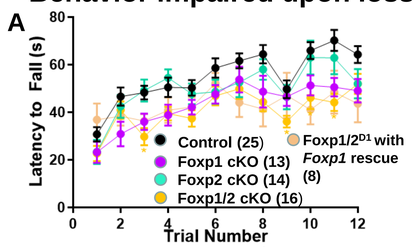
Genotype	# nuclei	# SPNs	# dSPNs
Control (n=3)	65,405	11,009	5,350
Foxp1^{D1} (n=3)	53,727	5,448	2,580
Foxp2^{D1} (n=2)	47,628	9,298	4,033
Foxp1/2^{D1} (n=3)	38,148	4,726	2,440

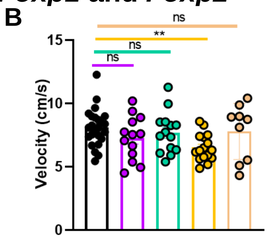


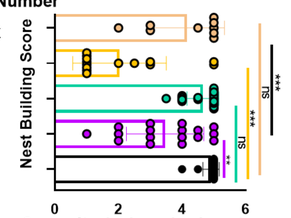


Striatal snRNA-seq data from adult Drd1cKO of Foxp1 and/or Foxp2. Same methods used as for analysis of juvenile samples. **A)** UMAP clustering after removal of non-neuronal cell-types. **B)** Table showing number of total nuclei and SPNs included in the analysis. **C)** Scaled Venn diagram with the number of overlapping and unique differentially expressed genes per genotype per MAST-GLM. **D)** Bar plot showing Gene Ontology terms enriched in Foxp1/2^{D1} D1-SPN DEGs.

Behavior impaired upon loss of *Foxp1* and *Foxp2*







Loss of both *Foxp1* and *Foxp2* results in impaired motor and social behavior. Foxp1/2^{D1} mice show motor learning deficits **(A)** hypoactivity in the open field **(B)**. **C)** Foxp1^{D1} mice show impairment as assessed in nest building; Foxp1/2^{D1} show further impairment. All behaviors in Foxp1/2^{D1} rescued by injection of *Foxp1*.* p <0.05, ** p < 0.01, *** p < 0.001. One- or Two-way ANOVA with Tukey's post-hoc analysis.

Conclusions

- Differentially expressed genes (DEGs) from D1-SPNs of both juvenile and adult knockout are involved in mediating electrophysiological properties and cell function.
  - The K_{Leak} channel, *Kcnk2 is* downregulated in Foxp1^{D1} and Foxp1/2^{D1} D1-SPNs.
- Loss of *Foxp1* results in hyperexcitability of the D1-SPNs; effect exacerbated with further loss of *Foxp2*. Loss of only *Foxp2* results in hypoexcitability.
  - Neuronal hyperexcitability driven by impairments in K_{Leak} channels.
  - Re-expression of *Foxp1* into Foxp1/2^{D1} D1-SPNs rescues excitability phenotype.
- Motor impairments in adult mice only seen upon the loss of both *Foxp1* and *Foxp2* from the D1-SPNs. Knockout of *Foxp1* results in a social behavior deficit. This impairment is amplified upon the further loss of *Foxp2*.
  - Exogenous expression of *Foxp1* rescues behavioral impairments.