High dimensional neuroanatomical and behavioral analysis for probing cerebellar involvement in nonmotor function

Talmo D. Pereira[1], Julia W. Metzger[1,2], Ben Deverett[1,2,3], Thomas J. Pisano[1,2,3], Aleksandra Badura[1,4], Samuel S.-H. Wang[1,2]

[1] Princeton Neurosci. Inst., [2] Mol. Biol., Princeton Univ., Princeton, NJ; [3] Rutgers-Robert Wood Johnson-Princeton MD/PhD Program, Rutgers Univ., New Brunswick, NJ; [4] Netherlands Inst. for Neurosci., Amsterdam Zuidoost, Netherlands

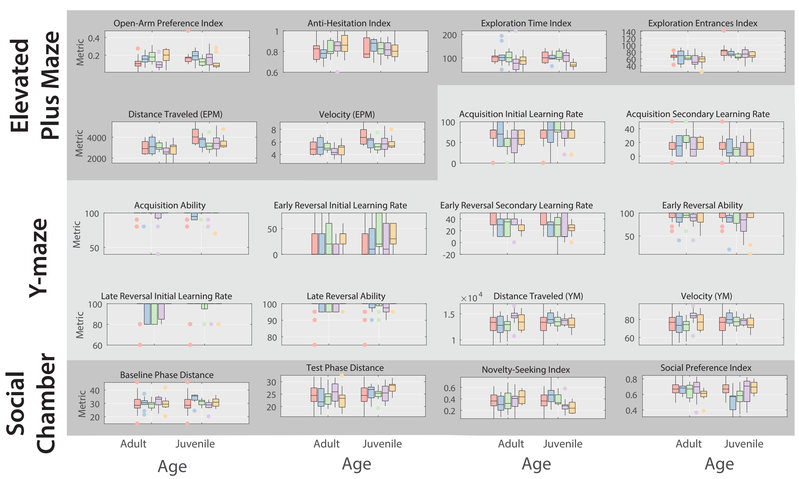
Abstract

Genetically encodable Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) allow reversible inactivation of identified cell types in freely-moving animals on a time scale of hours. To understand the role of specific cerebellar regions in guiding behavior during development and adulthood, we have developed detailed quantitative approaches for analyzing the extent of DREADD inactivation and the consequent pattern of behavioral perturbation.

In both juvenile and adult mice, we performed cerebellar lobule-specific injections of AAV8-hSyn-hM4D(Gi)-mCherry to co-express an inhibitory DREADD (hM4D) and a fluorescent reporter (mCherry). After administration of DREADD agonist clozapine-N-oxide (CNO), mice were tested sequentially in five behavioral paradigms commonly used to model autism in mice: an elevated-plus maze, reversal in a swimming Y-maze, self-grooming, three-chamber social preference, and a virtual reality-based working memory task. In each case, direct monitoring and video recording were used to acquire subsecond-resolution measurements of animal trajectory for offline analysis. To define different dimensions of autism-like phenotypes, we used principal components analysis to identify patterns of animal-to-animal covariation encompassing multiple behavioral measurements. In this way we created a basis set of "eigenbehaviors" constructed from performance in unperturbed mice, which we used to quantify lobule-specific inactivation. These behaviors constitute a multidimensional autism-like phenotype.

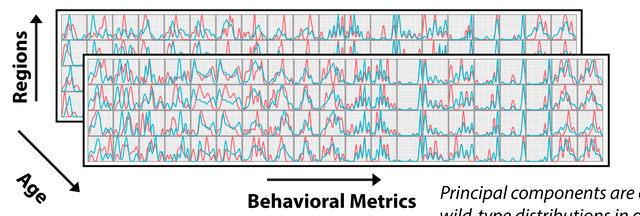
We next sought to associate behavioral phenotypes with spatial patterns of DREADD expression. We used serial two photon (STP) reconstruction to render anatomical volumes complete with precisely mapped regions of expression. Our developed image processing pipeline builds aligned volumes from these STP stacks and registers the volumes to a standard mouse brain coordinate system (Allen CCFv2). Comparison of anatomical representations to behavioral phenotypes will test the hypothesis that lobule-specific perturbation of cerebellar regions leads to multi-dimensional effects on behavior. Our experimental and analytical framework enables quantitative linkage of neuroanatomy to behavior.

1 Behavioral effects of cerebellar perturbations



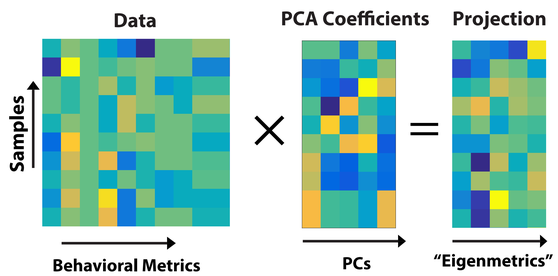
2 Dimensionality reduction of behavioral metrics

i) Raw behavioral metric distributions



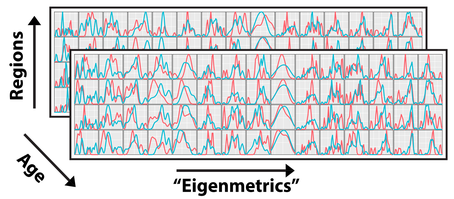
Principal components are computed from the wild-type distributions in order to find natural correlations between behavioral metrics.

ii) Principal components analysis



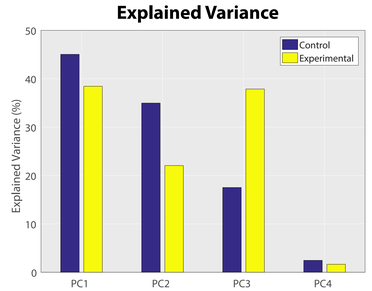
The experimental data is then projected onto these "eigenmetrics" and only dimensions that capture significant variance in both are kept.

The reprojected data recapitulates the raw behavioral data across a reduced set of dimensions. Differences in control versus experimental distributions suggest effects of DREADD inactivation.



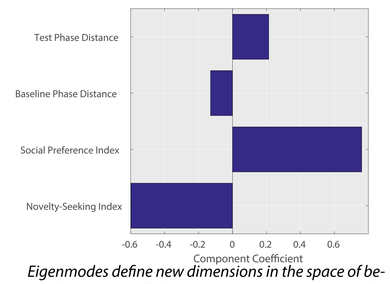
iii) Example: Social Chamber Eigenmetrics

Explained Variance



Eigenmodes capture variance in behavioral data for both wild-type and cerebellar DREADD-inactivated mice, despite being computed from only the former.

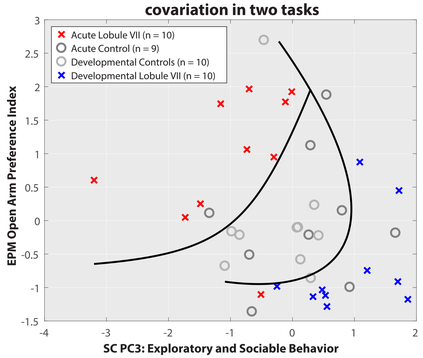
PC3: "Exploratory and Sociable Behavior"



Eigenmodes define new dimensions in the space of behavioral metrics. This component suggests that social preference and novelty-seeking measures are anticorrelated.

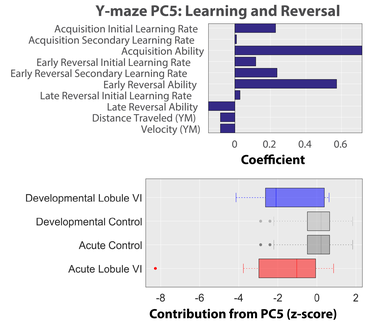
3 Behavioral metrics affected by cerebellar inactivation

a) Lobule VII DREADD induces development-dependent covariation in two tasks



Two metrics from different assays (EPM and Social Chamber) exhibit a clear separation between acute (red), developmental (blue) and control groups. Boundary lines (black) derived from fitting quadratic SVM. This suggests opposing effects in social and anxiety-related behavior resulting from Lobule VII inactivation at different developmental stages.

b) Lobule VI DREADD induces a specific reversal learning deficit



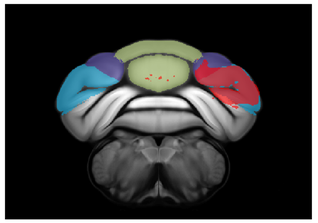
A principal component derived from Y-maze appears to capture learning and reversal metrics (top). Interestingly, the distributions across this dimension appear shifted in Lobule VI-inactivated mice, but not controls, independent of chronic vs acute treatment.

4 *Preliminary*: Anatomical localization of behavioral effects

i) Allen Brain Atlas registration (see also: 719.11 / RR12)

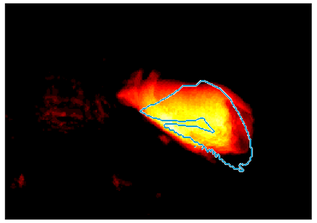
Serial two-photon stacks of DREADD-expressing brains are registered to a common coordinate framework (Allen CCFv2).

ii) Localization of expression



Anatomical data confirms expression (red) overlaps with cerebellar ROIs (e.g., blue: Crus II).

iii) Behavior metrics projection



(Preliminary): Projecting behavioral metrics to expression voxels (red; average score) associates behavior to anatomical regions (blue trace: Crus II)

Acknowledgements: This work is supported by NIH R01NS045193, Nancy Lurie Marks Family Foundation, F31 NS08930301, and National Science Foundation Graduate Research Fellowship under Grant No. DGE1148900.