Disruption of cerebellar activity causes autism-like mature phenotypes

A. Badura[1,2], J.W. Metzger[2], T.D. Pereira[2], B. Deverett[2,4], T.J. Pisano[2,4], S. Koay[2], D.W. Tank[2,5], C.D. Brody[2,4], J.L. Verpeut[2], S.S.-H. Wang[2]

[1]Netherlands Institute For Neuroscience, Amsterdam, The Netherlands; [2]Princeton Neuroscience Institute and Department of Molecular Biology Princeton University, Princeton, NJ, USA; [3]Rutgers RWJMS, Rutgers University, New Brunswick, NJ, USA; [4]Howard Hughes Medical Institute; [5]Bezos Center for Neural Dynamics, Princeton University, Princeton, NJ, USA;

719.10/ RR11

ABSTRACT

Cerebellar lobules VI/VII and crus I/II form reciprocal loops with neocortical regions associated with executive functions (Wang et al, 2014). To test the functional significance of those connections we used DREADDs to disrupt neural activity of these cerebellar regions during adulthood or postnatal development, and measured the consequences in two major domains: (1) social choice and behavioral inhibition; and (2) cognitive flexibility.

We made lobule-specific injections of AAV8-hSyn-hM4D(Gi)-mCherry to achieve expression in molecular layer interneurons (MLIs), a major source of inhibition to Purkinje cells. For developmental inactivation, mice were injected with the virus at P21, and received DREADD agonist clozapine-N-oxide (CNO) from P30 through P56. For acute inactivation, mice were injected at P42, followed by testing at P56 with CNO administered on the day of testing. The inhibitory action of CNO on DREADD-expressing MLIs was confirmed in acute cerebellar brain slices, as well as through indirect effects on Purkinje cell firing in vivo in awake mice.

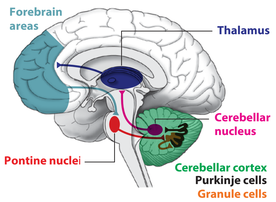
To analyze behavioral data, we used principal component analysis (see Poster by Pereira, Metzger et al.). In lobule VI, CNO administration in adult mice led to increased perseveration. The mice also showed reduced performance and bias in a virtual reality-based working memory task. These results suggest that lobule VI is an active part of brainwide circuitry for cognitive flexibility.

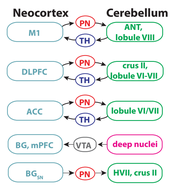
Developmental effects were seen from perturbations of crus I, crus II, and lobule VII. CNO administration during development led to reduced social interaction in a three-chamber test and impaired reversal learning in a Y-maze in mice injected in crus I. Unilateral crus II perturbation led to reductions in movement in the elevated plus maze and three-chamber task, as well as reduced social preference in the three-chamber test. None of these effects were seen with adult CNO treatment. In lobule VII, juvenile treatment with CNO decreased exploratory activity in the elevated-plus maze and increased social behavior in the threechamber test. Opposite effects were seen in acute adult treatment with CNO, suggesting that lobule VII contributes to maturation and acute function of exploratory behavior.

These experiments provide the first direct evidence for the developmental diaschisis hypothesis of cerebellar function.

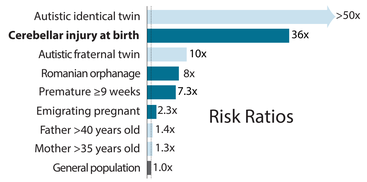
1 CEREBELLAR INFLUENCES: ADULT AND DEVELOPMENTAL ROLES

NEOCORTICAL LOOPS

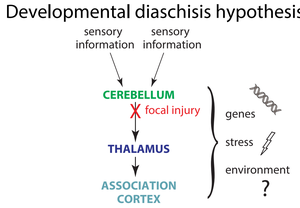




DEVELOPMENTAL INFLUENCES

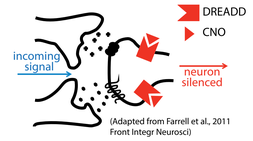


Developmental diaschisis hypothesis



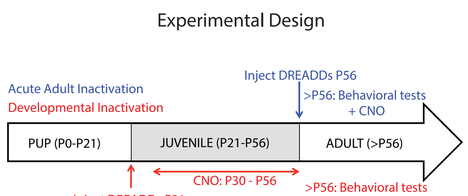
2 DEVELOPMENTAL AND ADULT CEREBELLAR INACTIVATION

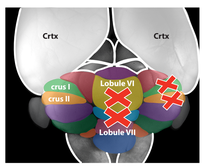
Designer Receptors Exclusively Activated by Designer Drugs



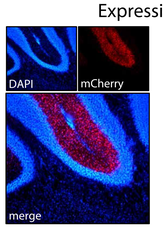
(Adapted from Farrell et al., 2011 Front Integr Neurosci)

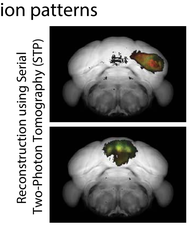
Experimental Design





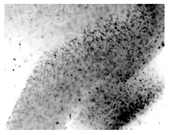
Expression patterns



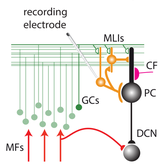


3 CNO-INDUCED CHANGES IN MOLECULAR LAYER INTERNEURONS (MLIs)

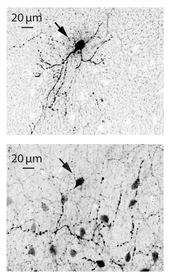
Expression in cerebellar acute slices at 32°C



Local circuitry

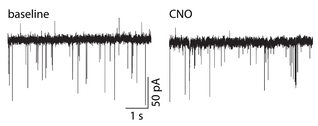


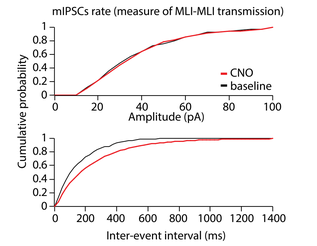
Patched MLI filled with Alexa 488



DREADD-mCherry expression

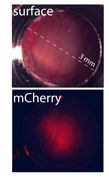
Intracellular MLI signals

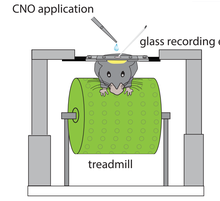




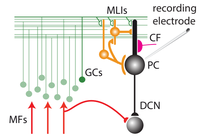
4 INHIBITION OF MLIs AFFECTS PURKINJE CELL SIMPLE SPIKE FIRING *IN VIVO*

In vivo awake recording setup

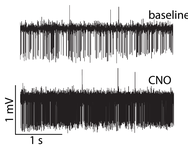




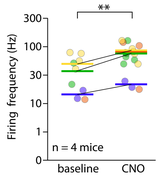
Local circuitry



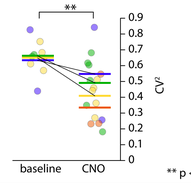
Extracellular PC signals



Spike frequency increases



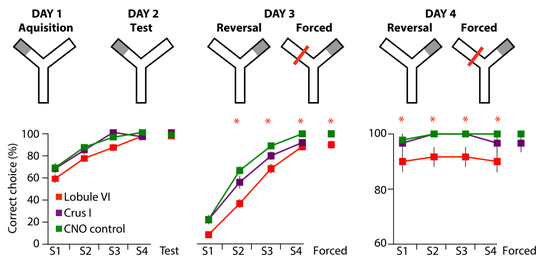
Regularity increases



** p < 0.01

5 CEREBELLAR CONTRIBUTIONS TO MOUSE BEHAVIOR - ACUTE INACTIVATION DURING ADULTHOOD

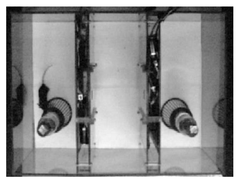
Repetitive Behavior : Y-Maze



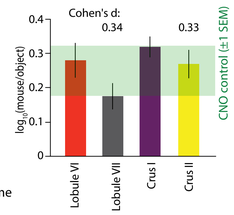
Region	Measure	Δ
Lobule VI	Acquisition Initial Learning Rate	↓
Lobule VI	Early Reversal Initial Learning Rate	↓
Lobule VI	Early Reversal Ability	↓
Lobule VI	Early Reversal Secondary Learning Rate	↓
Lobule VI	Distance	↑
Lobule VI	Velocity	↑

Social Behavior : Three-Chamber

(see also poster 719.12/RR13)



Mouse vs. object preference was quantified as ratio of time spent in proximity using automated tracking methods.



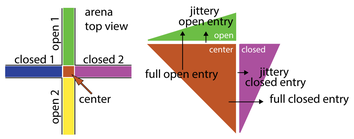
Region	Measure	Δ
Lobule VI	Baseline Distance	↑
Lobule VI	Lingering	↓

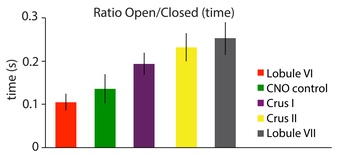
Repetitive Behavior : Grooming

Berridge et al. 2005 (BMC Biology)

No significant diffreneces

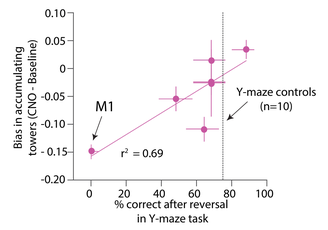
Anxiety : Elevated Plus-Maze



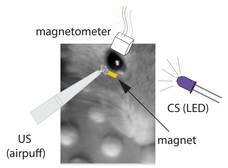


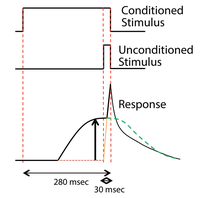
Region	Measure	Δ
Crus I	Open Arm Preference Index	↑
Lobule VI	Anti-Hesitation Index	↑
Lobule VI	Exploration Time	↓
Lobule VI	Exploration Entrance Index	↓
Lobule VII	Open Arm Preference Index	↑

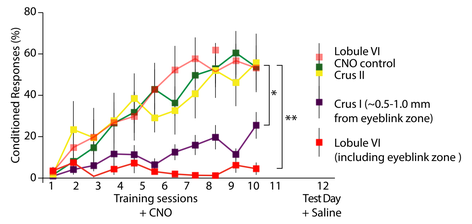
Working Memory



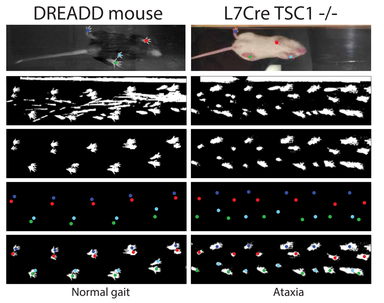
Motor Behavior : Eyeblink Conditioning







Motor Behavior : Gait analysis



No significant differences in paw-stride length, distance between forepaws, or distance betweenrear paws.

ACKNOWLEDGMENTS: This work is supported by NIH R01NS045193, R21NS092320, BRAIN U01NS090541, McKnight Technology Innovation and NancyLurie Marks Family Foundation. A.Badura is funded by Innovational Research Incentives Scheme VENI (The Netherlands).