CEREBELLAR LEARNING IN MOUSE MODELS OF AUTISM

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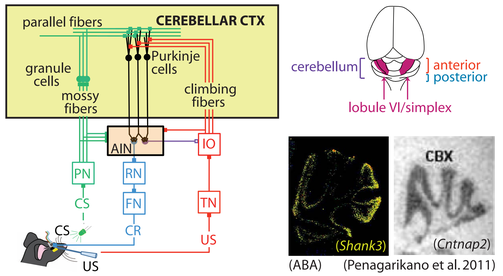
Overview

Although the cerebellum is the most frequently observed site of dysmorphism in autistic persons, cerebellar function has been investigated very little in animal models of autism. Cerebellar function is of significance because autism commonly includes motor deficits and because the cerebellum may additionally serve cognitive and affective functions. As a test of cerebellar dysfunction, we are using delay eyeblink conditioning, an associative learning task that requires the cerebellum and is aberrant in persons with autism spectrum disorder.



(ABA) (Penagarikano et al. 2011)

We previously developed a system for delay eyeblink conditioning in head-fixed, freely locomoting mice. Male mice are headplated and mounted on top of a cylindrical treadmill on which they can walk freely during testing. Eyeblink responses are measured using an electromagnetic sensor placed above the animal's upper eyelid to detect increases in voltage produced inductively by a magnet affixed to the lower eyelid. Training occurs by pairing a light-flash conditioned stimulus (CS) with a co-terminating, blink-evoking corneal airpuff unconditioned stimulus (US). After acquisition training for 12 days, a WT mouse learns to generate well-timed conditioned response (CR) blinks in response to the CS alone. Learning properties include trial-to-trial and day-to-day changes in CR probability and amplitude, as well as the latency to CR onset and the time of peak CR amplitude in individual trials. We are screening transgenic mice that show social defects, including Cntnap2-/- (Peñagarikano et al. 2011) and Shank3(+/ΔC) (Bangash et al. 2011).

Mouse models: connecting genetics and systems

The cerebellum and human autism

Anatomical evidence: consistent gross (Palmen *et al.*, 2004) and cellular (Amaral *et al.*, 2008) defects; reduced connectivity to other brain areas

Neuropsychological evidence: motor defects in autism (Ming *et al.*, 2007); cerebellar lesion patients frequently show social and cognitive symptoms (Schmahmann *et al.*, 2004)

Developmental evidence: perinatal cerebellar injury correlated with later autism and neocortical hypoplasia (Limperopoulos *et al.*, 2010)

Genetic mouse models

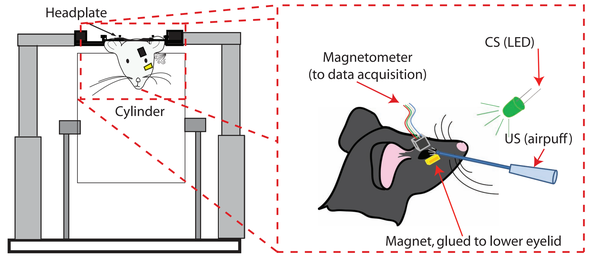
For this study, we have selected two validated mouse models of mutations affecting genes strongly expressed in the cerebellum with deficits in social behavior and other mouse behavior relevant to autism.:

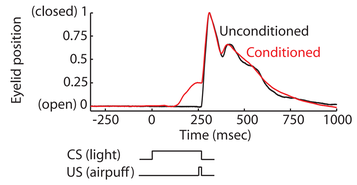
- **Cntnap2-/-**: *Cntnap2* is expressed in the cerebellar cortex; knockout disrupts brain maturation and neural migration of interneurons in neocortex. Ortholog of human *CNTNAP2* (Peñagarikano et al. 2011)

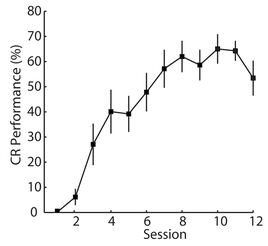
- **Shank3(+/ΔC)**: Mutation commonly seen with autism and Phelan-McDermid syndrome; *Shank3*, expressed in the postsynaptic density of granule cells is downregulated and is thought to perturb glutamatergic signalling and plas,ticity (Bangash et al. 2011).

However, behavioral correlates of autism that are isolated to cerebellar function have not yet been uncovered in mouse models.

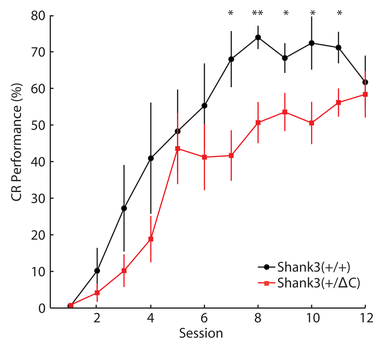
Eyeblink conditioning is a reliable screen for cerebellar function in head-restrained mice



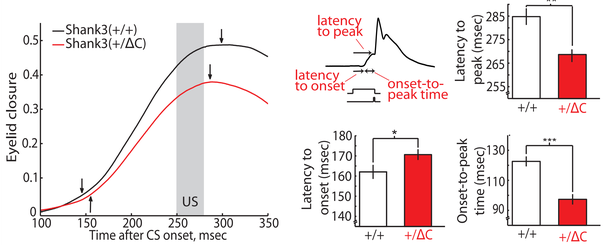




Shank3(+/ΔC) mice learn less than Shank3(+/+) over the training period...

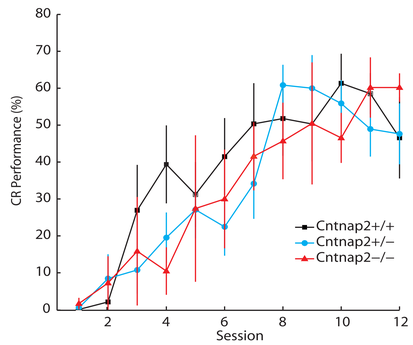


...and produce smaller and narrower CRs

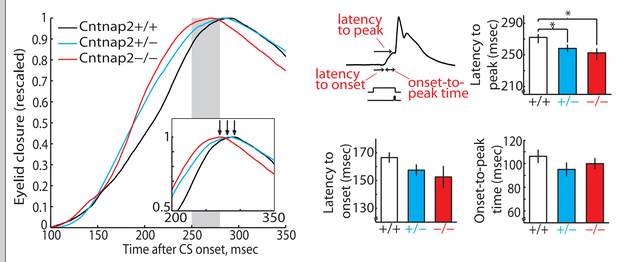


* p < 0.05; ** p < 0.01; *** p < 0.005

Cntnap2-/- has a similar timecourse of learning to Cntnap2+/+ and Cntnap2+/-...



...but tends to produce earlier CRs



* p < 0.05

Learning to produce well-timed responses of an appropriate magnitude is a **critical function** of the cerebellum. These models show performance and timing deficits, consistent with eyeblink conditioning studies in human patients.

Clinical studies of eyeblink conditioning with autism and related disorders have shown:
- Narrower responses with later onsets than control (autism; Sears, Finn, and Steinmetz 1994)
- Narrower responses and reduced performance(Fragile X syndrome; Tobia and Woodruff-Pak, 2009)

Discussion

Delay eyeblink conditioning in head-restrained mice produces well-timed conditioned responses. Magnetic tracking of eyelid deflection in a head-restrained mouse on a stationary wheel (Arlt et al., 2010) leads to reliable classical conditioning and well-timed blinks, providing a pipeline for fast screening of cerebellar learning.

Mouse models of autism show learning deficits and timing errors. Deficits in the timing and magnitude of conditioned eyeblinks are suggestive of disrupted cerebellar function. Delay eyeblink conditioning requires identified regions of cerebellum, but the extent of functional loss may be more widespread.

Disruptions of *Shank3* and *Cntnap2* expression may affect cerebellar information processing and plasticity. *Shank3* is expressed in cerebellar granule cells, and expression of a dominant negative allele (ΔC) disrupts transmission or plasticity in the mossy fiber pathway, as has been shown in other brain areas. *Cntnap2* knockout may also disrupt information processing in Purkinje cells.

Probing cerebellar function in autism models. Functional disruption of Purkinje cells leads to the core symptoms of autism (Tsc1: Tsai et al., 2012; ProSAP1/Shank2: Schmeisser et al., 2012). Delay eyeblink conditioning provides a biomarker for screening for cerebellar function.

Future Directions

Confirmation of the cerebellar locus via lesion or inactivation of the interpositus nucleus.

Whole-brain systems function. Other brain areas are known to modulate classical eyeblink conditioning, including hippocampus and amygdala (Lee and Kim, 2004). Increasing the CS-US delay interval (trace conditioning) introduces a requirement for the recruitment of additional brain areas.

Anatomical and physiological correlates. In each of these mouse models, we need to examine the gross morphology of cerebellum and the morphology and spine density of specific cell types at different locations throughout the cerebellar cortex. In addition, slice physiology may be necessary to characterize synaptic transmission at synapses affected by the present mutation. We anticipate that these experiments may yield different results as the mutation affect different cell-types.

Functional disruption during sensitive periods of development. The developmental time course of autism suggests the possibility that disrupted function in early life may lead to lasting functional consequences.

References

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This work is supported by Simons Foundation Autism Research Initiative 221582 (S.S.-H.W.), NIH R01 NS045193 (S.S.-H.W.), NIH R01 NS070301 (P.F.W.), NIH F31 MH098651 (A.D.K.), and Autism Speaks Translational Grant 7657 (O.P.).