Characterizing the neural circuits implicated in short term memory deficits of cholinergic *Mecp2* conditional knockout mice

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Novel Object Recognition Test

The Novel Object Recognition (NOR) Test is a quantitative assessment of mouse memory based on the premise that a given mouse will explore a novel object more than a familiar one, but only if the mouse remembers that the familiar object is indeed familiar, otherwise it will explore both the novel and familiar objects equally. To study the behavior/network relationship, I conducted NOR tests on 21 mice, split into four groups: WT exposed to only a familiar object, WT exposed to a novel object on day 4 of testing, CKO familiar, and CKO novel. The experimental procedures are outlined in **Figure 1**. Tests were performed on mice at 14 weeks of age, and each mouse was single-housed for three days prior to testing. During the assay, each mouse was exposed for five minutes to two identical objects for the first three days of the assay, with 24 hours between each test. On Day 4, each mouse was exposed to either the same two familiar objects (the familiar cohorts) or one familiar and one novel object (the novel cohorts).

Figure 1

Figure 1: Experimental Procedures.
1. NOR test: Day 1, Day 2, Day 3 (24 hours in between)
2. Day 4 - Either same 2 familiar objects, or 1 familiar and 1 novel
3. Mice sacrificed 30 minutes after final NOR task to allow for *Fos* mRNA expression
4. Brains dissected, embedded, 25 µm coronal sections, ≈100 sections/mouse.
   In situ hybridization using probes for ChAT and c-Fos mRNA follows.

Figure 2

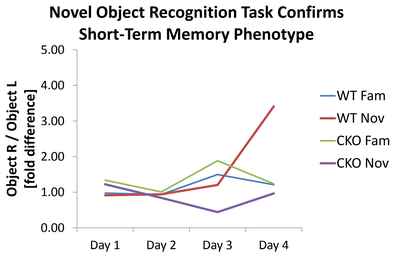

Figure 2: NOR test confirms the short term memory phenotype. These results demonstrate that WT mice have intact short term memory, with a novel object exploration time / familiar object exploration time ratio of 3.41 (SEM 1.32). I also confirmed that the CKO mice indeed demonstrate short term memory deficits, exploring the novel and familiar object at roughly the same rate, with a ratio of .97 (SEM .17).

Abstract

Rett Syndrome (RTT) is a progressive, postnatal neurological disorder caused by mutations in the gene encoding methyl-CpG binding protein 2 (*MECP2*). *Mecp2* null mice recapitulated the various phenotypes observed in human patients, including seizures, cognitive deficits, paw stereotypies, ataxia and other symptoms. MeCP2's role in contributing to these phenotypes is difficult to discern, leading us to ask whether individual RTT phenotypes result from MeCP2 dysfunction in specific neural networks. The Zoghbi lab used a Cre-lox approach to generate conditional knockout mice (CKO) mice in which *Mecp2* is deleted only in cholinergic neurons, which are important for learning and memory. We found that CKO mice are comparable to wild type (WT) mice, but exhibit short-term memory deficits.

Using the novel object recognition test, a quantitative assessment of mouse memory, I confirmed the short-term memory deficit in CKO mice, as measured by a novel/familiar object exploration time ratio of .97 (SEM .17) compared to that of WT mice 3.41 (SEM 1.32). Next, we hypothesized that loss of MeCP2 in central cholinergic neurons alters the activity of these neurons, and explored the possibility that *Fos*, a gene that marks clusters of neural activity, can indicate activation, or inactivation, of cholinergic neurons in our mice. We found that Fos activity in the lateral septal nucleus is increased in CKO brains even when exposed to a familiar object, indicating that CKO mice lack short-term memory, which may result from impaired cholinergic signaling caused by lack of MeCP2 in the respective neurons.

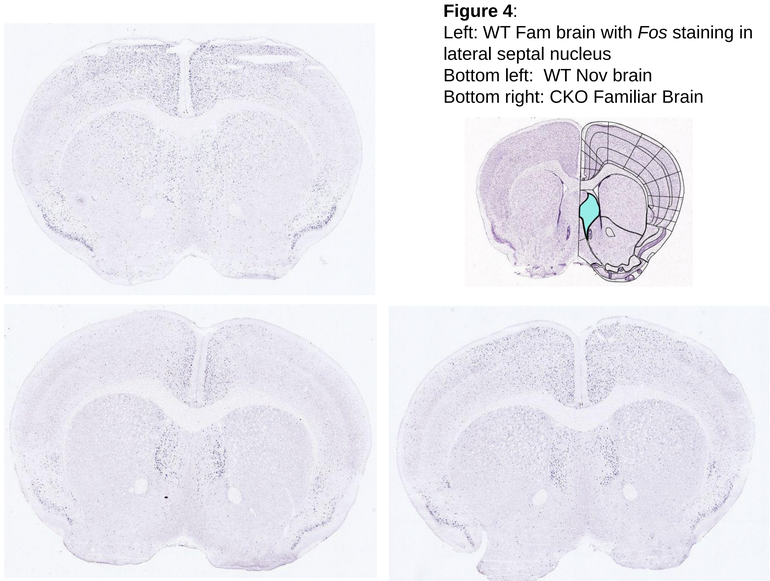

Figure 4:
Left: WT Fam brain with *Fos* staining in lateral septal nucleus
Bottom left: WT Nov brain
Bottom right: CKO Familiar Brain

References:
[1]Winters DB and Bussey TJ (2005) Removal of cholinergic input to perirhinal cortex disrupts object recognition but not spatial working memory in the rat. *European Journal of Neuroscience.* 21: 2263-2270.

Acknowledgements:
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Characterizing the Neural Circuit

The central cholinergic nervous system consists of a network of neurons that secrete the neurotransmitter acetylcholine and the neurons that they innervate. The cholinergic neurons we are interested in reside in the basal forebrain and project to the hippocampus, an important region for learning and memory. Recent literature suggests that cholinergic neurons of the basal forebrain are crucial for object recognition memory[1]. Therefore, these neurons served as regions of interest in probing for *Fos* immunoreactivity in coronal sections of my mouse brains. We hypothesized that loss of MeCP2 in central cholinergic neurons alters the activity of these neurons, and explored the possibility that *Fos*, a gene that marks clusters of neural activity, can indicate activation, or inactivation, of cholinergic neurons in our mice.

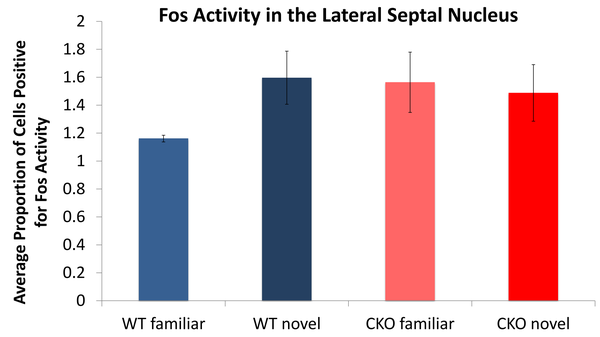

Figure 3: Fos activity in the lateral septal nucleus is increased in CKO brains even when exposed to a familiar object, indicating that CKO mice lack short-term memory, which may result from impaired cholinergic signaling caused by lack of MeCP2 in the respective neurons. WT familiar: 1.16; WT novel: 1.59; CKO familiar 1.56; CKO Novel 1.48.

Conclusions:

In this study we demonstrate that the deletion of *Mecp2* in the cholinergic neurons results in a short term memory phenotype using the NOR task. Furthermore, our investigation of the molecular mechanisms involved in the phenotype showed that Fos activity in the lateral septal nucleus of CKO mice is increased, even when exposed to a familiar object. This finding suggests that loss of MeCP2 in cholinergic neurons of the basal forebrain can result in impaired cholinergic signaling, resulting in CKO mice lacking short-term memory.

Future Directions:

We plan to test more animals to better define the impaired neural circuit implicated in short term memory deficits of CKO mice. If MeCP2 in cholinergic neurons is proved to be necessary for short term memory, the next step will be to show sufficiency. This could be done via an optogenetic approach, in which genetically engineered cholinergic neurons can be activated by light to show in real time the effects of hippocampal cholinergic innervation on learning and memory in behavioral assays such as NOR. This work should reveal important insights into the neural circuits that are involved in novel object recognition, and ultimately, learning and memory. Further research into the molecular mechanisms of how *Mecp2* loss leads to reduced cholinergic signaling will be necessary to elucidate its role in learning and memory, cognitive deficits, and autonomic dysfunction.