Final report: Neurocognitive impairments and the role of MeCP2 in ASD

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by social interaction and social communication deficits as well as sensory and motor impairments [1]. Given the polygenic nature of ASD in addition to its increasing prevalence [2; 3], understanding the neurobiological effects of early behavioral intervention is of paramount importance when implementing these types of treatments in clinical populations. The current proposal sought to determine the therapeutic effectiveness of early behavioral interventions in a mouse model of ASD.

Our mouse model of ASD uses a knockout of methyl-CpG binding protein 2 (MeCP2), a neuroepigenetic factor implicated in the etiology of ASD [4; 5; 6; 7]. Previously published work has demonstrated that loss-of-*Mecp2* function mutations results in aberrant dendritic and axonal processes in mouse cortical neurons [8] while clinical studies have shown decreased *Mecp2* mRNA expression in the frontal cortices of ASD patients [6]. Collectively, these data suggest that dysregulation of MeCP2 levels leads to impairments in neuroplasticity, which may underlie the learning and memory deficits that are often associated with ASD [5]. Brain-derived neurotrophic factor (BDNF) is a protein that regulates neuroplasticity as well as neuronal health and is highly sensitive to environmental perturbations [9; 10; 11]. Past studies have revealed that chronic stress reduces while environmental enrichment enhances levels of BDNF expression in an area of the brain associated with learning and memory, the hippocampus [9; 10].

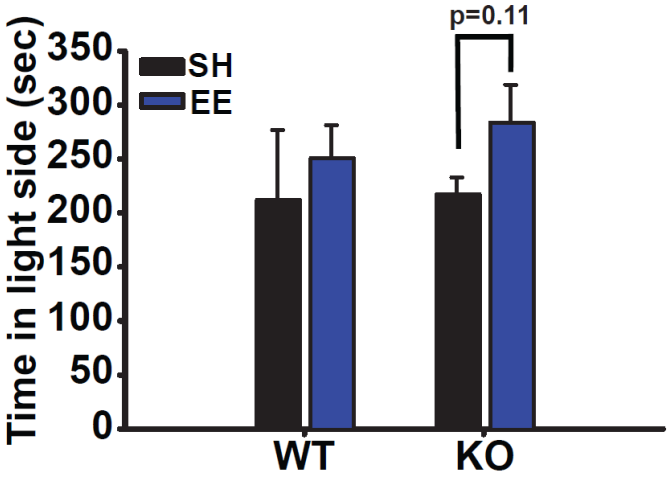


Figure 1. Dark-light test. MeCP2 KO mice given EE show increased time in the light side of a dark-light box, compared to MeCP2 KO mice exposed to SH. WT: wildtype; KO: knockout; SH: single-housed; EE: environmental enrichment

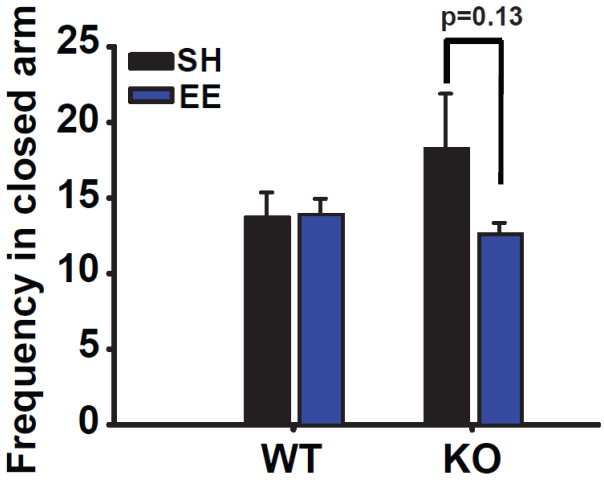
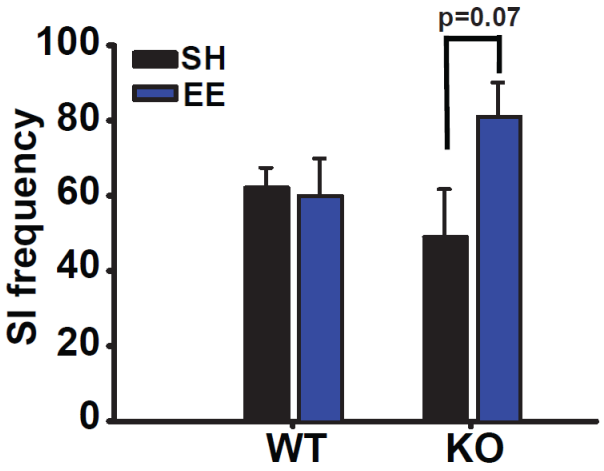


Figure 2. Elevated plus maze (EPM). MeCP2 KO mice given EE visited the closed arm of an EPM less frequently than MeCP2 KO mice exposed to SH. WT: wildtype; KO: knockout; SH: single-housed; EE: environmental enrichment

In the current study, we used transgenic mice in which MeCP2 was knocked out (KO) globally throughout the brain and then randomly assigned knockout mice to either environmental enrichment (EE) or a single housed (SH) condition. Wildtype (WT) mice, which served as our control group, were also randomly assigned to either a SH or EE condition. EE mice were chronically exposed to a number of different EE apparatuses such as a running wheel, mouse hut, specialized bedding, etc [10] for 12 weeks. After 12 weeks of SH or EE exposure, several different behavioral tests were administered. Behavioral tests assayed for anxiety-like behavior, social interaction, as well as episodic memory to determine if EE could mitigate deficits in these phenotypes in our MeCP2 KO mice. We also assayed for levels of BDNF protein in the hippocampus after behavioral testing was complete.

Based on our findings, we show that long-term exposure to EE can ameliorate anxiety-like behaviors and reverse social interaction deficits. MeCP2 KO mice spent more time on the light side of a dark-light box (Figure 1) and traveled to the closed arm of the elevated plus maze less than their SH KO counterparts (Figure 2). These data suggest that EE may lead to reductions in anxiety-



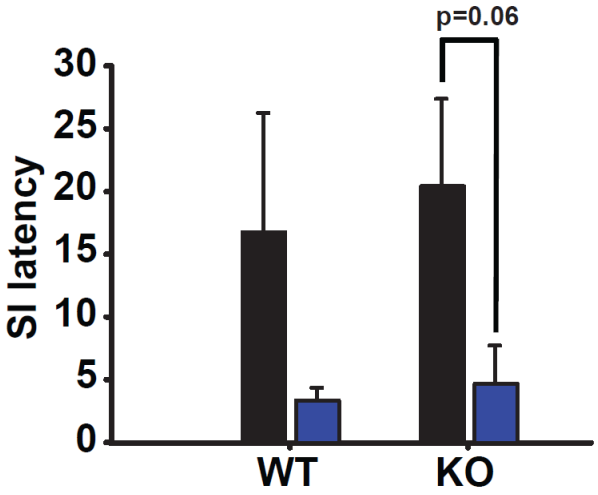
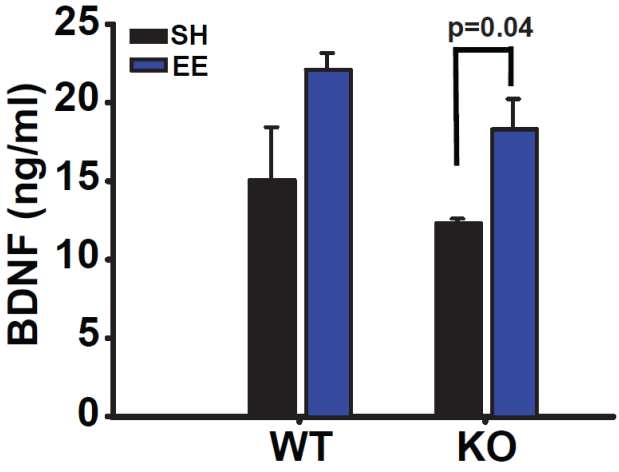


Figure 3. Social interaction (SI) test. MeCP2 KO mice given EE showed increased SI frequency and decreased latency to interact with conspecific relative to MeCP2 KO mice exposed to SH. WT: wildtype; KO: knockout; SH: single-housed; EE: environmental enrichment. N's per group WT SH: 11; WT EE: 10; KO SH: 4; KO EE: 5



like behavior. While p values are not statistically significant, they are trending towards significance and given the low n's, we are confident that these data will hold until statistical power is achieved.

Interestingly, MeCP2 KO mice exposed to EE also increased social interaction frequency and decreased latency to interact with a male conspecific relative to SH KO mice (Figure 3), demonstrating that EE improves social interaction in KO mice. Once again, these data are trending towards significance but given the robustness of the data, we believe that adding additional cohorts to these behavioral experiments will yield statistically significant results.

Lastly, with regard to BDNF protein expression, EE + MeCP2 KO mice show increased hippocampal BDNF expression (Figure 4) compared to SH + MeCP2 KO mice. These results indicate that EE may improve hippocampal function by way of increasing BDNF expression. Future experiments could address the nature of this improvement by examining processes associated with neuroplasticity such as hippocampal long-term potentiation [12].

Taken together, our results suggest that long-term exposure to environmental enrichment can improve several symptoms associated with ASD, including anxiety, social interaction as well as neural mechanisms that underlie neuroplasticity. The funding of this work through the Woodcock Institute has provided the foundation with which to build a successful research program that will continue to explore therapies that have significant potential to bring insight into how these therapies work to benefit brain health and ASD symptoms.

Dissemination of research

We are presenting our findings at the Society for Neuroscience conference, October 2024 after which we will continue to collect data to complete the proposed experiments. After completing our studies, we will prepare our current data set for publication in a reputable peer-reviewed Neuroscience journal.

Figure 4. Hippocampal BDNF protein expression. MeCP2 KO mice given EE have increased hippocampal BDNF expression compared to MeCP2 KO mice exposed to SH. WT: wildtype; KO: knockout; SH: single-housed; EE: environmental enrichment. N's per group WT SH: 4; WT EE: 4; KO SH: 3; KO EE: 3

Budget

The funds awarded by the Woodcock Institute were spent on BDNF ELISA kits, per diems for animal housing, as well as the environmental enrichment apparatuses.

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