Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

MeCP2 Function in the Basolateral Amygdala in Rett Syndrome

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Review of Adachi et al.

Two parents watch with wonder as their first child, a daughter, develops. As an infant, she charms them with her smiles and babbling. By 9 months, she is excellent company, able to sit in her highchair for meals and stuff her mouth with animal crackers. Peek-a-boo is her favorite game, and when her parents laugh, she joins in. Then, over a few months, everything changes. Normally happy and alert, she becomes withdrawn, sometimes crying for prolonged periods of time. Before, she loved grabbing at her parents' thumbs. Now she fixates on her own hands, often rubbing them together intently. She no longer speaks the simple words she had learned. Soon she will not be able to hold a cracker or reach for her toys. Her parents know something is very wrong, and after many doctors' visits and tests, they receive a diagnosis: Rett syndrome.

This devastating progression of developmental milestones gained then dramatically lost is the picture of Rett syndrome, one of the Autism Spectrum Disorders. First described by the Austrian neurologist Andreas Rett in 1966, the collection of symptoms received attention as a clinical presentation in 1983 (Hagberg et al., 1983). Because predominantly females present with the disorder, which is characterized by stereotypic hand motions, a search for a sex-linked genetic basis was initiated. In 1999, mutations in a single gene on the X chromosome—*MECP2*—was discovered to be the basis of the disease (Amir et al., 1999). The methyl CpG-binding protein 2 (MeCP2) binds to methylated CpGs in DNA and was known at the time to associate with the Sin3 homolog A protein (Sin3A) and histone deacetylase, chromatin regulators that repress transcription. Based on these observations, the neurological dysfunction seen in Rett syndrome was hypothesized to result from the loss of MeCP2-mediated repression of critical target genes in neurons.

To examine the functions of MeCP2 *in vivo*, knock-out mice were generated by two groups of investigators (Chen et al., 2001; Guy et al., 2001). These mice recapitulated neurological degeneration similar to that observed in patients with Rett syndrome. Additionally, conditional knock-out mice were generated to induce tissue-specific deletion of MeCP2. Mice in which *Mecp2* was flanked by loxP sites in all cells were crossed to mice expressing Cre recombinase under the control of tissue-specific promoters, allowing Cre-mediated recombination of the loxP sites, excision of *Mecp2* in Cre-expressing cells, and deletion of MeCP2 from those tissues. Mice that lacked MeCP2 in forebrain structures (hippocampus, cortex, striatum, and amygdala) recapitulated much of the neurological dysfunction of the full knock out and demonstrated impaired motor coordination, increased anxiety-like behaviors, impaired cue-dependent fear conditioning, and abnormal social interactions, indicating that loss of forebrain MeCP2 contributes to a wide spectrum of Rett syndrome symptoms (Chen et al., 2001, Gemelli et al., 2006).

To link behavioral symptoms with specific brain regions, Adachi et al. (2009) sought to lower MeCP2 levels in the basolateral amygdala (BLA), a region that is central to emotional responses. Since there are no known markers for BLA neurons that allow genetic knockdown in that particular subset of cells, the authors targeted the region using an adeno-associated virus that expresses Cre. In floxed *Mecp2* mice, the virus induces expression of the recombinase and knock out of MeCP2 in infected cells. The authors injected the Cre-expressing virus into the BLA of the previously generated floxed *Mecp2* mice (Chen et al., 2001) and confirmed transgene expression using fluorescent *in situ* hybridization and BLA microdissection followed by mRNA and protein analysis. Cre expression led to the knockdown of MeCP2 to ~50% of normal levels for the region as a whole.

Mice were subjected to a battery of motor and behavior tests. In two tests of anxiety, the open field test and the elevated plus maze, mice with BLA-specific MeCP2 knockdown exhibited heightened anxiety-like behavior. In fear-conditioning assays, the mice demonstrated impaired

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cue-dependent fear learning but intact context-dependent fear learning. These findings were similar to those seen in a previous study using forebrain-specific knock-out mice (Gemelli et al., 2006). However, in tests of motor skills and social interactions, BLA-specific deletion of MeCP2 did not produce any changes. From these findings, the authors conclude that the BLA is involved in the anxiety and fear-learning phenotypes but not the motor or social interaction deficits of the forebrain conditional knock out.

The authors determined that these behavioral phenotypes were attributable to loss of MeCP2-mediated repression because histone H3 acetylation, a mark of activated genes, was increased. They hypothesized that infusion of the BLA with a histone deacetylase inhibitor would recapitulate the loss of MeCP2 by relieving genes of repression. They chronically infused SAHA, an inhibitor of class I and II histone deacetylases, into the BLA bilaterally. To confirm that gene transcription was active, they probed for acetylated histone H3 and histone H4, both of which were increased.

The motor skills and behavior of these mice were similar to those of the mice in which MeCP2 was knocked down specifically in the BLA. SAHA-infused mice demonstrated increased anxiety-like behavior and impaired cue-dependent fear learning with no significant difference in context-dependent fear learning. Additionally, there were no deficits in social interaction, motor locomotion, or motor coordination. Therefore, the authors conclude that MeCP2 mediates these behaviors in the BLA by acting as a repressor.

Although MeCP2 was initially described as a global repressor of transcription *in vitro*, this function has been questioned. Gene array studies do not show a global increase of transcription in Rett syndrome patients nor in *Mecp2* knock-out mice. On the contrary, a microarray analysis comparing the hypothalamus from *Mecp2* null and overexpressing mice found that 85% of the genes were downregulated in null animals and upregulated in overexpressing animals (Chahrour et al., 2008). These results suggest that MeCP2 may predominantly activate genes. In support of this, MeCP2 associated with CREB1 in the promoters of activated genes.

The findings of Adachi et al. (2009) might be reconciled with these other studies if the role of MeCP2 in gene transcription is context dependent, as determined by the methylated status of a genomic region and also by the interactions with transcriptional modulators like CREB1 or Sin3A. MeCP2 might act mainly as a repressor in the amygdala while acting mainly as activator in the hypothalamus.

Adachi et al. (2009) argue in favor of MeCP2 as a repressor based on two findings: (1) After virus-mediated knockdown of MeCP2 in BLA, there is an increased amount of acetylated histone H3, and (2) infusion of histone deacetylase inhibitor caused anxiety-like behavior that mimicked the phenotype seen in BLA-specific MeCP2 knockdown mice. One caveat to the first argument is that although an increase of acetylated histone H3 suggests an increase in transcription, the finding is not specific. When the authors examined the levels of several genes—Crh, Sgk, FK506, gephyrin, α-actinin and BDNF—they were unable to detect increased amounts of transcription. Moreover, they did not detect an increase in histone H4 acetylation, which is also found in active genes. Although SAHA infusion recapitulated the anxiety phenotype of BLA-specific MeCP2 knockdown mice, anxiety is a complex behavior that is mediated by a combination of genes, and SAHA infusion is likely to target genes that are not regulated by MeCP2. Indicative of this broader effect of SAHA, histone deacetylase inhibition led to acetylation of histone H4, which was not seen in the BLA-specific MeCP2 knockdown mouse. Also, the authors do not demonstrate that the structures around the BLA were not affected by the SAHA infusion.

The findings of Adachi et al. (2009) are important because they not only link certain behaviors to the BLA, but they also exclude motor behavior and social interaction from BLA regulation. The virus-mediated technique they use to deliver Cre recombinase could be applied generally to knock down genes in regions for which there are no good genetic markers. Additionally, the anxiety effects produced by SAHA infusion may have implications for histone deacetylase inhibitor use as a potential therapeutic for other conditions. The controversial issue of whether MeCP2 is acting as a repressor or an activator in the BLA can be clarified by more specific analysis to determine which target genes are controlled by MeCP2, and what their roles are in anxiety.

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