Review

The role of MeCP2 in learning and memory

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Gene transcription is a crucial step in the sequence of molecular, synaptic, cellular, and systems mechanisms underlying learning and memory. Here, we review the experimental evidence demonstrating that alterations in the levels and functionality of the methylated DNA-binding transcriptional regulator MeCP2 are implicated in the learning and memory deficits present in mouse models of Rett syndrome and *MECP2* duplication syndrome. The significant impact that MeCP2 has on gene transcription through a variety of mechanisms, combined with well-defined models of learning and memory, make MeCP2 an excellent candidate to exemplify the role of gene transcription in learning and memory. Together, these studies have strengthened the concept that precise control of activity-dependent gene transcription is a fundamental mechanism that ensures long-term adaptive behaviors necessary for the survival of individuals interacting with their congeners in an ever-changing environment.

The roles of gene transcription and mRNA translation in learning and memory throughout the animal kingdom have been extensively and very well defined over the past five decades. Studies inhibiting gene transcription demonstrate its necessity for learning and memory in both invertebrates and vertebrates (Brink et al. 1966; Thut and Lindell 1974; Wetzel et al. 1976; Pedreira et al. 1996). In rats, during both memory consolidation (Taubenfeld et al. 2001; Garcia-Osta et al. 2006; Bekinschtein et al. 2007; Chen et al. 2011) and memory reconsolidation (Da Silva et al. 2008), at least one phase of gene transcription is required, and in some cases, like avoidance memory formation, multiple phases of transcription are required (Igaz et al. 2002). Gene transcription also plays a fundamental role in long-term plasticity of excitatory synaptic transmission (i.e., long-term potentiation and long-term depression) in invertebrates and vertebrates via the transcriptional regulation of genes encoding proteins responsible for the formation of new synapses and the structural remodeling of existing ones, which results in lasting changes in synaptic strength (Davis and Squire 1984; Tully et al. 1994).

Methyl CpG binding protein 2 (MeCP2) is an X-linked global transcriptional regulator that binds methylated sites in DNA, and whose dysfunction is implicated in two severe neurological disorders: Rett syndrome and *MECP2* duplication syndrome. Rett syndrome is caused by loss-of-function mutations in *MECP2* (Amir et al. 1999), while *MECP2* duplication syndrome is caused by a gain-of-function due to the overexpression of *MECP2* (Van Esch et al. 2005). Because several mouse models of both syndromes show altered learning and memory, MeCP2 is an excellent candidate for the examination of the role of epigenetic regulation of gene transcription in learning and memory. Here, we review experimental observations on the role of MeCP2 in the regulation of gene transcription and learning and memory in rodent models.

Molecular mechanisms of MeCP2 function

In addition to its strict definition by Waddington (i.e., "*the various developmental pathways a cell might take toward differentiation*"), the term epigenetics is also used to describe the tagging of DNA and DNA-associated histones, which leads to transient or enduring regulation of gene transcription without changes in the gene coding sequence itself. The most common occurrence of epigenetic modification is during the differentiation of specific cell types by the regulation of the chromatin structure (Rakyan et al. 2001). Epigenetic modifications include methylation of DNA, as well as acetylation, phosphorylation, methylation, ubiquitylation, and SUMOylation of histones (Hebbes et al. 1988; Zhang 2003; Miller et al. 2010; Sánchez-Álvarez et al. 2010; Zovkic et al. 2013; Meas and Mao 2015). Histone acetylation and phosphorylation both typically function as transcriptional activators (Hebbes et al. 1988; Mujtaba et al. 2007). Histone methylation and SUMOylation act as transcriptional repressors (Whetstine 2009; Sánchez-Álvarez et al. 2010). Both DNA methylation (Chahrour et al. 2008) and ubiquitylation (Zhang 2003; Meas and Mao 2015) can result in either transcriptional activation or repression. MeCP2 is the founding member of the methyl-DNA binding proteins (MBD), and was discovered several years before its role in Rett syndrome and *MECP2* duplication syndrome was recognized (Lewis et al. 1992; Amir and Zoghbi 2000).

DNA methylation adds a methyl group at the 5′ position on the cytosine pyrimidine rings (Holliday and Pugh 1975), creating a carbon–carbon bond that is very stable. MeCP2 binding to methylated CpG sites causes the interaction with the histone deacetylase complex (Maunakea et al. 2013). It is important to note that MeCP2 is preferentially localized to the pericentromeric heterochromatin, which has the highest concentration of 5-methylcytosine (Nan et al. 1996). This carbon–carbon bond stability helps to prevent demethylation (Wolffe et al. 1999); however, in rare cases, spontaneous demethylation can occur. In the event of spontaneous demethylation, the methylated compound is resynthesized (Ma et al. 2009). When MeCP2 binds to methylated DNA it interacts with either the mSin3A/HDAC corepressor complex (Nan et al. 1998) or CREB (Chahrour et al. 2008), which results in transcriptional repression or transcriptional activation, respectively (discussed in further detail below) (Fig. 1). The NCoR/SMRT

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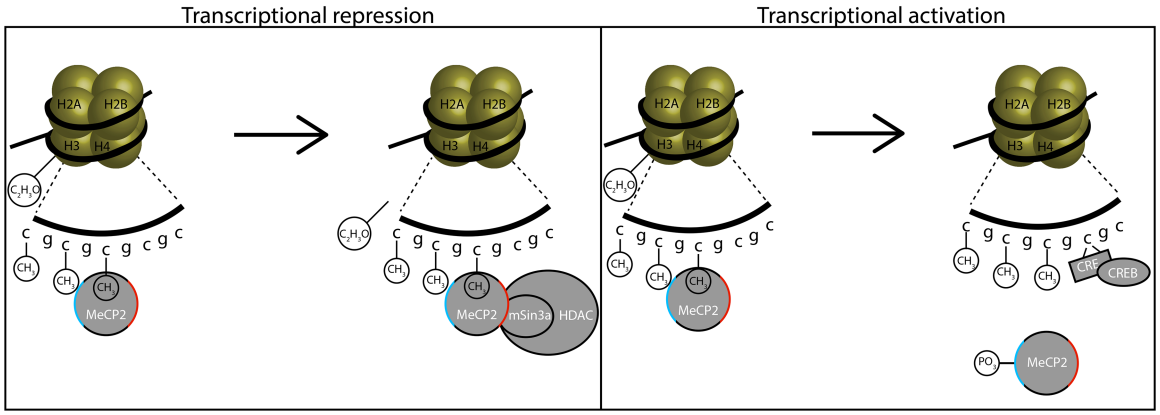

Figure 1. The role of MeCP2 in learning and memory through transcriptional repression and activation. Altered deacetylation results in altered transcriptional repression, resulting in impaired spatial memory, fear learning, and social memory. Preventing MeCP2 phosphorylation results in no release from *Bdnf*, resulting in improved spatial memory and fear learning.

complexes are known corepressors needed for transcriptional repression that have also been found to directly bind to MeCP2. Additionally, the cSki/Sno complex is found to directly bind to MeCP2. Successful binding of cSki/Sno to MeCP2 is necessary for transcriptional repression (Kokura et al. 2001). These interactions alter the reading of the DNA by RNA polymerase II. Altering the conformation of the DNA being read by RNA polymerase II results in either the silencing of mRNA transcription or the transcription of the mRNA transcript (Kriaucionis and Bird 2003).

MeCP2 is highly localized to pericentromeric heterochromatin due to its binding to methylated cytosine (mC) in CG (mCG) and, to a less extent, mCH (H: A, C, or T) pairs. While MeCP2 has the propensity to interact with any cytosine rich region, interactions with methylated CpG islands are the most thoroughly studied, and will be the focus of this review (Lewis et al. 1992; Hendrich and Tweedie 2003). MeCP2 interaction with methylated sites throughout the gene body results in both transcriptional activation and repression (Chahrour et al. 2008). When an organized chromatin structure is not present, MeCP2 selectively represses transcription of methylated templates (Nan et al. 1997; Kaludov and Wolffe 2000). One hypothesis for the pathogenesis of Rett syndrome is that loss-of-function mutations in the *MECP2* gene are detrimental because they result in the lack of MeCP2-mediated silencing of transcriptional noise (Bird and Tweedie 1995). It has also been suggested that MeCP2 controls the expression of long genes (more than 100 kb) in a length-dependent manner (Gabel et al. 2015), although this view is controversial (Raman et al. 2018). Regardless of the specific molecular mechanism(s) of MeCP2 regulation of gene expression (Guy et al. 2011), there is ample consensus that MeCP2 is a critical component of the gene structure required for proper transcription. Considering its stoichiometry to DNA (Nan et al. 1997), its abundance significantly affects gene transcription (Fig. 1).

Mechanisms of MeCP2 mediated transcriptional repression

In vitro experiments have shown that transcriptional repression occurs via the formation of aggregates of MeCP2 on the DNA template, preventing transcription complexes from forming (Kaludov and Wolffe 2000). Similar aggregation is observed in the nuclei at foci containing methylated DNA (Nan et al. 1996). After binding methylated DNA, MeCP2 impacts transcription by restricting access to RNA polymerase II, as well as by splicing mRNA and association with mRNA already spliced by MeCP2 (Long et al. 2011).

MeCP2 also represses transcription by the interaction between chromatin, DNA methylation, and histone deacetylation. In MeCP2, the chromatin infrastructure recognizes methylated CpG dinucleotides in the nucleosome (Chandler et al. 1999). When bound to DNA, MeCP2 can displace histone H1 from chromatin, which allows MeCP2 access to its binding sites (Nan et al. 1997). In short, MeCP2 is involved in the modification of the chromatin structure directly or through other repressive enzymes (Robertson and Wolffe 2000). Alteration of the chromatin structure interacts with islands of CpG, allowing for transcriptional repression (Nan et al. 1997). One region of MeCP2 contains a transcriptional repression domain that allows binding to the corepressor complex mSin3A/HDAC through interaction with mSin3A. This indicates that both histone deacetylation and DNA methylation are necessary for MeCP2 to repress transcription (Nan et al. 1998). Additionally, it is likely that MeCP2 interactions with NCoR/SMRT and cSki/Sno complexes, in concert with the mSin3A/HDAC complex, are necessary for successful MeCP2-mediated transcriptional repression (Kokura et al. 2001).

Mechanisms of MeCP2 mediated transcriptional activation

MeCP2 can also function as a transcriptional activator: MeCP2 copurifies with multiple proteins including the well-known transcriptional activator, CREB1. In addition, MeCP2 binds to the promoter region of *Creb1*. While deletion of *Mecp2* causes more transcriptional repression than activation, duplication of *Mecp2* results in more activation than repression (Chahrour et al. 2008). Binding of MeCP2 has been observed at the promoter region of *Sst*, *Oprk1*, *Gamt*, *Gprin1*, *Mef2c*, and *A2bp1*, all genes that show a bimodal expression between mouse models of *Mecp2* deletion and *MECP2* overexpression. Binding of MeCP2 was not detected at the promoter region of genes which are down-regulated in both mouse models. The genes that show MeCP2 binding also show increased binding in *Mecp2* overexpressing mice as compared to wild-type mice (Chahrour et al. 2008). In addition, *Mecp2* binding to *RNASEH2A* is responsible for up-regulation of its transcription, while *Mecp2* deletion results in lower *RNASEH2A* transcription (Yasui et al. 2007). Together, these findings suggest that MeCP2 does not prevent the binding of repressors, but rather recruits transcriptional activators (Yasui et al. 2007; Chahrour et al. 2008).

Phosphorylation of MeCP2

Posttranslational modifications like phosphorylation are well-characterized mechanisms that alter protein function. MeCP2 is

phosphorylated at serine 80 (S80) and serine 421 (S421). When S80 is dephosphorylated, MeCP2 binds less strongly to chromatin regions. While neuronal activity induces phosphorylation at S421, it reduces phosphorylation at S80. It has been suggested that phosphorylation of MeCP2 at these sites promotes the transition between the resting state and the depolarized state of the neuron (Tao et al. 2009). S80 phosphorylation allows for binding of MeCP2 to chromatin during resting states, while S421 phosphorylation allows for disassociation from the chromatin during neuronal depolarizations. Alterations of phosphorylation of S80 can cause transcriptional changes to a small number of genes but does not impair global gene transcription. However, the transition between resting and depolarized states that is assisted by phosphorylation of MeCP2 causes shifts in gene transcription (Tao et al. 2009).

Experimental animal models to characterize MeCP2 function

Rett syndrome is modeled in mice carrying a constitutive deletion of one or two of the coding exons in the *Mecp2* gene, or the insertion of a premature STOP codon at specific sites to generate a truncated MeCP2 protein, as well as by cell type-specific or brain region-specific deletions of *Mecp2* exons using the Cre-loxP system (for review, see Li and Pozzo-Miller 2012).

One of the two constitutive *Mecp2* knockout (KO) mouse models was generated using the Cre recombinase-loxP system, inserting loxP sites flanking exons 3 and 4 of the *Mecp2* gene, which results in their deletion in Cre-expressing cells ("Bird" line) (Guy et al. 2001). The other constitutive *Mecp2* KO mouse model carries a Cre-loxP deletion of exon 3 ("Jaenisch" line) (Chen et al. 2001). While these *Mecp2* exon deletions result in truncated peptides, these are nonfunctional. Male mice of both these constitutive *Mecp2* KO lines develop a combination of neurological phenotypes at around 2 mo of age, which are reminiscent of the symptoms presented by Rett syndrome individuals. Due to the mosaic pattern of mutant and nonmutant cells resulting from random X-chromosome inactivation, female heterozygous (Het) mice of both lines show a delayed onset of the same Rett-like neurological phenotypes.

Mice carrying a premature STOP codon at position 308 were generated to model the consequences of *MECP2* mutations that result in truncated MeCP2 protein in some Rett syndrome individuals (Shahbazian et al. 2002). Similar to the mild clinical presentation in these Rett syndrome individuals, male *Mecp2*^{308} mice have milder phenotypes and longer lifespan than constitutive KO mice (Shahbazian et al. 2002).

Conditional *Mecp2* KO mice were later developed using the Cre-loxP system, with Cre expression driven by promoters that are expressed in specific cell types or different brain regions, including *Camkii*-expressing excitatory forebrain neurons (Chen et al. 2001), *Sim1*-expressing hypothalamic neurons (Fyffe et al. 2008), *Th*-expressing dopaminergic/noradrenergic neurons (Samaco et al. 2009), *Pet1*-expressing serotonergic neurons (Samaco et al. 2009), *Viaat*-expressing inhibitory interneurons (Chao et al. 2010), *Dlx5/6*-expressing forebrain inhibitory interneurons (Chao et al. 2010), and *Gfap*-expressing astrocytes (Lioy et al. 2011).

The combination of Cre-loxP deletions of *Mecp2* with the inducible expression of Cre recombinase using the estrogen receptor system allowed the demonstration that some Rett-like phenotypes are reversible in fully symptomatic adult mice (Guy et al. 2007). A similar inducible Cre-loxP approach also demonstrated that MeCP2 is continuously required for proper function because its deletion in asymptomatic adult mice result in the abrupt appearance of Rett-like phenotypes (McGraw et al. 2011).

Because almost a third of Rett syndrome individuals carry nonsense mutations (Neul et al. 2008), mouse models with similar mutations were generated for preclinical studies of read-through compounds that skip premature STOP codons and produce full-length mRNA transcripts and proteins, including R168X (Brendel et al. 2011) and R255X (Pitcher et al. 2015). It is interesting to note that MeCP2 binds to the NCoR/SMRT complex at amino acids 269–309, a series which overlaps with the cluster of Rett syndrome missense mutations. Loss of NCoR/SMRT-MeCP2 interaction has been implicated in several Rett syndrome phenotypes (Lyst et al. 2013), making this model particularly useful for clinical research.

In an effort to increase translation from the bench to the clinic, new experimental models of Rett syndrome have been developed using rats and nonhuman primates. A rat model of Rett syndrome was created using the zinc-finger nuclease strategy (*Mecp2*^{ZFN}), which resulted in the deletion of 71 base pairs in exon 4 that caused posttranscriptional loss of *Mecp2* (Veeraragavan et al. 2016). CRISPR/Cas9 and TALEN-mediated mutagenesis were used to edit the *Mecp2* gene in rhesus and cynomolgus monkeys to generate models of Rett syndrome (Liu et al. 2014; Niu et al. 2014).

MECP2 duplication syndrome was discovered in humans after a distinct neurological phenotype was described in *MECP2* overexpressing mice (Collins et al. 2004). These mice were generated through the microinjection of linearized PAC671D9 into fertilized oocytes. This is a 99 kb human clone which contains all exons of the human *MECP2* gene. These observations led to the discovery of *MECP2* duplication syndrome as a new clinical entity in humans (Ramocki et al. 2010). More recently, another mouse model of *MECP2* duplication syndrome was generated by targeting *Mecp2* expression in the locus of the neuron-specific gene *Tau*. This model exhibits a wide array of phenotypes seen in *MECP2* duplication syndrome (Na et al. 2012).

In addition, the consequences of posttranslational modifications of MeCP2 have been studied in transgenic knock-in mice. For example, *Mecp2*$^{S421A;S424A}$ knock-in mice carry phosphorylation sites that result in *Mecp2* overexpression (Li et al. 2011). However, it is important to note that these mutations have not been discovered yet in humans.

Deficits in learning and memory are consistently found in all the mutant mouse lines that carry loss-of-function mutations, truncations, and deletions of the *Mecp2* gene as well as in all models of *MECP2* duplication syndrome. These impairments vary in a way that allows for identifying specific molecular mechanisms and cell types, as well as microcircuits and long-range projections responsible for specific behaviors (Adachi et al. 2009; Chao et al. 2010; Li et al. 2011; Durand et al. 2012; Ito-Ishida et al. 2015; Krishnan et al. 2017; Phillips et al. 2019). However, it is important to note that the behavioral phenotypes expressed in these mouse models depend critically on the genetic background of the mouse, the sex of the mouse, and the assay used for behavioral testing which results in some degree of variance between experiments; this will be exemplified in the Consequences of MeCP2 dysfunction in learning and memory section. The next sections will describe these processes and mechanisms in more detail.

Consequences of MeCP2 dysfunction in learning and memory

Spatial memory

Spatial memory is defined as the recognition, encoding, storage, and retrieval of information involving the arrangement of objects within an area or pathways through the area (Kessels et al. 2001). Spatial memory is tested in rodents using mazes such as Y-mazes,

T-mazes, circular mazes, radial mazes, arena mazes, and the Morris water maze. While each of these methods have their own advantages and disadvantages, the fundamental purpose of each maze is to test how well animals can remember the location of safety or rewards, such as food or water, through visual-spatial signals (Paul et al. 2009). While more recent functional imaging and neuronal inactivation experiments have shown that the prefrontal and anterior cingulate cortex, the parietal cortex, and the retrosplenial cortex are involved in the storage and retrieval of spatial memory (Maviel et al. 2004), the hippocampus has been shown to be critical for the formation of spatial memory for over four decades (Olton et al. 1978). This has allowed spatial memory tasks to be a prime marker for testing hippocampal-dependent memory.

Female *Mecp2* Het mice show deficits in spatial memory using the Morris water maze (Hao et al. 2015), the "novel object location" task (Li et al. 2017), as well as using a rectangular tract maze with spatial cues (Kee et al. 2018). Mice with conditional deletion of *Mecp2* from GABAergic inhibitory interneurons show impairments in spatial memory tasks using the Morris water maze (Chao et al. 2010). There have been attempts to test constitutive male *Mecp2* KO mice using the Morris water maze, but because they have hindered motor performance and have difficulties swimming, cognitive performance is difficult to assess using this test (Stearns et al. 2007). The first report of male *Mecp2*^{308} mice described typical performance in the Morris water maze (Shahbazian et al. 2002), which was attributed to residual function of the truncated MeCP2 protein being expressed. Later it was found that male *Mecp2*^{308} mice on a pure 129 background did show impairments in the Morris water maze (Moretti et al. 2006). This contrasting phenotype can be contributed to the difference in genetic background and will be discussed again in the sections Contextual and cued fear conditioning and Social memory.

Additional evidence of the role of MeCP2 in spatial memory was obtained from phospho-mutant knock-in mice (Li et al. 2011). As discussed above, neuronal activity induces phosphorylation of MeCP2 at S421, which causes its release from chromatin (Tao et al. 2009). In addition, MeCP2 phosphorylation at S421 precedes its release from the promoter region of the gene encoding brain-derived neurotrophic factor (*Bdnf*), which results in its transcription (Chen et al. 2003; Zhou et al. 2006). *Mecp2*$^{S421A;S424A}$ knock-in mice were generated to study the impact of activity-dependent MeCP2 phosphorylation in vivo. These MeCP2 phospho-mutant mice do not show overt neurological phenotypes, and have a typical healthy lifespan, which makes them amenable for multiple behavioral tests that other *Mecp2* mutant mice do not withstand. Intriguingly, male *Mecp2*$^{S421A;S424A}$ knock-in mice spent significantly more time in the target region during the hidden platform session of the Morris water maze as compared to wild-type littermates, indicating improved spatial memory (Li et al. 2011). These mice also show larger long-term potentiation, higher numbers of excitatory synapses, and *Bdnf* mRNA levels in the hippocampus. These results are in agreement with earlier work demonstrating the role of BDNF in hippocampal long-term potentiation and hippocampal-dependent spatial memory (Figurov et al. 1996; Ma et al. 1998).

Contextual and cued fear conditioning

Contextual fear learning is a learning task that requires the hippocampus and the amygdala. Contextual fear learning tests the ability of a subject to associate an unpleasant stimulus with a specific environment (Phillips and LeDoux 1992). Learning is measured by freezing behaviors in the environment where the mouse received the footshock as compared to other environments. Cued-fear learning is amygdala-dependent learning activity in which mice receive a footshock after a given cue (Blanchard and Blanchard 1972; Phillips and LeDoux 1992). Learning is measured by freezing behavior given the cue associated with the footshock as compared to other cues. Contextual fear conditioning is often used to evaluate hippocampal-dependent memory, while cued fear conditioning is used as an evaluation of amygdala-dependent memory.

Constitutive male *Mecp2* KO mice show impaired cued fear memory, while female *Mecp2* Het mice do not (Stearns et al. 2007). However, female *Mecp2* Het mice do show deficits in contextual fear memory (Samaco et al. 2013), while male *Mecp2* KO mice do not (Stearns et al. 2007). These results suggest that there is a large sex-dependent component in hippocampal function, where the mosaic pattern of mutant *Mecp2* expression in different cells in female *Mecp2* Het mice contributes to hippocampal-dependent memory. It was later described that female *Mecp2* Het mice show intact contextual fear memory over short periods of time, but impaired contextual fear memory over longer time spans (Kee et al. 2018).

Similar to deficits in hippocampal-dependent spatial memory, *Mecp2*^{308} mice in a mixed C57 and 129 genetic background show normal contextual fear conditioning (Shahbazian et al. 2002), while the same allele in a pure 129 genetic background results in impaired contextual fear memory (Moretti et al. 2006), once more demonstrating the importance of taking into account the genetic background of a model used for behavioral testing. On the other hand, *MECP2* overexpression results in increased freezing in both contextual and cued fear conditioning (Collins et al. 2004). Conditional *Mecp2* deletion in *Sim1*-expressing neurons in the hypothalamus does not affect fear learning (Fyffe et al. 2008), confirming that the behavioral consequences of *Mecp2* deletion reflect its necessity for proper neuronal function in the brain regions responsible for those behaviors.

To determine the role of activity-dependent phosphorylation of MeCP2 in different types of memory tasks, the phospho-mutant *Mecp2*$^{S421A;S424A}$ knock-in mice discussed above were also tested in fear learning. These mice exhibit increased freezing compared to wild-type littermates in contextual fear learning, but not in cued fear learning (Li et al. 2011). Much like with spatial memory, the increased freezing observed in *Mecp2*$^{S421A;S424A}$ knock-in mice during contextual fear learning is likely due to increased hippocampal activity resulting from the absence of activity-dependent S421 phosphorylation (Li et al. 2011).

Targeted *Mecp2* deletion in the basolateral amygdala of male mice causes increased freezing during cued fear learning, which is thought to result from increased H3 acetylation, due to loss of transcriptional repression by MeCP2 (Adachi et al. 2009). This type of experiment exemplifies the specificity yielded by targeted deletions of *Mecp2* in selected brain regions, which provide complementary information to that obtained from constitutive *Mecp2* KO mice throughout the brain.

Social memory

Studies focusing on social behaviors in mouse models of autism spectrum disorders are becoming increasingly useful because deficits of social interactions are often early markers of these neurodevelopmental disorders. In mouse studies, sociability is defined as the preference of the test mouse for another mouse over an inanimate object or an empty side chamber. Such preference is most commonly tested using a 3-chamber assay in which the test mouse is placed in a center chamber connected to a side chamber containing a target mouse restrained in an inverted pencil cup on one side, and to another side chamber containing an empty inverted pencil cup (Moy et al. 2004) or an inanimate object (Silverman et al. 2010). In the slightly different "partition test," a cage is divided in half with a perforated transparent wall that allows the test

mouse to see, hear, and smell the target mouse but cannot physically interact with it (Silverman et al. 2010). In these tests, social memory is defined as the time-delayed recognition of a familiar mouse, meaning that a mouse can distinguish a familiar mouse from a novel mouse (Silverman et al. 2010). Therefore, social memory can be measured as the preference for interaction with a novel mouse over a familiar mouse using a 3-chamber assay with one chamber containing a novel mouse and a second chamber containing a familiar mouse (Moy et al. 2004). Similarly, social memory can be assayed with a partition test with a familiar and novel mouse with separate partitions, as well as in Y-mazes in which a novel and familiar mouse are contained in cages in the two arms of the maze. In addition, the novel and familiar mice can be introduced to the test simultaneously or sequentially with a time delay (Silverman et al. 2010). Recent advances in computer vision and machine learning now allow quantitative and unbiased analyses of individual mouse behaviors as well as social interactions between test and target mice within an open field, which allow assessing sociability and social memory with unrestricted familiar and novel target mice (Kabra et al. 2013; Ohayon et al. 2013; Robie et al. 2017; Phillips et al. 2019).

Several studies have investigated social behaviors in *Mecp2* mutant mice, with seemingly contrasting results (for review, see Pearson et al. 2012). Constitutive male *Mecp2* KO mice of the "Jaenisch" line show increased sociability and social memory in the 3-chamber assay (Schaevitz et al. 2010), while a recent study using the same mouse line describes normal social preference but impaired social memory in both the 3-chamber test and in unrestricted social interactions with familiar and novel mice (Phillips et al. 2019). It is important to consider variations in how "interaction" is defined, as Pearson et al. considered interaction as time spent in the chamber, while Phillips et al. considered interaction as active investigation of the pencil cup by the test subject; additionally Phillips et al. only used the first 4 min out of the 10 min unrestricted social interaction as even wild-type mice show a loss of interest in either mouse after 4 min.

Consistent with a slower progression of Rett-like phenotypes, female *Mecp2* Het mice show normal sociability in the 3-chamber assay at 12 wk of age, but they develop impaired sociability at 22 wk of age (McGraw et al. 2011). Male *Mecp2*^{308} mice expressing a truncated protein (in a pure 129 background) showed reduced sociability in the partition test, without apparent deficits in the recognition of familiar mice (Moretti et al. 2005), in addition to impaired long-term social memory (Moretti et al. 2006). On the other hand, male *Mecp2*^{308} mice in a pure C57/BL6J background showed typical sociability in the 3-chamber test (Pearson et al. 2012). The differing results between male and female models show the importance of taking into account sex-specific differences in all studies, particularly those involving models with *Mecp2* mutations. The opposing results between the same mouse line in different genetic backgrounds once more exemplifies how great of an impact background can have on all types of behavior.

The ~40% reduction in *Mecp2* expression that results from the insertion of loxP sites flanking exons 3 and 4 (without Cre expression) also causes increased sociability in the partition test (Samaco et al. 2008). However, another study using the same *Mecp2* hypomorphic mice described increased social memory with normal sociability using a 3-chamber assay (Kerr et al. 2008). When these floxed *Mecp2* mice were crossed with mice expressing Cre under control of the *Sim1* promoter (to delete *Mecp2* in hypothalamic neurons), both the conditional *Sim1 Mecp2* KO mice and the floxed *Mecp2* hypomorphs spent more time on the partition facing the novel mice than the wild-type and *Sim1*-Cre controls (Fyffe et al. 2008), again indicating that a 40% reduction in *Mecp2* levels affects certain behaviors and not others.

Similarly, conditional deletion of *Mecp2* in forebrain GABAergic inhibitory interneurons results in increased sociability in both the 3-chamber assay and the partition test (Chao et al. 2010). In contrast, conditional deletion of *Mecp2* in forebrain excitatory neurons of postnatal mice causes reduced sociability using a modified partition test, in addition to impaired social memory in an unrestricted behavioral assay in which mice habituated to a target mouse for 4 d and the target mouse is replaced with a novel mouse on the fifth day (Gemelli et al. 2006), as well as lower social preference in the 3-chamber test (Chin et al. 2018). This exemplifies the usefulness of conditional KOs in behavioral testing.

Finally, both male *Mecp2* KO rats (Wu et al. 2016) and female *Mecp2* Het rats (Veeraragavan et al. 2016) show impaired sociability in the 3-chamber assay. Taken together, these studies suggest sex-specific differences in the consequences of *Mecp2* deletion on social behavior. Furthermore, the mosaic pattern of mutant *Mecp2* expression in different cells in female *Mecp2* Het mice contributes to those differences in social behaviors.

Conclusions

Through many decades of rigorous and exhaustive research, it has become widely accepted that proper gene transcription is critical for learning and memory (Brink et al. 1966; Thut and Lindell 1974; Wetzel et al. 1976; Pedreira et al. 1996) at multiple timepoints during this complex process (Taubenfeld et al. 2001; Garcia-Osta et al. 2006; Bekinschtein et al. 2007; Chen et al. 2011). Transcriptional regulators such as MeCP2 are critical for ensuring that gene transcription proceeds successfully. MeCP2 has complex functions in brain cells including mediating both transcriptional activation and repression (Fig. 1; Guy et al. 2011). Different manipulations of the *Mecp2* gene locus results in different levels of MeCP2 protein expression, in addition to the expression of nonfunctional truncated peptides, with all leading to altered levels of gene transcription, resulting in impairments of learning and memory. Together, these studies provide further evidence to support that precise control of activity-dependent gene transcription is necessary to ensure long-term adaptive behaviors required for the survival of individuals interacting with their conspecifics in a fluid world.

Competing interest statement

The authors do not have competing financial interests.

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