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Experience-Dependent Retinogeniculate Synapse Remodeling is Abnormal in MeCP2 deficient mice

Joao Noutel[1,2], **Y. Kate Hong**[1], **Byunghee Leu**[1], **Erin Kang**[1], and **Chinfei Chen**[1,*]

[1]Department of Neurology F.M. Kirby Neurobiology Center Children's Hospital, Boston Harvard Medical School 300 Longwood Avenue Boston, MA 02115

[2]Doctorate Program in Biomedicine and Experimental Biology Center for Neuroscience and Cell Biology University of Coimbra, 3004-517 Coimbra, Portugal

Summary

Mutations in *MECP2* underlie the neurodevelopmental disorder Rett (RTT) syndrome. One hallmark of RTT is relatively normal development followed by a later onset of symptoms. Growing evidence suggests an etiology of disrupted synaptic function, yet it is unclear how these abnormalities explain the clinical presentation of RTT. Here we investigate synapse maturation in *Mecp2*-deficient mice at a circuit with distinct developmental phases– the retinogeniculate synapse. We find that synapse development in mutants is comparable to that of WT littermates between postnatal days 9–21, indicating that initial phases of synapse formation, elimination and strengthening are not significantly affected by MeCP2 absence. However, during the subsequent experience-dependent phase of synapse remodeling, the circuit becomes abnormal in mutants as retinal innervation of relay neurons increases and retinal inputs fail to strengthen further. Moreover, synaptic plasticity in response to visual deprivation is disrupted in mutants. These results suggest a crucial role for *Mecp2* in experience-dependent refinement of synaptic circuits.

Introduction

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the transcriptional regulator *MECP2* (Methyl-CpG-binding protein 2) (Amir et al., 1999; Lewis et al., 1992). Growing evidence implicates MeCP2 in synaptic development and function suggesting a possible etiology for RTT. MeCP2 expression in the brain correlates with the period of synapse formation and maturation (Shahbazian et al., 2002). Mouse models with disrupted *Mecp2* function exhibit abnormalities in dendritic arborization (Fukuda et al., 2005), synaptic strength and excitatory-inhibitory balance (Chao et al., 2007; Dani et al., 2005; Dani and Nelson, 2009; Nelson et al., 2006; Wood et al., 2009; Zhang et al., 2010), and long-term potentiation (Asaka et al., 2006; Moretti et al., 2006). Strikingly, RTT children reach developmental milestones such as smiling, standing, and speaking before developmental stagnation or regression characterized by loss of cognitive, social, and language skills sets (Zoghbi, 2003). It is unclear how synaptic defects described in the *Mecp2* mouse models could explain these clinical sequelae. Moreover, to understand RTT, it

*To whom correspondence should be addressed: The Children's Hospital F.M. Kirby Neurobiology Center 300 Longwood Avenue, CLS 12250 Boston, MA 02115 Phone: 617-919-2685, Fax: 617-919-2772 chinfei.chen@childrens.harvard.edu.

will be critical to determine whether the synaptic defects are due to disruption in the formation, elimination, or strengthening of synaptic connections.

To examine the role of MeCP2 in the context of developing synaptic circuits, we studied the connection between retinal ganglion cells (RGC) and relay neurons in the dorsal lateral geniculate nucleus (LGN) of the thalamus. Development of the murine retinogeniculate synapse involves at least three phases. During the first phase, RGC axons project to the LGN, form initial synaptic contacts, and then segregate into eye-specific zones by postnatal day (P)8 (Godement et al., 1984). Subsequently, between P8–16, many connections are functionally eliminated while others are strengthened (Chen and Regehr, 2000; Jaubert-Miazza et al., 2005). The bulk of synaptic refinement during this second phase occurs around eye opening (P12), however, this process requires spontaneous activity, not vision. A third phase of synaptic plasticity occurs following a week of visual experience (P20–34). This developmental phase represents a sensitive period: a time window during which experience is necessary to maintain the refined retinogeniculate circuit, and visual deprivation elicits weakening of RGC inputs and increase in afferent innervation (Hooks and Chen, 2006, 2008).

Here, we examined retinogeniculate synapse development in *Mecp2* null mice (Guy et al., 2001). We found that initial synapse formation, strengthening and input elimination during the experience-independent phase of development proceeds in a manner similar to WT mice. During the later vision-dependent phase, however, retinal inputs fail to strengthen further, and afferent innervation of relay neurons increases. Moreover, the synaptic response to deprivation is abnormal in these mutants. These results suggest that mice lacking MeCP2 fail to properly incorporate sensory information into neuronal circuits during the experience-dependent critical period.

Results

Retinogeniculate Synapse is Abnormal in *Mecp2* null Mice

To assess a possible role for MeCP2 at the retinogeniculate synapse, we first confirmed the protein is present in retina and LGN of WT mice over development (Figure S1). Next, we examined synaptic strength and connectivity in *Mecp2* null (−/y) mice at P27–34, when this connection is relatively mature. Figure 1 shows excitatory postsynaptic currents (EPSCs) recorded from relay neurons of −/y and WT littermates (+/y) while increasing optic tract stimulation intensities incrementally. Comparison of the recordings suggested a disruption in the synaptic circuit of mutants. To further understand the nature of this defect, we quantified the properties of this synapse in mutants.

To test whether synaptic strength in −/y mice is affected we examined single retinal fiber response to minimal stimulation at P27–34 (supplemental experimental procedures). Comparison of the distributions of peak single fiber (SF) AMPAR EPSC amplitudes of +/y and −/y littermates revealed clear differences (Figure 2A). Overlay of the cumulative probability plots (far right panel) shows that synaptic strength is significantly weaker in mutant mice when compared to their WT littermates ($p<0.01$). Thus, MeCP2 plays an important role in normal strengthening of this synapse.

Initial Synapse Formation and Strengthening Occurs in −/y mice

We next asked whether RGC inputs of −/y mice are weak due to abnormal synapse formation. We reasoned that if synapse formation is disrupted, then differences in strength should present earlier in development. In mice, RGCs innervate the LGN by P0 (Godement et al., 1984) and functional connections are clearly measurable by voltage-clamp recordings at P9 (Hooks and Chen, 2006). Thus we examined synaptic strength at intermediate ages

P19–21, P15–16 and P9–12 (Figure 2B–D, respectively). At P9–12, AMPAR SF strength is similar in −/y and +/y mice (Figure 2D). NMDAR SF strength, as well as AMPAR and NMDAR maximal EPSC currents, is also not significantly different between WT and mutants at P9–12 (Figure S3). These results suggest that initial formation of the retinogeniculate synapse is not significantly affected in −/y mice.

While RGC synapse formation occurs normally in −/y mice, subsequent strengthening might depend on proper expression of MeCP2. RGC inputs strengthen more than 10-fold during a period when synapse refinement is driven by spontaneous activity (P9–20) (Hooks and Chen, 2006). Our recordings reveal that this strengthening also occurs in −/y mice. In mutant mice, the median AMPAR SF EPSC amplitude increases from 19.6 to 60.2 pA between P9–12 and P15–16, and to 181.6 pA by P21. Comparison of SF input strength distributions show no significant difference at P15–16 or at P19–21 in −/y vs. +/y mice (Figure 2C, B; $p>0.05$). Only after P21 is a significant deviation seen in AMPAR SF current amplitude ($p<0.01$). In contrast, maximal currents and SF NMDAR currents are not significantly different between +/y and −/y mice throughout development (Figure S3).

Early Synapse Elimination is not Significantly Disrupted in −/y mice

Developmental synaptic strengthening is often accompanied by synaptic pruning at many CNS synapses. At the retinogeniculate synapse, 50% of the afferent RGC inputs found at P9 are eliminated by P15–16 (Hooks and Chen, 2006). To address whether synapse elimination is affected in −/y mice, we compared fiber fraction ratios (FF). This ratio approximates the number of retinal inputs that innervate a relay neuron by quantifying the contribution of each SF EPSC to the maximal evoked response (Hooks and Chen, 2008). A small FF suggests many weak synapses while a larger FF indicates a few strong synapses. We found that the median FF increases more than 2-fold between P9–12 and P19–21 in both −/y and +/y mice (Figure 2E). Thus, early retinogeniculate synapse elimination occurs relatively normally in mutant mice.

Synaptic Connectivity Becomes Aberrant During a Later Phase of Development

While early development is similar between WT and mutant mice, the FF for −/y mice becomes significantly smaller than that of +/y mice after P21 (Figure 2E). By P27–34, the median RGC input contributes about 6% of the total synaptic current evoked by retinal inputs in mutant mice, as compared to 23% in WT littermates. This deviation is not simply due to stagnation of synaptic pruning during the later phase of development, rather, the FF actually decreases after P21 ($p<0.05$). Thus, after initial pruning of inputs during the earlier phase of synaptic refinement, RGC innervation of a given relay neuron increases in mutant mice. Thus, both synaptic strength and afferent innervation becomes significantly disrupted during the later, sensory-dependent phase of synapse development.

Quantal Size and Probability of Release in −/y mice

Mechanisms that can contribute to the observation of weaker retinal inputs in the P27−34 mutants include a reduced quantal response, a decreased probability of release (Pr), or a reduced number of release sites. Because relay neurons receive glutamatergic input from both retina and cortex, we examined the evoked mEPSCs rather than spontaneous mEPSCs. Substitution of extracellular Ca^{2+} with Sr^{2+} desynchronizes evoked release of vesicles from retinal inputs, and allows for resolution of quantal events (Chen and Regehr, 2000). Figure 3A shows representative recordings from −/y and +/y mice in the presence of Sr^{2+}. Comparison of the cumulative probability distribution of quantal amplitudes reveals a small but significant shift to the left for the mutant when compared to that of WT littermates (Figure 3B, $p<0.001$).

The reduction in the quantal amplitude in mutant mice is relatively small when compared to their ~80% decrease in retinal input strength at P27–34 (median SF AMPAR amplitude: 90.5 pA in −/y vs. 428.5 pA in +/y). Thus we asked whether a reduction in Pr could also contribute to the decrease in synaptic strength. The synaptic response to pairs of stimuli (PPR, see supplemental methods) can be used as an indirect measure of Pr. We found that PPR is not significantly different between genotypes (Figure 3C). Taken together, our results demonstrate that reduction in quantal size contributes to only a fraction of the reduced synaptic strength in −/y mice. Since Pr is not altered, we conclude that there must also be a significant decrease in the number of release sites that each RGC makes onto relay neurons of −/y mice. This mechanism is similar to that described at autaptic hippocampal synapses (Chao et al., 2007), although other studies using densely cultured hippocampal neurons or hippocampal slices from *Mecp2* mutant mice find a disruption in the Pr (Asaka et al., 2006; Nelson et al., 2006). Mechanisms underlying synaptic weakening may vary depending on culture conditions and the specific synapse studied.

Eye-specific segregation in *Mecp2* −/y mice becomes abnormal after P34

Our physiological data show that the retinogeniculate circuit becomes abnormal in −/*y* mice after P21. We asked whether these changes are a result of failure to maintain refined axon projections, a process that has been described in mice with disrupted retinal activity (Demas et al., 2006). Retinal axons organize into eye-specific regions in the LGN in a process that is thought to be largely complete by P8–10 in mouse (Godement et al., 1984; Jaubert-Miazza et al., 2005). To address whether eye-specific segregation is disrupted in the mutant, we injected both eyes with two different β-choleratoxin-conjugated fluorescent dyes to visualize the terminal fields of ipsi- and contra-lateral retinal projections to the LGN. We quantified segregation using an unbiased assay that analyzes, for each pixel, the logarithm of the ratio of fluorescence intensity from each fluorescence channel (R-value) (Torborg and Feller, 2004). The variance of R, defined as the width of the histogram distribution of R-values, can be used to compare segregation patterns. High variance indicates a high degree of segregation, whereas low variance indicates a high degree of overlap (supplemental information).

Using this analysis, we did not observe a significant difference in the segregation pattern of retinogeniculate projections between −/y and +/y mice at P27–34. However, by P46–51, a modest but significant difference in segregation was noted (Figure 4). These results are consistent with our physiological findings that the initial formation and refinement of this synaptic circuit is relatively normal in mutant mice and functional defects arise only during a later, experience-dependent period of development.

Experience-dependent Synapse Remodeling is Disrupted in −/y Mice

At the mouse retinogeniculate synapse a vision-dependent sensitive period for synaptic remodeling occurs around the age of P20. Dark rearing from P20 for more than 6 days (Late Dark Rearing, LDR) results in an approximate halving of retinal input strength and a reduction in FF (Hooks and Chen, 2006). The striking similarity between the mutant phenotype after P21 and LDR WT mice raised the question of whether MeCP2 plays a role in experience-dependent plasticity. To address this question, we examined the synaptic response of −/y mice to LDR. Although retinal input strength is weaker in normally-reared mutants at P27–34 when compared to WT mice, they are still much stronger than retinal inputs at P9–12 (Figure 2). Thus we reasoned that we could still detect a reduction in strength in response to sensory deprivation. Consistent with previous results in C57BL/6 mice, LDR results in a decrease in SF AMPAR and NMDAR strength in +/y mice (Figure 5A). Cumulative probability plots of the SF peak AMPAR current show the expected shift to the left consistent with weaker retinal inputs in LDR +/y mice (dashed black line) when

compared to light-reared +/y mice (solid black line) (Figure 5B). Moreover, FF decreases from a median of 0.23 to 0.06 in LDR +/y mice, consistent with a decrease in the amplitude of individual RGC inputs without a change in the maximal synaptic current (Figure 5A,C). In contrast, SF strength of AMPAR and NMDAR currents and FF of −/y mice do not change significantly when compared to normally-reared −/y mice. Thus, the retinogeniculate synapse of −/y mice does not respond in the typical manner to changes in sensory experience during the thalamic sensitive period.

DISCUSSION

The Thalamus as a Model System for RTT Syndrome

A distinct feature of many patients with RTT is that developmental milestones of the first 6–12 months are met, followed by stagnation or regression. These clinical manifestations are consistent with a disruption of synaptic circuits occurring during later phases of development, after the initial formation of synaptic contacts (Zoghbi, 2003). To gain insight into aspects of synapse development that are disrupted in RTT, we studied the development of the retinogeniculate synapse in *Mecp2* null mice for several reasons. First, this synapse matures over many weeks, allowing for experimental dissection of periods of axon mapping, synapse formation, strengthening, elimination and experience-dependent plasticity. Second, MeCP2 is strongly expressed in the rodent visual thalamus (Shahbazian et al., 2002) at a time when synapse remodeling is robust. Interestingly, the thalamus, which processes and relays sensory information to the cortex, is one of the regions where reduction in MeCP2 levels is most prominent in RTT patients (Armstrong et al., 2003). Finally, although visual acuity is not affected, several studies have reported abnormal visual processing in RTT patients (Bader et al., 1989; Stauder et al., 2006; von Tetzchner et al., 1996). Thus the general principles learned from the retinogeniculate synapse can enhance our understanding of the synaptic defects that occur in RTT.

Aberrant Synapse Remodeling in Developing −/y mice

We show that during the earlier, spontaneous activity-driven phase of synapse refinement, synapses in −/*y* mice form, strengthen and eliminate similarly to those of their WT littermates. Although no differences in synaptic parameters were statistically significant between mutants and WT during early development, this does not exclude the possibility that there are real but small differences between the two genotypes. Only after P21, during the vision-dependent phase of development, do differences in strength and connectivity in −/y and +/y mice become statistically significant. Consistent with the late onset of synaptic defects, analysis of eye-specific segregation indicates that large-scale anatomical changes are not detectable at P27–34, but become significant at P46–51. The electrophysiological assay is likely more sensitive than the anatomical assay of bulk axon mapping. Thus changes in segregation are only detectable with progressive circuit disruption, consistent with the manifestation of symptoms in the mouse (Guy et al., 2001). Due to difficulties in preparing viable brain slices at older ages, we were unable to record at P46–51 to validate this functionally. Nevertheless, the anatomical data are consistent with a role for MeCP2 in the experience-dependent phase of retinogeniculate remodeling.

During the later sensory-dependent phase of development, SF strength does not continue to increase between P19–21 and P27–34 in mutants. Moreover, FF measurements show that afferent inputs to a relay neuron initially prune, only to increase in number during the vision-dependent phase. At this age (P27–34) mutant mice become symptomatic (Guy et al., 2001). However, changes in circuitry during the late developmental age are not likely due to a failure to thrive or to metabolically unhealthy neurons because maximal evoked currents continue to increase in mutants. Instead, the phenotypes of reduced synaptic strength and

recruitment of additional afferents are strikingly similar to those of WT littermates when deprived of visual experience during the thalamic sensitive period. Consistent with a role for MeCP2 in experience-dependent plasticity, deprivation-induced synaptic remodeling is disrupted in −/y mice. Our data show that changes in the sensory environment elicit some plasticity in −/y mice, as there is a significant decrease in AMPAR maximal currents (Figure 5A). However, this plasticity does not include the changes in SF strength and FF seen in +/y mice. It is still unclear whether defects seen at the retinogeniculate synapse in −/y mice result from cell autonomous, circuit-dependent or compensatory mechanisms. Regardless of the mechanism, disrupting sensory information processing in the thalamus will have global effects, as the information is propagated to many circuits in the cortex.

Comparison to Other CNS synapses

We explored whether previously proposed synaptic models for the role of MeCP2 may explain our results. Several studies have reported defects in hippocampal long-term potentiation and depression (LTP and LTD)(Monteggia and Kavalali, 2009). At the retinogeniculate synapse, LTD is thought to play a role in eye-specific segregation and synaptic elimination prior to eye-opening, and LTP correlates with synaptic strengthening (Mooney et al., 1993; Ziburkus et al., 2009). However, since segregation and initial synaptic strengthening and elimination still occur in −/y mice, disruption in LTP and LTD alone cannot explain all of our findings.

Another model proposes that synaptic circuits in *Mecp2* mutant mice remain immature. Consistent with this model, cortical ocular dominance plasticity is still present in mutant mice at ages when the critical period is normally closed, although this plasticity was only tested at one age (P60) (Tropea et al., 2009). While our studies show that the −/y retinogeniculate synapse is not mature at P27–34, the phenotype is not simply developmental stagnation. The immature circuit model cannot explain the increase in afferent innervation of relay neurons following initial pruning. Moreover, the retinogeniculate synapse in −/y mice exhibits altered plasticity in response to visual deprivation.

Our data suggest that the retinogeniculate circuit in −/y mice becomes aberrant during the developmental phase when experience is incorporated into synaptic circuits, and loss of vision results in weakening and rearrangement of RGC inputs. Based on our findings, we propose a model, not mutually exclusive of previous models, in which the retinogeniculate circuit in −/y mice is responding as if it is deprived. That is, −/y mice fail to incorporate sensory experience into the synaptic circuit during the thalamic critical period, resulting in a failure to further strengthen afferent inputs and maintain the refined retinal innervation of relay neurons (Hooks and Chen, 2008).

Consistent with our findings at the retinogeniculate synapse, studies of somatosensory cortical circuits of *Mecp2* mutant mice show reduced strength and connectivity at synapses between layer 5 (L5) neurons at older (P26–29) but not younger ages (P16–19)(Dani and Nelson, 2009). However, it remains unclear whether these findings reflect a loss of synaptic strength, a regression in development, or conceivably a sensory-dependent critical period during which the L5 synapses respond abnormally to sensory experience. Notably, the excitatory-inhibitory balance that is important for cortical critical periods is disrupted in L5 neurons of *Mecp2* mutant mice (Dani et al., 2005; Hensch and Fagiolini, 2005). Moreover, disruption of *Mecp2* expression in cortical inhibitory neurons recapitulates many features of RTT (Chao et al., 2010). It will be interesting to see whether other changes in synaptic function seen in *Mecp2* mutants are a result of disruptions in experience-dependent critical periods.

Comparison to Other Mouse Models of Autism Spectrum Disorders

Aberrant synaptic plasticity during critical periods has also been proposed in other mouse models of autism spectrum disorders, although the relationship between experience and synapse development appears distinct among the models. The somatosensory system of the Fmr1 KO mouse model for Fragile ***X syndrome exhibits delayed plasticity at the thalamocortical synapse, and abnormal cortical connectivity and plasticity during the sensory-dependent critical period (Bureau et al., 2008; Harlow et al., 2010). Another model for autism spectrum disorders, the Ube3a mouse model for Angelman syndrome, also shows abnormal synaptic plasticity during experience-dependent maturation of sensory cortical circuits (Sato and Stryker, 2010; Yashiro et al., 2009). In this case, however, visual deprivation restores plasticity. In contrast to the Ube3a mouse model, we show that abnormal plasticity is elicited with deprivation in *Mecp2* null mice. The difference in findings between these mouse models for autism are likely due to the distinct molecular mechanisms involved, the area of the brain studied or the age range examined. Yet, a common emerging theme among mouse models for autism spectrum disorders is a disruption in experience-dependent synaptic plasticity.

Our results from *Mecp2* null mice support the idea that distinct phases of synapse development are driven by different molecular mechanisms. We find that Mecp2 has a more prominent role in experience-dependent vs. -independent synapse remodeling. The mechanism by which visual experience, as opposed to spontaneous activity, imparts changes in synaptic circuits is still not clear. The MeCP2 protein has a number of phosphorylation sites that can be modulated in an activity- and experience-dependent manner (Chen et al., 2003; Tao et al., 2009; Zhou et al., 2006). Specific phosphorylation patterns may mediate distinct forms of plasticity. Moreover, MeCP2 regulates chromatin structure and function and thus the expression of thousands of genes (Chahrour et al., 2008; Skene et al., 2010). In the future it will be interesting to examine how different forms of activity influence neuronal chromatin structure, DNA methylation profiles and MeCP2 phosphorylation during the various stages of synapse development.

Experimental Procedures

Animals—Mecp2 −/+ female mice (MeCP2$^{tm1.1Bird}$, Jackson Laboratories, Bar Harbor, ME (Guy et al., 2001)) were mated with C57BL/6 males. Only homozygote and WT males were used in this study because heterozygote females are phenotypically variable due to X-chromosome inactivation. For dark rearing experiments, mothers with P20 litters were placed for 7–14 days in a light-tight container in which temperature, humidity and luminance were continually monitored (Hooks and Chen, 2006). Control (normally-reared) animals were raised under a 12 hr light-dark cycle. All the procedures were reviewed and approved by the IACUC at Children's Hospital, Boston.

Electrophysiology—Detailed descriptions of the preparation of LGN brain slices and the electrophysiological methods used to study development of the retinogeniculate synapse, including the measurement of single fiber, fiber fraction and quantal events are published (Chen and Regehr, 2000; Hooks and Chen, 2006, 2008) and are also elaborated on in Supplemental Experimental Procedures. All recordings were performed blind to the genotype.

Labeling of retinal projections—Mice anesthetized with 2% isofluorane were injected with 2–3μl of a 2% solution of cholera toxin β subunit conjugated with Alexa 488 (Green) or 594 (Red) (Invitrogen, Carlsbad, CA) using a glass pipette and a picospritzer (Picospritzer III, Parker Hannifin Corp, Cleveland, OH). After 2–4 days, mice were deeply anesthethized with Avertin (200 mg/kg I.P.) and transcardially perfused with PBS followed by 4%

paraformaldehyde. After postfixation, 60–70μm thick coronal sections of the brains were mounted and allowed to absorb the mounting medium overnight before fluorescence imaging. Slices showing the largest projections were used. Generally, 1–3 slices were analyzed per animal. Images were analyzed using the previously described threshold-independent quantitative measure of eye-specific layer segregation ((Torborg and Feller, 2004), supplemental information).

Statistics—The majority of our data did not follow a normal distribution as determined by the Kolmogorov-Smirnov test. Thus, unless otherwise noted, we used the nonparametric two-tail Mann-Whitney test. Box and whisker plots are shown as medians (white lines), with 25^{th} to 75^{th} percentile bars and 10^{th} and 90^{th} percentiles whiskers. Statistical significance in graphs were indicated as: *p<0.05; **p<0.01; ***p<0.001.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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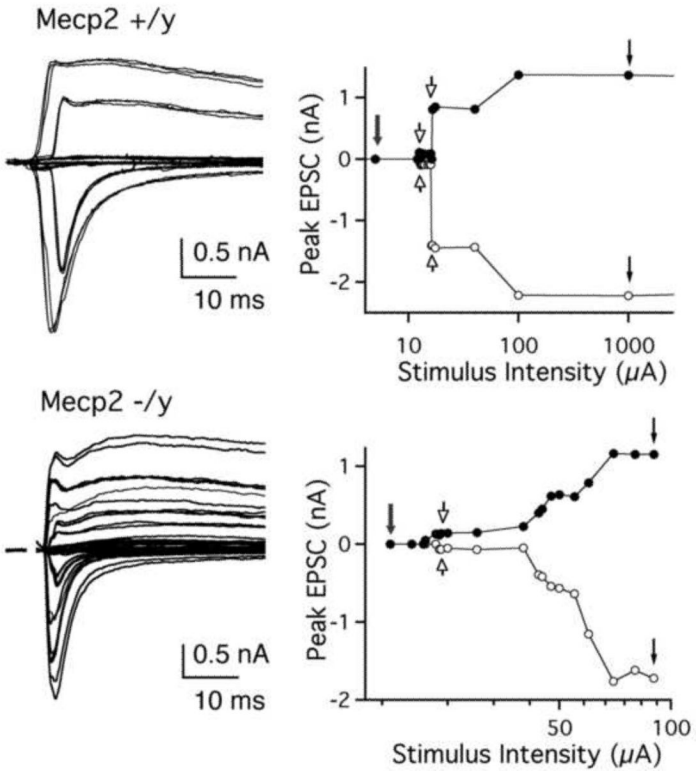

Figure 1. Comparison of Synaptic Remodeling in −/y and +/y mice

(*Left*) Superimposed traces of EPSCs evoked by optic tract stimulation at increasing intensities while alternating the holding potential (HP) between −70 mV (inward currents) and +40 mV (outward currents) from +/y (P30, top) and −/y (P28, bottom) mice. (Right) The peaks of the inward (white circles) and outward (black circles) currents (through AMPA and NMDA receptors, respectively) are plotted as a function of stimulus intensity on a log scale. Grey arrows: baseline failures; white arrows: SF responses; black arrows: maximal currents (see supplemental information).

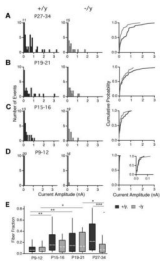

Figure 2. Sensitive Period Synaptic Remodeling is Impaired in *Mecp2* −/y mice

(A–D) SF AMPAR EPSC amplitudes (HP=−70) recorded from a population of relay neurons from +/y and −/y mice over development. Amplitude histograms in 100 pA bins (left, middle panels) and cumulative probability plots (right) for ages P27–34 (A; n= 42 & 29 SF inputs for +/y and −/y, respectively), P19–21 (B; n=26 & 23), P15–16 (C; n=30 & 28) and P9–12 (D; 20 & 17). Inset in P9–12 cumulative plot shows expanded scale of the distribution. Numbers above first bin indicate total points less than 100 pA (off scale). This number includes silent inputs (SF responses with 0 AMPAR current but a measurable NMDAR current (Chen and Regehr, 2000)). The ratio of silent inputs/total inputs for P15–16 was (4/28 vs 2/30) for −/y and +/y, respectively, P19–21: (1/23 vs 0/26), and P27–34: (2/29 and 0/43). (E) Estimation of synaptic connectivity by FF for WT and −/y mice over development. For (A–E) +/y: (P9–12) 20 cells from 11 mice; (P15–16) 23 from 6; (P19–21) 22 from 14; (P27–32) 28 from 8; −/y: (P9–12) 17 from 9; (P15–16) 22 from 7; (P19–21) 17 from 10; (P27–32) 20 from 12.

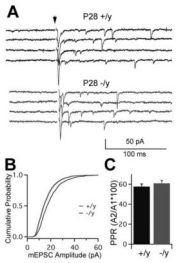

Figure 3. Comparison of Synaptic Properties in +/y and −/y mice

(A) Representative recordings of evoked quantal events from P27–34 animals in a saline solution containing 3 mM $SrCl_2$ and 2 mM $MgCl_2$. Arrowhead indicates time of optic tract stimulation. Stimulus artifact blanked for clarity. (B) Thousands of quantal events from many experiments were used to build cumulative probability distributions of quantal amplitudes (+/y: n=1097 events from 5 cells; −/y: n=1010 from 3 cells). The median mEPSC amplitude in −/y mice is ~17% smaller than that of +/y mice (14 pA vs. 17 pA, respectively). (C) Plot of average PPR of +/y (58±3%, n=4) and −/y (61±3%, n=6) mice (p>0.4, student t-test). A1 and A2 correspond to the peak amplitude of the first and second EPSC, respectively.

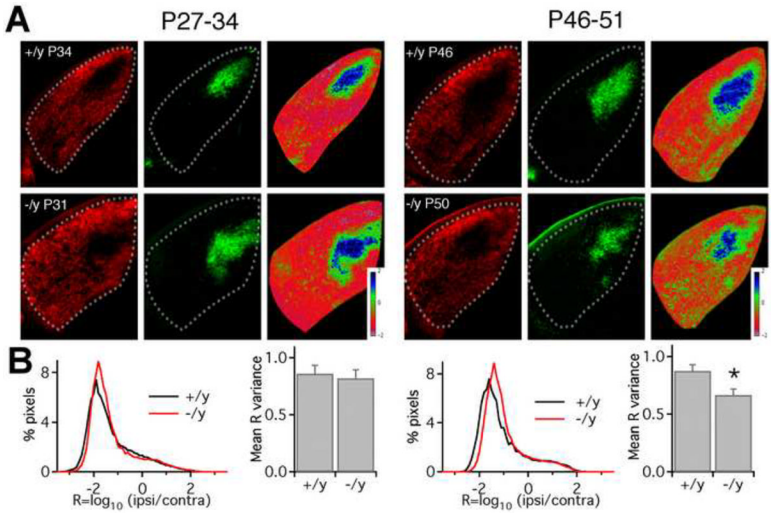

Figure 4. Abnormalities in eye-specific segregation are detectable after P34

Eye-specific segregation in +/y and −/y mice at P27–34 (*left*) and P46–51 (*right*). (A) Fluorescently labeled contra- (red) and ipsi-lateral (green) retinal projections in coronal sections of LGN. Far right panels show pseudocolored R-values where contra- and ipsi-dominant pixels are red and blue, respectively (see pseudocolor scale). (B) Mean R-distributions (*left*) and mean R-variance (*right*) for +/y and −/y mice. P27–34: +/y, n=11 sections from 5 animals; −/y, n=14 from 5. P46–51: +/y, n=12 from 4; −/y, n=12 from 4. Mean R-variance was significantly different between +/y and −/y at P46–51 but not at P27–34 when comparing the distributions of R-variance values of all sections ($p<0.05$ Mann-Whitney U test) as well the average within animals ($p<0.01$, student t-test).

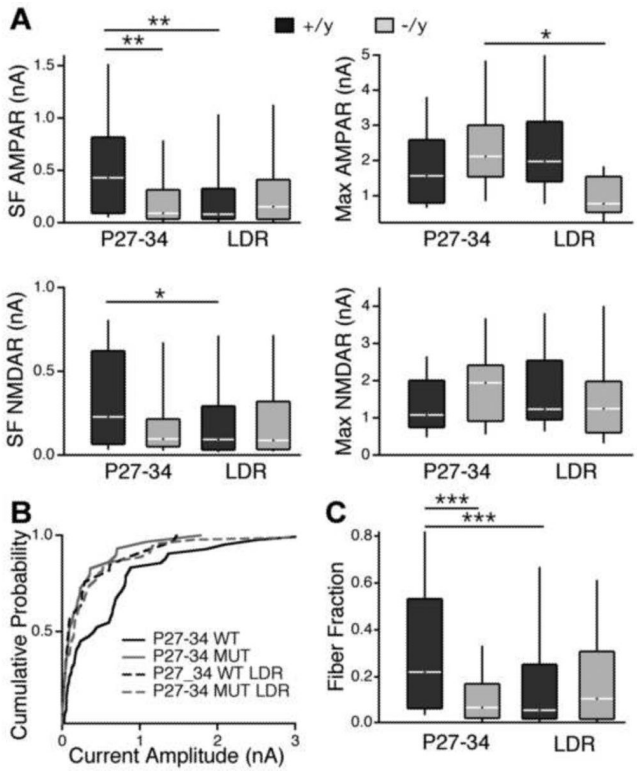

Figure 5. Experience-dependent Plasticity is Reduced in −/y mice

(A) Summary of SF (left) and maximal (right) amplitude data for AMPAR (top) and NMDAR (bottom) EPSCs in P27–34 +/y and −/y LDR mice. Data for normally-reared mice are the same as that in Figure 2. (B) Comparison of SF AMPAR current amplitudes cumulative probability plots (P27–34 LDR: n=33 and 44 for +/y and −/y, respectively). (C) Summary of FF data in LDR mice. (n=66 and 84, for WT and −/y littermates, respectively). Recordings were from 23 cells from 9 mice (+/y) and 34 cells from 12 mice (−/y).