MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice

Keerthi Krishnan1,*, Billy Y.B. Lau1,*, Gabrielle Ewall1, Z. Josh Huang1 & Stephen D. Shea1

Neurodevelopmental disorders are marked by inappropriate synaptic connectivity early in life, but how disruption of experience-dependent plasticity contributes to cognitive and behavioural decline in adulthood is unclear. Here we show that pup gathering behaviour and associated auditory cortical plasticity are impaired in female Mecp2het mice, a model of Rett syndrome. In response to learned maternal experience, Mecp2het females exhibited transient changes to cortical inhibitory networks typically associated with limited plasticity. Averting these changes in Mecp2het through genetic or pharmacological manipulations targeting the GABAergic network restored gathering behaviour. We propose that pup gathering triggers a transient epoch of inhibitory plasticity in auditory cortex that is dysregulated in Mecp2het. In this window of heightened sensitivity to sensory and social cues, Mecp2 mutations suppress adult plasticity independently from their effects on early development.
ett syndrome (RTT) is a neuropsychiatric disorder predominantly caused by mutations in the X-linked gene methyl CpG-binding protein 2 (MECP2). Males with mutations of their single copy of the gene suffer neonatal encephalopathy and die in infancy, and most surviving patients with RTT are females that are heterozygous for MeCP2 mutations. In these females, random X-chromosome inactivation leads to mosaic wild type MECP2 expression and consequently a syndromic phenotype. Patients with RTT achieve early postnatal developmental milestones, but experience an abrupt developmental regression around 6–12 months. They typically survive into middle age, exhibiting sensory, cognitive and motor deficits throughout life.

MECP2 is broadly expressed in the developing and adult brain and is continually required to maintain adult neural function. Moreover, restoration of normal MECP2 expression in adult mice improves symptoms. These observations establish that MECP2 is necessary to regulate brain function in adulthood. However, the specific function of MECP2 in the mature brain remains unclear, despite its widely studied role in development.

MECP2 regulates neuronal chromatin architecture and gene transcription in response to neural activity and experience during postnatal life. The known cellular function of MECP2 and the characteristic timing of disease progression raise the possibility that the regulation of neural circuits by MECP2 is increased during specific windows of enhanced sensory and social experience throughout life. We therefore hypothesized that continued disruptions of experience-dependent plasticity in female mice heterozygous for MeCP2 (MeCP2het) hinders learning during adulthood. We tested this hypothesis in adult female MeCP2het mice using pup retrieval, a learned natural maternal behaviour, which is known to induce experience-dependent auditory cortical plasticity. First-time mother mice respond to their pups’ ultrasonic distress vocalizations by gathering the pups back to the nest, an essential aspect of maternal care. Virgin females with no previous maternal experience (‘surrogates’) can acquire this behaviour when co-housed with a first-time mother and her pups. Single-unit neural recordings show that proficient pup gathering behaviour is correlated with neurophysiological plasticity in the auditory cortex in both surrogates and mothers.

Here we report that adult MeCP2het surrogates, and surrogates with conditional knockout of MeCP2 in auditory cortex, exhibit impaired pup retrieval behaviour. Maternal experience-triggered changes in GABAergic interneurons occur in wild-type surrogates, but we found that additional changes were observed in MeCP2het surrogates. Specifically, we observed elevated expression of parvalbumin (PV) and perineuronal nets (PNNs). Increases in expression of these markers are associated with the termination or suppression of plasticity in development and adulthood. Genetic manipulation of GAD67, the primary synthetic enzyme for GABA, suppressed increases in PV and PNNs and restored gathering in MeCP2het. Furthermore, specific depletion of the PNNs into the auditory cortex also restored efficient pup retrieval behaviour in MeCP2het. Finally, we found that specific knockout of MeCP2 in PV neurons was sufficient to transiently interfere with pup retrieval behaviour. Altogether, our results show that MECP2 regulates experience-dependent plasticity in the adult auditory cortex.

**Results**

Pup gathering behaviour requires auditory cortex. To assess the efficacy of cortical plasticity underlying pup gathering learning, we devised an assay for gathering behaviour in nulliparous surrogates (Sur). We chose to examine cortical plasticity underlying the acquisition of gathering behaviour in Sur to eliminate the influence of pregnancy. Our intent was not to study maternal behaviour per se or plasticity in males, but to use this assay to study the function of MECP2 in adult experience-dependent plasticity in Sur at the neural circuit and behavioural levels.

Assaying the effects of heterozygous deletion of MeCP2 on gathering behaviour presents several advantages. First, the vast majority of patients with RTT are females and heterozygous for mutations of MeCP2 who exhibit mosaic expression of the wild type protein. Thus, female MeCP2het (ref. 27) are a particularly appropriate model of RTT. Second, we can directly relate a natural, learned adult behaviour to specific, experience-dependent changes in the underlying neural circuitry. Third, we can observe effects on adult learning and plasticity that are distinct from developmentally programmed events in the Sur by studying a window of heightened plasticity that is triggered by exposure to a mother and her pups.

Two 7–10 week old matched female littermates (Sur) were co-housed with a first time mother and her pups from late pregnancy until the fifth day following birth (D5). Sur were virgins with no prior exposure to pups. All three adults (the mother and both Sur) were subjected to a retrieval assay (see Materials and Methods) on D0 (day of birth), D3 and D5.

We confirmed the experience-dependent nature of gathering behaviour by comparing performance of maternally-naive WT (NaiveWT) females with that of SurWT on D5. Performance was assessed by computing a normalized measure of latency (latency index, see Methods) and by counting the number of gathering errors (instances of interacting with a pup and failing to gather it to the nest). SurWT performed significantly better than NaiveWT by both measures (Fig. 1b,c) (NaiveWT: N = 9 mice; SurWT: N = 18 mice; Mann–Whitney: P = 0.027), presumably reflecting maternal experience-dependent plasticity.

Several lines of evidence suggest that auditory cortical responses to ultrasonic distress vocalizations facilitate performance of pup gathering behaviour. We confirmed this by making bilateral excitotoxic (ibotenic acid) lesions of the auditory cortex in wild type mice. Compared with saline-injected mice, mice with lesions exhibited significantly larger latency indices (Saline: 0.20 ± 0.034, N = 6 mice; Lesion: 0.66 ± 0.033, N = 6 mice; Mann–Whitney: P = 0.0022) and made more errors (Saline: 1.33 ± 0.95 errors, N = 6 mice; Lesion: 6.64 ± 0.91 errors, N = 6 mice; Mann–Whitney: P = 0.015).

**MECP2 is required for efficient pup gathering behaviour.** Next, we compared the pup gathering performance of SurHet with that of mothers and SurWT. SurWT retrieved pups to the nest with efficiency (as measured by latency index in Fig. 1d,f) and accuracy (as measured by errors in Fig. 1e,g) that were indistinguishable from the mother (Supplementary Movie 1). By contrast, SurHet exhibited dramatic impairment in gathering behaviour, retrieving pups with significantly longer latency and more errors when compared with the SurWT or mothers (Fig. 1d–g). Moreover, this behaviour did not improve with subsequent testing on D3 and D5 (Fig. 1d,e) (N = 13–24 mice; Kruskal–Wallis with Bonferroni correction: H values for latency – D0 = 9.4, D3 = 13.05, D5 = 21.68; H values for error – D0 = 26.07, D3 = 26.31, D5 = 24.32; *post-hoc P < 0.055). The variability in behaviour in SurHet can be partly explained by the variability in MECP2 expression in the auditory cortex because of random X-chromosome inactivation. Specifically, SurHet with fewer cells expressing MECP2 performed worse in latency and errors than SurHet with more cells expressing MECP2, showing that the range of variability in SurHet behaviour is correlated with MECP2 expression.
MECP2 expression in the auditory cortex (Fig. 1h,i) (N = 10 mice; Pearson’s r). Taken together, the results demonstrate that MECP2 expression is required for successful acquisition of this learned behaviour.

In these experiments, we used a germline Mecp2 knockout that affects MECP2 expression throughout the animal. Therefore, the poor pup gathering performance of SurHet could, in principle, be because of motor deficits or deafness. We found no significant difference in movement during behaviour trials between the genotypes (SurWT: 2,059 ± 216.5 significant motion pixels (SMP), N = 8 mice; SurHet: 2,139 ± 259.9 SMP, N = 8 mice; Mann–Whitney: P = 0.78), consistent with previous findings that Mecp2het lack robust motor impairments29.

We also found no evidence that Mecp2het are deaf or otherwise insensitive to sound, consistent with a previous study30. Neurons in the auditory cortex of NaiveHet exhibited widespread and robust responses to auditory stimuli. Baseline spontaneous activity was comparable between NaiveWT and NaiveHet (Fig. 2a) (WT: n = 99 cells, 11 mice; Het: n = 87 cells, 13 mice; Mann–Whitney, P = 0.70). Analysis of stimulus-evoked responses showed that auditory cortex neurons of NaiveHet were excited by a small but significantly greater number of stimuli (WT: n = 56 cells, 11 mice; Het: n = 66 cells, 13 mice; Mann–Whitney, P = 0.047), but inhibited by a similar number of stimuli compared with NaiveWT (WT: n = 47 cells, 11 mice; Het: n = 24 cells, 13 mice; Mann–Whitney, P = 0.33). Response strength, measured as a z score, was not significantly different between NaiveWT and NaiveHet, for excitation (d) but was significantly increased in NaiveHet for inhibition (e) (Excitation: WT: n = 136 responses, 56 cells, 11 mice; Het: n = 192 responses, 66 cells, 13 mice; Mann–Whitney, P = 0.43; Inhibition: WT: n = 133 responses, 47 cells, 11 mice; Het: n = 59 responses, 24 cells, 13 mice; Mann–Whitney, P = 0.0054). Bar graphs represent mean ± s.e.m.
MECP2 in adult auditory cortex is required for pup gathering. Measuring behavioural effects in germline mutants leaves open the possibility of a requirement for MECP2 in early postnatal development and/or in other brain regions. Therefore, we used a conditional deletion approach to specifically delete MECP2 expression in the auditory cortex by bilaterally injecting AAV-GFP-Cre (adeno-associated virus expressing CRE recombinase) in 4-week old Mecp2<sup>flox/flox</sup> mice<sup>31</sup> (Fig. 3a). Histological analysis of sections from SurMecp2<sup>flox/flox</sup> five weeks after injection with AAV-Cre showed >91% of GFP expressing (GFP<sup>+</sup>) nuclei in the auditory cortex (n = 685 GFP<sup>+</sup> cells, 12 images, 3 mice) (see methods) were devoid of MECP2 expression (Fig. 3b–f). We counted non-GFP expressing (GFP<sup>−</sup>) and GFP<sup>+</sup> cells to determine the extent of MECP2 knock-down in the GFP<sup>+</sup> cells and found significant reduction of MECP2 expression in the Mecp2<sup>het</sup> is not caused by frank deafness or insensitivity of the auditory system in naive females.

SurHet exhibit altered plasticity of GABAergic interneurons. The regional requirement for MECP2 led us to examine maternal experience-dependent molecular events in the auditory cortex. Recent data on the neurophysiological correlates of maternal learning suggest that there are changes in inhibitory responses of vocalizations in the auditory cortex of mothers and surrogates<sup>17,32</sup>. There is also evidence that inhibitory networks are particularly vulnerable to MeCP2 mutation<sup>33–35</sup>. For these reasons, we focused our attention on experience-dependent dynamics of molecular markers associated with inhibitory circuits.

We used immunostaining of brain sections from the auditory cortex of Sur and naive females to examine experience-induced molecular events in inhibitory networks of Mecp2<sup>het</sup> and

![Figure 3](https://example.com/figure3.png)
Mature neural circuits are often stabilized by perineuronal nets (PNNs), which are composed of extracellular matrix proteins such as chondroitin sulfate proteoglycans \(^\text{37}\), and mainly surround PV\(^+\) GABAergic interneurons in the cortex\(^\text{38}\). We observed a dramatic experience-dependent increase in the number of high-intensity PNNs in SurHet but not in SurWT (Fig. 5c,g) \((n = 292–1,735 \text{ PNN}^+ \text{ cells}, 12–38 \text{ images}, 3–9 \text{ mice}; \text{ANOVA: Tukey's post-hoc test, } *P < 0.05 \text{ compared with all other groups})\). Importantly, both PV and PNNs returned to baseline levels in surrogates by weaning age of the pups (D21) (Fig. 5b,c). In addition, the percentage of PNN that co-localized with PV\(^+\) cells was unchanged among all groups of mice (Fig. 5d,h) \((n = 1–107 \text{ PNN}^+ \text{ cells}, 1–103 \text{ PV}^+ \text{ cells}, 6 \text{ images}, 3 \text{ mice}; \text{ANOVA: Tukey's post-hoc test, } *P < 0.05 \text{ compared with all other groups except SurWT P21})\). To test this idea, we crossed germline Mecp2\(^{\text{het}}\) null male mice into the Mecp2\(^{\text{het}}\) background and examined the effects on maternal experience-dependent changes in PV and PNNs. As expected, naive WT and Mecp2\(^{\text{het}}\) carrying the Gad1\(^{\text{het}}\) allele (NaiveWT;Gad1\(^{\text{het}}\) and NaiveHet;Gad1\(^{\text{het}}\), respectively) showed half the GAD67 expression seen in WT and Mecp2\(^{\text{het}}\) (NaiveWT: 458.9 \pm 60.6 \text{ cells per mm}^3, NaiveHet: 393.5 \pm 73.3 \text{ cells per mm}^3, NaiveWT;Gad1\(^{\text{het}}\): 174.6 \pm 60.9 \text{ cells per mm}^3, NaiveHet;Gad1\(^{\text{het}}\): 193.2 \pm 41.3 \text{ cells per mm}^3; \text{n = 92–334 cells, 20–32 images, 5–8 mice; T-test: } *P < 0.05 \text{ NaiveHet;Gad1\(^{\text{het}}\) compared with NaiveWT and NaiveHet}; \text{T-test: } *P < 0.05 \text{ NaiveWT;Gad1\(^{\text{het}}\) compared with NaiveWT and NaiveHet})\). In contrast to SurHet, SurHet;Gad1\(^{\text{het}}\) exhibited a correction in the maternal experience-dependent increase in PV expression levels (Fig. 6a,b) and had a significantly lower proportion of high-intensity PV\(^+\) cells (Fig. 6b) \((n = 4,353–5,079 \text{ cells}, 16–20 \text{ images}, 4–5 \text{ mice}; \text{ANOVA: Tukey's post-hoc test, } *P = 0.02)\). We also saw significantly fewer PNNs in the double mutants (Fig. 6d) \((n = 196–1,735 \text{ PNN}^+ \text{ cells}, 17–38 \text{ images}, 4–9 \text{ mice}; \text{ANOVA: Tukey's post-hoc test, } *P = 0.01)\). NaiveWT;Gad1\(^{\text{het}}\) exhibited a significantly elevated percentage of high-intensity PV\(^+\) cells, compared with NaiveWT (Fig. 6c), likely because of compensatory effects of long-term genetic reduction of GAD67.

Figure 4 | Maternal experience transiently enhances GAD67 expression level in the auditory cortex of wild-type and Mecp2\(^{\text{het}}\) mice. (a) The density of high-intensity GAD67 cells was significantly increased in both SurWT (dark blue) and SurHet (red) at D5, and returned to naive levels at D21 \((n = 36–451 \text{ cells}, 12–32 \text{ images}, 4–8 \text{ mice}; \text{ANOVA: Tukey's post-hoc test, } *P < 0.05)\). Bar graphs represent mean ± s.e.m. (b) Representative confocal images taken from the auditory cortex of a NaiveWT and NaiveHet (top row) and SurWT and SurHet at D5 (bottom row). Arrows point to high-intensity GAD67 cells. Scale bar, 100 \(\mu\)m, applies to all images. Dashed lines delineate cortical layers with layers III and V indicated.
Interestingly, this increase was not seen after maternal experience (Fig. 6c), returning to the appropriate activity-dependent expression of PV that was not significantly different from the WT (n = 2704–4906 PV⁺ cells, 19–20 images, 5 mice for each group). The solid line and shaded region represent mean ± s.e.m. respectively, in both panels. (b) The shift reflects a significant transient increase in high-PV expressing cells at D5 that returned to baseline at D21 in SurHet (ANOVA: Tukey’s post-hoc test, *P < 0.05 compared with all other groups). (c) The density of high-intensity perineuronal nets (PNNs) was significantly increased only in SurHet at D5 (n = 292–1735 PNN⁺ cells, 12–38 images, 3–9 mice; ANOVA: Tukey’s post-hoc test, *P < 0.05 compared with all other groups), and returned to baseline at D21. (d) The percentage of PNN co-localizing with PV-expressing cells was not significantly different across genotypes and conditions (n = 1–107 PNN⁺ cells, 1–103 PV cells, 6 images, 3 mice; ANOVA: Tukey’s post-hoc test, P > 0.05). (e) However, the percentage of PV cells co-localizing with PNN was significantly higher in SurHet at D5 (n = 92–319 PV cells, 1–103 PNN⁺ cells, 6 images, 3 mice; ANOVA: Tukey’s post-hoc test, *P < 0.05 compared with all other groups except SurWT P21). (b–e) Bar graphs represent mean ± s.e.m. (f–h) Representative confocal images taken from the auditory cortex of a SurWT and SurHet showing relative expression of PV (f) and PNN (g). Arrowheads indicate high-intensity PV cells. Arrows point to co-localization of PV and PNN. Scale bar, 50 μm, applies to all images. Dashed lines delineate cortical layers with layers III and V indicated.

We next assessed whether the corrective effect of GAD67 reduction on inhibitory markers in SurHet reinstated learning. Remarkably, SurHet;Gad1het exhibited significant decreases in latency index (Fig. 6f) and the number of errors (Fig. 6g) (SurHet;Gad1het, N = 7 mice; SurWT: N = 18 mice; SurHet: N = 18 mice; ANOVA: Tukey’s post-hoc test, *P < 0.05 when compared with SurHet. In fact, the gathering performance of SurHet;Gad1het was indistinguishable from that of SurWT or SurWT;Gad1het (Fig. 6f,g) (SurWT;Gad1het, N = 7 mice). These results show that manipulating GABAergic neurons in the Mecp2-deficient background alleviates
learning deficits, potentially through effects on levels of PV and PNNs.

**Suppressing PNN formation of SurHet improves pup gathering.** PNNs are known to act as barriers to structural plasticity. Thus, relief from the excessive formation of PNNs in SurHet;Gad1het could be a critical factor allowing efficient pup gathering. We speculated that suppressing PNN formation selectively in the auditory cortex just before maternal experience is sufficient to improve behavioural performance of

---

**Figure 6 | Genetic manipulation of the GABA synthesizing enzyme Gad1 rescues cellular and behavioural phenotypes in Mecp2het.** (a) Histograms showing the mean distribution of PV cell intensity comparing SurHet (left, red), SurHet;Gad1het (middle, purple) and SurWT;Gad1het (right, orange) at D5 to their respective naive genotypes (grey). SurHet;Gad1het showed a smaller shift toward elevated PV expression after maternal experience (n = 4353–5079 PV+ cells, 16–20 images, 4–5 mice). The solid line and shaded region represent mean ± s.e.m., respectively. (b) SurHet;Gad1het showed a significant decrease in the high-intensity PV population compared with SurHet at D5 (ANOVA: Tukey’s post-hoc test, *P = 0.02). (c) NaiveWT;Gad1het showed significantly more high-intensity PV cells compared with NaiveWT and SurWT. Upon maternal experience, the PV population of SurWT;Gad1het shifted towards WT PV expression levels (n = 3561–4782 PV+ cells, 16–20 images, 4–5 mice; ANOVA: Tukey’s post-hoc test, *P < 0.05). (d) At D5, high-intensity PNN densities were significantly reduced in SurHet;Gad1het, compared with SurHet (n = 196–1735 PNN+ cells, 17–38 images, 4–9 mice; ANOVA: Tukey’s post-hoc test, *P = 0.01). (e) High-intensity PNN densities were not significantly different between WT;Gad1het and WT mice, before and 5 days after maternal experience (n = 319–780 PNN+ cells, 16–28 images, 4–7 mice; ANOVA: Tukey’s post-hoc test, *P > 0.05). (f–g) Bar graphs represent mean ± s.e.m. (f) Pup retrieval behaviour is significantly improved in SurHet;Gad1het (purple) (N = 7 mice) as measured by normalized latency (f) and errors (g) averaged across three sessions (SurWT: N = 18 mice; SurHet: N = 18 mice; SurWT;Gad1het: N = 7 mice. ANOVA: Tukey’s post-hoc test, *P < 0.05). Mean ± s.e.m. are shown.
SurHet. We therefore made bilateral auditory cortical injections of chondroitinase ABC (ChABC), which dissolves and suppresses the formation of PNNs (ref. 24), thereby allowing for the formation of new synaptic contacts37. Two sites of injection were made into each hemisphere one to three days before initiating assessment of retrieval performance (see Materials and Methods). Injection of ChABC into the auditory cortex of Het and WT significantly reduced high-intensity PNN counts compared with their respective controls: penicillinase-injected mice (Fig. 7a-d) (Het-Pen: n = 710 PNN+ cells, 31 images, 8 mice; Het-ChABC: n = 273 PNN+ cells, 24 images, 6 mice; Mann Whitney, \( P = 0.0003 \); WT-Pen: n = 455 PNN+ cells, 32 images, 8 mice; WT-ChABC: n = 108 PNN+ cells, 32 images, 8 mice; Mann Whitney: \( P < 0.0001 \)). SurHet mice that received bilateral injections of ChABC in the auditory cortex showed significantly improved gathering performance of D5 pups. ChABC-injected SurHet retrieved pups with lower latency index (Fig. 7e-g) and fewer errors (Fig. 7h) compared with SurHet injected with the control enzyme, penicillinase (at D5: Het-Pen: grey line, \( N = 12 \) mice; Het-ChABC: red line, \( N = 10 \) mice; Mann–Whitney, \( P < 0.05 \). ChABC-injected WT performed similarly to the penicillinase-injected WT, with a small significant decrease in latency index at Day 3 (Fig. 7e-h) (For clarity, only WT-ChABC data are shown in the blue line, \( N = 5 \) mice; WT-Pen: normalized latency index – D0 = 0.43 ± 0.13, D3 = 0.29 ± 0.12, D5 = 0.19 ± 0.07, \( N = 7 \) mice, at D3: Mann–Whitney, \( P = 0.048 \); WT-Pen: errors – D0 = 2.3 ± 1.4 errors, D3 = 1.6 ± 0.78 errors, D5 = 1.57 ± 0.81 errors, \( N = 7 \) mice; Mann–Whitney, \( P > 0.05 \)).

Not all injections covered the entire auditory cortex, because of technical issues. Hence, we correlated the percentage of the region affected by the injection with gathering performance. In SurHet, the proportion of auditory cortex bilaterally encompassed by the injection site was significantly negatively correlated with latency index (Fig. 7i) (\( N = 13 \) mice, \( r = -0.75, P = 0.0033 \), Pearson’s \( r \) and number of errors (Fig. 7j) (\( N = 13 \) mice, \( r = -0.75, P = 0.0034 \), Pearson’s \( r \) exhibited on D5 pups. Interestingly, this relationship did not emerge until day 5 of maternal experience. Therefore, increased PNNs in SurHet inhibit auditory cortical plasticity that is required for rapid and accurate pup gathering.

Knocking out Mecp2 in PV neurons affects early learning. Lack of MEC2 expression in PV+ neurons contributes to distinct RTT-like phenotypes35 and affects critical period plasticity in the visual cortex43. To determine the role of MEC2 in PV neurons in the pup retrieval behaviour, we crossed Mecp2lox/PVcre (ref. 31) mice with PV-Cre mice44. Mecp2lox/PVcre (PV-KO) mice displayed significant impairment in latency and errors on D0, but improved significantly to WT performance by D3 and D5 (Fig. 8a–d; Normalized Latency: CTRL: \( N = 11 \) mice; PV-KO: \( N = 9 \) mice; Mann–Whitney, \( P = 0.020 \); Errors: CTRL: \( N = 11 \) mice; PV-KO: \( N = 9 \) mice; Mann–Whitney, \( P = 0.010 \). In agreement with the behaviour results, PNN numbers were similar between WT and PV-KO at D5 (Fig. 8e; CTRL: \( n = 514 \) PNN+ cells, 36 images, 9 mice; PV-KO: \( n = 344 \) PNN+ cells, 34 images, 9 mice; Mann–Whitney, \( P = 0.064 \). These results potentially reveal a

**Figure 7 | Pharmacological suppression of PNN formation in the auditory cortex restores wild-type behaviour in Mecp2Het.** (a,b) Samples of low (left panel) and high (right panel) magnification confocal images taken at D5, from the auditory cortex of SurHet that received injections of either a control enzyme (a, penicillinase) or an enzyme that dissolves PNNs (b, chondroitinase ABC). Arrows indicate high-intensity PNNs. Dashed lines delineate cortical layers with layers III and V indicated. Scale bars in a: left image = 1 mm, right = 50 μm, which also apply to the respective images in b. (c,d) At D5, chondroitinase ABC (ChABC) significantly dissolved PNNs in the injected brains of Het (c) and WT (d) compared with their respective penicillinase (Pen) -injected genotypes (Het-Pen: n = 710 PNN+ cells, 31 images, 8 mice; Het-ChABC: n = 273 PNN+ cells, 24 images, 6 mice; Mann Whitney, \( P = 0.0003 \); WT-Pen: n = 455 PNN+ cells, 32 images, 8 mice; WT-ChABC: n = 108 PNN+ cells, 32 images, 8 mice; Mann Whitney: \( P < 0.0001 \). Bar graphs represent mean ± s.e.m. (e–h) Pup retrieval behaviour improved significantly on D5 in SurHet injected with ChABC (orange), as measured by normalized latency (e,g) and errors (f,h) compared with penicillinase-injected SurHet (grey) (Het-Pen: \( N = 12 \) mice; Het-ChABC: \( N = 10 \) mice; ANOVA: Tukey’s post-hoc test, \( P < 0.05 \). No significant differences in latency and errors were observed between ChABC-injected and penicillinase-injected WT except at D3 (Mann–Whitney, \( P = 0.048 \). For simpler graphic presentation, only ChABC-injected WT data are shown in blue (WT-Pen: \( N = 7 \) mice; WT-ChABC: \( N = 5 \) mice). Mean ± s.e.m. are shown. (i,j) Correlation analysis showed a significant negative relationship between the proportion of auditory cortex encompassed by chondroitinase ABC injection for both latency (i) and number of errors (j) at Day 5 (\( N = 13 \) mice; Pearson’s \( r \) for latency: \( r = -0.75, P = 0.0033 \); for errors: \( r = -0.75, P = 0.0034 \)).
We find evidence of dysregulated cortical inhibitory networks during maternal experience in Mecp2<sup>Het</sup>. This is consistent with increasing evidence that dysfunction of GABA signalling is associated with autism disorders and RTT (refs 33–35,51). Importantly, disruption of MECP2 in GABAergic neurons recapitulates multiple aspects of RTT including repetitive behaviours and early lethality<sup>34</sup>, although the pathogenic mechanisms remain unclear.

Figure 8 | Knocking out Mecp2 in PV neurons affects early learning.

(a–d) Mice with PV cells lacking MECP2 (PV-KO) behaved significantly worse than their control littersmates (CTRL) at Day 0 (D0) by measure of latency (a,c) and errors (b,d) (CTRL: N = 11 mice; PV-KO: N = 9 mice; At D0: Latency: Mann–Whitney, *P = 0.020; errors: Mann–Whitney, *P = 0.010). However, PV-KO mice behaved equally as well as their control littersmates at Day 3 and 5 (D3 and D5, respectively (c,d: Mann–Whitney, P > 0.05). Lines represent mean ± s.e.m. (e) Density of high-intensity PNN<sup>+</sup> cells were comparable between PV-KO mice and their control littersmates assessed at Day 5 (D5) (CTRL: n = 514 PNN<sup>+</sup> cells; 36 images, 9 mice; PV-KO: n = 344 PNN<sup>+</sup> cells, 34 images, 9 mice; Mann–Whitney, P = 0.064). Bar graphs represent mean ± s.e.m.

Discussion

A key challenge for understanding the pathogenesis of RTT and neuropsychiatric disorders in general is to identify the associated molecular and cellular changes and trace the resulting circuit alterations that underlie behaviour deficits. It is also critical to differentiate between impairment of developmental programs and effects on experience-dependent neural plasticity. Here we take advantage of a robust natural behaviour in female mice that relies on a known cortical region, and link molecular events in that region and behaviour. Our data identify a specific critical role for MECP2 in experience-dependent plasticity of cortical inhibitory networks in adults.

Most previous studies in mouse models of RTT were conducted in Mecp2-null male mice, because they exhibit earlier and more severe phenotypes in many assays. Therefore, with the exception of a few studies<sup>29,45,46</sup>, the molecular, circuit, and behavioural defects in Mecp2<sup>Het</sup> female mice are largely unknown. Since RTT affects more females, Mecp2<sup>Het</sup> female mice represent a more translationally-relevant model of RTT than Mecp2-null male mice.

We found a robust behavioural phenotype in the Mecp2<sup>Het</sup> mice, suggesting impairment of adult experience-dependent plasticity. We conclude that dysregulated auditory processing in the cortex, because of impaired inhibitory neuronal plasticity, leads to altered learned behaviour. We also showed that when normal plasticity is restored, even acutely during adulthood, this behavioural deficit is improved. These results suggest that Mecp2 deficiency impairs not only developing neural circuits, but also the function and plasticity of adult circuits, via mechanisms involving PV<sup>+</sup> GABAergic networks. GABAergic interneurons are basic components of cortical microcircuits that are conserved across brain areas. The same mechanisms that underlie experience-triggered and MECP2-dependent PV interneuron function during development and adulthood may also apply to other functional modalities affected in RTT.

Emerging evidence indicates that the appropriate expression and function of MECP2 is required in adulthood for normal plasticity and behaviour<sup>5,9</sup>. Remarkably, restoring normal MECP2 expression in adulthood improves behaviour deficits in mice<sup>45,47</sup>. These observations have several implications. First, they indicate that some cellular functions of MECP2 are involved in the maintenance and adult plasticity of neural circuitry, not only its development. Second, they raise the possibility that in humans it may be beneficial to therapeutically restore MECP2 levels at later stages. Nevertheless, the specific mechanisms by which Mecp2 mutations impair adult neural function need to be elucidated.

Our data demonstrate that heterozygous mutations in Mecp2 (Mecp2<sup>Het</sup>) interfere with auditory cortical plasticity that occurs in adult mice during initial maternal experience. Mothers and wild type virgin surrogates achieve proficiency in pup retrieval behaviour by an experience-dependent learning process<sup>16,19,20,32,48,49</sup>, that is correlated with neurophysiological plasticity in the auditory cortex<sup>16,17,18,50</sup>. We used gathering behaviour to assay defects in this sensory plasticity. Our results show that Mecp2<sup>Het</sup> have markedly impaired ability to learn appropriate gathering responses to pup calls. This interference is in large part because of a specific requirement for MECP2 in the adult auditory cortex. Deletion of MECP2 in adult mice selectively in the auditory cortex also produced inefficient retrieval. We saw no improvement in the behaviour of the mutants over the first five days post birth. At that point, pups were sufficiently mobile that they no longer required gathering. However, it is tempting to speculate that the Mecp2<sup>Het</sup> might improve with more practice, such as with subsequent litters.

Electrophysiological recordings from naive mice of both genotypes demonstrate that there are no gross deficits in basic auditory cortex function in heterozygous mutants and that they are not deaf. We speculate instead that there are more subtle and context-specific impairments of intra-cortical processing and plasticity in the auditory cortex of Mecp2<sup>Het</sup>.

Dynamic role for MECP2 in PV neurons during pup retrieval behaviour. Further work will be required to define the time course and molecular mechanisms mediating the change in plasticity between D0 and D3.
Our data suggest that an important aspect of the pathology associated with heterozygous Mecp2 mutations is impaired plasticity of cortical inhibitory networks. Pup exposure and maternal experience trigger an episode of heightened auditory cortical inhibitory plasticity. For example, GAD67 levels are roughly doubled in the auditory cortex of both wild type and Mecp2<sup>h&lt;/sup> five days after the birth of the litter. This result suggests a reorganization of the cortical GABAergic network triggered by maternal experience. Although this feature of auditory cortex plasticity is shared between SurWT and SurHet, SurHet also show large increases in expression of PV and PNNs on the fifth day of pup exposure. Notably, initial levels of these inhibitory markers in NaiveWT and NaiveHet, and levels in Sur after pups are weaned, are identical. Therefore, potentially crucial features of Mecp2<sup>h&lt;/sup> pathology may only be revealed by the commencement of an episode of heightened sensory and social experience, as occurs with first-time pup exposure. We speculate that this may be a general phenomenon wherein exposure to salient sensory stimuli may define a particularly vulnerable point for Mecp2<sup>h&lt;/sup>. Further assessment using natural stimuli targeting motor and social circuits that challenge network plasticity mechanisms may reveal endo-phenotypes.

Both WT and Mecp2<sup>h&lt;/sup> female mice exhibit low GAD67 expression as maternally-naive adults. Expression sharply increases after exposure to a mother and her pups, and returns to baseline levels when the pups are weaned. This is correlated with a surge in the expression of PV and PNNs in Mecp2<sup>h&lt;/sup> only. This result is consistent with increased PV (ref. 33) and PNN expression observed in the developing Mecp2-null visual cortex.<sup>39</sup> Several lines of evidence implicate elevated expression of PV and PNN as brakes that terminate episodes of plasticity in development and adulthood. In the developing cortex, maturation of GABAergic inhibition mediated by the fast-spiking PV interneuron network is a crucial mechanism for regulating the onset and progression of critical periods.<sup>36</sup> During postnatal development, PV interneurons undergo substantial changes in morphology, connectivity, intrinsic and synaptic properties<sup>32–55</sup>, and they form extensive reciprocal chemical and electrical synapses<sup>32,56,57</sup>. Learning associated with a range of adult behaviours might rely on similar local circuit mechanisms observed in the developing cortex.<sup>25,58</sup> This model is supported by our finding that knockout of Mecp2 specifically in PV neurons is sufficient to impair pup gathering behaviour. From these results, we speculate that increased PV and PNN expression might support an enhanced inhibitory function that might lead to reduced neuronal activation of excitatory circuits in a stimulus-specific manner, in agreement with previously published reports.<sup>30,59,60</sup>

PNNs inhibit adult experience-dependent plasticity in the visual cortex<sup>24</sup>, and in consolidating fear memories in the amygdala.<sup>61</sup> PNN assembly in the SurHet tracks with changes in PV expression after maternal experience, suggesting there is remodelling of the extracellular matrix during natural behaviour. This is an interesting observation as the prevailing notion of PNNs during adulthood is as a stable, structural barrier which needs to be removed with chondroitinase ABC to reactivate plasticity. Related to this, there was no further improvement in WT that received ChABC injection possibly revealing a ceiling effect.

We demonstrate that manipulating GAD67 expression using Gad1 heterozygotes is sufficient to restore normal PV and PNN expression patterns and behaviour. This result suggests a critical role for Gad1 in regulating MECP2-mediated experience-driven cellular and circuit operations. MECP2 directly occupies the promoter regions of Gad1 and PV (refs 33,34), thus potentially configuring chromatin in these promoter and enhancer regions for appropriate activity- and experience-dependent regulation. We speculate that MECP2 regulates specific enzymes of genes and the temporal profile of their expression to control the tempo of plasticity. MECP2 regulates many genes<sup>15,62</sup>, therefore there are likely other as yet unappreciated targets that could contribute to this control.

Our data are consistent with an emerging body of literature that suggests that auditory cortical plasticity is triggered in adult female virgin mice by pup exposure. By using pup gathering behaviour as readout of the efficacy of this plasticity, we observe that impaired MECP2 expression disrupts both behaviour and the underlying auditory cortical plasticity. This is consistent with recent data revealing sensory impairments in individuals with RTT, which may contribute to behavioural symptoms.<sup>63,64</sup> We further speculate that MECP2 deficiency results in suppressed (‘negative’) experience-dependent plasticity that may act at other brain regions and time points to contribute to a range of altered behaviours.

**Methods**

**Animals.** All experiments were performed in adult female mice (7–10 weeks old) that were maintained on a 12-h light–dark cycle (lights on 07:00 h) and received food ad libitum. Genotypes used were CBA/Ca, Mecp2<sup>h&lt;/sup> (C57BL/6 background; B6.129P2(C)-Mecp2<sup>tm1(cre)Arbr/J</sup>), Mecp2<sup>h&lt;/sup>, Mecp2<sup>h&lt;/sup>/null (B6.129P2(Mecp2<sup>2<sub>h&lt;/sub>tm1(cre)Arbr/J)-Mecp2<sup>2<sub>h&lt;/sub>tm1(cre)Arbr/J)).</sup></ref> Further assessment using natural stimuli targeting motor and social circuits that challenge network plasticity mechanisms may reveal endo-phenotypes.

We further speculate that MECP2 deficiency results in suppressed (‘negative’) experience-dependent plasticity that may act at other brain regions and time points to contribute to a range of altered behaviours.

**Pup gathering behaviour and movement analysis.** We housed two virgin female mice (one control and one experimental mouse; termed ‘surrogates’) with a primiparous CBA/Ca female beginning 1–5 days before birth. Pup retrieval behaviour was assessed starting on the day the pups were born (postnatal day 0; D0) as follows: (1) one female was habituated with 3–5 pups in the nest of the home cage for 5 min, (2) pups were removed from the cage for 2 min and (3) one pup was placed at each corner and one in the center of the home cage (the nest was left empty if there were fewer than 5 pups). Each adult female had maximum of 5 min to gather the pups to the original nest. After testing, all animals and pups were returned to the home cage. The same procedure was performed again at D3 and D5. All behaviours were performed in the dark, during the light cycle (between 10:30 AM and 4:00 PM) and were video recorded. For analysis, an experimenter who was blind to genotype and experimental condition counted the number of errors and measured the latency of each mouse to gather all five pups. An error was scored for each instance of gathering of pups to the wrong location or of interacting with the pups (for example, licking or grooming) without carrying them to the nest. Normalized latency was calculated using the following formula:

\[
\text{latency index} = \frac{\left| (t_1 - t_0) + (t_1 - t_0) + ... + (t_n - t_0) \right|}{(n \times L)}
\]

where \(n\) = # of pups outside the nest, \(t_0\) = start of trial, \(t_n\) = time of rth pup gathered, and \(L\) = trial length.

Movement was measured while the animal was performing pup retrieval behaviour, using Matlab-based software (MultiWorks).<sup>68</sup>

**Injections.** Mice were anesthetized with ketamine (100 mg kg<sup>−1</sup>) and xylazine (5 mg kg<sup>−1</sup>) and stabilized in a stereotaxic frame. Lesions in the auditory cortex of CBA/Ca mice were performed by injection of ibotenic acid (0.5 μl of 10 mg ml<sup>−1</sup>).
To knock down MECP2 expression, we injected AAV9-GFP-IRES-Cre (0.3 μl per site; Tocris Bioscience). Control animals were injected with the solvent only.

To degrade PNNs, we injected chondroitinase ABC (0.3 μl (50 U ml⁻¹) into each auditory cortical hemisphere of each animal to degrade PNNs.

Fluoromount-G (Southern Biotech) was used to block the sections. The sections were incubated with the following primary antibodies overnight: MECP2 (1:1,000; rabbit; Cell Signaling), PV (1:1,000; mouse; Sigma-Aldrich) and biotin-conjugated Labels (labeled PNNs; 1:500; Sigma-Aldrich).

Sections were incubated with appropriate AlexaFluor dye-conjugated secondary antibodies (1:1,000; Molecular Probes) and mounted in Fluoromount-G (Southern Biotech). To obtain GAD67 staining in soma, three confocal images from each auditory cortical region of each AAV-GFP-Cre injected mouse were acquired using the Zeiss LSM710 confocal microscope (20 objective; 1 zoom) for each mouse.

In vivo physiology. For awake-state recordings, we anesthetized Mecp₂⁻/⁻ mice and age-matched Mecp₂⁺/⁺ mice with an 80 mg ml⁻¹ (1.00 mg kg⁻¹ i.p.) of ketamine (100 mg ml⁻¹) and xylazine (20 mg ml⁻¹) (KX) and stabilized in a stereotaxic frame. A head bar was affixed above the cerebellum with RelY X Luting Cement (3M) and methyl methacrylate-based dental cement (TEETS). For additional support, five machine screws (Amazon Supply) were placed in the skull before cement application. After one day of recovery, mice were anesthetized with isoflurane (Fliuriso; Vet One) and small craniotomies were made to expose the left hemisphere of auditory cortex. Mice were then head-fixed via the attached head bar over a foam wheel that was suspended above the air table. The foam wheel allowed mice to walk and run in one dimension (forward-reverse).

Baseline stimuli were presented via Ed1 Electrostatic Speaker Driver and ESI Electrostic Speaker (TDT), in a sound attenuation chamber (Industrial Acoustics) at 65 dB SPL. RMS measured at the animal’s head. Stimuli consisted of 100 ms presentation of broadband noise, four logarithmically-spaced tones ranging between 4 and 32 kHz, and ultrasound noise bandpassed between 40 and 60 kHz.

To analyse GAD67 expression, two confocal images from each auditory cortical hemisphere of each animal were acquired using the Zeiss LSM710 confocal microscope (20 objective; 1 zoom) for each mouse.

The intensity of GAD67 expression in soma was measured based on Allen brain atlas boundaries (Version 1, NIH), a region of 100 μm² in the neuronal soma visible in the confocal images were used for the analysis.

To analyse PV expression, two confocal images from each auditory cortical hemisphere of each animal were acquired using the Zeiss LSM710 confocal microscope (20 objective; 1 zoom) for each mouse.

The intensity of PV expression was measured based on Allen brain atlas boundaries (Version 1, NIH), a region of 100 μm² in the neuronal soma visible in the confocal images were used for the analysis.

The percentage of cell population with MECP2 expression by the total number of infected cells was determined to be either positive or negative for MECP2 expression. The percentage was calculated by dividing the number of DAPI cells with MECP2 expression by the total number of DAPI cells. Each data point in Fig. 1h,i, represents an average percentage value calculated from four × 20 projection images from each mouse.

To analyse knockdown of the auditory cortex by AAV-GFP-Cre or degradation of PNNs by chondroitinase ABC, 4–5 single-plane images per auditory cortical hemisphere from each animal were acquired using Olympus BX43 microscope (×4 objective, UPlanFL.N) and analysed using ImageJ (NIH). To calculate percentage infection/degradation in each image, the area of the entire auditory cortex was determined using a previously published Allen Brain Atlas boundary (Allen Brain Atlas, 2008). Then, the area containing GFP cells or reduced PNNs was measured and divided by the total auditory cortical area. For non-auditory cortical region analysis, cumulative regions included temporal association cortex, entorhinal cortex and parahippocampal cortex. Each correlation data point represents the percentage infection or degradation per animal per animal.

The intensity of GAD67 expression in soma was measured based on Allen brain atlas boundaries (Version 1, NIH), a region of 100 μm² in the neuronal soma visible in the confocal images were used for the analysis.

The intensity of PV expression was measured based on Allen brain atlas boundaries (Version 1, NIH), a region of 100 μm² in the neuronal soma visible in the confocal images were used for the analysis.

To analyse knockdown of the auditory cortex by AAV-GFP-Cre or degradation of PNNs by chondroitinase ABC, 4–5 single-plane images per auditory cortical hemisphere from each animal were acquired using Olympus BX43 microscope (×4 objective, UPlanFL.N) and analysed using ImageJ (NIH). To calculate percentage infection/degradation in each image, the area of the entire auditory cortex was determined using a previously published Allen Brain Atlas boundary (Allen Brain Atlas, 2008). Then, the area containing GFP cells or reduced PNNs was measured and divided by the total auditory cortical area. For non-auditory cortical region analysis, cumulative regions included temporal association cortex, entorhinal cortex and parahippocampal cortex. Each correlation data point represents the percentage infection or degradation per animal per animal.
13. Chahrouh, M. et al. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320, 1224–1229 (2008).

14. Zhou, Z. et al. Extracellular phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. Neuron 52, 255–269 (2006).

15. Ebert, D. H. et al. Activity-dependent phosphorylation of MeCP2 threonine 308 regulates interaction with NCoR. Nature 499, 341–345 (2013).

16. Cohen, L., Rothschild, G. & Muzrai, A. Multisensory integration of natural odors and sounds in the auditory cortex. Neuron 72, 357–369 (2011).

17. Galindo-León, E. E., Lin, F.-C. & Liu, R. C. Inhibitory plasticity in a lateral band improves cortical detection of natural vocalizations. Neuron 62, 705–716 (2009).

18. Liu, R. C. & Schreiner, C. E. Auditory cortical detection and discrimination correlates with communicative significance. PLoS Biol. 3, e159 (2005).

19. Galtrey, C. M. & Fawcett, J. W. The role of chondroitin sulfate proteoglycans in plasticity in murine barrel cortex. Nat. Neurosci. 13, 478–484 (2010).

20. Ehret, G., Koch, M., Haack, B. & Markl, H. Sex and parental experience determine the onset of an instinctive behaviour in mice. Naturwissenschaften 74, 47 (1987).

21. Nowicka, D., Solsby, S., Skangiel-Kramska, J. & Glazewski, S. Parvalbumin-containing neurons, perineuronal nets and experience-dependent plasticity in murine barrel cortex. Eur. J. Neurosci. 30, 2053–2063 (2009).

22. Miyata, S., Komatsu, Y., Yoshimura, Y., Taya, C. & Kitagawa, H. Persistent plasticity in murine barrel cortex. Science 333, 1411–1413 (2011).

23. Galtrey, C. M. & Fawcett, J. W. The role of chondroitin sulfate proteoglycans in plasticity in murine barrel cortex. Nat. Neurosci. 13, 478–484 (2010).

24. Pizzorusso, T. et al. Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298, 1248–1251 (2002).

25. Donato, F., Rompani, S. B. & Caroni, P. Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. Nature 504, 272–276 (2013).

26. Bavelier, D., Levi, D. M., Li, R. W., Dan, Y. & Hensch, T. K. Removing brakes on adult brain plasticity: from molecular to behavioural interventions. J. Neurosci. 30, 14964–14971 (2010).

27. Guy, J., Hendrich, B., Holmes, M., Martin, J. E. & Bird, A. A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat. Genet. 27, 322–326 (2001).

28. Marín, P. J., Mitre, M., D’Amour, J. A., Chao, M. V. & Froemke, R. C. Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature 520, 499–504 (2015).

29. Samaco, R. C. et al. Female Mecp2(+/−) mice display robust behavioural deficits on two different genetic backgrounds providing a framework for preclinical studies. Hum. Mol. Genet. 22, 96–109 (2013).

30. Godin, D., Brodkin, E. S., Bendley, J. A., Siegel, S. J. & Zhou, Z. Cellular origins of auditory event-related potential deficits in Rett syndrome. Nat. Neurosci. 17, 804–806 (2014).

31. Chen, R. Z., Akbarian, S., Tudor, M. & Jaenisch, R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. Nat. Genet. 27, 327–331 (2001).

32. Lin, F.-C., Galindo-León, E. E., Ivanova, T. N., Mappus, R. C. & Liu, R. C. A role for maternal physiological state in preserving auditory cortical plasticity for lactating females. Naturwissenschaften 68, 208–209 (1981).

33. Smith, J. C. Responses of adult mice to models of infant calls. J. Comp. Physiol. Psychol. 90, 1105–1115 (1976).

34. Cohen, L. & Muzrai, A. Plasticity during motherhood: changes in excitatory and inhibitory layer 2/3 neurons in auditory cortex. J. Neurosci. 35, 1806–1815 (2015).

35. Han, S. et al. Autistic-like behaviour in Scn1a−/− mice and rescue by enhanced GABA-mediated neurotransmission. Nature 489, 385–390 (2012).

36. Doischer, D. et al. Postnatal differentiation of basket cells from slow to fast firing devices. J. Neurosci. 28, 12956–12968 (2008).

37. Huang, Z. et al. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98, 739–755 (1999).

38. Lazarus, S. M. & Huang, Z. J. Distinct maturation profiles of perisomatic and dendritic targeting GABAergic interneurons in the mouse primary visual cortex during the critical period of ocular dominance plasticity. J. Neurophysiol. 106, 775–787 (2011).

39. Pangratz-Fuehrer, S. & Hestrin, S. Synaptogenesis of electrical and GABAergic synapses of fast-spiking inhibitory neurons in the neocortex. J. Neurosci. 31, 10767–10775 (2011).

40. Galaretz, M. & Hestrin, S. Electrical synapses between GABA-releasing interneurons. Nat. Rev. 2, 425–431 (2001).

41. Bartos, M. et al. Fast synaptic inhibition promotes synchronized gamma oscillations in hippocampal interneuron networks. Proc. Natl Acad. Sci. USA 99, 13222–13227 (2002).

42. Hensch, T. K. Bistable parvalbumin circuits pivotal for brain plasticity. Cell 156, 17–19 (2014).

43. Kron, M. et al. Brain activity mapping in Mecp2 mutant mice reveals functional deficits in forebrain circuits, including key nodes in the default mode network, that are reversed with ketamine treatment. J. Neurosci. 32, 13860–13872 (2012).

44. Panerai, A. et al. Chronic administration of the N-Methyl-D-Aspartate receptor antagonist ketamine improves rett syndrome phenotype. Biol. Psychiatry. 79, 755–764 (2016).

45. Gogolla, N., Caroni, P., Luthi, A. & Herry, C. Perineuronal nets protect fear memories from erasure. Science 325, 1258–1261 (2009).

46. Gabell, H. W. et al. Disruption of DNA-methylation-dependent long gene repression in Rett syndrome. Nature 522, 89–93 (2015).

47. Peters, S. U., Gordon, R. L. & Key, A. P. Induced gamma oscillations differentiate familiar and novel voices in children with MECP2 duplication and Rett syndromes. J. Child. Neurol. 30, 145–152 (2015).

48. LeBlanc, L. J. et al. Visual evoked potentials detect cortical processing deficits in Rett syndrome. Ann. Neurol. 78, 775–786 (2015).

49. Merzenich, M. M., Nahum, M. & Van Vleet, T. M. Neuroplasticity: introduction. Prog. Brain. Res. 207, xxi–xxvi (2013).

50. He, M. et al. Cell-type-based analysis of microRNA profiles in the mouse brain. Neuron 73, 35–48 (2012).

51. Tamamaki, N. et al. Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. J. Comp. Neurol. 467, 60–79 (2003).

52. Koepe, C. D. et al. A robust automated method to analyze rodent motion during fear conditioning. Neuropearmacology 52, 228–233 (2007).

53. Eslapez, M., Tillakaratne, N. J., Kaufman, D. L., Tobin, A. J. & Houser, C. R. Comparative localization of two forms of glutamic acid decarboxylase and their mRNAs in rat brain supports the concept of functional differences between the forms. J. Neurosci. 14, 1834–1855 (1994).

Acknowledgements
We wish to thank D. Huang and A. Chandrasekhar for data collection and analysis assistance. Alexandra Nowlan for help with the mouse drawing and Stephen Hearn at the NSF stereotaxic facility for assistance with Velocity software. We would also like to thank A. Zador, B. Li, R. Froemke, J. Tollkuhn, J. Morgan, D. Eckmeier, B. Cazakoff, A. Fleischmann and A. Maffei for helpful comments and discussion. This work was supported by grants to S.D.S. from the Simons Foundation Autism Research Initiative
Author contributions
S.D.S. and Z.J.H. supervised the project. K.K., B.Y.B.L. and S.D.S. designed the experiments and developed the methods. K.K., B.Y.B.L., G.E. and S.D.S. collected and analysed the data. K.K., B.Y.B.L., Z.J.H. and S.D.S. wrote and edited the paper.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Krishnan, K et al. MECP2 regulates cortical plasticity underlying a learned behaviour in adult female mice. Nat. Commun. 8, 14077 doi: 10.1038/ncomms14077 (2017).

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2017