Deep brain stimulation (DBS) has improved the prospects for many individuals with diseases affecting motor control, and recently it has shown promise for improving cognitive function as well. Several studies in individuals with Alzheimer disease and in amnesic rats have demonstrated that DBS targeted to the fimbria–fornix, the region that appears to regulate hippocampal activity, can mitigate defects in hippocampus-dependent memory. Despite these promising results, DBS has not been tested for its ability to improve cognition in any childhood intellectual disability disorder. Such disorders are a pressing concern: they affect as much as 3% of the population and involve hundreds of different genes. We proposed that stimulating the neural circuits that underlie learning and memory might provide a more promising route to treating these otherwise intractable disorders than seeking to adjust levels of one molecule at a time. We therefore studied the effects of fornix DBS in a well-characterized mouse model of Rett syndrome (RTT), which is a leading cause of intellectual disability in females. Caused by mutations that impair the function of MeCP2 (ref. 6), RTT appears by the second year of life in humans, causing profound impairment in cognitive, motor and social skills, along with an array of neurological features. RTT mice, which reproduce the broad phenotype of this disorder, also show clear deficits in hippocampus-dependent learning and memory and hippocampal synaptic plasticity. Here we show that fornix DBS in RTT mice rescues contextual fear memory as well as spatial learning and memory. In parallel, fornix DBS restores in vivo hippocampal long-term potentiation and hippocampal neurogenesis. These results indicate that fornix DBS might mitigate cognitive dysfunction in RTT.

A deficit in contextual fear memory is one of the most reproducible and reliable outcome measures among RTT mouse models. Specifically, female Mecp2+/− mice (hereafter referred to as RTT mice) have impaired contextual fear memory when tested 24 h after training. Because this deficit is readily quantifiable and accessible, we used fear memory as our first test of the effect of fornix DBS in freely moving RTT mice. We implanted DBS electrodes in the fimbria–fornix (FFx) of 6- to 8-week-old RTT mice and wild-type controls (Extended Data Fig. 1), guiding electrode placement with FFx-evoked potentials in the dentate gyrus (Fig. 1a–c). We divided mice into four groups after recovery: wild-type, sham; wild-type, DBS; RTT, sham; and RTT, DBS. Mice in both DBS groups received daily DBS treatment for 14 days while the two sham groups experienced the same procedures except for DBS. Based on widely used parameters for DBS in the clinic and in rodents, along with our own pilot testing, we set DBS at 130 Hz, 60 μs pulse duration, and 1 h per day. Stimulus intensities were individually optimized to 80% of the threshold that elicited an instance of afterdischarge in the hippocampus. No seizures appeared under these DBS parameters. Three weeks after completing the two-week DBS protocol, we performed behavioural testing and subjected the mice (now aged 14 weeks, Extended Data Fig. 1) to a fear conditioning paradigm to examine contextual fear memory and cued fear memory (see Methods).

Fornix DBS significantly enhanced contextual fear memory in both wild-type (3 h, day 1 and day 3, P < 0.05; RTT mice (3 h and day 1, P < 0.05; Fig. 1d). In fact, DBS restored contextual fear memory in RTT mice to wild-type levels: there was no difference between the DBS-treated RTT mice (3 h, 47.56 ± 4.22%; day 1, 47.84 ± 4.16%) and sham-treated wild-type mice (3 h, 4.48 ± 3.60%; day 1, 45.97 ± 3.69%). Notably, fornix DBS did not alter cued fear memory (Fig. 1e), even though the FFx also projects to the amygdala. All the mice that received DBS/sham treatment responded to tone presentation (Extended Data Fig. 2e–h), but less than the animals that were implanted and did not experience the 2-week DBS/sham procedures (Extended Data Fig. 2b–d). Further analysis indicated that the longer period of handling and exposure (for example, daily transportation, connection/disconnection of the wires, and staying in the DBS/sham chamber for 1 h per day) increased the motor activity and decreased the anxiety levels in DBS/sham-treated mice (Extended Data Fig. 3). These changes likely reduced the fear responses to the tone, and the conditioning context, in general (Fig. 1d, e and Extended Data Fig. 2a, b).

Fornix DBS did not improve levels of locomotion, anxiety, pain threshold or motor learning (Extended Data Figs 3 and 4a, b) as well as motorcoordination, social behaviour and body weight in RTT mice, although there were differences between RTT mice and wild-type controls in these features (Extended Data Figs 4c–f and 5a, b). Fornix DBS thus specifically rescued contextual memory impairment in RTT mice without evident off-target effects.

To determine whether fornix DBS would improve spatial cognition, which is also hippocampus-dependent, we trained new cohorts of mice, who received the same DBS/sham procedures, in a hidden platform version of the water maze task. Sham-treated RTT mice needed more time than sham-treated wild-type mice to locate the hidden platform across the training trials, spent less time in the target quadrant, and had fewer platform area crossings in the probe test (Fig. 2a). In wild-type mice, DBS significantly enhanced spatial learning compared to the sham group (Fig. 2b). Treatment made no difference during the probe test, probably because of a ceiling effect in sham-treated wild-type animals. We observed an even stronger effect of DBS in RTT mice: fornix DBS enhanced not only spatial learning but also spatial memory retrieval (Fig. 2c). Again, the rescue was so strong that there was no difference between DBS-treated RTT and sham-treated wild-type groups in latencies to the hidden platform, time in target quadrant, or platform area crossings (Fig. 2d). Visible platform training confirmed that neither MeCP2 level nor fornix DBS altered visual or sensorimotor skills (Extended Data Fig. 5c–e).

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Since the RTT mice used in this study are impaired in both hippocampus-dependent memory\(^1\) and in vitro hippocampal long-term potentiation (LTP)\(^2\), they provide an ideal setting in which to examine whether DBS alters synaptic plasticity. We implanted RTT and wild-type mice with DBS electrodes in the FFX, stimulation electrodes in the perforant path for the LTP test, and a recording electrode in the dentate gyrus (using evoked potentials as a guide). As with the behavioural studies described above, the mice underwent 2 weeks of DBS.

Figure 1 | Forniceal DBS restores contextual fear memory in RTT mice. a, b, Photomicrographs illustrating DBS electrode placement (arrowheads) in the FFx (a) and the recording electrode in the dentate gyrus (b). c, c, Representative evoked potential trace of the FFx pathway recorded in the dentate. d, Forniceal DBS enhanced contextual fear memory in both wild-type (WT) and RTT mice (DBS-treated wild-type mice, \(n = 21\); sham-treated wild-type mice, \(n = 21\); DBS-treated RTT mice, \(n = 17\); sham-treated RTT mice, \(n = 14\)). There were significant main effects on freezing time among the four groups (two-way repeated-measures ANOVA: group, \(F_{3,89} = 5.67, P = 0.002\); day, \(F_{2,156} = 6.44, P < 0.001\); group \times day interaction, \(F_{4,180} = 2.15, P = 0.027\)). Within-genotype analysis revealed a significant DBS effect in both wild-type (\(F_{1,49} = 8.50, P = 0.006\)) and RTT mice (\(F_{1,29} = 6.44, P = 0.016\)). DBS in RTT mice restores contextual fear memory to wild-type levels (DBS-treated RTT mice vs. sham-treated wild-type mice: group, \(F_{1,38} = 3.90, P = 0.055\); treatment, \(F_{1,38} = 11.41, P = 0.001\)). e, Cued fear memory of mice tested in d. There was no difference in cued fear memory between groups over any time point (main effect: group, \(F_{1,38} = 0.45; P = 0.48;\) day, \(F_{2,76} = 1.65, P = 0.179;\) group \times day interaction, \(F_{2,76} = 0.89, P = 0.538\)) or on day 1 (genotype, \(F_{1,69} = 0.64, P = 0.428;\) treatment, \(F_{1,69} = 0.11, P = 0.741\). \(*P < 0.05;\) NS, not significant. Data presented as mean ± s.e.m. Scattergrams show individual values.

Figure 2 | Forniceal DBS rescues spatial learning and memory in RTT mice. In the water maze task, all mice were trained with a hidden platform for 9 days followed by a probe test without the platform 24 h after the last training. There were significant main effects of escape latencies among the four groups (\(n = 18\) mice per group) during acquisition training (two-way repeated-measures ANOVA: group, \(F_{3,89} = 20.74, P < 0.001\); day, \(F_{8,272} = 17.10, P < 0.001\); treatment, \(F_{1,29} = 10.31, P = 0.003\); day \times treatment, \(F_{8,272} = 6.13, P < 0.001\)) but not during the probe test (\(P > 0.05\)) or in the number of platform area crossings (\(P > 0.05\)) between DBS and sham groups during the probe test. a, In the DBS-treated RTT mice showed increased escape latencies during training (genotype, \(F_{1,34} = 35.30, P < 0.001\); day, \(F_{8,272} = 7.06, P < 0.001\)) but decreased time in target quadrant (\(P < 0.01\)) and fewer platform area crossings during the probe test (\(P < 0.001\)) than sham-treated wild-type controls. b, Forniceal DBS decreased escape latencies during training in DBS-treated wild-type mice compared to sham-treated wild-type controls (treatment, \(F_{1,34} = 5.94, P = 0.020\); day, \(F_{8,272} = 17.10, P < 0.001\); treatment \times day, \(F_{8,272} = 2.19, P = 0.028\)). There was no difference in time spent in the target quadrant (\(P > 0.05\)) or in the number of platform area crossings (\(P > 0.05\)) between DBS and sham groups during the probe test. c, The DBS-treated RTT mice showed shorter escape latencies during training (treatment, \(F_{1,34} = 10.31, P = 0.003\); day, \(F_{8,272} = 6.13, P < 0.001\)) but more time in the target quadrant (\(P < 0.05\)) and fewer platform area crossings (\(P < 0.05\)) during the probe test than sham-treated RTT controls. d, There was no difference between DBS-treated RTT mice and sham-treated wild-type controls in escape latencies during training (genotype, \(F_{1,34} = 2.91, P = 0.097;\) group \times day interaction, \(F_{8,272} = 0.80, P = 0.606\), time in the target quadrant (\(P > 0.05\)), or number of crossings of the platform area (\(P > 0.05\)) during the probe test. *\(P < 0.05;\) **\(P < 0.01;\) ***\(P < 0.001;\) NS, not significant (Tukey’s post hoc test during acquisition; two-tailed unpaired t-test between groups and two-tailed paired t-test within group during probe). Data presented as mean ± s.e.m. Scattergrams show individual values.
followed by a 3-week interval before LTP testing (Extended Data Fig. 1). These DBS/sham procedures did not alter the hippocampal neural excitability (Extended Data Fig. 6a). LTP was induced on day 0 and monitored across 5 days after induction in awake, freely moving mice. We quantified the population spike amplitude in each of the four groups of animals\(^1\) (Fig. 3a). Neither DBS nor sham treatment altered LTP and hippocampus-dependent memory\(^18\)–\(^20\), and because DBS in wild-type mice (Fig. 3c; DBS, 381.75 ± 99.84; sham, 160.37 ± 14.08%) compared to sham-treated wild-type controls (BrdU, 1,218 ± 175.75; DCX, 2,131 ± 381.49) were significantly lower than in sham-treated wild-type controls (BrdU, 1,218 ± 175.75; DCX, 2,131 ± 381.49) (Fig. 3e, f). Forniceal DBS, however, bilaterally enhanced dentate neurogenesis by the numbers of cells positive for BrdU, DCX, 1,526 ± 426.92; sham-treated wild-type controls (BrdU, 1,218 ± 175.75; DCX, 2,131 ± 381.49) to the degree that there was no difference in the stimulus intensities used for LTP induction among the four groups (wild-type, sham, 38.91 ± 13.29 μA; wild-type, DBS, 69.50 ± 7.58 μA; RTT, sham, 73.50 ± 14.58 μA; RTT, DBS, 68.62 ± 13.87 μA; P > 0.05). As expected, sham-treated RTT mice showed impaired in vivo LTP (1 h after induction, 160.37 ± 14.08%) compared to sham-treated wild-type controls (256.06 ± 27.50%) (Fig. 3b). Forniceal DBS, however, enhanced LTP in both wild-type mice (Fig. 3c; DBS, 381.75 ± 26.13%; sham, 26.13%; P = 0.001; genotypes, F, 21 = 7.46; P = 0.01; day 1; (genotypes, F, 21 = 12.16, P = 0.002; time, F, 15,315 = 121.93, P < 0.001; treatment, F, 11, 121 = 18.99, P < 0.001; time, F, 15,315 = 4.77, P = 0.005), day 2 (treatment, F, 11, 121 = 11.25, P = 0.003; time, F, 15,315 = 3.72, P = 0.016) and day 5 (treatment, F, 11, 121 = 9.44, P = 0.006; time, F, 15,315 = 6.73, P = 0.001) (NS, not significant. Data presented as mean ± s.e.m.) by stimulating hippocampal neurogenesis. We first observed that two-hit unilateral fornical DBS stimulated the activity of dentate neurons, as indicated by increased expression of the immediate early gene Fos (Extended Data Fig. 7a). Each day after DBS over the 2 weeks of DBS/sham treatment, we injected RTT and wild-type mice with 5-bromo-2′-deoxyuridine (BrdU) to mark newborn cells. We quantified dentate neurogenesis by the numbers of cells positive for BrdU, DCX (doublecortin, to label the immature neurons), or double labelled for BrdU and DCX (Extended Data Fig. 7a). Each day after DBS over the 2 weeks of DBS/sham treatment, we injected RTT and wild-type mice with 5-bromo-2′-deoxyuridine (BrdU) to mark newborn cells. We quantified dentate neurogenesis by the numbers of cells positive for BrdU, DCX (doublecortin, to label the immature neurons), or double labelled for BrdU and DCX. Forniceal DBS thus restored hippocampal LTP in the perfusion path/dentate pathway in RTT mice.

Because hippocampal neurogenesis contributes to hippocampal LTP and hippocampus-dependent memory\(^21\)–\(^23\), we explored whether fornical DBS might exert its effects through cholinergic modulation in rodents\(^3\). Therefore, we examined the effect of hippocampal infusion of muscarinic acetylcholine receptor antagonist atropine on the DBS benefit. There was no difference of fear memory between atropine and vehicle-treated groups in either RTT or wild-type mice, suggesting that DBS must benefit memory via additional mechanisms (Extended Data Fig. 8).

**Figure 3** Forniceal DBS rescues hippocampal synaptic plasticity in freely moving RTT mice. a, Superimposed traces of the perforant path recorded in the dentate gyrus 5 min before (grey) and 55 min after (black or red) tetani. b, Sham-treated RTT mice (n = 12) showed impaired LTP compared to the sham-treated wild-type group (n = 11) on day 0 (two-way repeated-measures ANOVA: genotype, F, 21 = 11.34; P = 0.003; time, F, 3,44 = 40.51; P < 0.001; genotype × time interaction, F, 21 = 9.36; P < 0.001), day 1 (genotype, F, 21 = 7.46; P = 0.012; time, F, 21 = 5.15; P = 0.003), and day 2 (genotype, F, 21 = 6.50; P = 0.019). c, d, Forniceal DBS enhanced LTP in both wild-type and RTT mice (DBS-treated wild-type, n = 12; sham-treated wild-type, n = 11; DBS-treated RTT, n = 13; sham-treated RTT, n = 12). Two-way repeated-measures ANOVA revealed significant main effects of population spike amplitudes among the four groups on day 0 (group, F, 6,60 = 17.25, P < 0.001; time, F, 15,315 = 167.28, P < 0.001; group × time interaction, F, 6,60 = 14.50, P < 0.001), day 1 (group, F, 21 = 21.53, P < 0.001; time, F, 15,315 = 7.69, P < 0.001), day 2 (group, F, 21 = 16.21, P < 0.001; time, F, 15,315 = 8.96, P < 0.001), and day 5 (group, F, 21 = 8.42, P < 0.001; time, F, 15,315 = 8.35, P < 0.001). e, Forniceal DBS enhanced LTP in wild-type controls on day 0 (treatment, F, 21 = 12.16, P = 0.002; time, F, 3,132 = 121.93, P < 0.001; treatment × time interaction, F, 3,132 = 10.91, P < 0.001), day 1 (treatment, F, 21 = 18.99, P < 0.001; time, F, 3,132 = 4.77, P = 0.005), day 2 (treatment, F, 21 = 11.25, P = 0.003; time, F, 3,132 = 3.72, P = 0.016) and day 5 (treatment, F, 21 = 9.44, P = 0.006; time, F, 3,132 = 6.73, P = 0.001) (NS, not significant. Data presented as mean ± s.e.m.)
treatment, fornical DBS clearly enhanced contextual fear memory as well as spatial learning and memory in both wild-type and RTT mice. DBS in the fimbria–fornix is so effective in the RTT mice that it restores hippocampus-dependent memory in both tasks to wild-type levels. We also found that fornical DBS increases hippocampal synaptic plasticity and hippocampal neurogenesis, both of which are central to hippocampal learning and memory.23,29

Although this study was limited to hippocampus-based learning and memory, it is remarkable that DBS could exert any benefit in the face of such profound cognitive impairments as caused by RTT. Future work will explore additional DBS targets to determine the possible benefits of DBS on other RTT features such as dystonia and motor incoordination. Our studies lead us to suggest that DBS should be explored in other models of intellectual disabilities and eventually in human patients, particularly those conditions that cause more focal deficits in learning and memory. Intellectual disabilities as a group affect 2–3% of the population30 and are at present untreatable; their molecular heterogeneity poses a daunting challenge to those looking for viable therapies. The fact that DBS is able to be modulated, reversible, and safe makes it an appealing candidate treatment that could potentially relieve a great deal of suffering.

Online Content

Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions

J.T. and H.Y.Z. designed the experiments. S.H., B.T., Z.W., Y.S., H.T., Y.G., K.U. and J.T. performed the research. S.H., B.T., K.U., H.Y.Z. and J.T. analysed and interpreted the data. R.C.S., A.J.P. and D.J.C. provided comments on the manuscript. S.H., H.Y.Z. and J.T. wrote and edited the paper.

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**METHODS**

**Animals.** Adult female *Mecp2*−/− mice (14–16 weeks of age at the time of fear conditioning, water maze or LTP test) (Extended Data Fig. 1) on an FVB.129 background were maintained on a 12 h light:12 h dark cycle (light on at 7:00) with standard mouse chow and water *ad libitum* in our on-site AAALAS-accredited facility. They were group-housed up to five mice per cage before surgery and individually housed with nesting material in the cage after surgery in a room maintained at 22 °C. All the experimental procedures and tests were conducted during the light cycle. Behavioural, electrophysiological, immunohistochemical, and pharmacological characterizations of the mice were performed and analysed blind to genotypes and/or treatments. All research and animal care procedures were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee.

**Surgery and DBS.** Mice were secured on a stereotaxic frame (David Kopf) under 1–2% isoflurane anaesthesia. Bipolar DBS electrodes were constructed with Teflon-coated tungsten wire (bare diameter 50 μm, A-M Systems) and the two tips were horizontally separated by 0.1–0.15 mm. The electrodes were targeted unilaterally to the fimbria–fornix (0.2 mm posterior, 0.2 mm lateral, and 2.3–2.4 mm below the bregma) under the guidance of evoked potentials recorded in the ipsilateral dentate gyrus (1.8–2.0 mm posterior, 1.4–1.6 mm lateral of bregma, 2.2–2.3 mm below the skull)13. All the electrodes together with the attached connector sockets were fixed on the skull by dental cement. Animals were given at least 2 weeks to recover.

After recovery, mice were assigned into four groups (randomly to DBS or sham groups within the same genotype): wild-type, sham; DBS, sham; and RTT, DBS. Animals in both DBS groups received 1 h DBS daily for 14 consecutive days. Based on DBS parameters widely used in the clinic12 and the cognitive assessment of DBS in rodents13, the DBS was biphasic rectangular pulses (130 Hz, 60 μs pulse duration). This DBS pattern is used both in human subjects12,13 and rodents12. The stimulus intensities were individually optimized to 80% of the threshold that elicits an afterdischarge in the hippocampus14. Animals in the sham groups experienced the same procedures as those in the DBS groups except without DBS. There was a 3-week interval between the last DBS administration and the beginning of fear conditioning, water maze or in vivo LTP test5. All the mice received DBS/sham treatment and those used for fear conditioning and EEG recordings were habituated to the headstage/wiring system in an environment different from both conditioning chamber and the cue memory test cage for 3 days (20 min per day) immediately before these tests.

After finishing all experiments, mice were euthanized with an overdose of isoflurane. An anodal current (30 μA, 10 s) was passed through the electrode wire to verify the electrode placements. Frozen 30-μm coronal sections were cut and stained with cresyl violet.

**Behavioural tests.** Tests of light/dark chamber and open field were conducted 1 week before fear conditioning or water maze. Wire-hang, dowel-walk, rotarod and three-chamber tests, as well as a test of pain threshold, were performed 1–2 weeks after fear conditioning or water maze. (Extended Data Fig. 1) For each test, mice were given at least 30 min to habituate after transport to the behavioural testing room before any tests were conducted. The light intensity of 150±1 lux and the background white noise at 60 dB were presented during the habituation and throughout the testing periods.

**Fear conditioning.** A delayed fear conditioning protocol was employed to evaluate hippocampus-dependent contextual fear memory and hippocampal-independent cue fear memory. On day 0 animals were trained in a mouse fear conditioning chamber with a grid floor that could deliver an electric shock (Med Associates, Inc.). This enclosure was located in a sound-attenuating box that contained a digital camera, a loudspeaker and a house light. Each mouse was initially placed in the chamber and left undisturbed for 2 min, after which a tone (30s, 5 kHz, 80 dB) coincided with a scrambled foot shock (2 s, 0.7 mA). The tone/foot-shock stimuli were repeated after 1 min. After an additional 1 min, the mouse was removed and returned to its home cage. Fear memory retention was evaluated 3 h, 1 d, 3 days, and 7 days after training unless stated otherwise. At each time point, mice were first recorded for 3 min in the same chamber (cleaned with 70% ethanol) without tone. The mice were then tested in a novel cage (cleaned with 1% acetic acid) where a 3-min tone was presented after the animals had acclimated to the cage for 3 min. Mouse behaviour was recorded and scored automatically by ANY-maze (Stoelting). Freezing, defined as an absence of all movement except for respiration23, was scored only if the animal was immobile for at least 1 s. The percentage of time spent freezing during the tests serves as an index of fear memory. Cued fear memory was the subtraction of freezing time between the tone phase and the no-tone phase. Data are shown as mean ± s.e.m. and analysed by two-way ANOVA followed by Tukey’s post hoc analysis.

Morris water maze. The Morris water maze was used to assess spatial learning and memory in RTT mice and the effect of fornical DBS. This assay was performed as previously described with a few modifications16,19. The pool (120 cm in diameter) was filled with water (50 cm deep, 22–24 °C) made opaque with non-toxic white tempera paint. Visual cues were set on the wall of the testing room, at least 1 m from the pool edge. The ANY-maze tracking system (Stoelting) was used to track and analyse animal swimming. Mice were tested for their ability to find an escape platform (10 cm in diameter) on three different components of the test: visible platform acquisition, hidden platform acquisition, and subsequent probe trial in the absence of the platform. In each case, the criterion for learning is an average latency of 15 s to less than locate the platform across a block of four consecutive trials (15 s interval) per day. Mice are given up to 9 days to reach this criterion for learning. Twenty-four hours after the last training trial the mice were given a probe trial. During the probe trial, the platform was removed, and each animal was allowed 60 s to search the pool. The amount of time and distance that each animal spent in each quadrant was recorded. The number of times a subject crossed the imaging location of the platform during training (platform crossing) was determined. Data of memory acquisition are expressed as mean ± s.e.m. and analysed by two-way ANOVA with repeated-measures followed by Tukey’s post hoc analysis. Data of probe tests are shown as individual values and two-tailed t-test is used to compare the searching time in the target quadrants and the platform crossing numbers between groups, and two-tailed paired t-test for comparing the searching time in quadrants within groups.

**Open field.** The open field apparatus consists of a clear, open Plexiglas box (40 × 40 × 30 cm, Stoelting) with overhead camera and photo beams to record horizontal and vertical movements. Activity was quantified over a 30-min period by ANY-maze (Stoelting). Data are shown as mean ± s.e.m. and analysed by two-way ANOVA followed by Tukey’s post hoc analysis.

**Light–dark box.** The light–dark box assay was performed as published with few modifications38. The box consisted of a clear Plexiglas chamber (40 × 20 × 30 cm) with an open top separated from a covered black chamber (40 × 20 × 30 cm) by a black partition with a small opening (Stoelting). RTT and wild-type mice were placed into the illuminated side and allowed to explore freely for 10 min. An ANY-maze system with photo beam and overhead camera (Stoelting) was used to score the mice for the number and latency of entries and the time spent in each compartment. The mouse must place 50% of body length into either the light or dark compartment to be scored as an entry. Data are shown as mean ± s.e.m. and analysed by two-way ANOVA with Tukey’s post hoc analysis.

**Wire hang and dowel walk.** These assays were performed as previously published with a few modifications44. RTT and wild-type mice were tested for motor coordination. For the wire-hang test the mouse was held by the tail and allowed to grasp with its forepaws the middle of a single 3-mm plastic-coated wire suspended 15 inches above a plastic-covered foam pad and the total number of side touches and latency to fall were measured with a 120 s cut-off. Data are shown as mean ± s.e.m. and analysed using two-way ANOVA with Tukey’s post hoc analysis.

**Accelerating rotarod.** This assay was performed as previously published with a few modifications44. RTT and wild-type mice were placed on the rotating cylinder of an accelerating rotarod apparatus (Ugo Basile) and allowed to move freely as the rotation increased from 5 r.p.m. to 40 r.p.m. over a 5-min period. Latency to fall was recorded when the mouse fell from the rod or when the mouse had ridden the rotarod for two revolutions without regaining control. Data are shown as mean ± s.e.m. Latency to fall was analysed by two-way repeated-measures ANOVA with Tukey’s post hoc analysis.

**Three-chamber interaction.** The test was performed as previously described15,19. RTT and wild-type mice were used in this assay. For the habituation stage, test mice were placed in the middle chamber of the three-chamber apparatus (Ugo Basile) equipped with two empty, barred cages in the corners of the left and right chambers. They were allowed to explore freely for 10 min, with their movement tracked and recorded using the ANY-maze software pack (Stoelting), and interaction time with each cage scored by an investigator blind to genotype and treatment group. For the social versus object stage, an age- and size-matched C57BL/6 female mouse was placed in one cage and a Lego block of similar size was placed in the other cage. The test mouse was again placed in the middle chamber and allowed to explore freely for 10 min, with movement and interaction time recorded as before. Interaction time and time in each zone are shown as mean ± s.e.m. and analysed by two-way ANOVA with Tukey’s post hoc analysis.

**Pain threshold.** The test was performed as previously published with a few modifications44. At the end of the test battery, animals were placed into the conditioning chamber. Every 30 s, a 2-s scrambled electric foot shock with 0.05 mA increments (starting from 0 mA) was applied. The shock current thresholds of...
flinch, vocalization, and jumping were each recorded. Data are shown as mean ± s.e.m. and analysed by two-way ANOVA with Tukey’s post hoc analysis.

**Induction and recording of hippocampal synaptic plasticity in vivo.** To determine the effect of fornical DBS on hippocampal synaptic plasticity, an additional concentric stimulating electrode was implanted ipsilaterally in the medial perforant path (0.2 mm posterior and 2.8–3.0 mm lateral of lambda, 1.0–1.3 mm below the dura). The stimulating and recording electrodes were surgically implanted as previously described with the following modifications. The final depth of the electrodes was determined by electrophysiological guidance. A cortical silver ball, placed contralaterally, served as a recording reference as well as ground. Dental cement was used to anchor the electrode assembly that is connected to a unity gain preamplifier, and the connecting device for chronic recordings. After recovery from surgical implantation, mice were transported and habituated to the recording system during each of the 4 days before starting the LTP test. Signals were amplified (100×), filtered (bandpass, 0.1–5 kHz), digitized at 10 kHz, and stored on disk for off-line analysis (pClamp 10 and 1440A; Molecular Devices). To evaluate whether fornical DBS influence the input-output (I/O) relation in the perforant path–dentate pathway, I/O curves were generated for each mouse 1 day before and 3 weeks after the DBS/sham treatment. For LTP evaluation, test responses elicited by 0.033 Hz monophasic pulses (0.1 ms duration) were recorded for 20-min periods on consecutive days at an intensity that evoked 40% of the maximal population spike. Following 20 min of stable baseline, a tetanus was delivered to the perforant path for LTP induction. Pulse width was doubled during tetanization, which consisted of six series of six stimuli at 400 Hz, 200 ms between trains, 20 s between series. Responses were measured for 60 min after tetanization and again for 20 min at 24 h, 48 h and 120 h after tetanization. Since the latency of the population spike usually decreases following LTP induction, it is impractical to compare the initial slope of the IEPSP (field excitatory postsynaptic potential) before and after LTP induction in awake animals. Accordingly, we quantified the amplitude of the population spikes. Data were averaged every 5 min and normalized to the baseline measured over the 10 min before tetanic stimulation and presented as mean ± s.e.m. Two-way repeated-measures ANOVA was used for data analysis.

**Recordings of hippocampal local field potentials (LFPs) and data analysis.** The recording electrode of LFPs was targeted to the upper molecular layer of the dentate gyrus with the reference electrode in the corpus callosum. Recording of LFPs was conducted under matched behavioural states for RTT and wild-type mice. Signals were amplified (100×), filtered (bandpass, 0.1–5 kHz), digitized at 2 kHz, and stored on disk for off-line analysis (pClamp 10 and 1440A; Molecular Devices). The power spectrum of the LFPs was calculated at 0.244 Hz resolution, using the built-in function of pClamp 10. Then the relative power of hippocampal theta activity was normalized as the ratio between the power of the theta signal at 0.033 Hz and the power of the baseline measured over the 10 min before tetanic stimulation and presented as mean ± s.e.m. Two-way repeated-measures ANOVA was used for data analysis. Statistical analyses. Sample sizes of mice were determined based on prior data and statistical power to detect the LTP mice. Animals with disconnected electrode implants before the completion of the experiments were excluded from data analyses. Data were analysed using two-way repeated-measures ANOVA. If any of the main effects were significant, Tukey’s post hoc analysis was used for all pairwise multiple comparisons unless otherwise specified. In all cases, P < 0.05 was set as the cut-off for statistical significance. SigmaPlot 12 was used to create all the summarized plots as well as all the statistical tests in this study.

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Extended Data Figure 1 | Timeline of fornical DBS tests in RTT and wild-type mice.
Extended Data Figure 2 | Fear memory in RTT mice and wild-type control animals. All mice were trained with tone–foot-shock pairings on day 0. Memory retention was tested 3 h, 1 day, 3 day, and 7 day after training. 

a, b, Impaired fear memory in RTT mice (n = 20) compared to wild-type (WT) littermates (n = 20). These animals were implanted with electrodes but did not receive DBS or sham treatment. A significant main effect of genotype was observed (two-way repeated-measures ANOVA followed by Tukey’s post hoc test: context, F1,38 = 15.32, P < 0.001; cue, F1,38 = 20.70, P < 0.001). *P < 0.05; **P < 0.01; ***P < 0.001 versus wild type. 

c, d, Cued fear memory in RTT mice (n = 20) and wild-type littermates (n = 20) that were implanted with electrodes but without DBS or sham treatment. During the retention test, freezing in the tone phase (T) was significantly more than in the no tone phase (NT) across all the test time points in both wild-type (c) and RTT mice (d). 

e–h, Retrieval of cue fear memory in DBS- or sham-treated RTT and wild-type mice. During the cued memory test, all four groups of animals actively responded to the tone presentation (WT-sham, n = 21; WT-DBS, n = 21; RTT-sham, n = 14; RTT-DBS, n = 17). There was a significant increase of freezing time in the tone phase (T) compared to the no-tone phase (NT) at each of the test time points over all the groups, *P < 0.05, **P < 0.01, ***P < 0.001 (two-tailed paired t-test). All data are presented as mean ± s.e.m.
Extended Data Figure 3 | Increased handling, but not fornical DBS, increased locomotor activity and decreased the anxiety level in RTT and wild-type mice. 

**a**, There was no difference among the four DBS/sham-treated groups in the total distance travelled in the open-field test (WT-sham, n = 20; WT-DBS, n = 20; RTT-sham, n = 17; RTT-DBS, n = 18; genotype, F_{1,71} = 1.13, P = 0.292; treatment, F_{1,71} = 0.13, P = 0.724; genotype × treatment, F_{1,71} = 0.063, P = 0.803). RTT and wild-type mice that received DBS/sham treatment travelled longer distances than RTT (n = 20) and wild-type (n = 20) animals that were implanted with electrodes but did not experience DBS/sham procedures, respectively. **b**, During the open-field test, there was no difference in the centerto total distance ratio among the four DBS groups (genotype, F_{1,71} = 1.22, P = 0.273; treatment, F_{1,71} = 0.0079, P = 0.93; genotype × treatment, F_{1,71} = 0.081, P = 0.777). Both RTT and wild-type mice that received DBS/sham treatment travelled more in the centre area compared to implanted RTT and wild-type animals that did not receive DBS/sham procedures. **c**, In the light/dark test there was no difference in the amount of time spent in the light compartment among the four chronically treated groups (n = 12 per group; two-way ANOVA: genotype, F_{1,44} = 1.83, P = 0.183; treatment, F_{1,44} = 0.057, P = 0.813; genotype × treatment, F_{1,44} = 0.33, P = 0.567). Both RTT and wild-type mice that received DBS/sham treatment spent more time in the light compartment than implanted RTT (n = 15) and wild-type (n = 14) animals that did not receive DBS/sham procedures. *P < 0.05, **P < 0.01, ***P < 0.001 (two-tailed t-test). All data are presented as mean ± s.e.m.
Extended Data Figure 4 | Forniceal DBS did not alter the pain threshold, motor function or social behaviour in RTT or wild-type mice. a, There was no group difference in foot shock threshold intensities to evoke flinch, vocalization or jumping (WT-sham, n = 14; WT-DBS, n = 14; RTT-sham, n = 11; RTT-DBS, n = 12; two-way ANOVA, no significant main effect of genotype, treatment, or genotype × treatment interaction, \( P > 0.05 \)). b, In a rotarod test (n = 12 mice per group), latency to fall increased over trials but there was no difference among the four groups (two-way repeated measures ANOVA: group, \( F_{3,44} = 1.68, P = 0.184 \); trial, \( F_{7,308} = 34.26, P < 0.001 \); group × trial interaction, \( F_{21,308} = 1.22, P = 0.230 \)). c, RTT mice showed decreased latency to fall in the wire-hang test compared to wild-type animals, but there was no difference between DBS- and sham-treated groups for either RTT or wild-type mice (n = 12 per group; two-way ANOVA: genotype, \( F_{1,44} = 10.41, P = 0.002 \); treatment, \( F_{1,44} = 0.33, P = 0.566 \); genotype × treatment interaction, \( F_{1,44} = 0.75, P = 0.392 \)). d, RTT mice showed a decreased latency to fall in the dowel test compared to wild-type animals, but there was no difference between DBS- and sham-treated groups for either genotype (n = 12 per group; genotype, \( F_{1,44} = 23.63, P < 0.001 \); treatment, \( F_{1,44} = 0.0018, P = 0.966 \); genotype × treatment interaction, \( F_{1,44} = 0.83, P = 0.367 \)). e, f, In the three chamber test, all four groups of animals (n = 12 per group) showed a clear preference for the partner mice compared to the object (e). Two-way ANOVA revealed a significant genotype main effect of the interaction time with the partner mice (\( F_{1,44} = 4.56, P = 0.038 \)), indicating altered social behaviour in RTT mice (\( P = 0.063, \) RTT-sham versus WT-sham, Tukey’s post hoc). However, DBS did not change the interaction time with the partners (treatment, \( F_{1,44} = 0.28, P = 0.597 \); genotype × treatment interaction, \( F_{1,44} = 0.31, P = 0.579 \)) or the object (treatment, \( F_{1,44} = 2.64, P = 0.111 \); genotype × treatment interaction, \( F_{1,44} = 0.015, P = 0.905 \)) (f). **\( P < 0.01 \), ***\( P < 0.001 \) (Tukey’s post hoc in e). All data are presented as mean ± s.e.m.
Extended Data Figure 5 | Forniceal DBS did not alter the body weight, visual or sensorimotor skills in RTT or wild-type mice. a, All four groups (n = 12 mice per group) showed changes in body weight over time. Two-way repeated measure ANOVA revealed a significant main effect of group (F$_{3,44}$ = 6.73, P < 0.001) and age (F$_{4,176}$ = 89.32, P < 0.001). Tukey’s post hoc showed that sham-treated RTT mice were significantly heavier than sham-treated wild-type mice (P = 0.015), but there was no difference in body weight between sham-treated and DBS-treated wild-type mice (P = 0.861) or between sham-treated and DBS-treated RTT mice (P = 0.099). b, Comparison of body weight at the age of 23 weeks among the four groups (two-way ANOVA: genotype, F$_{1,44}$ = 10.06, P = 0.003; treatment: F$_{1,44}$ = 1.93, P = 0.172). c–e, Swimming test in the water maze task with a flagged platform (n = 18 mice per group). Sham-treated RTT mice did not have different escape latencies than sham-treated wild-type controls (c, two-way repeated-measures ANOVA: genotype, F$_{1,34}$ = 1.73, P = 0.197; genotype × treatment interaction, F$_{1,34}$ = 0.133, P = 0.718). DBS did not change the escape latencies in either wild-type controls (d, treatment, F$_{1,34}$ = 0.44, P = 0.513; treatment × day interaction, F$_{1,34}$ = 1.24, P = 0.273) or RTT mice (e, treatment, F$_{1,34}$ = 2.36, P = 0.134; treatment × day interaction, F$_{1,34}$ = 0.41, P = 0.524). *P < 0.05; n.s., not significant (Tukey’s post hoc). All data are presented as mean ± s.e.m.
Extended Data Figure 6 | Effect of fornical DBS on hippocampal electrophysiological signatures. a, Representative traces of LFPs recorded in the dentate gyrus 1 day before and 3 weeks after DBS/sham treatment. There were no electrographic seizure spikes in any of the four groups of mice after DBS/sham treatment. Scale bars: 10 s, 1 mV. b, Input–output (I/O) curves of the evoked responses of the perforant path recorded in the dentate gyrus in DBS/sham-treated mice. For each of the four groups, I/O curves were generated 1 day before and 3 weeks after fornical DBS. All data points were normalized to the maximum value of the population spike amplitude before DBS/sham and the abscissa represents the seven increments used in each mouse. The I/O relationship was not altered by DBS in sham-treated wild-type mice (WT-sham; n = 5, F_{1,4} = 0.062, P = 0.818), DBS-treated wild-type mice (WT-DBS; n = 4, F_{1,3} = 0.036, P = 0.861), or sham-treated RTT mice (RTT-sham; n = 5, F_{1,4} = 0.018, P = 0.901). DBS reduced the amplitude of the evoked population spikes from the baseline test in DBS-treated RTT mice (RTT-DBS; n = 5, F_{1,4} = 6.73, P = 0.060). *P < 0.05 (Tukey’s post hoc). All data are presented as mean ± s.e.m.
Extended Data Figure 7 | Unilateral fornical DBS induces neuronal activity and stimulates neurogenesis bilaterally in the dentate gyrus.

a. Representative images showing that expression of the Fos gene was increased following fornical DBS in wild-type and RTT mice compared to their sham controls, respectively (percentage of ipsilateral c-Fos-positive cells over the dentate granule cells: WT-sham, 0.26 ± 0.04%; WT-DBS, 34.52 ± 4.62%; RTT-sham, 0.30 ± 0.05%; RTT-DBS, 32.55 ± 3.74%).

b. Representative images showing that there were more BrdU⁺ (green), DCX⁺ (red), and merged (yellow) cells in the dentate gyrus in fornical DBS-treated wild-type and RTT mice than in their respective sham controls. Scale bar, 100 μm. Con, contralateral; Ips, ipsilateral.
Extended Data Figure 8 | The cholinergic antagonist atropine did not alter fornical DBS-induced enhancement of fear memory. a, Placement of guide cannula and recording electrode into the dorsal hippocampus.
b, Hippocampal infusion of 1.0 μg atropine did not change the amplitudes of the evoked potentials of the FFx recorded in the dentate gyrus in both RTT and wild-type mice. There was no difference of the population spike amplitudes before or after atropine infusion in both RTT mice (n = 5; one-way ANOVA, F(9,36) = 0.69, P = 0.715) and wild-type controls (n = 3; F(9,24) = 0.99, P = 0.485).
c, Representative hippocampal EEG traces before and after vehicle (V) or atropine (A) infusion. Scale bars: 0.5 s, 0.2 mV.
d, RTT mice (n = 17) showed less spontaneous hippocampal theta activity than wild-type animals (n = 20) (**P < 0.01, two-tailed t-test). e, Hippocampal infusion of atropine, but not vehicle, reduced hippocampal theta oscillation in both RTT and wild-type mice compared to their pre-infusion baselines (WT-V, n = 9; WT-A, n = 11; RTT-V, n = 8; RTT-A, n = 9; *P < 0.05, two-tailed paired t-test; n.s., not significant).
f, Hippocampal microinfusion of atropine before fear conditioning training did not alter fear memory in fornical DBS treated RTT mice or wild-type controls. Mice in all four groups (WT-V, n = 10; WT-A, n = 11; RTT-V, n = 12; RTT-A, n = 13) experienced 2 weeks of fornical DBS that was finished 3 weeks before fear conditioning training. Atropine or vehicle was bilaterally infused into the dorsal hippocampus before training. Memory retention was tested 24 h after training. Two-way ANOVA revealed a significant main effect of genotype (F(1,42) = 10.27, P = 0.003), but there was no difference between atropine- and vehicle-treated mice (treatment, F(1,42) = 0.34, P = 0.562; genotype × treatment interaction, F(1,42) = 0.069, P = 0.794). Atropine did not change cued fear memory, either: two-way ANOVA revealed no difference between genotypes (F(1,42) = 2.99, P = 0.091) or between atropine- and vehicle-treated mice (treatment, F(1,42) = 0.046, P = 0.831; genotype × treatment interaction, F(1,42) = 0.154, P = 0.697).
*P < 0.05; n.s., not significant (Tukey's post hoc). g, Intra-hippocampal atropine infusion alone did not change the basal level of freezing in the contextual test environment in either wild-type or RTT mice. There was no difference between vehicle- (n = 9) or atropine-treated (n = 6) mice (P > 0.05, two-tailed t-test).

h, Schematic representation of the dorsal hippocampus at seven rostral-caudal planes (according to ref. 31) for the microinfusion sites in DBS-treatment experiments. The numbers on the left represent the posterior coordinate from the bregma. All data are presented as mean ± s.e.m.