Published in final edited form as:

Nature. 2015 October 15; 526(7573): 430–434. doi:10.1038/nature15694.

Forniceal deep brain stimulation rescues hippocampal memory in Rett syndrome mice

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Abstract

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 amnestic rats have demonstrated that DBS targeted to the fimbria-fornix[1-3], the region that appears to regulate hippocampal activity, can mitigate defects in hippocampus-dependent memory[3-5]. Despite these promising results, DBS has not been tested for its ability to improve cognition in any childhood intellectual disability disorder (IDD). IDDs are a pressing concern: they affect as much as 3% of the population and involve hundreds of different genes. We hypothesized 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 forniceal 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

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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. analyzed and interpreted the data. R.C.S., A.J.P., D.J.C. provided comments to the manuscript. S.H., H.Y.Z. and J.T. wrote and edited the paper.

The authors declare no competing financial interests.

function of MeCP2[6], RTT appears by the second year of life, causing profound impairment in cognitive, motor, and social skills along with an array of neurological features[7]; RTT mice, which reproduce the broad phenotype of this disorder, also show clear deficits in hippocampus-dependent learning and memory and hippocampal synaptic plasticity[8-11]. Here we show that forniceal DBS in RTT mice rescued contextual fear memory as well as spatial learning and memory. In parallel, forniceal DBS restored *in vivo* hippocampal long-term potentiation (LTP) and hippocampal neurogenesis. These results indicate that forniceal 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[9-11]. Specifically, female *Mecp2*$^{+/-}$ mice (hereafter referred to as RTT mice) have impaired contextual fear memory when tested 24 h after training[11]. Because this deficit is readily quantifiable and accessible, we used fear memory as our first test of the effect of forniceal DBS in freely-moving RTT mice. We implanted DBS electrodes in the fimbria-fornix (FFx) of 6- to 8-week-old RTT mice and WT controls (Extended Data Fig. 1), guiding electrode placement with FFx-evoked potentials in the dentate gyrus (DG) (Fig. 1a-c). We divided mice into 4 groups after recovery: WT-sham, WT-DBS, RTT-sham, and RTT-DBS. Mice in both DBS groups received daily DBS treatment for 14 days while the 2 sham groups experienced the same procedures except for DBS. Based on widely used parameters for DBS in the clinic[12] and in rodents[13] 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 elicits an after-discharge in the hippocampus[3,14]. No seizures appeared under these DBS parameters. Three weeks after completing the two-week DBS protocol, we performed behavioral 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).

Forniceal DBS significantly enhanced contextual fear memory in both WT (3h, d1 and d3, $P < 0.05$) and RTT mice (3h and d1, $P < 0.05$; Fig. 1d). In fact, DBS restored contextual fear memory in RTT mice to WT levels: there was no difference between the DBS-treated RTT mice (RTT-DBS, 3h: 47.56 ± 4.22%; d1: 47.84 ± 4.16%) and sham-treated WT mice (WT-sham, 3h: 44.87 ± 3.60%; d1: 45.97 ± 3.69%). Interestingly, forniceal DBS did not alter cued fear memory (Fig. 1e), even though the FFx also projects to the amygdala[15]. All the mice that received DBS/sham treatment responded to the tone presentation (Extended Data Fig. 2e-h), but less than the animals that were implanted and did not experience the two-week DBS/sham procedures (Extended Data Fig. 2b-d). Further analysis indicated that the longer period of handling and exposure (e.g., 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).

Forniceal DBS did not improve levels of locomotion, anxiety, pain threshold or motor learning (Extended Data Fig. 3, 4a, b) as well as motor coordination, social behavior, and body weight in RTT mice although there were differences between RTT mice and WT

controls in these features (Extended Data Fig. 4c-d, 5a, b). Forniceal DBS thus specifically rescued contextual memory impairment in RTT mice without evident off-target effects.

To determine whether forniceal 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[16] (Extended Data Fig. 1). RTT-sham mice needed more time than WT-sham 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 WT mice, DBS significantly enhanced spatial learning compared to the sham group (Fig. 2b). Treatment made no difference during the probe test, likely because of a ceiling effect in WT-sham animals. We observed an even stronger effect of DBS in RTT mice: forniceal 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 RTT-DBS and WT-sham treated 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 forniceal DBS altered visual or sensorimotor skills (Extended Data Fig. 5c-e).

Since the RTT mice used in this study are impaired in both hippocampus-dependent memory[11] and *in vitro* hippocampal long-term plasticity (LTP)[8], they provide an ideal setting in which to examine whether DBS alters synaptic plasticity. We implanted RTT and WT mice with DBS electrodes in the FFx, stimulation electrodes in the perforant path for the LTP test, and a recording electrode in the DG (using evoked potentials as a guide). As with the behavioral studies described above, the mice underwent two weeks of DBS followed by a three-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 five days after induction in awake, freely-moving mice. We quantified the population spike amplitude in each of the 4 groups of animals[17] (Fig. 3a). Neither DBS nor sham treatment altered baseline synaptic transmission in WT-sham, WT-DBS, or RTT-sham mice, but DBS slightly reduced the magnitude of the evoked responses in RTT-DBS animals (Extended Data Fig. 6b). There was no difference in the stimulus intensities used for LTP induction among the 4 groups (WT-sham, 83.91 ± 13.29 μA; WT-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, RTT-sham mice showed impaired *in vivo* LTP (1 h after induction: 160.37 ± 14.08%) compared to WT-sham controls (256.06 ± 27.50%) (Fig. 3b). Forniceal DBS, however, enhanced LTP in both WT mice (Fig. 3c; DBS: 381.75 ± 26.13%; sham: 256.06 ± 27.50%) and RTT animals (Fig. 3d; DBS: 254.93 ± 22.96%; sham: 160.37 ± 14.08%), to the degree that there was no difference between RTT-DBS and WT-sham groups (Fig. 3e). Forniceal DBS thus restored hippocampal LTP in the perforant path/dentate pathway in RTT mice.

Because hippocampal neurogenesis contributes to hippocampal LTP and hippocampus-dependent memory[18-20], and because DBS in other afferent pathways of the hippocampus increases dentate neurogenesis[21-23], we explored whether forniceal DBS might exert its effects by stimulating hippocampal neurogenesis. We first observed that two-hour unilateral forniceal DBS stimulated the activity of dentate neurons, as indicated by increased

expression of the immediate early gene *c*-fos (Extended Data Fig. 7a). Each day after DBS over the two weeks of DBS/sham treatment, we injected RTT and WT 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 labeled in each of the 4 groups (Fig. 4a-d). We found that baseline levels of dentate neurogenesis in RTT-sham mice (BrdU, 571 ± 99.84; DCX, 914 ± 242.76) were significantly lower than in WT-sham controls (BrdU, 1218 ± 175.57; DCX, 2059 ± 381.49) (Fig. 4e, f). Forniceal DBS, however, bilaterally enhanced dentate neurogenesis in both RTT (RTT-DBS: BrdU, 2012 ± 269.24; DCX, 3146 ± 340.04; BrdU/DCX, 1453 ± 187.69) and WT mice (WT-DBS: BrdU, 1833 ± 274.79; DCX, 3686 ± 426.92, BrdU/DCX, 1526 ± 257.46) (Fig. 4e-g; Extended Data Fig. 7b) such that levels of neurogenesis in RTT-DBS mice were even higher than those in WT-sham controls.

Data suggest that decreased cholinergic signaling plays a role in RTT[24] and that forniceal stimulation enhances hippocampal memory 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 WT mice suggesting that DBS must benefit memory via additional mechanisms (Extended Data Fig. 8).

DBS has been used to treat both motor and neuropsychiatric disorders in children[25]. There are a few case reports showing that pallidal DBS can resolve self-injurious behavior in Lesch-Nyhan syndrome[26,27], and hypothalamic DBS reduces aggressive behaviors in patients with intellectual disability[28]. To our knowledge, however, DBS has never been examined for cognitive benefits in the context of a childhood intellectual disability. In this study, we demonstrate that DBS improves contextual memory retrieval in a mouse model of Rett syndrome. Using a stimulation protocol that mimics clinical treatment, forniceal DBS clearly enhanced contextual fear memory as well as spatial learning and memory in both WT 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 WT levels. We also found that forniceal 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 population[30] and are at present untreatable; their molecular heterogeneity poses a daunting challenge to those looking for viable therapies. The fact that DBS is modulatable, reversible, and safe makes it an appealing candidate treatment that could potentially relieve a great deal of suffering.

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 7:00am) with standard mouse chow and water *ad libitum* in our onsite AAALAS-accredited facility. They were group-housed up to 5 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. Behavioral, electrophysiological, immunohistochemical, and pharmacological characterizations of the mice were performed and analyzed 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 anesthesia. 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[31]). 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: WT-sham, WT-DBS, RTT-sham, and RTT-DBS (randomly to DBS or sham groups within the same genotype). Animals in both DBS groups received 1 h DBS daily for 14 consecutive days. Based on DBS parameters widely used in the clinic[12] and the cognitive assessment of DBS in rodents[13], the DBS was biphasic rectangular pulses (130 Hz, 60 μs pulse duration). This DBS pattern is used both in human subjects[2,12,32] and rodents[13]. The stimulus intensities were individually optimized to 80% of the threshold that elicits an after discharge in the hippocampus[3,14]. 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 tests[23]. All the mice received DBS/sham treatment and 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.

Behavioral tests

Tests of Light/dark chamber and open field were conducted one week before fear conditioning or water maze. Tests of wire and dowel, rotarod, 3-chamber, and 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 behavioral testing room before any tests were conducted. The light intensity of 150 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 hippocampus-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 (30 s, 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 3h, d1, d3, and d7 after training if not 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 behavior was recorded and scored automatically by ANY-maze (Stoelting). Freezing, defined as an absence of all movement except for respiration[33], was scored only if the animal was immobile for at least 1 s[34]. 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 ± standard error of mean and analyzed 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 forniceal DBS. This assay was performed as previously described with a few modifications[35,36]. 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 meter from the pool edge. The ANY-maze tracking system (Stoelting) was used to track and analyze 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 or less to locate the platform across a block of 4 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 are shown as mean ± standard error of mean and analyzed by two-way ANOVA with repeated measures (training) or one-way ANOVA with repeated measures (probe) followed by Tukey's *post hoc* analysis. Student's two-tailed *t*-test

is used to compare the searching time in the target quadrants and the platform crossing numbers during the probe test.

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 ± standard error of mean and analyzed 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 modifications[37]. 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 WT 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 lehgth into either the light or dark compartment to be scored as an entry. Data are shown as mean ± standard error of mean and analyzed 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 modifications[38]. RTT and WT 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 released. For the dowel test the mouse was placed onto a 0.5-inch wooden dowel 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 cutoff. Data are shown as mean ± standard error of mean and analyzed using two-way ANOVA with Tukey's *post hoc* analysis.

Accelerating rotarod—This assay was performed as previously published with a few modifications[38]. RTT and WT 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 rpm to 40 rpm over a five-minute period. Latency to fall was recorded when the mouse fell from the rod or when the mouse had ridden the rotating rod for two revolutions without regaining control. Data are shown as mean ± standard error of mean. Latency to fall was analyzed by two-way repeated measures ANOVA with Tukey's *post hoc* analysis.

3-Chamber interaction—The test was performed as previously described[35,39]. RTT and WT 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 ten minutes, 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 vs. 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 ten minutes, with movement and interaction time recorded as before. Interaction

time and time in each zone are shown as mean ± standard error of mean and analyzed by two-way ANOVA with Tukey's *post hoc* analysis.

Pain threshold—The test was performed as previously published with a few modifications[40]. 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 increment (starting from 0 mA) was applied. The shock current thresholds of flinch, vocalization, and jumping were each recorded. Data are shown as mean ± standard error of mean and analyzed by two-way ANOVA followed with Tukey's *post hoc* analysis.

Induction and recording of hippocampal synaptic plasticity *in vivo*

To determine the effect of forniceal 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[41,42] 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 electrodes, 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 prior to starting the LTP test. Signals were amplified (100x), filtered (bandpass, 0.1-5 kHz), digitized at 10 kHz, and stored on disk for off-line analysis (pClamp10 and 1440A; Molecular Devices). To evaluate whether forniceal DBS influence the input-output (I/O) relation in the performant path-dentate pathway, I/O curves were generated for each mouse one day before and 3 weeks after the DBS/sham treatment. For LTP evaluation, test responses elicited by 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 two days of stable baseline, a tetanus was delivered to the perforant path for LTP induction. Pulse width was doubled during tetani, which consisted of 6 series of 6 trains of 6 stimuli at 400 Hz, 200 ms between trains, 20 s between series. Responses were measured for 60 min after tetanus and again, for 20 min at 24 h, 48 h, and 120 h after tetanus. Since the latency of the population spike usually decreases following LTP induction, it is impractical to compare the initial slope of the fEPSP (field excitatory postsynaptic potential) before and after LTP induction in awake animals[17,43]. Accordingly, we quantified the amplitude of the population spikes[42]. Data were averaged every 5 min and normalized to the baseline measured over the 10 min before tetanic stimulation and presented as mean ± standard error of mean. 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 behavioral states for RTT and WT mice. Signals were amplified (100x), filtered (bandpass, 0.1-5 kHz), digitized at 2 kHz, and stored on disk for off-line analysis (pClamp10 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 4.15 - 11.96 Hz and the power of the signal at 0-100 Hz[44,45]. Large-movement or electrical artifacts trials of the LFP recordings were excluded from further analysis[3].

Intracranial drug infusion and histology

Under isoflurane anesthesia, custom-made 26G guide cannulas were implanted bilaterally (1.8 mm posterior, 1.2 mm lateral, and 1.2 mm below the bregma) in RTT mice and littermate WT controls. Other than the DBS electrode, a recording electrode (Teflon-coated tungsten wire, 50 μm bare diameter) was unilaterally attached to the guide cannula for the recordings of evoked potential during surgical implantation and LFPs in the dentate. After 2 weeks of recovery, mice were randomly assigned into drug or vehicle-treated groups. Atropine sulfate (Sigma, St. Louis, MO) was dissolved in PBS (pH 7.4) and the solution was back-filled into a 33G injector. The solution of 0.5 μl drug (1.0 μg atropine)[46,47] or PBS vehicle per side was microinfused over 1 min into the dorsal hippocampus (dHP) through a pump (Harvard Apparatus)[42]. The tip of the injector was 1.0 mm below the guide tip and ~0.6 mm from the tip of the recording electrode. Following each injection, the injector was left in place for an additional minute to allow drug diffusion. To determine the effect of atropine on hippocampal potentials evoked by FFx stimulation, atropine was infused into the dHP after 20 min of baseline test (0.033 Hz) and the responses were followed up for another 30 min in freely-moving mice. To determine the effect of atropine on hippocampal theta activity, dentate LFPs were recorded for 5 min immediately before and another 5 min after atropine/vehicle infusion (10-15 min after the infusion). To evaluate the influence of atropine on DBS effect, RTT and WT mice receiving forniceal DBS were infused with atropine or vehicle 15 ± 2 min before fear conditioning training. Then fear memory was tested 24 h after training.

At the end of the experiment, 4% methylene blue in PBS (0.2 μl) was injected to each injection site. Mice were euthanized with an overdose of isoflurane. An anodal current (30 μA, 10 s) was passed through the electrode wire to verify electrode placements. Frozen 30 μm coronal sections were cut and stained with hematoxylin. Mice with blocked guide cannula(s) or with injection cite outside of the dorsal hippocampus were excluded from data analysis.

Immunohistochemistry

To check whether forniceal DBS increase the activity of dentate neurons, we assesssed expression of the *c*-fos gene 60 min after 2 h of DBS or sham treatment. To evaluate whether DBS increases neurogenesis in the dentate gyrus, mice were injected with 5-Bromo-2′-deoxyuridine (BrdU, Sigma-Aldrich, 75 mg/kg, i.p.) immediately after daily DBS or sham treatment for 12 days from the third day of the 2 weeks of DBS/sham treatment.

Immunohistochemical staining—Mice were deeply anesthetized with isoflurane and subsequently perfused with 50 ml 0.1 M Phosphate-buffered saline (PBS, PH 7.4) followed by 60 ml cold 4% paraformaldehyde in 0.1 M PBS. Brains were removed, post fixed with

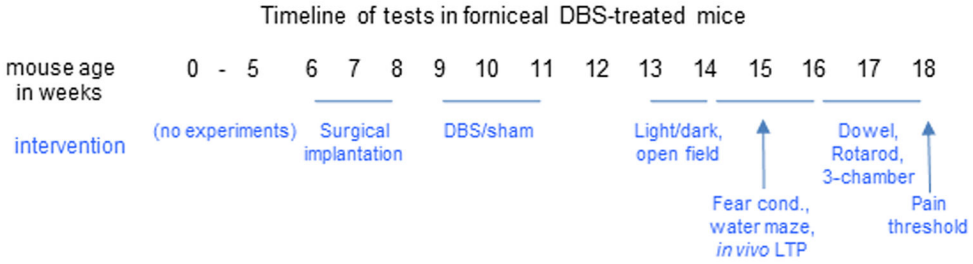
same fixative for 12 h, and then transferred to 30% sucrose at 4 °C. One-in-six of the floating serial 50 μm coronal sections throughout the hippocampus were processed for *c*-fos, DCX, and BrdU immunohistochemistry, respectively. After three rinses with 0.1 M PBS, the sections were incubated in blocking solution (0.3% Triton X-100, 5% normal goat serum in 0.1 M PBS) for 1 h at room temperature followed by 48 h incubation with primary antibody at 4 °C. Primary antibodies and their final concentrations were as follows: rabbit polyclonal anti-c-Fos (1: 10000, PC38 Millipore, USA), rabbit anti-Doublecortin (DCX) (1:400, Cell Signaling Technology, USA) and rat monoclonal anti-Bromodexyuridine (BrdU) (1:140, OBT0030, Accurte Chemical & Scientific Corporation, USA). Sections were incubated with Alex fluor 568 goat anti-rabbit IgG (1:500, A11036, Invitrogen, USA) and/or Alex fluor 488 goat anti-rat IgG (1:500, A11006,Invitrogen,USA) for 2 h and counterstained with DAPI (900 nM, D1036, Invitrogen, USA) for 15 min at room temperature in darkness. The sections were washed 3 times with 0.1 M PBS and mounted using Fluoromount-G (Southern Biotech, USA). For *c*-fos staining, sections were pretreated with 0.3% H_2O_2 in PBS for 30 min at room temperature. For BrdU detection, sections were pretreated with 2 N HCl for 30 min at 37 °C and washed in 0.1 M borate buffer, pH 8.4, for 10 min.

Imaging and quantification—Z stacks of 2 μm thick single-plane images at 20 x magnifications were collected through the entire thickness of the slice by employing a laser scanning microscope LSM 710 (Carl Zeiss). Each slice has 12-13 z-axis optical slices; the 6^{th} optical imaging was selected for counting c-fos-, BrdU- and DCX-positive cells. Digital images were routed into a Windows PC for quantitative analyses using ImageJ software (NIH). Double labeling for BrdU- and DCX-positive cells was assessed through the entire *z*-axis of each cell. For quantification, six sections per mouse brain were counted. Resulting cell numbers were multiplied by 6 to obtain the estimated total number of positive cells per DG.

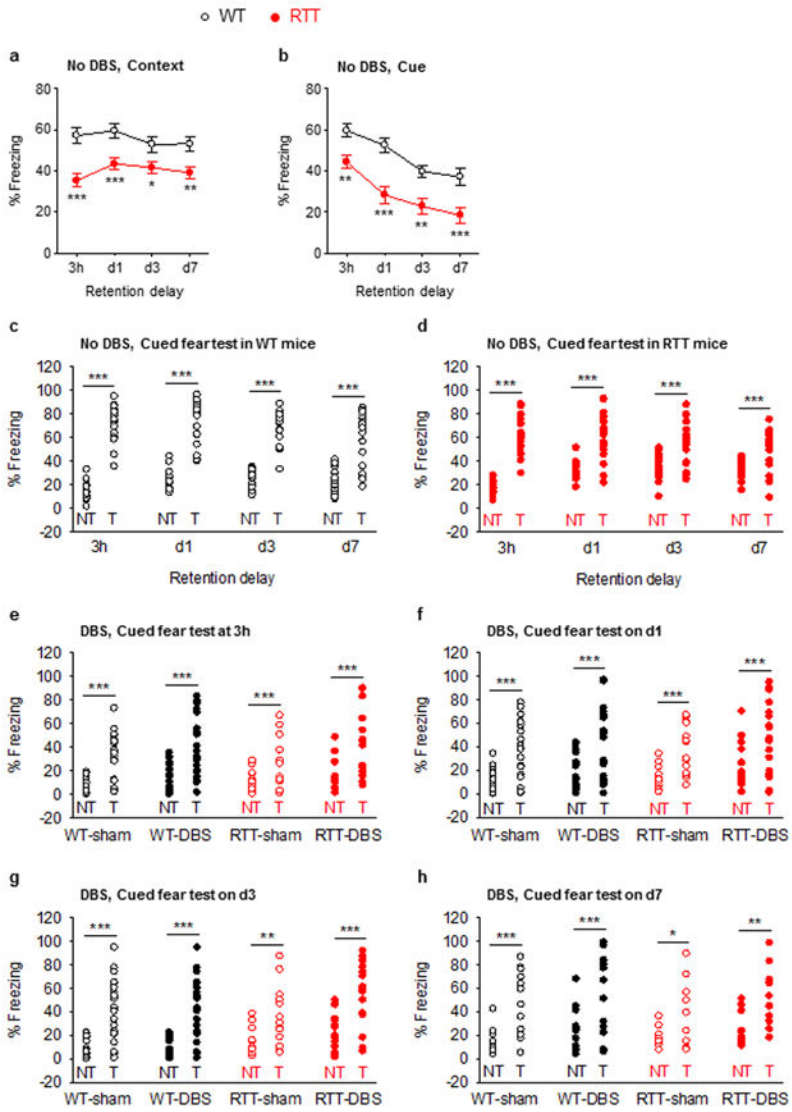
Statistical analyses

Sample sizes of mice were chosen to mitigate genetic background variance. Animals with disconnected electrode implants before the completion of the experiments were excluded from data analyses. Data were analyzed using two-way repeated measures analysis of variance (ANOVA). If any of the main effects were significant, Tukey *post hoc* was used for all pairwise multiple comparisons unless otherwise specified. In all cases, $P < 0.05$ was set as the cutoff for statistical significance. SigmaPlot 12 was used to create all the summarized plots as well as all the statistic tests in this study.

Extended Data

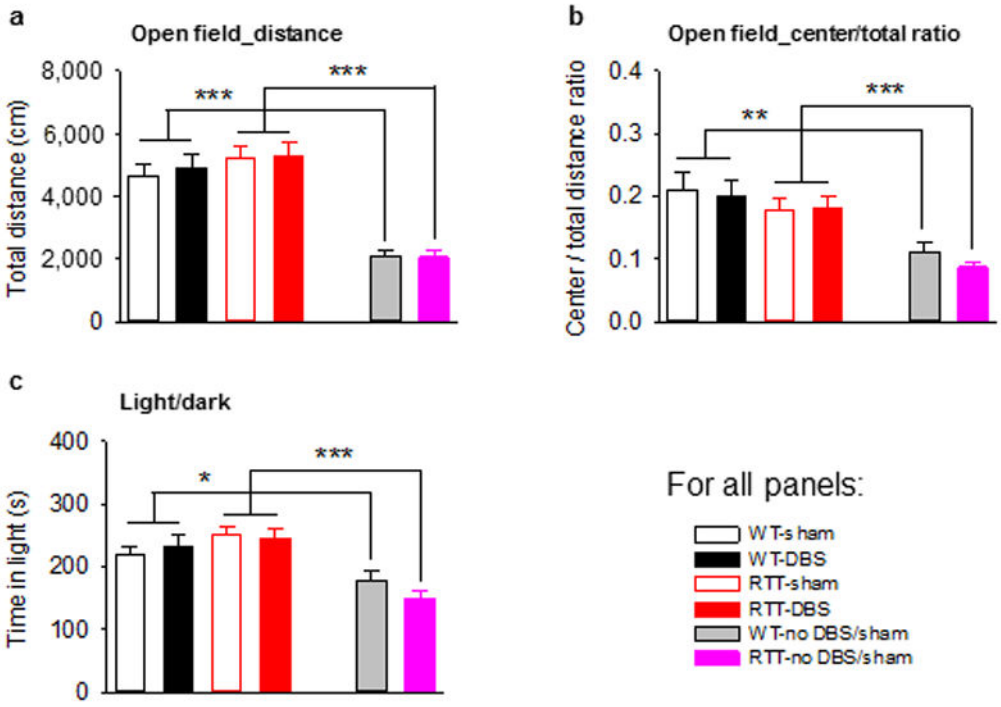


Extended Data Figure 1. Time line of forniceal DBS tests in RTT and WT mice



Extended Data Figure 2. Fear memory in RTT mice and WT control animals

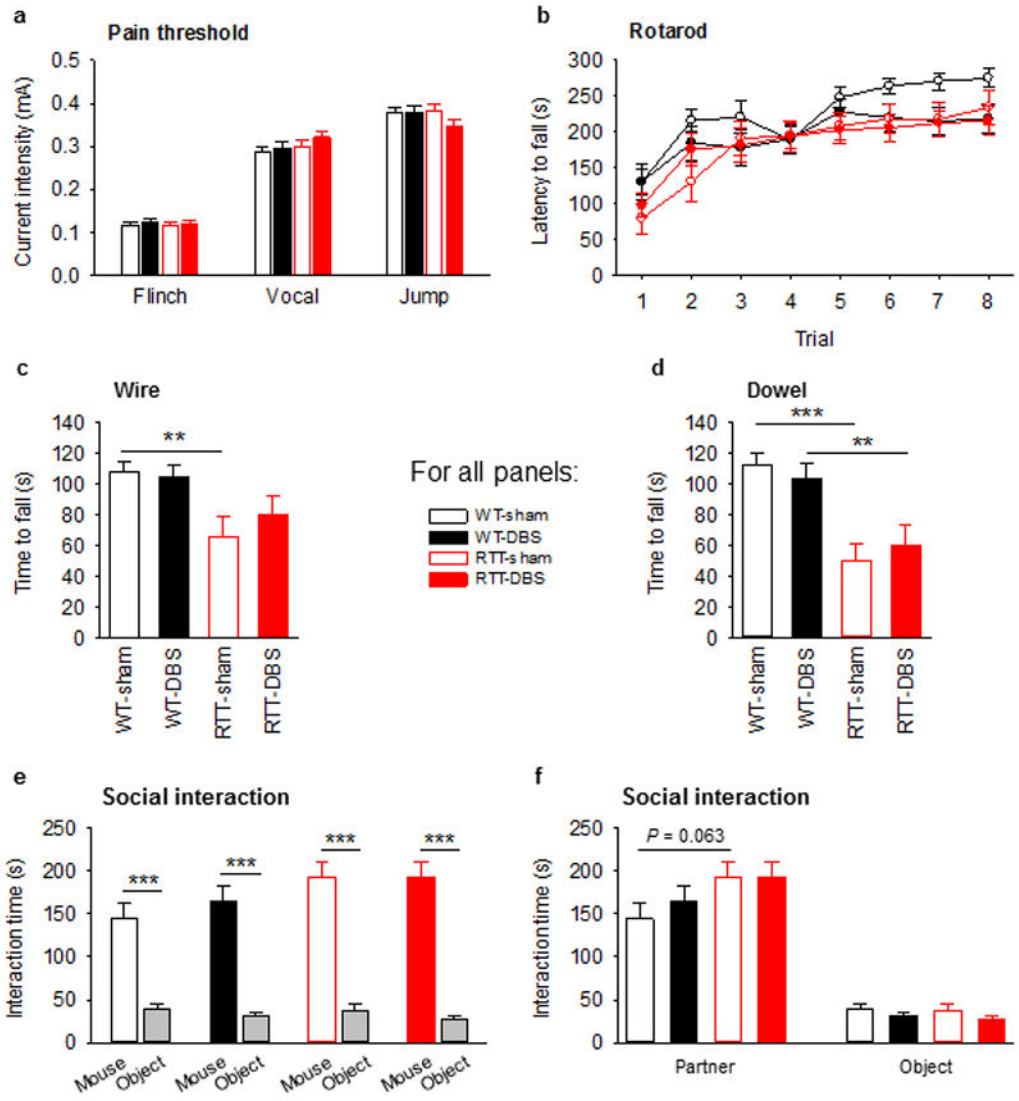
All mice were trained with tone-foot shock pairings on day 0. Memory retention was tested 3h, 1d, 3d, and 7d after training. **a**, **b**, Impaired fear memory in RTT mice (n = 20) compared to WT littermates (n = 20). These animals were implanted with electrodes but without DBS or sham treatment. A significant main effect of genotype was observed (two-way repeated measures ANOVA followed by Tukey *post hoc*: context, $F_{1,38} = 15.32$, $P < 0.001$; cue, $F_{1,38} = 20.70$, $P < 0.001$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ vs. WT. **c**, **d**, Cued fear memory in RTT mice (n = 20) and WT 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 WT (**a**) and RTT mice (**b**). **e-h**, Retrieval of cue fear memory in DBS or sham treated RTT and WT 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. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (paired *t*-test). All data are presented as mean ± s.e.m.



Extended Data Figure 3. Increased handling, but not forniceal DBS, increased locomotor activity and decreased the anxiety level in RTT and WT mice

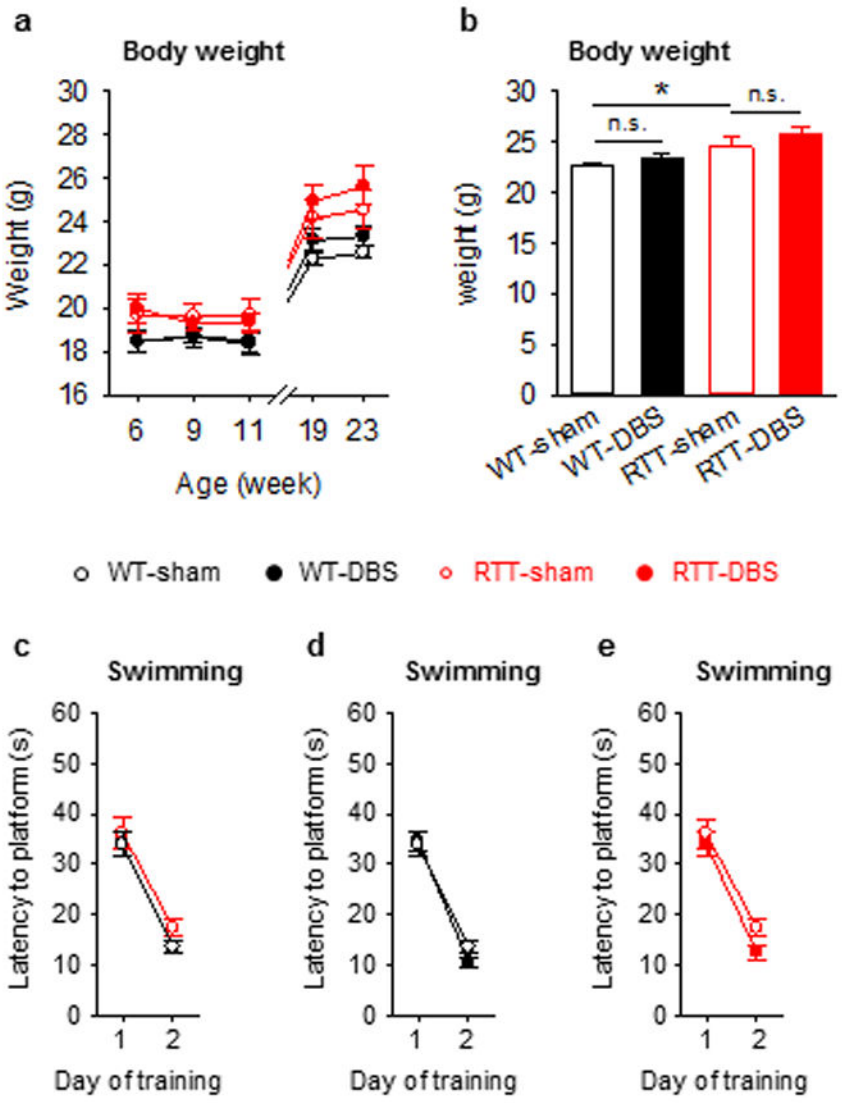
a, There was no difference among the four DBS-treated groups in the total distance traveled in the open field test (WT-sham, n = 20; WT-DBS, n = 20; RTT-sham, n = 17; RTT-DBS, n = 18; genotype, $F_{1,71} = 1.13$, $P = 0.292$; treatment, $F_{1,71} = 0.13$, $P = 0.724$; genotype × treatment, $F_{1,71} = 0.063$, $P = 0.803$). RTT and WT mice that received DBS/sham treatment traveled longer distances than RTT (n = 20) and WT (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 center/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 WT mice that received DBS/sham treatment traveled more in the center area compared to implanted RTT and WT animals without 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 4 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 WT mice that received DBS/sham treatment spent more time in the light compartment than implanted RTT (n = 15) and WT (n = 14) animals without DBS/sham procedures. *$P < 0.05$,0 **$P < 0.01$, ***$P < 0.001$ (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 behavior in RTT or WT mice

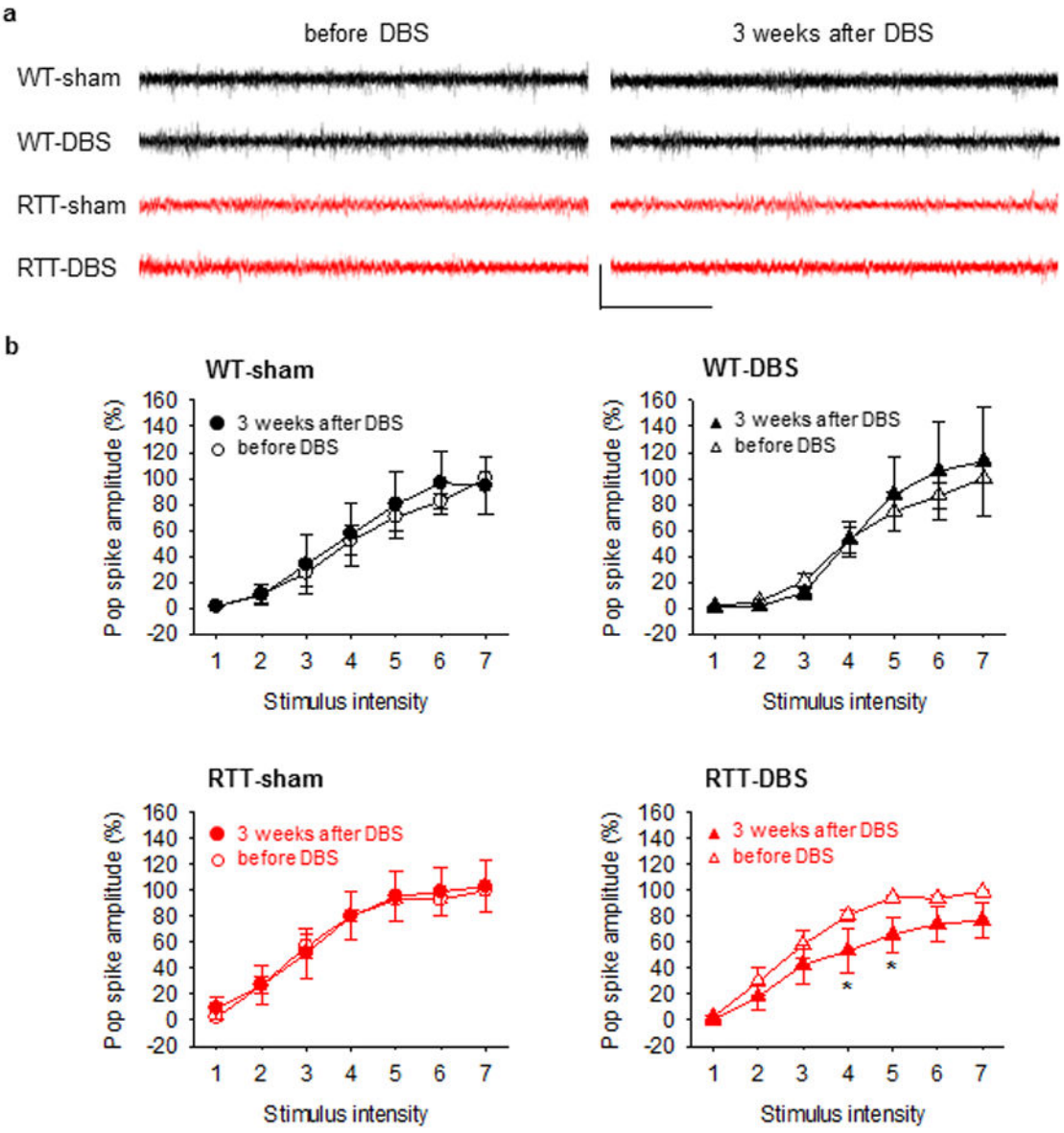
a, There was no group difference in foot shock threshold intensities to evoke flinch, vocalization, or jumping (WT-sham, n = 14; WT-DBS, n = 14; RTT-sham, n = 11; RTT-DBS, n = 12; two-way ANOVA, no significant main effect of genotype, treatment, or genotype × treatment interaction, $P > 0.05$). **b**, In a rotarod test (n = 12 mice per group), latency to fall increased over trials but there was no difference among the 4 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 WT animals, but there was no difference between DBS and sham treated groups for either RTT or WT 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 WT 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 4 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 behavior in RTT mice ($P = 0.063$, RTT-sham vs. WT-sham, Tukey *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 *post hoc* in **c**, **d**; paired *t*-test 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 WT 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 *post hoc* showed that RTT-sham mice were significantly heavier than WT-sham mice (P = 0.015), but there was no difference in body weight between WT-sham and WT-DBS (P = 0.861) or between RTT-sham and RTT-DBS (P = 0.099) mice. **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). RTT-sham mice did not have different escape latencies than WT-sham 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 WT controls (**d**, treatment, $F_{1,34}$ = 0.44, P = 0.513; treatment × day interaction, $F_{1,34}$

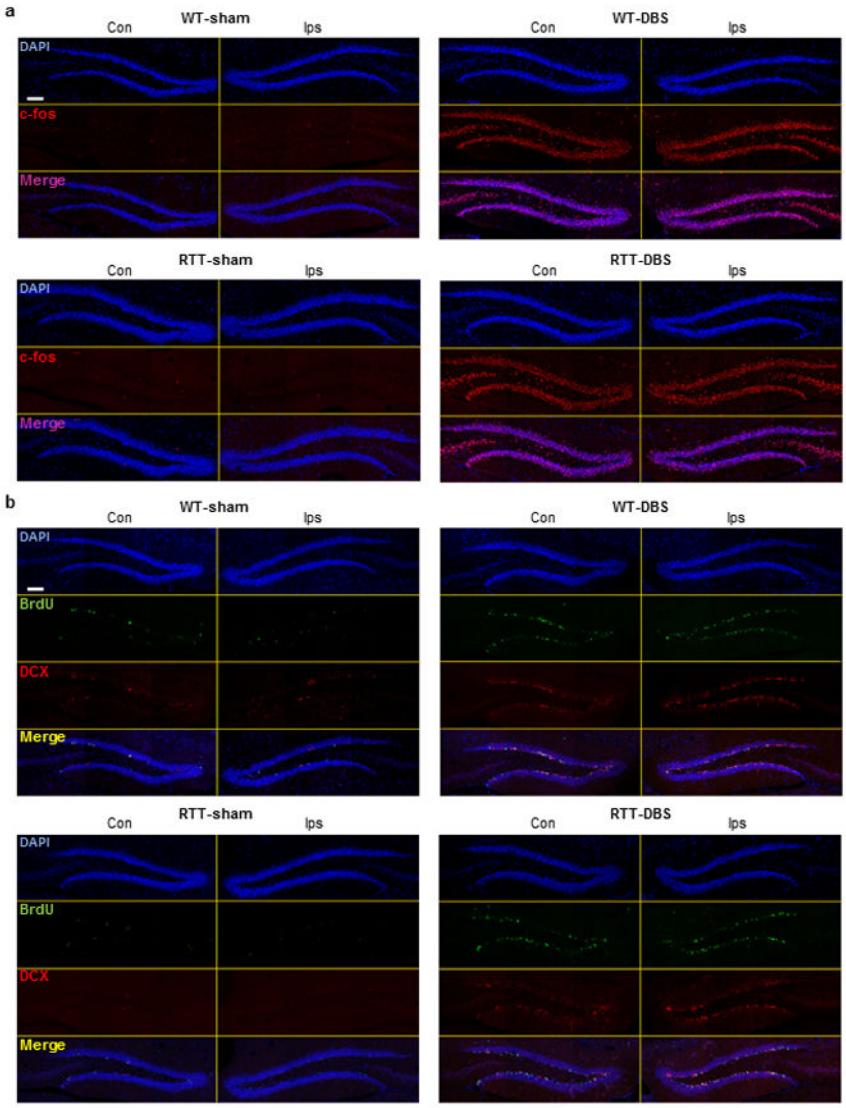
= 1.24, $P = 0.273$) or RTT mice (**e**, treatment, $F_{1,34} = 2.36$, $P = 0.134$; treatment × day interaction, $F_{1,34} = 0.41$, $P = 0.524$). *$P < 0.05$; n.s., not significant. (Tukey *post hoc*). All data are presented as mean ± s.e.m.



Extended Data Figure 6. Effect of forniceal DBS on hippocampal electrophysiological signatures

a, Representative traces of local field potentials 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 forniceal DBS. All data points were normalized to the maximum value of

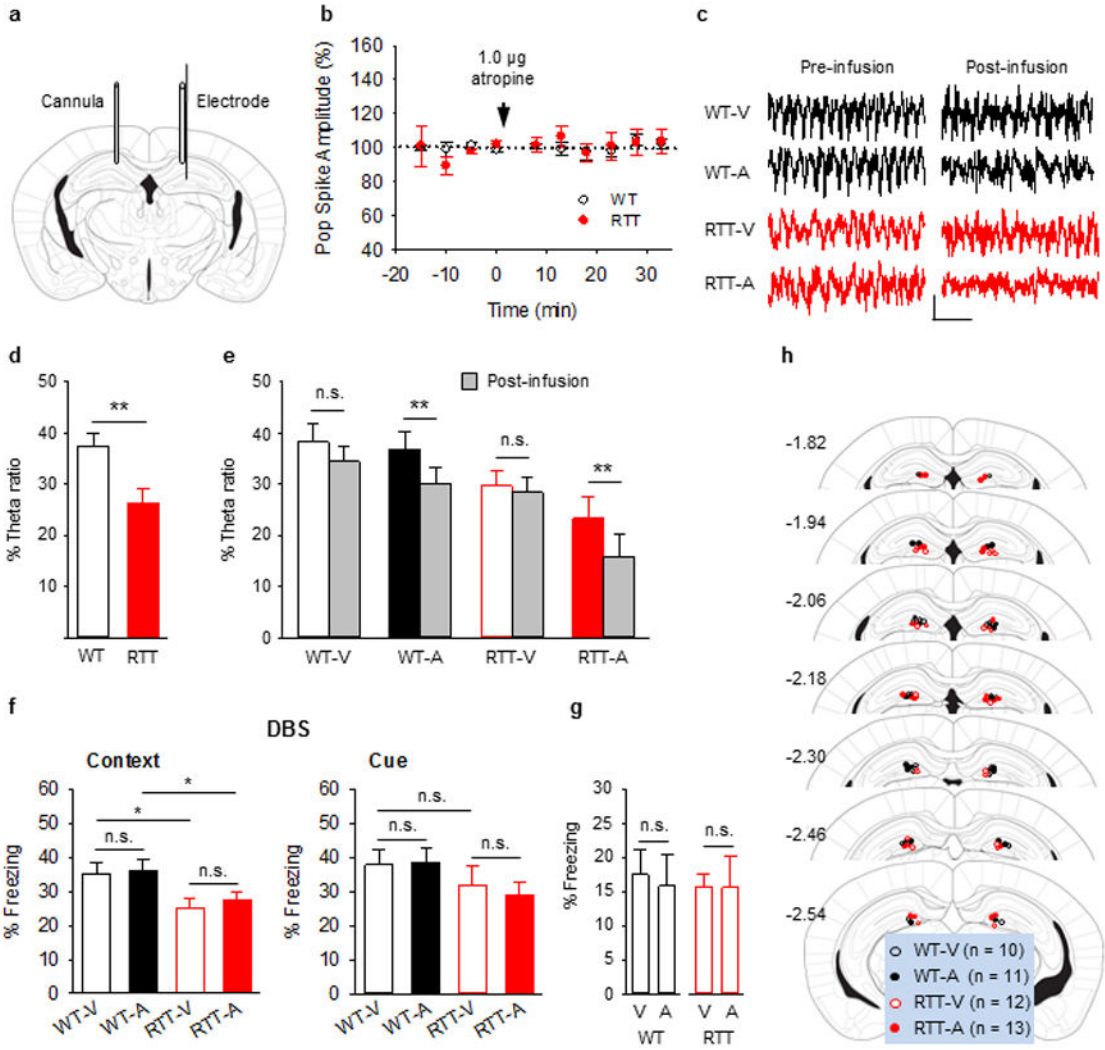
the population spike amplitude before DBS/sham and the abscissa represents the 7 increments used in each mouse. The I/O relationship was not altered by DBS in WT-sham (n = 5, $F_{1,4}$ = 0.062, P = 0.818), WT-DBS (n = 4, $F_{1,3}$ = 0.036, P = 0.861), or RTT-sham (n = 5, $F_{1,4}$ = 0.018, P = 0.901). DBS reduced the amplitude of the evoked population spikes from the baseline test in RTT-DBS mice (n = 5, $F_{1,4}$ = 6.73, P = 0.060). *P < 0.05 (Tukey *post hoc*). All data are presented as mean ± s.e.m.



Extended Data Figure 7. Unilateral forniceal DBS induces neuronal activity and stimulates neurogenesis bilaterally in the dentate gyrus

a, Representative images showing that the expression of *c*-fos gene was increased following forniceal DBS in WT 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 forniceal DBS-treated WT 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 forniceal 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 WT 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 WT controls (n = 3; $F_{9,18} = 0.99$, $P = 0.485$). **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 WT 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 WT 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 forniceal DBS treated RTT mice or WT controls. Mice in all 4 groups (WT-V, n = 10; WT-A, n = 11; RTT-V, n = 12; RTT-A, n = 13) experienced two weeks of forniceal DBS that was finished three 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 *post hoc*). **g**, Intrahippocampal atropine infusion alone did not change the basal level of freezing in the contextual test environment in either WT 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 Paxinos and Franklin, 2001) for the microinfusion cites in DBS treated experiments. The numbers on the left represent the posterior coordinate from the bregma. All data are presented as mean ± s.e.m.

Acknowledgments

We thank Mingshan Xue, Matthew C. Weston, and Vicky Brandt for comments on the manuscript, members of the Zoghbi laboratory for helpful discussions, Corinne M. Spencer, Carsten T. Wotjak, Feng Wei, and Dinghui Yu for technical suggestions. This work was supported by the W. M. Keck Foundation (H.Y.Z. and J.T.), the Cockrell Family Foundation, the Rett Syndrome Research Trust, Carl. C. Anderson Foundation, Sr. and Marie Jo Anderson Charitable Foundation, R01NS057819 (H.Y.Z.), and the Howard Hughes Medical Institute (H.Y.Z.), DP5OD009134 (R.C.S), and in part by the Neuroconnectivity Core, Mouse Neurobehavioral Core, and Neurovisualization Core of IDDRC at Baylor College of Medicine (U54 HD083092 from the Eunice Kennedy Shriver National Institute of Child Health & Human Development), and the C06RR029965 grant from the National Center for Research Resources.

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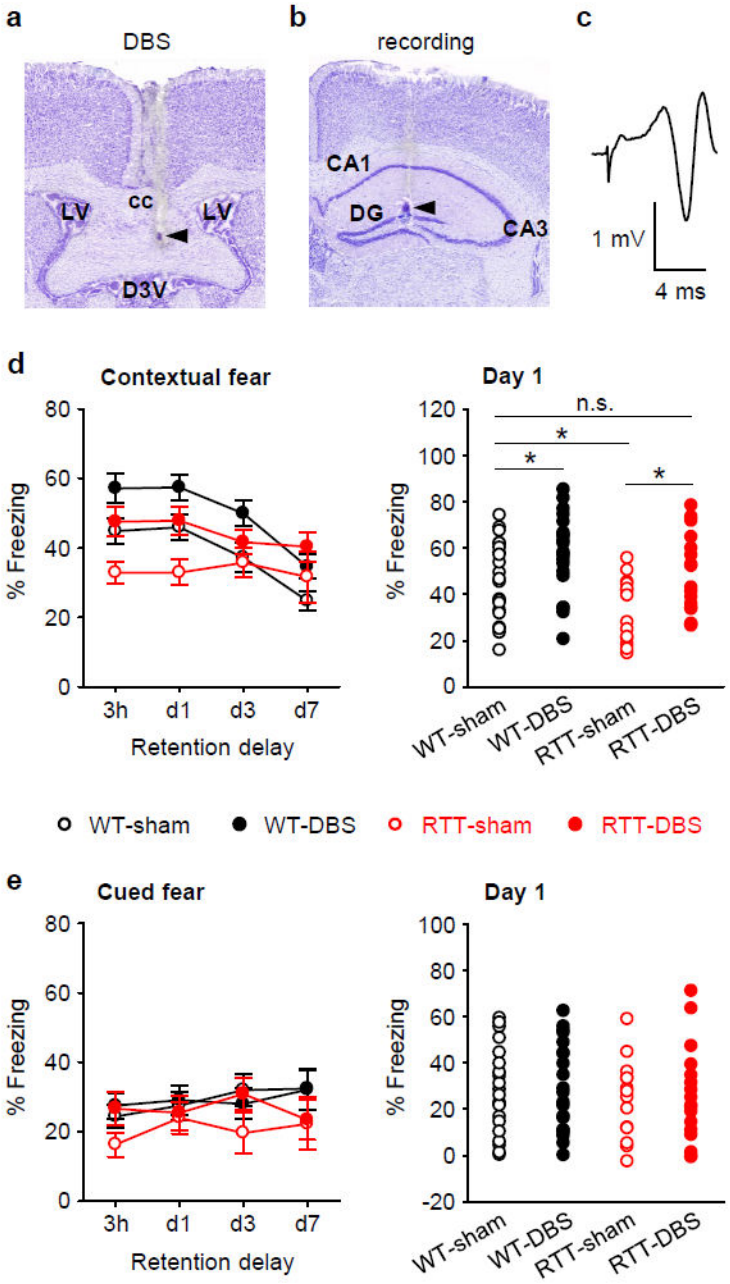

Figure 1. Forniceal DBS restores contextual fear memory in RTT mice

a, **b**, Photomicrographs illustrating DBS electrode placement (arrowheads) in the FFx (**a**) and the recording electrode in the DG (**b**). cc: corpus callosum; LV: lateral ventricle; D3V: dorsal 3rd ventricle; DG: dentate gyrus. **c**, Representative evoked potential trace of the FFx pathway recorded in the dentate. **d**, Forniceal DBS enhanced contextual fear memory in both WT and RTT mice (WT-DBS, n = 21; WT-sham, n = 21; RTT-DBS, n = 17; RTT-sham, n = 14). There were significant main effects on freezing time among the 4 groups (two-way repeated measures ANOVA: group, $F_{3,69} = 5.67$, $P = 0.002$; day, $F_{3,180} = 6.44$, $P < 0.001$; group × day interaction, $F_{9,180} = 2.15$, $P = 0.027$). Within-genotype analysis revealed a

significant DBS effect in both WT ($F_{1,40}$ = 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 WT level (RTT-DBS vs. WT-sham: group, $F_{1,36}$ = 2.76, P = 0.105). Comparison of contextual fear memory on d1 among the 4 groups revealed a significant main effect (two-way ANOVA followed by Tukey *post hoc*: genotype, $F_{1,69}$ = 8.39, P = 0.005; treatment, $F_{1,69}$ = 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, $F_{3,69}$ = 0.88, P = 0.456; day, $F_{3,180}$ = 1.65, P = 0.179; group × day interaction, $F_{9,180}$ = 0.89, P = 0.538) or on d1 (genotype, $F_{1,69}$ = 0.64, P = 0.428; treatment, $F_{1,69}$ = 0.11, P = 0.741). *P < 0.05. n.s., not significant. Data presented as mean ± s.e.m. Scattergrams show individual values.

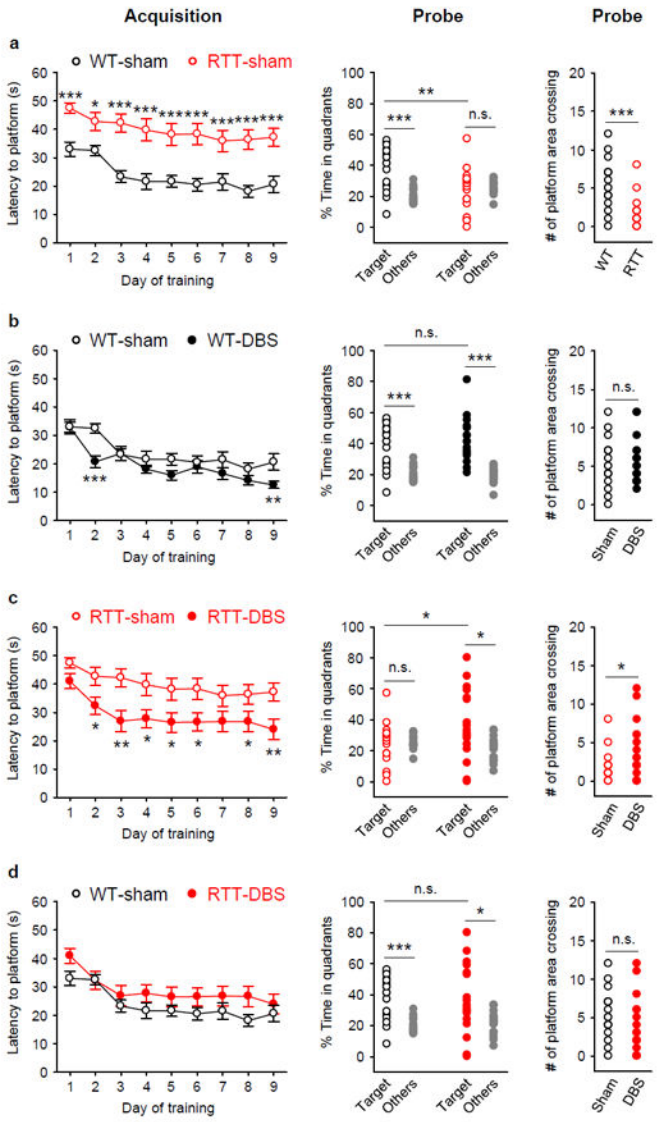

Figure 2. Forniceal DBS rescues spatial learning and memory in RTT mice

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 4 groups (n = 18 mice per group) during acquisition training (two-way repeated measures ANOVA: group, $F_{3,68} = 20.74$, $P < 0.001$; day, $F_{8,544} = 19.72$, $P < 0.001$). **a**, RTT-sham 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 WT-sham controls. **b**, Forniceal DBS decreased escape latencies during training in WT-DBS mice compared to WT-sham controls (treatment, $F_{1,34} = 5.94$, $P = 0.020$; day, $F_{8,272} = 17.10$, $P < 0.001$; treatment × 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 RTT-DBS

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 platform area crossings ($P < 0.05$) during the probe test than RTT-sham controls. **d**, There was no difference between RTT-DBS mice and WT-sham controls in escape latencies during training (group, $F_{1,34} = 2.91$, $P = 0.097$; group × 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. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. n.s., not significant (Tukey *post hoc in acquisition;* two-tailed unpaired *t*-test between groups and paired *t*-test within group in probe). Data presented as mean ± s.e.m. Scattergrams show individual values.

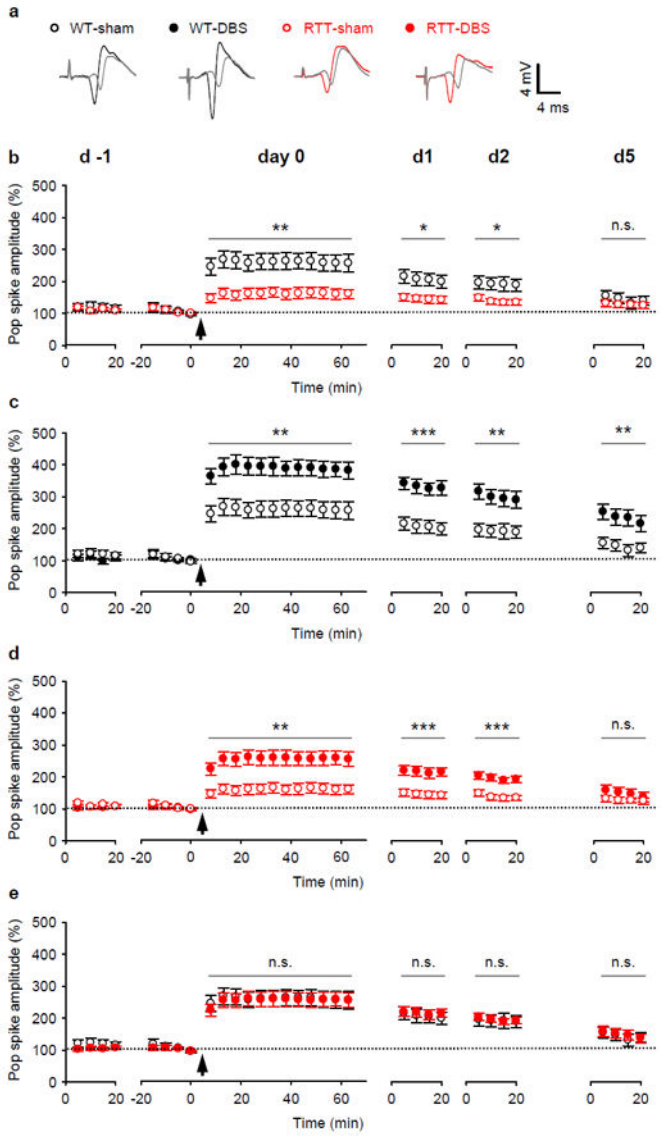

Figure 3. Forniceal DBS rescues hippocampal synaptic plasticity in freely-moving RTT mice

a, Superimposed traces of the perforant path recorded in the DG 5 min before (gray) and 55 min after (black or red) tetani. **b**, RTT-sham mice (n = 12) showed impaired LTP compared to WT-sham group (n = 11) on day 0 (two-way repeated measures ANOVA: genotype, $F_{1,21}$ = 11.34, P = 0.003; time, $F_{15,315}$ = 40.51, P < 0.001; genotype × time interaction, $F_{15,315}$ = 9.36, P < 0.001), d1 (genotype, $F_{1,21}$ = 7.46, P = 0.012; time, $F_{3,63}$ = 5.15, P = 0.003), and d2 (genotype, $F_{1,21}$ = 6.50, P = 0.019). **c**-**d**, Forniceal DBS enhanced LTP in both WT and RTT mice (WT-DBS, n = 12; WT-sham, n = 11; RTT-DBS, n = 13; RTT-sham, n = 12). Two-way repeated measures ANOVA revealed significant main effects of population spike amplitudes among the 4 groups on day 0 (group, $F_{3,44}$ = 17.25, P < 0.001; time, $F_{15,660}$ = 167.28, P < 0.001; group × time interaction, $F_{45,660}$ = 14.50, P < 0.001), d1 (group, $F_{3,44}$ = 21.53, P < 0.001; time, $F_{3,132}$ = 7.69, P < 0.001), d2 (group, $F_{3,44}$ = 16.21, P < 0.001; time, $F_{3,132}$ = 8.96, P < 0.001), and d5 (group, $F_{3,44}$ = 8.42, P < 0.001; time, $F_{3,132}$ = 8.35, P <

0.001). **c**, Forniceal DBS enhanced LTP in WT controls on day 0 (treatment, $F_{1,21} = 12.16$, $P = 0.002$; time, $F_{15,315} = 121.93$, $P < 0.001$; treatment × time interaction, $F_{15,315} = 10.91$, $P < 0.001$), d1 (treatment, $F_{1,21} = 18.99$, $P < 0.001$; time, $F_{3,63} = 4.77$, $P = 0.005$), d2 (treatment, $F_{1,21} = 11.25$, $P = 0.003$; time, $F_{3,63} = 3.72$, $P = 0.016$), and d5 (treatment, $F_{1,21} = 9.44$, $P = 0.006$; time, $F_{3,63} = 6.73$, $P < 0.001$). **d**, Forniceal DBS enhanced LTP in RTT mice on day 0 (treatment, $F_{1,23} = 11.86$, $P = 0.002$; time, $F_{15,345} = 45.02$, $P < 0.001$; treatment × time interaction, $F_{15,345} = 10.31$, $P < 0.001$), d1 (treatment, $F_{1,23} = 14.60$, $P < 0.001$; time, $F_{3,69} = 2.91$, $P = 0.041$), and d2 (treatment, $F_{1,23} = 19.45$, $P < 0.001$; time, $F_{3,69} = 6.09$, $P < 0.001$). **e**, There was no difference of LTP between RTT-DBS and WT-sham ($P > 0.05$ for all the test days). Arrow, LTP induction. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. n.s., not significant. Data presented as mean ± s.e.m.

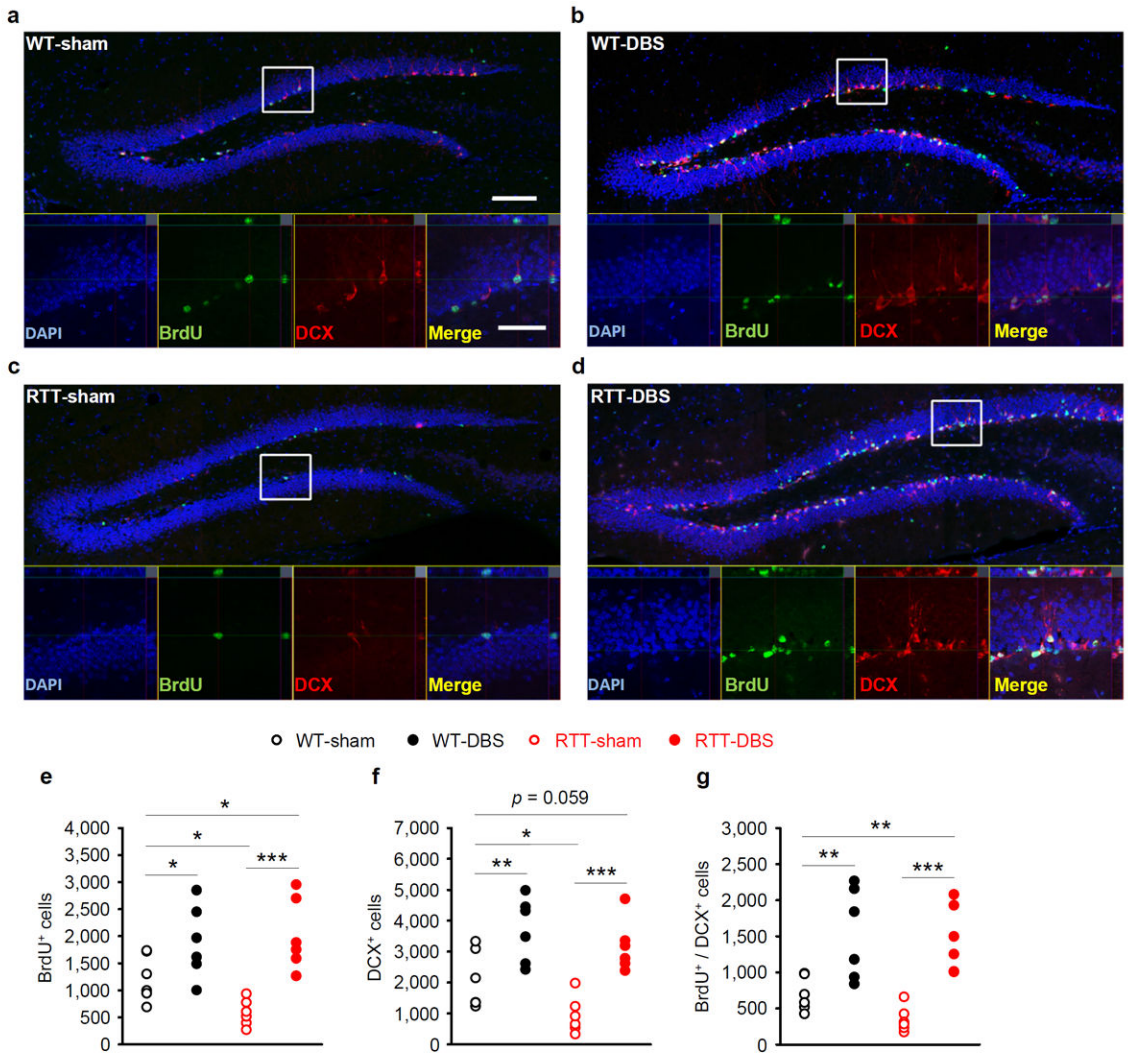

Figure 4. Forniceal DBS stimulates hippocampal neurogenesis in WT and RTT mice

One day after completing the two weeks of forniceal DBS/sham treatment, animals were perfused for immunohistochemical detection of BrdU- and DCX-positive cells in the DG. **a-d**, Representative images ipsilateral to the DBS/sham at low (top; scale bar, 100 μm) and high magnification (bottom; scale bar, 50 μm) showing $BrdU^+$ cells (green), DCX^+ cells (red), and the merge (yellow) from each of the four groups. New neurons were located in the innermost layer of the dentate gyrus. **e-g**, Summary of immunoreactive cell counting (n = 6 mice per group). Two-way ANOVA revealed a significant main effect on the numbers of $BrdU^+$ cells (**e**; treatment, $F_{1,20} = 23.49$, $P < 0.001$), DCX^+ cells (**f**; genotype, $F_{1,20} = 5.65$, $P = 0.028$; treatment, $F_{1,20} = 29.65$, $P < 0.001$), and $BrdU^+/DCX^+$ double staining cells (**g**; treatment, $F_{1,20} = 32.99$, $P < 0.001$). Tukey *post hoc* indicated that RTT-sham mice had fewer $BrdU^+$ (**e**) and DCX^+ (**f**) cells than WT-sham controls. Forniceal DBS increased the numbers of $BrdU^+$ (**e**), DCX^+ (**f**), and $BrdU^+/DCX^+$ (**g**) double staining cells in WT-DBS and RTT-DBS mice compared to their respective sham controls. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Scattergrams show individual values.