(Fig. 4F). Thus, we propose a model in which EFF-1 autonomously induces retraction of branches to simplify menorahs.

EFF-1 is both a sculptor of epithelial organs by cell fusion (*10*) and a menorah sculptor by controlling dendrite bending, retraction, and fusion. The activities of this fusogen may be due to its ability to induce membrane curvature, a process that is thought to constitute a major driving force in membrane fusion and fission (*12*–*14*). Proteins capable of bending membranes, such as atlastins (*15*, *16*) and dynamins (*17*), can induce tubulation, fusion, and fission (*12*, *13*). Three mechanistic principles may form and maintain branched tubes in the cytoplasm and in extracellular branched filopodia or neuronal arbors such as menorahs: first, assembly of specialized proteins on membranes; second, membranous tube formation involving growth and bifurcation of tubes; and third, membrane bending followed by membrane fusion and fission restricts excessive branching. How can EFF-1 control mechanistically different processes such as dendrite fusion and retraction? Different isoforms and interactions may account for diverse activities. For example, trans interactions between EFF-1 on dendrites will cause autofusion, whereas assembly of large EFF-1 complexes on dendrites may induce actin-mediated retraction.

Note added in proof: After we submitted this report, Ghosh-Roy *et al.* (*18*) showed that axotomized PLM sensory neurons fail to reconnect in *eff-1* mutants.

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Induction of Fear Extinction with Hippocampal-Infralimbic BDNF

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The extinction of conditioned fear memories requires plasticity in the infralimbic medial prefrontal cortex (IL mPFC), but little is known about the molecular mechanisms involved. Brain-derived neurotrophic factor (BDNF) is a key mediator of synaptic plasticity in multiple brain areas. In rats subjected to auditory fear conditioning, BDNF infused into the IL mPFC reduced conditioned fear for up to 48 hours, even in the absence of extinction training, which suggests that BDNF substituted for extinction. Similar to extinction, BDNF-induced reduction in fear required *N*-methyl-D-aspartate receptors and did not erase the original fear memory. Rats failing to learn extinction showed reduced BDNF in hippocampal inputs to the IL mPFC, and augmenting BDNF in this pathway prevented extinction failure. Hence, boosting BDNF activity in hippocampal-infralimbic circuits may ameliorate disorders of learned fear.

Extinction of conditioned fear forms a new memory in the infralimbic medial prefrontal cortex (IL mPFC) that is critical for the retrieval of extinction (*1*, *2*). IL single-unit responses correlate with the successful retrieval of such extinction memories (*3*), and IL stimulation strengthens these memories (*3*). Consolidation of extinction requires plasticity within the IL mPFC, which in turn depends on *N*-methyl-D-aspartate (NMDA) receptors, mitogen-activated protein kinase, and protein synthesis (*2*, *4*). Understanding the molecular mechanisms that support this extinction-related plasticity could lead to pharmacological approaches for enhancing extinction memory, which might facilitate the treatment of anxiety disorders.

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Epigenetic regulation within the IL mPFC of the gene encoding BDNF correlates with fear extinction (*5*). Because BDNF is a major molecular mediator of memory consolidation (*6*), we hypothesized that BDNF is responsible for consolidating extinction memory within the IL mPFC. If true, it should be possible to enhance extinction via direct application of BDNF to the IL mPFC. Accordingly, rats were subjected to auditory fear conditioning and, the following day, received bilateral IL mPFC infusion of human recombinant BDNF protein (0.75 μg per side) 60 min before extinction training. Conditioned freezing in BDNF-treated rats was significantly reduced relative to saline-infused rats (main effect of drug $F_{1,14} = 28.359$, $P < 0.001$, Fig. 1A; for suppression of food seeking, see fig. S1). This effect persisted in an extinction test the following day (day 3, main effect of drug $F_{1,14} = 11.029$, $P = 0.005$, Fig. 1A), which indicated that BDNF strengthened extinction memory.

Freezing was significantly reduced in BDNF rats from the first extinction trial [$t(14) = 3.335$, $P = 0.005$], which suggested that BDNF reduced fear independent of extinction training. We therefore repeated the previous experiment but omitted extinction training from day 2. Conditioned rats were infused with BDNF or saline and returned to their home cages. The following day, freezing was again reduced in BDNF-treated rats from the first trial [$t(10) = 4.476$, $P = 0.001$, Fig. 1B] and throughout the extinction session (main effect of drug $F_{1,10} = 27.220$, $P < 0.001$). Although the effect of BDNF on fear did not require extinction training, it did require conditioning, because BDNF infused 1 day before conditioning did not significantly reduce freezing (Fig. 1C). BDNF infusions did not alter locomotion, anxiety, or motivation to seek food reward (fig. S2, A to C). The lack of effect on conditioning and open-field anxiety suggests that BDNF infusions did not decrease amygdala activity nonspecifically. Nor could BDNF's effects be attributed to potentiation of latent inhibition, because removing habituation trials did not prevent the effect (fig. S2D).

There are two interpretations for these results. BDNF could inhibit fear expression (similar to extinction), or it could have degraded the original fear memory. To distinguish between these possibilities, we determined the extent to which freezing could be reinstated after unsignaled footshocks, which can reveal the underlying fear memory (*7*). One day after infusions, rats were given extinction training followed by two unsignaled shocks. Replicating our previous experiment, BDNF rats showed reduced fear throughout the extinction session (main effect of drug $F_{1,21} = 7.337$, $P = 0.013$, Fig. 2A). On day 4, however, both saline- and BDNF-treated rats froze equivalently to the tone (78% and 80%, respectively; Fig. 2A), indicating that BDNF left the original fear memory intact. The return of freezing on day 4 was not due

to BDNF "wearing off" (fig. S3A) or contextual conditioning (fig. S3B).

One hallmark of extinction memory is its dependence on NMDA receptors (*4*, *8*, *9*). For example, systemic administration of the NMDA receptor antagonist 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) prevents long-term extinction memory (*10*). The BDNF receptor TrkB interacts with the NMDA receptor in vivo (*11*), and BDNF enhances NMDA currents in vitro (*12*). It is possible, therefore, that IL BDNF mediates its extinction-like effects through NMDA receptors. To test this, we conditioned rats as previously on day 1. On day 2, in the absence of training, rats received one of the following treatment combinations: (i) saline injection (intraperitoneally) + saline infusion into IL (SAL + SAL), (ii) saline injection + BDNF infusion (SAL + BDNF), or (iii) CPP injection + BDNF infusion (CPP + BDNF). On day 3, all rats were returned to the chambers for a single-tone test. As before, SAL + BDNF rats showed significantly reduced fear relative to SAL + SAL rats (main effect of drug $F_{2,25} = 4.597$, $P = 0.020$, post hoc $P = 0.046$; Fig. 2B). However, CPP + BDNF rats were indistinguishable from SAL + SAL rats in their freezing level (post hoc $P = 0.828$; Fig. 2B), which demonstrated that NMDA receptors are necessary for BDNF-induced reductions in fear.

Does extinction depend on endogenous BDNF levels in the IL mPFC or its inputs? We addressed this question by capitalizing on the fact that there can be considerable variability in extinction memory across rats (*8*, *13*). Rats were conditioned and extinguished on days 1 and 2, respectively, as above. We then selected two subgroups on the basis of their ability to successfully recall extinction on day 3. "Extinction Failure" and "Extinction Success" rats had freezing values in the top or bottom 44%, respectively (i.e., the middle 12% was excluded). These two subgroups differed significantly on test day [$t(10) = 4.728$, $P = 0.001$] but showed no significant differences during conditioning or extinction training (Fig. 3A). Normal extinction training followed by poor retrieval of extinction is consistent with impaired infralimbic function (*1*, *2*).

For each subgroup, brain tissue from the mPFC, amygdala, and hippocampus was dissected 24 hours after the extinction test to determine BDNF levels. The amygdala and hippocampus were chosen as putative BDNF-containing inputs that might be important for supplying BDNF to the IL mPFC to facilitate extinction recall (*14*–*16*). Indeed, hippocampal CA1 neurons produce BDNF (*16*, *17*) and project to the IL mPFC (*14*). BDNF protein levels in the Success group were elevated relative to the Failure group in the hippocampus [$t(9) = 4.370$, $P = 0.002$], but not the mPFC or

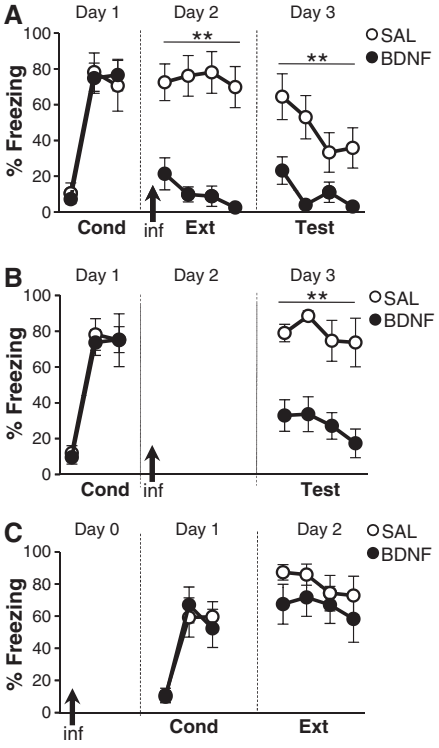

Fig. 1. BDNF infused into the infralimbic cortex substitutes for behavioral extinction. (**A**) Rats' freezing levels in response to tones that were paired with footshocks (Cond) or given alone during extinction (Ext) and Test sessions. BDNF infusions into the IL mPFC before extinction (arrow) reduced freezing on days 2 and 3 relative to saline-infused (SAL) controls ($n = 8$ per group). (**B**) A similar effect was observed when BDNF was infused in the absence of training on day 2 (SAL, $n = 5$; BDNF, $n = 7$). (**C**) Infusing BDNF 24 hours before conditioning had no effect (SAL, $n = 9$; BDNF, $n = 7$). Trials are shown in blocks of two. $^{**}P < 0.01$, repeated-measures analysis of variance (ANOVA). Error bars represent SEM.

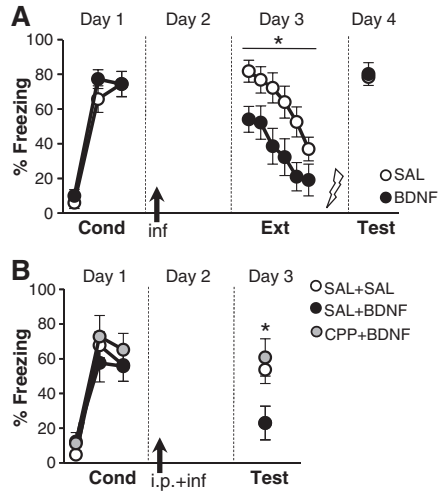

Fig. 2. Similar to extinction, the BDNF effect does not degrade the original fear memory and requires NMDA receptors. (**A**) Conditioned rats received BDNF or saline infusions into the IL mPFC on day 2 (SAL, $n = 12$; BDNF, $n = 11$). On day 3, both groups were extinguished, followed by two shocks, resulting in a complete return of freezing in the BDNF group. (**B**) IL infusion of BDNF was combined with a systemic injection of the NMDA antagonist CPP (CPP + BDNF, $n = 8$). Controls were infused with BDNF and given a saline injection (SAL + BDNF, $n = 10$) or were both infused and injected with saline (SAL + SAL, $n = 10$). On day 3, all groups underwent a single-tone extinction test. $^{*}P < 0.05$, two-way repeated-measures ANOVA, main effect of drug; $^{*}P < 0.05$, Student's t test, SAL + SAL compared to SAL + BDNF.

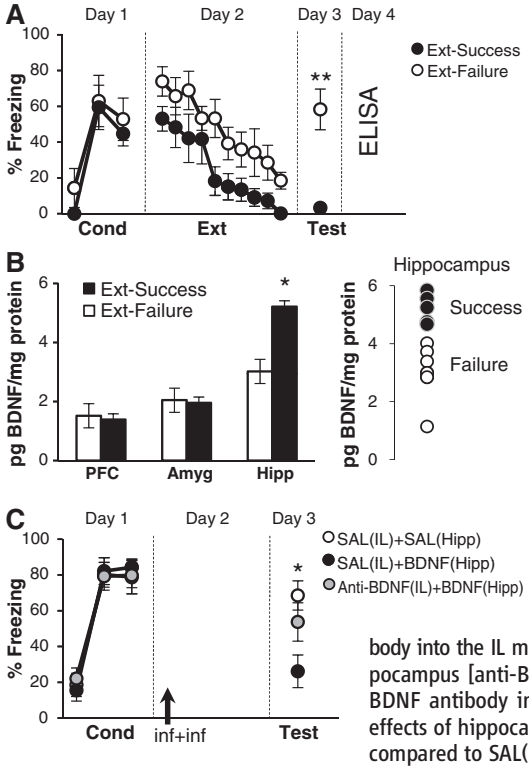

Fig. 3. Hippocampal projections to the infralimbic cortex mediate BDNF-extinction. (**A**) One day after extinction training, rats were divided into two groups on the basis of their ability to retrieve extinction (Ext-Success, $n = 6$; Ext-Failure, $n = 6$; $^{**}P < 0.01$). The following day, BDNF protein concentrations were determined by enzyme-linked immunosorbent assay (ELISA). (**B**) Ext-Success rats showed elevated levels of BDNF in the hippocampus but not in the mPFC or amygdala ($^{*}P < 0.05$, Ext-Success versus Ext-Failure). Hippocampal BDNF levels in individual Success and Failure rats were nonoverlapping. (**C**) Conditioned rats were divided into three groups. Controls received a saline infusion into the IL mPFC followed by a saline infusion into the hippocampus [SAL(IL) + SAL(Hipp), $n = 7$]. Another group received a saline infusion into the IL mPFC followed by a BDNF infusion into Hipp [SAL(IL) + BDNF(Hipp), $n = 8$]. A third group received an infusion of a BDNF-sequestering antibody into the IL mPFC followed by BDNF infusion into the hippocampus [anti-BDNF(IL) + BDNF(Hipp), $n = 9$]. Infusion of BDNF antibody into the IL mPFC blocked the fear-reducing effects of hippocampal BDNF. $^{*}P < 0.05$, SAL(IL) + SAL(Hipp) compared to SAL(IL) + BDNF(Hipp).

amygdala (Fig. 3B). These data are consistent with previous studies in which genetic knockdown of hippocampal BDNF impaired fear extinction (*17*).

If the hippocampus is the source of IL BDNF, then increasing the available supply of hippocampal BDNF should have similar effects. We took advantage of the fact that BDNF infusions increase BDNF levels in efferent targets (*18*). There were three treatment groups in this experiment. After conditioning, one group received a hippocampal infusion of BDNF immediately after a saline infusion into the IL mPFC [SAL(IL) + BDNF(Hipp)]. A second group also received a hippocampal BDNF infusion, but this was preceded by infusion of a BDNF-inactivating antibody into the IL mPFC [anti-BDNF(IL) + BDNF(Hipp)] to test the hypothesis that Hipp-applied BDNF works via the IL mPFC. A control group received SAL infusions into both structures [SAL(IL) + SAL(Hipp)].

Similar to its effect on the IL mPFC, BDNF infused into the hippocampus reduced fear, as measured by both freezing [main effect of drug $F_{2,21}$ = 4.715, P = 0.020, post hoc P = 0.013 comparing SAL(IL) + SAL(Hipp) to SAL(IL) + BDNF(Hipp)] (Fig. 3C) and conditioned suppression of food seeking (fig. S4). The effect of hippocampal BDNF could be prevented by coadministration of a BDNF-inactivating antibody in the IL mPFC [P = 0.461 comparing SAL(IL) + SAL(Hipp) to Anti-BDNF(IL) + BDNF(Hipp)], which suggests that the IL mPFC is the primary site of action for hippocampal BDNF.

We were able to pharmacologically induce extinction with a single infusion of BDNF into the hippocampal-infralimbic pathway, a key projection for extinction memory. This effect was not a facilitation of extinction, as no extinction training was required. We have adopted the term "BDNF-extinction" to parallel the term "BDNF-LTP" used to describe BDNF induction of hippocampal LTP in the absence of electrical stimulation (*19*). Extinction potentiates the hippocampal-prefrontal pathway, and disrupting this potentiation disrupts extinction recall (*20*). Our results provide further support for the importance of this pathway in extinction and extend these findings by identifying BDNF as a key molecular mediator.

In our experiments, BDNF-extinction required NMDA receptors, which are also necessary for extinction-related bursting in IL neurons (*8*). Because BDNF facilitates NMDA receptor currents (*11*, *12*), exogenously applied BDNF may simulate extinction by inducing bursting in the IL mPFC. Additionally, BDNF-extinction may involve IL targets, such as intercalated (*21*) or basolateral amygdala (*9*, *15*) neurons, which also participate in extinction.

Because the behavioral effects of BDNF were observed only when BDNF was infused after conditioning, it is possible that BDNF treatment may lead to partial reversal of conditioning-induced changes. Conditioning induces a rapid reduction in hippocampal BDNF, which reverts in 2 days (*22*). Extinction failure then may arise from a delayed normalization of BDNF levels after conditioning. If so, application of BDNF to the hippocampus (or to the IL mPFC) may work to reduce fear by restoring BDNF to preconditioning levels and/or reversing conditioning-induced reductions in IL excitability (*23*).

Recall of extinction in healthy human subjects activates the ventromedial PFC and hippocampus (*24*), both of which are deficient in posttraumatic stress disorder (*25*). A single-nucleotide polymorphism in the gene encoding human BDNF ($Val^{66} \rightarrow$ Met) results in extinction impairment (*26*) and decreases the release of BDNF from hippocampal neurons (*27*). Pharmacotherapies that increase hippocampal BDNF may prove to be efficacious treatments for fear disorders characterized by extinction impairments. BDNF-extinction is complementary to reconsolidation blockade, in which pharmacological agents are used to eliminate the original fear memory (*7*). Both approaches represent potentially powerful strategies to treat anxiety disorders by manipulating traumatic memories within fear circuits.

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Supporting Online Material

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SphK1 Regulates Proinflammatory Responses Associated with Endotoxin and Polymicrobial Sepsis

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During sepsis, activation of phagocytes leads to the overproduction of proinflammatory cytokines, causing systemic inflammation. Despite substantial information regarding the underlying molecular mechanisms that lead to sepsis, several elements in the pathway remain to be elucidated. We found that the enzyme sphingosine kinase 1 (SphK1) is up-regulated in stimulated human phagocytes and in peritoneal phagocytes of patients with severe sepsis. Blockade of SphK1 inhibited phagocyte production of endotoxin-induced proinflammatory cytokines. We observed protection against sepsis in mice treated with a specific SphK1 inhibitor that was enhanced by treatment with a broad-spectrum antibiotic. These results demonstrated a critical role for SphK1 in endotoxin signaling and sepsis-induced inflammatory responses and suggest that inhibition of SphK1 is a potential therapy for septic shock.

The incidence of sepsis, and death from septic shock, has increased over the past few decades (*1*, *2*). During sepsis, the host's innate immune response to bacterial infection is primarily mediated by neutrophils and monocytes/macrophages (*3*). These cells express pattern-recognition receptors (PRRs) that bind conserved molecular structures shared by groups of microorganisms (*3*). Upon stimulation, PRRs initiate inflammatory signaling pathways leading to secretion of proinflammatory mediators, which promote the elimination of infectious agents and the

Supporting Online Material for

Induction of Fear Extinction with Hippocampal-Infralimbic BDNF

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This PDF file includes:

Materials and Methods

Subjects. Male Sprague-Dawley rats weighing 250-300g were housed individually and received water ad libitum throughout the experiment. Food was restricted to 18g/d of standard laboratory rat chow (~85% free-feeding weight). Rats were trained to lever press for 45mg food pellets in standard operant chambers located inside sound-attenuating boxes. Rats pressed on a variable interval schedule of reinforcement (initially on a VI-15, then VI-30, and finally VI-60, until 10 presses/min were achieved on VI-60). Throughout fear conditioning procedures, animals were allowed to press for food on this VI-60 schedule in order to maintain a baseline level of activity against which freezing can be reliably measured (*S1*). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Puerto Rico in compliance with NIH guidelines (Publication no. DHHS NIH 86-23).

Surgery. After rats were trained to lever press for food, they were surgically implanted with bilateral, chronic indwelling, intracranial guide cannulae directed at infralimbic cortex (sterotaxic coordinates according to the atlas of Paxinos & Watson were: anteroposterior, AP, +3.0mm; mediolateral, ML, ±0.6mm; dorsoventral, DV, -4.2mm), or hippocampus (AP, -4.4mm; ML, ±3.6mm; DV, -2.3mm, angled 13° toward the midline in the coronal plane). Dummies (33 gauge) were cut flush with the 26 gauge-cannulae, and injectors (33 gauge) extended 1mm beyond the cannulae. Anaesthesia was a ketamine (80 mg/kg, i.p.)/xylazine (5 mg/kg, i.p.) mixture. Buprenorphine hydrochloride (Buprenex, 0.05 mg/kg, s.c.) was administered during acute recovery to reduce post-

operative pain. Rats were allowed to recover from surgery 5-7 days prior to initiation of fear conditioning procedures.

Fear Conditioning. Rats were fear conditioned and extinguished in standard operant chambers inside sound-attenuating boxes in an isolated behavioral room. On Day 1, rats received five habituation tones (4kHz, 30s, 78dB), followed immediately by seven conditioning tones that co-terminated with footshock (0.5s, 0.5mA). In all phases of the experiment, the intertone interval varied around 3 minutes. Tone-only controls shown in Fig. S3B received no footshock. On Day 2, extinction training was conducted in the same context used for conditioning on Day 1. Extinction consisted of 8 to 20 tones (same frequency as conditioning), depending on the experiment. In some cases, extinction recall was tested on Day 3, under conditions identical to extinction (Day 2), except the number of tone presentations varied from as few as one tone (CPP experiment shown in Fig. 2B and IL-Hipp dual infusion study shown in Fig.3C) to 8 tones (as in Fig. 1). For reinstatement (Fig. 2A), extinction was conducted on Day 3, and consisted of 12 tones for the BDNF group and 20 tones for the saline group, to match extinction levels between groups. Animals were then returned to the home cage for at least 45 minutes before being returned to the operant chambers for two non-contingent footshocks (same shock level as in conditioning) presented 1 min apart, after 3 min in the chamber. On Day 4, animals returned to the chambers for the reinstatement test, which consisted of two tone-presentations under extinction conditions.

Freezing was measured via automated analysis of video files, and expressed as a

percentage of the 30 sec tone. Conditioned suppression of food seeking was also calculated by comparing press rates in the 1-min pre-tone period to those during tone presentation, using the following formula: Suppression Ratio = (pretone rate - tone rate)/(pretone rate + tone rate). A value of 0 indicates no suppression of food seeking (no fear), whereas a value of 1 indicates complete suppression (high fear).

Open field testing. To test for non-specific effects of the BDNF infusions on locomotion or anxiety, behavior was measured in a 20-min open field session within an isolated testing room. The dimensions of the open field were 91.5 x 91.5 x 61cm (l x w x h), and the area was subdivided into a central area (61 x 61cm) and a perimeter (within 15.25cm of walls), with additional grid lines demarcating smaller subdivisions within these areas. A video camera was mounted above the apparatus, and behavior was recorded for later scoring by an experimenter blind to treatment condition. The total number of line crosses was tallied, along with the time (in sec) spent within the center vs. perimeter of the apparatus.

Intracranial drug infusions and systemic treatment. BDNF was administered at a dose of 0.75 μg/0.5μl/side, a dose which has been shown previously to inhibit cocaine-seeking behavior when infused into medial prefrontal cortex (*S2*), and a volume which has been used successfully in discriminating effects between structures in close neuroanatomical proximity to one another (eg. the ventral tegmental area and substantia nigra) (*S3*). Anti-BDNF antibody was administered at a dose of 0.5 μg/0.5μl/side, a dose which has been shown previously to block BDNF signaling (*S4*). Both drugs were dissolved in

physiological saline, and infused at a rate of 0.25 μl/min. The NMDA receptor antagonist CPP was dissolved in saline and administered intraperitoneally at a dose of 10 mg/kg, which was previously shown to be sufficient for blocking bursting in medial prefrontal neurons (*S5*).

Enzyme-linked immunosorbent assay (ELISA). The BDNF E_{max}® ImmunoAssay system for the detection and quantification of endogenous BDNF protein was performed according to the manufacturer's protocol. This assay can reliably detect quantities as low as 15.6 pg/ml BDNF. Samples from the PFC, amygdala, and hippocampus from extinction-success vs. extinction-failure rats (behavior shown in Fig. 3A) were run in duplicate. In some cases (n = 1 per brain region), the values comprising the duplicate varied by more than 200%, and were eliminated from the analysis shown in Fig. 3B. Data was normalized to mg of total protein in each sample.

Histology. At the end of each experiment, when necessary, rats were transcardially perfused with 10% formalin and the brains were removed. Brains were post-fixed in 10% formalin/20% sucrose and subsequently sectioned at 40 μm increments through the region of interest. Sections were stained with cresyl violet, and examined at 10x magnification under a microscrope. The most ventral location of the injector tip was used to identify the location of the infusion site within the anatomical boundaries of interest (indicated by black dots in Fig. S5).

Statistics. Freezing and suppression data were analyzed with a 2-way repeated measures ANOVA, with tones as the within-subject factor and group (SAL/BDNF or Ext Success/Failure) as the between subjects factor. In some cases, when only a two-tone test was administered (Fig. 2A, 3A), freezing across the two tones was averaged and a Student's t-test was used for statistical comparison of groups. A planned comparison t-test was conducted for the first tone only following BDNF infusion in some experiments (Fig. 1A, 1B). For the two, three treatment group studies that used a single tone test (Fig. 2B, 3C), a one-way ANOVA across treatment followed by a Dunnett's post-hoc was used for statistical comparison. Student's t-tests (two-tailed) were used for all other analyses.

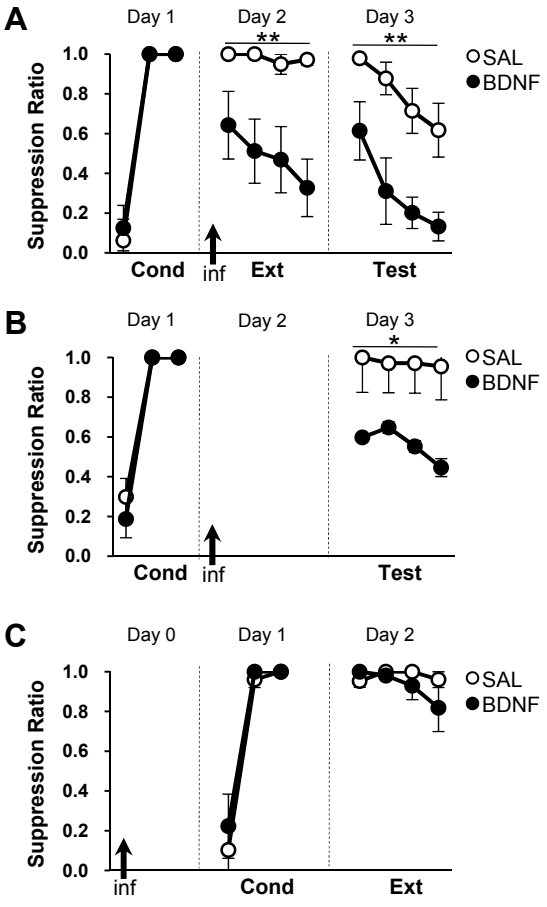

Fig S1. Conditioned suppression of bar pressing, corresponding to freezing data shown in Figure 1. (A) Pre-extinction infusion of BDNF into IL (arrow) reduced suppression ratios throughout the extinction session on Day 2 (main effect of drug $F[1,14] = 12.134$, $p = 0.004$), and persisted throughout the recall test conducted on Day 3 (main effect of drug $F[1,14] = 17.189$, $p = 0.001$). (B) Infusion of BDNF into IL on a blank day between conditioning and test also reduced suppression throughout the test on Day 3 (main effect of drug $F[1,10] = 5.480$, $p = 0.041$). (C) Pre-conditioning infusion of BDNF into IL did not alter suppression during conditioning or extinction training conducted the day after conditioning. Data are shown in blocks of two trials. ** $p < 0.01$, * $p < 0.05$, 2-way repeated measures ANOVA, main effect of drug. Error bars represent SEM.

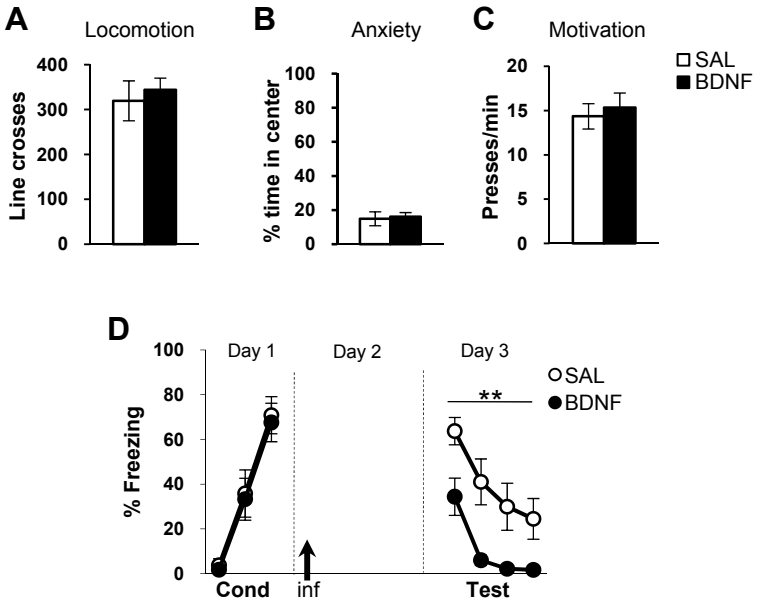

Fig. S2. Reduced fear with BDNF is not due to effects on locomotion, anxiety, motivation, or latent inhibition. In A-C, rats were infused with BDNF or SAL in IL, and tested 24 hrs later. Locomotion was defined as total line crosses in an open field, and anxiety was defined as the percent time spent in the center of the apparatus. For C, rats were placed in the testing chamber and allowed to press for food on a VI-60 schedule of reinforcement. The prior day's infusion of BDNF did not significantly alter rats' behavior in any of these measures. (D) On Day 1, naïve rats were given 3 conditioning trials, without any preceding habituation trials. On Days 2 and 3, they were infused and tested as in Fig. 1B. Despite the elimination of habituation trials, BDNF-infused rats showed significantly reduced freezing at test (two-way repeated measures ANOVA on Day 3, main effect of drug $F(1,26) = 12.385$, $p = 0.002$). Data are shown as single trials on Day 1, and blocks of two trials on Day 3.

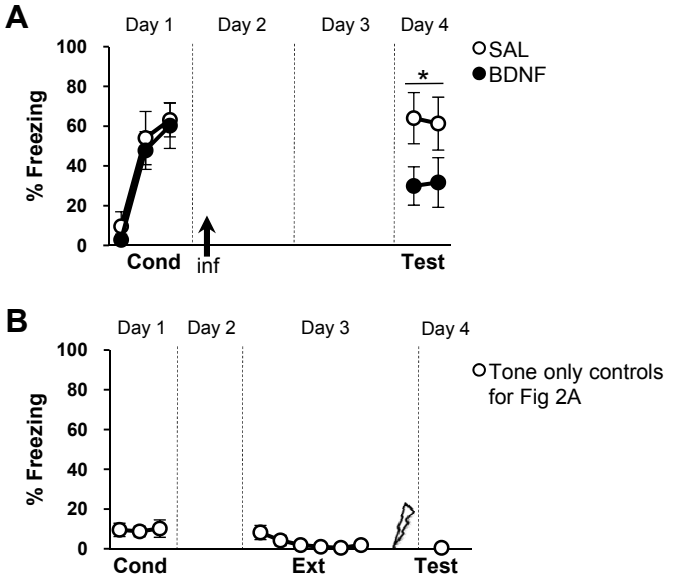

Fig S3. Reinstatement of freezing in BDNF-infused rats cannot be attributed to the BDNF effect "wearing off" or to contextual conditioning. (A) Rats were conditioned on Day 1, and on Day 2 received a BDNF (n = 8) or SAL (n = 7) infusion into IL. Rats remained in their home cages for Days 2-3, and were tested in Day 4. BDNF-extinction was still apparent at this 48-hour timepoint (two-way repeated measures ANOVA on Day 4, main effect of drug $F(1,13) = 5.072$, $p = 0.042$). (B) A tone only control group was examined under an experimental protocol comparable to that of the reinstatement experiment (Fig. 2A). These rats received tones during conditioning and extinction, but were never shocked. Exposure to reinstating shocks on Day 3 did not increase freezing on Day 4. Thus, no significant contextual conditioning was produced by the non-contingent shocks. * $p < 0.05$ BDNF compared to SAL.

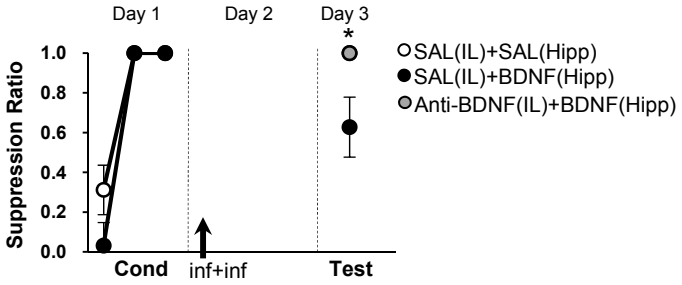

Fig S4. Conditioned suppression data for the same animals shown in Fig. 3C. BDNF infusion into hippocampus on Day 2 reduced suppression ratios on Day 3 (two-way repeated measures ANOVA, main effect of drug $F(2,21) = 6.080$, $p = 0.008$), but co-infusion of anti-BDNF into IL prevented this effect. Data are shown in blocks of two trials. * $p < 0.05$ comparing SAL(IL) + SAL(HIPP) to SAL(IL) + BDNF(HIPP).

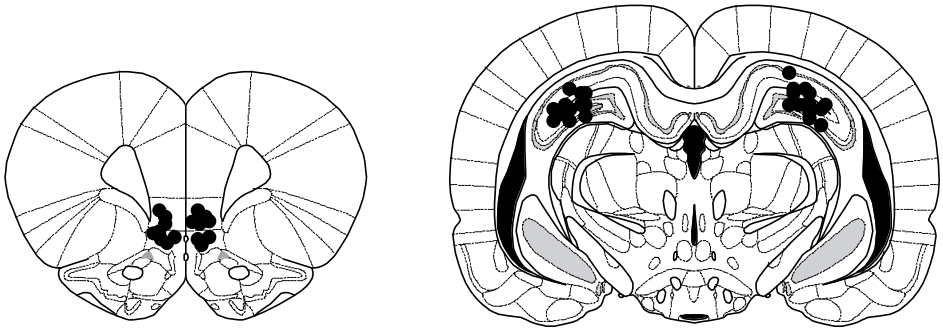

Fig. S5. Histological verification of BDNF infusion sites. Black dots indicate the lowest point of the injector tip for each rat. BDNF infusions spanned both infralimbic cortex and dorsopeduncular regions within the medial prefrontal cortex. For the hippocampal experiment, BDNF infusions were located within the posterior part of dorsal hippocampus, spanning CA1, dentate gyrus, and CA3.

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