Neuritin produces antidepressant actions and blocks the neuronal and behavioral deficits caused by chronic stress

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Decreased neuronal dendrite branching and plasticity of the hippocampus, a limbic structure implicated in mood disorders, is thought to contribute to the symptoms of depression. However, the mechanisms underlying this effect, as well as the actions of antidepressant treatment, remain poorly characterized. Here, we show that hippocampal expression of neuritin, an activity-dependent gene that regulates neuronal plasticity, is decreased by chronic unpredictable stress (CUS) and that antidepressant treatment reverses this effect. We also show that viral-mediated expression of neuritin in the hippocampus produces antidepressant actions and prevents the atrophy of dendrites and spines, as well as depressive and anxiety behaviors caused by CUS. Conversely, neuritin knockdown produces depressive-like behaviors, similar to CUS exposure. The ability of neuritin to increase neuroplasticity is confirmed in models of learning and memory. Our results reveal a unique action of neuritin in models of stress and depression, and demonstrate a role for neuroplasticity in antidepressant treatment response and related behaviors.

despair | anhedonia | BDNF | synaptogenesis

Major depressive disorder (MDD) is a devastating and recurrent illness affecting up to 17% of the population, resulting in personal disability, increased rates of suicide, and socioeconomic loss (1). Moreover, currently available antidepressants are only effective in approximately one-third of patients with MDD and in up to two-thirds after multiple trials, and they take weeks to months to produce a response (2, 3). In addition, the mechanisms underlying the therapeutic actions of antidepressants are poorly understood. New targets beyond monoamine signaling are now emerging in both preclinical and clinical reports of MDD (4, 5). These studies have focused on key limbic brain structures, including the hippocampus, that are significantly altered by chronic stress and depression and that are known to regulate mood, anxiety, and cognition (6, 7). Hippocampal synaptic plasticity has received much attention in recent years because human imaging and rodent studies demonstrate that stress and depression are associated with decreased hippocampal volume and atrophy of neurons (6, 8).

Neuritin, also known as candidate plasticity gene 15 (CPG15), encodes a small, extracellular GPI-anchored protein critical for dendritic outgrowth, maturation, and axonal regeneration (9–13). Neuritin expression in the hippocampus is induced by neuronal activity following chemical- or electrical-induced seizures (9, 14, 15), ischemia (16), and exercise (5, 17). Neuritin has been implicated in the actions of BDNF (9, 18), which is up-regulated in the hippocampus by antidepressant treatment and is sufficient to produce antidepressant behavioral responses (19, 20). Moreover, chronic antidepressant treatment has been shown to increase neuritin expression in rat brain (21). The current study was conducted to test the hypothesis that neuritin is a critical downstream mediator of antidepressant/BDNF-mediated plasticity and, conversely, that loss of neuritin could contribute to depressive symptoms caused by stress exposure.

Results

Neuritin Is Down-Regulated by Chronic Stress: Reversal by Antidepressant Treatment. Models of chronic unpredictable stress (CUS), which can precipitate or worsen depression, are typically used for preclinical studies of mood disorders. We have adopted a CUS model that results in a spectrum of cellular and behavioral abnormalities, notably anhedonia, a core symptom of patients with MDD that is reversed with chronic antidepressant treatment (22, 23).

Here, we show that neuritin mRNA levels are significantly decreased in the major subregions of the dorsal hippocampus, including CA1 and CA3 pyramidal and dentate gyrus (DG) granule cell layers [Fig. 1 *A* and *B*; Fisher's least significant difference (LSD), $P < 0.01$ compared with the nonstressed control]. There was no significant effect in the adjacent parietal cortex (Fig. 1*B*). In contrast, chronic administration (3 wk, initiated at day 15 of CUS) of the 5-hydroxytryptamine selective reuptake inhibitor, fluoxetine, significantly reversed the effects of CUS exposure in all hippocampal subregions (Fig. 1*B*; Fisher's LSD, $P < 0.05$ compared with CUS). Chronic fluoxetine treatment alone had no significant effect on neuritin expression in nonstressed animals ($P > 0.05$). Similar effects were found with quantitative real-time PCR of dissected hippocampus, although the combination of CUS + fluoxetine increased neuritin mRNA compared with fluoxetine alone (Fig. 1*C*; $P < 0.05$). Why fluoxetine alone did not produce a significant effect as previously reported (21) could be due to different doses (5 mg/kg vs. 10 mg/kg) or conditions resulting from the CUS handling paradigm.

Neuritin Increases Hippocampal Dendrite Complexity and Spine Density. Recent studies demonstrate that atrophy of neuronal processes contributes to the negative effects of stress/depression and can be reversed by certain antidepressants (24, 25). We investigated the effects of viral neuritin expression in the dorsal hippocampus on DG granule neuron dendrite branching, spine number, and spine head diameter. An adenoassociated virus (AAV) vector was designed to coexpress GFP and CMV promoter-driven AAV-neuritin (AAV-Nrn). Control AAV (AAV-

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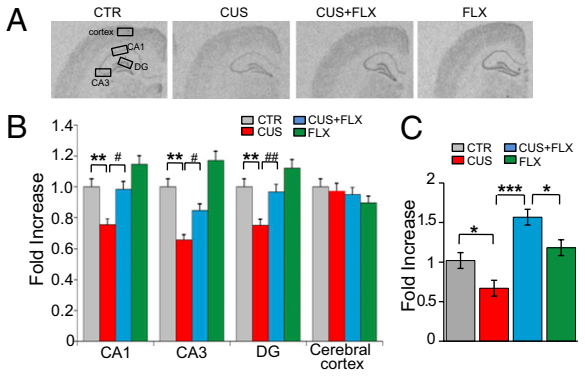

Fig. 1. Effects of CUS on neuritin expression, determined by in situ hybridization and quantitative real-time (RT) PCR. (*A*) Rats were exposed to CUS for 35 d and received either saline or fluoxetine (CUS + FLX) for the last 21 d, and sections were subjected to quantitative in situ hybridization. Representative autoradiograms from hippocampal sections from in situ hybridization are shown for home cage control (CTR), CUS, CUS + FLX-, and FLX-treated animals (n = 5 per group). (*B*) Quantified expression of neuritin mRNA from the indicated subregions in *A*. Results are expressed as a ratio of CTR and are the mean ± SEM, each analyzed in duplicate brain sections. Neuritin mRNA was decreased in CUS animals compared with CTR animals (CA1: $F_{3,\ 15} = 9.27$, $^{**}P < 0.01$; CA3: $F_{3,\ 15} = 13.45$, $^{**}P < 0.001$; DG: $F_{3,\ 15} = 11.96$, $^{**}P < 0.001$). CUS animals injected with FLX showed an increase in neuritin mRNA compared with CUS animals (CA1: $P = 0.011$; CA3: $P = 0.04$; DG: $P = 0.006$). FLX in the nonstressed CTR did not increase neuritin mRNA compared with CTR (CA1: $P = 0.075$; CA3: $P = 0.062$; DG: $P = 0.078$). In the cortex, there were no differences between groups ($P > 0.2$). $^{**}P < 0.01$ compared with CTR and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with CUS (two-way ANOVA, Fisher's LSD post hoc analysis). (*C*) Rats were exposed to CUS and FLX as described above, and whole hippocampus was subjected to quantitative RT-PCR analysis. Results are normalized to cyclophilin and expressed as the mean ratio of fold change ± SEM of six individual animals. $^{*}P < 0.05$; $^{***}P < 0.001$, two-way ANOVA.

ctl) expresses only GFP. Neuritin in situ hybridization and EGFP fluorescence immunostaining demonstrated a localized increase of neuritin in the DG of animals infected with AAV-Nrn relative to AAV-ctl animals (Fig. 2 *A* and *B*). Confocal microscopy revealed a striking remodeling of granule cell dendrites following neuritin overexpression (>4 dendritic branch points) relative to AAV-ctl (Fig. 2 *C* and *D*; $P < 0.001$). In addition, AAV-Nrn infusion significantly increased spine density and head diameter of mushroom-like spines by ∼70% (Fig. 2 *E*–*G*; $P < 0.001$ compared with the AAV-ctl group).

Synaptogenesis is accompanied by up-regulation of postsynaptic proteins, including PSD-95 and GluR1, as previously reported (25, 26). Studies in cultured hippocampal neurons demonstrate that AAV-Nrn increases the levels of PSD-95 (Fig. S1*A*). Similar effects were observed in response to AAV-Nrn infusions into hippocampal DG by immunohistochemical analysis of PSD-95 (Fig. S1 *B* and *C*).

Neuritin Produces Antidepressant-Like Behavioral Effects and Prevents the Effects of CUS. To test directly whether neuritin is sufficient to produce antidepressant-like actions, we investigated the influence of AAV-Nrn on rodent behavioral models of depression, anxiety, and antidepressant response. Behavioral testing was conducted 5 wk after viral infusion into dorsal DG, a time when AAV is fully expressed and stable (27) (Fig. 3*A*). In the novelty suppressed feeding test (28–30), AAV-Nrn significantly decreased the latency to feed, similar to the actions of antidepressant treatments (Fig. 3*B*; $P < 0.05$ compared with AAV-ctl). There was no effect on home cage feeding, indicating that there is no general effect on metabolic status (Fig. 3*D*). In the forced swim test (FST), which responds to acute antidepressant treatment, AAV-Nrn infusions markedly decreased immobility, a typical antidepressant

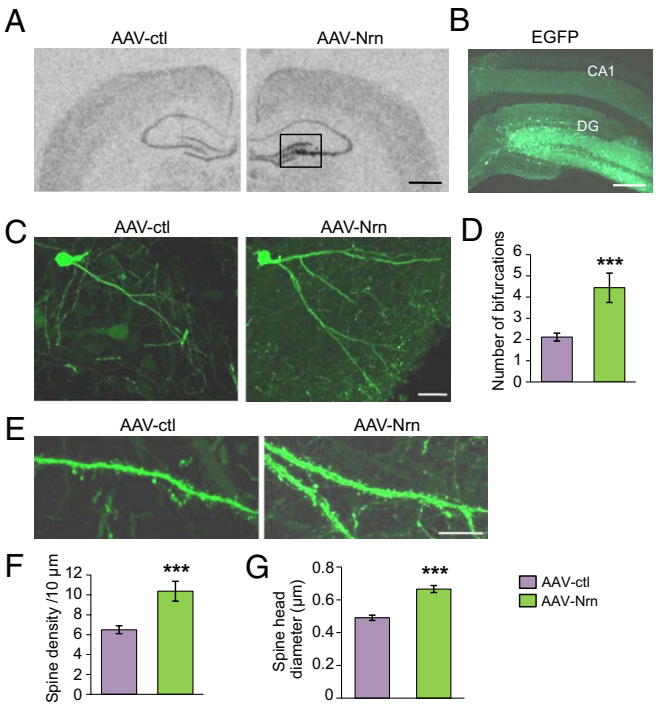

Fig. 2. Neuritin increases dendritic arborization and synaptogenesis. (*A*) In situ hybridization analysis was conducted to examine the expression of neuritin mRNA, and representative autoradiograms are shown for AAV-ctl and AAV-Nrn. (*B*) GFP staining is also shown and further demonstrates localization of AAV infection of the DG. (*C*) Representative images of GFP(+) cells from AAV-ctl– and AAV-Nrn–injected animals. A continuous stretch of dendritic shaft localized in the outer half of the granular layer was identified in the outer molecular layer and imaged. (*D*) Immunocytochemical analysis showing that the dendritic arborization was increased in AAV-Nrn–injected rats compared with AAV-ctl–injected rats ($^{***}P < 0.001$). The data were expressed as the number of bifurcations per GFP(+) neurons (n = 26 cells and n = 16 cells for AAV-ctl– and AAV-Nrn–injected groups, respectively). (*E*) Representative images are shown of high-magnification Z-stack projections of apical tuft segments of GFP(+) DG granule cells from AAV-ctl– and AAV-Nrn–injected rats. (*F*) Density of dendritic spines was significantly increased in AAV-Nrn–injected rats compared with AAV-ctl–injected rats. ($^{***}P < 0.001$) (n = 10 neurons from 6 rats in each group). The data were expressed as the number of spines per 10 μm. (*G*) Spine head diameter of mushroom spines was increased in AAV-Nrn–injected rats compared with AAV-ctl–injected rats ($^{***}P < 0.001$) (n = 12–15 neurons from 6 rats in each group). Results are the mean ± SEM. (Scale bars: *A*, 200 μm; *B*, 50 μm; *C* and *E*, 10 μm.) Student t test.

response (Fig. 3*C*; $P < 0.001$). AAV-Nrn had no effect on sucrose preference or active avoidance (Fig. S2 *A* and *B*). Spontaneous locomotor activity (LMA) was not different between these two groups (Fig. 3*E*).

To examine the antidepressant actions of neuritin further, we used a CUS model that decreases sucrose preference and impairs active avoidance, a measure of despair (25, 31). AAV-Nrn– and AAV-ctl–infused rats were randomly assigned to nonstressed control or CUS exposure for 21 d (Fig. 4*A*). CUS caused the predicted decrease in sucrose preference in control animals (AAV-ctl) (Fig. 4*B*; $P < 0.001$), and this effect was blocked by AAV-Nrn infusions ($P < 0.001$). There was no difference between AAV-Nrn–infused CUS rats and either of the nonstressed groups ($P > 0.05$). AAV-Nrn infusion had no effect in nonstressed rats. There were no differences in total fluid consumption in any of the groups (Fig. S3*C*).

In animals infused with AAV-ctl, CUS also increased the number of escape failures (Fig. 4*C*; $P < 0.01$), a despair phenotype consistent with previous findings (32). This effect was also

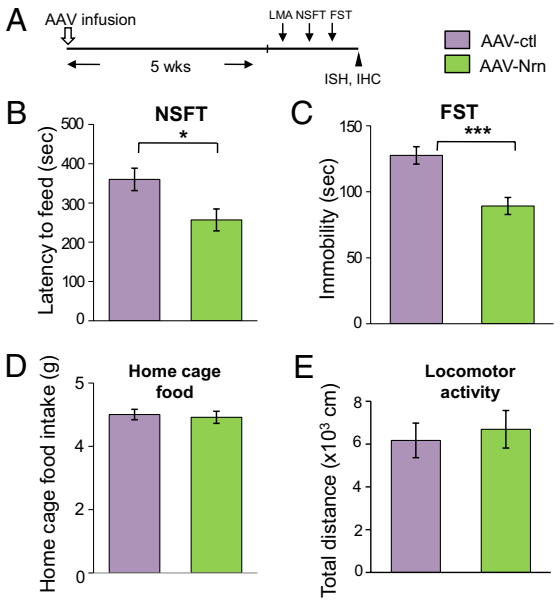

Fig. 3. AAV-Nrn infusions into the hippocampus produce antidepressant behavioral actions. (*A*) Experimental design. Animals were injected with AAV-ctl or AAV-Nrn; 5 wk later, they were tested in behavioral paradigms, and hippocampal sections were then harvested for in situ hybridization (ISH) and immunohistochemistry (IHC). (*B*) NSFT. A significant decrease in the latency to feed was shown in AAV-Nrn–injected animals compared with AAV-ctl–injected animals [$t_{(19)} = 2.57$, $^{*}P < 0.05$]. (*C*) FST. AAV-Nrn–injected rats had a shorter immobility score (time in seconds) than AAV-ctl–injected rats [$t_{(19)} = 4.14$, $^{***}P < 0.001$]. (*D*) Home cage feeding. There was no difference in the home cage food intake between AAV-ctl–injected and AAV-Nrn–injected rats [$t_{(30)} = 0.35$, $P = 0.73$]. (*E*) LMA. The total distance moved in the box was similar between groups [$t_{(19)} = 0.17$, $P > 0.5$]. Results are the mean ± SEM averaged from AAV-ctl–injected ($n = 10$) vs. AAV-Nrn–injected ($n = 11$) rats. Student *t* test.

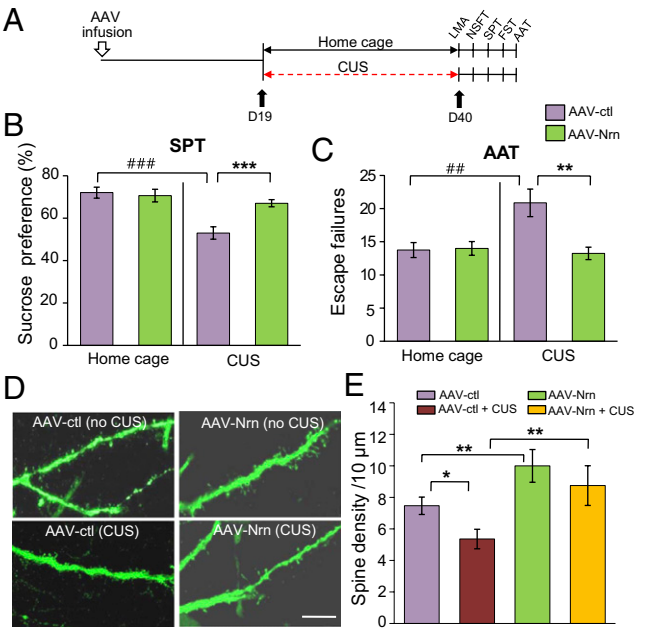

Fig. 4. AAV-Nrn infusions into the hippocampus block the behavioral deficits caused by CUS exposure. (*A*) Experimental design. Rats were injected with AAV-ctl ($n = 16$) or AAV-Nrn ($n = 16$). Each of the virus-infected cohorts was split into two experimental groups and exposed to home cage or CUS for 21 d. The efficacy of neuritin overexpression and CUS on behavioral performances of the animals was measured for 3–4 consecutive days starting on day 40 (D40). (*B*) SPT. Two-way ANOVA revealed that there was a main effect of virus ($F_{1, 28} = 5.91$, $P < 0.05$), a main effect of stress ($F_{1, 28} = 18.92$, $P < 0.001$), and interaction ($F_{1, 28} = 8.8$, $P < 0.01$). Further analysis indicates that CUS decreased sucrose preference compared with home cage in AAV-ctl–injected animals ($^{\#\#\#}P < 0.001$). Neuritin increased sucrose preference in CUS rats ($^{***}P < 0.001$) but not in home-caged rats ($P = 0.73$). There was no difference in AAV-Nrn–injected CUS animals and AAV-ctl–injected home cage animals ($P = 0.13$). (*C*) Active avoidance test (AAT). Two-way ANOVA: main effect of virus, $F_{1, 28} = 7.19$, $P < 0.05$; main effect of stress, $F_{1, 28} = 5.37$, $P < 0.05$; interaction $F_{1, 28} = 8.19$, $P < 0.01$. Further analysis indicates that CUS-exposed rats had more escape failures than home cage rats in AAV-ctl animals ($^{\#\#}P < 0.01$) and that neuritin overexpression decreased escape failures only in CUS rats ($^{**}P < 0.01$) but not in nonstressed rats ($P = 0.87$). There was no difference in AAV-Nrn–injected CUS animals and AAV-ctl–injected home cage animals ($P = 0.74$). (*D*) Representative images of high-magnification Z-stack projections of apical tuft segments of GFP(+) DG granule cells. (*E*) Density of dendritic spines. Two-way ANOVA: main effect of virus, $F_{3, 42} = 19.05$, $P < 0.001$; main effect of stress, $F_{3, 42} = 7.45$, $P < 0.01$; interaction $F_{3, 42} = 0.001$, $P > 0.05$. The density of dendritic spines was significantly decreased in CUS rats ($^{*}P < 0.05$), and the effects were blocked in AAV-Nrn–injected rats ($^{**}P < 0.01$). $n = 10$–19 GFP(+) neurons from four rats in each group. The data were expressed as the number of spines per 10 µm. (Scale bar: 10 µm.)

completely prevented by AAV-Nrn infusion ($P < 0.01$). CUS exposure also increased the latency to feed in the novelty suppressed feeding test (NSFT) (Fig. S3*A*; $P < 0.05$) and immobility in the FST (Fig. S3*B*; $P < 0.01$), and these effects were blocked by infusions of AAV-Nrn (Fig. 4*E*). There were no changes in spontaneous LMA after stress (Fig. S3*D*). In addition to these behavioral effects, infusion of AAV-Nrn before CUS exposure completely prevented the CUS-induced spine deficit (Fig. 4 *D* and *E*; $P < 0.01$).

Neuritin Knockdown Produces Depressive-Like Behaviors. Because CUS exposure decreases levels of neuritin in the hippocampus, we wanted to determine if knockdown of neuritin is sufficient to cause depressive behaviors. We used a lentivirus expressing shRNAs targeted against rat neuritin (lenti-shNrn), and lenti-EGFP as a control. Studies of hippocampal neurons in vitro demonstrate that lenti-shNrn decreases both basal and BDNF-induced neuritin levels (Fig. 5*B*). Infusion of lenti-shNrn into the hippocampus also decreased levels of neuritin mRNA in microdissections of the infused DG area (Fig. 5*C*; $P < 0.001$).

Lenti-shNrn produced behavioral effects opposite to antidepressant treatment, significantly increasing the latency to feed in the NSFT (Fig. 5*D*; $P < 0.05$), increasing immobility in the FST (Fig. 5*E*; $P < 0.001$), and decreasing sucrose preference (Fig. 5*F*; $P < 0.05$). These effects were observed in the absence of changes in home cage food intake or total LMA (Fig. 5 G and *H*). Together, the results demonstrate that neuritin knockdown in the DG is sufficient to cause depressive behaviors similar to those observed after CUS exposure for 3 wk.

We also examined the influence of neuritin knockdown on the antidepressant response to BDNF in the FST, as previously reported (33). Infusion of BDNF (0.25 µg per side) into DG resulted in the expected antidepressant-like decrease in immobility, and this effect was blocked by lenti-shNrn (Fig. 5*I*; BDNF vs. lenti-shNrn + BDNF; $P < 0.001$). BDNF did not produce a significant decrease in immobility in rats infused with lenti-shNrn (Fig. 5*I*; lenti-shNrn ± BDNF; $P > 0.05$).

Hippocampal-Dependent Learning Is Enhanced by Neuritin. Previous studies have implicated neural plasticity in the pathophysiology and treatment of depression (31). To test the role of neuritin in behavioral models of synaptic plasticity, we examined the influence of neuritin on two hippocampal-dependent learning tasks, object recognition and contextual fear conditioning (Fig. 6).

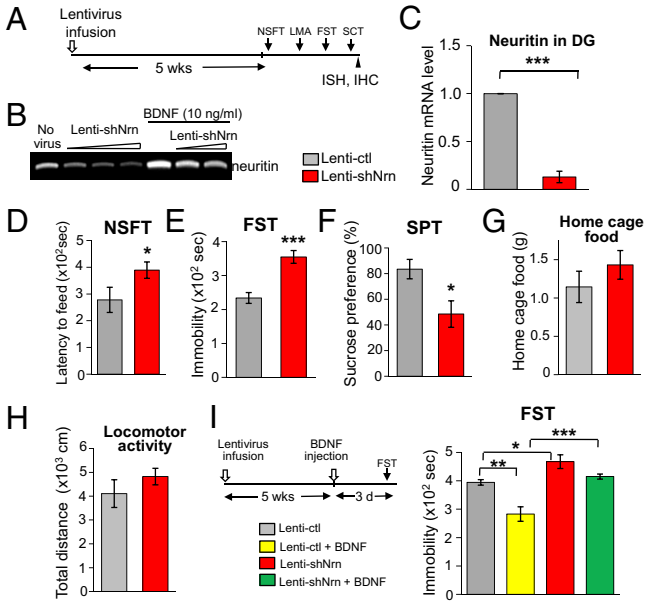

Fig. 5. Neuritin knockdown causes depressive behaviors. (*A*) Experimental design. Lenti-shNrn was infused into the hippocampal DG, and behavioral testing was initiated 5 wk later. (*B*) Knockdown efficiency of lenti-shNrn. Lenti-shNrn was transfected into rat primary hippocampal neuronal cells, and neuritin and β-actin mRNA levels were measured by real-time (RT) PCR. Treatment of cells with BDNF (10 ng/mL) for 6 h induced neuritin mRNA expression, and the increase was reduced by lenti-shNrn in vitro in a concentration-dependent manner. (*C*) Knockdown efficiency of lenti-shNrn. Quantitative RT-PCR of neuritin mRNA in microdissected DG from hippocampal sections taken from lenti-ctl–injected and lenti-shNrn–injected animals confirms that neuritin knockdown decreases neuritin mRNA in the DG (***$P < 0.001$). (*D*) NSFT. Lenti-shNrn infusion increased the latency to feed compared with lenti-EGFP–injected control animals (*$P < 0.05$, Student *t* test). (*E*) FST. Animals injected with lenti-shNrn had higher immobility times than those injected with lenti-EGFP when assessed by means of a 15-min test (***$P < 0.001$, Student *t* test). (*F*) SPT. Lenti-shNrn decreased sucrose preference compared with lenti-EGFP–injected animals (*$P < 0.05$, Student *t* test). (*G*) Home cage feeding. There was no difference in the amount in the home cages between groups ($P = 0.8$). (*H*) LMA. Lenti-shNrn did not influence LMA ($P = 0.16$, Student *t* test). Results are the mean ± SEM averaged from seven animals per group. (*I*) FST. (*Left*) Experimental design. Two-way ANOVA, main effect of virus: $F_{3, 21} = 25.79$, $P < 0.001$; main effect of BDNF: $F_{3, 21} = 16.50$, $P < 0.001$; higher immobility time by lenti-shNrn (*$P < 0.05$). BDNF-infused rats had a shorter immobility score than lenti-ctl–injected rats (**$P < 0.01$) and the effect of BDNF on immobility was blocked by lenti-shNrn (***$P < 0.001$). Scores are measured by means of a 15-min test. Results are the mean ± SEM averaged from seven animals per each group.

In object recognition, both groups readily discriminated the novel object from the familiar object during testing (Fig. 6*A*; familiar vs. novel object: AAV-ctl, $P < 0.05$; AAV-Nrn, $P < 0.05$). The time spent exploring the novel object was comparable between AAV-ctl and AAV-Nrn groups when tested 1.5 h after training, but when the retention interval was delayed for 24 h, the discrimination index was significantly higher for the AAV-Nrn rats (Fig. 6*B*; $P < 0.001$). There was also a strong exploration preference for the novel object in the AAV-Nrn rats at the 24-h time point (Fig. 6*B*; familiar vs. novel object, $P < 0.002$). In fear conditioning, there was no difference between the groups in postshock (immediate) freezing (Fig. 6*C*; $P > 0.1$). When the rats were returned to the original training context 24 h later, the AAV-Nrn group showed significantly higher levels of freezing compared with the AAV-ctl group, indicative of enhanced contextual fear conditioning (Fig. 6*D*; $P < 0.05$).

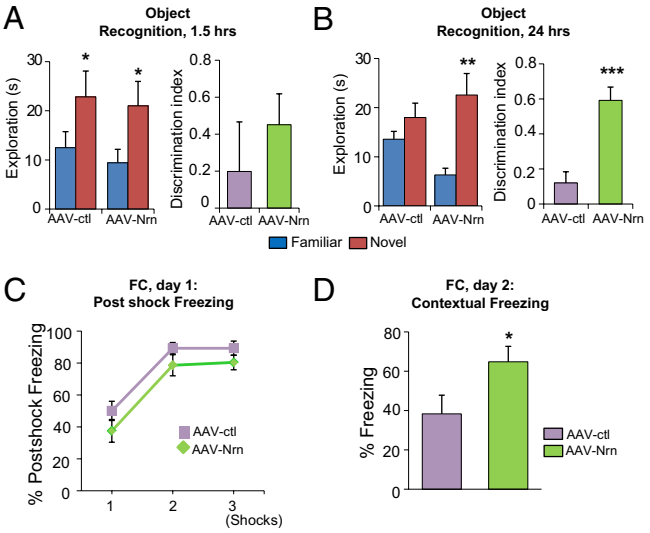

Fig. 6. AAV-Nrn infusion into the hippocampus improves hippocampal-dependent memory. (*A*) Rats were injected with AAV-ctl or AAV-Nrn and then tested in object recognition 30 d later. Both groups show comparable exploration of the novel object at 1.5 h after training (familiar vs. novel object, $P < 0.05$; $t < 1.00$, $P = 0.769$). The discrimination index was not different between the two groups ($t < 1.00$, $P = 0.427$). (*B*) In a separate experiment (45 d after virus infusion), where testing was conducted 24 h after training, rats injected with AAV-Nrn show significantly higher levels of discrimination between the familiar and novel objects [unpaired *t* test, familiar vs. novel object: $t_{(12)} = 3.47$, **$P < 0.005$]) compared with AAV-ctl (familiar vs. novel object: $t_{(12)} = 0.871$, $P = 0.4$). The discrimination index was significantly higher in animals injected with AAV-Nrn than in controls [$t_{(12)} = 4.76$, ***$P < 0.001$]. (*C*) Fear conditioning (FC). The freezing behavior of animals treated with AAV-ctl and AAV-Nrn (35 d after virus infusion) is shown. On the training day, both groups showed similar levels of freezing in the context after each single 2-s, 0.55-mA foot shock was delivered ($P > 0.05$). (*D*) FC, contextual test. AAV-Nrn animals exhibit significantly higher levels of freezing when reexposed to the fear-conditioned context 24 h after training [$t_{(14)} = 2.14$, *$P < 0.05$]. Results are presented as the mean ± SEM averaged from seven animals per each group. Unpaired *t*-test.

Discussion

The results of the present study demonstrate that CUS decreases hippocampal levels of neuritin and that viral expression of neuritin is sufficient to produce an antidepressant response and to prevent the morphological and behavioral deficits caused by CUS. Moreover, shRNA knockdown of neuritin in the hippocampus causes depressive behaviors. The mechanisms underlying the regulation of neuritin are unclear, but neuritin expression is regulated by pathways that mediate neuronal plasticity, including induction of BDNF expression and signaling (9), and are decreased by stress and increased by antidepressant treatment (19, 24).

Neuritin is enriched in synapses (10, 11), and increased expression could underlie the enhanced synaptic plasticity and dendrite morphology reported for antidepressants (24, 34). The induction of spine density and dendrite branching in response to increased neuritin expression is consistent with this hypothesis. Induction of PSD-95 is also consistent with increased synapse formation and function. Conversely, chronic corticosterone, which is increased by stress, reduces PSD-95 levels in the stratum lucidum of CA3 in mouse hippocampus (35), raising the possibility that neuritin induction of PSD-95 is involved in blockade of stress-induced deficits. Although the exact mechanisms are unknown, it is possible that a soluble form of neuritin might act as an extracellular signal to stimulate synaptogenesis as previously demonstrated (36). Neuritin expression occurs in progenitor populations in the developing brain and in some differentiated neurons during target selection and circuit formation (36). The

results of the current study indicate that neuritin is sufficient to induce synaptic remodeling of differentiated neurons in adult brain. Further studies will be required to determine if the soluble form of neuritin mediates this effect and serves as an activity-dependent differentiation factor.

The results of our behavioral studies demonstrate that the actions of viral neuritin expression are dependent on the type of test and prior stress exposure. Neuritin expression is sufficient to produce an antidepressant response in the FST and NSFT, models that are responsive to acute or chronic antidepressant administration in unstressed animals. However, there was no effect in either the number of escapes in the active avoidance test or preference in the sucrose preference test (SPT), models in which the behavioral deficits are produced by prior chronic stress and are reversed by antidepressant treatments. Only after CUS exposure did neuritin produce an antidepressant response in these models. These findings indicate that neuritin expression is sufficient to produce an antidepressant response in the absence of stress and to prevent or block the deficits caused by chronic stress exposure, presumably by compensating for the neuritin and synaptogenic deficits caused by stress. Conversely, the results of the lenti-shNrn knockdown experiments demonstrate that neuritin is required for normal responding in the NSFT, FST, and SPT consistent with the possibility that neuritin loss could underlie the behavioral deficits caused by CUS exposure. Moreover, analysis of postmortem tissue from depressed subjects previously described (37) demonstrates that levels of neuritin are decreased by 57 percent in the DG of the hippocampus compared to controls ($P < 0.05$), raising the possibility that neuritin deficits could contribute to neuronal atrophy and behavioral symptoms in depressed patients. We also found that lenti-shNrn knockdown of neuritin reverses the antidepressant effect of BDNF in the FST, supporting the hypothesis that neuritin is induced by and contributes to the antidepressant actions of BDNF.

The results also demonstrate that neuritin enhances memory retention but not immediate acquisition in both the object recognition and contextual fear conditioning tasks. These effects may be related to the role of neuritin in neurite outgrowth (9–13) and spine formation (present study), which could facilitate synaptic plasticity. The ability of neuritin to enhance memory in these models is consistent with the hypothesis that the actions of antidepressant treatment are mediated by increasing neural plasticity (6, 31). This may be particularly true for hippocampal-dependent plasticity, given the enrichment of neuritin in this region. Our findings are consistent with recent data that neuritin regulates synapse stabilization, resulting in efficient learning (38). Together, the results suggest overlap of the cellular, neuroplasticity-related mechanisms underlying the antidepressant and memory-enhancing actions of neuritin.

Elucidating the mechanisms for the antidepressant actions of neuritin in the hippocampus is an important avenue of research for future investigations. One possibility is that neuritin is involved in regulation of newborn neurons in the adult hippocampus (39). Recently, it has been shown that adult-born hippocampal granule neurons buffer stress responses at both the endocrine and behavioral levels (40). Increased expression of neuritin in the hippocampus could play a role in adult neurogenesis, and thereby regulate stress and antidepressant behaviors in addition to learning and memory.

Based on the findings presented here, it is possible that neuritin deficits contribute to the atrophy of hippocampal neurons during the course of lifetime stress exposures, or even during a critical developmental period, and thereby lead to increased vulnerability to anxiety and mood disorders. Development of strategies that target neuritin or related signaling pathways could represent unique approaches for improved antidepressant therapy.

Materials and Methods

A detailed description of the materials and methods used in this study is provided in *SI Materials and Methods*.

Animals and Drug Treatment. Male Sprague–Dawley rats weighing 160–250 g (Charles River Laboratories) were used. All procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Yale University Animal Care and Use Committee.

CUS Procedure. The CUS animals were subjected to exactly the same sequence of 12 stressors (2 per day for 21–35 d) described by Banasr et al. (23).

Behavioral Experiments. The FST (26) and NSFT (23), as well as learned helplessness (33) and LMA (6) tests, were conducted as previously described. Behavioral tests were analyzed by an experimenter blinded to the study code.

shRNA Preparation and Stereotaxic Surgery. We used shRNA constructs for neuritin (36) and a control nontargeting shRNA (pll3.7; American Type Culture Collection). Bilateral viral injections were performed with coordinates −4.1 mm (anterior/posterior), ±2.4 mm (lateral), and −4.1 mm (dorsal/ventral) relative to the bregma.

Spine Density, Spine Head Diameter, and Dendritic Arborization Analysis. Images were acquired through Z-stacks, which typically consisted of 10 scans at high zoom at 1-μm steps in the *z* axis. Each GFP(+) granule neuron was clearly distinguishable from other cells. Spine density, spine head diameter, and dendritic arborization were analyzed in each section by an experimenter blinded to the study code.

Statistical Analysis. Statistical differences were determined by ANOVA (StatView 5; SAS Software) followed by Fisher's LSD post hoc analysis. The *F* values and group and experimental degrees of freedom are included in the legends of the figures. For experiments with two groups, the Student *t* test was used. The level of statistical significance was set at $P < 0.05$ using two-tailed tests.

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