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A role for p11 in the antidepressant action of brain-derived neurotrophic factor

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Abstract

Background—p11 (also called S100A10) is down-regulated in human and rodent depressive-like states. Considerable experimental evidence also implicates p11 in the mechanism of action of antidepressant drugs and electroconvulsive seizures, in part due to its interaction with specific serotonin receptors. Brain-derived neurotrophic factor (BDNF) has been linked to the therapeutic activity of antidepressants in rodent models and humans. In the current study, we investigated whether BDNF regulates p11 *in vitro* and *in vivo*.

Methods—We utilized primary neuronal cultures, *in vivo* analyses of transgenic mice, and behavioral techniques to assess the effects of BDNF on p11.

Results—Results indicate that BDNF stimulates p11 expression through trkB receptors and via the MAPK signaling pathway. BDNF-induced changes in p11 *in vivo* correlate with changes in ligand binding to the 5-HT1B receptor, the subcellular localization of which is known to be regulated by p11. Behavioral studies demonstrate that p11 KO mice are insensitive to the antidepressant actions of BDNF.

Conclusions—Taken together, our data demonstrate that p11 levels are regulated by BDNF *in vitro* and *in vivo*, and that the antidepressant-like effect of BDNF in two well-established behavioral models requires p11. These data support a role for p11 in the antidepressant activity of neurotrophins.

Keywords

S100A10; BDNF; 5-HT1B; depression; neurotrophin; neurogenesis

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Introduction

Major depression is currently treated with agents that increase neurotransmission via the monoamines, serotonin and/or noradrenaline. It is generally agreed that increased monoamine transmission initiates cellular adaptations that ultimately lead to a therapeutic action. Identification, and ultimately more direct targeting, of these downstream mediators could lead to novel therapeutics that are both faster acting and produce fewer off-target effects.

p11 has recently been described as a key factor involved in both depressive-like states and antidepressant responses(1–3). Specifically, p11 is down-regulated in post-mortem tissue from depressed individuals(1,2,4) and is a mediator of responses to antidepressant drugs in rodents(2,3). P11 is a member of the S100 family of calcium effector proteins. Like other S100 proteins, the localization and function of p11 depend heavily on the tissue and/or cell-type in which the protein is expressed (for review, see (5)). However, p11 is unique among its family members since it is insensitive to calcium due to mutations in both EF-hand calcium binding motifs(6).

p11 interacts with a subset of serotonin receptors(2,3) to regulate the localization of these receptors at the cell surface. Our previous work has shown that p11 knockout mice have a depression-like phenotype and show a diminished behavioral response to antidepressants(2,3). Furthermore, transgenic over-expression of p11 produces antidepressant-like effects in mice(2). Both electroconvulsive seizures and chemical antidepressants increase p11(2,3), but the underlying mechanism has not been elucidated.

A neurotrophic hypothesis of depression supports a role for brain-derived neurotrophic factor (BDNF) in the therapeutic activity of antidepressants(7,8). Two recent meta-analyses of the clinical literature report positive correlations between serum levels of BDNF and antidepressant responses in depressed individuals(9,10). BDNF modulates synaptic plasticity, promotes neuronal cell survival, and influences adult hippocampal neurogenesis, all of which are linked to the cellular actions of antidepressants(8,11). Furthermore, BDNF can mimic the action of an antidepressant in several well-established behavioral paradigms(12–14).

Mature BDNF is released by neurons and other cell-types and binds specifically to its receptor, tropomyosin-related kinase B (trkB). The activation of trkB receptors is amplified by both mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3'-kinase (PI3K) signal transduction pathways. Mature and immature BDNF also activate the p75 neurotrophin receptors (p75NTR) and the nuclear-factor kappa B (NFkB) pathway to mediate functions including cell survival(15). BDNF influences cell growth, survival, and synaptic plasticity through regulation of gene transcription. Serotonin and BDNF can also influence the development of a serotonergic cell phenotype through a positive feedback loop(16–18).

Antidepressants increase BDNF in rodents with a time course resembling the delayed therapeutic onset in humans. For example, two to three weeks are required for clinical effectiveness of selective serotonin reuptake inhibitors (SSRIs) in humans. A similar time course is required for an SSRI to increase BDNF mRNA and protein in the rodent frontal cortex and hippocampus(19,20). Correspondingly, in the current report, we found that treatment with citalopram increased p11 in these brain regions with a similar time course to SSRI-induced BDNF. This temporal correlation led us to examine the possibility that BDNF could regulate p11.

Methods and Materials

Animals and drug treatments

8–10 week old male mice were used for all experiments, and were housed 4–5 per cage with *ad libitum* access to food and water. C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA), BDNF transgenic mice that over-express BDNF under a CAMK2a promoter and wild-type (WT) C57BL/6J controls were purchased from the Jackson Laboratory (Bar Harbor, ME). Floxed BDNF knockout mice that lack BDNF in neurons expressing CRE under the CAMK2 promoter and wild-type (WT) C57BL/6J controls were produced as previously described(21) (KO used in Figures 4–5 and S1). BDNF heterozygous knockout mice were purchased from The Jackson Laboratory and backcrossed to the C57BL/6 strain to generate mice used for primary neuronal cultures. P11 knockout (KO) mice (derived and maintained at The Rockefeller University) were also used(2). Citalopram hydrobromide (Sigma, St. Louis, MO) was administered in the drinking water (0.16 g/L), which contained 1% saccharin to mask any taste of the drug, for 1 or 14 days. Mice drank approximately 4–5 ml per day, resulting in an average daily oral dose of 20–25 mg/kg/day. Control groups drank 1% saccharin solution. Animal use and procedures were in accordance with the National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committees.

Primary neuronal cultures

Dissociated neuronal cultures express the cellular machinery (including trkB receptors and p11) needed to study these signal transduction pathways without the complexity of intact brain circuitry. Mixed cortical primary neuronal cultures were derived from E18 timed pregnant female C57BL/6 mice (Charles River Laboratories, Wilmington, MA). Since BDNF knockout mice (The Jackson Laboratory) do not survive past postnatal day 14, heterozygous mice were mated and single pup cultures were derived from E18 embryos. Genotypes were determined before stimulation with 5-HT. Cultures were prepared as described(3). Cells were used at 5–7 days *in vitro*.

Reagents for cell culture studies

BDNF, cycloheximide solution, LY294002, and serotonin hydrochloride were purchased from Sigma (St. Louis, MO). U0124, U0126, SN50, SN50M were purchased from Calbiochem (EMD Chemicals Inc., Gibbstown, NJ). K252a was purchased from Invitrogen (Carlsbad, CA). 1000× stock solutions were prepared immediately before use to achieve final concentrations of 10 μ M for U0124/U0126, 50 μ M for LY294002, 18 μ M for SN50/SN50M, and 500 nM for K252a. Inhibitors were applied 30 minutes prior to BDNF (50 ng/ml) or vehicle stimulation. Doses were chosen based on previous literature.

Sample preparation and Western blotting

Mice were anesthetized with carbon dioxide and brains were rapidly dissected and frozen at -80°C until use. Hippocampus or frontal cortex was sonicated in lysis buffer (PBS with 2mM EDTA, 2mM EGTA, 1% Triton $\times$ 100, 0.5% SDS, and protease inhibitors). Primary neuronal cultures were rinsed in ice-cold PBS, lysed, sonicated, and centrifuged before use. Protein concentrations were measured by BCA assay (Pierce) according to the manufacturer's instruction. All samples were separated on 16% Tricine gels (Invitrogen, Carlsbad, CA), transferred to PVDF membrane, blocked in 5% non-fat dry milk in Tris-buffered saline + 0.1% Tween-20 (TBST), and incubated with primary antibodies diluted in milk overnight at 4°C (anti-S100A10, 1:200, R&D Systems; anti- β actin, 1:2,000, Cell Signaling Technologies). Membranes were washed in TBST and incubated with HRP-conjugated secondary antibodies for 1 hour at 4°C (rabbit anti-goat 1:5,000; goat anti-rabbit

1:5,000). Blots were incubated in ECL reagent (Perkin Elmer, Waltham, MA), exposed to film (Kodak), and optical densities were quantified using NIH Image software.

Quantitative real-time RT-PCR (qPCR)

Total RNA was isolated from primary neuronal cultures using the RNAqueous kit (Ambion) according to the manufacturer's instructions. 25 ng of total RNA was used to synthesize cDNA (Superscript III kit, Invitrogen, Carlsbad, CA) using oligo(dT)₂₀ primers according to the manufacturer's instructions. Each biological sample was assayed in triplicate and was normalized to β -actin. Primers (500 nm final concentration) were obtained from Primer Bank. Using iQ syber green supermix (Biorad, Hercules, CA) and iQ5 multiplex real-time detection hardware (Biorad), samples were subjected to 45 cycles (94°C, 30 s; 63°C, 30 s; 72°C, 30 s) followed by a melt-curve. iQ5 optical system software was used to analyze the data.

In situ hybridization (ISH)

Radiolabeled riboprobes for p11 were used for ISH as described(2). After hybridization, the sections were exposed to Biomax MR film (Kodak) and were analyzed using NIH Image software. Optical density was measured in the indicated regions and data are expressed as a percent of the WT control group.

Ligand binding

Ligand binding was performed on 12 μ m thick brain sections as described(2).

Surgical procedures

Stereotaxic surgeries were performed on p11 KO and WT mice under ketamine (60 mg/kg) and xylazine (3 mg/kg) anesthesia. A cannula (Plastics One, Roanoke, VA) was implanted into the lateral ventricle (coordinates from bregma: +0.2 mm anterior/posterior, -0.75 mm mediolateral, and -1.8 dorsoventral from dura according to the atlas of Franklin and Paxinos, 2008). Recombinant BDNF (100 ng/ μ l) was delivered i.c.v. via subcutaneous microosmotic pump (Durect Corp., Cupertino, CA) at a rate of 1 μ l/h for 3 days, resulting in a final dose of 2.4 μ g/day.

Behavioral assays

The Tail Suspension Test (TST), Forced Swim Test (FST) and Open Field locomotor activity were performed as described(3).

Statistical Analyses

Unless otherwise noted, all comparisons were made by Student's t-test. Statistical significance was set at $p < 0.05$.

Results

Fourteen days of treatment with citalopram (for dose, please see *Methods and Materials*) significantly increased p11 protein (Fig. 1A) in the mouse hippocampus (to $162.7\% \pm 10.5$, $n=5$, $p < 0.05$) and frontal cortex (to $144\% \pm 13.6$, $n=9$, $p < 0.05$) compared to vehicle, while 1 day of citalopram had no effect on p11 (not shown). This time course was consistent with previous reports of antidepressant-induced BDNF(19,20).

To test whether BDNF stimulates p11 expression, we applied varying doses of BDNF to primary mixed cortical neuronal cultures derived from E18 mouse embryos. Western blotting analysis revealed that p11 protein was significantly increased by BDNF at

concentrations of 10–50 ng/ml (Fig. 1B). At a 50 ng/ml concentration, the time course for this effect indicated that p11 was increased within 3 hours of stimulation and sustained for at least 24 hours (Fig. 1C). BDNF stimulation also significantly increased p11 mRNA (Fig. 1D).

The increase in p11 mRNA following BDNF stimulation indicated an increase in p11 synthesis. Next, we evaluated whether there was an effect of BDNF on the breakdown of p11. Cycloheximide, a protein synthesis inhibitor, was first added to neuronal cultures in order to prevent any additional protein (e.g., p11) from being produced. Then, we applied vehicle or BDNF, lysed neurons at different time points (from 15 min to 24 hours), and measured p11 protein levels in order to assess the degradation of p11 and whether BDNF slowed its degradation. Western blotting analysis of p11 indicated no differences between vehicle and BDNF stimulated cultures at any time point tested (Figure S1 in the Supplement). This also allowed us to detect the time course of p11 degradation, estimating its half-life at 2–3 hours in our neuronal culture system. These results show that BDNF induces expression of p11 mainly by increasing its synthesis and not by inhibiting its breakdown.

Next, we investigated the signal transduction pathway that mediates this effect. Mature BDNF binds specifically to the trkB receptor and activates both MAPK and PI3K pathways. Multiple neurotrophins, including both mature and immature BDNF, bind to p75NTR and promote neuronal survival through activation of NFkB(15). A previous publication reported a potential NFkB binding site in the human p11 promoter sequence(22). To first determine if the effect of BDNF on p11 was mediated through trkB, we applied BDNF to primary cultured neurons in the presence or absence of the trkB inhibitor K252a. K252a completely abolished the induction of p11 protein by BDNF (Fig. 2A). To identify which of the three signal transduction pathways mediated the induction of p11 by BDNF, we applied BDNF to primary cultured neurons in the presence of an inhibitor of MAPK kinase (U0126) or inactive analog (U0124), an inhibitor of PI3K (LY294002), an inhibitor of NFkB (SN50) or inactive analog (SN50M), or vehicle. U0126 abolished the induction of p11 by BDNF stimulation (Fig. 2B), while other inhibitors had no effect.

If antidepressants, by increasing extra-synaptic 5-HT, increase p11 in a BDNF-dependent way, then 5-HT should increase p11 only if BDNF is present. To test this possibility, we stimulated primary neuronal cultures derived from wild-type (WT) or BDNF knockout (KO) mouse embryos with 5-HT. 5-HT increased p11 in WT neurons, but not in neurons derived from BDNF KO mice (Fig. 3). Cultures derived from BDNF KO mice had significantly lower basal levels of p11 compared to WT (Fig. 3).

To test the effects of BDNF on p11 *in vivo*, we took advantage of transgenic mice that either lack BDNF (KO) or over-express BDNF (OE) in the forebrain under a CAMK2 α promoter. *In situ* hybridization analyses revealed that there is a complete KO of BDNF in KO mice, and that in OE mice, BDNF over-expression was most prominent in the cortex compared to WT littermates (see Figure S2 in the Supplement). p11 mRNA and protein were decreased in the cortex of BDNF KO mice compared to WT littermates (Fig. 4A,B). There was also less p11 mRNA detected in the striatum of BDNF KO mice ($52.53\% \pm 3.118$) compared to WT ($100\% \pm 7.893$, $p < 0.001$). BDNF OE mice showed significantly increased cortical p11 mRNA and protein compared to WT (Fig. 4C,D).

We previously reported that p11 interacts with 5-HT_{1B} receptors to increase receptor expression at the cell surface(2). To test whether BDNF also increased receptor expression at the cell surface, we used the radioligand [¹²⁵I] cyanopindolol to measure surface levels of the 5-HT_{1B} receptor in brain slices. There was significantly less 5-HT_{1B} (Fig. 5A) ligand

binding in the cortex (Fig. 5A) and striatum ($87 \pm 2\%$ vs. $100 \pm 4\%$, OD (% control) $\pm$ SEM, $p < 0.05$) of BDNF KO mice compared to WT controls. Conversely, in BDNF OE mice, where p11 is elevated, there was a corresponding increase in 5-HT_{1B} ligand binding in the cortex (Fig. 5B) compared to WT mice. The 11% increase in 5-HT_{1B} ligand binding observed in the striatum of BDNF OE mice did not reach statistical significance. There was no effect of BDNF KO or OE on 5-HT_{1B} ligand binding in the hippocampus. 8-OH-DPAT is a ligand for the 5-HT_{1A} receptor, a receptor that does not interact with p11(2,3). There was no effect of BDNF over-expression on 8-OH-DPAT binding (Fig. 5C).

Both BDNF and p11 produce robust antidepressant-like effects in rodent models of depression(12–14) and both are required for antidepressant activity(23). To determine if p11 is required for behavioral responses to BDNF, WT and p11 KO mice were injected intracerebroventricularly with BDNF before testing in the tail suspension test (TST) or forced swim test (FST), two well-established paradigms in which reductions in immobility reflect antidepressant activity. BDNF produced a statistically significant antidepressant-like effect in WT mice that was not observed in p11 KO mice in both the TST (Fig. 6A) and the FST (Fig. 6B), suggesting that the antidepressant activity of BDNF requires p11. There were no effects of p11 KO and/or BDNF on locomotor activity (Fig. 6C).

Discussion

We have previously shown in rodents that (1) cortical p11 expression is increased by antidepressant drugs or electroconvulsive seizure and that (2) p11 is necessary for antidepressant responses(2,3). In the present study, we have investigated the mechanism by which antidepressants increase p11. Electroconvulsive seizures and three classes of antidepressant drugs increase BDNF protein levels in the rodent frontal cortex(24). Chronic, but not acute, treatment with antidepressants increases BDNF, correlating with the time course required for therapeutic efficacy(19,20). Antidepressant-induced increases in p11 follow a similar time course (see reference² and current report). Here we report that BDNF increases p11 mRNA and protein in neurons via trkB receptor activation and a MAPK signal transduction pathway.

Antidepressants increase extra-synaptic levels of serotonin, which initiates a cascade of events that culminate in a therapeutic antidepressant response. The details of this cascade have not yet been fully elucidated, but the involvement of BDNF has been supported by a large numbers of studies (for review, see(25)). Serotonin and its receptors have previously been shown to mediate antidepressant-induced increases in BDNF(26–29). Here we find that serotonin also increases p11 levels, but that serotonin has no effect on p11 in neurons cultured from BDNF KO mice, suggesting that BDNF is required for serotonin-induced increases in p11. We cannot exclude the possibility that serotonin receptors are desensitized in BDNF KO cultures since alterations in the serotonin system have been reported in adult BDNF KO mice(18,30). However, in the context of our other results, the most straightforward interpretation is that serotonin-induced increases in p11 are BDNF dependent. A previous report has shown that BDNF heterozygous mice are less responsive to an SSRI and have increased 5-HT_{1B} and 5-HT_{2A} receptor mRNA expression in the cortex compared to WT mice(31). The diminished SSRI response is consistent with our findings, since p11 KO mice also have a diminished response to antidepressants. The reported increase in 5-HT_{1B} mRNA detected in BDNF heterozygous mice could be compensating for reduced p11 levels and 5-HT_{1B} surface expression, which we report here. In addition, we find that neurons from BDNF KO mice express less p11 than WT cultures, suggesting that BDNF could maintain endogenous levels of p11.

To determine the effects of BDNF on p11 *in vivo*, we took advantage of transgenic mice which lack (KO) or over-express (OE) BDNF in the forebrain. Since p11 interacts directly with certain 5-HT receptors, including 5-HT1B receptors to increase their expression at the cell surface(2), we investigated whether expression of 5-HT1B receptors was affected in BDNF transgenic mice. We found a direct relationship between BDNF, p11 mRNA and protein, and cell surface expression of 5-HT1B in the cortex of adult mice. Specifically, BDNF KO mice had decreased p11 expression and 5-HT1B ligand binding in the cortex compared to WT littermates, and BDNF OE mice had increased p11 levels and 5-HT1B ligand binding compared to WT littermates. Therefore, it appears that 5-HT, through increasing BDNF, increases p11 levels and results in the redistribution of 5-HT receptors to the plasma membrane of nerve cells.

We also found that BDNF KO mice had decreased p11 expression in the striatum. Corticostriatal projection neurons are known to express and release BDNF(32). Since the striatum has emerged as an important substrate for p11 effects on depressive-like states,(4) these projection neurons could be important for (1) preventing depressive-like states and (2) mediating the therapeutic effect of antidepressants. For example, loss of corticostriatal BDNF could lead to reduced levels of p11 in the striatum, which induces depressive-like behavior. In addition, replacing BDNF in the cortex could reverse the depressive-like effect of striatal p11 loss. Future studies will address this issue *in vivo*.

Finally, we report that p11 KO mice do not respond to BDNF in two paradigms that measure antidepressant efficacy: the TST and the FST. We cannot exclude the possibility that p11 KO mice could respond to a higher concentration of BDNF. However, the effective dose for WT mice has no effect on p11 KO mice, suggesting that p11 is required to either mediate or amplify the actions of BDNF in these paradigms. Intracerebroventricular delivery of BDNF produces a behavioral antidepressant response(12) that could be due to its activity in the cortex and/or hippocampus. BDNF infusions directly into the hippocampus also cause antidepressant activity(13). Conversely, conditional BDNF KO mice show attenuated antidepressant responses measured by the FST(33). Selective regional loss of BDNF or trkB receptors in the hippocampal dentate gyrus also attenuates the response to antidepressants in rodent models(23,34). Our results provide strong evidence that behavioral responses to BDNF require p11.

A leading hypothesis of depression supports a role for neurotrophins such as BDNF in the cellular adaptations that underlie the therapeutic actions of antidepressants(8,11,25). BDNF influences adult hippocampal neurogenesis, which is increased by antidepressant treatments (35,36) and is required for the behavioral response to an SSRI(37). Our results suggest that the level of p11 is regulated by BDNF, so the potential effect of p11 on neurogenesis is an important avenue of investigation. In fact, we have now found that p11 is required for the effects of antidepressants on neurogenesis(38).

We conclude that p11 levels are regulated by BDNF *in vitro* and *in vivo*, and that the behavioral antidepressant-like effect of BDNF in the TST and FST requires p11. Work is ongoing to identify the circuit(s) and cell-type(s) that are involved. In addition, preliminary *in silico* analysis of the p11 promoter region has identified a number of putative transcription factor binding sites in the p11 promoter. Several of these transcription factors are known to be regulated by the MAPK signaling pathway. Current studies are also aimed at determining whether if these factors mediate the effects of BDNF on p11 transcription.

Taken together, our data support a role for p11 in the antidepressant activity of neurotrophins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1. BDNF induces p11 expression

Representative western blot from mice treated with 0 or 14 days of citalopram (CIT) (A). Primary mixed cortical neuronal cultures were stimulated with BDNF, and p11 protein (B,C) and mRNA (D) was measured by western blotting or quantitative RT-PCR analyses, respectively. (B) Dose response: representative blot (top) and quantification of at least three independent experiments (bottom). (C) Time course: representative blot (top) and quantification of at least three independent experiments (bottom). (D) p11 mRNA measured by quantitative RT-PCR. All data are presented as means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

Fig 2. BDNF increases p11 through trkB receptors and a MAPK signal transduction pathway
 (A) Western blotting analyses of p11 or actin loading control in primary neuronal cultures stimulated with BDNF in the presence of a trkB inhibitor (K252a) or vehicle. Representative blot (top) and quantification of at least three independent experiments (bottom). (B) Stimulation with BDNF in the presence of a MAPK inhibitor (U0126) or inactive analog (U0124), a PI3K inhibitor (LY294002), an NFkB inhibitor (SN50) or inactive analog (SN50M), or vehicle. Representative blot (top) and quantification of at least three independent experiments (bottom). All data are presented as means $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ (ANOVA and post-hoc Fisher's LSD test).

Fig 3. 5-HT increases p11 in WT, but not BDNF KO mice

Primary cultures derived from WT or BDNF KO mouse embryos (single pup cultures, see *Methods*) were stimulated with 5-HT for the indicated times. Representative western blot for p11 or actin loading control (top) and quantification of at least three independent experiments (bottom). All data are presented as means $\pm$ SEM. * $p < 0.05$ (ANOVA and post-hoc Fisher's LSD test).

Fig 4. Regulation of p11 by BDNF in mouse cortex

(A) ISH analyses reveal less p11 mRNA in BDNF KO mice (representative autoradiography, top; quantification of p11 mRNA, $n=6$, bottom) and (B) corresponding decrease in p11 protein (representative western blot, top; quantification of p11 protein, $n=6$, bottom). (C) Analyses of p11 mRNA in BDNF-OE mice reveal a significant increase in cortex compared to WT (representative autoradiography, top; quantification of p11 mRNA, $n=6$, bottom). (D) Western blotting analyses show corresponding increase of p11 protein in these mice (representative western blot, top; quantification of p11 protein, $n=6$, bottom). Arrows in (A) and (C) indicate region of cortex measured for quantification. All data are presented as means $\pm$ SEM. * $p<0.05$, ** $p<0.01$.

Fig 5. BDNF transgenic mice show changes in 5-HT_{1B} ligand binding

Regulation of $[^{125}\text{I}]$ cyanopindolol (5-HT_{1B}) ligand binding in BDNF KO mice (A) and BDNF-OE mice (B), but not of $[^3\text{H}]$ 8-OH-DPAT (5-HT_{1A}) ligand binding in BDNF-OE mice (C). Representative images (top) and quantification (bottom) of 6 mice per group. Arrows indicate region of cortex measured for quantification. All data are presented as means $\pm$ SEM. * $p < 0.05$.

Fig 6. p11 mediates the behavioral antidepressant action of BDNF

BDNF reduces immobility in WT but not p11 KO mice in the Tail Suspension Test (A, n=21–23), or the Forced Swim Test (B, n=8–10). (C) No differences in locomotor activity measured in the Open Field (C, n=8–10). All data are presented as means ± SEM. *p<0.05, ** p<0.01 (ANOVA and post-hoc Fisher's LSD test).