Corrections

CELL BIOLOGY


The authors note that, due to a printer’s error, the author name Eric H. Baehreck should have appeared as Eric H. Baehrecke. Additionally, the author name Christina McPhee should have appeared as Christina K. McPhee. The corrected author line appears below. The online version has been corrected.

Yueguang Rong, Christina K. McPhee, Shuangshen Deng, Lei Huang, Lilian Chen, Mei Liu, Kirsten Tracy, Eric H. Baehrecke, Li Yu, and Michael J. Lenardo

DEVELOPMENTAL BIOLOGY


The authors note that on page 8693, left column, first paragraph, line 9, “T-to-C” should instead appear as “T-to-A.” The authors note that on page 8693, right column, first paragraph, lines 3 and 4, “Axin2smp/Axin2null mice (n=15)” should instead appear as “Axin2smp/Axin2null mice (n=15).”

NEUROSCIENCE


The authors note that the following acknowledgment was omitted from the article: “This work was supported by NIH Grant MH090963.”

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NEUROSCIENCE

The authors note that Figures 2, 3, and 4 appeared incorrectly. The corrected figures and their legends appear below.

**Fig. 2.** RR blocks inward currents of AgOrco alone and in complex. (A–C) Representative traces of RR-blocked inward currents in AgOrco (A) and AgOrco+AgOr10 (B and C) cells. Holding potential was −60 mV for A–C. (D) Analysis of RR blockage of VUAA1 and BA-induced currents from A (n = 5), B (n = 5), and C (n = 4).

**Fig. 3.** AgOrco is a functional channel and responds to VUAA1 in outside-out membrane patches. (A) Single-channel recording from an outside-out excised patch pulled from a cell expressing AgOrco. (B–D) Expansions of trace A before (B), during (C), and after (D) a 5-s application of −4.0 logM VUAA1. All-point current histograms of trace expansions are on the right sides of B–D. Excised membrane patch was held at −60 mV.

**Fig. 4.** 8-Br-cAMP and 8-Br-cGMP did not elicit currents in AgOrco or AgOrco+AgOr10 cells. (A) Representative trace from whole-cell recordings from cells expressing AgOrco with application of 8-Br-cAMP, 8-Br-cGMP, and VUAA1. (B) Representative trace from cells expressing AgOrco+AgOr10 with application of 8-Br-cAMP, 8-Br-cGMP, BA, and VUAA1. (C) Representative trace from cells expressing rCNGA2 with application of 8-Br-cGMP. Holding potentials for all recordings were −60 mV. (D) Histogram of normalized currents from cyclic nucleotide and control responses (n = 4). All currents normalized to VUAA1 responses.

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Antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) are attenuated by antiinflammatory drugs in mice and humans

Jennifer L. Warner-Schmidt1, Kimberly E. Vanover2, Emily Y. Chen3, John J. Marshall3, and Paul Greengard3,4

*Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10065; and *Intra-Cellular Therapies, New York, NY 10032

Contributed by Paul Greengard, March 28, 2011 (sent for review March 10, 2011)

Antinflammatory drugs achieve their therapeutic actions at least in part by regulation of cytokine formation. A “cytokine hypothesis” of depression is supported by the observation that depressed individuals have elevated plasma levels of certain cytokines compared with healthy controls. Here we investigated a possible interaction between antidepressant agents and antiinflammatory agents on antidepressant-induced behaviors and on p11, a biochemical marker of depressive-like states and antidepressant responses. We found that widely used antiinflammatory drugs antagonize both biochemical and behavioral responses to selective serotonin reuptake inhibitors (SSRIs). In contrast to the levels detected in serum, we found that frontal cortical levels of certain cytokines (e.g., TNFα and IFNγ) were increased by serotonergic antidepressants and that these effects were inhibited by antiinflammatory agents. The antagonistic effect of antiinflammatory agents on antidepressant-induced behaviors was confirmed by analysis of a dataset from a large-scale real-world human study, “sequenced treatment alternatives to relieve depression” (STAR*D), underscoring the clinical significance of our findings. Our data indicate that clinicians should carefully balance the therapeutic benefits of antiinflammatory agents versus the potentially negative consequences of antagonizing the therapeutic efficacy of antidepressant agents in patients suffering from depression.

citalopram | fluoxetine | S100A10

Mood disorders including major depressive disorder (MDD) affect as many as one in five individuals and are the most prevalent psychiatric conditions (1). Approximately one-third of patients suffering from MDD are refractory to any kind of antidepressant treatment including selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and electroconvulsive therapy (ECT) (2).

The hypothesis that cytokines play a role in depression is based in part on the observations that (i) many patients undergoing treatment involving IFNα or interleukin-2 develop depressive symptoms; (ii) sickness behavior induced by the endotoxin lipopolysaccharide (LPS) or interleukin-1 shares some common features with depression; (iii) several cytokines can activate the hypothalamic–pituitary–adrenal (HPA) axis, which is commonly activated in depressed individuals; and (iv) some cytokines regulate brain norepinephrine or serotonin systems, which are linked to MDD and its treatment (3–6).

p11, a member of the S100 family of proteins, is a small acidic protein that interacts with specific serotonin receptors to regulate their trafficking and influence their localization at the cell surface (7, 9). This action affects the excitability of the cells and leads to profound behavioral responses. p11 knockout (KO) mice display a depressive-like phenotype and p11 overexpressing mice display antidepressant-like responses in classical behavioral paradigms, including the tail suspension and forced swim tests (7). Genetically “helpless” mice, which exhibit some symptoms of depression, as well as humans suffering with MDD, have reduced levels of p11 mRNA and protein in the cerebral cortex and striatum (7, 10). In rodents, three classes of antidepressants (SSRIs, TCAs, and ECT) increase p11 levels in the cerebral cortex and hippocampus (7, 8). Here we report an investigation of antiinflammatory drugs and of antidepressant agents on p11 expression and antidepressant behavior.

The “sequenced treatment alternatives to relieve depression” (STAR*D) clinical trial was a large, real-world study of treatment-resistant depression that evaluated a series of treatments and clinical outcomes. Many patients continue to suffer residual symptoms after weeks of treatment with a single agent and even after trying many different antidepressants and combination therapies. The STAR*D study found that only 36.8% of patients exhibited remission after treatment with the SSRI, citalopram (CT), and found a cumulative remission rate of 67% after multiple treatments were attempted (11). The underlying factors contributing to treatment resistance remain unclear. We used the STAR*D dataset to determine whether nonsteroidal antiinflammatory drugs (NSAIDs) could play a role in the treatment outcome of depressed individuals taking SSRIs.

Here we test a model in which SSRI antidepressants increase brain levels of certain cytokines, which in turn regulate p11 levels and ultimately control the behavioral response to an SSRI antidepressant (Fig. 1). Importantly, we have identified an antagonism by NSAIDs of SSRI responsiveness, which is likely mediated through the action of certain cytokines and p11 in the brain. We believe that this antagonism contributes, in part, to the high resistance rates to SSRIs seen in MDD. We believe that reduced use of NSAIDs by physicians in severely depressed patients being treated with SSRIs would significantly improve positive outcomes from this major class of antidepressant.

Results

Effects of SSRIs and NSAIDs on Certain Cytokines and p11. We measured mouse brain levels of cytokines using a bead-based ELISA following chronic treatment with the SSRI citalopram in the presence or absence of ibuprofen (IBU) cotreatment (SI Materials and Methods). We focused on the frontal cortex, a brain area that is strongly linked to antidepressant responses in mice and humans (12, 13). Results identified cytokines that fell into one of three major categories: (i) cytokines that were increased by citalopram, the effect of which was abolished by IBU cotreatment (Fig. 2A); (ii) cytokines that were increased by citalopram, the effect of which was not affected by IBU cotreatment (Fig. 2B); or (iii) cytokines that were not changed by either

Author contributions: J.L.W.-S., K.E.V., and P.G. designed research; J.L.W.-S., K.E.V., E.Y.C., and J.J.M. performed research; J.L.W.-S., K.E.V., E.Y.C., and J.J.M. analyzed data; and J.L.W.-S., K.E.V., and P.G. wrote the paper.

The authors declare no conflict of interest.

See Commentary on page 8923.

1To whom correspondence may be addressed. E-mail: jschmidt@rockefeller.edu or greengard@rockefeller.edu.

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citalopram or IBU (IL-1α, IL-4, and IL-13). IBU reduced plasma levels of both citalopram and its metabolite dideethylsamethyl citalopram (ddCIT) compared with mice that received citalopram alone (CIT: 1508.36 ± 282.7 versus 537.03 ± 65.8 pg/μL, P < 0.05; ddCIT: 128.0 ± 21.0 versus 34.5 ± 10.8 pg/μL, P < 0.01).

We have shown previously that p11 is increased in the mouse frontal cortex by multiple classes of antidepressants (7, 8). Here we investigated whether antiinflammatory agents alone or in combination with antidepressants regulated p11 levels. Interestingly, coadministration of either ibuprofen (IBU) or another NSAID, acetylsalicylic acid (ASA), with antidepressants for 14 d blocked the increase in p11 caused by the two different SSRIs, citalopram or fluoxetine (Fig. 2B). The tricyclic antidepressant (TCA) desipramine induced a small increase in p11, and this increase was not significantly affected by IBU or ASA [Fig. 2B, interaction NSAID × antidepressant: F(6, 52) = 4.48, P < 0.01]. Taken together, these experiments show that SSRI antidepressants increase brain levels of certain cytokines and p11, the effect of which is abolished by NSAID cotreatment.

Antidepressant-Induced Increases in p11 Levels Are Cytokine Dependent. Two of the cytokines that were regulated by both citalopram and ibuprofen, namely, IFNγ and TNFα, were further studied as possible mediators of the inhibitory effects of NSAIDS on SSRI-induced p11 levels described above. First, we investigated whether IFNγ or TNFα signaling is required for antidepressant-induced increases in p11, using IFNGR1 KO or TNFR1 KO mice. Western blotting analysis of frontal cortex from IFNGR1 KO, TNFR1 KO, or WT control mice treated with chronic citalopram revealed that both IFNGR1 and TNFR1 signaling are necessary for the increase in p11 by citalopram. Citalopram significantly increased p11 in WT, but not IFNGR1 KO or TNFR1 KO mice [Fig. 3A, interaction genotype × treatment F(1, 22) = 6.22, P < 0.05].

To examine whether IFNγ or TNFα was sufficient to increase p11 in vivo, mice were injected with recombinant IFNγ, TNFα, or vehicle and euthanized 4 h later. Western blot analysis revealed that both cytokines significantly increased p11 protein in the frontal cortex compared with vehicle injected controls [Fig. 3B, F(2, 16) = 8.519, P < 0.01].

Immunohistochemical detection of IFNγ receptor 1 (IFNGR1), p11, and the neuronal marker NeuN demonstrated that neurons in layer 5 of the mouse cortex express both p11 and IFNGR1 (Fig. S1). TNF receptor 1 (TNFR1) was also coexpressed with p11 in cortical neurons (Fig. S1). These data support the idea that these cytokines may regulate p11 levels.

NSAIDs Prevent the Antidepressant-Like Effect of SSRIss in Classical Behavioral Paradigms. Because p11 has been shown to be both necessary and sufficient for behavioral antidepressant responses (7–9) and IBU potently inhibited antidepressant-induced increases in p11 (Fig. 2B), we examined the possibility that IBU might inhibit the behavioral response to antidepressant drugs. We tested various classes of antidepressants including SSRIss (citalopram and fluoxetine), TCAs (imipramine and desipramine), a MAOI (tranylcypromine), and an atypical antidepressant (bupropion) in two well-established mouse models of depression: the tail suspension test (TST) and the forced swim test (FST).

Antidepressant-induced increases significantly reduced immobility time in both the TST [Fig. 4A, interaction antidepressant × NSAID: F(4, 95) = 6.11, P < 0.001] and the FST [Fig. 4B, interaction antidepressant × NSAID: F(6, 106) = 5.07, P < 0.001]. IBU significantly attenuated the antidepressant-like effects of SSRIs in both tests (Fig. 4A and B). IBU was less effective in altering the behavioral response to TCAs and failed to alter the behavioral response to other classes of antidepressant drugs.

To examine the specificity of the effect of IBU on SSRI-induced behavioral changes, we tested the effect of three different NSAIDs and an analgesic on the behavioral response to citalopram. Mice were treated with IBU (1 mg/mL), naproxen (2 mg/mL), acetylsalicylic acid (3 mg/mL), or acetaminophen (3 mg/mL) in their drinking water for 5–7 d and received a single injection of citalopram (20 mg/kg, i.p.) or saline before testing in the TST or FST. All of the drugs tested significantly blocked the antidepressant effect of citalopram on immobility time in both tests (Fig. 4 C and D) compared with mice that received citalopram alone [TST interaction antidepressant × NSAID: F(6, 129) = 3.25, P < 0.01; FST interaction antidepressant × NSAID: F(6, 106) = 5.07, P < 0.005].

To determine whether ibuprofen inhibits the behavioral response to chronic SSRI administration, we coadministered citalopram and ibuprofen for 2 wk before testing mice in the tail suspension test (TST) and the forced swim test (FST). All of the drugs tested significantly reduced immobility time in both the TST [Fig. 4A, interaction antidepressant × NSAID: F(4, 95) = 6.11, P < 0.001] and the FST [Fig. 4B, interaction antidepressant × NSAID: F(6, 106) = 5.07, P < 0.001]. IBU significantly attenuated the antidepressant-like effects of SSRIs in both tests (Fig. 4A and B). IBU was less effective in altering the behavioral response to TCAs and failed to alter the behavioral response to other classes of antidepressant drugs.

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novelty suppressed feeding (NSF) test or TST. In both tests, ibuprofen blocked the behavioral antidepressant-like response to citalopram [Fig. 4E and F, NSF interaction antidepressant × NSAID: $F(1, 56) = 4.23, P < 0.05$; TST interaction antidepressant × NSAID: $F(1, 56) = 4.23, P < 0.05$]. There were no differences among groups tested in home cage feeding (vehicle 0.08 ± 0.024; citalopram 0.1 ± 0.033; ibuprofen 0.08 ± 0.02; ibuprofen and citalopram 0.08 ± 0.02). Citalopram or ibuprofen and citalopram significantly increased bodyweight (vehicle 23.3g ± 0.27; citalopram 24.7 ± 0.23; ibuprofen 23.4 ± 0.35; ibuprofen and citalopram 25.0 ± 0.20, $P < 0.01$).

**Effects of Cytokines and p11 on Behavioral Responses.** Cytokines, their receptors, and p11 are expressed by neurons, glia, and endothelial cells. To determine whether the effects of antidepressants, IFN$\gamma$, or TNF$\alpha$ on immobility required neuronal

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**Fig. 3.** IFN$\gamma$ and TNF$\alpha$ are necessary and sufficient for antidepressant-induced increases in p11 levels. (A) Western blot analysis of p11 protein or actin loading control in the frontal cortex of WT, IFNGR1 KO, or TNFR1 KO mice treated for 3 wk with citalopram. Representative blots (Upper); quantification of 8–10 mice per group (Lower). All data are presented as means ± SEM. Statistically significant effects of antidepressants (**$P < 0.01$) or genotype (*$P < 0.05$) are noted. (B) Western blot analysis of p11 or actin loading control reveals that acute i.p. injection of IFN$\gamma$ (10 μg/kg bodyweight) or TNF$\alpha$ (10 μg/kg bodyweight) significantly increases p11 protein in mouse cortex compared with vehicle injected controls. Representative blots (Upper); quantification of 5–6 mice per group (Lower). All data are presented as means ± SEM. (*$P < 0.05$).

**Fig. 4.** Effects of antidepressants and NSAIDs on behavioral responses. NSAIDs and other analgesics attenuate the behavioral response to SSRIs. IBU (5–7 d) diminished the behavioral response to the selective serotonin reuptake inhibitors citalopram (CIT) and fluoxetine (FLX), was less effective in altering behavioral responses to the tricyclic antidepressants imipramine (IMI) and desipramine (DMI), and did not affect responses to other classes of antidepressants, including the monoamine oxidase inhibitor inhibitor transcytropic (TCP) and the atypical antidepressant bupropion (BUP) in two tests of antidepressant activity, the tail suspension test (A) and forced swim test (B). Mice receiving 5–7 d of nonsteroidal antiinflammatory drugs ibuprofen (IBU), naproxen (NPX), acetylsalicylic acid (ASA), or the analgesic acetaminophen (ACE) showed diminished response to citalopram (CIT) in the tail suspension test (C) and forced swim test (D). There was no response to chronic citalopram treatment when ibuprofen was coadministered before testing in the tail suspension test (E) or the novelty suppressed feeding test (F). All data are presented as means ± SEM. Statistically significant effects of antidepressants (**$P < 0.05$) or NSAIDs/analgesics (*$P < 0.05$, **$P < 0.01$) are noted. $n = 8–16$ per group.

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p11 expression, we generated a conditional p11 KO mouse by crossing a mouse with lox-P sites flanking exon 2 of the p11 gene with a mouse expressing the Cre recombinase under the CAMK2α promoter (14, 15). The CAMK2α promoter was chosen because it is expressed only in neurons of the forebrain. The resulting p11 KO mouse lacked p11 only in cells that expressed the CAMK2α-Cre. Immunohistochemical analysis of the p11 KO mice showed a complete lack of p11 protein expression in the neurons of the cortex and hippocampus, and lessened expression in the striatum (Fig. S2). Citalopram had no effect on TST immobility in p11 KO mice (Fig. S4). In contrast, p11 KO mice responded normally to a tricyclic antidepressant, desipramine, underscoring the specificity of our results for serotonergic antidepressants (Fig. S4).

Acute injection of IFNγ (10 μg/kg) or TNFα (10 μg/kg) before testing in the TST significantly reduced immobility in wild-type mice, consistent with an antidepressant-like effect [Fig. 5B, interaction genotype × treatment F(2, 64) = 3.55, P < 0.05]. A lower dose of IFNγ (1 μg/kg) or TNFα (1 μg/kg) had no effect on TST immobility (IFNγ 107.9% ± 9.6, n = 6; TNFα 98.6% ± 7.3, n = 5). IFNγ or TNFα had no effect on TST immobility in p11 KO mice [Fig. 5B, interaction genotype × treatment F(2, 77) = 5.18, P < 0.01], suggesting that the antidepressant-like actions of IFNγ and TNFα are mediated by p11 in neurons of the cortex.

We tested whether behavioral responses to chronic SSRI treatment were absent in IFNGR1 KO or TNFR1 KO mice. The effect of citalopram on TST immobility was abolished in IFNGR1 KO mice, but not in TNFR1 KO mice [Fig. 5C, interaction genotype × treatment F(2, 71) = 93.32, P < 0.0001]. Citalopram significantly reduced the latency to feed in WT mice but not in IFNGR1 KO or TNFR1 KO mice in the NSF test [Fig. 5D, interaction genotype × treatment F(4, 107) = 8.39, P < 0.0001]. Both KO mouse lines responded normally to desipramine in the TST (WT: 61.03% ± 9.9; IFNGR1 KO: 71.56% ± 9.3; TNFR1 KO: 65.4% ± 8.1, percentage of vehicle control group ± SEM), demonstrating the specificity for serotonergic antidepressants.

**NSAIDs and Other Analgesics Inhibit SSR1 Efficacy in a Clinical Population.** The TST and FST are two well-established rodent behavioral paradigms that predict antidepressant efficacy in humans (16, 17). To determine whether NSAIDs influence SSR1 efficacy in clinically depressed individuals, we took advantage of the STAR*D dataset. The first level of this large scale clinical trial investigated remission rates in depressed patients taking citalopram for 12 wk (Materials and Methods). Contingency tables and analyses were performed on 1,546 human subjects for which there were concomitant medication data and a data point at week 12 for presence/absence of clinical “remission” from depressive symptoms (Table 1). These data show that 182 subjects were in remission at the end of 12 wk of treatment with citalopram and had taken an NSAID at least once during those 12 wk. There were 628 subjects in remission who had not taken an NSAID. There were 227 subjects who were treatment resistant (i.e., did not experience remission) and had taken an NSAID at least once during the 12 wk of treatment. Finally, there were 509 subjects who were treatment resistant and had not taken any NSAID.

Of those subjects who took an NSAID, 45% were in remission, and 55% were treatment resistant. Of those subjects who did not take an NSAID, the reverse relationship was observed with 55% in remission and 45% being treatment resistant. In other words, a higher percentage of patients were treatment resistant to citalopram if they had taken an NSAID than if they had not taken an NSAID. This relationship was statistically significant (P = 0.0002). Similar analyses were conducted for other analgesics and similar results were found. Subjects taking other analgesics were less likely to undergo remission (37% in remission) compared with those not taking analgesics (54% in remission, P = 0.0002). Another analysis was conducted to determine whether the relationship between remission and concomitant medication was strongest for subjects who were taking both NSAIDs and other analgesics. Despite the relatively fewer number of subjects taking both NSAIDs and other analgesics, the relationship appeared to be quite strong with 63% of subjects who were taking both types of concomitant medication.

### Table 1. Effects of NSAIDs and other analgesics on treatment response to citalopram in humans

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Remission</th>
<th>No remission</th>
<th>Remission rate (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSAID</td>
<td>182</td>
<td>227</td>
<td>44.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>No NSAID</td>
<td>628</td>
<td>509</td>
<td>55.2</td>
<td></td>
</tr>
<tr>
<td>Analgesic</td>
<td>52</td>
<td>88</td>
<td>37.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>No analgesic</td>
<td>758</td>
<td>648</td>
<td>53.9</td>
<td></td>
</tr>
<tr>
<td>Both (NSAID and analgesic)</td>
<td>23</td>
<td>40</td>
<td>36.5</td>
<td>0.0138</td>
</tr>
</tbody>
</table>

| None | 787 | 696 | 53.1 |
| Either (NSAID or analgesic) | 211 | 275 | 43.4 |
| None | 599 | 461 | 56.5 |
| Vitamins | 44 | 31 | 58.7 |
| No vitamins | 766 | 705 | 52.1 |

Number of patients in remission or not in remission at the end of 12 wk of treatment with citalopram shown as a function of presence or absence of various concomitant treatments. Data were analyzed using Fisher’s exact test; P < 0.05 indicated a statistically significant relationship between the concomitant medication and the treatment outcome.
of medication failing to show remission (P = 0.0138) in contrast to 47% failure for patients taking neither.

Moreover, a contingency table was set up for subjects who had taken either NSAIDs or analgesics. The relationship between taking either of these medications and being more likely to show treatment resistance was highly statistically significant (P < 0.0001).

Importantly, there seemed to be specificity in the relationship between the presence of an NSAID and/or other analgesics with treatment resistance (lack of remission) because there was no relationship between treatment remission and other concomitant medications such as vitamins.

**Discussion**

Previous work from our laboratory indicated that p11 is a determinant of depressive-like states and antidepressant responses (7–9). Here we provide evidence that antidepressants increase brain levels of certain cytokines, which increase p11 levels, which then induce antidepressant-like behavioral responses (Fig. 1). We have further shown that IFNγ and TNFα are two cytokines that may be involved in this process. Anti-inflammatory drugs antagonized both the induction of p11 by and the behavioral response to SSRI antidepressants. We used the STAR*D dataset to confirm our results in a clinical population. Consistent with our mouse studies, we found that human patients reporting concomitant NSAID or other analgesic treatments showed a reduced therapeutic response to citalopram. Concomitant use of NSAIDs may be an important reason for high SSRI treatment resistance rates. We suggest that NSAIDs and other analgesics may potentially interfere with the therapeutic efficacy of SSRIs. P11 expression is detected in various brain areas including the frontal cortex, hippocampus, striatum, amygdala, and dorsal raphe nucleus (7). Overexpression of p11 in the forebrain mimics the action of an antidepressant (7). Here we show that p11 in forebrain neurons is necessary for the action of an SSRI antidepressant, but not a tricyclic antidepressant, suggesting that SSRI and noradrenergic antidepressants might act through different mechanisms and that p11 is selectively involved in pathways related to SSRI activity.

The antidepressant-like effects of TNFα on p11 and behavior could be mediated by direct effects of TNFα on neurons or indirectly through neurotrophic factors. TNFα increases production by astrocytes of neurotrophic factors, including nerve growth factor (NGF), glia-derived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF) (18, 19). TNFα also increases BDNF in human cerebral endothelial cells (20), and BDNF (i) increases p11 in the mouse cerebral cortex and (ii) has antidepressant-like and neurogenic effects that require p11 (8, 21). The effects of TNFα on levels of neurotrophins may be an important component of the mechanism by which TNFα produces an antidepressant-like response in rodent models of depression.

Our results further suggest that, like TNFα, IFNγ mediates its antidepressant activity through its action on p11. IFNγ increases p11 through a direct interaction with IFNγ binding sites on the p11 promoter (22). IFNγ increases neurite outgrowth (23), promotes neuronal differentiation (24), and enhances neurogenesis (25), all of which are consistent with antidepressant-like activity (26). However, it has been suggested that IFNγ mediates depression-behaviors caused by immune activation (27). Others have suggested that IFNγ plays a role in depression-like behaviors, but find that IFNγ KO mice respond normally to chronic unpredictable stress in the FST (28). These results are not inconsistent with our findings. It is well established that there is an increase in the level of plasma cytokines in depressed subjects (4). We speculate that the production of these cytokines and their actions in the periphery may be distinct from those local effects observed in brain areas like the frontal cortex. It is also possible that the increased levels of certain cytokines in the periphery of depressed individuals are involved in efforts by the brain to compensate for depression.

Future studies will be necessary to determine the mechanism by which NSAIDs inhibit SSRI efficacy. Acetaminophen is not generally thought to be antiinflammatory, so perhaps the anti-pyretic actions of the NSAIDs and analgesics is more related to their antagonism of SSRI potency. The two drugs could interfere with each other in the periphery, because most NSAIDs do not readily cross the blood brain barrier and because blood levels of citalopram and its metabolite were decreased in mice that also received ibuprofen. We cannot exclude the possibility that NSAIDs are having a direct effect on the interaction between SSRIs and the serotonin transporter, even though the involvement of p11 in antidepressant activity is mediated by neurons in the forebrain.

Analysis of our clinical data strongly suggests that remission rates among depressed individuals may be improved by avoiding certain common over-the-counter medications such as ibuprofen, aspirin, and acetaminophen. Depressed patients treated for 12 wk with citalopram are significantly less likely to show full remission if they are concomitantly taking NSAID and/or other analgesics.

The data suggest that treatment with NSAIDs prevents clinical responses to antidepressants. However, it is possible that underlying condition(s) contribute to treatment resistance rather than any one particular mechanism of action of concomitant medication. Indeed, it has been reported that depressed patients with painful physical symptoms took longer to achieve remission from depressive symptoms and were less likely to achieve remission than patients without pain (29). We cannot exclude the possibility that severity of depression and accompanying pain symptoms could be associated with antidepressant treatment resistance. However, in one study, Leuchter and colleagues (29) found that statistically for potential confounding factors such as race, sex, ethnicity, and severity of depression at baseline, and report that the statistical significance of the relationship between pain and remission from depression was lost. They concluded that the presence and severity of physical pain are not predictors of poor antidepressant treatment outcome, but that physical pain is associated with some factors that are predictors. Our present data suggest that at least one of the factors associated with physical pain that is a predictor of SSRI treatment outcome is concomitant therapy with NSAIDs and other analgesics. Despite the lack of understanding of causality associated with our clinical data analysis, our animal data strongly suggest that treatment with NSAIDs and/or other analgesics prevents antidepressant action of SSRIs.

Because the clinical analyses were conducted as post hoc analyses, it would be informative to evaluate the effects of NSAIDs and other analgesics on SSRI antidepressant response in a prospective, double-blind, randomized clinical study. Specifically, it will be important to standardize medications to better evaluate their role in determining treatment outcome. In the present study, no adjustments were made for multiple comparisons in the analyses. However, the effects were highly statistically significant such that small statistical adjustments would not affect the overall statistical significance or interpretation of the data. Moreover, the lack of a significant relationship between vitamins and clinical response suggests specificity to the underlying mechanisms by which NSAIDs and other analgesics prevent clinical remission. In addition, medical coding for concomitant medication in the database may not have been consistent across subjects or medications. Individuals taking many prescription medications may have been less likely to report over-the-counter medications, so reports of over-the-counter NSAIDs and analgesics may be underrepresented in the STAR*D dataset. Also, there are no data regarding dose of concomitant medications in the database and there is little information regarding duration of use (i.e., duration is only
available for some medications). The utility of the data shown in Table 1 may be limited by not being able to differentiate between subjects taking concomitant medications for a single administration or only rarely and subjects taking the medications chronically. The magnitude of effect for the relationship between major depression and treatment resistance may be even greater for those subjects who chronically take NSAIDs and/or other analgesics. Lastly, any subjects who discontinued before week 12 may have discontinued due to lack of efficacy. The percentage of subjects in remission may be overrepresented at week 12. However, many subjects (26%) dropped out of the acute phase of the trial due to nonmedical reasons (2). Therefore, subject discontinuation may or may not have affected the outcome of the present results. Despite these weaknesses, our findings indicate that many common over-the-counter medications greatly affect treatment response to SSRI antidepressants.

NSAIDs have been reported to increase the efficacy of some antidepressant treatments, but those reports focus on tricyclic or noradrenergic antidepressants (30) and not SSRIs. Our data indicate that the antagonism by NSAIDs of antidepressant responses is specific for serotonergic antidepressants. It is important to emphasize that the interaction between antidepressants and antiinflammatory agents appears to be specific to the efficacy of SSRIs and not a general effect on all classes of antidepressants. Furthermore, there is no evidence from our studies that NSAID administration alone has any effect on depressive-like states.

We report here a robust inhibitory effect of NSAIDs on SSRI-induced increases in p11 and on antidepressant-like behaviors in rodents. We confirmed the association in a dataset from a large-scale real-world human study (STAR*D), underscoring the clinical significance of these results. Work is ongoing to understand the cellular and molecular mechanism(s) underlying these effects, but the clinical implications of our findings are clear. With that, we urge the medical community to consider these findings when designing treatment strategies for their patients that include SSRIs.

**Materials and Methods**

**Animals.** Eight- to 10-wk-old male mice were used for all experiments, and were housed four to five per cage with ad libitum access to food and water. C57BL/6 mice were purchased from Charles River Laboratories, p11 KO mice (derived and maintained at The Rockefeller University) were also used (7). CAMK2α–p11 conditional knockout mice were generated for these studies (SI Materials and Methods). Animal use and procedures were in accordance with the National Institutes of Health guidelines and approved by the institutional animal care and use committees.

**Sample Preparation and Western Blotting.** Western blotting was performed using standard procedures as described (9) (SI Materials and Methods).

**Behavioral Assays.** The TST, FST, and open field locomotor activity were performed as described (9). Novelty suppressed feeding was performed as described (21).

**Clinical Data Analyses.** Please see SI Materials and Methods.

**Statistical Analyses.** All comparisons were made by ANOVA using Prism 5 software (GraphPad). In experiments composed of more than two groups, data were first analyzed by two-way ANOVA followed by a post hoc Bonferroni test. Statistical significance was set at P < 0.05.

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Supporting Information

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SI Materials and Methods

Drug Treatments. All drugs, including recombinant cytokines, were purchased from Sigma. Antidepressants included: citalopram hydrobromide, fluoxetine hydrochloride, desipramine hydrochloride, imipramine hydrochloride, bupropion hydrochloride, and trans-2-phenylecllopromamine (tranylcypromine) hydrochloride. Chronic antidepressant drug treatments (14 d) were administered in the drinking water (0.16 g/L), which contained 1% saccharin to mask any taste of the drug. Control groups drank a 1% saccharin solution. Antiinflammatory drugs included: ibuprofen sodium salt (1 g/L), naproxen sodium (2 g/L), acetylsalicylic acid (3 g/L), and acetaminophen (3 g/L) and were coadministered in the drinking water. On the basis of water consumption and bodyweight, we estimate an approximate daily dose of ibuprofen (70 mg·kg\(^{-1}\)·d\(^{-1}\)), naproxen (140 mg·kg\(^{-1}\)·d\(^{-1}\)), acetylsalicylic acid (210 mg·kg\(^{-1}\)·d\(^{-1}\)), or acetaminophen (210 mg·kg\(^{-1}\)·d\(^{-1}\)) (1–3). For acute behavioral studies, mice drank antiinflammatory drugs at doses listed above for 5–7 d before receiving a single i.p. injection of antidepressant (20 mg/kg bodyweight) prepared in sterile saline. Control mice received an equivalent volume of saline injected i.p. Antidepressants were injected 30 min before behavioral testing. Recombinant murine TNF\(\alpha\) (10 or 1 pg/kg bodyweight), recombinant murine IFN\(\gamma\) (10 or 1 pg/kg bodyweight), or saline vehicle were injected i.p. 30 min before testing in the tail suspension test. For chronic behavioral experiments, mice drank citalopram (0.16 g/L) dissolved in 1% saccharin solution or saccharin vehicle for at least 14 d before behavioral testing.

Sample Preparation and Western Blotting. Mice were anesthetized with carbon dioxide and brains were rapidly dissected and frozen at \(-80^\circ\)C until use. Frontal cortex, defined as the first 2 mm of cortex after olfactory bulbs were removed, was sonicated in lysis buffer (PBS with 2 mM EDTA, 2 mM EGTA, 1% Triton X-100, 0.5% SDS, and protease inhibitors). Protein concentrations were measured by BCA assay (Pierce, Thermo Scientific) according to manufacturer’s instruction. All samples were separated on 16% tricine gels (Invitrogen), transferred to PVDF membrane, blocked in 5% nonfat 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 and anti-b actin, 1:2,000; Cell Signaling Technologies). Blots were incubated in ECL reagent (Perkin-Elmer), exposed to film (Kodak), and optical densities were quantified using National Institutes of Health (NIH) Image software.

Cytokine Arrays. Mice were treated with antidepressants and/or NSAIDs for 14 d. Mice were anesthetized with carbon dioxide and frontal cortex was rapidly dissected and frozen at \(-80^\circ\)C. Samples were shipped on dry ice to Assayagge where protein concentrations and bead-based suspension protein arrays were analyzed to measure levels of various mouse cytokine/chemokine and growth factor analytes as described (4). The detection limit (pg/mg), intrassay CV%, and interassay CV% are noted here for each cytokine in Fig. 3 (IL-1b: 0.40 pg/mL, 8.1%, 7.0%; IL-3: 0.12 pg/mg, 9.1%, 9.5%; IL-6: 0.88 pg/mg, 10.4%, 15.2%; IL-12 (p70): 0.9 pg/mL, 7.6%, 8.9%; IFN\(\gamma\): 0.19 pg/mg, 8.2%, 12.5%; TNF\(\alpha\): 5.5 pg/mg, 16.3%, 18.2%; IL-10: 3.8 pg/mg, 7.0%, 10.1%; and IL-12(p40): 2.3 pg/mg, 8.7%, 9.0%).

Plasma Analysis of Citalopram Levels. Citalopram and metabolite levels were analyzed on an Applied Biosystems 4000 QTRAP mass spectrometer. Trunk blood was collected in EDTA-containing tubes. Samples were centrifuged at 13,000 rpm for 25 min at 4 °C. Plasma was removed and stored at \(-80^\circ\)C until use. A total of 100 μL of each sample was spiked with 10 μL of 20 ng/mL citalopram-d₄, or 0.2 ng total. The samples were liquid-liquid extracted, the organic layer pipetted off and dried down in a glass tube. This was dissolved in 60 μL of 98% water, 2% acetonitrile, 0.1% formic acid, and was pipetted to a vial for the ultra performance liquid chromatography (UPLC) system. The UPLC injected 4 μL onto the column for each of three replicates, and the samples were run in positive ion mode. All multiple reaction monitoring (MRM) spectra were quantitated using the Multiquant 1.1 software package. The standard curve is quantitated first, and then a calibration is exported and applied to the unknowns to give the calculated concentrations.

Behavioral Assays. The tail suspension test (TST), forced swim test (FST), and open field locomotor activity were performed as described (5). Novelty suppressed feeding (NSF) was performed as described (6). WT/vehicle control groups took \(~200\) s to approach food in the NSF test and spent \(~150\) s immobile in the TST. Each experiment included positive (e.g., WT antidepressant-treated) and negative control groups (e.g., WT vehicle-treated mice). Data are expressed as the percentage of WT/vehicle controls to combine data from multiple experiments for analysis because there was some variation in baseline values across experiments.

Clinical Data Analyses. Data were obtained from the limited access dataset (version 4) distributed from the NIH-supported “sequenced treatment alternatives to relieve depression” (STARplusD). The STAR*D focused on nonpsychotic major depressive disorder in adults seen in outpatient settings. The primary purpose of the STAR*D study was to determine which treatments work best if the first treatment with medication does not produce an acceptable response.

For the purpose of the present post hoc analysis, data from “level 1” of the STAR*D protocol were extracted. All subjects received treatment with citalopram for a minimum of 8 wk, with strong encouragement to complete 12 wk. Remission from depression was defined by a QIDS-C16 score of 5 or less. Concomitant medications were recorded and coded. Treatment with NSAIDs or other analgesics were coded separately. NSAIDs included ibuprofen, Relafen, Motrin, Advil, Naproxen, Celebrex, Vioxx, Indocin, and others, as well as all salicylates including acetylsalicylic acid, aspirin, salsalate, enteric coated acetylsalicylic acid, and baby aspirin. Analgesics included acetaminophen, Tylenol, Tylenol PM, Excedrin, and others, but excluded opiates with acetaminophen (e.g., Percocet and Vicodin, which were usually coded separately as opiates).

For the initial analysis, the concomitant medication from any visit during level 1 (first 12 wk of the study) was extracted and the clinical response at week 12 (“remission?” yes or no). Using Fisher’s exact test on 2 × 2 contingency tables, a relationship between a given concomitant medication (presence/absence) and clinical status at week 12 (remission/no) was analyzed separately for each of the concomitant medications. As a test for specificity, other medication codes including vitamins were evaluated for a relationship with clinical status using Fisher’s exact test.
There were 4,173 subjects in the concomitant medications list at level 1. Many subjects had multiple medications listed such that there were a total of 14,554 entries for 4,173 subjects. Of the 14,554 entries, there were 1,302 instances of NSAID codings, 401 instances of analgesic codings, 259 instances of vitamin codings, and 100 instances of antacid codings. Every subject who had at least one treatment visit had at least one entry; there were 4,028 subjects with entries. Subjects who did not have any concomitant medications were listed as “none.” Subjects may have had medications entered on more than one visit, so some medications may have been duplicate listings. Of these 4,028 subjects, only 1,546 had a week 12 data entry for remission. Many subjects discontinued the study before week 12 and some who may have continued past week 12 may have had missing data at week 12 or lacked a data entry specifically for remission. Contingency tables and analyses were performed on the 1,546 subjects for whom there were concomitant medication data and a week 12 data point for remission.


Fig. S1. Confocal micrographs illustrating the immunohistochemical detection of NeuN (blue), p11 (red), and IFNGR1 (green, Upper) or TNFR1 (green, Lower) in the cortex of adult WT mice. Colocalization of p11 and IFNGR1 or TNFR1 in NeuN positive neurons indicated by white arrows in “merged” images. Regions in white box magnified (4x) below.
**Fig. S2.** Immunohistochemical characterization of CAMK2α p11 knockout mice. CAMK2α conditional p11 KO mice were generated by crossing a floxed-p11 mouse with a mouse that expresses the cre-recombinase under the CAMK2α promoter. Immunohistochemical detection of NeuN (green) and p11 (red) in wildtype or p11 KO mice indicated that p11 was deleted from cells that expressed CAMK2α in forebrain neurons. (A) Representative confocal micrographs indicated that p11 is absent in layer 5 cortical neurons in the adult KO mouse brain compared with WT. Open arrows indicate cortical layer 5 at 10× magnification; closed arrows indicate p11 positive cells at 40× magnification. (B) In CAMK2α conditional p11 KO mice, p11 is absent from most cells in the dentate gyrus (DG), CA1 and CA3 subfields of the hippocampus compared with WT littermate controls. Open arrow heads indicate cells expressing p11 in a WT mouse; closed arrows indicate interneurons, which do not express CAMK2α-Cre and therefore express p11 in both WT and KO mice. (C) In the striatum, p11 is expressed by both medium spiny neurons (MSNs, open arrowheads) and interneurons (solid arrows). KO mice have lessened expression of p11 in the MSNs, but still express p11 in striatal interneurons, due to the expression of the CAMK2α-Cre. (Scale bar, 20 μm.)