Supporting Information

Warner-Schmidt et al. 10.1073/pnas.1104836108

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-phenylcyclopropylamine (tranylcipromine) 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 $mg \cdot kg^{-1} \cdot d^{-1}$), naproxen (140 $mg \cdot kg^{-1} \cdot d^{-1}$), acetylsalicylic acid (210 $mg \cdot kg^{-1} \cdot d^{-1}$), or acetaminophen (210 $mg \cdot kg^{-1} \cdot d^{-1}$) (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 TNFα (10 or 1 μg/kg bodyweight), recombinant murine IFNγ (10 or 1 μg/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 °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). Membranes were washed in TBST and incubated with HRP-conjugated secondary antibodies for 1 h at 4 °C (rabbit antigoat 1:5,000; goat antirabbit 1:5,000). 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 °C. Samples were shipped on dry ice to Assaygate 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), intraassay 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%; IFNγ: 0.19 pg/mg, 8.2%, 12.5%; TNFα: 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 °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" (STAR*D). 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.

1. Hoving S, et al. (2010) NO-donating aspirin and aspirin partially inhibit age-related atherosclerosis but not radiation-induced atherosclerosis in ApoE null mice. *PLoS ONE* 5:e12874.
2. Ishida T, et al. (2007) Effect of acetaminophen, a cyclooxygenase inhibitor, on Morris water maze task performance in mice. *J Psychopharmacol* 21:757–767.
3. Miranda HF, Sierralta F, Prieto JC (2009) Synergism between NSAIDs in the orofacial formalin test in mice. *Pharmacol Biochem Behav* 92:314–318.
4. Opalka D, et al. (2003) Simultaneous quantitation of antibodies to neutralizing epitopes on virus-like particles for human papillomavirus types 6, 11, 16, and 18 by a multiplexed luminex assay. *Clin Diagn Lab Immunol* 10:108–115.
5. Warner-Schmidt JL, et al. (2009) Role of p11 in cellular and behavioral effects of 5-HT4 receptor stimulation. *J Neurosci* 29:1937–1946.
6. Egeland M, Warner-Schmidt J, Greengard P, Svenningsson P (2010) Neurogenic effects of fluoxetine are attenuated in p11 (S100A10) knockout mice. *Biol Psychiatry* 67: 1048–1056.

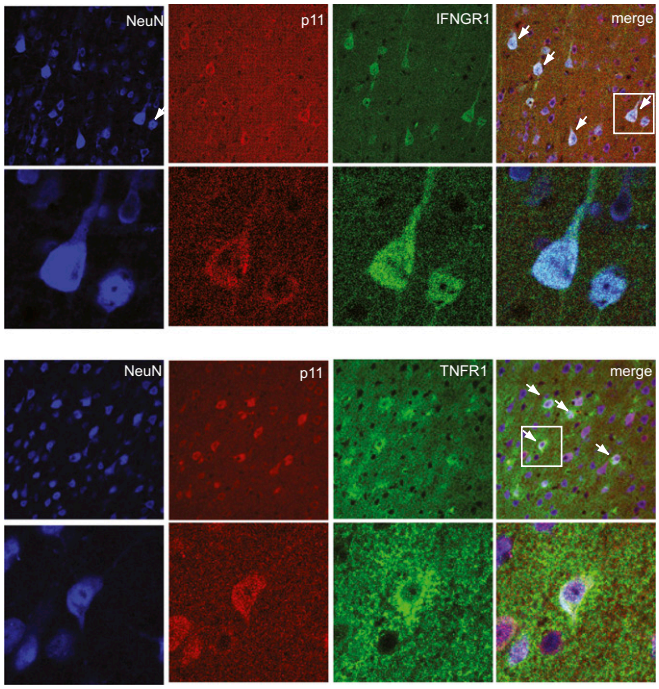

Fig. S1. Confocal micrographs illustrating the immunohistochemical detection of NeuN (blue), p11 (red), and IFNGR1 (green, *Upper*) or TNFR1 (green, *Lower*) in the cortex of adult WT mice. Colocalization of p11 and IFNGR1 or TNFR1 in NeuN positive neurons indicated by white arrows in "merged" images. Regions in white box magnified (4×) below.

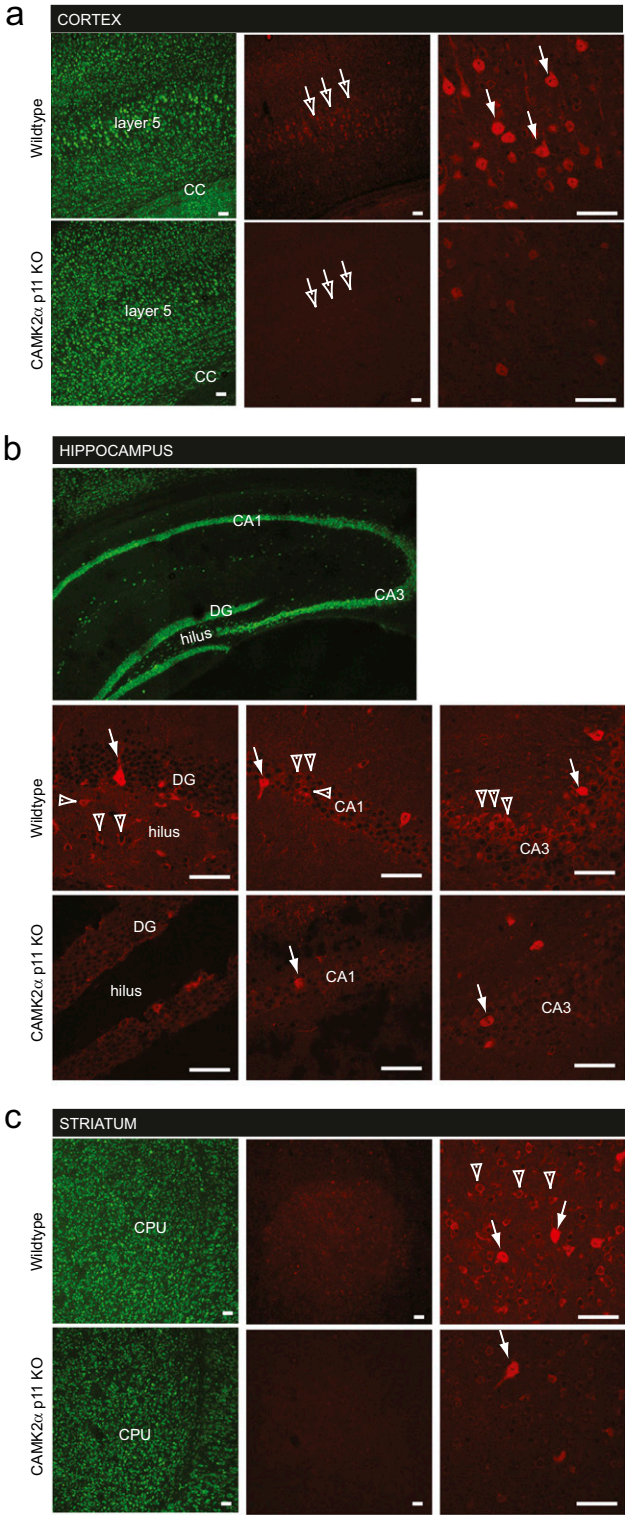

Fig. S2. Immunohistochemical characterization of CAMK2α p11 knockout mice. CAMK2α conditional p11 KO mice were generated by crossing a floxed-p11 mouse with a mouse that expresses the cre-recombinase under the CAMK2α promoter. Immunohistochemical detection of NeuN (green) and p11 (red) in wild-type or 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.)