Disinhibition of somatostatin-positive GABAergic interneurons results in an anxiolytic and antidepressant-like brain state

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INTRODUCTION

Major depressive disorder (MDD) is a highly disabling and phenotypically heterogeneous psychiatric syndrome that affects up to 17% of the worldwide population at least once in their lives.1

The biology underlying depressive brain states remains poorly understood. However, studies of patients and animal models increasingly suggest a key role for functional imbalances between the major excitatory and inhibitory neurotransmitters, glutamate and γ-aminobutyric acid (GABA) and their respective receptors. Evidence from analyses of depressed patients includes reduced expression of plasma membrane glutamate transporters2,3 and elevated brain concentrations of glutamate.4 Conversely, MDD is also associated with reduced concentrations of GABA,5–10 reduced expression of GABA type A receptors (GABA_A_Rs),11 reduced expression of glutamic acid decarboxylase12,13 and impaired function of certain subtypes of GABAergic interneurons.14–16

Glutamic acid decarboxylase functions as a key enzyme in the synthesis of GABA from glutamate; its reduced expression therefore could account for both the reduced concentrations of GABA and elevated glutamate. Such neurochemically based excitation–inhibition (E:I) imbalances may directly cause MDD, or they may increase the vulnerability to environmental factors that precipitate depressive episodes, such as uncontrollable stress.

Studies in rodents indicate that chronic and excessive stress can lead to impaired inhibition of neural circuits by a shift in the chloride reversal potential (E_Cl) of neurons, which renders GABA ineffective as an inhibitory neurotransmitter, as shown for principal neurons of the paraventricular nucleus of the hypothalamus that control the stress axis, as well as the hippocampus.17–19 Intriguingly, the cellular vulnerability to stress is exacerbated in a major subclass of GABAergic interneurons that express the neuropeptide somatostatin (SST, also known as SOM or SRIF)20 (see also below). Thus E:I imbalances and uncontrollable stress are subject to reciprocal positive reinforcement and have been implicated independently as causal factors for MDD (for reviews, see Maguire,21 Luscher and Fuchs22 and Bains et al.23).

The vast majority of currently used antidepressants are designed to modulate monoaminergic neurotransmitter systems. Their therapeutic effectiveness is delayed by several weeks, indicating that the drug-induced changes that ameliorate
depressive brain states are only remotely related to changes in the function of monoamine transmitters. Instead these antidepressants ultimately may affect the functional balance of the major amino-acid neurotransmitters glutamate and GABA. Indeed, successful antidepressant drug treatment and electroconvulsive therapy of patients are associated with a normalization of GABA concentrations in the brain. Moreover, anxious-depressive-like phenotypes of mice induced by globally reduced function of GABAARs (GABAAR y2/−/− mice) are normalized by chronic treatment with antidepressant drugs, such as the tricyclic desipramine. Similar to the delayed effects of conventional antidepressants, the rapidly acting experimental antidepressant, ketamine, normalizes the depressive-like phenotypes of GABAAR y2/−/− mice, along with prominent potentiation of inhibitory synapses in the medial prefrontal cortex (mPFC). Collectively, these observations indicate that antidepressant therapies act by normalizing chronic E/I imbalances, perhaps through potentiation of GABAergic synaptic inhibition. However, none of these data demonstrate a causative relationship between enhanced GABAergic synaptic transmission and effective antidepressant therapies.

Ketamine’s remarkable efficacy as a rapidly acting antidepressant has spurred interest into understanding the underlying mechanism. Ketamine acts by promoting the formation and function of synapses by triggering a cascade of phosphorylation events involving activation of mammalian target of rapamy cin (mTOR), activation of its target p70S6K (S6K), inhibition of the eukaryotic translational elongation factor 2 kinase (eEF2K) and reduced inhibitory phosphorylation of the single eEF2K target, eEF2, which ultimately leads to enhanced activity of eEF2 and increased mRNA translation elongation (reviewed in Duman et al; however, see also Zanos et al). Notably, mTOR and eEF2 are also activated downstream of antagonists of the 5-HT2C receptor, which have antidepressant-like effects in rodents that are also activated downstream of antagonists of the 5-HT2C receptor. This mechanism could underlie the development of antidepressant-like effects in rodent models of depression. A more detailed description is found in Supplementary Information.

Materials and Methods
A more detailed description is found in Supplementary Information.

Animals
All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUC) of The Pennsylvania State University or Tufts University and performed in accordance with all relevant guidelines and regulations of the National Institute of Health (NIH). SSTCre mice were obtained from JAX mice (Stock no. 013044, Jackson Laboratory, Bar Harbor, ME, USA). The y2/−/− mice were generated in house. The LSL-YFP Cre-reporter strain also known as ROSA26-EYFP was obtained from JAX mice (Stock no. 006148). All mice were backcrossed to the 129X1/sv genetic background for at least six generations. The genotypes compared were produced as littermates and identified by PCR analyses of tail DNA as described. The mice were maintained on a 12:12 h light–dark cycle with food and water available ad libitum.

Electrophysiological recordings
Electrophysiological recordings were carried out essentially as described. Behavioral testing
Behavioral testing started at 9 weeks of age, with one test per week and starting with the open field test (OFT), followed by elevated plus maze (EPM), novelty suppressed feeding test (NSFT) and forced swimming test (FST). The learned helplessness test (LHT) was conducted with a separate
cohort of mice. All testing was performed under red light, between 2 and 6 h after the beginning of the dark phase and scored by investigators blind to genotype. The OFT was used to assess locomotion in a novel environment, under red light. The behavior was video recorded and motor activity (path length) was analyzed in 5 min bins using the EthoVision XT video tracking system (Noldus Information Technologies, Leesburg, VA, USA). For the EPM,69 mice were placed into the center square of the maze facing a closed arm. Behavior was video recorded for 5 min using EthoVision XT. For the NSF,60 mice were food deprived for 18 h. The latency to feed in a novel arena was hand scored. In the FST,61 we assessed the average swim speed during the first minute, the latency to the first episode of passive floating (time to first immobility) and the total time immobile during the last 4 min of a 6 min test, using Ethovision XT. The LHT was modified from reference.64 Mice were exposed to 120 inescapable foot shocks (0.3 mA, 15 s) at an average interval of 45 s in a two-compartment shuttle box with the connecting gate closed (SanDiego Instruments, San Diego, CA, USA). Twenty-four hours later, mice were tested in the same shuttle box in a 30-trial active avoidance task (escapable foot shock). Escape failures were recorded automatically.

Western blotting
Tissue extracts in 50 μl Tris-HCl (pH 8.0), 150 μl NaCl, 2 mM EDTA, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 1 mM NaVO₃, 5 mM NaF and 1× protease inhibitor cocktail (Roche, Basel, Switzerland) were analyzed by 4–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes and probed with mouse anti β-tubulin (1:10 000, no. 798238, Sigma-Aldrich, St Louis, MO, USA), rabbit anti-eEF2K (1:500, no. 4661), rabbit anti-phospho-eEF2K (Thr348) (1:500, no. 4411), rabbit anti-phospho-eEF2K (Ser500) (1:500, no. 4451, all from Cell Signaling, Danvers, MA, USA). The blots were incubated with 1% bovine serum albumin (BSA) in PBS and probed with rabbit anti-GABA (1:1000, no. A1220, Sigma-Aldrich, St Louis, MO, USA) and rabbit anti-MAP2 (1:5000, no. 22022, Sigma-Aldrich, St Louis, MO, USA). The bands were visualized using a horseradish peroxidase (HRP)–conjugated secondary antibody and enhanced chemiluminescence (ECL) Western blotting kit (GE Healthcare, Uppsala, Sweden). All Western blot bands were quantified using ImageJ software (National Institute of Health, Bethesda, MD, USA) and normalized against β-tubulin. Western blotting was conducted in biological triplicates. Densities for both proteins were significantly lower in the 2f/f:LSL-YFP mutant vs control mice in both brain areas (P < 0.001; L2/3, P = 0.01, Mann–Whitney, n = 3–4 mice/genotype).

**Figure 1.** Deletion of postsynaptic γ-aminobutyric acid type A receptors (GABA_{A/R}s) and gephyrin from somatostatin-positive (SST⁺) neurons of SSTCre;γ2flo mice. (a) Strategy for γ2 subunit knockout-mediated disinhibition of SST⁺ interneurons. Loss of synaptic GABA_{A/R}s removes inhibitory input (IN) to SST⁺ neurons and increases excitability of these neurons. Increased excitability of SST⁺ neurons strengthens inhibitory synaptic inputs to apical dendrites and spines of pyramidal cells (PN). (b) Schematic of Cre-mediated inactivation of the ‘floxed’ γ2 locus. (c) Representative micrographs of the soma of an SST⁺ neuron from a SSTCre; γ2 f/f:LSL-YFP control mouse (left column) compared with a SST⁺ neuron from a SSTCre; γ2 f/f:LSL-YFP mutant animal, immunostained for the γ2 subunit (top row, green), gephyrin (second row, red) and yellow fluorescent protein (YFP; third row; blue) with merged images showing colocalization of γ2 and gephyrin in yellow in the bottom row. Note the drastic reduction in punctate staining for both the γ2 subunit and gephyrin, indicative of loss of functional synapses. Residual staining for γ2 is likely attributable to dendrites of Cre-lacking neighboring neurons. (d) Quantification of puncta densities overlapping with YFP⁺ cell somata (puncta per μm²) in S. pyramidale and S. radiale of the hippocampus. Densities for both proteins were significantly reduced in both areas (P < 0.001, respectively). ***P < 0.001, Mann–Whitney, n = 30–40 cells, 2 mice/genotype.

**Figure 2.** Recordings from somatostatin-positive (SST⁺) neurons. (a–f) Spontaneous inhibitory synaptic current (sIPSC), miniature inhibitory synaptic current (mIPSC) and spontaneous excitatory postsynaptic current (sEPSC) recordings from SST⁺ neurons of SSTCre;γ2flo:LSL-YFP mutants and SSTCre;γ2f/f:LSL-YFP control mice in hippocampus CA1 (a–c) and L2/3 cingulate cortex (d–f). Representative traces are shown on top of the summary statistics. Note the significant reductions in both sIPSC and mIPSC frequencies and amplitudes recorded from CA1 (a, b) and L2/3 (d, e) SST⁺ neurons of mutant vs control mice (P < 0.05 for all eight measures, n = 6 and 8 cells, one cell/slice, three mice/genotype). sEPSCs recorded from SST⁺ neurons were unaffected by genotype independent of brain area (c, f, CA1, L2/3 frequency, P, NS (non-significant); amplitude, P, NS; n = 6 and 8 cells and slices, 3 mice/genotype). (g–n) Current injection data from SST⁺ neurons in CA1 and L2/3 cingulate cortex of SSTCre;γ2flo:LSL-YFP mutants and controls. Representative traces are shown in panels (g) (CA1) and (k) (L2/3) with summary data in panels (h–j) (CA1) and (l–n) (L2/3). Note the increased number of action potentials recorded from SST⁺ neurons in mutant vs control mice in both CA1 and L2/3 (h, l) (P < 0.05 for both comparisons, Bolzman fit, W50, t-tests). The input resistance (Rin; j, n) was significantly reduced in SSTCre;γ2flo:LSL-YFP mutant vs control mice in both brain areas (Rin, CA1, P < 0.05; L2/3, P < 0.01, n = 9 and 10), while the resting membrane potentials (RMP; j, n) of SST⁺ cells were unaffected by genotype independent of brain area (RMP, P, NS). Data represent means ± s.e. *P < 0.05, **P < 0.01, t-tests.
from ECM Biosciences, Versailles, KY, USA), rabbit anti-phospho-eEF2 (Thr56) (1:500, no. 2331), rabbit anti-eEF2 (1:500, no. 2332), rabbit anti-phosphor-mTOR (Ser2448) (1:500, no. 5536), mouse anti-mTOR (1:500, no. 4517), rabbit anti-phosphor-p70S6K (Thr389) (1:500, no. 9205) and rabbit anti-p70S6K (1:500, no. 9202) (all from Cell Signaling Technology, Danvers, MA, USA). Immunoreactive bands were developed and quantitated using IRDye secondary antibodies and an Odyssey CLx infrared imager (LI-COR) using conditions recommended by LI-COR (Lincoln, NE, USA).
Enzyme-linked immunosorbent assay
Brain tissue was extracted by sonication in 20 mM Tris-HCl, 137 mM NaCl, 0.2% Triton X-100, 10% glycerol and 1 x protease inhibitor cocktail (Roche) and cleared by centrifugation. The SST concentration in supernatants was measured using a Mouse Somatostatin ELISA Kit (F12622, LifeSpan Biosciences, Seattle, WA, USA).

Real-time quantitative PCR
Total RNA was extracted from brain tissue using the GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich). Reverse transcription was performed using the qScript cDNA Supermix (Quanta Biosciences, Gaithersburg, MD, USA). PCR reactions were performed in triplicate and normalized to a β-actin internal control, using primers designed with the Primer Express software (Thermo Fisher Scientific, Waltham, MA, USA). Quantification was performed using the comparative threshold cycle (Ct) measurement with SYBR green fluorescence signal (Quanta Biosciences).

Statistics
All statistical analyses were based on biological replicates. Group means that satisfied the normality assumption (D’Agostino and Pearson) were compared by two-tailed unpaired t-tests or, in case of multiple group means, by analysis of variance followed by planned comparisons using t-tests, as detailed in figure legends (PRISM software, Graphpad, La Jolla, CA, USA). Data subjected to analyses of variance (latency to feed, time to first immobility and eEFKT348 phosphorylation) were log transformed to satisfy the normality assumption (D’Agostino and Pearson) were analyzed by Mann-Whitney U-test. Outliers that deviated from the mean by > 2 s.d. were removed from analyses.

RESULTS
Deletion of postsynaptic GABAARs from SST⁺ neurons results in increased excitability of these neurons
To examine whether increasing the excitability of SST⁺ interneurons results in an antidepressant phenotype, we chose to reduce GABAergic inhibitory synaptic input to these neurons (Figure 1a). The γ2 subunit of GABAARs is essential for postsynaptic accumulation of GABAARs and the subsynaptic scaffolding protein gephyrin, for normal channel conductance of GABAARs, and for GABAergic synaptic function.63,52,54 To selectively inactivate the γ2 gene (Gabrg2) in SST⁺ interneurons we crossed mice carrying the Cre-driver STTCre64 and the Cre-reporter LSL-YFP with ‘floxed’ γ2 mice (γ2fl mice)53 (Figure 1b). As expected, the punctate immunoreactivity for GABAARs that overlapped with YFP⁺ somata of SST⁺ neurons was drastically reduced in STTCreγ2fl/LSL-YFP mice compared with STTCreγ2f/+−LSL-YFP (heterozygous) controls, as demonstrated for S. pyramidale and S. radiatum (Figures 1c–e). The punctate immunoreactivity for gephyrin was similarly reduced, consistent with the loss of postsynaptic GABAARs (Figures 1c–e). Punctate immunoreactivity remaining for γ2 was likely due to dendrites of Cre-lacking neighboring neurons that invaded the optic plane imaged of YFP⁺ somata, indicating that the loss of inhibitory synapses may be more drastic than these data suggest.

We next performed V-clamp recordings from SST⁺ (YFP⁺) neurons to assess predicted changes in inhibitory synaptic currents. Recordings from SST⁺ neurons of the CA1 region of

Figure 3. Recordings from pyramidal cells. (a–h) Spontaneous inhibitory synaptic current (sIPSC), miniature inhibitory synaptic current (mIPSC), tonic inhibition and spontaneous excitatory postsynaptic current (sEPSC) recordings from pyramidal neurons of STTCreγ2fl/ compared with control mice (STTCreγ2fl/+−/ and γ2fl+) in CA1 hippocampus (a–d) and L2/3 cingulate cortex (e–h). Representative traces are shown on top of the bar graph summary statistics. Note the significant increases in sIPSC frequency and amplitude recorded from neurons of STTCreγ2fl/ mice in both brain areas (a, e, P < 0.05 for all four measures; n = 8 and 15 neurons, recording 1 neuron/slice, from 4 mice/genotype). By contrast, mIPSCs showed a moderate increase in amplitude for STTCreγ2fl/ mice in CA1 only (b, f) [CA1 amplitude, P < 0.05, frequency, P, NS (non-significant); for L2/3 frequency, L2/3 amplitude and L2/3 frequency]. Tonic inhibition (c, g) was unaffected by genotype (P, NS; for all comparisons, n = 8 and 11 neurons and slices, 3 mice/genotype). Similarly, the sEPSCs (d, h) did not differ between genotypes independent of brain area (P, NS; all comparisons, n = 8 and 15 neurons and slices, 4 mice/genotype). Data represent means ± s.e. *P < 0.05, t-tests.
the hippocampus revealed drastic reductions in the frequencies and amplitudes of both spontaneous and miniature inhibitory synaptic currents (sIPSCs and mIPSCs, respectively) (Figures 2a and b) in SSTCre;γ2f/f:LSL-YFP mutants, consistent with the loss of postsynaptic GABAARs. Virtually identical results were obtained for SST+ neurons of L2/3 cingulate cortex (Figures 2d and e). By contrast, spontaneous and miniature excitatory postsynaptic currents (sEPSCs and mEPSCs, respectively) remained unaffected, independent of brain region (Figures 2c and f, Supplementary Figures S1A and B).

To assess the changes in excitability of SST+ neurons, we performed current clamp recordings in response to a series of current injections, with amplitudes ranging from 20 to 300 pA. The number of action potentials recorded from SST+ neurons was significantly increased in SSTCre;γ2f/f:LSL-YFP mutants vs SSTCre;γ2f/f mutants in CA1 hippocampus (Figures 2g and h) and L2/3 cingulate cortex (Figures 2k and l) (P < 0.05, both comparisons). The input resistance of SST+ neurons was increased in mutant vs control mice, independent of brain region and consistent with the reduced number of chloride channels expressed by these cells (Figures 2i and m). By contrast, the resting membrane potential was unaffected, indicating that increased excitability did not adversely affect the energy balance of mutant SST+ neurons (Figures 2j and n).

To assess possible secondary changes induced by chronic loss of GABAergic inhibition, we compared the effects of genetic deletion of GABAARs with those of pharmacological blockade of GABAARs. Treatment of slices with bicucullin (25 μM) increased the number of current-induced action potentials in control mice to levels recorded from untreated CA1 SST+ cells of SSTCre;γ2f/+;LSL-YFP mutant mice (Supplementary Figures S1C and D, P, NS; Bolzmann fit, W50, t-test). Moreover, the bicucullin effect on the maximum number of action potentials was occluded in the mutants (Supplementary Figures S1C and D). Similarly, the input resistance of SST+ neurons recorded from control mice was increased by bicucullin to the levels of mutant mice, without additional drug effect in the mutants (Supplementary Figure S1E). Thus, the effect of pharmacological blockade of GABAARs on excitability of SST+ neurons was indistinguishable from that of genetic inactivation of GABAARs and there is no evidence for secondary changes that contributed to increased excitability of mutant SST+ neurons.

Increased excitability of SST+ neurons results in increased GABAergic synaptic inhibition of principal cells

To assess functional consequences of SST+ cell hyperexcitability on putative target cells, we performed V-clamp recordings of pyramidal cells. The frequency and amplitude of sIPSCs recorded from pyramidal cells in CA1 was significantly increased in SSTCre;γ2f/+ mice vs controls (SSTCre;γ2f/+;y2f/+ and y2f/y2f) (Figure 3a). By contrast, the frequency of mIPSCs remained unaffected (Figure 3b), consistent with frequency changes being presynaptic action potential dependent. Nevertheless, the mIPSC amplitude was elevated (Figure 3b), indicating that GABAergic synapses of CA1 pyramidal cells were potentiated both presynaptically and postsynaptically. Strengthening of the postsynaptic apparatus is consistent with recent evidence for presynaptic activity-dependent plasticity of inhibitory synapses (see Discussion). Tonic GABAAR currents remained unaffected by genotype (Figure 3c), in keeping with the absence of spontaneous activity of SST+ neurons.

**Figure 4.** SSTCre;γ2f/+ mice show an anxiolytic- and antidepressant-like behavioral phenotype. (a) Elevated Plus Maze: Percentage of open arm entries of SSTCre;γ2f/+ mutants was increased compared with γ2f/+ controls (sexes combined (M&F), F(1, 60) = 6.34, P < 0.05, n = 29 and 35) with similar trends in females (P = 0.06, n = 14 and 16) and males (P = 0.08, n = 15 and 19). The percentage of time spent in open arms was increased in mutants vs controls in both sexes (F(1, 57) = 19.16, P < 0.001; male: P < 0.05, n = 14 and 19; female: P < 0.001, n = 16 and 12). The number of closed arm entries did not differ between genotypes (P > 0.05, NS (non-significant), t-test). (b) Novelty Suppressed Feeding Test: SSTCre;γ2f/+ mutants showed a reduced latency to feed vs controls (F(1, 60) = 6.4, P = 0.014), with significant effects in females (P < 0.05, n = 16) and a tendency in the same direction in males (P, NS; n = 15 and 19). (c) Forced Swim Test: SSTCre;γ2f/+ mice of both sexes showed increased latencies to their first bout of immobility (F(1, 62) = 15.41, P = 0.0002; female: P < 0.01, n = 16; male: P < 0.05, n = 15 and 19) and a reduced total time immobile vs controls (F(1, 57) = 15.98, P < 0.0001; female: P < 0.05; male: P < 0.01). The average swim speed during the first minute did not differ between genotypes (M&F: P, NS; n = 32 and 34, t-test). (d) Learned Helplessness Test: Male SSTCre;γ2f/+ mice showed fewer escape failures than controls (P < 0.05, n = 17 and 19, Mann–Whitney). Data represent means ± s.e. *P < 0.05, **P < 0.01, ***P < 0.001, analyses of variance and posthoc t-tests, t-tests or Mann–Whitney.
Figure 5. SSTCre:γ2f/f mice show biochemical changes indicative of reduced intracellular Ca2+ signaling and increased dendritic translational elongation. (a–c) Representative western blottings and summary statistics for phospho-eukaryotic translational elongation factor 2 (p-eEF2), phospho-mammalian target of rapamycin (p-mTOR) and p-S6K in the hippocampus (Hipp) and medial prefrontal cortex (mPFC) normalized to levels of the respective total proteins in the same samples. Western blottings show a reduction of p-eEF2/total eEF2 in the Hipp and mPFC of SSTCre:γ2f/f vs γ2f/f control mice (P<0.05, n = 10 and 11, for both hipp and mPFC, t-tests) (a). The ratios of p-mTOR/S6K/mTOR (n = 6 and 6; both brain regions) (b) and of p-S6K/S6K (c) were unaffected by genotype (P, NS (non-significant); all four comparisons, n = 6 and 7, both brain regions, Mann–Whitney). (d) Representative western blottings and summary statistics of phosphorylation of eEF2 kinase (eEF2K) at T348 and S500, normalized to total eEF2. A two-way analysis of variance for auto-phosphorylation at T348 revealed significant main effects for genotype (F(1,22) = 10.50, P<0.01), brain region (F(1,22) = 10.35, P<0.01) and interaction among the two (F(1,22) = 7.69, P<0.05). Posthoc tests showed significantly reduced eEF2K/T348 auto-phosphorylation in the hippocampus of SSTCre:γ2f/f vs γ2f/f controls (P<0.01, n = 5 and 8) with a tendency in the same direction in mPFC (P, NS; n = 5 and 7, t-tests). By contrast, no genotype-dependent changes were evident for eEF2KS500 (P, NS; for both brain regions; n = 5 and 6 (hipp) and 6 and 8 (mPFC), Mann–Whitney). (e) Schematic of signaling cascades converging on the phospho-state and activity of eEF2K as a target downstream of (i) mTOR- and S6K-mediated inhibitory phosphorylation, (ii) excitation–inhibition (E:I) ratio and calmodulin (CaM)-dependent auto-phosphorylation at T348 (activating, black arrow) and (iii) Ca2+-insensitive activating protein kinase A-mediated phosphorylation at S500. Small color-coded arrows illustrate previously reported alterations in phospho-state induced by ketamine (blue29,30) and 5-HT2C antagonists (green33) and reported here owing to a reduced synaptic E:I ratio (red, unaltered phospho-states are indicated by a horizontal dash). The unaltered phospho-states of mTOR, S6K and eEF2K of SSTCre:γ2f/f mice indicate that reduced phosphorylation of eEF2 involves reduced E:I ratio and reduced CaM-mediated auto-phosphorylation of eEF2K at T348. (f) Somatostatin (SST) mRNA levels quantitated by reverse transcriptase–PCR were reduced in hippocampus (2−ΔΔCt, P<0.05) with a trend in the same direction in mPFC (P, NS; n = 5 and 6, Mann–Whitney). (g) SST protein levels quantitated by enzyme-linked immunosorbent assay were not measurably affected by genotype, independent of brain region (P, NS; n = 5 and 6 (hipp), 5 and 5 (mPFC), Mann–Whitney). Data represent means ± s.e. *P<0.05, **P<0.01, t-tests or Mann–Whitney.
in slices even in the mutants (Figures 2h and I). Interestingly, increased inhibitory synaptic input to CA1 pyramidal cells had no secondary effects on spontaneous EPSCs (Figure 3d). Virtually identical results were observed in L2/3 cingulate cortex (Figures 3e, g and h), except that the amplitude of mIPSCs was unaffected by genotype (Figure 3f). The passive membrane properties of pyramidal cells assessed by current clamp were unaffected in SSTCre\(^{2f/f}\) mice compared with controls, as evidenced by the unaltered number of action potentials elicited across the entire range of currents injected (20–300 pA) (Supplementary Figures S2A–C and E–G), as well as the unaltered resting membrane potentials, independent of brain region (Supplementary Figures S2D and H). Thus increasing the excitability of SST\(^{+}\) neurons results in a marked and selective increase of GABAergic inhibitory synaptic input to pyramidal target cells.

The behavior of SSTCre\(^{2f/f}\) mice mimics the effects of antidepressant drug treatment in anxiety- and depression-related behavioral tests

Before assessing the behavioral consequences of a reduced synaptic E/I ratio in SSTCre\(^{2f/f}\) mice, we assessed the emotion-related behavior of mice that served as controls. The behavior of SSTCre male and female mice analyzed in OFT, FST and EPM was largely indistinguishable from wild-type controls (Supplementary Figures S3A–C), except for modestly reduced locomotion in an OFT of female mice (Supplementary Figure S3A) and an anxiety-like reduction in the time spent in the open arms of an EPM for male mice (Supplementary Figure S3C). These changes occurred in a direction opposite to those found later for SSTCre\(^{2f/f}\) mutant vs \(\gamma_{2f/f}\) control mice (see below) and therefore did not interfere with interpretation of behavior of these mutants. SSTCre\(^{2f/+}\) mice, which are heterozygous for \(\gamma_{2f/+}\) pseudo wild-type controls in the same three test paradigms, indicating that inactivation of a single copy of the \(\gamma_{2}\) gene in SST\(^{+}\) cells was insufficient to affect behavior and that unspecific Cre activity did not interfere with the behavioral assessment of SSTCre\(^{2f/+}\) mice (Supplementary Figures S3D–F).

Hyperexcitability of SST\(^{+}\) neurons in SSTCre\(^{2f/+}\) mice had no overt effects on body weight (Supplementary Figure S4A), fertility or overall health, as evidenced by normal gait, posture and lifespan. However, when examined in a 10 min OFT, SSTCre\(^{2f/+}\) mice of both sexes showed increased locomotion vs \(\gamma_{2f/+}\) controls (Supplementary Figure S1B). This effect was more pronounced in female SSTCre\(^{2f/+}\) mice and did not result in altered home cage activity (Supplementary Figure S1B).

To assess the emotional behavioral consequences of SST\(^{+}\) cell hyperexcitability, we subjected SSTCre\(^{2f/+}\) mutant mice to a battery of tests sensitive to anxiolytic and antidepressant drug treatment. In the EPM, the mutants showed an increased percentage of open arm entries and an increased percentage of time spent in the open arms compared with \(\gamma_{2f/+}\) littermate controls, independent of sex (Figure 4a). In light of the increased motor activity described above, it is worth noting that the percentage of open arm entries and the percentage of time spent in open arms are largely independent of locomotion. Moreover, the number of closed arm entries was unaltered by genotype (Figure 4a), indicating that differences on the open arms were due to differences in anxiety and not locomotion. Thus the behavior of SSTCre\(^{2f/+}\) vs \(\gamma_{2f/+}\) mice in the EPM mimics the effects of classic anxiolytics (that is, benzodiazepines\(^{65}\)), as well as of the rapidly acting antidepressant, ketamine.\(^{28}\) In the NSFT, SSTCre \(\gamma_{2f/+}\) mice took less time to feed in a novel environment than littermate controls (analysis of variance, \(F_{(1, 62)} = 6.45, P < 0.05\)). Posthoc tests showed that feeding latencies of female SSTCre \(\gamma_{2f/+}\) mice were significantly reduced relative to controls, with a similar but non-significant trend also in males (Figure 4b).

Behavioral changes in the NSFT analogous to those of SSTCre\(^{2f/+}\) mice are observed following chronic but not acute administration of conventional antidepressants\(^{66,67}\) as well as after an acute dose of ketamine.\(^{29,32}\) In the FST, SSTCre\(^{2f/+}\) mice showed a greater latency to assume an immobile position and spent less time in an immobile position than \(\gamma_{2f/+}\) controls, independent of sex (Figure 4c). Notably, the average swim speed assessed during the first minute of the two sexes combined did not differ between genotypes (Figure 4c). Finally, in the LHT, a male cohort of SSTCre: \(\gamma_{2f/+}\) mice showed fewer escape failures compared with \(\gamma_{2f/+}\) littermate controls (Figure 4d). The FST and LHT have strong predictive validity for antidepressant drug activity in patients.\(^{68}\) In summary, the behavior of SSTCre\(^{2f/+}\) mice mimics antidepressant drug treatment-induced behavior in four different test paradigms.

The behavior of SSTCre\(^{2f/+}\) mice in a sucrose preference test was unchanged, most likely due to the very high sucrose prevalence (~98%) already in control mice (Supplementary Figure S5A). Learning and memory of SSTCre\(^{2f/+}\) mice in the Morris water maze was unaffected (Supplementary Figure S5B), suggesting that the behavioral changes of SSTCre\(^{2f/+}\) mice were limited to emotional domains and confirming that differences in the FST behavior were not due to altered overall swim speed or motor coordination.

SSTCre\(^{2f/+}\) mice reproduce biochemical end points of treatment with rapidly acting antidepressants, independent of mTOR activation and altered SST expression

To gain mechanistic insights into the antidepressant-like behavioral phenotype of SSTCre\(^{2f/+}\) mice, we examined whether they might show biochemical changes associated with the mechanisms of rapidly acting antidepressants. Interestingly, brain extracts from hippocampus and mPFC of SSTCre\(^{2f/+}\) mice showed prominently reduced phosphorylation of eEF2 at T56 (Figure 5a), reminiscent of similar but transient changes in eEF2\(^{29,30}\) phosphorylation induced in rodents by the experimental antidepressant ketamine\(^{29}\) and 5-HT2C antagonists.\(^{33}\) Phosphorylation of eEF2 inhibits its function with respect to most mRNA targets. Reduced eEF2 phosphorylation in SSTCre\(^{2f/+}\) mice therefore is consistent with increased translation elongation of eEF2-target mRNAs as reported following ketamine treatment.\(^{29}\) Importantly, phosphorylation of mTOR and S6K was unchanged in SSTCre\(^{2f/+}\) mice independent of brain region (Figures 5b and c), suggesting that in SSTCre\(^{2f/+}\) mice eEF2 activity was controlled by pathways distinct from those activated by rapidly acting antidepressants. Indeed, the key kinase that controls the eEF2 phospho-state and activity, eEF2K, is a target not only of S6K-mediated inhibitory phosphorylation downstream of mTOR but also activated by protein kinase A and CaM-dependent auto-phosphorylation, through non-overlapping phosphorylation sites.\(^{69}\) Interestingly, CaM-dependent auto-phosphorylation of eEF2K at T438 was reduced in SSTCre\(^{2f/+}\) vs \(\gamma_{2f/+}\) mice with a significant effect in hippocampus and a non-significant trend in the same direction also in the mPFC (Figure 2d). By contrast, phosphorylation of eEF2K at S500, which is mediated by protein kinase A and insensitive to Ca\(^{2+}\) (Ref 70, 71) remained unaffected, independent of brain region (Figure 2d). Collectively, the data suggest that the reduced synaptic E/I ratio of principal cells of SSTCre\(^{2f/+}\) mice leads to reduced intracellular Ca\(^{2+}\) levels, reduced Ca\(^{2+}\)-dependent activation of CaM-dependent auto-phosphorylation of eEF2K and reduced eEF2K-mediated inhibitory phosphorylation of eEF2 (Figure 2e). Notably, the expression of SST mRNA was reduced in the hippocampus and trended lower in mPFC (Figure 5f), while SST protein levels remained unchanged in SSTCre\(^{2f/+}\) mice compared with \(\gamma_{2f/+}\) controls independent of the brain regions analyzed (Figure 5g). The data suggest that the antidepressant-like brain state of SSTCre\(^{2f/+}\) mice that is caused by increased excitability of SST\(^{+}\) interneurons did not involve increased SST expression (see Discussion).
**DISCUSSION**

We here provide direct evidence that reducing the synaptic E/I ratio of principal neurons by functional potentiation of a GABAAergic inhibitory synaptic input to principal cells is sufficient to induce a sustained antidepressant-like phenotype that includes both behavioral and biochemical end points of antidepressant drug treatment. Our findings are consistent with the GABAAergic deficit hypothesis of MDD, which posits that defects in GABAAergic transmission may be causal for depressive disorders and that antidepressant therapies involve enhancement of GABAAergic synaptic transmission.22

Intracerebroventricular infusion of an SST peptide has anxiolytic- and antidepressant-like behavioral consequences,72 raising the question of whether hyperexcitability of SST+ neurons leads to increased expression and release of SST that might contribute to the behavioral phenotype of SSTCreγ/2f/f mice. However, SST mRNA was reduced rather than increased and we found no measurable change in the expression of SST+ protein in the brain of SSTCreγ/2f/f mice (Figures 5f and g). Furthermore, treatment of cultured neurons with purified SST peptide leads to profound reduction in the density and function of excitatory synapses without changing the function of inhibitory synapses,73 which is opposite to the synaptic alterations observed in SSTCreγ/2f/f mice. Acute silencing of frontal cortex SST+ interneurons results in an anxious-depressive-like phenotype in mice, while chronic silencing or ablation has the opposite effect.74 Although these experiments point to a central role of SST+ neurons in emotion regulation, they were not designed to discriminate between loss of GABAAergic inhibition and loss of SST function. Thus the collective evidence is consistent with the antidepressant phenotype of SSTCreγ/2f/f mice owing to increased inhibitory synaptic input to principal cells.

SST+ neurons are known to target preferentially the dendrites of principal neurons. However, they also provide inhibitory input to parvalbumin-positive (PV+) interneurons. In addition to innervating each other, PV+ interneurons synapse preferentially onto the perisomatic region and axon initial segment of pyramidal cells, thereby controlling their spike output.75,76 Thus increased inhibition of PV+ cells by hyperactive SST+ neurons of SSTCreγ/2f/f mice might result in disinhibition of pyramidal cells. The increased miPSC amplitude observed in CA1 pyramidal cells of SSTCreγ/2f/f mice might reflect chronically reduced input at a subset of synaptic inputs originating from PV+ cells, as sustained activation of GABAAergic synapses is known to result in dispersal of GABAARs from synapses.27 However, the increased siPSC frequency of SSTCreγ/2f/f pyramidal cells is inconsistent with reduced overall inhibition of pyramidal cells by PV+ cells. Moreover, preliminary behavioral analyses of Nkx2.1Creγ/2f/f mice, which lack forebrain GABAARs in both PV+ and SST+ interneurons, show an antidepressive-like phenotype comparable to that of SSTCreγ/2f/f mice, indicating that disinhibition of PV+ cells does not contribute materially to the behavioral phenotype of SSTCreγ/2f/f mice (Fuchs and Luscher, unpublished). The collective evidence suggests that the behavioral phenotype of SSTCreγ/2f/f mice reflects increased GABAAergic inhibition of pyramidal cells by disinhibited SST+ cells.

The behavioral changes of SSTCreγ/2f/f mice were paralleled by reduced phosphorylation of eEF2, reminiscent of biochemical end points of treatments with antidepressant doses of ketamine29,30,36 and 5-HT2C receptor antagonists.33 In contrast to drug-induced reductions in eEF2 phosphorylation, which involve transient activation of mTOR29,33,78–80 (however, see Autry et al.30 and Zanos et al.32), this pathway was unaffected in SSTCreγ/2f/f mice, as evidenced by unaltered mTOR and S6K phosphorylation. Instead, reduced phosphorylation of eEF2 was likely a consequence of the reduced synaptic E/I ratio of principal cells (Figure 3), correspondingly reduced NMDA receptor-mediated Ca2+ influx42 and reduced CaM-dependent auto-phosphorylation of eEF2K (Figure 5d), which has eEF2 as a single known target71 (Figure 5e). CaM-dependent auto-phosphorylation of eEF2K73,84 can largely account for the reduced activity of this enzyme that is evident based on reduced phosphorylation of eEF2 (Figure 5a). Curiously, the change in eEF2K73,84 phosphorylation evident in the hippocampus was not significant in mPFC, perhaps owing to the higher interneuron content in that brain region. The E/I ratio change in SST+ interneurons of SSTCreγ/2f/f mice is inverse to that in principal cells (Figures 2 and 3), suggesting that opposite E/I ratio-dependent biochemical changes in these two cell types could hamper the detection of such cell-type-specific changes in brain extracts.

In vivo, SST+ interneurons often form inhibitory synapses onto dendritic spines of pyramidal cells. Moreover, they are subject to feedback excitation from local pyramidal cells, indicating that they are tailored to limit the gain in network activity.43 Consistent with such a role, SST+ interneurons have been shown to control compartmentalized NMDA receptor-mediated Ca2+ entry into individual spines, which is thought to control dendritic spike generation and the firing rate of glutamatergic cells.42,43 Pharmacological manipulation of the excitability of cultured neurons34 and the data presented here indicate that this same mechanism regulates local dendritic eEF2K activity and eEF2-dependent mRNA translation, a process that has also recently been implicated in antidepressant mechanisms of ketamine.36

**CONCLUSION**

Collectively, the data indicate that chronically increasing the inhibitory synaptic input from SST+ interneurons to dendrites of principal cells results in enduring behavioral alterations and biochemical changes that mimic the potent but transient effects of rapidly acting experimental antidepressants, such as ketamine. At the cellular level, this mechanism involves a chronically increased excitability of SST+ neurons, a correspondingly reduced E/I ratio of synaptic inputs from SST+ neurons to glutamatergic neurons, reduced CaM-mediated activation of eEF2K and reduced eEF2K-mediated phosphorylation of eEF2. eEF2K and eEF2 are established effectors of rapidly acting antidepressants and known to regulate dendritic mRNA translation. Identification of the eEF2-target mRNAs that are subject to chronically altered translation in SSTCreγ/2f/f mice should facilitate the identification of novel targets for chronically effective antidepressant therapies.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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