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ORIGINAL ARTICLE

Serotonin 2C receptor antagonists induce fast-onset antidepressant effects

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Current antidepressants must be administered for several weeks to produce therapeutic effects. We show that selective serotonin 2C (5-HT2C) antagonists exert antidepressant actions with a faster-onset (5 days) than that of current antidepressants (14 days) in mice. Subchronic (5 days) treatment with 5-HT2C antagonists induced antidepressant behavioral effects in the chronic forced swim test (cFST), chronic mild stress (CMS) paradigm and olfactory bulbectomy paradigm. This treatment regimen also induced classical markers of antidepressant action: activation of cAMP response element-binding protein (CREB) and induction of brain-derived neurotrophic factor (BDNF) in the medial prefrontal cortex (mPFC). None of these effects were induced by subchronic treatment with citalopram, a prototypical selective serotonin reuptake inhibitor (SSRI). Local infusion of 5-HT2C antagonists into the ventral tegmental area was sufficient to induce BDNF in the mPFC, and dopamine D1 receptor antagonist treatment blocked the antidepressant behavioral effects of 5-HT2C antagonists. 5-HT2C antagonists also activated mammalian target of rapamycin (mTOR) and eukaryotic elongation factor 2 (eEF2) in the mPFC, effects recently linked to rapid antidepressant action. Furthermore, 5-HT2C antagonists reversed CMS-induced atrophy of mPFC pyramidal neurons. Subchronic SSRI treatment, which does not induce antidepressant behavioral effects, also activated mTOR and eEF2 and reversed CMS-induced neuronal atrophy, indicating that these effects are not sufficient for antidepressant onset. Our findings reveal that 5-HT2C antagonists are putative fast-onset antidepressants, which act through enhancement of mesocortical dopaminergic signaling.

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Keywords: antidepressant; BDNF; eEF; mTOR; neuronal remodeling

INTRODUCTION

Major depression is among the leading causes of disability worldwide and affects roughly 17% of the population.[1] Current antidepressants, including selective serotonin reuptake inhibitors (SSRIs), tricyclics and monoamine oxidase inhibitors, have major limitations. These agents must be continuously administered for a minimum of 2–4 weeks to produce therapeutic effects[2] and only 30–40% of patients respond to first-line treatment.[3] Faster-onset antidepressant treatments are greatly needed to improve the treatment of depression.

Few pharmacological treatments have been identified, which exert fast-acting antidepressant effects in humans. Two such examples are ketamine, a non-competitive *n*-methyl-D-aspartate receptor antagonist,[4,5] and scopolamine, a competitive muscarinic acetylcholine receptor antagonist,[6,7] which induce antidepressant responses within hours to days, respectively. However, these agents remain unapproved for the treatment of depression owing to serious side effects. Identifying the mechanism of action of agents with fast-onset antidepressant properties could yield novel therapeutic targets for depressive disorders.

Recent studies in rodents suggest that acute ketamine treatment induces rapid-onset antidepressant effects through rapid activation of extracellular signal-regulated kinase (ERK) and protein kinase B/Akt, which activate the mammalian target of rapamycin (mTOR) pathway;[8] activation of mTOR then leads to rapid synaptic spine formation on apical dendritic tufts of pyramidal neurons in the prefrontal cortex (PFC).[8]

Other work has shown that acute ketamine treatment deactivates eukaryotic elongation factor 2 (eEF2) kinase,[9] resulting in desuppression of brain-derived neurotrophic factor (BDNF) translation,[9] which is required for onset of antidepressant behavioral effects. These recently identified molecular mechanisms underlying rapid-onset antidepressant effects are thought to be distinct from those underlying the effects of current antidepressants.

Here, we examined whether selective serotonin 2C (5-HT2C) receptor antagonists produce fast-onset antidepressant effects using mouse models. Although selective 5-HT2C receptor antagonists have not been tested in clinical trials, they test positive in antidepressant assay models.[10] In addition, 5-HT2C antagonism is one component of the pharmacological action of many current antidepressants.[11] For example, the SSRI fluoxetine[12] and several tricyclic antidepressants antagonize 5-HT2C receptors with nanimolar affinity.[12–14] Also, the atypical antidepressant agomelatine, a melatonergic agonist and selective 5-HT2C antagonist, was reported to induce faster therapeutic onset than SSRIs in depressed patients.[15–17] We assessed whether selective 5-HT2C receptor antagonists induce faster-onset antidepressant effects (5 days) than current antidepressants (14 days) using chronic models of antidepressant action: the chronic FST (cFST),[18–21] the olfactory bulbectomy (OBX) model[22] and the unpredictable chronic mild

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stress (CMS) paradigm.[23,24] Then, we investigated whether subchronic (5 days) 5-HT2C antagonist treatment induces molecular and morphological changes elicited by fast-acting agents (ketamine) or current antidepressants (citalopram), including activation of ERK, Akt, mTOR, eEF2 or cAMP response element-binding protein (CREB) signaling, increases in BDNF expression or reversal of CMS-induced atrophy of layer II/III pyramidal neurons in the medial (mPFC).

MATERIALS AND METHODS

Animals

Experiments were carried out in 6- to 10-week-old female BALB/cJ mice, except that CMS and neuronal morphology studies were conducted using male C57Bl/6 mice (The Jackson Laboratory, Bar Harbor, ME, USA). Mice were housed under standard conditions (12:12 light–dark cycle; food and water *ad libitum*). Separate groups of mice were used for cFST, OBX and CMS. All animals were handled in accordance with the guidelines approved by The University of Chicago Institutional Animal Care and Use Committee.

Drugs and chemicals

The following compounds were used: RS 10221 hydrochloride (Tocris Bioscience, Minneapolis, MN, USA), SB 242084 (Tocris Bioscience), citalopram hydrobromide (Biotrend, Destin, FL, USA), fluoxetine hydrochloride (LKT Laboratories, St Paul, MN, USA), and SCH 39166 (Tocris Bioscience). All compounds were diluted in 5% dimethyl sulfoxide/saline (0.9% NaCl) solution. For details, see Supplementary 1.

Assessment of mTOR signaling and neurotrophic proteins

The following proteins were measured by western blot: CREB/pCREB (Cell Signalling, Danvers, MA, USA), BDNF (Santa Cruz Biotechnology, Santa Cruz, CA, USA), ERK/pERK (Cell Signaling), AKT/pAKT (Cell Signaling), glycogen synthase kinase 3 (GSK3)/pGSK3 (Cell Signaling), mTOR/pmTOR (Cell Signaling), P70S6 kinase (P70S6K)/pP70S6K (Cell Signaling) and eEF2/peEF2 (Cell Signaling). pCREB/CREB were detected using secondary anti-mouse antibody (Cell Signaling). BDNF, ERK/pERK, AKT/pAKT, GSK3/pGSK3, mTOR/pmTOR, P70S6K/pP70S6K and eEF2/peEF2 were detected using secondary anti-rabbit antibody (Cell Signaling). Bands were captured using a CCD lens (Nikon, Melville, NY, USA; no. 365451, Melville, NY, USA) and relative densities quantified using the program NIH ImageJ (http://rsbweb.nih.gov/ij/). For details, see Supplementary 1.

Local infusion experiments

Mice were anesthetized with Nembutal (intraperitoneally 55 mg kg^{-1}), placed in a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA) and implanted with 28-G bilateral cannula (Plastics One, Roanoke, VA, USA) into the ventral tegmental area (VTA) (anterior–posterior (AP): −3.3 mm from bregma; mediolateral (ML): ±0.5 mm midsaggital line; dorsoventral (DV): −4.4 mm from dura matter).[25] Continuous infusion of vehicle, 0.3 μg per day SB 242084 or 1.0 μg per day SB 242084 into VTA was administered by connecting bilateral cannulae to osmotic minipumps (Alzet, San Diego, CA, USA) via plastic tubing (28 gauge). At the end of the experiment, tissue was collected from the mPFC and expression of mTOR and BDNF protein measured via western blot. Control infusion studies were conducted in the substantia nigra (SN) and BDNF protein measured in the mPFC. For details, see Supplementary 1.

Structural analysis of dendritic morphology

Dendritic length and arborization, and spine number and shape were analyzed in pyramidal neurons of layer II/III of the prelimbic area of the mPFC. A three-dimensional analysis of the reconstructed neurons was performed using the NeuroExplorer software (Microbrightfield, Williston, VT, USA). For details, see Supplementary 1.

Behavioral studies

Details regarding the performance of the cFST, OBX, CMS and sucrose preference test are provided in Supplementary 1.

Statistical analysis

Dependent measures were analyzed using analysis of variance (ANOVA). Significant interactions were resolved using Newman–Keuls *post hoc* tests. Differences were considered statistically significant when $P<0.05$. For details, see Supplementary 1.

RESULTS

5-HT2C antagonists induce fast-onset antidepressant behavioral effects

We examined whether the selective 5-HT2C antagonists RS 102221 and SB 242084 could produce faster-onset antidepressant effects in the chronic forced swim test (cFST) compared with the prototypical SSRI citalopram. Subchronic treatment with 1 mg kg^{-1} per day RS 102221 or 1 mg kg^{-1} per day SB 242084 reduced time spent immobile (Figure 1a). In addition, subchronic treatment with 1 or 5 mg kg^{-1} per day RS 102221 or 1 mg kg^{-1} per day SB 242084 increased time spent swimming (Figure 1b). No effects of any drug treatment on time spent climbing were observed (Figure 1c). Subchronic treatment with 10 mg kg^{-1} per day citalopram had no effect on any behavior in the cFST (Figures 1a–c). Rather, chronic treatment with 10 mg kg^{-1} per day citalopram was required to reduce time spent immobile (Supplementary Figure 1b) and increase time spent swimming (Supplementary Figure 1c), consistent with previous reports using fluoxetine.[19,21] We also assessed locomotor activity to ensure that reductions in immobility during the cFST were not due to nonspecific increases in activity levels. RS 102221, SB 242084 or citalopram had no effect on overall locomotion at any dose after subchronic or chronic treatment, as measured by total distance traveled in the open field (Supplementary Figure 1a). In summary, 5-HT2C antagonists induced fast-onset antidepressant effects in the cFST.

Rodents subjected to OBX develop profound hyperlocomotion that can only be reversed by chronic treatment with current antidepressants including SSRIs.[26,27] We tested whether subchronic treatment with 5-HT2C antagonists would attenuate OBX-induced hyperlocomotion in the open field. As reported previously, OBX mice displayed increased locomotor activity compared with sham-operated mice 14 days after surgery (Figure 2a). Subchronic treatment with 1 mg kg^{-1} per day SB 242084 attenuated locomotor activity in OBX mice at the 20, 25 and 30 min time points, but not SHAM mice (Figure 2a). RS 102221 (5 mg kg^{-1} per day) reduced locomotor activity across SHAM and OBX groups (Figure 2b). Subchronic treatment with 5 mg kg^{-1} per day SB 242084 or 1 mg kg^{-1} per day RS 102221 had no effect on OBX-induced hyperlocomotion (Supplementary Figure 2b). As expected, subchronic treatment with 10 mg kg^{-1} per day citalopram did not alter locomotor activity levels (Figure 2c). Overall, these data indicate that 1 mg kg^{-1} per day SB 242084 exerts fast-onset antidepressant effects in the OBX model.

The unpredictable CMS paradigm induces depression-like behaviors that can be reversed by chronic treatment with current antidepressants.[23,28] We evaluated whether subchronic treatment with 5-HT2C antagonists could ameliorate CMS-induced anhedonia, a core symptom of depression. We assessed sucrose preference, an operational measure of anhedonia, weekly during 4 weeks of CMS exposure, and then again after 5 days of treatment with vehicle, SB 242084, RS 102221 or citalopram. CMS mice exhibited significantly reduced sucrose preference levels compared with controls at the end of weeks 1, 2, 3 and 4 (Figure 3). Four weeks into the study, CMS and control mice received subchronic treatment with vehicle, 1 mg kg^{-1} per day SB 242084, 3 mg kg^{-1} per day RS 102221 or 10 mg kg^{-1} per day citalopram; exposure to CMS continued during the 5-day period of drug treatment. Although no drug treatments altered sucrose preference under control conditions, 1 mg kg^{-1} per day SB 242084 and 3 mg kg^{-1} per day RS 102221 restored sucrose

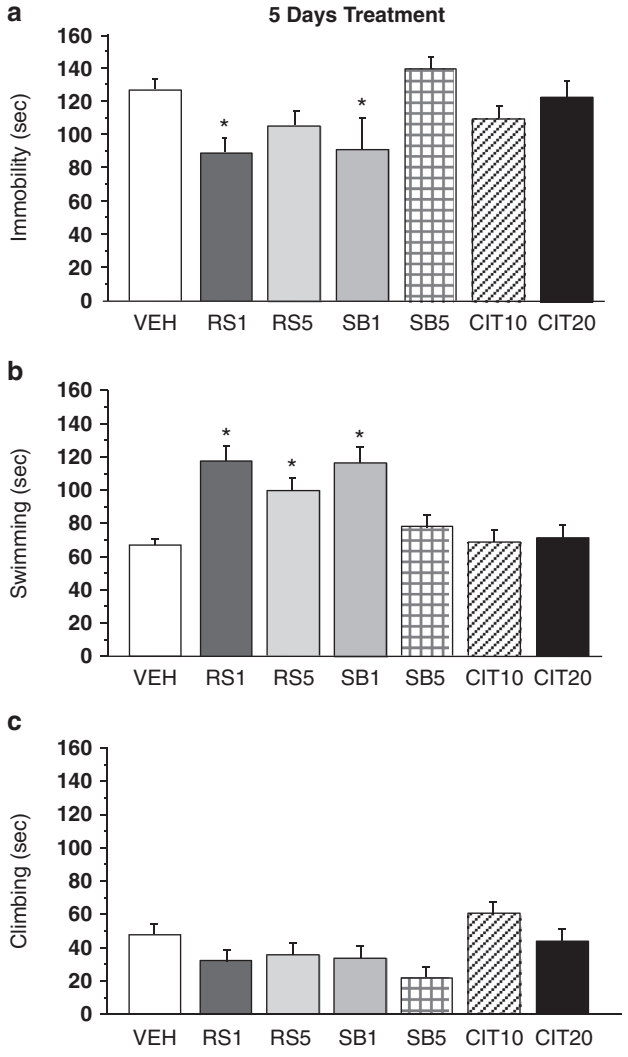

Figure 1. Effects of serotonin 2C (5-HT2C) antagonists and citalopram in the chronic forced swim test. (**a**) Subchronic treatment (5 days) with 1 mg kg^{-1} per day RS 102221 or 1 mg kg^{-1} per day SB 242084 reduced immobility; analysis of variance (ANOVA) $F_{(6,89)} = 2.38$, $^*P < 0.05$, Student–Newman–Keuls *post hoc* analysis. (**b**) RS 102221 (1 and 5 mg kg^{-1} per day) and SB 242084 (1 mg kg^{-1} per day) increased swimming behavior; ANOVA $F_{(6,89)} = 3.58$, $^*P < 0.01$, Newman–Keuls *post hoc* analysis. (**c**) Neither 5-HT2C antagonists nor citalopram affected climbing behavior; ANOVA $F_{(6,89)} = 1.10$, $P = 0.37$ ($n = 12$–15 mice per group for all experiments here; error bars represent s.e.m.).

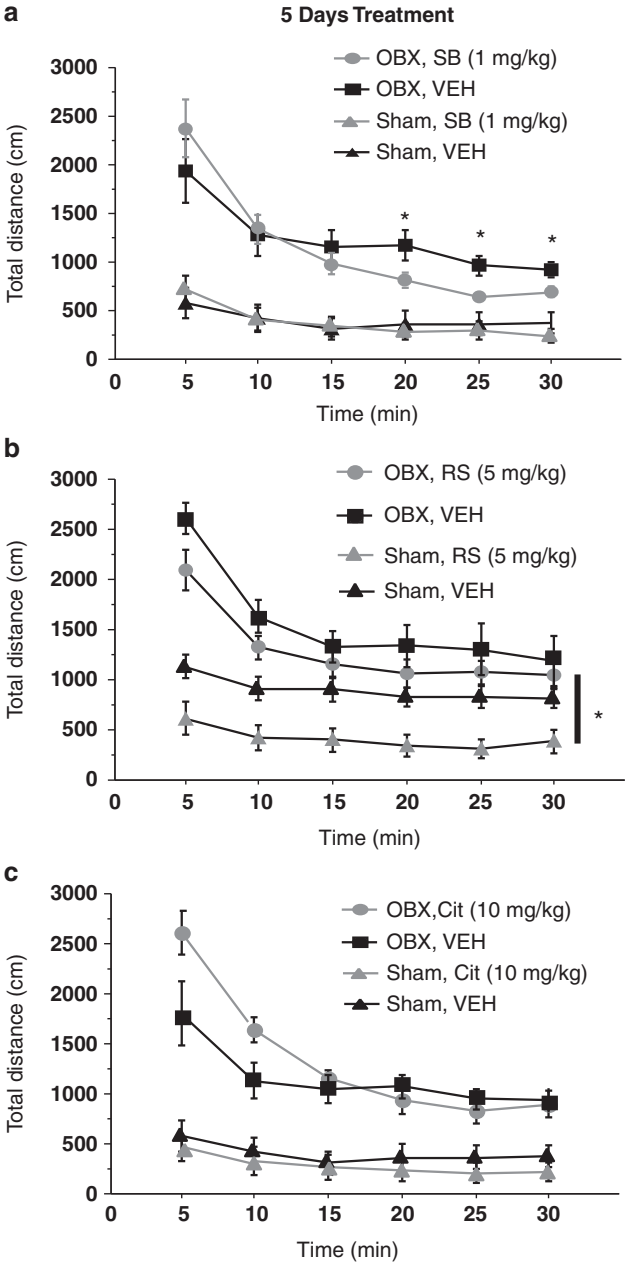

Figure 2. Effects of serotonin 2C (5-HT2C) antagonists and citalopram in the olfactory bulbectomy model. (**a**) Subchronic treatment (5 days) with 1 mg kg^{-1} per day SB 242084 (SB) reduced olfactory bulbectomy (OBX)-induced hyperactivity; analysis of variance (ANOVA) $F_{(5,80)} = 3.22$; $^*P < 0.01$, Newman–Keuls *post hoc* analysis. (**b**) Subchronic (5 days) treatment with 5 mg kg^{-1} per day RS 102221 (RS) reduced locomotion across OBX and Sham groups; ANOVA $F_{(1,326)} = 30.51$; $^*P < 0.0001$. (**c**) Subchronic (5 days) treatment with 10 mg kg^{-1} per day citalopram did not reduce OBX-induced hyperactivity ($n = 12$–15 mice per group for all experiments here; error bars represent s.e.m.). Veh, vehicle.

preference to baseline levels compared with vehicle in CMS mice (Figure 3). Subchronic treatment with 10 mg kg^{-1} per day citalopram did not affect sucrose preference in either condition (Figure 3). There were no consistent effects of drug treatments or CMS on total fluid consumption (Supplementary Figure 3). These data indicate that 5-HT2C antagonists induce fast-onset antidepressant effects in the CMS paradigm.

5-HT2C antagonists activate CREB and induce BDNF in the mPFC

Having identified fast-onset antidepressant behavioral effects of 5-HT2C antagonists, we then investigated the molecular mechanisms underlying this effect. Acute treatment with fast-acting antidepressants and chronic treatment with current antidepressants consistently increases pCREB and BDNF expression in the PFC.[29–31] Subchronic treatment with 1 mg kg^{-1} per day RS 102221 or 1 mg kg^{-1} per day SB 242084 significantly increased pCREB and BDNF expression in the mPFC (Figures 4a and b). In contrast, subchronic treatment with 10 mg kg^{-1} per day citalopram had no effect on BDNF or pCREB expression (Figures 4a and b). Thus, 5-HT2C antagonists induce rapid expression of molecular

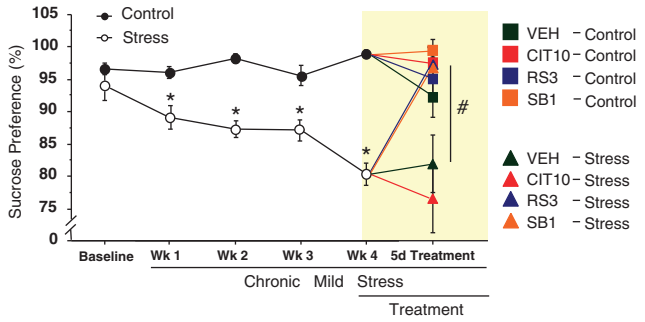

Figure 3. Effects of serotonin 2C (5-HT2C) antagonists and citalopram (CIT) on sucrose preference. In stressed mice, preference for 2% sucrose solution was significantly reduced across weeks 1–4; analysis of variance (ANOVA) $F_{(4,350)} = 8.53$, $^{*}P < 0.0001$ Stress vs Control, Newman–Keuls *post hoc* analysis. Subchronic treatment (5 days) with 3 mg kg^{-1} per day RS 102221 (RS) or 1 mg kg^{-1} per day SB 242084 (SB) restored sucrose preference to baseline levels; ANOVA $F_{(3,62)} = 4.31$; $^{\#}P < 0.01$ VEH Stress vs VEH Control, CIT Control, RS Control and SB Control; Newman–Keuls *post hoc* analysis ($n = 8$–10 mice per group for all experiments here; error bars represent s.e.m.).

markers known to be regulated by other fast-acting antidepressants[8,32,33] and current antidepressants.[29]

Next, we explored a role for mesocortical dopaminergic signaling in the induction of BDNF by 5-HT2C antagonists. We directly infused SB 242084 into the VTA for 5 days and assessed BDNF protein expression in the mPFC. Subchronic infusion of 0.3 μg per day or 1.0 μg per day SB 242084 into VTA significantly increased BDNF expression in the mPFC (Figure 4c and Supplementary Figures 6a and b). As a control, we directly infused SB 242084 into the SN for 5 days and measured BDNF protein expression. Subchronic infusion of 1.0 μg kg^{-1} per day SB 242084 into the SN did not alter BDNF expression in the mPFC (Supplementary Figures 7 and 8a, b). These findings indicate that blockade of 5-HT2C receptors in the VTA is sufficient to induce BDNF expression in the mPFC.

To further examine the role of dopaminergic signaling in the antidepressant effects of 5-HT2C antagonists, we tested whether systemic treatment with the D1-like antagonist, SCH 39166, could reduce the antidepressant effects of SB 242084 in the cFST. Planned comparisons and Newman–Keuls *post hoc* tests found that subchronic treatment with 0.3 mg kg^{-1} per day SCH 39166 blocked SB 242084-induced reductions in immobility and increases in swimming (Figure 4d). These findings show that signaling at D1 receptors is required for 5-HT2C antagonist-induced antidepressant effects.

5-HT2C antagonists activate mTOR and eEF2 in the mPFC

Next, we investigated the effects of subchronic treatment with 5-HT2C antagonists on intracellular signaling cascades implicated in fast onset antidepressant action. We found that subchronic treatment with 1 mg kg^{-1} per day RS 102221 and 1 mg kg^{-1} per day SB 242084 significantly activated mTOR (Figure 4e). These treatments also activated eEF2, which is activated in the dephosphorylated state (Figure 4f). However, no consistent effects of 5-HT2C antagonist treatment were observed on the activation states of ERK, protein kinase B/AKT, GSK3 or P70S6K (Supplementary Figures 4a–d).

Interestingly, either subchronic or chronic treatment with 10 mg kg^{-1} per day citalopram also significantly activated mTOR and eEF2 in the mPFC (Figures 4e and f). Furthermore, subchronic treatment with 10 mg kg^{-1} per day fluoxetine also activated mTOR in the mPFC (Supplementary Figure 5). No consistent effects of SSRI treatment were found on the activation state of ERK, protein kinase B/AKT, GSK3 or P70S6K (Supplementary Figures 4a–d). Thus, SSRIs activate mTOR and eEF2 in the mPFC following subchronic treatment, before antidepressant behavioral effects emerge.

We then examined whether mesocortical dopaminergic signaling regulates mTOR activation in the mPFC. We directly infused SB 242084 into the VTA for 5 days and then measured mTOR activation in the mPFC. Subchronic infusion of 0.3 or 1.0 μg per day SB 242084 into VTA significantly increased activated mTOR in the mPFC (Figure 4g and Supplementary Figures 6a and b). These data indicate that 5-HT2C antagonists activate mTOR in the mPFC through a mesocortical dopaminergic mechanism.

5-HT2C antagonists induce neuronal remodeling in mPFC pyramidal neurons

Chronic treatment with current antidepressants reverses CMS-induced atrophy of layer II/III pyramidal neurons in the mPFC of rodents.[23] Moreover, recent reports have shown that acute treatment with ketamine rapidly increases dendritic spine density on pyramidal neurons in the mPFC.[8,32] Therefore, we assessed whether subchronic treatment with 5-HT2C antagonists could reverse stress-induced alterations in dendritic morphology after CMS. Three-dimensional morphometric analysis of Golgi-impregnated neurons revealed that CMS induced significant atrophy of layer II/III pyramidal neurons in the mPFC, as measured by total dendritic length (Figure 5a). *Post hoc* analysis showed that CMS reduced total dendritic length in the vehicle group, but not the 1 mg kg^{-1} per day SB 242084, 3 mg kg^{-1} per day RS 102221 or 10 mg kg^{-1} per day citalopram-treated groups. In addition, CMS induced significant atrophy of apical dendrites of layer II/III pyramidal neurons (Figure 5b). Planned comparisons and *post hoc* analysis showed that CMS reduced apical dendritic length in the vehicle group, but not the SB 242084-, RS 10221- or citalopram-treated groups. Thus, subchronic treatment with SB 242084, RS 102221 or citalopram reversed CMS-induced reductions in both total and apical dendritic length. The length of basal dendrites was unaffected by CMS or drug treatment (data not shown).

The CMS protocol did not induce changes in the spine density of apical dendrites of mPFC pyramidal neurons. However, subchronic treatment with 1 mg kg^{-1} per day SB 242084 significantly increased spine density on distal apical dendrites across CMS and control conditions (Supplementary Figures 9a and b). Subchronic treatment with 1 mg kg^{-1} per day SB 242084 also reduced the ratio of thin:mushroom spines on apical dendrites across CMS and control conditions (Supplementary Figure 10c). In addition, subchronic treatment with 10 mg kg^{-1} per day citalopram significantly increased the number of proximal (Supplementary Figure 10b) and distal (Supplementary Figure 10e) apical mushroom spines.

Sholl analysis of dendritic distribution revealed an interaction of stress condition and drug treatment on the number of dendritic branch points. CMS reduced the number of dendritic branch points in vehicle-treated mice (Figure 5d and Supplementary Figures 11a and b); however, subchronic treatment with 1 mg kg^{-1} per day SB 242084 or 3 mg kg^{-1} per day RS 102221 reversed this effect of CMS on dendritic branching (Figure 5d and Supplementary Figure 11a). In addition, subchronic treatment with 10 mg kg^{-1} per day citalopram also restored dendritic branching in pyramidal mPFC neurons (Figure 5d and Supplementary Figure 11b). Thus, subchronic treatment with 5-HT2C antagonists or citalopram reversed CMS-induced reductions in dendritic branching.

DISCUSSION

Here, we show for the first time that selective 5-HT2C antagonists induce fast-onset (5 days) antidepressant effects using mouse

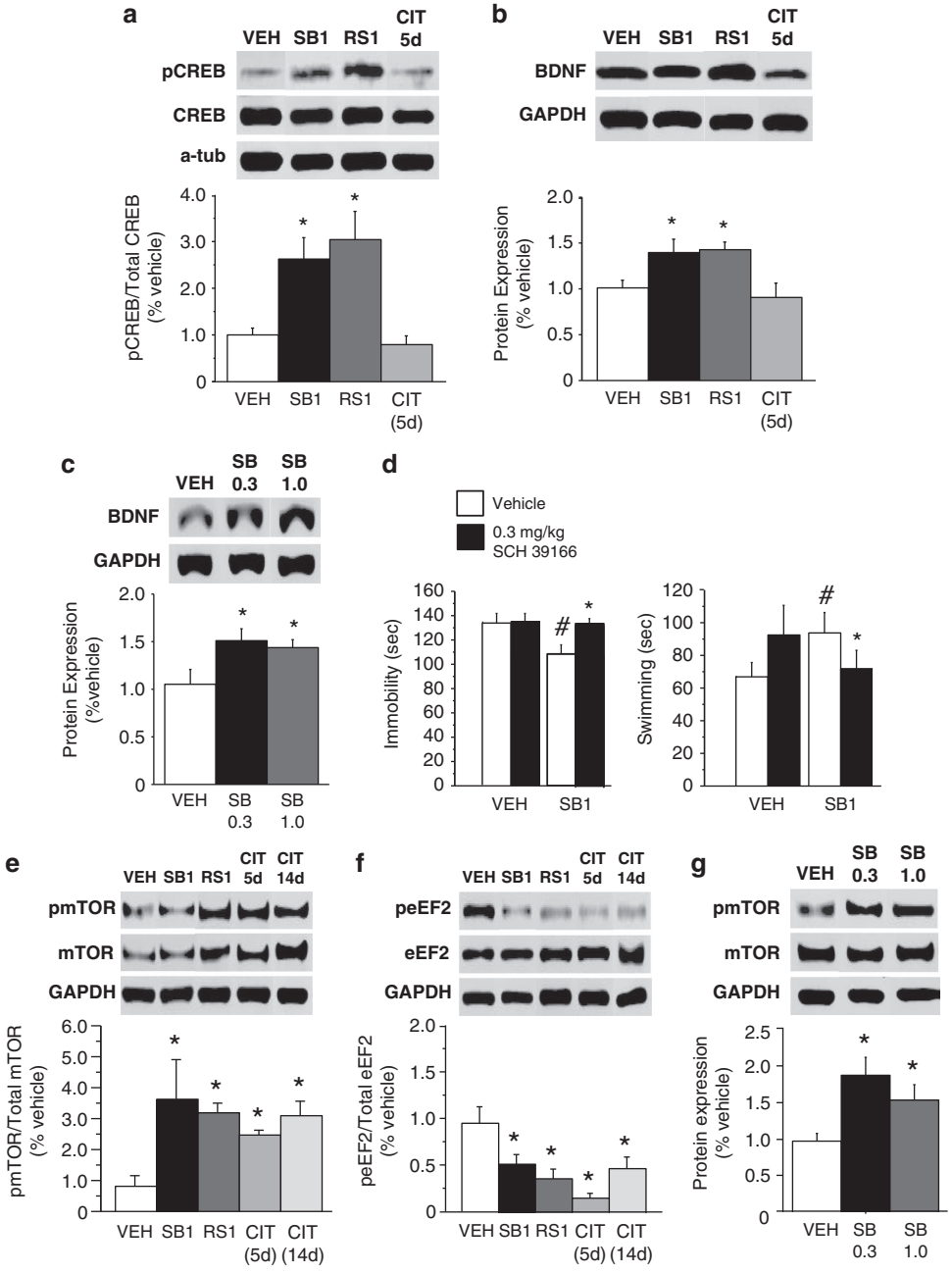

Figure 4. Effects of serotonin 2C (5-HT2C) antagonists and citalopram (CIT) on CREB, mammalian target of rapamycin (mTOR) and eukaryotic elongation factor 2 (eEF2) activation and brain-derived neurotrophic factor (BDNF) induction in the medial prefrontal cortex (mPFC). (**a**) Subchronic systemic treatment with 1 mg kg^{-1} per day RS 102221 (RS) or 1 mg kg^{-1} per day SB 242084 (SB) significantly increased pCREB expression; analysis of variance (ANOVA) $F_{(3,34)} = 10.21$; $^{*}P < 0.0001$, Newman–Keuls *post hoc* analysis, and (**b**) BDNF levels in the mPFC; ANOVA $F_{(3,39)} = 5.163$; $^{*}P < 0.005$, Newman–Keuls *post hoc* analysis. Relative optical densities were calculated for each band of interest and normalized to optical density of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and α-tubulin to account for differences in amount of protein loaded ($n = 8$–12 mice per group for all experiments here; error bars represent s.e.m.). (**c**) Subchronic infusion of 0.3 or 1.0 μg per day into the ventral tegmental area (VTA) significantly increased BDNF levels in the mPFC; ANOVA $F_{(2,20)} = 4.070$; $^{*}P < 0.05$, Newman–Keuls *post hoc* analysis ($n = 7$–8 mice per group; error bars represent s.e.m.). (**d**) Subchronic systemic treatment with 1 mg kg^{-1} per day SB 242084 reduced immobility and increased swimming behaviors in the chronic forced swim test, whereas SCH 39166 blocked these effects; ANOVA; $^{*}P < 0.05$, $^{\#}P = 0.05$, Newman–Keuls *post hoc* analysis. (**e**) Subchronic systemic treatment (5 days) with 1 mg kg^{-1} per day SB 242084, 1 mg kg^{-1} per day RS 102221, 10 mg kg^{-1} per day citalopram, or chronic treatment with 10 mg kg^{-1} per day citalopram significantly increased pmTOR expression; ANOVA $F_{(4,33)} = 3.91$, $^{*}P < 0.05$, Newman–Keuls *post hoc* analysis, and (**f**) decreased peEF2 expression; ANOVA $F_{(4,33)} = 5.81$, $^{*}P < 0.005$, Newman–Keuls *post hoc* analysis. (**g**) Subchronic infusion of 0.3 μg kg^{-1} per day SB 242084 or 1 μg kg^{-1} per day SB 242084 into the VTA significantly increased pmTOR expression in the mPFC; ANOVA $F_{(2,19)} = 5.999$; $^{*}P < 0.01$, Newman–Keuls *post hoc* analysis ($n = 7$–8 mice per group; error bars represent s.e.m.).

models. Antidepressant behavioral effects were not observed after 5 days (subchronic) of treatment with the prototypical SSRI citalopram, which requires chronic treatment to produce antidepressant actions.[33,34] The two 5-HT2C antagonists used in this study, SB 242084 and RS 102221, each induced antidepressant behavioral effects in the cFST, OBX model and the CMS paradigm.

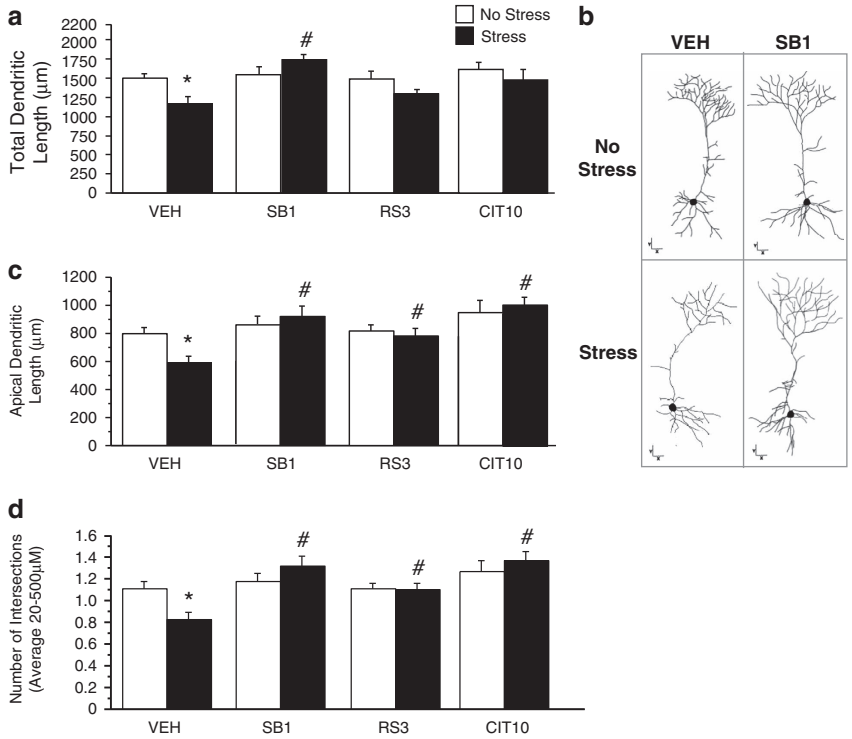

Figure 5. Effects of serotonin 2C (5-HT2C) antagonists and citalopram (CIT) on neuronal remodeling of pyramidal neurons in the medial prefrontal cortex. (**a**) Chronic mild stress (CMS) reduced total dendritic length of medial prefrontal cortex (mPFC) pyramidal neurons only in vehicle-treated mice; analysis of variance (ANOVA) $F_{(3,40)} = 2.82$; $^{*}P = 0.05$, Newman–Keuls *post hoc* analysis. Within CMS groups, subchronic systemic treatment with 1 mg kg^{-1} per day SB 242084 (SB) increased total dendritic length compared with vehicle; $^{\#}P = 0.05$, Newman–Keuls *post hoc* analysis. (**b**) Representative neuronal reconstructions showing the effects of CMS and 1 mg kg^{-1} per day SB 242084 treatment on dendritic length. (**c**) CMS decreased apical dendritic length only in vehicle-treated mice; planned comparisons, Newman–Keuls *post hoc* analysis. Within CMS groups, subchronic systemic treatment with 1 mg kg^{-1} per day SB 242084, 3 mg kg^{-1} per day RS 102221 (RS) or 10 mg kg^{-1} per day increased apical dendritic length compared with vehicle; $^{\#}P = 0.05$, Newman–Keuls *post hoc* analysis. (**d**) CMS signifcantly reduced the number of dendritic intersections only within vehicle-treated mice; ANOVA $F_{(3,40)} = 3.12$, $^{*}P < 0.05$, Newman–Keuls *post hoc* analysis. Within CMS groups, subchronic systemic treatment with 1 mg kg^{-1} per day SB 242084, 3 mg kg^{-1} per day RS 102221 or 10 mg kg^{-1} per day citalopram significantly increased apical dendritic length compared with vehicle; Newman–Keuls *post hoc* analysis ($n = 6$ mice per group for all experiments here; error bars represent s.e.m.).

Both drugs also activated CREB, mTOR and eEF2 signaling and increased BDNF protein levels in the mPFC. Local infusion of 5-HT2C antagonist into the VTA was sufficient for BDNF induction and mTOR activation in the mPFC. In addition, 5-HT2C antagonist-induced antidepressant behavioral effects were blocked by treatment with a dopamine D1 receptor antagonist. Both 5-HT2C antagonists also produced similar changes in the morphology of stress-sensitive pyramidal neurons in layer II/III of the mPFC. Surprisingly, subchronic SSRI treatment also activated mTOR and eEF2 and induced neuronal remodeling, suggesting that these effects are not sufficient for antidepressant onset. The present findings suggest that 5-HT2C antagonists induce fast-onset antidepressant effects by increasing mesocortical dopaminergic signaling onto dopamine D1 receptors.

Subchronic treatment with RS 102221 or SB 242084 reduced immobility, and increased swimming in the cFST (Figure 1). The effect sizes for RS 102221 and SB 242084 to increase swimming were large (Cohen's $d = 1.1$ and.9, respectively), suggesting that this effect may have clinical relevance. Chronic, but not subchronic, treatment with citalopram was required to produce antidepressant effects in the cFST (Supplementary Figure 1), consistent with previous work examining the onset of SSRIs in this test.[13,21,35] None of the drug treatments altered locomotion, indicating that reductions in immobility were not due to general increases in motor activity. Steady-state levels of citalopram are achieved in <2 days of treatment in mice,[36] suggesting that insufficient citalopram levels do not explain the lack of behavioral response after subchronic treatment. Thus, selective 5-HT2C antagonists induce a substantially faster-onset antidepressant response than SSRIs in the cFST.

Subchronic treatment with RS 102221 or SB 242084 also reversed OBX-induced hyperactivity. Specifically, 1 mg kg^{-1} per day SB 24084 reversed OBX-induced hyperlocomotion in the latter half of the open field session, indicating fast-onset antidepressant potential. The effect size for 1 mg kg^{-1} per day SB 242084 to attenuate OBX-induced hyperlocomotion was large (Cohen's $d = 1.2$, 0.9 and 1.1 for the 20, 25 and 30 min time points, respectively). RS 102221 (5 mg kg^{-1} per day) also attenuated OBX-induced hyperactivity; however, this dose of RS 102221 also reduced locomotion in sham mice, suggesting that the effects of RS 102221 were nonspecific. As chronic treatment with current antidepressants is required to attenuate OBX-induced hyperactivity in rodents,[37] our results indicate that SB 242084 produces fast-onset antidepressant effects in the OBX model.

Subchronic treatment with SB 242084 or RS 102221 reversed CMS-induced anhedonia, whereas 2 weeks of treatment with SSRIs are required for this effect.[23,38] The effect size for RS 102221 or SB 242084 to reverse CMS-induced reductions in sucrose preference was large (Cohen's $d = 1.4$); importantly, subchronic treatment with citalopram did not reverse CMS-induced anhedonia. We measured sucrose preference using a two-bottle test, rather than sucrose solution consumption using a one-bottle test, to avoid the potential confound of altered fluid intake. We found that CMS did not alter fluid intake during baseline, or weeks 1 or 4, indicating

that increased overall fluid intake did not reduce sucrose preference in the CMS group. In addition, no drug treatment altered overall fluid intake. Our results suggest that selective 5-HT2C antagonists induce fast-onset antidepressant effects in the CMS paradigm.

Like all known antidepressants, 5-HT2C antagonists activated CREB (pCREB) and induced BDNF expression in the mPFC. These effects require chronic treatment (2 weeks) with current antidepressants, including SSRIs.[29,39–41] Consistent with these reports, 5 days of treatment with citalopram did not increase pCREB or BDNF protein levels in the mPFC. However, subchronic SB 242084 or RS 102221 treatment robustly increased pCREB and induced BDNF expression in the mPFC (Figures 4a and b). The activation of CREB increases BDNF transcription,[42,43] and increased BDNF expression is required for the antidepressant behavioral response to current antidepressants[44–46] and acute ketamine treatment[9] in rodent models. Future studies will determine whether BDNF induction is required for 5-HT2C antagonist-induced fast-onset antidepressant effects.

The antidepressant behavioral effects of current antidepressants and ketamine depend upon BDNF induction.[9,45,46] Therefore, a better understanding of the mechanisms mediating BDNF induction will be critical for identifying novel antidepressant agents. We examined the mechanisms underlying BDNF induction in the mPFC by 5-HT2C antagonists. In rodents, systemic treatment with 5-HT2C antagonists increases the basal firing rate and bursting activity of dopamine neurons in the VTA, but not the SN, at the present doses.[47,48] As a consequence, 5-HT2C antagonist treatment increases dopamine levels in the frontal cortex ($\sim$50–70%), but not the striatum.[47] 5-HT2C antagonist-induced dopamine release is mediated by blockade of 5-HT2C receptors on GABAergic interneurons in the VTA, which tonically inhibit dopamine neurons.[47,48] We found that subchronic local infusion of SB 242084 into the VTA, but not the SN, increased BDNF protein levels in the mPFC (Figure 4c), implicating increased mesocoritcal dopaminergic transmission in BDNF induction. These findings show for the first time that increased dopaminergic signaling can induce BDNF expression *in vivo*. Furthermore, we found that the dopamine D1 receptor antagonist SCH 39166 blocked the antidepressant behavioral effects of SB 242084 in the cFST (Figure 4d). Thus, our findings suggest that 5-HT2C antagonists induce fast-onset antidepressant behavioral effects through the enhancement of mesocortical dopaminergic signaling onto D1 receptors.

Recent reports have identified some of the intracellular signaling pathways that are recruited by fast-onset antidepressant agents such as ketamine[8,32] and scopolamine.[49] For example, ketamine treatment rapidly induces BDNF, which then activates mTOR through the Akt and ERK pathways; mTOR activation ultimately leads to eEF2 activation and neuronal remodeling in the PFC.[8,9] We found that subchronic 5-HT2C antagonist treatment did not consistently alter the activation state of ERK (ERK1/2), Akt, GSK3, or P70S6K in the mPFC (Supplementary Figure 4), which are all altered by acute ketamine treatment.[8] Nevertheless, we found that subchronic 5-HT2C antagonist treatment activated mTOR (Figure 4e) and eEF2 (Figure 4f), two effects suggested to be essential for the rapid-onset antidepressant effects of ketamine.[8,9] 5-HT2C antagonists likely activate mTOR through molecular pathways downstream of dopaminergic signaling, as we found that intra-VTA infusion of SB 242084 activates mTOR in the mPFC (Figure 4g).

The intracellular signaling pathways engaged by fast-onset antidepressant agents are thought to be distinct from those recruited by current antidepressants.[42] For example, recent reports suggest that ketamine, but not SSRIs, activate mTOR and eEF2.[8] Surprisingly, we found that subchronic treatment with the SSRIs citalopram or fluoxetine (Supplementary Figure 5), or chronic treatment (14 days) with citalopram, both activate mTOR in the mPFC (Figure 4e). A recent report found no effect of 21 days of chronic fluoxetine treatment on mTOR activation in the PFC of rats.[8] Thus, either differences in treatment duration or species used could account for the discrepancy. Other work suggests that eEF2K inhibitors, which indirectly activate eEF2, provide novel rapid-acting therapeutics for depression.[9] However, we found that subchronic SSRI treatment activates eEF2 (Figure 4f), indicating that eEF2 activation is not sufficient for antidepressant onset. Our present findings suggest that BDNF induction remains the molecular marker that correlates most consistently with antidepressant onset. Our findings also indicate substantial overlap, rather than segregation, of the molecular signaling pathways mediating fast-onset versus current antidepressant effects.

Additional pharmacological agents that target serotonergic receptors have been reported to induce fast-onset antidepressant effects in rodents, including 5-HT4 agonists[33] and 5-HT7 antagonists.[50] For example, 3 days of treatment with 5-HT4 agonists reduces immobility in the FST, reverses OBX-induced hyperlocomotion and increases pCREB levels in the hippocampus of rats.[33] However, 5-HT4 agonists and 5-HT2C antagonists likely mediate their fast-onset antidepressant effects through largely different mechanisms. 5-HT4 agonists increase firing rates of serotonergic neurons in the dorsal raphe nucleus,[51] whereas 5-HT2C antagonists increase dopaminergic, but not serotonergic, cell firing.[52,53] These findings show that agents acting on neuromodulatory systems (e.g. monoamines) are capable of inducing fast-onset antidepressant effects in addition to those acting on major excitatory neurotransmitter systems (e.g. glutamate). However, fast-acting antidepressant agents acting on neuromodulatory systems, like 5-HT2C antagonists, might also indirectly influence glutamatergic signaling.

The onset of the antidepressant response has been associated temporally with the growth and remodeling of stress-sensitive neurons in prefrontal cortical circuits. Specifically, chronic treatment with current antidepressants induces synaptic spine formation and maturation, dendritic growth and increased dendritic branching of layer II/III pyramidal neurons in the mPFC following CMS-induced atrophy of these neurons.[23] We found that CMS reduced the total dendritic length and complexity of layer II/III pyramidal neurons, as reported previously.[23] Reductions in total dendritic length resulted from reductions in apical, not basal, dendritic length (Figure 5). Like current antidepressants, subchronic treatment with RS 102221 or SB 242084 reversed CMS-induced reductions in dendritic length and branching (Figure 5). Furthermore, subchronic SB 242084 treatment increased spine density on the apical tufts of layer II/III pyramidal neurons irrespective of stress condition (Supplementary Figures 9a and b), an effect induced on layer V pyramidal neurons by acute ketamine treatment.[8,32] Similar to the effects of chronic SSRI or acute ketamine treatment,[8,23] SB 242084 treatment also reduced the ratio of thin/mushroom spines on proximal apical dendrites on pyramidal neurons, suggesting an increase in spine function (Supplementary Figures 10a and c). RS 102221 treatment also increased apical spine density and reduced the ratio of proximal thin/mushroom spines, but these effects did not achieve statistical significance (Supplementary Figures 10a and c). These observations demonstrate that 5-HT2C antagonist treatment remodels mPFC pyramidal neurons in a manner similar to current antidepressant and ketamine treatment, and provide further evidence that 5-HT2C antagonists represent novel fast-onset antidepressant agents.

Few studies have examined the effects of subchronic treatment with current antidepressants on neuronal remodeling. Surprisingly, we found that subchronic citalopram treatment reversed CMS-induced reductions in dendritic length and complexity in layer II/III pyramidal neurons in the mPFC (Figure 5), effects thought to require chronic treatment. Subchronic citalopram treatment also increased apical spine density and reduced the

ratio of proximal thin/mushroom spines, although these effects did not reach statistical significance. As subchronic citalopram treatment does not induce antidepressant behavioral effects,[18,20,35] our findings indicate that these morphological effects are not sufficient for antidepressant onset. Future work should determine which morphological changes to neurons are required to induce antidepressant onset.

In conclusion, our findings show that selective 5-HT2C antagonists are putative fast-onset antidepressants. Five days of treatment with 5-HT2C antagonists induced antidepressant behavioral, molecular and morphological effects that are comparable to those of current antidepressants and the fast-acting agent ketamine. We show that 5-HT2C antagonists induce fast-onset antidepressant effects by increasing mesocortical dopaminergic signaling onto D1 receptors. We also show that the signaling pathways recruited by fast-onset versus current (slow-onset) antidepressant agents are more similar than recently suggested. Selective 5-HT2C antagonists may not produce serious side effects compared with ketamine and scopolamine, as many approved antidepressants antagonize 5-HT2C receptors. The putative fast-onset antidepressant effects of selective 5-HT2C antagonists should be examined in clinical trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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