Role of 5-HT\textsubscript{1A} Receptor Stimulation in the Medial Prefrontal Cortex in the Sustained Antidepressant Effects of Ketamine

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Abstract

Background: We previously reported that serotonergic transmission plays an important role in antidepressant effects of ketamine. However, detailed mechanisms have not been elucidated. Among the serotonin receptor subtypes, the serotonin\textsubscript{1A} receptor in the medial prefrontal cortex has an important role in depression. Here, we investigated the role of the medial prefrontal cortex serotonin\textsubscript{1A} receptor and its signaling mechanism in the antidepressant effects of ketamine.

Methods: The role of serotonin\textsubscript{1A} receptor-mediated signaling mechanism (phosphoinositide-3 kinase/Akt) in the medial prefrontal cortex was examined in the mouse forced swimming test and western blotting.

Results: Ketamine exerted antidepressant effects that lasted for 24 hours, and the sustained antidepressant effects were attenuated by intra-medial prefrontal cortex injection of a serotonin\textsubscript{1A} receptor antagonist, WAY100635. The sustained antidepressant effects were mimicked by intra-medial prefrontal cortex injection of (±)-8-hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT). The sustained antidepressant effects of ketamine and 8-OH-DPAT were abrogated by intra-medial prefrontal cortex injection of a phosphoinositide-3 kinase inhibitor.

Ketamine increased the phosphorylation of Akt in the medial prefrontal cortex at 60 minutes after administration, which was blocked by a serotonin\textsubscript{1A} receptor antagonist and a phosphoinositide-3 kinase inhibitor. Furthermore, the sustained antidepressant effects of ketamine and 8-OH-DPAT were attenuated by pretreatment of intra-medial prefrontal cortex injection of a mechanistic target of rapamycin complex-1 inhibitor.

Conclusions: These results indicate that selective stimulation of the medial prefrontal cortex serotonin\textsubscript{1A} receptor and subsequent activation of the phosphoinositide-3 kinase/Akt/mechanistic target of rapamycin complex-1 pathway may be necessary for ketamine to exert the sustained antidepressant effects, and that this mechanism could be targeted to develop a novel and effective approach for treating depression.

Keywords: ketamine, 5-HT\textsubscript{1A} receptor, antidepressant, phosphoinositide-3 kinase, Akt

Introduction

Major depressive disorder (MDD) represents a major social problem, with an estimated lifetime prevalence rate in the United States of approximately 17% (Kessler et al., 2003). With the currently available antidepressants, all of which act on the monoaminergic system, it takes several weeks before the antidepressant effects are manifested, and more than 30% of patients remain resistant to a series of treatments (Rush et al., 2006).
Significance Statement

Discovery of the antidepressant effects of ketamine opened up a new opportunity for the treatment of depression. However, mechanisms underlying the antidepressant effects of ketamine still remain largely unexplored. Recent evidence has indicated that serotonergic transmission mediates the antidepressant effects of ketamine. The present study demonstrated the importance of selective stimulation of the postsynaptic 5-HT\(_{1A}\) receptor in the medial prefrontal cortex and subsequent activation of phosphoinositide-3 kinase/Akt/mechanistic target of rapamycin complex-1 signaling in the sustained antidepressant effects of ketamine. These findings show the important role of a particular 5-HT\(_{1A}\) receptor-mediated signaling cascade in the antidepressant actions of ketamine, which could facilitate the development of more effective antidepressants.

In the present study, we investigated the role of the mPFC 5-HT\(_{1A}\) receptor and its downstream signaling in the antidepressant effects of ketamine. Among 5-HT\(_{1A}\) receptor-mediated signaling mechanisms, we particularly focused on the involvement of 5-HT\(_{1A}\) receptor-mediated phosphoinositide-3 kinase (PI3K)/Akt signaling in the mPFC, because not only is this pathway known to be a critical signaling cascade for neural plasticity (Chilmonczyk et al., 2015), but also changes in neural plasticity have been suggested to play important roles in the antidepressant actions of ketamine (Li et al., 2010; Gerhard et al., 2016; Dong et al., 2017). Moreover, because PI3K/Akt signaling activates mechanistic target of rapamycin complex 1 (mTORC1) signaling, which has also been strongly implicated in synaptogenesis (Hoeffer and Klann, 2010; Duman and Voleti, 2012; Duman et al., 2016) and antidepressant actions of ketamine (Li et al., 2010; Kolke et al., 2011), we additionally investigated the involvement of mTORC1 signaling in the mPFC in the antidepressant actions of ketamine.

Materials and Methods

Animals and Housing

Eight- or 9-week-old male C57BL/6J mice (Charles River Laboratories) were used for all the experiments. The animals were maintained under controlled temperature (23°C ± 3°C) and humidity (50% ± 20%) conditions under a 12-h-light/-dark cycle (lights on at 7:00 am). Food and water were provided ad libitum. All the studies were performed according to the guidelines of the Taisho Pharmaceutical Co., Ltd. Animal Care Committee, and met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments.

Drug Administration

Ketamine (Veterinary Ketalar 50; Sankyo Yell Pharmaceutical Co., Ltd) was diluted with saline. N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl[ethyl]-N-2-pyrindiny]cyclohexane carboxamide (WAY100635, a 5-HT\(_{1A}\) receptor antagonist) maleate (Sigma-Aldrich Co) and (a)-8-hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT, a 5-HT\(_{1A}\) receptor agonist) (Sigma-Aldrich Co.) were dissolved in saline or Ringer’s solution (147 mM NaCl, 4 mM KCl, and 1.2 mM CaCl\(_2\)), 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002, a PI3K inhibitor) (Focus Biomolecules) and rapamycin, an mTORC inhibitor (Sigma-Aldrich Co.), were dissolved in 6.2% dimethylsulfoxide (DMSO)/Ringer’s solution and 1% DMSO/Ringer’s solution, respectively. All these solutions were diluted with Ringer’s solution prior to being used for the intracerebral microinjections. The schedules of administration of each compound are illustrated in Figure 1 (A, D, G), Figure 2 (A, D, G, I), Figure 3 (A), Figure 4 (A, C), and Figure 5 (A). In the case of ketamine administration,
the effects at 30 minutes after administration are defined as acute effects, while those at 24 hours after administration are defined as sustained effects. Doses of 8-OH-DPAT (0.1–3 mg/kg for s.c. administration, 1 and 3 nmol/side for intra-mPFC injection) and WAY100635 (0.3–3 mg/kg for s.c. administration, 0.3 and 1 nmol/side for intra-mPFC injection) were selected by previous reports (Matsuda et al., 1995).

**Microinjection**

Each mouse was anesthetized with pentobarbital (50 mg/kg, i.p.) and fixed to a brain stereotaxic apparatus (Narishige Instruments). For the injection into the mPFC, the brains were implanted with guide cannulas (Eicom) bilaterally, so that the tips were positioned near the mPFC (anteroposterior, 2.0 mm from bregma; lateral, ±1.4 mm; ventral, -2.3 mm; angle, 20°). The cannulas were held in place with dental cement. A dummy cannula was inserted into the guide cannula to prevent clogging. Microinjection of the compounds was performed on day 2 or 3 after the surgery. Before the microinjections, the dummy cannulas were removed from the guide cannula, and a 28-gauge injection cannula, extending 0.5 mm from the tip of the guide cannula, was inserted. The injection cannula was connected via a Teflon tubing to a micro syringe (Hamilton Co) driven by a micro infusion pump (Harvard Apparatus, Inc). Injections of the compounds were performed for 2 minutes at the rate of 0.05 μL/min. The injection cannulas were left in position for an additional 2 minutes before being withdrawn. After the behavioral test, Evans blue was infused, followed by preparation of coronal sections to confirm the locations of the cannula tips. The locations of the cannula tips are shown in supplementary Figures 1 to 12.

**Forced Swimming Test (FST)**

The FST was performed by a previously reported method (Fukumoto et al., 2016), a modified method reported by Dulawa et al. (2004) that includes 2 swimming sessions at day 1 and day...
2. In brief, the mice were placed in a swim tank for 6 minutes on day 1. By doing this procedure, a state of helplessness is induced to prolong immobility in a swimming session on day 2. On day 2, they were placed again in the swim tank for 6 minutes to measure the immobility time. The swimming session on day 2 was regarded as the test session, and drugs were administered after swimming session on day 1. The swimming sessions were conducted by placing the mice in cylinders (24 cm height × 17 cm diameter) containing water (25°C ± 1°C) up to a height of 13 cm, so that the mice could not support themselves by touching the bottom of the tank with their paws. The FST was conducted between 8:00 am and 5:00 pm. The test sessions were videotaped from the front of the cylinders for later scoring. The total duration of immobility during a 6-minute test session was measured by an observer blinded to the treatment conditions. Immobility was defined as floating in the water without struggling and only making movements that were necessary to keep the head above the water. Different from the original method (Porsort et al., 1977), we measured immobility for 6 minutes to evaluate the effects of drugs. In the present method, mice showed immobility soon after placing them in a swim tank in the test session, as shown in representative groups (supplementary Figure 13).

Measurement of the Spontaneous Locomotor Activity

Mice were housed individually in transparent acrylic cages (φ30 cm × 30 cm). The animals were injected with each of the compounds at the designated time points, and the spontaneous locomotor activities were recorded for 60 minutes after the injections using the SCANET apparatus (Neuroscience Inc) placed in a sound-proof box.

Western Blotting

According to the report that ketamine transiently increased phosphorylation of Akt in the PFC, which may trigger the subsequent events (Li et al., 2010), we measured phospho-Akt at 60 minutes after ketamine administration. Medial prefrontal cortical tissue specimens were homogenized and sonicated in cell lysis buffer (50 mM Tris-HCl buffer, pH 7.5, containing 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 2 mM EDTA, 1 mM NaVO₃, 10 mM NaF, and protease inhibitor cocktail (Sigma-Aldrich Co). For western blotting, equal amounts of protein (20 μg) from each sample were separated by SDS-PAGE. The proteins were transferred to PVDF membranes (PerkinElmer), and the membranes were blocked in Tris-buffered saline (TBS) containing 0.1% Tween.
and 5% bovine serum albumin for 60 minutes and incubated with the primary antibodies (phospho-Akt (Ser473), 1:1000; Cell Signaling Technology) overnight at 4°C. The following day, the blots were washed 3 times in TBS containing 0.1% Tween 20 and incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:10,000; GE Healthcare) for 60 minutes at room temperature. After 3 final washes with TBS containing 0.1% Tween 20, the immunoreactive bands were detected using enhanced chemiluminescence. The blots were then incubated in stripping buffer for 60 minutes at room temperature, followed by 3 washes with TBS containing 0.1% Tween 20. The stripped blots were blocked for 60 minutes and incubated with the primary antibody (Akt, 1:1000; Cell Signaling Technology) as the loading control.

### Statistical Analysis

The results were expressed as the mean ± SEM. Statistical significance was determined by a 1-way ANOVA or a 2-way ANOVA, followed by the Dunnett, Tukey, or LSD posthoc test for comparing the treated group with the control group and multi-group comparisons, respectively. Statistical differences between any 2 groups were determined using the Student’s t test; P < .05 was considered to indicate statistically significant difference.

## Results

### Role of the 5-HT<sub>1A</sub> Receptor in the mPFC in the Acute Antidepressant Effects of Ketamine

Following systemic administration, ketamine (30 mg/kg, i.p.) significantly reduced the immobility time in the FST at 30 minutes after the drug administration (acute antidepressant effect) (Figure 1B, 1E). We previously confirmed that ketamine did not significantly increase locomotor activity at the time of the FST in the present condition (Fukumoto et al., 2016). Subcutaneous administration of the 5-HT<sub>1A</sub> receptor antagonist, WAY100635, abrogated the reduction of the immobility time induced by ketamine [F(4, 27) = 11.4, P < .01] (Figure 1B). Involvement of the 5-HT<sub>1A</sub> receptor in the actions of ketamine was also reported from a previous study conducted using a novelty-suppressed feeding test (Fukumoto et al., 2014). Then, we investigated the involvement of the 5-HT<sub>1A</sub> receptor in the mPFC in the acute antidepressant effects of ketamine. Microinjection of WAY100635 into the mPFC attenuated the reduction of the immobility time induced by ketamine [F(3, 28) = 35.03, P < .01] (Figure 1E). On the other hand, s.c. [F(1, 14) = 0.80, P = 0.79] (Figure 1C) or intra-mPFC [F(1, 19) = 0.13, P = 0.73] (Figure 1F) administration of WAY100635 per se had no effect on the immobility time in the FST. Therefore, the 5-HT<sub>1A</sub> receptor stimulation in the mPFC is involved in the acute...
antidepressant effects of ketamine. Systemic administration of the 5-HT_1A receptor agonist, 8-OH-DPAT (0.3–3 mg/kg, s.c.), significantly reduced the immobility time in the FST at 60 minutes after administration \( [F(4,35) = 6.00, \, P < .01] \) (Figure 1H), while it did not affect the spontaneous locomotor activity \( [F(1,10) = 0.00, \, P = .97] \) (Figure 1I), confirming that 5-HT_1A receptor stimulation exerts the acute antidepressant effects.

Role of the 5-HT_1A Receptor in the mPFC in The Sustained Antidepressant Effects of Ketamine

Following systemic administration, ketamine (30 mg/kg, i.p.) exhibited a sustained antidepressant effect (effect lasting for at least 24 hours after the drug administration) (Figure 2B, 2E). Subcutaneous administration of WAY100635 abrogated the reduction of the immobility time induced by ketamine \( [F(4,27) = 12.65, \, P < .01] \) (Figure 2B). Microinjection of WAY100635 into the mPFC abrogated the reduction of the immobility time in the FST induced by ketamine \( [F(3,28) = 52.09, \, P < .01] \) (Figure 2E). Subcutaneous \( [F(1,14) = 0.00, \, P = .98] \) (Figure 2C) and intra-mPFC \( [F(1,14) = 0.00, \, P = .98] \) (Figure 2F) injections of WAY100635 per se had no effect on the immobility time in the FST. Therefore, the sustained antidepressant effects of ketamine are mediated through 5-HT_1A receptor stimulation in the mPFC. To confirm that stimulation of the 5-HT_1A receptor in the mPFC is associated with sustained antidepressant effects, the effect of 8-OH-DPAT was investigated. Systemic administration of 8-OH-DPAT did not result in sustained antidepressant effects in the FST \( [F(3,28) = 0.21, \, P = .89] \) (Figure 2H). Importantly, in contrast to the systemic administration of 8-OH-DPAT, local injection of 8-OH-DPAT into the mPFC was associated with sustained antidepressant effects \( [F(2,20) = 6.11, \, P < .01] \) (Figure 2J), with no effect on the spontaneous locomotor activity \( [F(1,10) = 4.26, \, P = .01] \) (Figure 2L). Microinjection of WAY100635 into the mPFC abrogated the reduction of the immobility time in the FST induced by 8-OH-DPAT \( [F(3,27) = 23.01, \, P < .01] \) (Figure 2K).

Role of PI3K/Akt Signaling in the mPFC in the Sustained Antidepressant Effects of Ketamine and 8-OH-DPAT

The sustained antidepressant effects of ketamine have been reported to be mediated via changes in synaptic plasticity (Li et al., 2010; Dong et al., 2017), and PI3K/Akt signaling is a critical signaling cascade involved in synaptic plasticity induced by 5-HT_1A receptor activation (Chilmonczyk et al., 2015). Therefore,
we investigated the involvement of PI3K/Akt signaling in the mPFC in the sustained antidepressant effects of ketamine. Microinjection of a PI3K inhibitor, LY294002, into the mPFC significantly abrogated the reduction of the immobility time in the FST induced by ketamine \([F(3,28) = 17.07, P < .01]\) (Figure 3B) and 8-OH-DPAT \([F(3,28) = 29.36, P < .01]\) (Figure 3C), only at the highest dose. Intra-mPFC injection of LY294002 per se had no effect on the immobility time in the FST \([F(1,9) = 0.00, P = .95]\) (Figure 3D).

Moreover, significant increase in the phosphorylation of Akt was observed in the mPFC at 60 minutes after systemic ketamine (30 mg/kg, i.p.) administration (Figure 4B, 4D). Importantly, the increase in Akt phosphorylation induced by ketamine was blocked by intra-mPFC injection of not only LY294002 (Figure 4B), but also that of WAY100635 (Figure 4D). These results indicate that PI3K activation is involved in the sustained antidepressant effects of ketamine and 8-OH-DPAT, and that ketamine activates Akt signaling via stimulation of the PI3K and 5-HT1A receptor in the mPFC.

**Role of mTORC1 in the mPFC in the Sustained Antidepressant Effects of Ketamine and 8-OH-DPAT**

We next investigated if mTORC1, which is downstream of PI3K/Akt, is involved in the antidepressant actions of ketamine. Microinjection of a mTORC1 inhibitor, rapamycin, into the mPFC abrogated the sustained antidepressant effects of ketamine \([F(3,28) = 35.13, P < .01]\) (Figure 5C), only at the highest dose. On the other hand, local injection of rapamycin into the mPFC per se had no effect on immobility time in the FST \([F(1,13) = 0.86, P = .37]\) (Figure 5D). These results suggest not only that stimulation of mTORC1 signaling in the mPFC mediates the sustained antidepressant effects of ketamine, but also that mTORC1 signaling is necessary to exert the mPFC 5-HT1A receptor stimulation-mediated antidepressant actions.

**Discussion**

The present results indicated that stimulation of 5-HT1A receptor and subsequent activation of PI3K/Akt/mTORC1 signaling in the mPFC may play an important role in the sustained antidepressant effects of ketamine.

In the present study, both the acute and sustained antidepressant effects of ketamine observed in the FST were attenuated by local injection of WAY100635, a 5-HT1A receptor antagonist, into the mPFC, indicating that stimulation of the mPFC 5-HT1A receptor plays an important role in both acute and sustained antidepressant actions of ketamine. Interestingly, acute, but not sustained, antidepressant effects were observed following systemic administration of a 5-HT1A receptor agonist, while local injection of a 5-HT1A receptor agonist into the mPFC was associated with the sustained antidepressant effects. Therefore, selective stimulation of the 5-HT1A receptor in the mPFC, and not generalized stimulation in all regions of the brain, may be
involved in a sustained antidepressant action of ketamine. 5-HT\textsubscript{1A} receptor is not only localized as a presynaptic inhibitory autoreceptor on the 5-HT neuronal cell bodies in the DRN, but also as postsynaptic receptors in brain regions associated with the control of mood and cognition, such as the PFC and hippocampus (Miquel et al., 1992). Thus, the present results indicate the importance of selective stimulation of the postsynaptic 5-HT\textsubscript{1A} receptor in the mPFC for obtaining sustained antidepressant actions. This is consistent with the previous findings that the postsynaptic 5-HT\textsubscript{1A} receptor in the mPFC and limbic areas is particularly important for the antidepressant response (Blier and de Montigny, 1994; Haddjeri et al., 1998), while the loss of 5-HT\textsubscript{1A} heteroreceptor in the mPFC during adolescence is also reported to be sufficient to exert the depressive-like behavior (Garcia-Garcia et al., 2017). The importance of selective stimulation of the postsynaptic 5-HT\textsubscript{1A} receptor is also underscored by the finding reported by Assié et al. (2010) that a selective postsynaptic 5-HT\textsubscript{1A} receptor agonist, F15599, exerted a more potent and sustained antidepressant effect than 5-HT\textsubscript{1A} receptor agonists that act on both the postsynaptic and presynaptic 5-HT\textsubscript{1A} receptor.

Ketamine is rapidly eliminated from the plasma within minutes to hours (Can et al., 2016; Maxwell et al., 2006). Therefore, the sustained antidepressant effects of ketamine (effects lasting for at least 24 hours after a single administration) may be ascribed to changes in the synaptic plasticity. Indeed, ketamine has been reported to enhance synaptic protein synthesis and dendritic spine density in the mPFC (Li et al., 2010; Dong et al., 2017). Among the intracellular signaling cascades induced by postsynaptic 5-HT\textsubscript{1A} receptor stimulation, PI3K/Akt signaling plays a crucial role in synaptic plasticity (Polter et al., 2012; Islam et al., 2014; Wang et al., 2016a). In the present study, the sustained antidepressant effects of ketamine were abrogated by intra-mPFC injection of a PI3K inhibitor. In addition, ketamine increased Akt phosphorylation in the mPFC, which was attenuated by local injection of a 5-HT\textsubscript{1A} receptor antagonist or a PI3K inhibitor into the mPFC. Therefore, the sustained antidepressant actions of ketamine were exerted, presumably through 5-HT\textsubscript{1A} receptor-mediated activation of PI3K/Akt signaling in the mPFC. This notion was supported by the finding that the sustained antidepressant effect of a 5-HT\textsubscript{1A} receptor agonist was also blocked by intra-mPFC injection of a PI3K inhibitor. Ketamine has been reported to increase synaptic protein synthesis and to induce the sustained antidepressant effects through activation of mTORC1 signaling (Li et al., 2010), downstream of PI3K/Akt (Duman and Voleti, 2012; Chilmonczyk et al., 2015). Interestingly, we obtained the results in the present study that mTORC1 signaling in the mPFC is involved in not only the sustained antidepressant effects of ketamine but also the sustained antidepressant effects of a 5-HT\textsubscript{1A} receptor agonist. Therefore, 5-HT\textsubscript{1A} receptor-mediated activation of mTORC1 signaling in the mPFC can induce the sustained antidepressant effects. Collectively, the present study suggests that 5-HT\textsubscript{1A} receptor activation and its downstream PI3K/Akt/mTORC1 signaling in the mPFC may have an important role in the sustained antidepressant actions of ketamine.

It is unknown how ketamine stimulates the postsynaptic 5-HT\textsubscript{1A} receptor in the mPFC to exert antidepressant effects. Because ketamine has negligible activity at 5-HT\textsubscript{1A} receptor (Roth et al., 2013), ketamine may indirectly activate 5-HT\textsubscript{1A} receptor and its signaling cascade. It has previously been reported that intra-mPFC injection or systemic administration of ketamine increases 5-HT release in the mPFC via activation of neurons of the DRN (Nishitani et al., 2014; Pham et al., 2017). We previously reported that ketamine activates a subpopulation of 5-HT neurons in the DRN via stimulation of AMPA receptor in the mPFC (Fukumoto et al., 2016). Therefore, it is hypothesized that ketamine increases 5-HT release in the mPFC through activation of the subpopulation of the DRN 5-HT neurons by stimulating the mPFC projections. To support this hypothesis, increase in glutamate transmission, including AMPA receptor activation, in the infralimbic cortex (a part of the mPFC) has also been shown to increase 5-HT release in the region, likely through increased activity of neurons of the DRN, resulting in an antidepressant effect (Gasull-Carnós et al., 2017). However, it has also been reported that ketamine decreased, at 24 hours after administration, the firing of 5-HT neurons in the DRN (Pham et al., 2017) and that ketamine increased 5-HT release in the PFC by inhibition of 5-HT transporters (Yamamoto et al., 2013). Therefore, it is also conceivable that ketamine may increase 5-HT release in the mPFC through the local mechanisms. In either case, increased 5-HT release in the mPFC by ketamine may subsequently stimulate postsynaptic 5-HT\textsubscript{1A} receptor in the region. Taking these findings and the present results into consideration, it is suggested that increase of 5-HT release in the mPFC by ketamine stimulates the postsynaptic 5-HT\textsubscript{1A} receptor-mediated activation of PI3K/Akt/mTORC1 cascade, leading to sustained antidepressant actions of the drugs. Nonetheless, this hypothesis remains to be further investigated. The above-mentioned hypothetical mechanisms are illustrated in Figure 6.

Figure 6. Hypothetical mechanisms underlying the sustained antidepressant effects of ketamine. Ketamine increases serotonin (5-HT) release in the medial prefrontal cortex (mPFC) through local mechanisms such as inhibition of 5-HT transporter or through activation of the dorsal raphe nucleus (DRN) neurons by mPFC projection. Increase in 5-HT release may stimulate postsynaptic 5-HT\textsubscript{1A} receptor in the mPFC, leading to activation of phosphoinositide-3 kinase (PI3K)/Akt/mTORC1 signaling. Activation of PI3K/Akt/mTORC1 signaling has been reported to mediate the sustained antidepressant effects of ketamine (Li et al., 2010).
There are some limitations in the present study. One of the limitations is doses of 5-HT_{1A} receptor ligands 8-OH-DPAT and WAY100635 used in this study. We used higher doses of both compounds than those used in the previous studies (Linge et al., 2016). 8-OH-DPAT has been reported to act on other receptors, particularly as an agonist for 5-HT, receptor (Sprouse et al., 2004; Thomas et al., 1998), while WAY100635 acts as an agonist for dopamine D₂ receptor (Chemel et al., 2006; Dilly and Liégeois, 2016). Therefore, roles of other receptors cannot be fully ruled out. The other limitation of the present study is lack of investigations on roles of postsynaptic 5-HT_{1A} receptor in other brain regions, particularly in the hippocampus. Because the postsynaptic 5-HT_{1A} receptor in the hippocampus has been implicated in depression and antidepressant actions (Santarelli et al., 2003; Samuels et al., 2015), and roles of the ventral hippocampus-mPFC pathway have been reported for antidepressant actions of ketamine (Carreno et al., 2016), it would be necessary to elucidate roles of postsynaptic 5-HT_{1A} receptor in the hippocampus in the actions of ketamine in future studies. Moreover, in the present study, we used only the FST of naïve mice. Although the FST has been widely used to evaluate antidepressant effects, the test does not mimic some of the modifications observed in depression. Therefore, investigations by using other models, particularly models in which chronic stress is exposed are needed to confirm the role of 5-HT_{1A} receptor and its signaling cascade in the sustained antidepressant effects of ketamine.

In conclusion, the present study demonstrated that selective stimulation of the postsynaptic 5-HT_{1A} receptor and the subsequent activation of PI3K/Akt/mTORC1 signaling in the mPFC may be responsible for the sustained antidepressant effect of ketamine. More precise elucidation of the mechanisms of regulation of the 5-HTergic systems by ketamine may pave the way for the development of new faster, effective, and longer-acting drugs for the treatment of depression.

Supplementary Material
Supplementary data are available at International Journal of Neuropsychopharmacology online.

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Statement of Interest
All authors are employees of Taisho Pharmaceutical Co., Ltd.

References
Fukumoto K, Iijima M, Chaki S (2016) The antidepressant effects of an mGlu2/3 receptor antagonist and ketamine require AMPA receptor stimulation in the mPFC and subsequent activation of the 5-HT neurons in the DRN. Neuropsychopharmacology 41:1046–1056.


