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Rapid antidepressant actions of scopolamine: Role of medial prefrontal cortex and M1-subtype muscarinic acetylcholine receptors

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ABSTRACT

Clinical studies demonstrate that scopolamine, a non-selective muscarinic acetylcholine receptor (mAchR) antagonist, produces rapid therapeutic effects in depressed patients, and preclinical studies report that the actions of scopolamine require glutamate receptor activation and the mechanistic target of rapamycin complex 1 (mTORC1). The present study extends these findings to determine the role of the medial prefrontal cortex (mPFC) and specific muscarinic acetylcholine receptor (M-AchR) subtypes in the actions of scopolamine. The administration of scopolamine increases the activity marker Fos in the mPFC, including the infralimbic (IL) and prelimbic (PrL) subregions. Microinfusions of scopolamine into either the IL or the PrL produced significant antidepressant responses in the forced swim test, and neuronal silencing of IL or PrL blocked the antidepressant effects of systemic scopolamine. The results also demonstrate that the systemic administration of a selective M1-AChR antagonist, VU0255035, produced an antidepressant response and stimulated mTORC1 signaling in the PFC, similar to the actions of scopolamine. Finally, we used a chronic unpredictable stress model as a more rigorous test of rapid antidepressant actions and found that a single dose of scopolamine or VU0255035 blocked the anhedonic response caused by CUS, an effect that requires the chronic administration of typical antidepressants. Taken together, these findings indicate that mPFC is a critical mediator of the behavioral actions of scopolamine and identify the M1-AChR as a therapeutic target for the development of novel and selective rapid-acting antidepressants.

Major depressive disorder (MDD) is a recurring, debilitating illness characterized by depressed mood, diminished self-esteem, decreased motivation, and inability to experience pleasure. Moreover, MDD is one of the leading causes of morbidity and mortality worldwide leading to a significant socioeconomic impact on health care systems (Kessler et al., 2003). Given the unmet needs in the treatment of this condition, including tolerability, slow onset of action, and low rates of efficacy (Fournier et al., 2010; Papakostas, 2007), the search for new therapies that can overcome these limitations has been a high priority of drug development programs. Recent clinical findings have provided several novel treatments that address these issues, including the NMDA receptor antagonist ketamine (Berman et al., 2000; Zarate et al., 2006) and the non-selective muscarinic acetylcholine receptor (M-AChR) antagonist scopolamine (Drevets and Furey, 2010; Furey and Drevets, 2006), both of which produce rapid antidepressant actions in depressed patients, including patients considered treatment resistant (i.e., have failed to respond to two or more typical antidepressant agents).

Recent mechanistic studies in rodent models have demonstrated similarities in the downstream cellular actions underlying the rapid antidepressant effects of ketamine and scopolamine (Duman and Aghajanian, 2012; Li et al., 2010, 2011; Voleti et al., 2013). We have reported that a single dose of scopolamine, like ketamine, rapidly increases the mechanistic target of rapamycin complex 1 (mTORC1) signaling in the medial prefrontal cortex (mPFC), and that the antidepressant behavioral actions of scopolamine are blocked by infusion of rapamycin, a selective inhibitor of mTORC1 (Voleti et al., 2013). These studies also show that the behavioral actions of scopolamine, like ketamine, are blocked by pretreatment with a glutamate AMPA receptor antagonist, consistent with evidence that scopolamine and ketamine cause a rapid, transient burst of glutamate in the mPFC (Li et al., 2010; Moghaddam et al., 1997; Voleti et al., 2013). This burst of glutamate is thought to occur via blockade of muscarinic or NMDA receptors by scopolamine or ketamine, respectively, on GABA interneurons, resulting

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in the disinhibition of glutamate transmission (see supplementary Fig. 1). Glutamate–AMPA receptor activation leads to stimulation of voltage-dependent calcium channels and release of BDNF, which then stimulates mTORC1 signaling and synaptogenesis (Duman and Aghajanian, 2012; Voleti et al., 2013). These studies also demonstrate that a single dose of scopolamine or ketamine rapidly increases the number and function of spine synapses on layer V pyramidal neurons in the mPFC (Li et al., 2010; Voleti et al., 2013). We have also reported that a single dose of ketamine rapidly reverses the anhedonia caused by exposure to chronic unpredictable stress (CUS) (Li et al., 2011). The effect of CUS and the resulting anhedonia, a core symptom of depression, is only blocked by the chronic (2 to 3 weeks) administration of a typical antidepressant (Willner, 1997) and therefore provides a rigorous test for putative rapid-acting antidepressants, although the effects of scopolamine have not been tested.

Based on our findings that scopolamine increases mTORC1 and spine synapses in the mPFC, the current studies were undertaken to examine the role of the mPFC in the antidepressant actions of scopolamine and to better understand the M-AChR subtype underlying these actions. We also determine if scopolamine administration can reverse the anhedonia caused by CUS exposure. We use a combination of scopolamine microinfusion, neuronal silencing, and selective M-AChR antagonist approaches to address these questions. Taken together, these findings highlight a role for M1-AChR in the mPFC as an integral mediator of the antidepressant effects following scopolamine.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 175–250 g were pair-housed and maintained in standard conditions with a 12-h light/dark cycle and *ad libitum* access to food and water. Animal use and procedures were in accordance with the National Institutes of Health guidelines and approved by the Yale University Animal Care and Use Committees.

Drug administration and surgical procedure

Animals received a single acute injection of vehicle, scopolamine (25 μg/kg, intra-peritoneal, i.p.), or the preferential M1-AChR antagonist VU0255035 (*N*-[3-oxo-3-[4-(4-pyridinyl)-1-piper azinyl]propyl]-2,1,3-benzothiadiazole-4-sulfonamide) (1 or 3 mg/kg, i.p.). In a separate series of studies, the influence of 3 doses of scopolamine or VU0255035 on alternate days was also examined. Tissue was collected from separate groups of animals for molecular or immunohistochemical studies, and separate cohorts were also used in behavioral experiments as described below. For experiments involving the central administration of scopolamine (30–100 pg, intra-cerebral-ventricular, i.c.v.), muscimol, or the selective M3-AChR antagonist 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP) (100 or 200 pmol, i.c.v.), rats were implanted with either intracortical or intracerebral ventricular (i.c.v.) guide cannulas under anesthesia with pentobarbital (50 mg/kg i.p.) as previously reported (Cota et al., 2006; Li et al., 2010). Coordinates for prelimbic (PrL) and infralimbic (IL) cortex were determined according to a stereotaxic atlas (Paxinos and Watson, 2007) and were +3.2 rostral-caudal (RC), ±1.0 medial-lateral (ML), and −2.8 dorsal–ventral (DV) or 2.8 RC, 3.1 ML, and 3.8 DV for PL or IL, respectively, using bilateral cannulas. For IL, cannulas were implanted with a medial–lateral angulation of 30° to avoid destruction of PrL due to cannula placement. After recovery for at least 7 days, PBS or scopolamine were infused in the PrL or IL cortex at 0.1 μl/min rate. For neuronal silencing prior to systemic scopolamine, muscimol was administered (1.25 μg in 0.2 μl) bilaterally using the same coordinates described for IL and PrL. Coordinates for i.c.v. infusion of 4-DAMP (unilateral) were −0.9 RC, ±1.5 ML, −3.5 DV; vehicle (DMSO) or 4-DAMP was delivered i.c.v. at 0.25 μl/min rate.

Brain slice preparation and immunohistochemistry

Brain slices were prepared as previously described (Peng et al., 1995). Briefly, rats were deeply anesthetized (chloral hydrate 400 mg/kg IP) and perfused transcardially with phosphate-buffered saline (PBS) followed by 4% phosphate-buffered paraformaldehyde (pH 7.4). The brains were then cut into serial 40 μm thick coronal sections that were then incubated for 36–40 h in anti-*c-Fos* rabbit polyclonal primary antibody (Calbiochem® PC38T) diluted 1:20,000 in blocking solution (5% normal goat serum and 0.2% Triton X-100 in PBS). Secondary anti-rabbit antibody (Vector Laboratories, BA-1000) was applied at 1:1.000 dilution and a standard avidin–biotin peroxidase protocol (ABC kit, Vector), and a heavy metal intensification of the 3-3′ diaminobenzidine (DAB Peroxidase Substrate Kit, Vector) was used to visualize *c-Fos* immunoreactivity.

Behavioral analysis: forced swim test (FST) and novelty suppressed feeding test (NSFT)

Behavioral responses in the FST were conducted as previously described (Li et al., 2010). Rats were exposed to a pre-swim 24 h prior to the treatment with scopolamine or selective M-AChR antagonists, for 15 min in a clear cylinder filled with water (24 ± 1 °C, 45 cm depth). Rats were administered scopolamine, 4-DAMP, or VU0255035 and were tested for immobilization in the FST 24 h later. In the blocking studies, muscimol was administered (0.2 nmol in 2 μl, i.c.v.) 1 h prior to scopolamine. Video-recorded sessions were scored for total immobility time during minutes 2–6 of the test by a blinded experimenter. Brains were collected, and cannula placement was determined by histology; only rats with correct cannula placement were included for analysis. NSFT was conducted as previously described (Elsayed et al., 2012). Rats were food deprived for 24 h and placed in an open field (76.5 cm × 76.5 cm × 40 cm, Plexiglas) with a small amount of food in the center. The latency to feed (time to approach and take first bite of food) was recorded; home cage food intake was measured right after the test as a control.

Chronic unpredictable stress and sucrose preference test

Chronic unpredictable stress (CUS) is an experimental procedure used to mimic re-occurring uncontrollable stressors that may lead to the pathophysiology of depression. In brief, animals were exposed to a variable sequence of mild and unpredictable stressors, 2 per day for 28 days. The stressors included 45° cage tilt, wet bedding, lights on overnight, lights off for 3 h, food and water deprivation overnight, isolation overnight, odor, 4 °C cold stress, 18 °C cold swim stress, stroboscope overnight, crowded housing and cage rotation. Control animals were handled daily but otherwise left undisturbed in their home cage. On day 21, the animals received 4 °C cold and then cage rotation, and 4 h after the last stress rats received either 1 or 3 doses of scopolamine, VU0255035, or saline/vehicle; 24 h after the first or third dose, rats were subjected to the sucrose preference test (SPT). Prior to the SPT, rats were habituated to a palatable solution containing sucrose (1%; Sigma, St Louis, MO, USA) and water for 72 h. The day of the test, rats were water deprived for 4 h and then the animals were exposed to two identical bottles, one containing the sucrose solution and the other containing water, for 1 h. This procedure has been adapted from previous studies executed in our lab (Li et al., 2011). Sucrose and water consumption were determined by measuring the change in the volume of fluid consumed. Sucrose preference was defined as the ratio of the volume of sucrose versus total volume of sucrose and water consumed during the 1-h test.

Statistical analysis

Statistical analysis was conducted by Student *t*-test (two groups) or one-way ANOVA (more than two groups) followed by the Newman–Keuls or the Fisher multiple comparisons test. Significance was determined at $p < 0.05$ or less, where specified.

Results

Scopolamine rapidly increases Fos immunoreactivity in the medial PFC

In a previous study, we found that scopolamine increases extracellular glutamate, mTORC1 signaling, and spine synapses in the mPFC (Voleti et al., 2013). The burst of glutamate indicates that scopolamine stimulates neuronal activity, and here we examined the influence of scopolamine on levels of Fos immunoreactivity as a marker to map the mPFC subregions that are activated by scopolamine. We used a dose of scopolamine, 25 μg/kg (i.p.) found to be optimal in our previous study (Voleti et al., 2013) and examined Fos-positive (Fos+) immunoreactivity 1 h after scopolamine administration. Fig. 1 shows examples of Fos immunoreactivity in PrL and IL PFC of rats administered saline or scopolamine. Fos+ neurons were counted across three coronal sections from bregma (+2.20, +3.20, and +4.20 mm) for PrL and IL, and the results are shown on the right panels of Fig. 1. Robust induction of Fos+ neurons was observed in both PrL and IL subregions of the mPFC after a single dose of scopolamine (Fig. 1a and b; $p = 0.002$ and $p = 0.007$ respectively, Student *t*-test). Values represent the result of an average of Fos+ cells from both the left and the right hemispheres from the three different sections relative to bregma. These findings indicate that systemic scopolamine administration rapidly increases neuronal activity in the PrL and IL subregions of the mPFC. We also found a significant scopolamine induction of Fos+ neurons in other brain regions, including the lateral habenula, nucleus accumbens shell, and medial amygdala, and trends in the nucleus accumbens core and central nucleus of the amygdala (Supplemental Fig. 1).

Direct infusions of scopolamine into mPFC produce antidepressant behavioral responses

To directly test the role of mPFC in the behavioral actions of scopolamine, animals were infused with scopolamine or vehicle into the IL or PrL subregions and tested in the FST and NSFT. Preliminary dose finding studies were conducted with infusions into the IL. Rats were first exposed to a pre-swim and 24 h later received infusions (bilateral) of scopolamine at doses of 30 and 100 pg into the IL, and then tested 24 h later for immobility in the FST. The lower dose had no effect, but the 100 pg dose of scopolamine resulted in a significant reduction of immobility time in the FST (Fig. 2a) ($F_{2,17} = 3.605$; $p = 0.04$). IL infusion of scopolamine (100 pg) also produced a trend for an anxiolytic-like effect in the NSFT, as it reduced the latency to feed in an open field (Fig. 2b). The higher dose (100 pg) was used for the PrL infusion experiment and was found to produce a similar reduction of immobility time in the FST ($p = 0.02$, Student *t*-test) and decreased latency to feed in the NSFT (Fig. 2c, d).

To determine if neuronal activation of mPFC is required for the antidepressant actions of scopolamine, we tested whether the local infusion of muscimol, a gamma-amino-butyric acid type A ($GABA\text{-}_A$) receptor agonist that silences neurons, blocks the effects of systemic scopolamine. The infusion sites and doses used for these studies were based on previous reports demonstrating restricted spread and subregion-specific inactivation following muscimol infusion in the IL or PrL (Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011). Muscimol (1.25 μg/side) or vehicle (PBS) was infused into the PrL or IL sub-regions 1 h prior to the systemic administration of scopolamine (25 μg/kg) injection (i.p.). Behavioral tests were performed 24 h after scopolamine injection, a time when the acute actions of muscimol and scopolamine

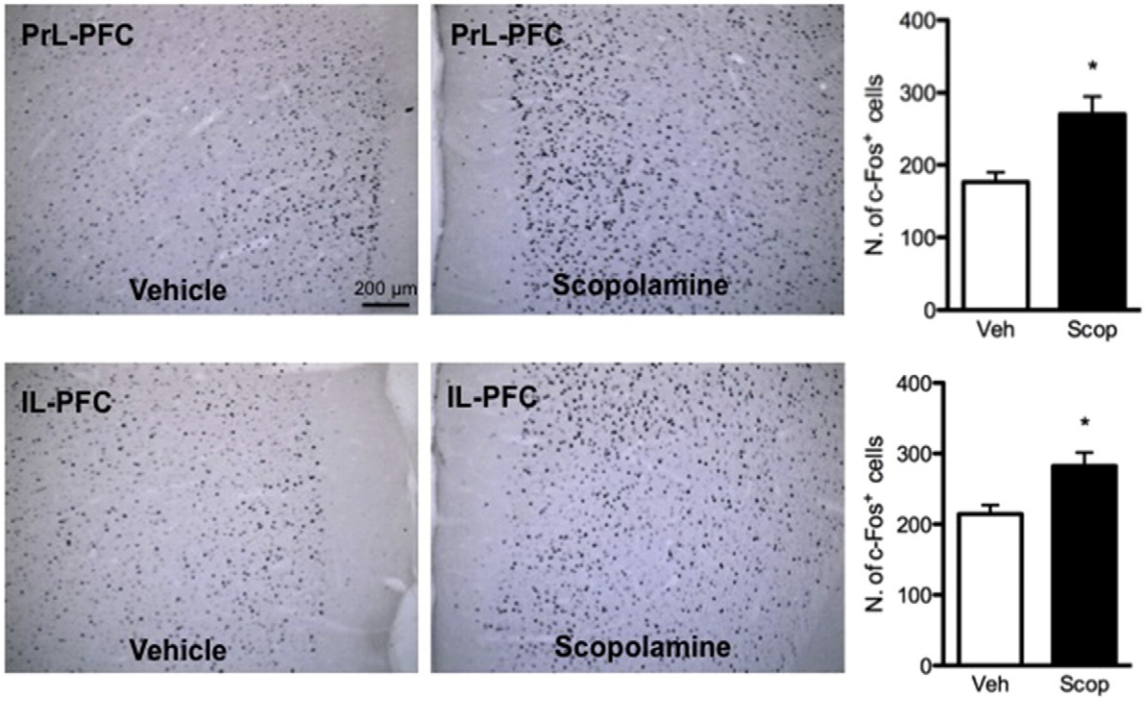

Fig. 1. Scopolamine increases Fos+ immunoreactivity in the mPFC. The influence of scopolamine administration (25 μg/kg, i.p.) on Fos+ immunoreactivity in mPFC of rat was determined 1 h after drug treatment. Shown in the panels to the left are representative images from vehicle and scopolamine-treated animals demonstrating induction of Fos+ neurons in the PrL and IL. The number of Fos+ neurons was analyzed, and the results are show in the bar graphs to the right for each region. The results are the mean ± SEM of 6 rats per group. *$p < 0.01$ compared to vehicle (Student *t*-test).

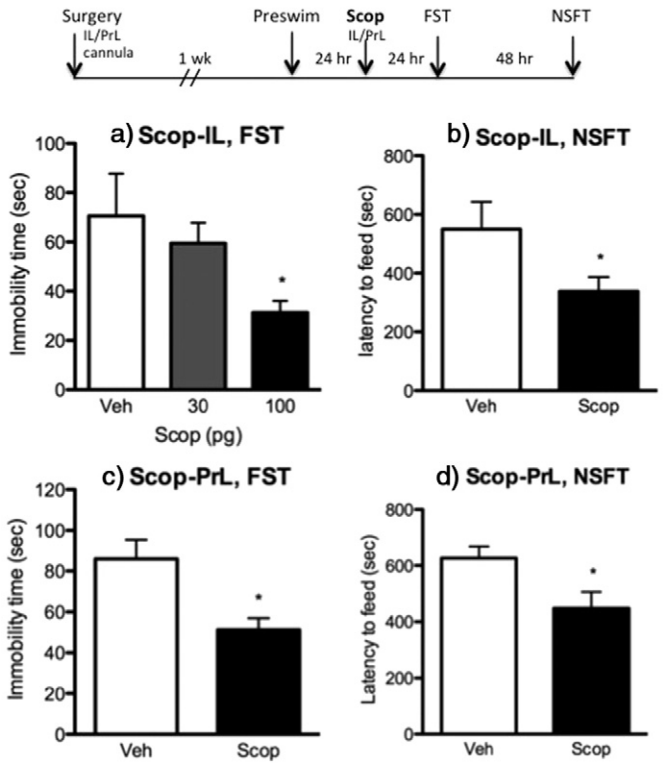

Fig. 2. Microinfusions of scopolamine into the mPFC produce antidepressant behavioral responses. The influence of scopolamine microinfusions into mPFC subregions of rat on behavior in the FST and NSFT was determined (see diagram at the top for experimental design). (a) For initial studies, we examined microinfusions of two doses of scopolamine, 30 and 100 pg, into the IL and determined immobility in the FST 24 h later. These studies demonstrate that the 100 pg dose of scopolamine significantly decreases immobility in the FST. (b) Only the 100 pg dose of scopolamine was used for subsequent studies, and infusions into IL and were found to decrease the latency to feed in the NSFT. (c, d) Microinfusions of scopolamine into the PrL also decreased immobility in the FST and latency to feed in the NSFT. Results are the mean ± SEM, $n = 7$–9 per group. $^{*}p < 0.05$ compared to controls (ANOVA and Newman–Keuls post hoc test for results in A, and Student *t*-test for B, C, and D).

have subsided but the more long-lasting antidepressant actions are observed (Voleti et al., 2013). The systemic administration of scopolamine produced a significant antidepressant response in the FST, and this effect was completely blocked by infusion of muscimol into either the IL or PrL (Figs. 3a and b, respectively, $F_{2,\ 26} = 4.552$, $p = 0.02$; $F_{2,\ 18} = 5.382$, $p = 0.01$). We have recently demonstrated that infusions of muscimol alone do not influence behavior in the FST at the time point examined, 25 h after infusions (Fuchikami et al., 2015); these data are included in Supplementary Fig. 2. Analysis of NFST conducted 72 h after scopolamine administration showed that muscimol infusion into the PrL prevented the decrease in latency to feed ($F_{2,\ 18} = 5.529$, $p = 0.01$). These data demonstrate that scopolamine infusions into the IL or PrL are sufficient to promote antidepressant behavioral effects, and that inactivation of these subregions can block the antidepressant effects of systemic scopolamine.

Selective M1-AChR antagonist administration produces antidepressant responses

Scopolamine is a non-selective M-AChR antagonist and may produce antidepressant effects through interactions with one of the five different M-AChR subtypes. In our previous study, we found that telenzepine, an antagonist with limited selectivity for M1-AChR (7-fold relative to other M-AChR subtypes), produced antidepressant effects in the FST and increased mTORC1 signaling (Voleti et al., 2013). Here we extend these studies by testing VU0255035, another M1-AChR antagonist that has approximately 70-fold selectivity for M1-AChR (Sheffler et al., 2009). We examined two doses, 1 and 3 mg/kg (i.p.), and found that the administration of VU0255035 at the higher dose significantly reduced immobility time compared to saline group (Fig. 4A) ($p < 0.02$, one-way ANOVA and Newman–Keuls post hoc test). For comparison, we also examined an M3-AChR-selective antagonist, 4-DAMP (Moriya et al., 1999). Infusion of 4-DAMP (100 or 200 pmol, i.c.v.) had no effect on immobility in the FST (Fig. 4b).

We also examined the ability of VU0255035 administration (3 mg/kg, i.p.) to activate the mTORC1 signaling pathway 1 h after drug treatment. Levels of the phosphorylated and activated forms of mTOR and the downstream target p70 S6 kinase (S6K) and two upstream kinases ERK and Akt were analyzed 1 h after VU0255035 administration. The results demonstrate that VU0255035 administration produces a significant increase in levels of phospho-Akt and phospho-S6K (Fig. 4C); there were no significant effects on levels of phospho-mTOR or phospho-ERK. Together these findings demonstrate that M1-AChR antagonism produces antidepressant behavioral and molecular signaling responses similar to scopolamine.

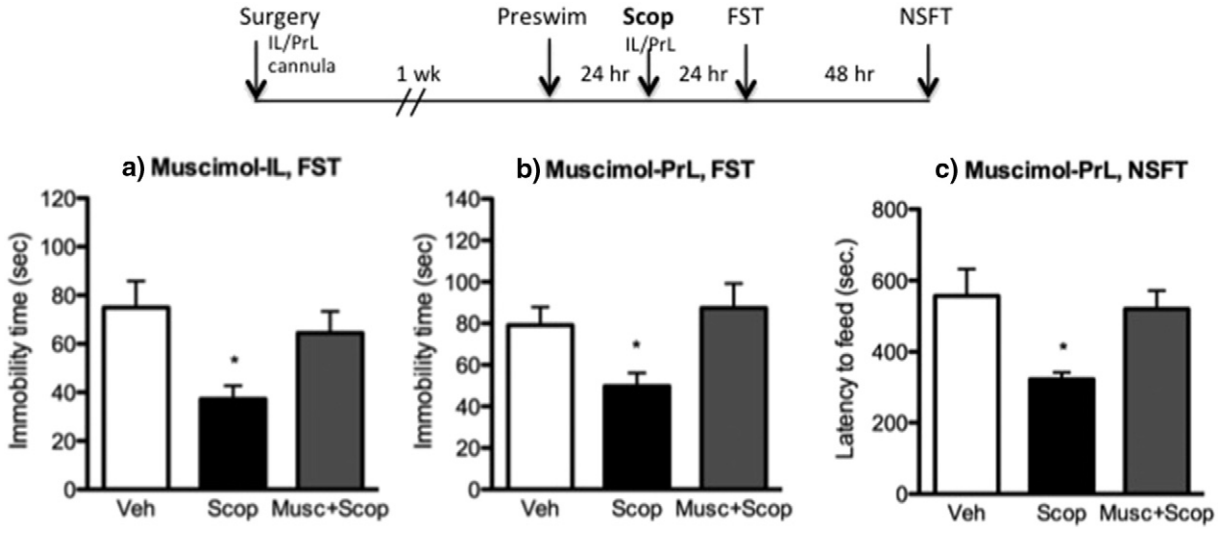

Fig. 3. Infusion of muscimol into mPFC blocks the antidepressant actions of systemic scopolamine. The influence of the neuronal silencing agent, muscimol, infused into mPFC subregions of rat on the antidepressant response to systemic scopolamine (i.p.) was determined. The experimental design is shown at the top. Muscimol was infused 1 h prior to scopolamine administration and immobility in the FST was determined 24 h later. (A) Infusion of muscimol into the IL (A) or PrL (B) significantly blocked the decrease in immobility resulting from scopolamine administration. Results are the mean ± SEM, $n = 10$ per group. $^{*}p < 0.05$ compared to controls (ANOVA and Newman–Keuls post hoc test).

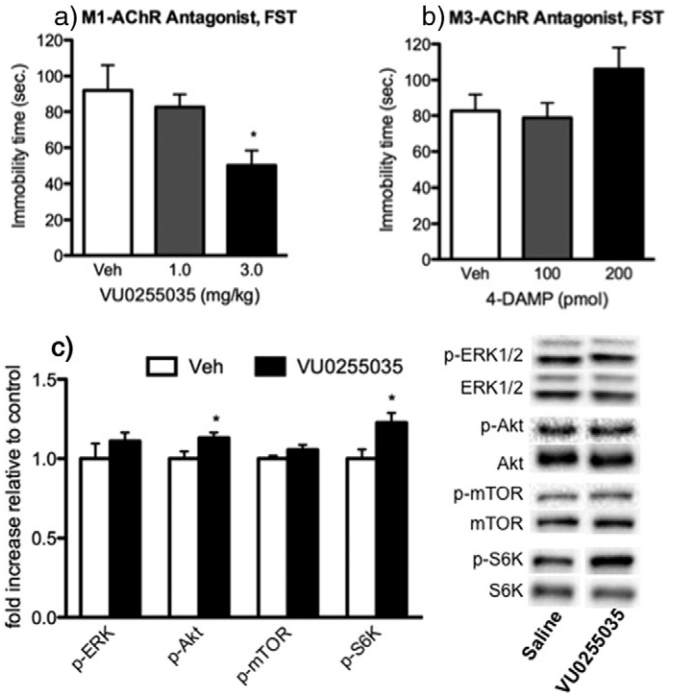

Fig. 4. Administration of a selective M1-AChR antagonist produces antidepressant-like effects in the FST and increases mTORC1 signaling. The influence of a selective M1-AChR antagonist, VU0255035, on immobility in the FST and mTORC1 signaling in the PFC was determined. (A) Rats were administered (i.p.) VU0255035 at two different doses, 1 and 3 mg/kg VU0255035, and 24 h later were tested in the FST. We found that the 3 mg/kg dose of VU0255035 significantly decreased immobility in the FST (ANOVA and Newman–Keuls post hoc test). (B) For comparison, the influence of an M3-AChR antagonist, 4-DAMP, infused (i.c.v.) at two different doses was examined, but no significant effects were observed. (C) The influence of VU0255035 (3 mg/kg, i.p.) on mTORC1 signaling in the PFC was determined by Western blot analysis. Shown on the right are representative immunoblots for each phosphorylated kinase, as well as total levels of the corresponding non-phosphorylated protein. Results are relative fold change and are the mean ± SEM, $n = 7$–9 per group. $p < 0.05$ compared to controls (Student t-test).

Scopolamine and VU0225035 attenuate CUS-induced deficits in sucrose preference

FST has been extensively used as a screen for the identification of putative antidepressant drugs but has limited validity since this test is responsive to acute or short-term administration of typical antidepressants that take weeks or months to produce a response in depressed patients. To more rigorously test the rapid antidepressant actions of scopolamine and VU0255035, we used a chronic unpredictable stress (CUS) model of depression. Exposure of rats to CUS produces anhedonia, a core symptom of depression that can be measured in a sucrose preference test (SPT) (Willner, 1997). The reduction in the SPT is only reversed after 2 to 3 weeks of treatment with a typical antidepressant (Willner et al., 1987), but we have reported that a single dose of ketamine produces a rapid reversal of anhedonia resulting from CUS exposure (Li et al., 2011). Here we tested the influence of scopolamine or VU0255035 in the CUS/SPT model. Rats exposed to CUS demonstrated a significant decrease in sucrose preference, and the administration of a single dose of scopolamine (25 μg/kg) or VU0255035 (3 mg/kg) 24 h before testing partially blocked this effect (Fig. 5a) ($F_{3,50} = 12.0$, $p < 0.0001$; $^{*}p < 0.005$ compared to control; $^{‡}p < 0.05$ compared to Sal/CUS; $^{\#}p = 0.08$ compared to Sal/CUS). CUS exposure resulted in a small decrease in body weight as expected after 3 weeks of stress exposure; this decrease was not influenced by either scopolamine or VU0255035 administration (Supplemental Fig. 3).

Because a single dose of scopolamine or VU0255035 only partially blocked the effects of CUS, we also tested the influence of a 3-dose regimen. The choice of this regimen is based on clinical findings demonstrating a more efficacious antidepressant response to a second and third dose of scopolamine administered over 5 to 7 days (Furey and Drevets, 2006; Drevets and Furey, 2010). Preliminary studies demonstrate that 3 doses of scopolamine or VU0255035 administered every other day for 5 days results in robust induction of mTORC1 signaling in the PFC (Fig. 6) that is greater than observed with a single dose of VU0255035 (Fig. 4) or scopolamine (Voleti et al., 2013). We also observed modest effects on mTORC1 signaling in the hippocampus (Supplemental Fig. 4). This 3-dose regimen of scopolamine also produced a significant antidepressant response in the FST and NSFT but had no effect on locomotor activity (Supplemental Fig. 5). Based on these findings, we tested the 3-dose regimen in the CUS/SPT paradigm. The results demonstrate that 3 doses of scopolamine completely blocked the effects of CUS in the SPT (Fig. 5b) ($F_{3,20} = 3.81$, $p < 0.02$; $^{*}p < 0.005$ compared to control; $^{‡}p < 0.02$ compared to Sal/CUS). The 3-dose VU0255035 regimen partially reversed the effects of CUS in the SPT, but the CUS + VU0255035 was not significantly different from CUS ± saline. Together, these findings are consistent with the hypothesis that scopolamine and VU0255035 produce rapid antidepressant actions in a rodent model that requires the chronic administration of a typical antidepressant.

Discussion

Previous studies demonstrating that scopolamine increases extracellular glutamate, mTORC1 signaling, and spine synapse number and function in the mPFC indicate that scopolamine increases neuronal activity and synaptogenesis (Voleti et al., 2013). This is thought to occur via blockade of muscarinic receptors on GABAergic interneurons, resulting in the disinhibition of neuronal pyramidal cell activity and a burst of glutamate transmission (Fig. 7). This possibility is supported by the current finding that scopolamine rapidly increases the neuronal activation marker Fos in the mPFC (Ceccatelli et al., 1989; Hoffman and Lyo, 2002; Sagar et al., 1988). A role for neuronal activation is further supported by studies demonstrating that local infusion of a neuronal silencing agent blocks the effects of systemic scopolamine. The rapid, transient burst of glutamate and neuronal activation then lead to more long-term changes in synapse number that may underlie the antidepressant behavioral responses to scopolamine.

The possibility that mPFC subregions are an important target region is supported by neuroimaging studies showing that depressed patients have reduced gray matter volume and altered blood flow in PFC (Drevets et al., 2008; MacQueen et al., 2008) and rodent chronic stress studies reporting reductions in dendrite complexity and spine synapse number and function in mPFC (Liu and Aghajanian, 2008; Radley et al., 2006, 2004). Conversely, scopolamine and other rapid acting antidepressants (i.e., ketamine) increase spine synapse number and function in the mPFC (Li et al., 2010; Voleti et al., 2013), and ketamine rapidly reverses the synaptic deficits caused by chronic stress exposure (Li et al., 2011). Here we directly test the importance of mPFC subregions and show that microinfusions of scopolamine produce antidepressant and anxiolytic responses similar to systemic scopolamine. Blockade of the antidepressant actions of systemic scopolamine by local infusions of a neuronal silencing agent provide further support for mPFC. The lack of differential effects of infusions into IL or PrL could be related to the diffusion of scopolamine, but this is less likely to explain the blocking effects of neuronal silencing as previous studies using the same dose and infusion coordinates for muscimol have reported differential effects of these subregions (Laurent and Westbrook, 2009; Sierra-Mercado et al., 2011; Fuchikami et al., 2015). Further studies will be needed to examine the role of these and other regions, as well as circuits underlying the actions of scopolamine. For example, we also observed increased Fos+ immunoreactivity in several other regions of interest that could be either directly or indirectly activated by scopolamine and that could contribute to the antidepressant behavioral responses.

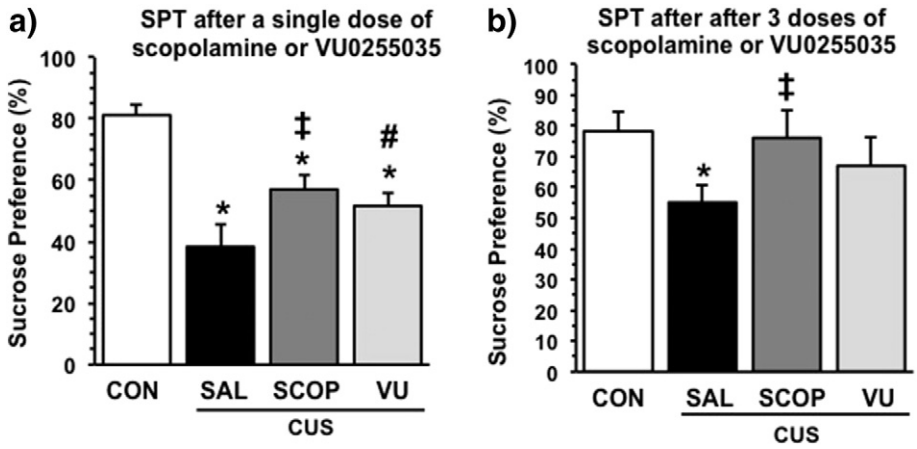

Fig. 5. Administration of scopolamine or VU0255035 blocks the influence of CUS exposure on sucrose preference. Rats were exposed to CUS (21 d) and were then received either one or three doses of vehicle, scopolamine (25 μg/kg), or VU0255035 (3 mg/kg), and levels of sucrose preference were determined 24 h later. Results are the mean ± SEM, $n = 8$ to 16 per group. (a) Results of the SPT after a single dose of scopolamine or VU0255035 ($F_{3,50} = 12.0$, $p < 0.0001$). $^{*}p < 0.005$ compared to control; $^{‡}p < 0.05$ compared to Sal/CUS; $^{\#}p = 0.08$ compared to Sal/CUS (Fisher's LSD). (b) Results of the SPT after three doses of scopolamine or VU0255035 ($F_{3,20} = 3.81$, $p < 0.02$). $^{*}p < 0.005$ compared to control; $^{‡}p < 0.02$ compared to Sal/CUS (Fisher's LSD).

Scopolamine is a non-selective antagonist of the five known muscarinic receptor subtypes (Golding and Stott, 1997). We previously demonstrated that telenzepine, an M1-AChR antagonist with limited selectivity produced antidepressant behavioral and mTORC1 signaling effects similar to scopolamine. Here we extend these findings and demonstrate that VU0255035, a muscarinic receptor antagonist with much higher selectivity for M1-AChR (~70 fold over M2) (Sheffler et al., 2009; Xiang et al., 2012), produces antidepressant behavioral responses in the FST. This is consistent with a recent report in mice demonstrating that scopolamine produces an antidepressant response in the FST and that this effect is blocked in M1-AChR knockout mice (Witkin et al., 2014). In contrast, infusions of a selective M3-AChR antagonist, 4-DAMP, did not influence behavior in the FST consistent with the results of Witkin et al. (2014), in which scopolamine caused antidepressant responses in M3-AChR knockout mice. In the present study, we also demonstrate that VU0255035 blockade of M1-AChR rapidly stimulates mTORC1 signaling in the mPFC, which has not been tested in previous studies. These studies support the possibility that a selective M1-AChR antagonist could be effective for the treatment of depression. Although M1-AChR blockade could have side effects, notably memory impairment reported in rats (Roldan et al., 1997), there is evidence that VU0255035 does not cause cognitive deficits (Sheffler et al., 2009). Finally, the study by Witkin and colleagues also reports that the antidepressant response to scopolamine is blocked in M2-AChR knockout mice. Thus, further studies will be required to examine the role of M2-AChR in the behavioral and mTORC1 signaling responses to scopolamine.

Although scopolamine is reported to produce antidepressant actions in rodent models, these studies have been limited to tests that are considered drug-screening paradigms, such as the FST, and do not have good validity as models of depression. An additional limitation is that the FST is responsive to the acute administration of a typical antidepressant and therefore cannot distinguish drugs that have rapid acting efficacy. To address these issues, we used a CUS model that results in anhedonia, a core symptom of depression, which is only responsive to the chronic administration of a typical antidepressant (Willner et al., 1987; Willner, 1997). The results demonstrate that a single dose of scopolamine or VU0255035 partially reverse the effects of CUS on sucrose preference. Moreover, using a 3-dose paradigm over 5 days (based on the clinical dose regimen), we found that scopolamine completely reversed the anhedonic effects of CUS. Using the same 3-dose regimen, VU0255035 produced a strong trend for a complete reversal, and further studies will be needed to determine if a higher dose of VU0255035 or additional doses produce a complete reversal. The 3-dose scopolamine or VU0255035 regimen also produced robust increases in levels of mTORC1 signaling in the PFC, providing further evidence of the efficacy of this dose regimen.

In conclusion, the results of this study further elucidate the rapid antidepressant properties of scopolamine, demonstrating that mPFC plays a key role in the behavioral responses to scopolamine and that the M1-AChR subtype strongly contributes to the behavioral and mTORC1 signaling actions of scopolamine. The antidepressant actions of scopolamine and importantly the selective M1-AChR antagonist are

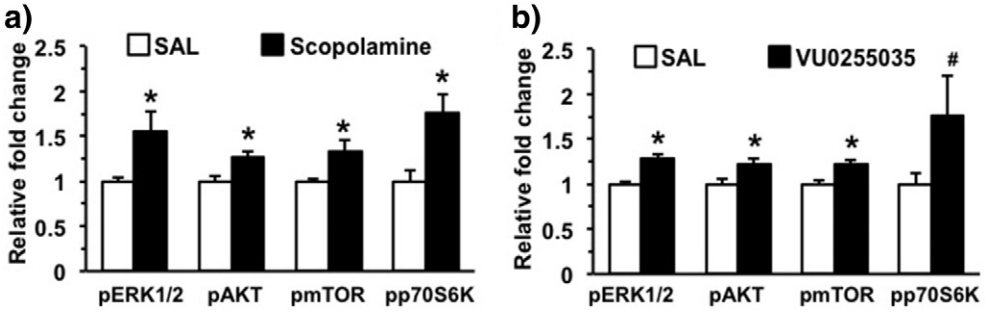

Fig. 6. Three-dose scopolamine or VU0255035 regimen causes robust stimulation of mTORC1 signaling in the PFC. Scopolamine (25 μg/kg) or VU0255035 (3 mg/kg) were administered in 3 doses over 5 days, and PFC levels of the phosphorylated forms of ERK, Akt, mTOR, and p70S6K was determined by Western blot. The levels of each phospho-protein were compared to total levels of the unphosphorylated kinase, as shown in Fig. 4. Results are presented as relative fold change and are the mean ± SEM, $n = 5$–7 per group. $^{*}p < 0.05$ compared to controls (Student t-test).

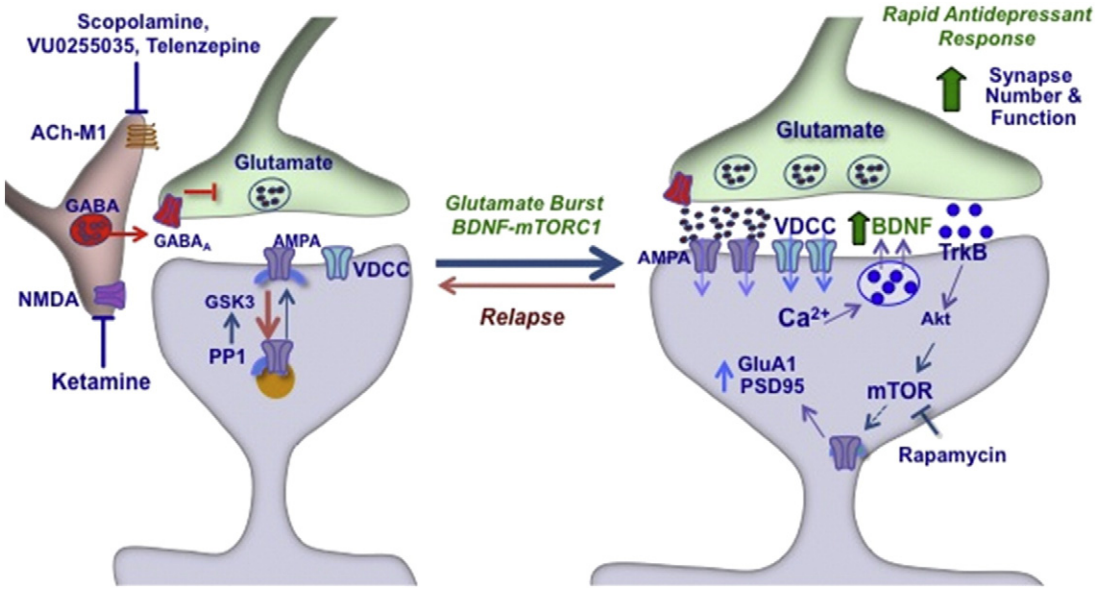

Fig. 7. Mechanism for scopolamine to cause a burst of glutamate, activation of mTORC1 signaling, and increased synapse formation. Scopolamine blockade of Ach-M1 receptors located on GABAergic interneurons results in disinhibition and a burst of glutamate transmission, activation of AMPA receptors, and depolarization of postsynaptic pyramidal neurons. This leads to the activation of voltage-dependent calcium channels (VDCC), which activates release of BDNF and the subsequent stimulation of TrkB receptors and the Akt-mTORC1 signaling pathway. This pathway controls the translation and synthesis of synaptic proteins, such as GluA1 and PSD95, that are required for the formation of new synapses that occur and are associated with the rapid antidepressant actions of scopolamine. Administration of rapamycin, a selective mTORC1 inhibitor, blocks the antidepressant actions scopolamine.

further strengthened by the results demonstrating reversal of the anhedonic effects of CUS exposure. Nevertheless, the role of other M-AChR subtypes still need to be investigated as some have modulatory activity over the cholinergic system in the brain (Medalla and Barbas, 2012). Moreover, characterization of the circuits connected with mPFC as well as other primary targets of scopolamine will require further investigation.

Conflict of interest

There are no competing financial interests in relation to the work described.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nbd.2015.06.012.

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