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Research paper

# Antidepressant-like effect of low dose ketamine and scopolamine co-treatment in mice

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## HIGHLIGHTS

- Low dose ketamine (3 mg/kg) or scopolamine (0.1 mg/kg) is ineffective in the mouse FST.
- Co-treatment with subeffective doses of both drugs has an antidepressant-like effect.
- Low dose ketamine and scopolamine co-treatment may be effective for human depression.

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## ABSTRACT

Current medications for depression typically require weeks of treatment before significant clinical improvement is observed, and are only effective in a relatively small subset of patients. Recent human clinical studies have demonstrated that ketamine, an NMDA receptor antagonist, and scopolamine, a muscarinic acetylcholine receptor antagonist, produce rapid antidepressant responses within hours of administration, and are effective in treatment-resistant patients. We hypothesize that efficacy and tolerability may be improved by combining lower doses of both drugs in the treatment of depression. We therefore conducted a preclinical study in mice to assess whether co-treatment of low doses of scopolamine and ketamine that alone are ineffective has antidepressant-like effects in the forced swim test (FST), an assay with predictive validity for antidepressant drugs. Whereas single administration of ketamine (3 mg/kg intraperitoneal [i.p.]) or scopolamine (0.1 mg/kg i.p.) did not reduce immobility time in the FST, co-administration of both drugs at these doses significantly reduced immobility time by 45% compared to vehicle treated controls. These results suggest that the combination of subeffective doses of ketamine and scopolamine may prove efficacious for the treatment of depression and should be evaluated in human clinical trials.



## 1. Introduction

Current medications for depression typically require weeks of treatment before significant clinical improvement is observed, and are only effective in a relatively small subset of patients [1]. Recent clinical studies have demonstrated that ketamine, an NMDA receptor antagonist, and scopolamine, a muscarinic acetylcholine receptor antagonist, produce rapid antidepressant responses within hours of administration and are effective in patients who are resistant to conventional treatments [2–8]. Ketamine's antidepressant action is observed after a single intravenous infusion at a dose (0.5 mg/kg) below its anesthetic range [2–5]. Significant improvement in depression symptoms typically occurs within 1–2 h and persists for approximately 1 week [4]. Scopolamine intravenous infusion at a low dose (0.004 mg/kg) produces clinical improvement within three days of treatment,

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although patients notice changes after 24 h, suggesting that earlier clinical assessment may reveal more rapid effects [7,8]. Elevated cortical activity has been correlated with response to both drugs, and therefore may be a marker of their antidepressant actions [9,10].

Both scopolamine and ketamine have significant effects in rodent behavioral assays that have predictive validity for antidepressant efficacy. Subanesthetic doses of ketamine in mice and rats reduce immobility time in the forced swim test (FST) and are effective in other depression paradigms compared to vehicle-treated controls [11–13]. The antidepressant-like effect has been correlated with increased hippocampal brain-derived neurotrophic factor (BDNF) levels, which clinical antidepressants are known to elevate [14], suggesting a shared neurotrophic mechanism of action. Recent studies of ketamine implicate activation of mammalian target of rapamycin (mTOR) signaling and inhibition of eukaryotic elongation factor 2 kinase (eEF2K), which increase synthesis of synaptic proteins (including BDNF) to modulate synaptic function [12,13,15,16]. Inhibition of glycogen synthase kinase 3 (GSK3), a target of lithium, is also implicated in the antidepressant-like effect of ketamine [17]. A single administration of scopolamine (0.1 or 0.2 mg/kg i.p.) produces reliable antidepressant effects in mice in the tail suspension test and the FST [18]. Scopolamine (0.5–1.0 mg/kg i.p.) is also reported to potentiate the antidepressant effects of desipramine and nomifensine in the FST in rats [19]. Although scopolamine's antidepressant mechanism of action has been less studied, it is reported to rapidly activate mTOR signaling [20] and decrease NMDA receptor subunit expression [21], potentially modulating circuit function in a similar manner to ketamine.

Since both ketamine and scopolamine produce rapid antidepressant effects in human clinical trials that may be mediated through a common neural mechanism, we hypothesized that there may be benefits in terms of efficacy and tolerability in combining lower doses of both drugs for depression treatment. We therefore investigated co-administration of scopolamine and ketamine in the FST in mice at doses that alone are ineffective in producing antidepressant-like effects. Our results indicate that the combination of subeffective doses of ketamine and scopolamine may prove efficacious for the treatment of depression in humans.

## 2. Materials and methods

### 2.1. Animals

Male C57BL/6N mice were purchased from Taconic Farms (Germantown, NY) at 10 weeks of age and acclimated to the vivarium for one week prior to behavior testing. Mice were group housed under a 12 h light/dark cycle with food and water provided ad libitum. Behavior testing was conducted during the light phase between 8am and 4pm. All procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Massachusetts Institute of Technology Committee on Animal Care.

### 2.2. Drug treatment

Ketamine hydrochloride (Sigma-Aldrich, St. Louis, USA) was dissolved in 10% DMSO, 45% polyethylene glycol 400 (Thermo Fisher Scientific, Waltham, USA), and 45% saline (0.9% sodium chloride) for experiments using a dose of 10 mg/kg, and in saline for experiments using a dose of 3 mg/kg. Mice treated with the different vehicles had very similar FST immobility times, therefore it is unlikely that the vehicle difference is a confounding factor. Scopolamine hydrochloride (Sigma-Aldrich, St. Louis, USA) was dissolved in saline. Drugs or vehicle were administered by i.p. injection 30 min prior to behavior testing. For ketamine and scopolamine co-treatment, each drug was administered by a separate injection.

### 2.3. Behavioral procedures

A two-day FST protocol was utilized in which mice were pre-exposed to the task 24 h before testing, which increases immobility time and sensitivity for detecting antidepressant-like effects [22]. On both days, mice were acclimated to the testing room for one hour prior to the start of the session. On the first day, mice were acclimated to mock i.p. injection 30 min prior to being placed in a cylindrical container (24 cm high × 15 cm diameter) filled halfway with warm water (26 ± 2 °C) for 10 min. The following day, mice were administered drug or vehicle 30 min prior to being placed in the cylinder for 6 min. Immobility (floating behavior) was scored automatically throughout the 6 min trial [22] using EthoVision XT software (Noldus Information Technology; Wageningen, Netherlands). While active behaviors of swimming and climbing can discern serotonin and norepinephrine action in rats [23], active behaviors are typically not assessed in mice [24], and climbing was not detected in our mice, therefore active behaviors were not scored.

### 2.4. Statistical analysis

All values are expressed as mean ± SEM. Statistical significance was determined using one-way analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) post-hoc test. P-values below 0.05 were considered significant. All statistical analyses were performed using SPSS v. 18 (IBM).

## 3. Results

### 3.1. Determination of subeffective doses of ketamine and scopolamine in the mouse forced swim test

We initially identified doses of ketamine and scopolamine that alone are ineffective in producing antidepressant-like effects in the FST in adult male C57BL/6N mice. Published mouse and rat studies of ketamine in the FST have used doses ranging from 3 to 20 mg/kg i.p. [12,13,25]. We initially tested a dose in the middle of this range, 10 mg/kg i.p., and determined that it significantly reduced immobility time by 44% ($p = 0.032$; Fig. 1a). We subsequently tested a lower dose of 3 mg/kg i.p., which did not produce a significant decrease in immobility ($p > 0.05$; Fig. 1b). As there are few published studies of scopolamine in rodent depression models, we performed a dose response experiment with three doses to identify a subeffective dose. Scopolamine at 0.1 mg/kg i.p. did not reduce immobility time versus vehicle (post hoc $p > 0.05$; Fig. 1c). Higher scopolamine doses (0.5 and 1.0 mg/kg i.p.) substantially reduced immobility time versus vehicle (post hoc $p < 0.001$ for both comparisons; Fig. 1c).

### 3.2. Ketamine and scopolamine interaction in the mouse forced swim test

We selected doses of ketamine (3 mg/kg i.p.) and scopolamine (0.1 mg/kg i.p.) that alone were ineffective in the previous experiments to evaluate whether co-treatment produced significant antidepressant-like effects in the FST. Replicating the previous results, administration of only ketamine or only scopolamine did not significantly reduce FST immobility time compared to vehicle (post hoc $p > 0.05$ for both comparisons; Fig. 2). In contrast, co-treatment with both drugs at these same doses significantly reduced immobility time by 45% compared to vehicle (post hoc $p = 0.016$; Fig. 2). The immobility time of mice co-treated with both drugs did not significantly differ from the immobility times