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# PIPE-307, a brain penetrant M1 selective antagonist, exhibits scopolamine-like antidepressive effects

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## Introduction

A recent CDC report suggests that 30.2% of adults aged >18 years report experiencing some type of depressive disorder (Vahratian et al., 2021). While treatments for major depressive disorder (MDD) alleviate symptoms, these are often impeded by factors such as unwanted side effects, time needed to generate a therapeutic response (weeks to months), as well as forms of depression that are treatment resistant (TRD). By contrast, fast-acting antidepressants, such as scopolamine or ketamine, act on the order of hours to days and are effective in patients with TRD (Jaffe et al., 2013 and Krystal JH, 2007). Although scopolamine is a non-selective muscarinic acetylcholine receptor (mAChR) antagonist, its antidepressive effects are mediated by the M1 receptor isoform (M1) as evidenced by knockout and pharmacological data (Witkin et al., 2014). Scopolamine (and ketamine) act on layer 5 pyramidal neurons in the medial prefrontal cortex (mPFC), antagonizing inhibitory neurons, resulting in enhanced excitatory, transmission. Concomitantly, BDNF release and spine density are also enhanced. Together, these effects likely contribute to scopolamine's antidepressive effect (Wohleb et al 2017). Because scopolamine does not selectively target the M1 receptor, clinical dosing is associated with adverse events common to all non-selective muscarinic antagonists.

We have identified and characterized an M1 selective, brain penetrant, small molecule antagonist, PIPE-307. Similar to scopolamine, we show that PIPE-307, 1) shows efficacy in the forced swim test, 2) increases mEPSC amplitude in pyramidal neurons in the mPFC, 3) increases dendritic spine density, and 4) enhances BDNF levels. We further show that M1 protein is present on GABAergic interneurons. Together, these data suggest that (like scopolamine) PIPE-307 may be beneficial as a fast-acting antidepressant, but with an improved tolerability profile.

## Results

### Proposed model

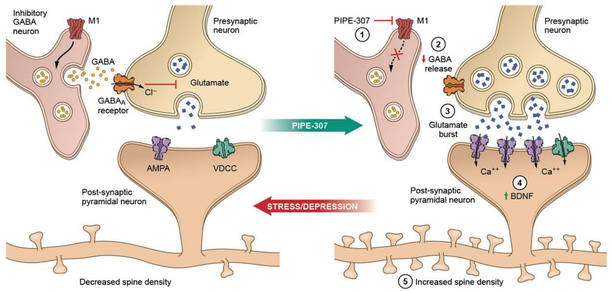



**Fig 1. Proposed M1 mechanism of action.** Based on previous literature (Duman et al., 2012, Witkin et al., 2014, Navarria et al., 2015), antidepressive activity can be achieved by inhibition of M1 by scopolamine or PIPE-307 which attenuates GABA release, leading to enhanced excitatory glutamatergic signaling via pre and postsynaptic mechanisms as well as inducing BDNF release.

### PIPE-307 improves performance in forced swim test

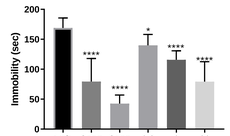
A.

PIPE-307 is selective for M1

| Muscarinic Receptor | $IC_{50}$ in nM (fold selectivity) |
|---|---|
| M1 | 3.78 |
| M2 | 1600 (424) |
| M3 | 207 (54.6) |
| M4 | 109 (28.9) |
| M5 | 3610 (953) |

C.

PIPE-307 improves performance in forced swim test




****=P<0.0001 compared to Veh one way ANOVA Dunnett's multiple
*=P0.0229 compared to Veh one way ANOVA Dunnett's multiple

B.

Mouse brain receptor occupancy

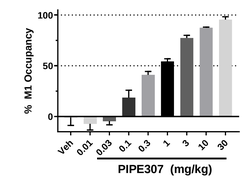



**Fig 2. M1 blockade with PIPE-307, a potent selective antagonist improves performance in the forced swim test.** PIPE-307 is a potent, selective M1 antagonist ($IC_{50}$ 3.78nM, *Fig 2A*). PIPE-307 occupies 100% of M1 in the brain at 30mg/kg. No binding is observed in the cerebellum, an M1-poor region (*Fig 2B*). Doses for PIPE-307 were selected using occupancy data. Mice habituated for 24h were dosed with PIPE-307 (2h prior, PO) or scopolamine (30m prior, IP) and tested. The last 4m of a 6m session were quantified. PIPE-307 significantly improved performance compared to vehicle in a dose dependent manner (*Fig 2C*).

### M1 is expressed on GAD67$^+$ neurons

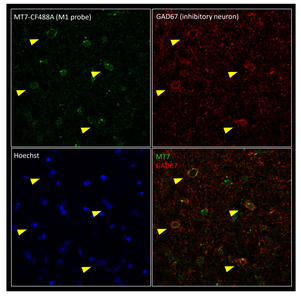
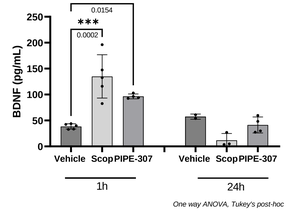



**Fig 3. M1 receptor co-localizes with GAD67$^+$ neurons.** Brain slices were stained with a fluorescently tagged M1 selective peptide, MT7-CF488A (green), a GAD67 antibody to identify GABAergic neurons (red), and counterstained Hoechst (blue). M1 expressed on non-GABAergic cells, presumably neurons, as well.

### M1 inhibition induces BDNF release in cortical culture

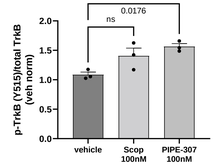
A.

Cortical culture (BDNF, 1 and 24h)




B.

pTrkB (Y515)




C.

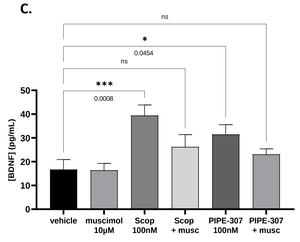
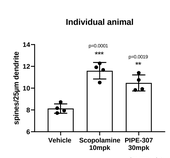



**Fig 4. BDNF is rapidly released after treatment with PIPE-307 in a GABA dependent manner.** Cultured cortical neurons (14 *div*) were treated with PIPE-307 (100nM) or scopolamine (100nM). Media was collected at 1 or 24h and BDNF analyzed by ELISA. After 1h, a significant increase in BDNF released was observed. No increase was observed by 24h, *Fig 4A*. After 6h of PIPE-307, an increase in phosphorylated TrkB was also observed, likely as a result of BDNF induction, *Fig 4B*. Muscimol addition reduces BDNF induction by PIPE-307 or scopolamine, consistent with model that reducing GABA release would increase BDNF release, *Fig 4C*.

### PIPE-307 induces dendritic spine formation *in vivo*

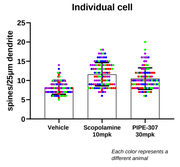
A.

Individual animal




B.

Individual cell




C.

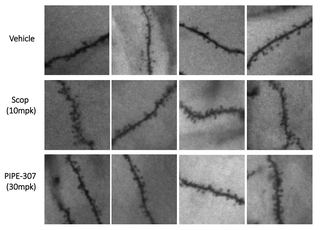
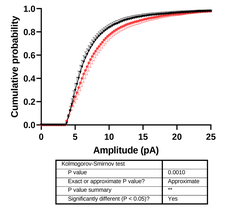



**Fig 5. PIPE-307 induces dendritic spines *in vivo*.** Mice were dosed with PIPE-307 (30mg/kg) or scopolamine (10mg/kg) for 24h and Golgi stained. Pyramidal neurons in layer V analyzed using 25μm dendritic segments (25 neurons/animal). Graphs by animal (*Fig 5A*) or individual cells where each color represents counts from a different animal (*Fig 5B*). Representative images (*Fig 5C*).

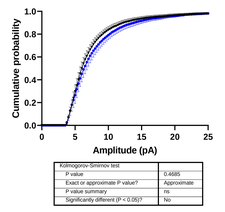
### PIPE-307 enhances mEPSC amplitude

PIPE-307
Cumulative histogram (mEPSC amplitude)




| Kolmogorov-Smirnov test | |
|---|---|
| P value | 0.0010 |
| Exact or approximate P value? | Approximate |
| P value summary | ** |
| Significantly different (P < 0.05)? | Yes |

Scopolamine
Cumulative histogram (mEPSC amplitude)

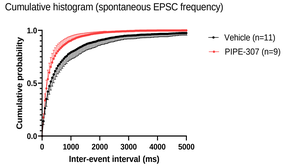



| Kolmogorov-Smirnov test | |
|---|---|
| P value | 0.4685 |
| Exact or approximate P value? | Approximate |
| P value summary | ns |
| Significantly different (P < 0.05)? | No |

**Fig 6. PIPE-307 enhances postsynaptic mEPSC amplitude.** Mice were treated with PIPE-307 (*left*) or scopolamine (*right*) for 24h. Slices were generated and whole cell patch clamp electrophysiology performed in the presence of TTX (Neuroservices Alliances). We observed a rightward shift in mEPSC amplitude, suggesting a postsynaptic mechanism, e.g., receptor insertion.

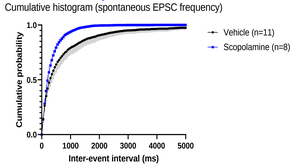
### PIPE-307 increases presynaptic activity

PIPE-307
Cumulative histogram (spontaneous EPSC frequency)




| Kolmogorov-Smirnov test | |
|---|---|
| P value | <0.0001 |
| Exact or approximate P value? | Approximate |
| P value summary | **** |
| Significantly different (P < 0.05)? | Yes |
| Kolmogorov-Smirnov D | 0.6567 |

Scopolamine
Cumulative histogram (spontaneous EPSC frequency)




| Kolmogorov-Smirnov test | |
|---|---|
| P value | <0.0001 |
| Exact or approximate P value? | Approximate |
| P value summary | **** |
| Significantly different (P < 0.05)? | Yes |
| Kolmogorov-Smirnov D | 0.7612 |

**Fig 7. PIPE-307 enhances spontaneous mEPSC frequency suggesting presynaptic involvement.** Mice were treated with PIPE-307 (*left*) or scopolamine (*right*) for 24h. Slices were generated and whole cell patch clamp electrophysiology performed in the absence of TTX (Neuroservices Alliances). We observed a leftward shift in interevent interval suggesting presynaptic involvement. This may be mediated by BDNF (Tyler and Pozzo-Miller, 2001)

## Conclusion

Scopolamine is a non-selective muscarinic antagonist that produces relatively fast-onset antidepressant effects in humans. Unfortunately, scopolamine is also associated with a heavy side effect burden which may be circumvented with selective (i.e., M1 or M2) muscarinic antagonists.

PIPE-307, a brain penetrant, selective small molecule inhibitor of M1 (currently in clinical trials), recapitulates both *in vitro* and *in vivo* antidepressant effects observed with scopolamine. Specifically, PIPE-307:

- Improves performance in the forced swim test
- Increases the number of dendritic spines *in vivo*
- Induces BDNF release in a GABA-dependent manner, i.e., can be inhibited by exogenous muscimol
- Enhances synaptic transmission, i.e., increases presynaptic release and postsynaptic response amplitude

Given these data, PIPE-307 may hold promise as a fast-acting antidepressant with a side effect burden that is less severe than that of scopolamine.

## References

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