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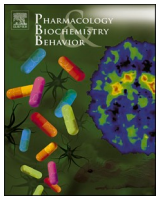

Coadministration of scopolamine and mGlu2 receptor negative allosteric modulator VU6001966 as a potential therapeutic approach for depression: Rat frontal cortex neurochemistry and behavior

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ABSTRACT

Clinical studies provide evidence that scopolamine, a nonselective antagonist of muscarinic cholinergic receptors, exerts rapid and prolonged antidepressant effects. However, its use as a psychiatric drug has been limited due to its significant adverse effects. A therapeutic option that could help reduce the adverse effects of scopolamine is its coadministration at lower doses with other substances with similar antidepressant properties. To address this issue, we have investigated the effect of a single acute coadministration of scopolamine and a negative allosteric modulator of the mGlu2 receptor VU6001966 on rat behavior using a forced swim test (FST) and locomotor activity test. The effect of given compounds on the extracellular levels of neurotransmitters in the rat frontal cortex (FCX) was examined using microdialysis in freely moving rats. Both scopolamine and VU6001966 induced dose-dependent antidepressant-like effects in the FST test without affecting locomotor activity. Furthermore, VU6001966 enhanced extracellular dopamine and serotonin levels while lowering glutamate, without affecting GABA level. Both scopolamine alone or in combination with VU6001966 increased dopamine, serotonin, and glutamate levels in the FCX, without affecting GABA levels. Our results suggest that coadministration of scopolamine with mGlu2 NAM might be a promising alternative to using scopolamine alone in depression therapy, potentially allowing for a lower therapeutically effective dose. The common mechanism underlying the observed behavioral effects of the tested drugs may be associated with the modulation of the serotoninergic, glutamatergic, and dopaminergic systems.

1. Introduction

Major depressive disorder (MDD) is a severe and recurrent condition that profoundly impacts patients' productivity and quality of life (World Health Organization, 2011). Etiologically and pathophysiologically, depression is highly heterogeneous, making it challenging to identify reliable biological markers applicable to most patients. Nonetheless, structural imaging consistently highlights abnormalities in the frontal cortex (FCX), with several studies reporting reduced volume and decreased activity in this region (Arnone et al., 2012). The FCX plays a critical role in processes involved in cognition, emotional processing, and motor performance, functions compromised in depression (Goodwin, 1997; Soares and Mann, 1997).

Conventional antidepressants primarily aim to enhance monoaminergic tone (Hirschfeld, 2000). However, despite their acute effects on the monoaminergic system, these drugs typically require several weeks to produce noticeable therapeutic benefits (Machado-Vieira et al., 2010). Moreover, only about 30 % of patients with MDD achieve full remission following adequate treatment (Gaynes et al., 2020). In contrast, clinical studies have shown that scopolamine, a nonselective muscarinic cholinergic receptor antagonist, can produce potent and rapid antidepressant effects within just a few days of treatment (Ellis et al., 2014; Furey and Drevets, 2006).

The adrenergic-cholinergic balance hypothesis of mania and depression, introduced by Janowsky and colleagues in 1972, suggests that increased cholinergic tone and decreased noradrenergic tone

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underlie depression (Janowsky et al., 1972). Supporting evidence includes findings that both direct-acting muscarinic agonists (e.g., arecoline and oxotremorine) and indirect-acting acetylcholinesterase inhibitors (AChEIs) (e.g., physostigmine), as well as acetylcholine precursors like choline, can worsen mood in both healthy individuals and bipolar patients, as well as exacerbate pre-existing depression (Davis et al., 1987; Janowsky et al., 1973; Modestin et al., 1973; Nurnberger et al., 1983; Tamminga et al., 1976). This suggests that reducing cholinergic tone with scopolamine may offer a viable treatment strategy for depression (Witkin et al., 2020).

However, scopolamine is associated with considerable adverse effects, including memory impairment, drowsiness, and visual disturbances (Renner et al., 2005), which limit its use as a psychiatric drug. One potential solution to mitigate these adverse effects is the coadministration of lower doses of scopolamine with other substances that possess similar antidepressant properties. Increasing evidence links the glutamatergic system to the pathophysiology of mood disorders, including depression. Given the limitations of current treatments based on the monoamine hypothesis, therapeutic strategies targeting the glutamatergic system are increasingly relevant. Clinical, postmortem and preclinical studies strongly implicate glutamatergic transmission dysregulation in MDD. Meta-analysis of proton magnetic resonance spectroscopy studies has revealed reduced cortical levels of glutamine and glutamate in patients with depression (Moriguchi et al., 2019). Postmortem studies have also shown elevated mGlu2/3 receptor levels in the prefrontal cortex (PFC) of individuals with MDD (Feyissa et al., 2010). These findings align with numerous preclinical studies demonstrating antidepressant-like effects of various compounds acting via mGlu receptors, particularly mGlu2/3 antagonists such as MGS0039, LY341495, TP0178894, VU6001966, and VU0650786 (Chaki et al., 2004; Dong et al., 2022; Joffe et al., 2020; Pałucha-Poniewiera et al., 2010), suggesting these receptors may be promising targets for novel antidepressants.

Previous studies revealed that antidepressant-like effects of subeffective doses of the rapid-acting drugs scopolamine and ketamine are enhanced by the nonselective mGlu2/3 receptor antagonist LY341495 (Podkowa et al., 2016a; Podkowa et al., 2016b). However, LY341495 also exhibits some affinity for other mGlu receptors (Kingston et al., 1998), making it necessary to define further the specific roles of mGlu2 and mGlu3 receptors in antidepressant-like action.

Experiments using mGlu2$^{-/-}$ and mGlu3$^{-/-}$ knockout (KO) mice have shown that the antidepressant-like properties of both ketamine and LY341495 involve mGlu2 but not mGlu3 receptors (Gleason et al., 2013; Zanos et al., 2019). These results support further investigation of mGlu2 receptor inhibitors, either as single-agent or combination, for depression treatment. The mGlu2 and mGlu3 receptors share a highly conserved sequence, making it challenging to develop a substance that selectively binds to the orthosteric site of the mGlu2 receptor. Hence, the use of mGlu2 allosteric negative modulators has become essential, as they additionally offer greater selectivity and preserve natural signaling dynamics compared to orthosteric agents, which are often associated with more side effects.

In this study, we extend this line of research by investigating whether the mGlu2 selective allosteric modulator VU6001966 can enhance the antidepressant-like effects of scopolamine at sub-effective doses. Furthermore, we examine the effects of these drugs on extracellular neurotransmitter levels in the FCX of freely moving rats.

2. Methods and materials

2.1. Animals

Male Sprague-Dawley rats (weighing 250–350 g) obtained from Charles River Laboratories, Germany, were housed in groups of four in polycarbonate cages. They were maintained under standard laboratory conditions of temperature (22 °C) and lighting (light phase 6 a.m. – 6 p.m.). Rats received free access to food and water and were handled for 3–5 days before behavioral testing. Each experimental group consisted of six to eight animals, and each rat was used only once. Behavioral experiments were performed between 9 a.m. and 2 p.m. All procedures were carried out in accordance with the criteria of the National Institutes of Health Animal Care and Use Committee and were approved by the Second Local Ethics Committee in Krakow at the Institute of Pharmacology, Polish Academy of Sciences.

2.2. Drugs and reagents

Muscarinic antagonist scopolamine hydrobromide (Tocris Cookson Ltd., Bristol, UK) was dissolved in 0.9 % NaCl, while mGlu2 NAM VU6001966 (Tocris Cookson Ltd., Bristol, UK) was prepared in 0.5 % methylcellulose with the addition of 2 % DMSO. Control animals received 0.9 % NaCl. Tested compounds were prepared immediately before administration and administered intraperitoneally (IP) at a constant volume of 2 ml/kg. The doses of compounds and treatment schedules were chosen on the basis of previous studies (Babii et al., 2024; Joffe et al., 2020; Podkowa et al., 2016b). All drugs were administered 45 min before the FST test and 60 and 100 min after the start of the locomotor activity test or microdialysis (after the fifth basal fraction collection), respectively. Ketamine and xylazine hydrochlorides used for anesthetizing the animals came from Biowet Puławy (Puławy, Poland). All necessary chemicals of the highest purity used for analysis by high-performance liquid chromatography (HPLC) were obtained from Merck (Warsaw, Poland). O-phthalaldehyde (OPA) from Sigma-Aldrich (Poznan, Poland) was used to derivatize glutamate to an electroactive compound.

2.3. Forced swim test

The studies were carried out according to the method of Porsolt et al. (1978). The rats were placed in glass cylinders (height 40 cm, diameter 20 cm) containing 15 cm of water, maintained at 24 °C. Two swim sessions were conducted: an initial 15-min pretest followed 24 h later by a 5-min test. Following both sessions, rats were removed from cylinders and returned to their home cages. Behavioral scoring was performed according to Detke et al. (1995) and during the 5 min test session three different behaviors were rated: 1) immobility – rat was judged to be immobile when it remained floating passively in the water; 2) swimming – rat was judged to be swimming if it was making active swimming motions, more than necessary to maintain its head above water solely; 3) climbing – rat was judged to be climbing when it was making active movements in and out of the water with its forepaws, usually directed against the walls.

2.4. Locomotor activity test

Spontaneous locomotor activity was recorded individually for each animal in Opto-Varimex (Columbus Instruments, Columbus, OH, USA) plexiglass chambers (43 × 43 cm), where the animals were individually placed for an acclimation period of 60 min. Then, they were administered an IP drug injection. Immediately after injection, each rat was returned to the chamber. The total distance traveled during a 120-min experimental session was measured and stored every 5 min. Each cage was surrounded by a 15 × 15 array of photocell beams located 3 cm from the floor surface. Interruptions of the photobeams were interpreted as horizontal activity, and the distance traveled was converted into centimeters. All the data were analyzed using Auto-track software (Columbus Instruments, USA).

2.5. Brain microdialysis procedure

Rats were anesthetized with 75 mg/kg ketamine and 10 mg/kg xylazine intramuscularly and placed on a stereotaxis apparatus.

Microdialysis probe (MAB 4.15.4Cu, AgnTho's AB, Sweden) was lowered slowly into the FCX at the following stereotaxis coordinates (mm): AP +2.7, L + 0.8, and V -6.5 from the bregma and dura surface according to the atlas (Paxinos and Watson, 1998). The active surface of the dialysis probe was 4 mm. Seven days after implantation, probe inlets were connected to a syringe pump (BAS, West Lafayette, IN, USA), which delivered artificial cerebrospinal fluid (aCSF) composed of 147 mM NaCl, 4 mM KCl, 2.2 mM CaCl2 and 1.0 mM MgCl2 at a constant flow rate of 2 µl/min. The monitoring of extracellular levels of neurotransmitters has been performed in freely moving animals. Five baseline samples were collected every 20 min after the washout period of 2 h. The respective drugs were administered, and dialysate fractions were collected for the next 120 min. As the experiment ended, the rats were terminated, and their brains underwent histological examination to validate probe placement.

2.6. Extracellular concentration of DA, 5-HT, glutamate, and GABA

Extracellular DA and 5-HT levels were analyzed using an UHPLC Ultimate 3000 system (Thermo Fisher Scientific, Sunnyvale, CA, USA), electrochemical detector ECD-3000 RS with a 6020RS-omni coulometric cell, 6011RS ultra coulometric analytical cell and a Hypersil Gold C18 analytical column (3 µm, 100 × 3 mm; Thermo Fisher Scientific, Sunnyvale, CA USA). The mobile phase consisted of 0.1 M KH_2PO_4 buffer at pH 3.8, 0.5 mM Na_2EDTA, 100 mg/L 1-octanesulfonic acid sodium salt, and 3.2 % methanol. The flow rate during the analysis was set at 0.6 mL/min, and the applied potential of an omni coulometric cell was 600 mV, whereas the potential of an ultra-coulometric analytical cell was E1 = −50 mV and E2 = 300 mV with a sensitivity set at 10 nA/V. The chromatographic data were processed by the Chromeleon v.7.280 (Thermo Fisher Scientific, Sunnyvale, CA, USA) software package run on a personal computer. The detection limit for DA was 0.002 pg/10 µl and 0.01 pg/10 µl for 5-HT. Glutamate and GABA levels in the extracellular fluid were measured by HPLC with electrochemical detection after the derivatization of samples with OPA/sulfite reagent to form isoindole-sulfonate derivatives (Rowley et al., 1995). The data were processed using Chromax 2005 (Pol-Lab, Warszawa, Poland) software on a personal computer. The limit of detection of glutamate and GABA in dialysates was 0.03 ng/10 µl and 6.4 pg/10 µl, respectively.

2.7. Statistical analysis

All statistical analyses were performed using GraphPad Prism 10.3.1 (GraphPad Software, San Diego, CA, USA) and STATISTICA v.13.3 StatSoft Inc. 1984–2011 (TIBCO Software Inc., Palo Alto, CA, USA). The results are expressed as the means ± SEM. In the FST, statistical significance was assessed using a one-way ANOVA followed by Dunnett's post hoc test (to evaluate dose-effect curves) or a two-way ANOVA followed by Bonferroni's post hoc test (to assess drug interaction). A two-way repeated measures ANOVA followed by Bonferroni's post hoc test was used to analyze the locomotor activity results. Drug effects on DA, 5-HT, glutamate, and GABA release in the FCX were analyzed with two-way repeated measures ANOVA on normalized responses followed by Tukey's post hoc test (time course) and a two-way ANOVA followed by Bonferroni's post hoc test (total effect). All obtained data were presented as a percentage of the basal level, assumed to be 100 %. The differences were considered statistically significant if $P < 0.05$.

3. Results

3.1. The antidepressant-like effects of scopolamine and VU6001966 in the FST

One-way ANOVA showed the dose-dependent effects of scopolamine (0.03–0.3 mg/kg, IP), administered 45 min before the FST, on immobility time [$F(3, 27) = 5.85$, $P = 0.003$; Fig. 1B], climbing time [$F(3, 28) = 11.22$, $P < 0.0001$; Fig. 1C], and swimming time [$F(3, 27) = 4.72$, $P = 0.009$; Fig. 1D]. Dunnett's post hoc tests revealed that scopolamine significantly decreased the immobility time (given at doses of 0.1 and 0.3 mg/kg, $P = 0.020$ and $P = 0.002$, respectively; Fig. 1B), the climbing time (given at a dose of 0.3 mg/kg, $P < 0.0001$; Fig. 1C), and the swimming time (given at doses of 0.1 and 0.3 mg/kg, $P = 0.009$ and $P = 0.043$, respectively; Fig. 1D). When applied at a dose of 0.03 mg/kg, scopolamine did not affect any of the tested parameters.

A one-way ANOVA revealed that VU6001966 (1–10 mg/kg, IP) administered 45 min before the FST significantly reduced the immobility time [$F(3, 25) = 3.06$, $P = 0.047$; Fig. 2B].

A tendency, though not statistically significant, to increase swimming time [$F(3, 26) = 2.55$, $P = 0.078$; Fig. 2D] was observed, with having no impact on climbing [$F(3, 26) = 2.00$, $P = 0.139$; Fig. 2C]. The post hoc Dunnett's test showed that VU6001966 given at a dose of 10 mg/kg significantly decreased the time of immobility ($P = 0.037$;

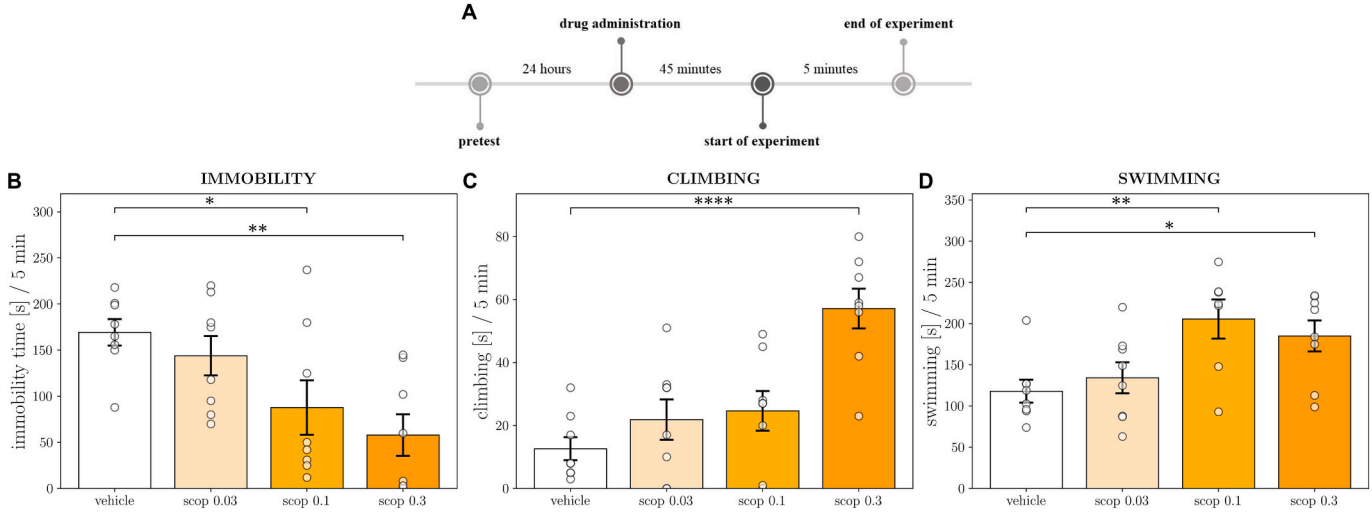

Fig. 1. The antidepressant-like effects of scopolamine administered IP 45 min before the FST in Sprague Dawley rats. (A) Schematic representation of the experimental schedule. The following three parameters were measured: (B) immobility, (C) climbing, and (D) swimming. Values are expressed as the mean ± SEM (N = 7–8) and were analyzed by one-way ANOVA followed by Dunnett's post hoc test (*$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$ vs. respective vehicle).

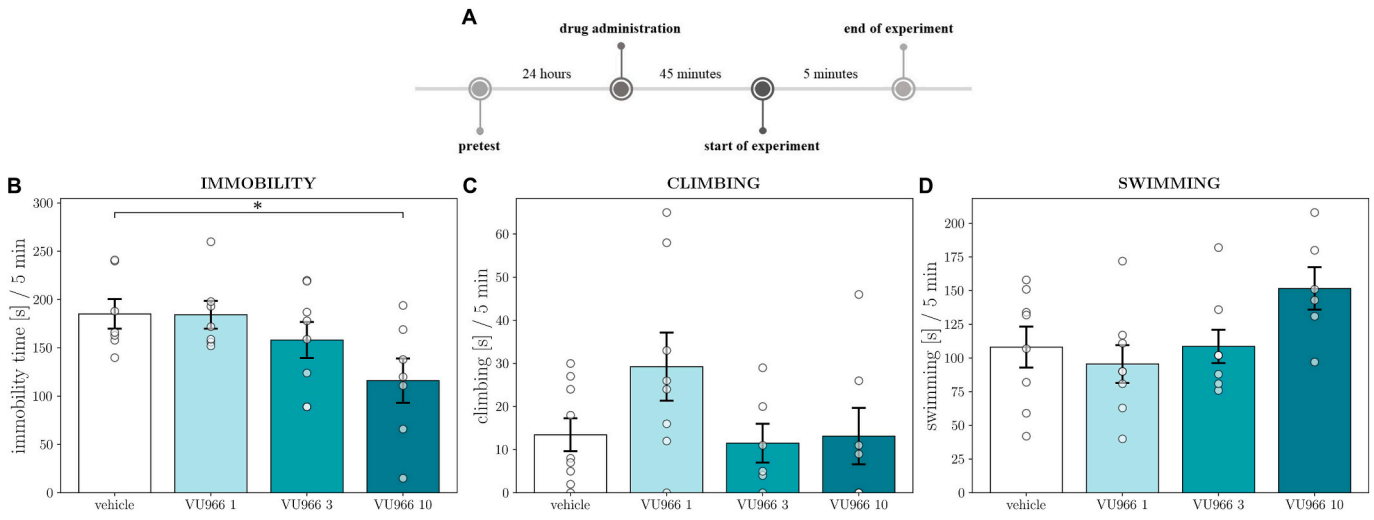

Fig. 2. The antidepressant-like effects of VU6001966 administered IP 45 min before the FST in Sprague Dawley rats. (A) Schematic representation of the experimental schedule. The following three parameters were measured: (B) immobility, (C) climbing, and (D) swimming. Values are expressed as the mean ± SEM (N = 7–8) and were analyzed by one-way ANOVA followed by Dunnett's post hoc test (*P < 0.05 vs. respective vehicle).

Fig. 2B). When applied at doses of 1 and 3 mg/kg, VU6001966 exert no effect on the tested parameters.

To study the rapid antidepressant-like effects of combined administration, the subeffective doses of 0.03 and 3 mg/kg for scopolamine and VU6001966, respectively, were selected for the experiment. Two-way ANOVA revealed that combined administration of scopolamine (0.03 mg/kg) and VU6001966 (3 mg/kg), given 45 min before the FST, induced a significant decrease of immobility [$F(1,22) = 4.35$, $P = 0.049$; Fig. 3B] and increase of climbing time [$F(1, 26) = 11.00$, $P = 0.003$; Fig. 3C], while no affecting the swimming time [$F(1, 28) = 0.13$, $P = 0.726$; Fig. 3D]. Above results indicate a significant enhancement of the rapid effect of scopolamine by VU6001966 in the FST.

3.2. The effects of scopolamine and VU6001966 on the spontaneous locomotor activity of rats

To eliminate any non-specific effects of tested compounds that could interfere with the results of the FST test based on measurement of immobility time following initial escape-oriented movements, as well as to examine the potential impact of dopaminergic system activation observed in the FCX after drugs administration on mobility of rats, the locomotor activity measurement was performed. Scopolamine, VU6001966, and a combination of these drugs were administered at doses used in microdialysis. We found no significant changes between control rats and rats injected with scopolamine (0.3 mg/kg) [$F(1,13) = 0.04$, $P = 0.853$], VU6001966 (3 mg/kg) [$F(1, 14) = 0.06$, $P = 0.807$], or the mixture of these drugs [$F(1, 14) = 0.22$, $P = 0.648$] (Fig. 4B).

3.3. The effects of scopolamine and VU6001966 on extracellular levels of 5-HT, DA, glutamate, and GABA in the rat frontal cortex

The 5-HT levels were elevated to approximately 300 % of the baseline when treated with either scopolamine or VU6001966 alone and up to ca. 400 % when the compounds were administered in combination (Fig. 5B). Repeated-measures ANOVA indicated a significant effect of treatment groups ($F(3, 17) = 218.45$, $P < 0.0001$), as well as a significant interaction between treatment groups and sampling period ($F(15, 85) = 30.71$, $P < 0.0001$). A two-way ANOVA revealed a significant

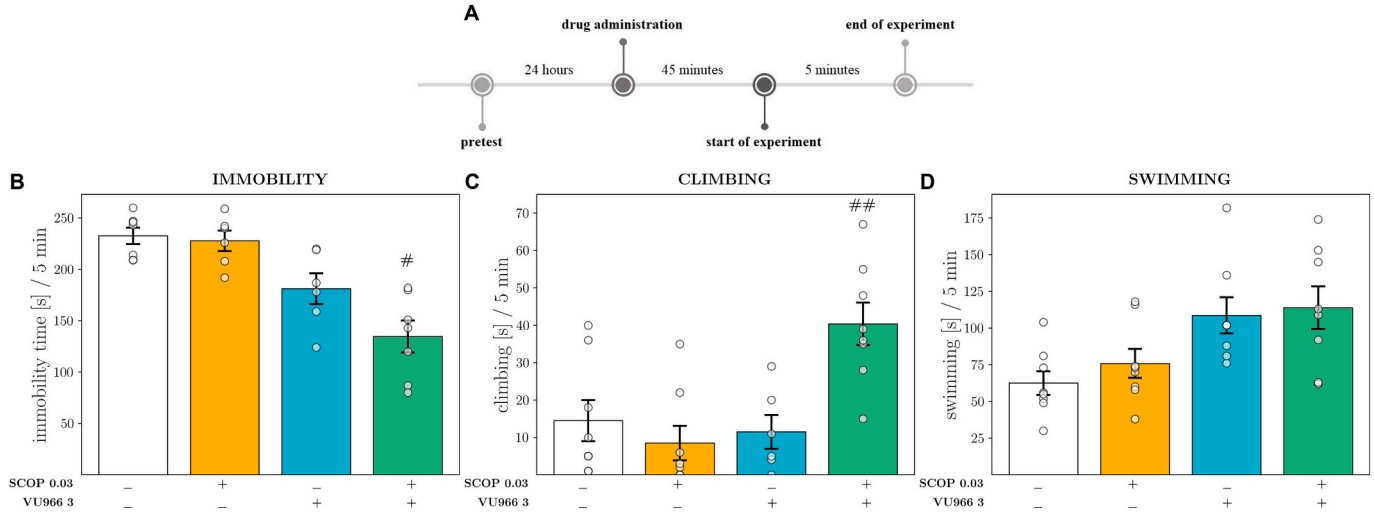

Fig. 3. The antidepressant-like effects of scopolamine and VU6001966 coadministered IP 45 min before the FST in Sprague Dawley rats. (A) Schematic representation of the experimental schedule. The following three parameters were measured: (B) immobility, (C) climbing, and (D) swimming. Values are expressed as the mean ± SEM (N = 7–8) and were analyzed by two-way ANOVA followed by Bonferroni's multiple comparisons test (#P < 0.05, ##P < 0.01; interaction: scopolamine × VU6001966).

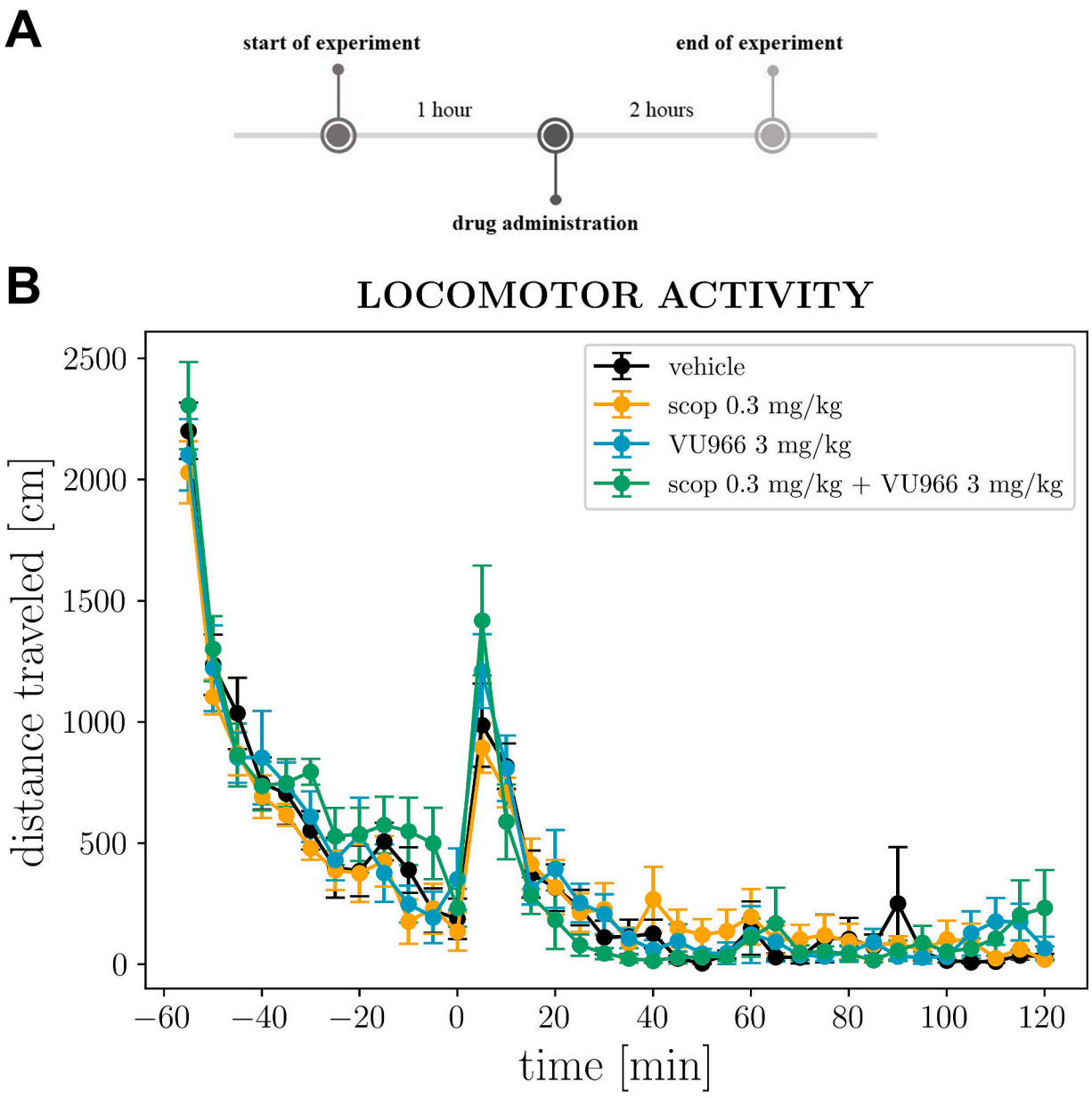

Fig. 4. The effects of scopolamine, VU6001966, and both in combination on the locomotor activity in Sprague Dawley rats. (A) Schematic representation of the experimental schedule. (B) The effect of a single administration of tested drugs on the spontaneous locomotor activity of rats during a 180-min experimental session. The drug injection corresponds to the time 0. The values are expressed as the mean ± SEM (N = 7–8) and were analyzed by two-way repeated measurements ANOVA followed by Bonferroni's post hoc test.

interaction between scopolamine (0.3 mg/kg) and VU6001966 (3 mg/kg) in their total effects on 5-HT levels, expressed as AUC [$F(1, 17) = 14.83$, $P = 0.001$; Fig. 5C]. Both scopolamine and VU6001966, alone or in combination, significantly increased 5-HT levels compared to the control group ($P < 0.0001$).

Both scopolamine at a dose of 0.3 mg/kg and VU6001966 at a dose of 3 mg/kg significantly increased extracellular levels of DA up to ca. 200 % of the baseline in the rat frontal cortex (Fig. 5D) when combined administration of given compounds enhanced DA up to ca. 400 % of the baseline. Repeated-measures ANOVA showed the significant effect of treatment groups ($F(3,20) = 38.66$, $P < 0.0001$) and the interaction between treatment groups and sampling period ($F(15, 100) = 9.25$, $P < 0.0001$). A two-way ANOVA revealed a significant interaction between scopolamine (0.3 mg/kg) and VU6001966 (3 mg/kg) in their total effects on DA levels, expressed as AUC [$F(1,20) = 4.86$, $P = 0.039$; Fig. 5E]. Both scopolamine and VU6001966, alone or in combination, significantly increased DA levels compared to the control group ($P < 0.0001$).

The extracellular glutamate level was increased in the rat frontal cortex by scopolamine at a dose of 0.3 mg/kg (up to ca. 150 % of the baseline) and more potently by the tested mixture of scopolamine and VU6001966 (up to ca. 220 % of the baseline), while decreased by VU6001966 given alone at a dose of 3 mg/kg (Fig. 5F). Repeated-measures ANOVA showed the significant effect of treatment groups ($F(3,22) = 193.84$, $P < 0.0001$) and the interaction between treatment groups and sampling period ($F(15, 110) = 24.46$, $P < 0.0001$). A two-way ANOVA revealed a significant interaction between scopolamine (0.3 mg/kg) and VU6001966 (3 mg/kg) in their total effects on glutamate levels, expressed as AUC [$F(1, 22) = 135.60$, $P < 0.0001$; Fig. 5G]. Both scopolamine and VU6001966, alone or in combination, significantly altered glutamate levels compared to the control group ($P < 0.0001$).

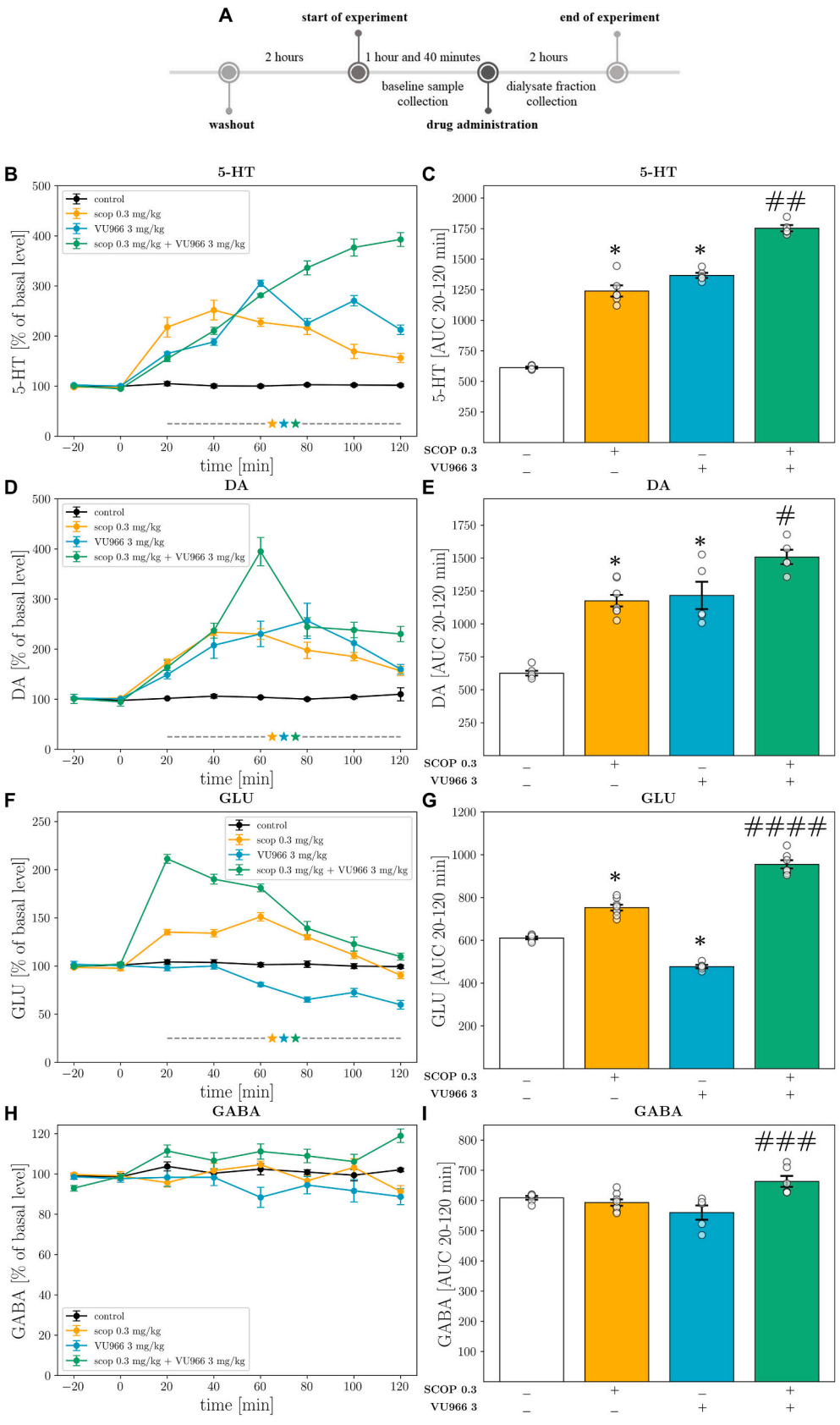

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Fig. 5. The effects of scopolamine (0.3 mg/kg), VU6001966 (3 mg/kg), and both in combination on extracellular levels of neurotransmitters in the Sprague Dawley rat frontal cortex. (A) Schematic representation of the experimental schedule. The time-course (B, D, F, H) and total effect (C, E, G, I) of tested compounds on the serotonin (5-HT), dopamine (DA), glutamate (GLU) and γ-aminobutyric acid (GABA) extracellular levels in the rat frontal cortex. The drug injection corresponds to the time 0. The statistical significance of time curves, compared to the control, within the time range of 20–120 min is indicated by dashed lines, with stars colored to match the corresponding curve. The total effect is calculated as an area under the concentration-time curve (AUC) and expressed as a percentage of the basal level. Values are expressed as the mean ± SEM ($N = 5–8$) and were analyzed by two-way repeated measures ANOVA on normalized responses followed by Tukey's post hoc test (time course) and a two-way ANOVA followed by Bonferroni's post hoc test (total effect). *$P < 0.001$ vs. respective vehicle; #P < 0.05, ##P < 0.01, ###P < 0.001, ####P < 0.0001; interaction: scopolamine × VU6001966.

All tested drugs had no effect on the extracellular level of GABA in the rat frontal cortex (Fig. 5H). Repeated-measures ANOVA showed the significant effect of treatment groups ($F(3,21) = 7.76$, $P = 0.001$) and the interaction between treatment groups and sampling period ($F(15, 105) = 3.86$, $P < 0.0001$). A two-way ANOVA revealed a significant interaction between scopolamine (0.3 mg/kg) and VU6001966 (3 mg/kg) in their total effects on GABA levels, expressed as AUC [$F(1,21) = 15.86$, $P = 0.001$; Fig. 5I]. However, the post hoc test did not reveal any significant changes compared to the control group ($P > 0.05$).

4. Discussion

Human studies have demonstrated that scopolamine exhibits a rapid and prolonged antidepressant effect. However, its considerable adverse effects limit its use as a psychiatric drug. In our previous studies, we have shown that combined administration of scopolamine with metabotropic glutamate receptor ligands can help overcome these limitations. Specifically, coadministration of low doses of scopolamine together with low doses of AMN082, a positive allosteric modulator (PAM) of mGlu7 receptors, or low doses of LY341495, an antagonist of group 2/3 mGlu receptors, enhances the antidepressant effects of scopolamine while reducing its adverse effects (Podkowa et al., 2018; Podkowa et al., 2016b). Here, we continued that line of research investigating whether the mGlu2 receptor subtype is involved in these interactions with scopolamine.

The results of this study show that a non-effective dose of mGlu2 NAM VU6001966 significantly enhanced the antidepressant-like effects of a non-effective dose of scopolamine in rats. Animal behavior in the FST test was evaluated in rats to investigate potential antidepressant-like activity and to preliminary assess the involvement of serotonergic and/or noradrenergic mechanisms. In the FST, classic antidepressants, including selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors (MAOIs), and tricyclic antidepressants (TCAs), typically reduce immobility time rapidly, with effects observable after a single injection administered 30–60 min prior to the test (Borsini and Meli, 1988). An increase in swimming time indicates serotonergic circuit involvement, while enhanced climbing behavior suggests engagement of noradrenergic and/or dopaminergic pathways (Detke et al., 1995). In the present study, the mGlu2 NAM VU6001966 dose-dependently reduced immobility time. Scopolamine not only reduced immobility time but also increased both swimming and climbing time. Notably, the coadministration of sub-effective doses of scopolamine (0.03 mg/kg) and VU6001966 (3 mg/kg) significantly reduced immobility time and increased climbing time. This specific increase in climbing, but not swimming, initially suggests that scopolamine and VU6001966 may share a mutual mechanism of antidepressant action, likely mediated via noradrenergic/dopaminergic rather than serotonergic pathways. Our findings align with our earlier studies conducted on mice, which suggest that activation of the noradrenergic system – rather than the serotonergic system – is responsible for the antidepressant-like effects of scopolamine and that scopolamine may potentiate the antidepressant effects of drugs that achieve their action through modulation of the noradrenergic system (Pałucha-Poniewiera et al., 2017).

To further elucidate the effects of the tested compounds on extracellular neurotransmitter levels, including DA, 5-HT, glutamate, and GABA, microdialysis of freely moving rats was performed. This technique allows the measurement of neurotransmitter fluctuations in the FCX by perfusing neurotransmitter-free artificial cerebrospinal fluid at a constant rate and collecting dialysate fractions, thereby reflecting the kinetics of in vivo neurotransmitter release (Di Chiara et al., 1996).

Unlike conventional antidepressants, scopolamine exerts rapid antidepressant effects after a few doses in patients with depression, including those with treatment-resistant depression (TRD) (Ellis et al., 2014; Furey and Drevets, 2006). Similarly, animal studies suggest that mGlu2/3 receptor antagonists may also produce rapid antidepressant-like effects (Chaki et al., 2004; Pałucha-Poniewiera et al., 2010; Podkowa et al., 2016b). This study aimed to examine the acute antidepressant-like effects of scopolamine and mGlu2 NAM VU6001966 coadministration, as well as its effect on rat frontal cortex neurochemistry within the first few hours after administration, to explore their shared mechanism of action.

Both scopolamine and mGlu2 NAM VU6001966, given alone or in combination, significantly increased 5-HT levels in the FCX. These findings align with the serotonergic effects observed with acute treatment using conventional antidepressants in the rat frontal cortex (Beyer and Cremers, 2008). While the serotonin hypothesis of depression has been influential for decades and serves as a foundation for the use of antidepressants (Lapin and Oxenkrug, 1969; Schildkraut, 1965), it has been increasingly questioned due to its limitations in explaining depression mechanisms (Moncrieff et al., 2023). Monoamine-based treatments are effective in addressing acute depressive episodes but often fail in long-term maintenance therapy (Fava, 2020). The delayed, and in many cases inadequate, response to current antidepressants suggests that additional neurotransmitters beyond those implicated in the monoamine theory may play a critical role in the pathophysiology of depression.

Second-generation antidepressants, such as SSRIs, are effective in improving general mood but often fail to enhance reward-related behaviors or motivation (Nutt et al., 2007). Several research studies support the involvement of dopamine dysfunction in depression, linking it to anhedonia and loss of motivation – two core symptoms of the disorder (Kapur and John Mann, 1992; Yadid and Friedman, 2008). Studies suggest that dopaminergic agents may be effective in addressing these symptoms. For example, both animal and clinical studies have reported positive effects of bupropion, a dopamine reuptake inhibitor, on reducing anhedonia (Paterson et al., 2007; Tomarken et al., 2004). Interestingly, SSRIs with some dopaminergic activity, such as sertraline, fluoxetine, and venlafaxine, appear to be more effective in treating anhedonia compared to other SSRIs (Boyer et al., 2000; Light et al., 2011). Our studies have shown that both scopolamine and mGlu2 NAM VU6001966, given alone or in combination, significantly increased DA levels in the rat frontal cortex. A similar increase in DA levels in FCX has been observed with the rapid-acting antidepressant ketamine, which also exhibits notable anti-anhedonic effects (Lally et al., 2014; Wojtas et al., 2022).

Our studies have shown that scopolamine, alone or in combination with mGlu2 NAM VU6001966, significantly increased glutamate levels in the rat frontal cortex. Glutamate is the principal excitatory neurotransmitter in the brain, which plays a key role in regulating cognitive and motor functions, pain perception, and mood (Sanacora et al., 2012; Wozniak et al., 2012). Depressive symptoms are associated with structural abnormalities and connectivity impairments in the cortical and limbic brain regions, linked to dysfunctions in excitatory glutamate neurons and inhibitory GABA interneurons. According to the

neurotrophic hypothesis of depression, which correlates lower neurotrophin levels with higher rates of depression (Duman and Monteggia, 2006), and evidence showing that acute glutamate release upregulates brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (Heese et al., 2000; Zafra et al., 1991), the tested drugs may promote synaptic plasticity by elevating glutamate levels.

4.1. Proposed mechanism of action

The effect of scopolamine on glutamate levels in the FCX aligns with previous studies suggesting that its antidepressant activity is mediated through two mechanisms: 1) indirect enhancement of pyramidal neuron activity and glutamate transmission in the PFC via the blockade of muscarinic stimulatory M1 receptors on GABAergic interneurons, and 2) direct enhancement of pyramidal neuron activity and glutamate transmission in the PFC via the blockade of muscarinic inhibitory M2 receptors on glutamatergic neurons (Navarria et al., 2015; Witkin et al., 2014). However, our findings suggest that scopolamine's antidepressant mechanism of action is more complex, involving the activation of the mesocortical pathway and DA release. Scopolamine administration not only increased glutamate levels but also elevated DA levels in the FCX. DA neuron projections from the medial ventral tegmental area (VTA) seem to be the main source of dopamine in the PFC (Zubair et al., 2021). The VTA, a midbrain structure, plays a critical role in motivation and reward processing. Dysfunction of VTA dopaminergic neurons has been implicated in several neuropsychiatric disorders, including depression and addiction (Polter and Kauer, 2014). Preclinical studies suggest that increased cholinergic tone in the VTA is associated with pro-depressive and anxiogenic behavioral effects (Small et al., 2016). Additionally, animal studies indicate that degeneration of DA neurons in the VTA enhances depressive-like behaviors, which can be alleviated by SSRIs and L-DOPA treatments (Winter et al., 2007). VTA infusion of M5 NAM has been shown to attenuate physostigmine-induced anhedonic, anxiogenic-like, and pro-depressive-like behaviors (Nunes et al., 2020), suggesting that scopolamine as a muscarinic antagonist may exert similar effects.

The proposed mechanism of action for mGlu2 NAM VU6001966 also implicates the VTA-PFC pathway. VU6001966 may enhance glutamate release by binding to mGlu2 on VTA glutamatergic neurons (Manzoni and Williams, 1999), thereby activating DA neurons and facilitating subsequent DA release in the FCX. Interestingly, mGlu2 NAM VU6001966 demonstrates dual effects on glutamate levels: a decrease is observed when VU6001966 is administered alone, whereas an increase occurs when coadministered with scopolamine. This discrepancy may be explained by VU6001966 modulatory properties, which are highly dependent on glutamate availability, which is elevated by scopolamine administration.

Dopamine can additionally modulate glutamate and GABA levels in the FCX. Pyramidal and GABAergic cells predominantly express D1 receptors rather than D2 receptors, and the absolute number of pyramidal neurons expressing D1 receptors exceeds that of GABAergic interneurons (Santana et al., 2009). Consequently, DA released from mesocortical terminals in the FCX predominantly stimulates pyramidal cells via D1 receptors, subsequently triggering the release of glutamate. Over time, strong activation of the VTA-PFC circuit gradually increases the excitability of parvalbumin-positive (PV+) interneurons in the PFC, which release GABA (Zhong et al., 2020). This may explain the lack of significant changes in GABA levels following administration of the tested drugs, with only a subtle increase observed when scopolamine and VU6001966 are coadministered.

4.2. Limitations and potential treatment alternatives

The main limitation of the present study is its focus solely on the acute effects of the tested drugs in naïve rats. Therefore, further investigation using rats with a depressive-like phenotype would be particularly important. Animal models of depression mirror core symptoms such as behavioral despair and anhedonia (Petković and Chaudhury, 2022), with the latter being especially relevant given the potential drug effect on the VTA suggested by this study. Furthermore, our findings open the door to future investigations exploring whether the observed behavioral effects are both rapid and sustained.

While an mGlu2 negative allosteric modulator is one potential add-on therapy, other combinations of scopolamine with currently available antidepressants should also be considered. To date, there have been a few clinical attempts using scopolamine augmentation. Combined treatment with scopolamine and the SSRI escitalopram failed to improve depressive symptoms in patients with MDD compared to placebo (Zhou et al., 2020). However, a pilot study by Taub showed that the combination of scopolamine and naltrexone provided significant benefits for MDD (Taub, 2019). Moreover, preclinical findings on ketamine and scopolamine coadministration suggest potential for clinical translation (Martin et al., 2017), highlighting the need for further investigation and offering hope for improved treatment strategies.

5. Conclusion

This research demonstrates that the combined administration of scopolamine and mGlu2 NAM VU6001966 significantly decreased immobility time in the forced swim test, a measure associated with “behavioral despair.” Additionally, this combination significantly enhanced serotonin, dopamine, and glutamate levels in the rat frontal cortex. Depression, as a highly heterogeneous disorder, is defined by a constellation of different classes of symptoms, making it challenging to identify the specific neural circuit dysfunctions underlying these symptoms. Therefore, therapeutic approaches that target multiple neural circuit processes appear particularly promising for the effective treatment of depression.

CRediT authorship contribution statement

Yana Babii: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Agnieszka Pałucha-Poniewiera:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Krystyna Gołembiowska:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Agnieszka Bysiek:** Investigation. **Izabela Szpręgiel:** Investigation. **Andrzej Pilc:** Writing – review & editing, Supervision, Funding acquisition.

Ethics approval statement

The experiments were carried out only after approval of the project by the Second Local Ethics Committee in Kraków, Poland (protocol code 248/2022, date of approval September 22, 2022).

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Declaration of competing interest

None.

Data availability

Data will be made available on request.

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