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The effects of a 5-HT$_{5A}$ receptor antagonist in a ketamine-based rat model of cognitive dysfunction and the negative symptoms of schizophrenia

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ABSTRACT

Serotonin (5-HT) receptors still represent promising targets for the development of novel multireceptor or stand-alone antipsychotic drugs with a potential to ameliorate cognitive impairments and negative symptoms in schizophrenia. The 5-HT$_{5A}$ receptor, one of the least known members of the serotonin receptor family, has also drawn attention in this regard. Although the antipsychotic efficacy of 5-HT$_{5A}$ antagonists is still equivocal, recent experimental data suggest the cognitive-enhancing activity of this strategy.

The aim of the present study was to evaluate pro-cognitive and pro-social efficacies of the 5-HT$_{5A}$ receptor antagonist in a rat pharmacological model of schizophrenia employing the administration of the NMDA receptor antagonist, ketamine. The ability of SB-699551 to reverse ketamine-induced cognitive deficits in the attentional set-shifting task (ASST) and novel object recognition task (NORT) was examined. The compound's efficacy against ketamine-induced social withdrawal was assessed in the social interaction test (SIT) and in the social choice test (SCT).

The results demonstrated the efficacy of SB-699551 in ameliorating ketamine-induced impairments on the ASST and NORT. Moreover, the tested compound also enhanced set-shifting performance in cognitively unimpaired control rats and improved object recognition memory in conditions of delay-induced natural forgetting. The pro-social activity of SB-699551 was demonstrated on both employed paradigms, the SIT and SCT.

The present study suggests the preclinical efficacy of a strategy based on the blockade of 5-HT$_{5A}$ receptors against schizophrenia-like cognitive deficits and negative symptoms. The utility of this receptor as a target for improvement of cognitive and social dysfunctions warrants further studies.

1. Introduction

Despite the fact that the 5-HT$_5$ receptor was cloned more than 20 years ago (Erlander et al., 1993; Plassat et al., 1992; Rees et al., 1994), it is still one of the least characterised subtypes among the serotonin receptor family. This subfamily comprises two receptors, the 5-HT$_{5A}$ and the 5-HT$_{5B}$. In humans, only the 5-HT$_{5A}$ receptor is functional (Grailhe et al., 2001). Although the physiological role of 5-HT$_{5A}$ receptors remain poorly understood, their role in the aetiology and/or therapy of CNS disorders, including schizophrenia, has been proposed (reviewed in (Nelson, 2004; Thomas, 2006; Volk et al., 2010)).

For example, polymorphism association studies of the 5-HT$_{5A}$ receptor gene demonstrated that substitutions in the 5′ untranslated region (-G19C) and in the coding region (C43T, which leads to a Pro15Ser amino acid change) of the 5-HT$_{5A}$ receptor gene are linked to schizophrenia (Birkett et al., 2000; Iwata et al., 2001). Furthermore, a silent substitution in the coding region (A12T) has also been associated with schizophrenia, and the 12T allele has been connected with a later age of onset of the disease (Dubertret et al., 2004). Moreover, an early study demonstrated that 5-HT$_{5A}$ receptor knock-out mice exhibited attenuated exploratory activity responses to lysergic acid diethylamide (LSD) (Grailhe et al., 1999), which produces a psychotic-like state in healthy people (Schmid et al., 2015). Given the strong binding of LSD to 5-HT$_{5A}$ receptors, these findings have suggested that some of the psychotic effects of

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LSD are mediated by this receptor (Grailhe et al., 1999). Of interest is also the finding that asenapine, a novel atypical antipsychotic drug with preclinical and clinical pro-cognitive efficacy (Elsworth et al., 2012; Fleming et al., 2007), is characterised by a relatively high affinity for 5-HT_{5A} receptors (pKi = 8.8, (Shahid et al., 2009)).

The limited number of studies that have assessed the antipsychotic efficacies of antagonists of 5-HT_{5A} receptors in animal models of schizophrenia has yielded equivocal results. In a study by Kassai et al. (2012), neither SB-699551 nor A-843277 counteracted phencyclidine (PCP)- or amphetamine-induced hyperlocomotion in rats. On the contrary, A843277 reversed dizocilpine-induced hyperlocomotion in mice and improved deficient prepulse inhibition (PPI) of the acoustic startle in DBA/2 mice, but was ineffective in classical dopaminergic models such as amphetamine-induced hyperlocomotion, apomorphine-induced climbing, apomorphine-disrupted PPI or conditioned avoidance response test (Jongen-Relo et al., 2006). On the contrary, another 5-HT_{5A} receptor antagonist ASP5736 attenuated methamphetamine-induced hyperactivity in mice (Yamazaki et al., 2014). In the latter study, co-administration of ASP5736 also increased the potency of an antipsychotic drug, olanzapine, to counteract the methamphetamine-evoked hyperlocomotion effect.

Although the available data do not unequivocally confirm the antipsychotic potential of 5-HT_{5A} receptor antagonists, the recent report has suggested that blockade of 5-HT_{5A} receptors may be effective against schizophrenia-like cognitive disturbances (Yamazaki et al., 2014). In this study, an antagonist of 5-HT_{5A} receptors, ASP5736, ameliorated an acute dizocilpine-induced working memory deficit in the Y-maze and a neonatal PCP-induced recognition memory deficit in mice (Yamazaki et al., 2014).

The aim of the present study was to further explore the pro-cognitive potential of blockade of 5-HT_{5A} receptors in a rat model of schizophrenia. Thus, we evaluated the ability of the 5-HT_{5A} receptor antagonist SB-699551 to reverse schizophrenia-like cognitive deficits that were assessed in the attentional set-shifting task (ASST) and in the novel object recognition task (NORT). As little is known about the involvement of 5-HT_{5A} receptors in the regulation of cognitive processes under physiological conditions, the impact of SB-699551 on delay-induced impairment in the NORT, which resembles natural forgetting, was also assessed. In addition to cognitive deficits, negative symptoms are also an important target for therapeutic intervention in schizophrenia because the efficacy of currently used antipsychotics remains ambiguous. Thus, the efficacy of SB-699551 against social withdrawal, which represents a key item of the cluster of negative symptoms, was evaluated using the social interaction test (SIT) and social choice test (SCT).

SB-699551 has been reported to display at least 30-fold selectivity for the human 5-HT_{5A} receptor versus a number of other 5-HT receptor subtypes and other CNS receptors (pKi values are 8.3, <6.0, <6.0, <6.0, <5.5 and <5.5 for 5-HT_{5A}, 5-$HT_{1B/D}$, 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A} and 5-HT_7 receptors, respectively) (Corbett et al., 2005; Thomas et al., 2006). The biological activity of SB-699551 has been demonstrated in several in vivo studies (Gonzalez et al., 2013; Kassai et al., 2012; Munoz-Islas et al., 2014).

In the current experiments, the pharmacological model based on administration of an antagonist of the N-methyl-D-aspartate receptor (NMDAR), ketamine, to rats was applied. Non-competitive antagonists of the NMDAR, such as ketamine, produce a behavioural syndrome in healthy humans that closely resembles the symptoms of schizophrenia (Newcomer et al., 1999). The administration of NMDAR antagonists evokes a broad range of schizophrenia-like disturbances, including not only psychotic-like behaviours but also negative symptoms and cognitive deficits. Therefore, NMDAR-based models are commonly used to mimic schizophrenia-like states in laboratory animals to identify possible treatments for this disorder (Meltzer et al., 2013). Because ketamine is commonly used in the clinic to model a transient schizophrenia-like state in healthy volunteers (Frohlich and Van Horn, 2014), the ketamine-based animal model might represent a valuable tool in preclinical research due to its translational value. Our previous studies have demonstrated that administration of ketamine produces distinct deficits in the ASST, NORT and SIT that may be ameliorated by antipsychotic drugs (Nikiforuk et al., 2013). The validation of a ketamine-based model of SCT was included in the present study with the use of amisulpride.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Sulzfeld, Germany) weighing 200–250 g (ASST, NORT, SCT) or 125–150 g (SIT) on arrival were housed in a temperature-controlled (21 ± 1 °C) and humidity-controlled (40–50%) colony room under a 12/12 h light/dark cycle (lights on at 06:00 h). For the NORT and SCT, the rats were group-housed (5 rats/cage) with free access to food and water. For the ASST, the rats were pair-housed with a mild food restriction (17 g of food pellets per day) for at least one week prior to testing. For the SIT, the rats were individually housed for 5 days prior to the start of the procedure with free access to food and water. Behavioural testing was performed during the light phase of the light/dark cycle.

The experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the II Local Ethics Committee for Animal Experiments at the Institute of Pharmacology, Polish Academy of Science, Krakow, Poland.

2.2. Attentional set-shifting task (ASST)

The ASST assesses cognitive flexibility, i.e., the ability to modify behaviour in response to the altering relevance of stimuli. In this paradigm, rats must select a bowl containing a food reward based on the ability to discriminate the odours or the media covering the bait (Birrell and Brown, 2000). The ASST requires rats to initially learn a rule and form an attentional “set” within the same stimulus dimensions. At the extra-dimensional (ED) shift stage, the essential phase of the task, animals must switch their attention to a previously irrelevant stimulus dimension and, for example, discriminate between the odours and not between the media covering the bait. The animal's performance at the ED stage is considered an index of cognitive flexibility.

2.2.1. Apparatus

Testing was conducted in a Plexiglas apparatus (length × width × height: 38 × 38 × 17 cm) with the grid floor and wall dividing half of the length of the cage into two sections. During testing, one ceramic digging pot (internal diameter of 10.5 cm and a depth of 4 cm) was placed in each section. Each pot was defined by a pair of cues along with two stimulus dimensions. To mark each pot with a distinct odour, 5 μl of a flavouring essence (Dr. Oetker®, Poland) was applied to a piece of blotting paper fixed to the external rim of the pot immediately prior to use. A different pot was used for each combination of digging medium and odour; only one odour was ever applied to a given pot. The bait (one-third of a Honey Nut Cheerio, Nestle®) was placed at the bottom of the “positive” pot and buried in the digging medium. A small amount of powdered Cheerio was added to the digging media to prevent the rat from trying to detect the buried reward by its smell.

2.2.2. Procedure

As described previously (Nikiforuk et al., 2013), the procedure lasted 3 days for each rat: habituation, training and testing. During a single test session, rats performed a series of seven discriminations. The first four trials at the beginning of each discrimination phase were a discovery period (not included in the six criterion trials). In subsequent trials, an incorrect choice was recorded as an error. Digging was defined as any distinct displacement of the digging media with either the paw or the nose; the rat could investigate a digging pot by sniffing or touching without displacing material. Testing was continued at each phase until the rat reached the criterion of six consecutive correct trials, after which testing proceeded to the next phase.

In the simple discrimination involving only one stimulus dimension, the pots differed along one of two dimensions (e.g., digging medium). For the compound discrimination (CD), the second (irrelevant) dimension (i.e., odour) was introduced but the correct and incorrect exemplars of the relevant dimension remained constant. For the reversal of this discrimination (Rev 1), the exemplars and relevant dimension were unchanged, but the previously correct exemplar was now incorrect and vice versa. The intra-dimensional (ID) shift was then presented, comprising new exemplars of both the relevant and irrelevant dimensions with the relevant dimension remaining the same as previously. The ID discrimination was then reversed (Rev 2) so that the formerly positive exemplar became the negative one. For the extra-dimensional (ED) shift, a new pair of exemplars was again introduced, but this time a relevant dimension was also changed. Finally, the last phase was the reversal (Rev 3) of the ED discrimination. The exemplars were always presented in pairs and varied so that only one animal within each treatment group received the same combination.

The following pairs of exemplars were used: Pair 1: odour: spicy vs. vanilla, medium: cotton wool vs. crumpled tissue; Pair 2: odour: lemon vs. almond, medium: shredded pipette tips vs. wooden sticks; and Pair 3: odour: rum vs. cream, medium: shredded papers vs. silk. The assignment of each exemplar in a pair as being positive or negative at a given phase and the left-right positioning of the pots in the test apparatus on each trial were randomised.

2.3. Novel object recognition task (NORT)

The NORT in rodents has been increasingly used as an ethologically relevant paradigm for the study of visual recognition memory (Ennaceur and Delacour, 1988). This paradigm is based on the spontaneous exploration of novel and familiar objects. Successful object recognition is indicated when an animal spends more time interacting with the novel object in the retention trial.

2.3.1. Apparatus

The rats were tested in a dimly lit (25 Lux) open field made of dull grey plastic (length × width × height: 66 × 56 × 30 cm). After each measurement, the floor was cleaned and dried.

2.3.2. Procedure

The rats were habituated to the arena (without any objects) for 5 min at 24 h prior to testing (Nikiforuk et al., 2013). The test comprised two 3-min trials separated by an inter-trial interval (ITI) of 1 h (in the ketamine experiment) or 24 h (in the natural forgetting experiment). During the first trial (familiarisation, T1), two identical objects (A1 and A2) were presented in opposite corners, approximately 10 cm from the walls of the open field. In the second trial (retention, T2), one of the objects was replaced with a novel object (A = familiar and B = novel). The animals were returned to the home cage after T1. The objects used included a glass bulb filled with gravel and a plastic bottle filled with sand. The heights of the objects were comparable (~12 cm), and both objects were heavy enough to not be displaced by the animals. Half of the animals from each group received the glass bulb as a novel object, and the other half received the plastic bottle. The location of the novel object in the recognition trial was randomly assigned for each rat. The exploration of an object was defined by looking, licking, sniffing or touching the object, while sniffing but not leaning against, standing or sitting on the object. Any rat spending less than 5 s exploring the two objects within 3 min of T1 or T2 was eliminated from the study. The behaviour of the rats was recorded using a camera placed above the arena and connected to the Any-maze® tracking system (Stoelting Co., Illinois, USA). An experimenter blinded to the treatment conditions manually assessed the exploration time. Additionally, the distance travelled was automatically measured using the Any-maze® tracking system. Based on the exploration time (E) of the two objects, a discrimination index was calculated as $DI = (E_B - E_A)/(E_A + E_B)$.

2.4. Social interaction test (SIT)

The analysis of social behaviours of pairs of unfamiliar rats in the open field arena might represent an ethologically valid approach for the preclinical assessment of social functions (Sams-Dodd, 1999). Specifically, perturbations in social functioning such as social withdrawal and asociality that represent key items of a cluster of negative symptoms of schizophrenia may be modelled using the SIT (Wilson and Koenig, 2014).

2.4.1. Apparatus

The experiments were conducted in an open field arena (length × width × height: 57 × 67 × 30 cm) made of black Plexiglas. The arena was dimly illuminated with an indirect light of 18 Lux. The behaviour of the rats was recorded using two cameras placed above the arena and connected to a Noldus MPEG recorder 2.1. An experimenter blinded to the treatment conditions analysed the videos off-line using Noldus Observer® XT, version 10.5.

2.4.2. Procedure

The rats were individually housed for 5 days prior to the start of the procedure. On the fifth day of social isolation, all rats were transferred to the experimental room and individually adapted to the open field arena for 7 min. The animals were subsequently handled and weighed, and the backsides of one half of the animals were dyed with a gentian violet (2% Methylrosanilinium chloride) solution. On the test day (the sixth day of social isolation), two unfamiliar rats of matched body weight (±5 g) were placed in the open field arena, and their behaviours were recorded for 10 min. Both rats in a given pair received the same treatment. The social interaction time was measured for each rat separately. The following active social behaviours were scored: sniffing (the rat sniffs the body of the conspecific), anogenital sniffing (the rat sniffs the anogenital region of the conspecific), social grooming (the rat licks and chews the fur of the conspecific), following (the rat moves toward and follows the other rat), mounting (the rat stands on the back of the conspecific) and climbing (the rat climbs over the back of the conspecific) (Holuj et al., 2015). No overt aggressive behaviours (such as biting, kicking, boxing and threatening behaviour) were observed in control animals or after treatment with ketamine, SB-699551 and the combination of both compounds. As the mean total time of aggressive behaviours was less that 3% of the session duration, aggression was not included in the analysis. The time of active social behaviours was summed to yield a total score. Because both animals in a pair yielded approximately equal scores (for either total time spent in social interactions or separate social

behaviours), social interaction time was expressed as a summed score for each pair of animals.

2.5. Social choice test (SCT)

This procedure provides an easily quantifiable measure of social approach/avoidance behaviour in rodents (Moy et al., 2004). Sociability is reflected as a tendency to spend more time in the compartment with an unfamiliar conspecific than in the empty compartment. Preference for the empty chamber reflects social avoidance/asociality.

2.5.1. Apparatus

The experiments were conducted in an open field arena (length × width × height: 100 × 60 × 30 cm) made of black Plexiglas that were divided into three compartments. Dividing walls were made from clear Plexiglas with (arched) openings (width × height: 10 × 12 cm) allowing access into each chamber. The apparatus was dimly illuminated with an indirect light of 18 Lux.

2.5.2. Procedure

The modified procedure was adapted from Moy et al. (2004) and started with a 10-min habituation to the testing apparatus 24 h before the test. Rats were placed in the middle part of the apparatus and allowed to explore all three chambers. In the test session, an unfamiliar rat (i.e., that had no previous contact with the tested rat) was enclosed in a cylindrical wire cage (height × diameter: 25 × 15 cm) that allowed nose contact between the bars but prevented fighting. The cage with a rat was placed in the middle of one of the outer compartments; the second outer compartment contained an empty wired cage. The tested rat was placed in the middle compartment of the apparatus and had free access to both outer compartments, i.e., the chamber with an empty wire cage (0) and the chamber with a stimulus rat (R) for 5 min. The amount of time spent in each chamber as well as the time spent sniffing (nose contact) each wire cage (empty and with a stimulus rat) was measured. The locations of both wire cages in the compartment on the left or right side of the apparatus were counterbalanced across the groups. Ten age-matched unfamiliar rats were acclimatised to the wire cage before the test, and they were alternatively used as a stimulus rat within the experiments. The behaviour of the rats was recorded by camera placed above the apparatus and connected to the Noldus MPEG. Videos were analysed manually off-line using the Noldus programme The Observer® XT, version 10.5. Based on exploration time (E) of two chambers, discrimination indexes were calculated in accordance with the equation: $DI = (E_R - E_0)/(E_R + E_0)$ and was used to measure rat's sociability. In the same way, the discrimination index was calculated on the basis of sniffing (nose contact) time with the empty cage and the rat-enclosed cage.

2.6. Drugs

Ketamine (aqueous solution (115.34 mg/ml), Vetoquinol Biowet, Gorzów Wielkoposki, Poland) was diluted in distilled water to the appropriate concentrations. SB-699551 (Tocris, Bristol, UK) was dissolved in an aqueous 10% Cremophor solution. Amisulpride (Tocris, Bristol, UK) was dissolved in distilled water with a drop of acetic acid, and the solution was neutralised with 0.1 N NaOH. Tested compounds or the vehicle (distilled water or 10% Cremophor solution) were administered at a volume of 1 ml/kg of body weight.

2.7. Drug administration

ASST. Ketamine at a dose of 10 mg/kg or vehicle was administered subcutaneously (SC) 75 min prior to the task, and SB-699551 (0, 0.3, 1 and 3 mg/kg) was administered intraperitoneally (IP) 30 min prior to the ketamine injection. The number of animals in each experimental group was N = 6. Each rat was tested only once.

NORT: ketamine-induced deficit. Ketamine, at a dose of 20 mg/kg (IP), was administered 45 min prior to the acquisition trial (T1), and SB-699551 (0, 0.3, 1 and 3 mg/kg; IP) was administered 30 min prior to the ketamine injection. The retention trial (T2) was performed 1 h after T1.

NORT: 24 h delay-induced deficit. SB-699551 (0, 0.3, 1 and 3 mg/kg; IP) was administered 30 min before T1. T2 was performed 24 h after T1. The number of animals in each experimental group was N = 9–10 (ketamine experiment) and N = 10 (24 h ITI experiment). Each rat was tested twice, with at least a 7-day washout period between each of two tests. No animal received the same treatment twice.

SIT. Ketamine, at a dose of 20 mg/kg or the vehicle was administered IP 30 min prior to the test. SB-699551 (0 and 3 mg/kg; IP) was administered 30 min prior to the ketamine injection. Both animals in a pair received the same treatment. The number of pairs of rats in each experimental group was N = 6–8. Each pair of animals was used only once.

SCT. Ketamine, at a dose of 20 mg/kg or vehicle was administered IP 30 min prior to the test. Amisulpride (0 and 3 mg/kg) or SB-699551 (0 and 3 mg/kg; IP) were administered 30 min prior to the ketamine injection. The number of animals in each experimental group was N = 5 (ketamine experiment), N = 6–8 (amisulpride experiment) and N = 7 (SB-699551 experiment). Each rat was tested twice with at least a 7-day washout period between each of the tests. No animal received the same treatment twice.

The doses of ketamine and schedules of administration, adopted from published protocols (Nikiforuk et al., 2013, 2016), have been demonstrated to produce reliable impairments in the ASST, NORT and SIT. The dose range of ketamine in a given task was adjusted in preliminary experiments to achieve distinct deficits in rat performance in the absence of confounding motivational and motor effects. The dose-range of SB-699551 was based on previous experiments in which the cognitive activity of this compound was assessed (Gonzalez et al., 2013). Amisulpride, which served as a positive control in the SCT, has been previously demonstrated to ameliorate ketamine-induced social withdrawal in the SIT (Holuj et al., 2015). Hence, this antipsychotic drug, administered at a dose of 3 mg/kg, was used to pharmacologically validate the SCT in the current study.

2.8. Statistical analysis

ASST. The number of trials required to achieve the criterion of 6 consecutive correct responses (i.e., trials to criterion, TTC) was recorded for each rat and for each discrimination phase of the ASST. The data were analysed using a three-way, mixed-design analysis of variance (ANOVA) with ketamine and SB-699551 treatment as between-subject factors and the discrimination phase as a repeated measure. For clarity of data presentation, only performance on the ED phase is shown in Fig. 1; TTC from all discrimination phases are presented in Table 1.

NORT. The data on exploratory preference were analysed using two-way, mixed-design ANOVAs with one between-subject factor, i.e., treatment, and object as a repeated measure. The DI data were analysed using one-way ANOVAs, and the distance travelled was analysed using mixed-design ANOVAs with treatment as a between-subject factor and trials as a repeated measure.

SIT. The total time of social interaction was analysed using a two-way ANOVA with two between-subject factors, ketamine and SB-

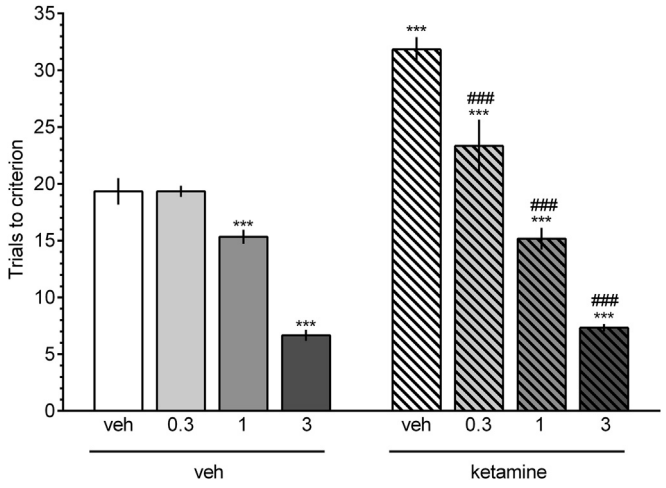

Fig. 1. The effect of SB-699551 on ketamine-induced cognitive impairment in the ED stage of the attentional set-shifting task. The results represent the means ± S.E.M. for the number of trials required to reach the criterion of 6 consecutive correct trials for the extradimensional (ED) shift phase of the ASST. N = 6 rats per group. Symbols: ***p < 0.001 vs ED performance in the vehicle-treated group; ###p < 0.001 vs ED performance in the ketamine-treated group.

699551 treatment.

SCT. The effect of ketamine on time of chamber exploration and time of cage sniffing were analysed using two-way, mixed-design ANOVAs with ketamine treatment as a between-subject factor and chamber (empty vs rat) or wired cage (empty vs rat), respectively, as a repeated measure. The effects of amisulpride or SB-699551 on ketamine-induced deficits were analysed by three-way, mixed-design ANOVAs with two between-subject factors, i.e., ketamine and the respective drug treatment, and chamber (or wired cage) as a repeated measure. The DI data were analysed using one-way ANOVAs when ketamine effects were assessed or two-way ANOVAs when the drugs' effects were evaluated in ketamine-disrupted conditions.

Post-hoc comparisons were performed using the Newman–Keuls test. The statistical analyses were performed using Statistica 10.0 for Windows. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Attentional set-shifting task (ASST)

The three-way, mixed-design ANOVA revealed significant interactions between SB-699551, ketamine and the discrimination phase: $F[18,240] = 7.42$, $p < 0.001$ (Table 1). Post-hoc analysis revealed that the acute administration of ketamine significantly and specifically impaired the performance of rats at the ED stage of the ASST, indicated by an increased number of trials to criterion during this phase (Table 1 and Fig. 1). No significant drug effect was observed during any other discrimination stage (Table 1).

The administration of SB-699551 (0.3, 1 and 3 mg/kg, Fig. 1) ameliorated ketamine-induced ED set-shifting impairment. Ketamine-treated rats co-administered with SB-699551 at a dose of 1 and 3 mg/kg also achieved the criterion during the ED stage in fewer trials compared with the vehicle-treated control group. Moreover, SB-699551 (1 and 3 mg/kg) also facilitated cognitive flexibility in vehicle-treated control rats.

3.2. Novel object recognition task (NORT)

Conditions of ketamine-induced deficits. As demonstrated in Table 2, no significant differences were observed in the time spent exploring the two identical objects in the acquisition phase (two-way ANOVA interactions: $F[4,43] = 0.56$, NS). However, post-hoc analysis after a significant treatment effect ($F[4,43] = 3.06$, $p < 0.05$) revealed that total exploration time in ketamine-treated rats that were co-administered with SB-699551 (3 mg/kg) was significantly lower compared to controls ($p < 0.05$).

A significant two-way ANOVA interaction ($F[4,43] = 20.15$, $p < 0.001$) was found for exploration time of novel and familiar object in the retention phase. As shown by post-hoc analysis, vehicle-treated, but not ketamine-treated, rats spent significantly more time exploring the novel object compared with the familiar object. Thus, the administration of ketamine abolished the ability to discriminate novel and familiar objects at T2. This ketamine-induced impairment was abolished by SB-699551 (1 and 3 mg/kg). Moreover, one-way ANOVA revealed a significant effect of SB-699551 on DI measures: $F[4,43] = 44.72$, $p < 0.001$ (Fig. 2a). Post-hoc analyses revealed that SB-699551 (1 and 3 mg/kg) significantly attenuated the ketamine-induced DI reduction. However, the DI measure in groups treated with SB-699551 at a dose of 1 mg/kg was still significantly lower compared with that of vehicle-treated rats.

No significant treatment effects were observed for the distance travelled by rats in the familiarisation and retention trials (data not shown), as revealed by the lack of significant two-way ANOVA interactions of trial and SB-699551 ($F[4,43] = 1.83$, NS).

Conditions of 24 h delay-induced deficits. As demonstrated in Table 2, no significant differences were observed in the time spent exploring the two identical objects in the acquisition phase (two-way ANOVA interactions: $F[3,36] = 0.29$, NS). A significant two-way ANOVA interaction ($F[3,36] = 15.29$, $p < 0.001$) was demonstrated for the retention phase. As shown by post-hoc analysis, the introduction of a 24-h ITI abolished the ability of control rats to

Table 1
The effect of SB-699551 on the performance of rats on the attentional set-shifting task.

Treatment (mg/kg)	Task stage (trials to criterion)						
	SD	CD	Rev 1	ID	Rev 2	ED	Rev 3
veh + veh	6.0 ± 0.0	6.3 ± 0.3	8.3 ± 1.0	6.5 ± 0.3	6.8 ± 0.5	19.3 ± 1.2	7.2 ± 0.6
SB-699551 (0.3)+veh	6.0 ± 0.0	6.3 ± 0.3	7.0 ± 0.8	6.5 ± 0.5	7.0 ± 0.6	19.3 ± 0.5	6.8 ± 0.4
SB-699551 (1)+veh	6.0 ± 0.0	6.8 ± 0.5	6.3 ± 0.3	6.7 ± 0.7	6.7 ± 0.4	15.3 ± 0.6*	6.7 ± 0.3
SB-699551 (3)+veh	6.0 ± 0.0	6.0 ± 0.0	8.2 ± 0.9	6.0 ± 0.0	6.7 ± 0.7	6.7 ± 0.5*	6.3 ± 0.2
veh + ket	6.2 ± 0.2	6.8 ± 0.5	9.7 ± 0.4	6.2 ± 0.2	6.3 ± 0.3	31.8 ± 1.1*	7.2 ± 0.7
SB-699551 (0.3)+ket	6.0 ± 0.0	6.2 ± 0.2	7.3 ± 0.8	6.2 ± 0.2	6.3 ± 0.3	23.3 ± 2.3*#	7.0 ± 0.5
SB-699551 (1)+ket	6.0 ± 0.0	6.0 ± 0.0	7.2 ± 0.7	6.8 ± 0.5	6.8 ± 0.5	15.2 ± 0.9*#	6.7 ± 0.3
SB-699551 (3)+ket	6.0 ± 0.0	6.0 ± 0.0	7.0 ± 0.5	6.9 ± 0.7	6.5 ± 0.3	7.3 ± 0.3*#	6.5 ± 0.3

The results represent the means ± S.E.M. for the number of trials required to reach the criterion of 6 consecutive correct trials for each of the discrimination phases. N = 6 rats per group. Symbols: *p < 0.001 vs ED performance in the vehicle-treated group; #p < 0.001 vs ED performance in the ketamine-treated group.

Table 2
Effects of SB-699551 on object exploration time in the novel object recognition task.

Treatment (mg/kg)	Acquisition trial (T1)		Retention trial (T2)	
	Object 1 (s)	Object 2 (s)	Familiar object (s)	Novel object (s)
Experiment 1: ketamine				
veh	11.9 ± 0.9	11.5 ± 0.9	3.5 ± 0.5	17.2 ± 1.9
veh + ket	10.3 ± 12	11.2 ± 1.3	9.5 ± 0.8	8.9 ± 0.9
SB-699551(0.3)+ket	11.6 ± 0.6	12.5 ± 1.2	7.9 ± 0.2	9.9 ± 1.2
SB-699551(1)+ket	9.7 ± 0.8	10.4 ± 0.4	3.5 ± 0.4	8.3 ± 0.9**
SB-699551(3)+ket	7.8 ± 0.7	8.8 ± 0.7	2.5 ± 0.3	11.4 ± 1.8***
Experiment 2: 24-h ITI				
veh	11.2 ± 0.9	12.1 ± 0.7	10.9 ± 10.5	10.5 ± 0.8
SB-699551(0.3)	10.3 ± 0.8	10.5 ± 0.3	5.5 ± 8.5	8.5 ± 1.18*
SB-699551(1)	11.5 ± 1.3	12.3 ± 0.8	6.4 ± 13.1	13.1 ± 1.6**
SB-699551(3)	10.6 ± 0.9	12.1 ± 1.1	3.2 ± 11.8	11.8 ± 0.9**

Data are shown as the mean ± S.E.M. Exploration time of two identical objects in the acquisition trial (T1) and of a novel and a familiar object in the retention trial (T2) conducted 1 h (experiment1) and 24 h (experiment 2) following T1. Symbols: ***p < 0.001, **p < 0.01, *p < 0.05: significant increase in time spent exploring the novel object compared with that for the familiar object.

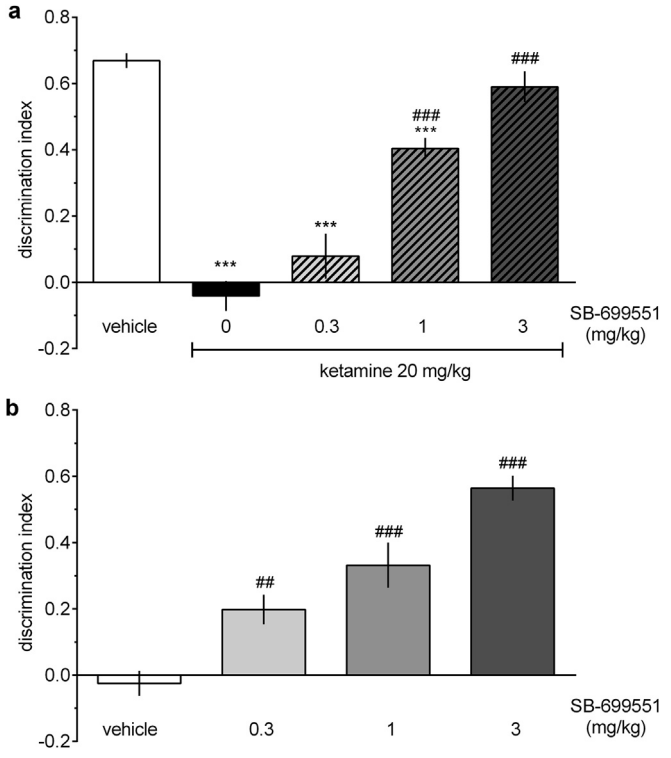

Fig. 2. The effects of SB-699551 on ketamine (a)- and delay (b)- induced cognitive impairment in the novel object recognition task. The data are shown as the mean ± S.E.M of the discrimination index (DI) in the retention trial (T2) conducted 1 h (a) or 24 h (b) following the acquisition trial (T1). N = 9–10 rats per group. Symbols: (a): ***p < 0.001, significant reduction in DI compared with the vehicle-treated group; ###p < 0.001, significant improvement in DI compared with the ketamine-treated group. (b): ##p < 0.01, ###p < 0.001, significant improvement in DI compared with the vehicle-treated group.

discriminate novel and familiar objects at T2. This delay-induced forgetting was ameliorated by administration of SB-699551 (0.3, 1 and 3 mg/kg). Moreover, a one-way ANOVA revealed a significant effect of SB-699551 on DI measures: F[3,36] = 25.74, p < 0.001 (Fig. 2b). Post-hoc analyses demonstrated that SB-699551 (0.3, 1 and 3 mg/kg) significantly increased DI compared with the controls.

No significant treatment effects were observed for the distance travelled by rats in the familiarisation and retention trials (data not shown), as revealed by the lack of significant two-way ANOVA interactions of trial and SB-699551 (F[3,36] = 0.64, NS).

3.3. Social interaction test (SIT)

As shown in Fig. 3, a two-way ANOVA revealed a significant interaction between ketamine and SB-699551 treatment (F[1,24] = 7.16, p < 0.05). Post-hoc analyses demonstrated that ketamine significantly reduced the total time of social interactions. This ketamine-induced social withdrawal was reversed after administration of SB-699551 (3 mg/kg).

3.4. Social choice test (SCT)

The effects of ketamine. Control rats spent significantly more time in the chamber containing the stimulus rat compared to the empty chamber. This preference was abolished after administration of ketamine at a dose of 20 mg/kg but not 10 mg/kg (Table 3, post-hoc tests following significant ketamine × chamber interaction: F[2,12] = 5.16, p < 0.05). Moreover, one-way ANOVA revealed a significant effect of ketamine on the DI measure: F[2,12] = 4.05, p < 0.05 (Fig. 4a). Post-hoc analyses demonstrated that ketamine (20 mg/kg) significantly decreased DI compared with the controls.

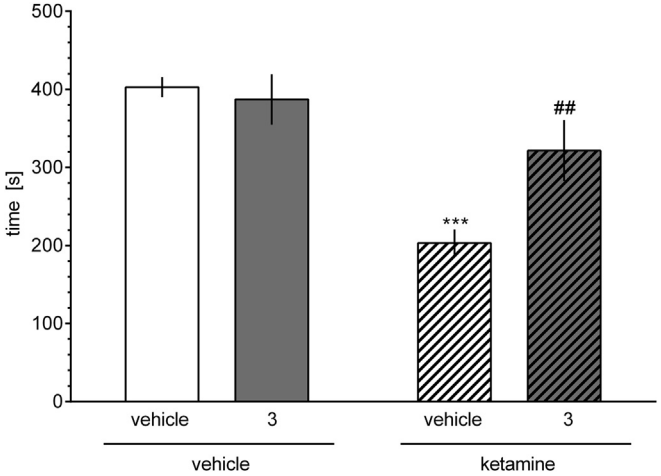

Fig. 3. The effects of SB-699551 on ketamine-induced social withdrawal in the social interaction test. The results represent the means ± S.E.M. of total time spent in active social interaction per pair of rats. N = 6–8 pairs of rats per group. Symbols: ***p < 0.001 significant reduction compared with the vehicle-treated group; ##p < 0.01 significant reversal of the ketamine-induced deficit.

Table 3
Effects of ketamine, amisulpride and SB-699551 on sociability in the social choice test.

Treatment (mg/kg)	Chamber exploration		Wired cage nose contact	
	Empty (s)	Rat (s)	Empty (s)	Rat (s)
Experiment 1: ketamine				
veh	78 ± 5	166 ± 6***	23 ± 3	83 ± 7***
ketamine (10)	81 ± 14	177 ± 12***	28 ± 7	81 ± 9***
ketamine (20)	155 ± 24	114 ± 26	47 ± 5	55 ± 13
Experiment 2: amisulpride				
veh + veh	68 ± 9	179 ± 12***	32 ± 15	92 ± 13**
amisulpride (3)+veh	76 ± 8	176 ± 9***	18 ± 3	77 ± 3**
veh + ketamine (20)	135 ± 21	133 ± 18	42 ± 5	56 ± 12
amisulpride (3)+ketamine (20)	64 ± 12	225 ± 19***	15 ± 4	93 ± 8***
Experiment 3: SB-699551				
veh + veh	77 ± 5	172 ± 9***	21 ± 3	87 ± 10***
SB-699551 (3)+veh	66 ± 13	196 ± 12***	14 ± 5	98 ± 10***
veh + ketamine (20)	140 ± 18	120 ± 15	49 ± 7	42 ± 9
SB-699551 (3)+ketamine (20)	56 ± 12	214 ± 13***	11 ± 3	74 ± 16***

Data are shown as the mean ± S.E.M. of time spent exploring empty vs rat chamber and time spent on sniffing empty vs rat-containing wired cage. N = 6–8 rats per group. Symbols: ***p < 0.001, **p < 0.01: significant increase in time spent exploring the rat chamber compared with that for the empty chamber and significant increase in time spent in nose contact with the rat cage compared with that for the empty cage.

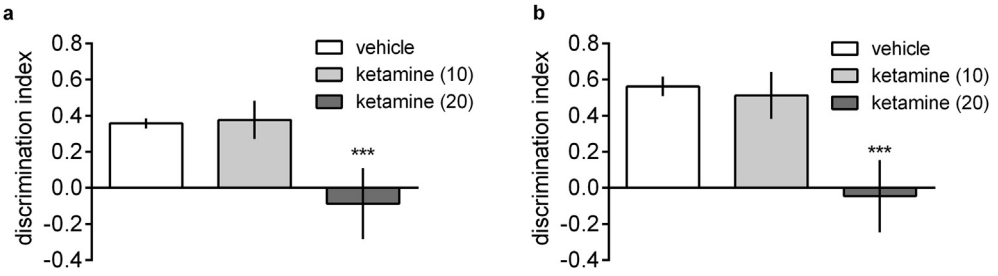

Fig. 4. The effects of ketamine in the social choice test. The data are shown as the mean ± S.E.M of discrimination index (DI) calculated for chamber exploration time (a) and for cage sniffing time (b). N = 5 rats per group. Symbols: ***p < 0.001, significant reduction in DI compared with the vehicle-treated group.

Similar results were obtained when the time of nose contact (i.e., sniffing) with the empty cage vs the stimulus rat cage was analysed (post-hoc tests following significant ketamine × cage interaction: $F[2,12] = 5.67$, $p < 0.05$). Ketamine (20 mg/kg) significantly reduced DI calculated on the basis of time of sniffing (post-hoc calculated following a significant one-way ANOVA effect: $F[2,12] = 5.67$, $p < 0.05$, Fig. 4b).

The effects of amisulpride. Amisulpride, administered at a dose of 3 mg/kg, reversed ketamine-induced deficits in sociability (Table 3). This effect was demonstrated when either time of chamber exploration (post-hoc tests following significant ketamine × amisulpride × cage interaction: $F[1,22] = 10.41$, $p < 0.01$) or time of wired cage sniffing (post-hoc tests following significant ketamine × amisulpride × cage interaction: $F[1,22] = 9.88$, $p < 0.01$) were analysed. Amisulpride reversed ketamine-evoked reduction in DI calculated for chamber exploration (post-hoc calculated following a significant ketamine × amisulpride interaction: $F[1,22] = 8.67$ $p < 0.01$, Fig. 5a) and for wired cage sniffing time (post-hoc calculated following a significant ketamine × amisulpride interaction: $F[1,22] = 8.50$, $p < 0.01$, Fig. 5b).

The effects of SB-699551. As demonstrated in Table 3, SB-699551 (3 mg/kg) significantly ameliorated the ketamine-induced reduction in social approach, as revealed by analysis of either chamber exploration times (post-hoc tests following a significant ketamine × SB-699551 × chamber interaction: $F[1,24] = 8.33$, $p < 0.01$) or cage wired sniffing times (post-hoc tests following significant ketamine × SB-699551 × cage interaction: $F[1,24] = 5.03$, $p < 0.05$). SB-699551 reversed ketamine-evoked reduction in DI calculated for chamber exploration (post-hoc calculated following a significant ketamine × SB-699551 interaction: $F[1,24] = 8.61$, $p < 0.01$, Fig. 6a) and for wired cage sniffing time (post-hoc calculated following a significant ketamine × SB-699551 interaction: $F[1,24] = 14.10$, $p < 0.001$, Fig. 6b).

4. Discussion

Our results demonstrated the pro-cognitive efficacy of the 5-HT_{5A} receptor antagonist SB-699551 in the NMDAR antagonist-based animal model of schizophrenia. Accordingly, SB-699551 reversed ketamine-induced deficits on the ASST and the NORT in rats. The cognitive-enhancing actions of SB-269970 were not restricted to the disease-like model as the compound also facilitated cognitive flexibility in cognitively unimpaired control animals and reversed a delay-induced deficit in object recognition. Moreover, SB-699551 was effective against ketamine-induced social withdrawal, a model of negative-like symptoms of schizophrenia.

The reversal of ketamine-induced NORT deficits after SB-699551 administration corroborates previous studies of Yamazaki et al. (2014) that demonstrated that other antagonists of 5-HT_{5A} receptor ASP5736 ameliorated the neonatal PCP treatment-induced impairment on that test in mice. In the same work, this compound also reversed acute dizocilpine-evoked working memory impairments in a mouse Y-maze test. Our results extended these observations by demonstrating for the first time that that the pharmacological blockade of the 5-HT_{5A} receptor is also effective in reversing ketamine-induced cognitive inflexibility as assessed in the ASST in rats. Importantly, this task measures specific frontal-dependent cognitive functions that are known to be primarily impaired in patients with schizophrenia (Keeler and Robbins,

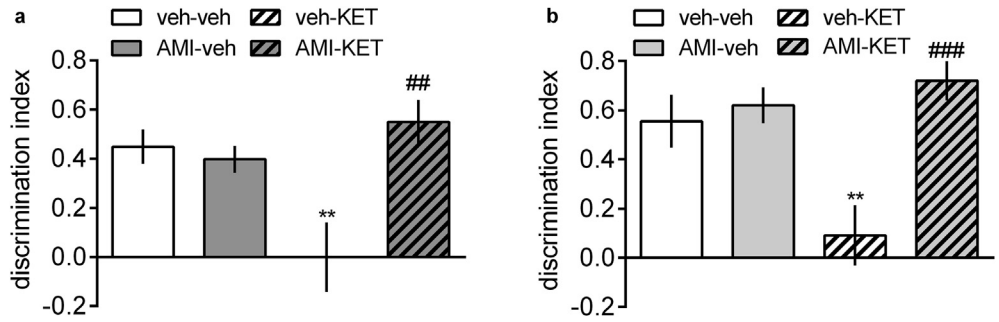

Fig. 5. The effects of amisulpride (3 mg/kg) on ketamine-induced social withdrawal in the social choice test. The data are shown as the mean ± S.E.M of the discrimination index (DI) calculated for chamber exploration time (a) and for cage sniffing time (b). N = 6–8 rats per group. Symbols: (a): $^{**}p < 0.01$, significant reduction in DI compared with the vehicle-treated group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, significant improvement in DI compared with the ketamine-treated group.

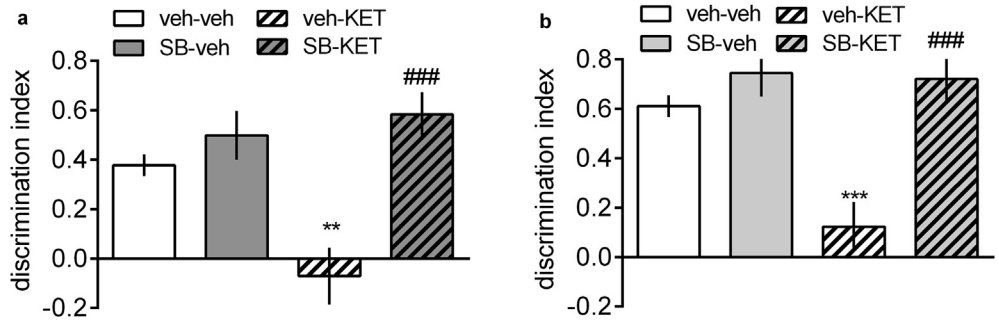

Fig. 6. The effects of SB-699551 (3 mg/kg) on ketamine-induced social withdrawal in the social choice test. The data are shown as the mean ± S.E.M of the discrimination index (DI) calculated for chamber exploration time (a) and for cage sniffing time (b). N = 7 rats per group. Symbols: (a): $^{**}p < 0.01$, $^{***}p < 0.001$, significant reduction in DI compared with the vehicle-treated group; $^{\#\#\#}p < 0.001$, significant improvement in DI compared with the ketamine-treated group.

2011). Moreover, this paradigm has been shown translational value in predicting pro-cognitive activity in schizophrenia (Goetghebeur and Dias, 2014). Thus, present results together with previous findings (Yamazaki et al., 2014) suggest that strategies that are based on the blockade of 5-HT_{5A} receptors may represent a therapeutic approach for cognitive enhancement in schizophrenia.

Nevertheless, the pro-cognitive efficacy of 5-HT_{5A} receptor antagonists may not be restricted to schizophrenia-like conditions, as this strategy has been suggested to also have utility in age-related dementia or Alzheimer's disease (AD). Indeed, ASP5736 and two related compounds (AS2030680 and AS2674723) were recently demonstrated to improve the performance of aged rats in the Morris water maze and to reverse spontaneous alternation deficits that were induced by scopolamine, a pharmacological model of the cholinergic deficits known to be associated with AD (Yamazaki et al., 2015).

Our results demonstrated for the first time that blockade of 5-HT_{5A} receptors may also facilitate cognitive performance in cognitively unimpaired control animals. Accordingly, administration of SB-699551 enhanced set-shifting performance compared to the vehicle-treated controls. The compound also reversed natural forgetting induced by an introduction of a 24-h ITI in the NORT. The role of 5-HT_{5A} receptors in the regulation of cognitive processes under physiological conditions has not been systematically explored. An early study with knock-out mice demonstrated that genetic deletion of 5-HT_{5A} receptors resulted in increased tendency to explore the novel object (Grailhe et al., 1999). This effect was not secondary to the decrease in fear of novelty, as these mice displayed no changes in anxiety-related behaviour. In the present study, however, administration of SB-699551 (3 mg/kg) resulted in a decrease in the time spent on object exploration in the acquisition trial. However, this reduction was observed only in ketamine- but not in vehicle-treated rats and, therefore, may represent the consequence of ketamine and SB-699551 interactions rather than the compound's effect *per se*. Nevertheless, if 5-HT_{5A} receptor blockade-induced enhancement of novelty exploration accounts for the successful novel object recognition in the present study remains only speculative.

Only one study assessed the effects of the pharmacological blockade of 5-HT_{5A} receptors in cognitively unimpaired rats. Using an autoshaping Pavlovian instrumental learning task, Gonzalez et al. (2013), demonstrated that SB-699551, administered at a dose range similar to the this used in the present study of 0.3–3 mg/kg, impaired short-term and long-term memory. Although these results are in contrast to the present demonstration of the cognitive-enhancing effects of SB-699551, the explanation of this discrepancy is currently unavailable. One may assume that the 5-HT_{5A} receptor plays diverse roles in regulation of associative learning and other cognitive processes. The different nature of behavioural memory tasks may also likely account for these discrepancies. For example, it cannot also be excluded the increasing the task complexity (e.g., ASST) allows for revealing the procognitive effects of 5-HT_{5A} receptor blockade. Alternatively, a recently suggested interplay between 5-HT_{5A} and 5-HT_{1A} receptors (Goodfellow et al., 2012) may also plausibly account for these effects. Notably, similar apparently contradictory findings regarding cognitive processes have been reported for other 5-HT receptors (reviewed in (Meneses, 2013)). The physiological role for the 5-HT_{5A} receptors in the regulation of learning and memory processes apparently awaits a further study.

Finally, the present study demonstrated that pharmacological blockade of 5-HT_{5A} receptors ameliorated negative-like symptoms

of schizophrenia as assessed in a model of NMDAR antagonist-induced social withdrawal. Accordingly, SB-699551 reversed ketamine-induced deficits in social interactions. The SIT is commonly used to model negative-like symptoms of schizophrenia (reviewed in (Gururajan et al., 2010; Neill et al., 2014; Wilson and Koenig, 2014)) and the procedure based on ketamine administration was pharmacologically validated and extensively discussed in our previous study (Holuj et al., 2015). To elucidate the pro-social action of SB-699551, a social choice paradigm was used. As compared to the standard social interaction, the social choice test allows for the control of non-social factors (reviewed in (Millan and Bales, 2013)). For example, social and non-social objects (i.e., the empty cage vs the stimulus rat cage) are matched by their physical features, the chamber conditions are constant and the location of a stimulus cage may be randomized. The tested animal has a choice not to interact socially when the stimulus animal is restrained, and thereby the active influence of the social object is minimized. Moreover, factors such as social motivation, emotional status and motor drive can be more easily distinguished than using the traditional SIT.

The measurement of sociability together with preference for social novelty was originally developed as a method to assess tendencies for autistic-like behaviours in mouse models (Moy et al., 2004). Nevertheless, deficits in sociability have also been observed in genetic mouse models of schizophrenia (Halene et al., 2009; Li et al., 2007). More recently, McKibben et al. (2014) employed this test to assess social withdrawal in an NMDAR antagonist-based model of schizophrenia. In this study, acute administration of PCP produced a reduction in measures of sociability. Our results corroborated this finding by also demonstrating that acute administration of another NMDAR antagonist ketamine evoked asociality on this task. Importantly, an antipsychotic medication, amisulpride reversed the ketamine-induced loss of preference for spending time with a conspecific as opposed to an empty chamber. These results agree with our previous study (Holuj et al., 2015), in which amisulpride also effectively counteracted the disruptive effects of ketamine on rats' social interactions. In line with preclinical data, the efficacy of amisulpride in the treatment of negative symptoms in patients with schizophrenia was demonstrated (Danion et al., 1999; Leucht et al., 2002; Moller, 2001). Thus, the efficacy of amisulpride in reversing the ketamine-induced SCT deficits may support the predictive validity of the applied model in finding novel compounds that ameliorate social withdrawal.

SB-699551 also reversed ketamine-induced asociality on the SCT. Notably, both measures of sociability, i.e., time of chamber exploration as well as time spend in nose contact with the wire cage, were equally affected. This finding supports the social nature of the observed effects. One may argue that potential ketamine-induced changes in motor activity are a confounding factor in measures such as both the SIT and SCT. However, our previous experiment demonstrated that ketamine did not affect locomotor activity at a time point corresponding to the behavioural assessment (Holuj et al., 2015).

It should be noted that SB-699551 also displays affinity for the rat serotonin transporter (Thomas et al., 2006). Interestingly, the selective serotonin reuptake inhibitor fluoxetine reversed social withdrawal in rats that were subchronically treated with PCP (Snigdha and Neill, 2008). On the contrary, fluoxetine did not affect the ketamine-induced disruption of social behaviour in the current experimental setup (Holuj et al., 2015). Similarly, Sams-Dodd (1998) reported that citalopram given in combination with PCP did not reverse the PCP-induced social interaction deficit. Thus, it seems unlikely that the effectiveness of SB-269970 in the present study resulted from the inhibition of serotonin reuptake.

While the precise mechanisms underlying the beneficial effects of antagonists of 5-HT_{5A} receptors are unknown, experimental data suggest some neurochemical mechanisms that could hypothetically account for these effects. In microdialysis in vivo experiments, for example, 5HT5A receptor antagonists, A763079, A843277 and A83355, induced a dose-dependent increase in acetylcholine levels in the rat medial prefrontal cortex (Drescher et al., 2006). It has been widely accepted that enhancement of cholinergic transmission may represent a useful strategy for cognitive enhancement in various conditions, including schizophrenia (Jones et al., 2012). Accordingly, our previous studies demonstrated that administration of either an acetylcholinesterase inhibitor galantamine or selective activators of alpha 7 nicotinic acetylcholine receptors (α7-nAchR) reversed ketamine-evoked impairments on the ASST and NORT as well as exerted pro-cognitive effects in control rats on these tasks (Nikiforuk et al., 2015, 2016). The α7-nAchR agents also reversed ketamine-induced social withdrawal (Nikiforuk et al., 2016). Thus, the beneficial action of SB-699551 in the present experiments may be presumably explained by an increase in prefrontal acetylcholine release. Alternative mechanisms of the pro-cognitive effects of 5-HT_{5A} receptor antagonists that has been proposed by Yamazaki et al. (2014), and assumes that the blockade of inhibitory 5-HT_{5A} receptors in the ventral tegmental area may lead to activation of dopamine neurons in the medial prefrontal cortex.

In summary, the present study demonstrated that the 5-HT_{5A} receptor antagonist, SB-699551 reversed ketamine-induced cognitive deficits and social withdrawal in rats. We suggest that the strategy based on the blockade of 5-HT_{5A} receptors may be a promising approach in treating cognitive deficits and negative symptoms of schizophrenia.

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