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ORIGINAL INVESTIGATION

SSR504734 enhances basal expression of prepulse inhibition but exacerbates the disruption of prepulse inhibition by apomorphine

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Abstract
Rationale Inhibition of glycine transporter 1 (GlyT1) elevates extracellular glycine and can thus increase *N*-methyl-D-aspartate receptor (NMDAR) excitability in the brain. The potent GlyT1 inhibitor, SSR504734, has also been shown to potentiate the behavioral effects of direct and indirect dopamine agonists. Thus, an acute systemic dose of SSR504734 was sufficient to exacerbate the motor-stimulant effect of the dopamine releaser amphetamine in C57BL/6 mice, even though SSR504734 alone exerted no significant effect on motor activity.
Objectives Here, we explore if SSR504734 might modulate dopamine-dependent sensory gating in the paradigm of prepulse inhibition (PPI) of the acoustic startle reflex.
Methods Experiment 1 characterized the effect of SSR504734 (10 and 30 mg/kg i.p.) on PPI expression when administered alone. Experiments 2 and 3 investigated the impact of SSR504734 when administered in conjunction with the dopamine receptor agonist, apomorphine (1 and 2 mg/kg s.c.), which is known to reliably disrupt PPI.
Results When administered alone, acute SSR504734 enhanced PPI only at 30 mg/kg—a dose that has been shown to improve cognitive functions including working memory, which has been linked to enhanced NMDAR function resulting from the elevation of extracellular glycine. However, this effect did not allow SSR504734 to antagonize the PPI-disruptive effect of apomorphine. At the lower dose of 10 mg/kg—that was insufficient to enhance PPI when administered alone—SSR504734 even exacerbated the deleterious effect of apomorphine on PPI.
Conclusions The therapeutic potential of GlyT1 inhibition against distinct behavioral/cognitive deficiency might require different magnitudes of GlyT1 inhibition.

Keywords Dopamine · Glutamate · Glycine transporter 1 · Positive symptoms · Schizophrenia · Sensorimotor gating

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Introduction

There is ample evidence that hypofunction of the *N*-methyl-D-aspartate receptor (NMDAR) contributes to the pathophysiology of schizophrenia, in particular to the genesis of the negative and cognitive symptoms of the disease that cannot be effectively treated with existing pharmacotherapy (Javitt 2010). New treatment strategies therefore aim to enhance NMDAR activity (Javitt 2009). To date, the glycine co-agonist site of the NMDAR complex (glycine-B site) is a new prime target of investigation (Wallace et al. 2011). However, despite some beneficial effects when added to conventional antipsychotics (Javitt 2010), the clinical use of glycine itself is limited due to poor bioavailability and brain penetration (Wallace et al. 2011). Extracellular glycine concentration in glutamatergic synapses is tightly controlled by the action of glycine transporter 1 (GlyT1), which prevents the saturation of the glycine-B site under physiological conditions (Berger et al. 1998; Smith et al. 1992). Inhibition of GlyT1 can effectively enhance NMDAR-dependent signaling through increasing synaptic glycine levels—a strategy that has been recognized as promising in the treatment of negative and

cognitive symptoms of schizophrenia (e.g., Javitt 2008; Wallace et al. 2011). Several synthetic GlyT1 inhibitors have been developed and are being evaluated clinically (Javitt 2009; Wallace et al. 2011).

Amongst the newly available GlyT1 inhibitors, we have shown that the compound SSR504734 (2-chloro-3-(trifluoromethyl)-*N*-((S)-phenyl((S)-piperidin-2-yl)methyl) benzamide hydrochloride) reversed NMDAR antagonist-induced hyperactivity and enhanced working memory in C57BL/6 mice, supporting its antipsychotic and procognitive potential (Singer et al. 2009b,c). However, SSR504734 at the same time enhanced the motor-stimulant effect of the dopamine releasing psychomimetic drug, amphetamine (Singer et al. 2009b). This effect may be mechanistically linked to a stimulation of the mesolimbic dopamine system, as suggested by the finding that SSR504734 facilitates electrically evoked dopamine release in the nucleus accumbens (NAC) (Leonetti et al. 2006). While pro-dopaminergic interventions have been reported to improve negative and cognitive symptoms in medicated schizophrenia patients (e.g., Barch and Carter 2005; Kirrane et al. 2000; Sanfilipo et al. 1996), concerns over a potential worsening of the acute psychotic (positive) symptoms, resulting from excessive subcortical dopaminergic activity (Carlsson 1988; Davis et al. 1991), cannot be ignored. To further address this issue, the present study evaluated the effect of SSR504734 on prepulse inhibition (PPI) of the acoustic startle reflex—one of the most widely used translational paradigms in schizophrenia research (Geyer and Moghaddam 2002). PPI is a cross-species measure of sensorimotor gating, defined as the attenuation of the startle response to an intense auditory pulse stimulus, when the stimulus is shortly preceded by a weak, nonstartling prepulse stimulus (Graham 1975). PPI is often attenuated in schizophrenia patients (e.g., Braff et al. 2001), and apomorphine-induced PPI disruption is a well-established animal model of the positive symptoms linked to hyperdopaminergia (for reviews, see Geyer et al. 2001; Swerdlow et al. 2008). Here, experiment 1 examined the effect of SSR504734 on PPI expression in normal C57BL/6 mice when administered alone, up to a dose of 30 mg/kg intraperitoneal (i.p.), which has been consistently shown to be behaviorally effective (Singer et al. 2009b, c). Experiments 2 and 3 went on to test whether SSR504734 pretreatment would interfere with the PPI-disruptive effect of acute apomorphine.

Methods

Subjects

Subjects were naïve adult male C57BL/6NCrl mice obtained from our in-house pathogen-free breeding colony (ETH Zurich Laboratory of Behavioral Neurobiology, Schwerzenbach, Switzerland). The offspring were weaned at postnatal day 21 and then kept in groups of 3–5 littermates in Makrolon® Type-III cages (Techniplast, Milan, Italy). The animals were maintained in a temperature (~21 °C) and humidity (~55 %) controlled vivarium under a 12:12 h reversed light–dark cycle (lights on at 1900 h) with ad libitum access to food and water. Behavioral testing took place in the dark phase of the circadian cycle, and commenced when the animals were 12 weeks old. All procedures described here were approved by the Cantonal Veterinary Office of Zurich and conformed to the ethical standards stipulated in the Swiss Federal Act on Animal Protection (1978) and Swiss Animal Protection Ordinance (1981) in accordance with the European Council Directive 86/609/EEC (1986). All efforts had been made to alleviate animal suffering and minimize the number of animals used.

Drugs

All administered solutions were freshly prepared on the day of testing. SSR504734 (gift from Sanofi-Aventis, Paris, France) was suspended in distilled water containing 5 % Tween 80 to obtain the appropriate concentrations to achieve the dosages of 10 and 30 mg/kg based on an injection volume of 10 ml/kg via the i.p. route. Apomorphine HCl (obtained from Sigma-Aldrich, Germany) was dissolved in 1 % sterile ascorbic acid (pH 3.2) to achieve the desired dosage of 1 or 2 mg/kg at an injection volume of 10 ml/kg via the subcutaneous (s.c.) route. The pH was adjusted to 5.5, with the addition of solid Na_2CO_3. SSR504734 and apomorphine (or their corresponding vehicle solutions) were administered 30 and 5 min prior to testing, respectively.

Prepulse inhibition

A full description of the apparatus and procedures is provided elsewhere (Yee et al. 2004a, b). In brief, four startle chambers for mice (SR-LAB, San Diego Instruments, San Diego, CA, USA) were used to measure PPI. All auditory stimuli were in the form of white noise. A test session began with a 2-min acclimatization period, followed by the presentation of six pulse-alone trials to habituate and stabilize the animals' startle response. The pulse stimulus was 120 dB_A in intensity and 40 ms in duration. Next, 12 blocks of discrete test trials were presented to assess PPI. Each block consisted of one trial of the following trial types: pulse-alone, prepulse-alone of each of five possible prepulse intensities (69, 73, 77, 81, and 85 dB_A), prepulse-plus-pulse of each of the five levels of prepulse, and no-stimulus (i.e., background noise alone). The 12 discrete trials within each block were presented in a pseudorandom order, with a variable intertrial interval averaging 15 s (ranging from 10 to 20 s). The

duration of the prepulse stimuli was 20 ms. All trials were presented against a constant level of background noise (65 dB_A). In prepulse-plus-pulse trials, the stimulus onset asynchrony between the two stimuli was 100 ms. Whole body acceleration on each trial was measured by a piezoelectric accelerometer within a 65-ms response window (from the onset of the pulse in pulse-alone and prepulse-plus-pulse trials or the onset of the prepulse on prepulse-alone trials). This output (in arbitrary units) was referred to as reactivity score. Because the distribution of the reactivity scores is highly positively skewed, we followed Csomor et al.'s (2008b) recommendations to perform a logarithmic transformation [*ln* (reactivity score+*e*) −1] prior to statistical analysis, in order to enhance data distribution and variance homogeneity. Pulse-alone trials and prepulse-alone trials (including no-stimulus trials) were separately analyzed to measure baseline startle reaction and prepulse-elicited reactivity, respectively.

PPI was specifically indexed by percent inhibition (% PPI), defined as the relative reduction in startle reaction on prepulse-plus-pulse trials relative to pulse-alone trials and calculated at each prepulse intensity as follows: [1−(reaction in prepulse-plus-pulse trials)/(reaction in pulse-alone trials)]×100 %. This calculation of % PPI was based on the untransformed reactivity scores according to convention.

Statistical analyses

All data were subjected to parametric analysis of variance (ANOVA) with the between-subject factor drug treatment and additional within-subject factors depending on the nature of the dependent variables. Statistically significant outcomes were verified by pairwise comparisons based on appropriate error variance obtained from the overall ANOVA (Fisher's least significant difference test). Reported *p* values were not adjusted for perceived inflation of familywise Type I error rate because the post hoc comparisons were confirmatory rather than exploratory in nature. All statistical analyses were carried out using IBM SPSS statistics (version 18, SPSS Inc. Chicago, IL, USA). A two-tailed criterion of $p<0.05$ was taken as the yardstick for statistical significance. All data illustrated in tables or figures refer to mean values± standard error.

Results

SSR504734 enhances PPI expression at 30 mg/kg but not at 10 mg/kg

PPI was observed in all groups, but the PPI magnitude as indexed by % PPI was larger in the SSR 30 mg condition, and this effect was more pronounced at high prepulse intensities (Fig. 1). By contrast, the expression of PPI did not substantially differ between the SSR 10 mg/kg condition and vehicle control except at the prepulse intensity of 77 dB (with background noise maintained at 65 dB), when % PPI was reduced in the SSR 10 mg condition. These interpretations were supported by a 3×5 (drug×prepulse intensity) ANOVA of % PPI, which yielded a significant effect of drug ($F(2, 35)=3.28$, $p<0.05$) and prepulse intensity ($F(4, 140)=58.84$, $p<0.001$) and their interaction ($F(8, 140)=4.42$, $p<0.001$). Pairwise post hoc comparisons revealed that the overall magnitude of % PPI significantly differed between the SSR 10 mg and SSR 30 mg groups ($p=0.02$), while both drug conditions did not significantly differ from the overall expression of PPI in vehicle controls. Closer examination of the two-way interaction provided clearer statistical evidence for enhanced PPI expression in the SSR 30 mg condition compared with controls at prepulse intensities of 81 and 88 dB ($p<0.05$). In addition, the comparison between SSR 10 mg and vehicle conditions at the 77 dB prepulse condition yielded a significant difference ($p<0.05$). We had subsequently addressed this unique effect of SSR504734 at 10 mg/kg in the same cohort of mice following a 2-week wash out period and drug experience fully counterbalanced but failed to observe the same effect. Results from this supplementary experiment (data not shown) confirm that SSR504734 at 10 mg/kg exerted minimal effect on the expression of PPI.

Finally, analysis of reactivity (logarithmically transformed) obtained on pulse-alone or prepulse-alone (including no-stimulus trials) trials did not yield any significant group differences (Fig. 1).

SSR504734 modulates the disruption of PPI induced by apomorphine

We have previously shown that SSR504734 (30 mg/kg, i.p.) enhanced the motor-depressant effect of apomorphine (0.75-mg/kg, s.c.) in C57BL/6 mice (Singer et al. 2009b). Here, we tested whether SSR504734 (10 or 30 mg/kg) might similarly exacerbate the PPI disruptive effect of apomorphine. A relatively low dose of apomorphine (1 mg/kg) was selected to avoid a potential floor effect that might prevent further attenuation of PPI. As illustrated in Fig. 2, the PPI-disruptive effect of apomorphine was relatively weak, while combining 10 mg/kg SSR504734 with apomorphine produced a visibly stronger disruption than apomorphine alone, which was most evident at low prepulse intensities. By contrast, 30 mg/kg SSR504734 did not substantially modify the PPI-disruptive effect of apomorphine.

Analysis of % PPI by a 4×5 (drug×prepulse intensity) repeated measures ANOVA yielded a significant effect of drug ($F(3, 36)=3.16$, $p<0.05$) and prepulse intensity ($F(4, 144)=77.61$, $p<0.001$) and a significant drug×prepulse intensity interaction ($F(12, 144)=2.56$, $p<0.05$). Post hoc

	Vehicle	SSR504734	
		10 mg/kg	30 mg/kg
	(n=13)	(n=13)	(n=12)
Pulse-alone trials (120dB$_A$)	4.41 ± 0.16	4.62 ± 0.16	4.94 ± 0.17
Prepulse-alone trials:			
No-stimulus*	2.37 ± 0.11	2.43 ± 0.11	2.21 ± 0.11
69 dB$_A$	2.38 ± 0.10	2.47 ± 0.10	2.22 ± 0.10
73 dB$_A$	2.45 ± 0.11	2.49 ± 0.11	2.32 ± 0.11
77 dB$_A$	2.49 ± 0.10	2.48 ± 0.10	2.34 ± 0.10
81 dB$_A$	2.50 ± 0.10	2.52 ± 0.10	2.54 ± 0.10
88 dB$_A$	2.62 ± 0.09	2.74 ± 0.09	2.65 ± 0.09

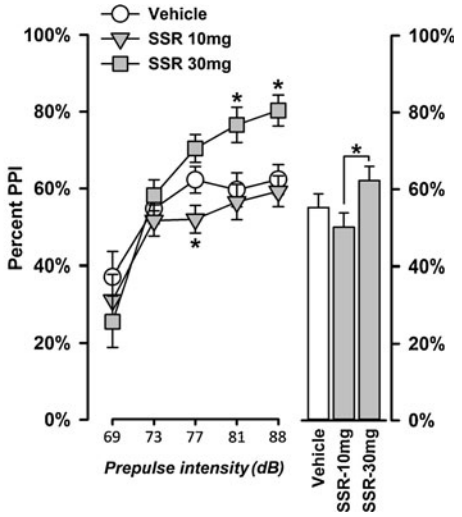

Fig. 1 Effect of SSR504734 (10 and 30 mg/kg) on PPI. 30 mg/kg SSR504734 (*SSR*) enhanced PPI as a function of increasing prepulse intensity. 10 mg/kg SSR had little effect on PPI except at a prepulse intensity of 77 dB$_A$ (with background noise maintained at 65 dB$_A$), when PPI levels were lower than in vehicle-treated animals. *Asterisk* indicates significant difference at $p<0.05$ based on Fisher's LSD post hoc pairwise comparisons. The table on the left shows the mean reactivity obtained on pulse-alone, prepulse-alone, and no-stimulus trials (in arbitrary units, after logarithmic transformation). "*No-stimulus**" refers to trials in which no discrete stimulus was presented except the background noise

comparisons indicated that PPI was significantly reduced by 10 mg/kg of SSR504734 in combination with apomorphine relative to vehicle control ($p<0.05$), while the disruption induced by apomorphine alone was weak as expected, yielding only a nonsignificant trend ($p=0.09$). However, the overall expression of PPI (averaged across all prepulse intensity) did not differ significantly between the Veh–APO and the SSR 10 mg-APO condition ($p>0.18$).

The significant two-way interaction was further investigated by analyzing each prepulse level using the error term associated with the interaction effect. At a prepulse intensity of 73 dB, combined treatment of 10 mg/kg SSR504734 and apomorphine produced a significantly stronger decrease of % PPI compared with apomorphine alone ($p<0.05$), when apomorphine alone was also sufficient to significantly reduce PPI expression relative to vehicle control ($p<0.05$). These results support our impression that SSR504734 at the specific dose of 10 mg/kg exacerbated the PPI-disruptive effect of apomorphine, and the outcomes cannot be attributed to any confounding effects on startle reaction or the perception of the prepulse stimulus because no drug effect was obtained in the reactivity obtained on pulse-alone trials or prepulse-alone trials (Fig. 2). The functional interaction between SSR504734 and apomorphine revealed here was further evaluated in the next experiment.

Exacerbation of the PPI-disruptive effect of apomorphine by SSR504734 at 10 mg/kg

To further ascertain the ability of SSR504734, at 10 mg/kg, to exacerbate the PPI-disruptive effect of apomorphine, we performed another experiment of the same design with a higher dose of apomorphine (2 mg/kg, s.c.) that typically leads to a more substantial PPI disruption in C57BL/6 mice (e.g., Russig et al. 2004; Yee et al. 2004b).

As expected, 2 mg/kg apomorphine produced a more pronounced disruption of PPI across all prepulse intensities relative to vehicle control (Fig. 3). Co-treatment of 10 mg/kg SSR504734 and apomorphine led to a much stronger reduction in PPI compared with apomorphine alone. These two effects led to the emergence of a highly significant main effect of drug ($F(2, 20)=14.08$, $p<0.001$) in the 3×5 (drug×prepulse intensity) ANOVA of % PPI. Post hoc pairwise comparison confirmed that all three groups substantially differed from each other (Veh–Veh vs. Veh–APO: $p<0.05$; Veh–Veh vs. SSR 10 mg-APO: $p<0.001$; Veh–APO vs. SSR 10 mg-APO: $p<0.005$). As expected, the analysis also yielded a significant effect of prepulse intensity ($F(5, 100)=11.95$, $p<0.001$), reflecting the fact that a monotonic increase of % PPI as a function of prepulse intensity was consistently observed in all treatment groups.

Again, our finding that 10 mg/kg SSR504734 exacerbated the PPI-disruptive effect of apomorphine was not confounded by any significant treatment effects on the reaction to pulse-alone stimuli or prepulse-alone stimuli (Fig. 3).

Discussion

The present study demonstrated that the GlyT1 inhibitor, SSR504734, enhanced the magnitude of PPI expression at basal level when given at a dose of 30 mg/kg, but failed to

	Vehicle	1 mg/kg s.c. Apomorphine		
		0 mg/kg SSR	10 mg/kg SSR	30 mg/kg SSR
	(n=10)	(n=10)	(n=10)	(n=10)
Pulse-alone trials (120dB$_A$)	4.92 ± 0.16	4.80 ± 0.16	4.85 ± 0.16	4.66 ± 0.16
Prepulse-alone trials:				
No-stimulus*	2.11 ± 0.16	2.31 ± 0.16	2.43 ± 0.16	2.14 ± 0.16
69 dB$_A$	2.16 ± 0.15	2.28 ± 0.15	2.41 ± 0.15	2.18 ± 0.15
73 dB$_A$	2.28 ± 0.14	2.46 ± 0.14	2.51 ± 0.14	2.31 ± 0.14
77 dB$_A$	2.42 ± 0.13	2.45 ± 0.13	2.53 ± 0.13	2.39 ± 0.13
81 dB$_A$	2.38 ± 0.13	2.53 ± 0.13	2.60 ± 0.13	2.47 ± 0.13
88 dB$_A$	2.52 ± 0.13	2.62 ± 0.13	2.51 ± 0.13	2.42 ± 0.13

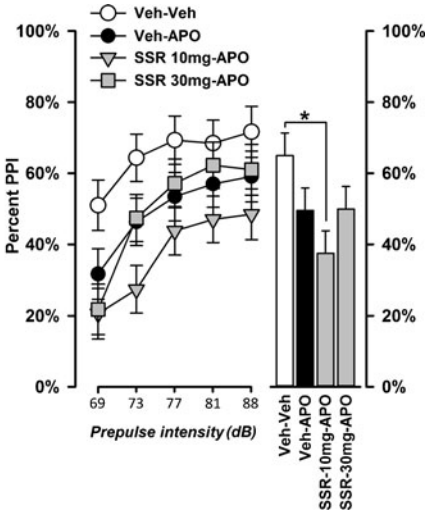

Fig. 2 Effect of SSR504734 (10 and 30 mg/kg) on apomorphine-induced PPI disruption. The co-administration of 10 mg/kg SSR504734 (*SSR*) with 1 mg/kg apomorphine (*APO*) significantly reduced PPI, while apomorphine alone led only to a moderate nonsignificant reduction compared with vehicle control. 30 mg/kg SSR504734 did not affect the PPI-disruptive effect of apomorphine. *Asterisk* indicates significant difference at $p<0.05$ based on Fisher's LSD post hoc comparisons. The table on the left shows the mean reactivity obtained on pulse-alone, prepulse-alone, and no-stimulus trials (in arbitrary units, after logarithmic transformation). "*No-stimulus**" refers to trials in which no discrete stimulus except the background noise (65 dB$_A$) was presented

antagonize the PPI-disruptive effect induced by the direct dopamine receptor agonist, apomorphine. Instead, when the drug was administered at the lower dose of 10 mg/kg, it exacerbated the negative impact of apomorphine on PPI. The drug's impact on PPI expression is unlikely attributable to any confounding effect on the generation of the whole-body startle response or the detection of the prepulse because neither the magnitude of the startle reaction nor the direct reaction recorded on prepulse-alone trials was significantly altered. Thus, SSR504734 seemingly modified sensorimotor gating underlying the interference of prepulse perception on pulse stimulus processing in a dose-dependent manner.

The dual effects of SSR504734 on prepulse inhibition

The enhancement of basal PPI was only evident with 30 mg/kg SSR504734—the same dose has been reported to robustly

	Vehicle	2 mg/kg s.c. Apomorphine	
		0 mg/kg SSR	10 mg/kg SSR
	(n=7)	(n=8)	(n=8)
Pulse-alone trials (120dB$_A$)	4.63 ± 0.23	4.23 ± 0.21	4.47 ± 0.21
Prepulse-alone trials:			
No-stimulus*	2.48 ± 0.11	2.67 ± 0.10	2.65 ± 0.11
69 dB$_A$	2.48 ± 0.09	2.60 ± 0.09	2.64 ± 0.09
73 dB$_A$	2.47 ± 0.12	2.71 ± 0.11	2.65 ± 0.12
77 dB$_A$	2.48 ± 0.12	2.80 ± 0.12	2.68 ± 0.12
81 dB$_A$	2.56 ± 0.10	2.81 ± 0.10	2.67 ± 0.10
88 dB$_A$	2.70 ± 0.12	2.95 ± 0.11	2.74 ± 0.12

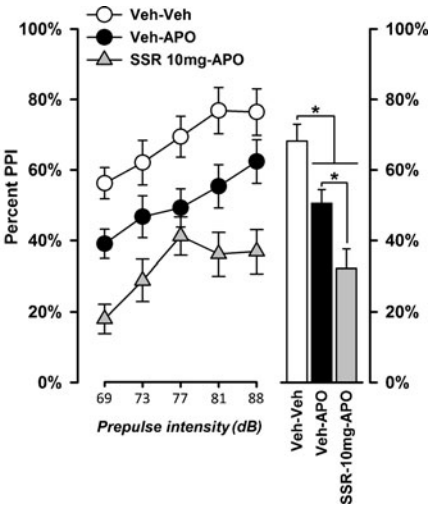

Fig. 3 SSR504734 (*SSR*) at 10 mg/kg exacerbated the significant disruption of PPI induced by 2 mg/kg apomorphine (*APO*). 2 mg/kg apomorphine (Veh-APO group) significantly disrupted PPI, and SSR pretreatment at 10 mg/kg led to a more severe disruption (SSR 10 mg/kg-APO group). *Asterisk* indicates significant difference at $p<0.05$ based on Fisher's LSD post hoc comparisons. The table on the left shows the mean reactivity obtained on pulse-alone, prepulse-alone, and no-stimulus trials (in arbitrary units, after logarithmic transformation). "*No-stimulus**" refers to trials in which no discrete stimulus except the background noise (65 dB$_A$) was presented

enhance working memory performance in normal C57BL/6 mice (Singer et al. 2009c). This coincidence may lead one to suspect that these two effects share similar neural mechanisms. This speculation is in line with reports of positive correlation between PPI and working memory performance in healthy subjects (Csomor et al. 2008a; Holstein et al. 2011; Singer et al. 2013), indicating that individual differences in these two tests are present in the absence of any drug manipulation. Hence, even if change in PPI performance as such is not directly responsible for specific cognitive function like working memory, it is nonetheless an instructive behavioral marker predictive of potential drug effect on cognitive performance (see Geyer 2006). Our results obtained with SSR504734 support this view.

At 10 mg/kg, PPI was, if anything, slightly reduced, albeit non-significantly so. Closer examination showed that the PPI-enhancing effect was the strongest at the highest prepulse intensity. If the potentiation of PPI were simply due to enhanced signal (prepulse) detection, it would have been the clearest at low (rather than high) prepulse intensity, when signal-to-noise ratio was low. The fact that the drug did not alter the magnitude of prepulse-elicited reaction provides further evidence against a sole effect on signal detection. Notwithstanding, this is the first report of a PPI potentiation by a GlyT1 inhibitor in a mouse strain other than DBA/J2—an inbred mouse strain with characteristically weak PPI expression (e.g., Bullock et al. 1997; Olivier et al. 2001). Although recent attempts in outbred CF-1 and Black Swiss mice have failed to identify a PPI-enhancing effect of SSR504734 (Flood et al. 2011), our results show that SSR504734's ability to enhance PPI is not unique to the DBA/J2 genetic background (also see Singer et al. 2009a). However, the PPI-enhancing effect by SSR504734 does not seem to be readily generalized to other GlyT1 inhibitors. For instance, Lipina et al. (2005) reported a dose-dependent PPI disruption (10 and 15 mg/kg) by the compound ALX 5407 administered to C57BL/6 mice. The divergent effects on PPI between SSR504734 and ALX 5407 might point to the relevance of their differing mode of pharmacological action: While ALX 5407 is a sarcosine-based noncompetitive GlyT1 inhibitor, which binds irreversibly to GlyT1, SSR504734—a piperidinebenzamide derivative—binds reversibly to GlyT1 as a competitive inhibitor (Atkinson et al. 2001; Depoortère et al. 2005; Mezler et al. 2008). The functional significance of this pharmacokinetic distinction certainly warrants further systematic investigation. Furthermore, apparently unique to ALX5407 is its potential to act like a NMDAR antagonist at sufficiently high doses—a property shared with another noncompetitive GlyT1 blocker, CP-802079, which has been shown to block NMDA-evoked currents at high doses (Martina et al. 2004), and the resulting impairment in NMDAR function might be responsible for the reported PPI disruption as well as the emergence of hyperactivity and stereotypy (Lipina et al. 2005). By contrast, SSR504734 did not produce hyperlocomotion or stereotypy up to 30 mg/kg (Singer et al. 2009b), and the compound enhanced NMDA-evoked currents with increasing concentrations (Depoortère et al. 2005). Thus, the discrepant PPI outcomes between GlyT1 inhibitors, at different doses and across mouse strains, might be explicable in terms of their associated modulatory effects on NMDAR function.

Although the exacerbation of apomorphine-induced PPI disruption by SSR504734 was not anticipated by the drug's effect on PPI when administered alone (at either dose), the pattern is similar to our earlier report of SSR504734's modulation of amphetamine-induced hyperlocomotor activity (Singer et al. 2009b). Together, our results demonstrate that SSR504734 not only interferes with presynaptic intervention of dopamine release (i.e., amphetamine) but also with postsynaptic intervention that directly acts on dopamine receptors (i.e., apomorphine). Mechanistically, the repeatedly observed behavioral pattern may be linked to reports that SSR504734 facilitated evoked dopamine release in the NAC, without altering basal accumbens dopamine levels (Depoortère et al. 2005; Leonetti et al. 2006). Since SSR504734 does not bind to dopamine receptors (Depoortère et al. 2005), it is unlikely that it can interfere with postsynaptic events following dopamine receptor activation. It may be worth considering the possibility that SSR504734 might influence upstream activities through its indirect influence on NMDAR or even glycinergic inhibitory signaling via strychnine-sensitive glycine receptors (GlyRs). However, whether the interaction with the dopamine system demonstrated here is unique to SSR504734 remains to be ascertained because the impacts of other GlyT1 inhibiting drugs on apomorphine-induced PPI disruption have not been systematically evaluated. Caution against overgeneralization is warranted here, especially when reports on amphetamine-induced hyperactivity have yielded inconsistent outcomes between different synthetic GlyT1 inhibitors (Boulay et al. 2008; Harsing et al. 2003).

Mechanistically, the facilitation of accumbal dopamine release by SSR504734 is reportedly linked to enhanced NMDAR activation, since it can be blocked by the NMDAR antagonist AP5 (2-amino-5-phosphonopentanoic acid) (Leonetti et al. 2006). Alternatively, GlyT1 blockade might potentiate inhibitory signaling via GlyRs in the NAC, which in turn leads to reduced GABAergic feedback inhibition of dopaminergic input into the NAC from the ventral tegmental area (Lidö et al. 2009, 2011).

SSR504734 as a modulator of dopamine function

Because dopamine receptor agonist-induced hyperlocomotion and PPI disruption are considered as animal correlates of positive schizophrenia symptoms, it is important to

consider whether SSR504734 may worsen positive psychotic symptoms in patients. The dopamine hypothesis of schizophrenia emphasizes that subcortical hyperdopaminergia coupled with prefrontal hypodopaminergia contributes to the emergence of the positive symptoms (Carlsson 1988; Davis et al. 1991). These hypothesized directions of dopaminergic dysfunction are distinct from those produced by SSR504734: the drug increased prefrontal dopamine levels without changing subcortical basal dopamine concentration (Depoortère et al. 2005; Leonetti et al. 2006) and antagonized hypersensitivity to amphetamine resulting from neonatal chronic phencyclidine exposure in rats (Depoortère et al. 2005)—an animal model that mimics neurodevelopmental abnormalities of schizophrenia (Wang et al. 2001). On the other hand, SSR504734 also tended to enhance the response to amphetamine in control rats without prior exposure to phencyclidine (Depoortère et al. 2005). Thus, depending on the differing physiological background of the subjects—perhaps, similar to that between schizophrenia patients and acute amphetamine abusers—SSR504734 might exert bidirectional effects on amphetamine-induced behavior.

Given that both under and overactivity of dopamine signaling have been implicated in schizophrenia, the therapeutic potential of the stimulating action of SSR504734 on dopaminergic activity also deserves further consideration. Of relevance is the finding that pro-dopaminergic stimulant drugs, including amphetamine, have been shown to improve the negative and cognitive symptoms in schizophrenia patients (e.g., Goldberg et al. 1991). In healthy subjects, such drugs could enhance working memory—an effect that has been linked to the stimulation of prefrontal dopamine D_1 receptors (Barch 2004; Barch and Carter 2005; Mehta and Riedel 2006). The possibility that such mechanisms might contribute to the reported procognitive (Singer et al. 2009b) and antinegative symptoms effects of SSR504734 (Black et al. 2009) has therefore been raised (Singer et al. 2009b, c). Given that hypofunction of prefrontal dopamine can impair PPI (Ellenbroek et al. 1996; Koch and Bubser 1994), the enhancement in basal PPI by SSR504734 at 30 mg/kg reported here might also share similar mechanisms.

Clinical perspectives on GlyT1 inhibition

Speculation that GlyT1 inhibition may be beneficial in the treatment of schizophrenia dates back to the late 1990s (Javitt 1997). Since then, several clinical trials have been performed with the naturally occurring GlyT1 inhibitor, sarcosine (see the meta-analysis by Singh and Singh 2011), which was invariably evaluated as adjunctive treatment in combination with conventional antipsychotic drugs (for reviews, see Javitt 2009; Lin et al. 2012). The most promising GlyT1 inhibitor developed by Hoffmann-La Roche, RG1678, which is the first-in-class compound currently in phase III trial, is no exception; RG1678 is evaluated strictly as an adjunctive treatment (Alberati et al. 2012; Umbricht et al. 2010). The effectiveness of GlyT1 inhibition as a monotherapy in patients, on the other hand, is poorly characterized. The only available study is a small-scale trial with sarcosine, which yielded highly ambiguous results (Lane et al. 2008). Large-scale studies with GlyT1 inhibitors as monotherapy are therefore urgently needed. Such studies would facilitate comparison with preclinical studies, which typically evaluate individual compounds in isolation (i.e., monotherapy) and would clarify the interaction between GlyT1 inhibitors and (typical) antipsychotic drugs.

One favored hypothesis is that the polytherapy approach might uniquely allow the simultaneous enhancement of cortical dopaminergic activity (by GlyT1 inhibiting drugs) and suppression of subcortical dopaminergic hyperactivity (by dopamine D_2 receptor blocking typical antipsychotics). The combined effects might be crucial for the effective control of positive, negative, and cognitive symptoms of schizophrenia. Notably, a similar idea underlies the proposal of co-administrating amphetamine with haloperidol; but chronically exposing schizophrenia patients to amphetamine—a known psychomimetic drug—could be a concern (e.g., Goldberg et al. 1991). Unlike amphetamine, which is a stimulant drug of abuse that directly facilitates the release of dopamine, the modulation of dopamine function by SSR504734 is mediated indirectly via its effects on NMDARs and possibly GlyRs. Thus, the overall psychopharmacological profile of SSR504734 does not resemble that of any psychostimulant drug, and its use might be safer compared with amphetamine, especially in a chronic setting. Continual efforts towards the further characterization of the interaction between GlyT1 and the dopaminergic system may aid future drug design (e.g., competitive vs. noncompetitive inhibition) and the determination of therapeutic regimes (e.g., poly vs. monotherapy) in order to maximize efficacy in the clinics.

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