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# Modulation of Striatal Dopamine Release by Glycine Transport Inhibitors

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Traditional models of schizophrenia have focused primarily upon dopaminergic (DA) dysregulation. In contrast, more recent models focus on dysfunction of glutamatergic systems, acting particularly through *N*-methyl-D-aspartate (NMDA) receptors. NMDA receptors in brain are regulated by glycine, acting via a strychnine-insensitive regulatory site, and by glycine (GlyT1) transporters that maintain low glycine levels in the immediate vicinity of the NMDA receptor complex. The present study investigates the role of NMDA receptors in the modulation of striatal dopamine release *in vitro*, and of glycine transport inhibitors (GTIs) as potential psychotherapeutic agents in schizophrenia. In striatum, NMDA receptors exert dual excitatory/inhibitory effects, with inhibition reflecting activity of local GABAergic feedback regulation. We have previously demonstrated effectiveness of glycine in regulating [$^3$H]DA release both *in vivo* and *in vitro*, consistent with its beneficial clinical effects. In the present study, similar effects were observed for the high-affinity GTI (+)*N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy-)propyl]sarcosine (NFPS), and for a range of high-affinity GTIs with appropriate rank order of potency. In addition, (+)NFPS significantly stimulated NMDA-induced [$^3$H]GABA release. Effects, of GTIs, were blocked by the glycine-site antagonists L689,560 and HA-966, and the $GABA_B$ antagonists phaclofen and CGP 52432, confirming the roles of both the NMDA-associated glycine-site and presynaptic $GABA_B$ receptors in NMDA receptor-mediated regulation of striatal DA release *in vitro*. Endogenous DA hyperactivity is associated with prominent positive symptoms in schizophrenia. The present results are consistent with recent clinical studies showing significant effectiveness of glycine-site agonists and GTIs in reduction of persistent positive, as well as negative, symptoms in schizophrenia.

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## INTRODUCTION

Schizophrenia is a severe brain disease that affects up to 1% of the world's population. Traditional models of schizophrenia emphasize the importance of dopaminergic (DA) dysregulation (eg, Davis *et al*, 1991; Moore *et al*, 1999), particularly in the etiology of positive symptoms. More recent models, in contrast, emphasize the potential role of *N*-methyl-D-aspartate (NMDA) receptor dysfunction as a basis for both persistent negative and cognitive symptoms, and DA dysregulation in schizophrenia (Javitt, 1987, 2004; Carlsson and Carlsson, 1990; Javitt and Zukin, 1991; Coyle, 1996; Hirsch *et al*, 1997; Abi-Saab *et al*, 1998; Olney *et al*, 1999; Moghaddam and Jackson, 2003). This reconceptualization of schizophrenia has led to efforts both to develop more effective NMDA-stimulating agents, and to define critical sites of interaction between dopamine and NMDA systems.

In schizophrenia, DA dysregulation, particularly in striatum, has been confirmed through *in vivo* neuroimaging (Abi-Dargham *et al*, 1998; Breier *et al*, 1997; Laruelle, 1998; Laruelle *et al*, 1996). NMDA receptors are present in high density in striatum, and are localized on both presynaptic DA terminals and on GABA interneurons where they serve to inhibit presynaptic DA release through local feedback regulation (Wu *et al*, 2000). NMDA receptor antagonists, such as phencyclidine (PCP) or ketamine, induce schizophrenia-like DA dysregulation in both animals (Balla *et al*, 2001, 2003; Miller and Abercrombie, 1996) and normal human volunteers (Kegeles *et al*, 2000). Further, mice with mutations of the NMDA ε1 subunit show both biochemical and behavioral evidence of DA dysregulation (Ballard *et al*, 2002; Miyamoto *et al*, 2001). Dysfunction or blockade of NMDA receptors particularly on GABA interneurons may, therefore, produce DA hyperactivity similar to that observed in schizophrenia.

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NMDA receptors in brain are regulated both by glutamate, which serves as the primary agonist, and glycine, which binds to an allosteric modulatory site (Dingledine *et al*, 1990; Reynolds and Miller, 1990). Glycine levels in brain, in turn, are regulated by glycine transporters including the glycine type I (GlyT1) transporter. GLYT1 transporters are colocalized with NMDA receptors and serve to 'protect' the NMDA site from high circulating levels of glycine (Lopez-Corcuera *et al*, 2001; Smith *et al*, 1992). One recent approach to NMDA potentiation, therefore, has been the development of glycine transport inhibitors (GTIs), which prevent glycine removal from the synaptic cleft thereby increasing occupancy of the NMDA/glycine-binding site (Javitt and Frusciante, 1997; Javitt *et al*, 1997; Supplisson and Bergman, 1997).

In initial studies, the prototypic glycine transport inhibitor glycyldodecylamide (GDA) significantly inhibited PCP-induced hyperactivity in rodents (Javitt *et al*, 1999; Toth *et al*, 1986) and reversed NMDA-induced DA release from rat striatal slices (Javitt *et al*, 2000). More recently, high-affinity glycine transporter inhibitors such as *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS, ALX5407) (Atkinson *et al*, 2001; Herdon *et al*, 2001) have been shown to potentiate NMDA receptor-mediated neurotransmission both *in vitro* (Bergeron *et al*, 1998) and *in vivo* (Chen *et al*, 2003); to normalize prepulse inhibition (Kinney *et al*, 2003; Le Pen *et al*, 2003); and to reverse PCP-induced DA dysregulation *in vivo* (Javitt *et al*, 2003). However, mechanisms underlying DA reregulation produced by NMDA agonists, including both glycine and newly developed glycine transport inhibitors, remain to be determined. In the present study, an *in vitro* striatal release assay, shown previously to be sensitive to effects of glycine and the prototype glycine transport inhibitor GDA (Javitt *et al*, 2000), was used to evaluate effects of newly developed glycine transport inhibitors on striatal DA and GABA release, and to investigate mechanisms underlying NMDA/DA/GABA interactions.

## METHODS

Mice were decapitated and the striatal tissue (approximately 8–10 mg) was dissected out and incubated for 60 min in $Mg^{2+}$-containing 0.5 ml of Krebs bicarbonate buffer containing 1.25–3.0 μCi [$^3$H]dopamine (New England Nuclear, Boston, MA) and, where applicable, 1.2 μCi [$^{14}$C]GABA. For striatal dissections, the mouse brain was partially dissected in half and the cortex carefully folded outwards with a small spatula to expose the striatum from each hemisphere. The area of the striatum was visible from the surrounding region by their striated texture. The spatula was used to undercut the region slightly below the surface and to remove the striatal tissue; approximately 15 mg for both left and right striatum. For experiments involving GABA, amino-oxyacetic acid (0.1 mM) was added to the incubation buffer. The reaction mixture was continuously gassed with an $O_2/CO_2$ mixture (95%/5%). After prelabeling, tissue was transferred to superfusion chambers ($12 \times 0.3$ ml chambers, Brandel Superfusion 1200, MD) and preperfused at a rate of 0.8 ml/min for 60 min in the above buffer minus $Mg^{2+}$. Effluent was discarded during this period and thereafter 3 or 4 min fractions were collected for an additional 45–60 min, depending on the experiment. Blocking drugs were added before indicated fractions and maintained until the end of the experiment.

Enhanced release was induced by addition of NMDA, at indicated doses, during fraction 5 (2 min exposure). Fractional release was defined as radioactivity content of each fraction divided by the amount of radioactivity remaining in the tissue at the time the fraction was collected. For studies using 25 μM NMDA, NMDA-induced release was defined as the area under the fractional release curve for 24 min following NMDA exposure (fractions 6–11), relative to surrounding fractions. For studies using 300 μM NMDA, NMDA-induced release was defined as the summed release during the post-NMDA exposure period (fractions 6–15), relative to baseline.

Following the perfusion period (fraction 15), the tissue was removed from the perfusion chamber along with the remaining perfusion buffer, sonicated, and assayed for tissue radioactivity. To determine radioactivity released from the tissue, the perfusate (2.4 ml) was mixed with 3 ml Liquiscint scintillation fluid (National Diagnostics, Atlanta, Georgia) and counted in a Packard Scintillation Counter (Model 1500).

Effects of GTIs on synaptosomal glycine transport were determined as previously described (Javitt and Frusciante, 1997). Assays were performed using a concentration of 1 μM of prospective GTIs.

Data in text represent mean ± SD. Two-tailed statistics with preset alpha level for significance of $p < 0.05$ are used throughout.

## RESULTS

The goal of this study was to investigate effects of newly developed GTIs on NMDA-stimulated DA release in rodent striatum. Initial studies were performed with enantiomers of ALX5311, a high-potency GTI structurally related to NFPS. Subsequent studies were performed with the resolved enantiomers (+) and (−)NFPS. L689,560, a high-affinity NMDA/glycine-site antagonist (Stauch Slusher *et al*, 1994) and HA-966 (Javitt *et al*, 2000), a partial agonist, were used to evaluate involvement of NMDA receptors in GTI-induced effects. The $GABA_B$ agonist baclofen, and antagonists phaclofen (Kerr *et al*, 1987) and CGP52432 (Teoh *et al*, 1996) were used to evaluate potential $GABA_B$ receptor involvement.

### Effect of Glycine Transport Inhibitors on NMDA-Induced Dopamine Release

An initial set of studies was performed in Balb/c mice. In the absence of added ligands, brief exposure of 25 μM NMDA induced a robust increase in dopamine efflux that peaked immediately following NMDA exposure and decayed over the ensuing 28 min (seven fractions). Initial studies were performed using 1 μM ALX5311, a potent, selective GTI (Figure 1). ALX5311 inhibited NMDA-induced dopamine release in a dose-dependent fashion ($F = 4.82$, $df = 3/126$, $p = 0.003$) with highly significant inhibition being observed at a dose of 1 μM ($t = 3.75$, $df = 116$, $p < 0.0001$) (Figure 2a).

Further, the (+) enantiomer exerted significantly greater inhibition of release than the (−) enantiomer ($t = 3.03$, df = 19, $p = 0.014$).

In order to determine role of the NMDA-associated glycine site in this effect, studies were repeated in the presence of the selective glycine-site antagonist L689,560 and the partial antagonist HA-966 (Figure 2b). NMDA-induced DA release was significantly inhibited by L689,560 itself in a dose-dependent fashion, with significant inhibition being observed at a dose of 100 nM ($t = 3.94$, df = 103, $p < 0.0001$). In the presence of L689,560, no significant effects of ALX5311 were observed ($t = 1.14$, df = 36, $p = 0.3$).

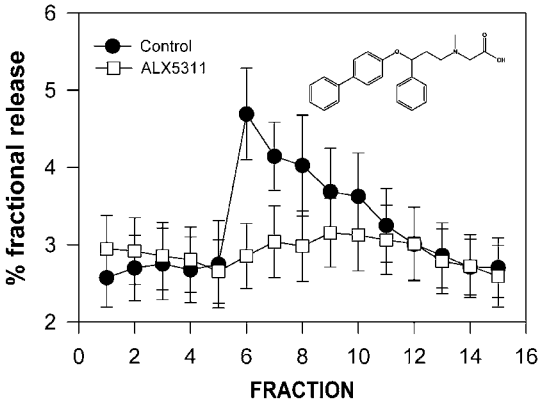


**Figure 1** Fractional NMDA-stimulated [$^3$H]DA release in the absence and presence of the high-affinity glycine transport inhibitor ALX5311 (1 μM). Structure of ALX5311 is shown in inset. Fractions were collected every 4 min. Data are mean ± SEM. *$p < 0.05$ *vs* control; **$p < 0.01$ *vs* control.

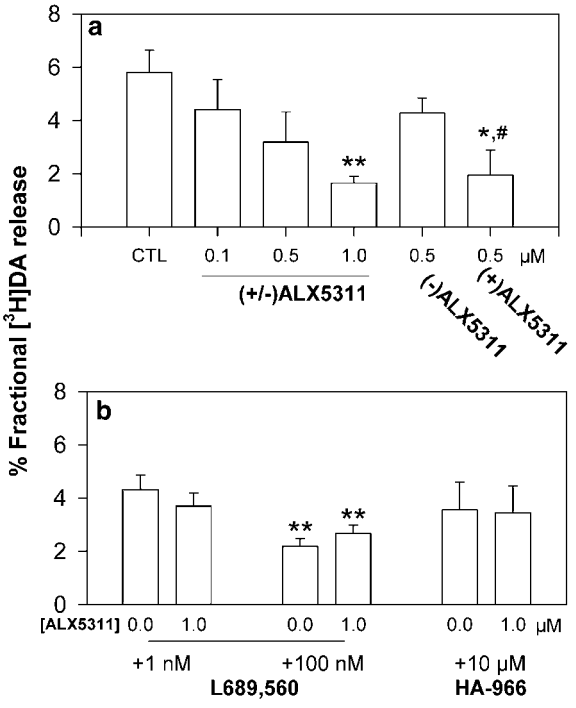


**Figure 2** Summed NMDA-stimulated [$^3$H]DA release in the absence and presence of (+) and (−) ALX5311 (a) and in the additional presence of the NMDA/glycine-site antagonists L689,560 and HA-966 (b). *$p < 0.01$ *vs* control, **$p < 0.001$ *vs* control; #$p < 0.05$ *vs* (−)ALX5311.

When data were entered into a 2 × 2 ANOVA, a significant L689,560 × ALX5311 interaction emerged ($F = 7.68$, df = 1/152, $p = 0.006$), reflecting the significant effects of ALX5311 in the absence but not the presence of L689,560. HA-966 alone did not significantly inhibit NMDA-induced DA release at a concentration of 10 μM. Nevertheless, in the presence of HA-966, no significant further effect of ALX5311 was observed.

In order to verify the effects of ALX5311, experiments were repeated with the subsequently developed, selective GLYT1 inhibitor (+) NFPS. As with ALX5311, the (+) isomer shows greater affinity for the GLYT1 transporter than the (−) isomer (Kinney *et al*, 2003; Mallorga *et al*, 2003), permitting differential effectiveness studies to be performed. (+)NFPS was significantly more effective than (−)NFPS ($t = 2.32$, df = 37, $p = 0.05$), which was without significant effect (Figure 3). Across this series of NFPS-type drugs, the degree of inhibition of [$^3$H]DA release correlated significantly with the degree of inhibition of synaptosomal glycine transport ($p = 0.01$).

Owing to the change in institutional breeding policies, a second set of studies with (+)NFPS was performed in C57BL/6 mice to verify stability of effect across strains (Figure 4). In order to optimize conditions, studies were performed in both the absence and presence of added glycine (100 μM). In tissue from these mice, (+)NFPS also induced significant, dose-dependent inhibition of NMDA-stimulated [$^3$H]DA release ($F = 5.0$, df = 2/334, $p = 0.007$). The effect of glycine was significant only at trend level ($F = 3.3$, df = 2/334, $p = 0.07$). No significant glycine × (+)NFPS interaction was observed ($F = 1.5$, df = 2/334, $p = 0.2$).

## Effect of GTIs on [$^3$H]GABA Release

One hypothetical mechanism by which GTIs might induce their effects is by modulation of GABAergic feedback regulation. In order to investigate effects of GTIs on striatal GABAergic function, striata were dually preincubated with [$^{14}$C]GABA and [$^3$H]DA. For these experiments, a higher

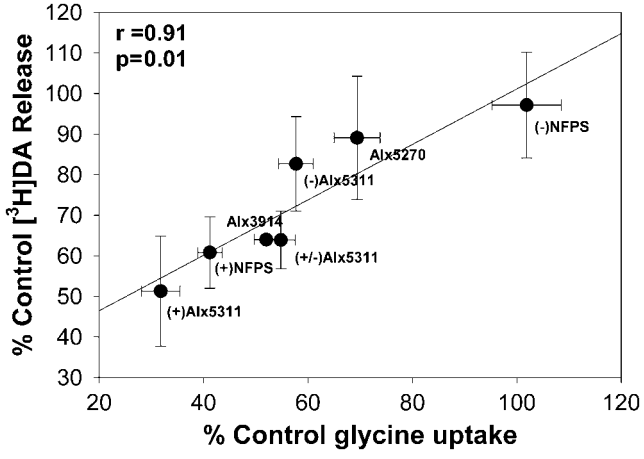


**Figure 3** Correlation between degree of inhibition of NMDA-stimulated DA release in mouse striatal release assay at concentrations of 1 μM and degree of inhibition of [$^3$H]glycine uptake in a rat synaptosomal [$^3$H]glycine uptake assay.

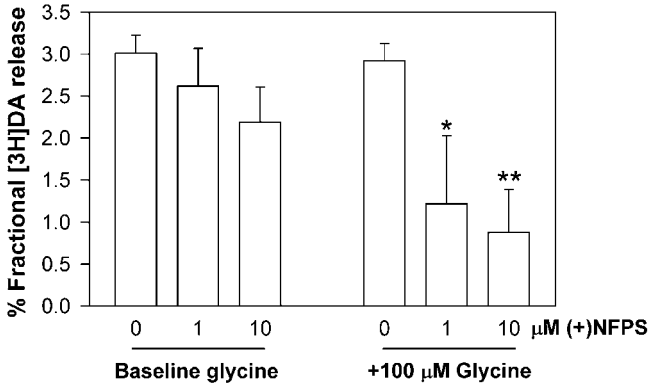


**Figure 4** Effect of the high-affinity GTI (+)NFPS on (a) basal, and (b) NMDA-stimulated [$^3$H] release in the absence and presence of added glycine (100 μM). Fractions were collected every 3 min. *$p<0.01$; **$p<0.001$ *vs* control.

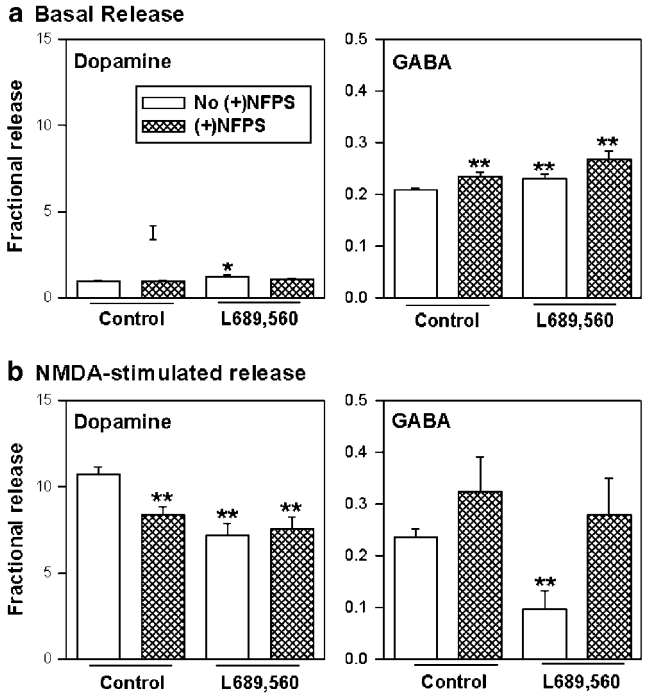


**Figure 5** Effect of (+)NFPS (10 μM ) on basal (a) and NMDA-stimulated (b) DA and GABA release in the absence and presence of L689,560 (100 nM). *$p<0.05$ *vs* ctl; **$p<0.01$ *vs* control.

dose (300 μM) of NMDA was used to increase release levels. (+)NFPS was employed at a dose of 10 μM. In this experiment, compounds were added at fraction 1 to permit greater evaluation of effects on basal release, as well as on NMDA-stimulated DA release. L689,560 (100 nM) was used to evaluate NMDA/glycine-site dependence of (+)NFPS effects.

As in prior studies, (+)NFPS had no effect on basal [$^3$H]DA release in either the absence ($t=.1$, df = 100, $p=0.9$) or presence ($t=1.35$, df = 52, $p=0.2$) of added L689,560 (Figure 5a). In contrast, L689,560 (100 nM) significantly enhanced basal release of [$^3$H]DA ($t=3.07$, df = 94, $p=0.003$). An ANOVA revealed a significant main effect of L689,560 (F = 9.4, df = 1/152, $p=0.003$), on basal DA release, but no significant main effect of (+)NFPS (F = 2.1, df = 1/152, $p=0.1$) or (+)NFPS × L689,560 interaction (F = 1.9, df = 1/152, $p=0.2$).

As in prior experiments, (+)NFPS significantly inhibited NMDA-induced release [$^3$H]DA in an L689,560-dependent fashion (Figure 5b). In the absence of added L689,560, significant inhibition by (+)NFPS was observed ($t=3.61$, df = 100, $p<0.001$). L689,560 by itself also inhibited NMDA-induced release ($t=4.68$, df = 94, $p<0.001$). However, in the presence of L689,560, (+)NFPS had no significant further inhibitory effect ($t=0.4$, df = 52, $p=0.7$). ANOVA, therefore, revealed a significant main inhibitory effect of L689,560 (F = 14.6, df = 1/152, $p<0.001$). Although (+)NFPS had no significant effect on its own (F = 3.1, df = 1/152, $p=0.08$), the (+)NFPS × L689,560 interaction effect was significant (F = 5.71, df = 1/152, $p=0.02$), reflecting the differential effect in the presence *vs* absence of added glycine-site antagonist and confirming prior observations.

Predicted effects of (+)NFPS were also observed on GABA release. Thus, both basal (F = 14.8, df = 1/224, $p<0.001$) (Figure 5c) and NMDA-stimulated (F = 9.97, df = 1/224, $p=0.002$) (Figure 5d) release of [$^{14}$C]GABA was significantly higher in the presence of (+)NFPS than in its absence. However, L689,560 had a more complex effect, in that it significantly increased basal release (F = 11.1, df = 1/224, $p=0.001$), but significantly inhibited NMDA-induced release (F = 4.74, df = 1/224, $p=0.03$). No significant (+)NFPS X L689,560 interaction were observed for either basal (F = .5, df = 1/224, $p=0.5$) or NMDA-stimulated (F = 1.26, df = 1/224, $p=0.3$) release. These findings demonstrate the predicted ability of GTIs to enhance NMDA-regulated GABA release in striatum, although it suggest the presence of multiple sites of action for L689,560.

## Effect of $GABA_B$ Ligands

A final series of experiments evaluated potential mechanisms by which GTIs mediate their effects. The most likely basis for the inhibitory effects is a modulation of GABAergic neurotransmission. GABA mediates its effects at $GABA_a$ receptors, which are sensitive to the selective $GABA_a$ antagonist bicuculline, and $GABA_b$ receptors, which are sensitive to the selective agonist baclofen and the selective antagonists phaclofen and CGP52432. Bicuculline had no significant effect on [$^3$H]DA release either on its own or in combination with (+)NFPS (data not shown). Subsequent studies, therefore, examined effects of $GABA_b$ ligands.

In this experiment, a dose of (+)NFPS of 25 μM was used (Figure 6). As in previous experiments, (+)NFPS produced a highly significant reduction in fractional [$^3$H]DA release ($t=4.54$, df = 262, $p<0.001$). Experiments were repeated in the presence of the $GABA_b$ receptor agonist baclofen (100 μM), and the antagonists phaclofen (50 μM) and CGP52432 (1 μM). Baclofen on its own significantly inhibited DA release ($t=4.91$, df = 166, $p<0.001$). Moreover, in the presence of baclofen, (+)NFPS produced no further significant inhibition, and even a tendency toward significant increase ($t=1.81$, df = 35, $p=0.08$), leading to a significant NFPS × baclofen interaction (F = 8.7, df = 1/164, $p=0.004$) (Figure 7).

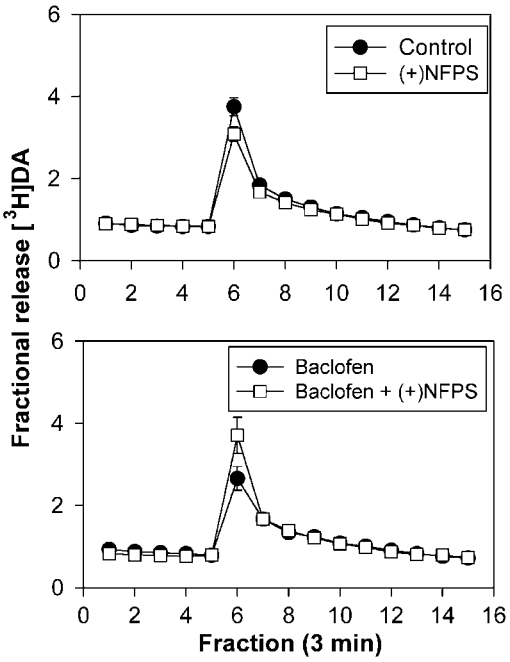


**Figure 6** Effect of (+)NFPS (25 μM) on NMDA (300 μM)-stimulated [$^3$H]DA release in the absence and presence of the $GABA_B$ agonist baclofen (100 μM).

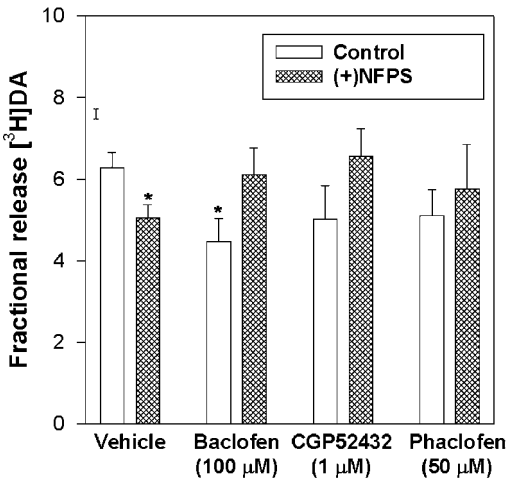


**Figure 7** Effects of (+)NFPS (25 μM) on NMDA (300 μM)-stimulated [$^3$H]release in the absence and presence of the $GABA_B$ agonist bacofen (100 μM) and the $GABA_B$ antagonists CGP52432 (1 μM) and phaclofen (50 μM). *$p<0.05$ *vs* control.

No significant reduction in [$^3$H]DA release was observed following treatment with the $GABA_b$ antagonists phaclofen ($t=1.07$, df = 76, $p=0.3$) or CGP52432 ($t=1.41$, df = 76, $p=0.6$) (Figure 7). Nevertheless, in the presence of $GABA_b$ antagonists, (+)NFPS was without effect. In the CGP52432 experiments, there was no significant effect of either CGP52432 ($F=0.9$, df = 1/211, $p=0.3$) or (+)NFPS ($F=0.8$, df = 1/211, $p=0.4$), but the CGP52432 × (+)NFPS interaction was significant ($F=6.1$, df = 1/211, $p=0.015$), reflecting the differential (+)NFPS effect in the absence *vs* presence of $GABA_b$ antagonists. Similarly, in the phaclofen experiments, an ANOVA revealed no significant effect of either phaclofen ($F=1.7$, df = 1/211, $p=0.2$) or (+)NFPS ($F=1.8$, df = 1/211, $p=0.2$). However, the phaclofen− × (+)NFPS interaction was significant ($F=4.0$, df = 1/211, $p=0.045$), replicating the CGP52432 results.

## DISCUSSION

Current treatments for schizophrenia target the dopamine system primarily by blockade of dopamine $D_2$ receptors. More recent theories have suggested alternative use of drugs targeted at the NMDA receptor (Javitt, 2001; Javitt and Zukin, 1991). 'First-generation' agents that have been used in clinical studies include both glycine (Heresco-Levy *et al*, 2004; Heresco-Levy *et al*, 1999; Javitt *et al*, 2001; Javitt *et al*, 1994) and D-serine (Tsai *et al*, 1998), which are endogenous agonists, as well as the synthetic glycine/D-serine derivative D-cycloserine, which is a partial agonist (Goff *et al*, 1999; Heresco-Levy *et al*, 1998). Development of high-affinity glycine-site agonists, however, is limited by the small molecular size of the target (McBain *et al*, 1989). A more recent approach has been the development of GTIs (Atkinson *et al*, 2000; Bergeron *et al*, 1998; Javitt, 2002; Javitt and Frusciante, 1997). This approach is analogous to use of selective noradrenaline or serotonin reuptake inhibitors, rather than noradrenaline or serotonin precursors, to influence brain monoamine levels in depression, or use of GABA transport inhibitors to elevate brain GABA levels in epilepsy or other brain hyperexcitability states (Dalby, 2003). The primary finding of the present study is that high-affinity GTIs show significant *in vitro* efficacy in potentiating NMDA-stimulated GABA release in striatum, and that this effect leads to significant inhibition of striatal DA release, an effect that would be expected to be therapeutically beneficial in schizophrenia.

The present results support a model in which NMDA receptors have dual excitatory–inhibitory function within striatum (Leviel *et al*, 1989; Taber *et al*, 1996). The excitatory effects of glutamate are most likely mediated at NMDA receptors located on presynaptic dopamine terminals. The existence of such receptors is well documented (Gracy and Pickel, 1996; Krebs *et al*, 1991; Wang, 1991). However, ultrastructual evidence of functional axoaxonal glutamatergic synapses on DA terminals is lacking, suggesting that the receptors may have limited physiological relevance. In the present study, NMDA stimulation of DA release via these receptors serves to mimic the impulse-dependent DA release that would normally be elicted by corticonigral activation in response to psychostimulant agents such as amphetamine, permitting analysis of feedback mechanisms within the system. Inhibitory effects of NMDA are mediated through NMDA receptors located on intrinsic GABAergic interneurons. Infusion of NMDA into striatum in intact animals increases both DA and GABA release (Hernandez *et al*, 2003), consistent with this model. Further, GABAergic inhibitory mechanisms in striatum have been demonstrated in both rodent (Hanania and Johnson, 1999; Hernandez *et al*, 2003) and primate (Schiffer *et al*, 2003) striatum, demonstrating *in vivo* relevance of this pathway to intrinsic DA regulation.

In brain, extracellular glycine levels are sufficient to fully saturate the NMDA/glycine-binding site. GTIs are

postulated to function by regulating synaptic, relative to extracellular, levels, leading to local protection of the NMDA/glycine-binding sites (Supplisson and Bergman, 1997). In the present study, GTIs did not affect presynaptic DA release, presumably due to the absence of functional synapses and of a protected intrasynaptic space , but did increase NMDA-stimulated GABA release consistent with the existence of ultrastructurally demonstrated glutamatergic synapses on GABAergic interneurons. In contrast, L689,560, which inhibits glycine binding at both presynaptic and postsynaptic receptors, inhibited both DA and GABA release. GTIs were without significant effect on DA release in the presence of L689,560, confirming the role of increased glycinergic stimulation at the NMDA/glycine site in mediating their effects. Interestingly, while GTIs stimulated striatal GABA release and L689,560 inhibited release, there was no significant interaction between the two treatments. This may indicate hetereogeneity either of GABA/NMDA interactions or NMDA subtypes in striatum (Nankai *et al*, 1995). Nevertheless, both effects were in the predicted direction, with presumed NMDA stimulators increasing striatal GABA release and the NMDA antagonist decreasing GABA release.

The role of dopamine in the pathophysiology of schizophrenia has been appreciated for at least the past 40 years. Recent *in vivo* imaging studies have confirmed the relation between striatal dopamine release and have suggested that increased striatal dopamine levels may serve as a final common mechanisms for generation of positive psychotic symptoms. Thus, striatal dopamine levels are increased not only by DA agents such as amphetamine (Laruelle *et al*, 1999) but also by NMDA antagonists such as PCP and ketamine (Breier *et al*, 1998), with the degree of increase correlating with the level of psychotic symptoms. Further, normalization of striatal dopamine release accompanies clinical remission of positive symptoms in schizophrenia despite persistence of negative symptoms (Laruelle *et al*, 1999). The ability of glycine (Javitt *et al*, 2000) and GTIs (this study) to reduced striatal dopamine release is consistent with recent observations that NMDA agonists such as glycine (Heresco-Levy *et al*, 2004), D-serine (Heresco-Levy *et al*, submitted; Tsai *et al*, 1998), and the naturally occurring GTI sarcosine (Tsai *et al*, 2004) significantly ameliorate persistent positive, as well as negative, psychotic symptoms in schizophrenia, with positive symptom effects most likely reflecting effects on striatal NMDA/DA interactions.

Finally, the present study demonstrates a significant involvement of $GABA_B$ receptors in mediating the effects of NMDA-stimulated GABA release on DA inhibition. This effect is consistent with known localization of $GABA_B$ receptors to presynaptic DA terminals (Smolders *et al*, 1995), and the ability of $GABA_B$ agonists to partially inhibit NMDA-induced striatal tyrosine hydroxylase stimulation in NMDA (Arias Montano *et al*, 1992; Arias-Montano *et al*, 1991).

Although the present study focuses on GTI potentiation of NMDA receptor-mediated neurotransmission within striatum, this is unlikely to be the sole site of psychotherapeutic action of these agents. GTIs, for example, have been shown to stimulate NMDA receptor-mediated neurotransmission in both hippocampus (Bergeron *et al*, 1998) and prefrontal cortex (Chen *et al*, 2003), areas of the brain associated with prominent disability and negative symptom generation in schizophrenia. The present results, however, suggest that *even* DA dysregulation in schizophrenia may be attributable to NMDA dysfunction, and that NMDA stimulatory agents may exert significant psychotherapeutic effects even for positive symptoms. A limitation of the study is that an *in vitro* model system was used. Replication of these results *in vivo* with more recently developed, systematically active GTIs, therefore, will be critical.

In summary, this is the first study to demonstrate significant modulation of striatal dopamine release by high-affinity GTIs, and one of small number of studies to date validating the GTI concept as a method for stimulating systems-level NMDA receptor-mediated neurotransmission. These studies are encouraging of continued development of GTIs as novel psychotherapeutic agents in schizophrenia and related neuropsychiatric disorders.

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