Induction of Spontaneous Tail-Flicks in Rats by Blockade of Transmission at \(N\)-Methyl-D-Aspartate Receptors: Roles of Multiple Monoaminergic Receptors in Relation to the Actions of Antipsychotic Agents

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ABSTRACT

We examined the involvement of multiple monoaminergic receptors in the induction of spontaneous tail-flicks (STFs) by the open channel blocker at \(N\)-methyl-D-aspartate (NMDA) receptors, dizocilpine, and the NMDA recognition site antagonist 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP). At doses eliciting a maximal STF response, dizocilpine and CPP elevated levels of norepinephrine, but not dopamine or serotonin, in dialysates of nucleus accumbens, their known locus of action in eliciting STFs. Chemically diverse \(\alpha_2\)-adrenergic receptor (AR) antagonists atipamezole, L745,743, RX821,002, idazoxan, and desfluparoxan abolished induction of STFs by dizocilpine, whereas the preferential \(\alpha_1\)-AR antagonists prazosin, WB4101, and ARC239 were weakly active: relative potencies in blocking STFs correlated significantly with affinity at \(\alpha_2\)-ARs. The D1/D5 receptor antagonists SCH23390, SCH39166, and NNC756 potently abolished STFs, whereas the D2 antagonist L741,626, the D3 antagonists GR218,231 and S14297, and the D2 antagonists S18126 and L745,870 were inactive. D1 and \(\alpha_2\)-AR antagonists also blocked induction of STFs by CPP. Blockade of dizocilpine-induced STFs was specific inasmuch as idazoxan and SCH 23390 did not modify induction of ataxia by dizocilpine. Antagonists at multiple 5-hydroxytryptamine receptors failed to modify induction of STFs. Finally, dizocilpine-induced STFs were blocked by clozapine and 11 other antipsychotics, the potency of which correlated significantly with affinity at \(\alpha_2\)-ARs. In conclusion, STFs evoked by interruption of transmission at NMDA receptors are dependent on D1 receptors and \(\alpha_2\)-ARs for their expression. Antagonism of the \(\alpha_2\)-ARs is involved in their blockade by antipsychotics. This model should facilitate exploration of interrelationships between glutamatergic and monoaminergic mechanisms involved in psychiatric and neurologic disorders.

Glutamatergic and monoaminergic networks in corticolimbic structures and the basal ganglia play an important role in the control of motor function. Correspondingly, an understanding of the mechanisms via which they exert their actions may lead to novel therapies for the improved management of neurological disorders, such as Parkinson’s disease, and psychiatric diseases, such as schizophrenia (Carlsson and Carlsson, 1990; Lange et al., 1997; Schmidt and Kretschmer, 1997). To this end, it is important to identify functional models that allow the exploration of interrelationships among glutamatergic and monoaminergic pathways. In previous studies, we demonstrated that the interruption of transmission at \(N\)-methyl-D-aspartate (NMDA) receptors by open channel blockers, such as dizocilpine, and antagonists of the NMDA receptor recognition site, such as 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), but not by glycine\(_B\) antagonists, such as L701,324, elicits STFs in rats (Millan et al., 1991). The population of NMDA receptors involved is localized in the nucleus accumbens (Millan et al., 1999a). This structure receives a pronounced glutamatergic input from frontal cortex, hippocampus, thalamus, and amygdala, and in interaction with monoaminergic pathways, glutamatergic mechanisms in the accumbens modulate motor function and mood (Carlsson and Carlsson, 1990; Meltzer et al., 1997; Schmidt and Kretschmer, 1997; Morari et al., 1998; Millan et al., 1999b).

The ventral tegmental area and the substantia nigra, pars compacta, the origin of ascending mesocorticolimbic and nigrostriatal dopaminergic projections, respectively, also possess a pronounced glutamatergic innervation from the frontal cortex, the subthalamic nucleus, and other regions

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ABBREVIATIONS: NMDA, \(N\)-methyl-D-aspartate; AR, adrenergic receptor; DA, dopamine; 5,7-DHT, 5,7-dihydroxytryptamine; CNS, central nervous system; 5-HT, 5-hydroxytryptamine (serotonin); NE, norepinephrine; CPP, 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; STF, spontaneous tail-flick; SSRI, selective 5-HT reuptake inhibitor; 8-OH-DPAT, 8-hydroxy-2-dipropylaminotetralin.
in single dialysate samples of the accumbens of freely moving rats. Second, we examined the influence of selective ligands at multiple dopaminergic, adrenergic, and serotonergic receptors on STFs elicited by the open channel blocker dizocilpine and, for certain drugs, the NMDA receptor antagonist CPP. Furthermore, we evaluated the specificity of antagonistic effects against dizocilpine-induced STFs by determining their comparative ability to block the induction of ataxia by dizocilpine. Third, we examined the actions of the neuroleptic haloperidol, the atypical antipsychotic clozapine, and a diversity of novel antipsychotic agents (Brunello et al., 1995; Meltzer, 1995; Millan et al., 1998a) on the induction of STFs by dizocilpine. The choice of drug doses used here was based on our extensive in vivo studies of models reflecting their actions at specific receptor types (for selective agents) and paradigms of potential therapeutic activity (for antipsychotic agents; Millan et al., 1991, 1994b, 1998b, 1999b; Schreiber et al., 1995; Gobert et al., 1998).

Materials and Methods

Measurement and Definition of STFs. Male Wistar rats of 220 to 230 g (Iffa Credo, L’Arbresle, France) were housed in sawdust-lined cages with unrestricted access to rat chow and water. There was a 12-h light/dark cycle with lights on at 7:00 AM and off at 7:00 PM. All experiments were undertaken during the light phase. STFs were determined exactly as detailed previously (Millan et al., 1991) in rats loosely restrained in horizontal, opaque, plastic cylinders with the tail emerging from the back to hang over the edge of the bench. One STF was defined as the elevation of the tail to a level higher than that of the body axis. The number of STFs emitted was recorded over 5 min. There was a 5-min adaptation period to the cylinder before the recording of STFs.

Drug Treatment for Inhibition and Induction of STFs. For interaction studies, dizocilpine (0.08 mg/kg s.c.) was administered 30 min before evaluation of STFs. This dose elicits a maximal STF response (Millan, 1991; Millan et al., 1999a), and this time corresponds to its peak effect. Drugs were injected 10 min before dizocilpine (i.e., 40 min before testing). For interaction studies with CPP (20.0 mg/kg s.c.), this NMDA receptor antagonist was administered 60 min before the evaluation of STFs: this corresponds to the maximally effective dose and its time of peak effect (Millan, 1991; Millan et al., 1999a). Drugs were injected 10 min before CPP (i.e., 70 min before testing). For evaluation of the ability of agonists at D1 and a2-ARs to elicit STFs, drugs were administered 30 min before testing. In the combination studies, they were administered via two injections given simultaneously 30 min before the evaluation of STFs. These doses and times correspond to those at which they maximally exert their effects at a2-AR and D1 receptors, respectively (Millan et al., 1994b).

5,7-Dihydroxytryptamine (5,7-DHT) Lesions of Serotonergic Pathways. The procedure was described previously (Bervoets et al., 1993). Briefly, rats were pretreated with desipramine (25 mg/kg i.p.) and anesthetized with pentobarbital (40.0 mg/kg i.p.), and 5,7-DHT (100 μg/10 μl) or vehicle (ascorbic acid) was injected over 1 min into the lateral ventricle at coordinates of AP = 0.0, L = −1.7, and DV = −3.1. The dose-response relationship for induction of STFs by dizocilpine was evaluated 1 week after the administration of 5,7-DHT. For confirmation of the neurochemical effects of 5,7-DHT, levels of 5-HT, DA, and NE were determined, as described previously (Bervoets et al., 1993), through HPLC and coulometric detection in several CNS regions.

Dialysis Studies. The procedure used was described in detail previously (Gobert et al., 1998). Briefly, male Wistar rats of 200 to 220 g were anesthetized with pentobarbital (60.0 mg/kg i.p.), and a guide cannula was implanted into the core of the nucleus accumbens.
hemifumurate, risperidone base, UK14304 [5-bromo-N-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoloxaline tartrate], ziprasidonechlorhydrate, (+)-S14297 [N,N-dipropylamino]-7-tetrahydro-5,6,7,8 naphto-[2,3b]dihydro-2,3 furane dibenzoxtartrate], (+)-S16924 [1-benzodioxanne-5y-3-[3-(4-fluorophenyl)piperazin]-1-oxapropanedi-2,3y-one base] was obtained from Servier chemicals. AR239 [2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-4,4-dimethyl-[2H,4H]-isoquinoline-1,3-dione HCl] was obtained from Boehringer (Ingelheim, France). L657,743 [1,3,4,5,6,7,12b-octahydro-1,3,3-dimethyl-spiro[2H-benzo[2,3]-quinolizine-2,4,1‘-pyrimidin]-2‘3‘-dione base] was obtained from Merck & Co. (Rahway, NJ). Omeprazole HCl was obtained from Janssen (Beerse, Belgium). Olanzapine base was obtained from Eli Lilly (Indianapolis, IN). NNC756 ([S,S]-8-chloro-2,3-dihydro-7-benzo[b]furanyl]-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepin-7-ol hemisuccinate) was obtained from Novo (Copenhagen, Denmark). OR35222 [(trans-5-chloro-2-methyl-2,3,3a,12b-tetrahydro-1H-dibenzo[2,3,6,7][oxepino[4,5-c]-pyrrole] fumarate] was obtained from Organon (Oss, Netherlands). Paroxetine HCl was obtained from Beecham (Brentford, England). SCH91666 [(-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzol[de]-naphto[2,1-b]azepine] HCl] was obtained from Schering-Plow (Kenilworth, NJ). Sertindole base was obtained form Lundbeck (Copenhagen, Denmark) and zotepine base was obtained from Fuji-sawa (Osaka, Japan).

Results

Influence of Dizocilpine and CPP Compared with L701,324 on Dialysate Levels of DA, NE, and 5-HT in Nucleus Accumbens. Administered to freely moving rats, dizocilpine elicited a dose-dependent, marked, and sustained elevation in extracellular levels of NE in the nucleus accumbens (Fig. 1). Indeed, at a dose (0.08) that elicits a maximal STF response (Millan, 1991; Millan et al., 1999a), dialysate levels of NE were increased above 2-fold relative to basal values. At this dose, there was, in contrast, no alteration in levels of either 5-HT or DA in the same dialysate samples (Fig. 1). At the highest dose of dizocilpine examined (0.63), there was, in fact, a significant elevation in extracellular levels of 5-HT. However, this response was transient, and its magnitude was substantially less pronounced than that for NE at this dose (Fig. 1). Furthermore, there was only a minor, variable, and nonsignificant increase in DA levels even at the highest dose (0.63; Fig. 1). In line with these observations, at a dose eliciting a maximal STF response (Millan, 1991; Millan et al., 1999a), CPP (20.0) provoked a significant elevation in levels of NE in nucleus accumbens without influencing those of 5-HT or DA (Fig. 1). The selective glycineB antagonist L701,324 (40.0), which does not evoke STFs (Millan et al., 1999a), did not influence accumbens levels of NE, DA, or 5-HT (not shown).

Influence of Antagonists at Multiple Dopaminergic Receptors on Induction of STFs by Dizocilpine. The selective antagonists at DA D1 receptors, SCH23390, SCH39166, and NNC756, all potently, dose dependently, and completely blocked the induction of STFs by dizocilpine (Fig. 3). In contrast, the selective DA D2 receptor antagonist L741,626 (10.0 mg/kg s.c.) and the selective antagonists at DA D3 receptors, GR218,231 (2.5 mg/kg s.c.) and S14297 (2.5 mg/kg s.c.), as well as the selective antagonists at DA D5 receptors, L745,870 (0.16 mg/kg s.c.) and S18126 (0.16 mg/kg s.c.), all failed to significantly modify the induction of STFs by dizocilpine at doses corresponding to those selectively
occupying their respective targets (Audinot et al., 1998; Millan et al., 1998b; STFs/5 min: vehicle/dizocilpine, 52.7 ± 6.8, L741,626/dizocilpine, 48.4 ± 5.0, P > .05; vehicle/dizocilpine, 43.2 ± 7.2, GR218,231/dizocilpine, 45.0 ± 8.0, P > .05; vehicle/dizocilpine, 45.3 ± 6.3, S14297/dizocilpine, 45.0 ± 8.0, P > .05, and L745,870/dizocilpine, 45.8 ± 14.1, P > .05; vehicle/dizocilpine, 54.0 ± 6.2, S18126/dizocilpine, 47.8 ± 10.6, P > .05). None of these antagonists elicited STFs on administration alone (not shown).

Influence of α1- and α2-AR Antagonists on Induction of STFs by Dizocilpine. Figure 4 illustrates the influence of drugs interacting with α1- and α2-ARs on the induction of STFs by dizocilpine. Several structurally diverse and preferential antagonists at α2- versus α1-ARs, RX821,002 (a benzodioxane), L657,743 (a benzofuroquinolizine), atipamezole and idazoxan (imidazolines), and desfluparoxan (a benzopyrrolidine), all potently, dose dependently, and completely blocked the induction of STFs by dizocilpine. In distinction, the preferential α1- versus α2-AR antagonists, prazosin (a quinazolinylpiperazine), WB4101 (a benzodioxide) and ARC239 (an isoquinolinepiperazine), only weakly inhibited the action of dizocilpine. ID50 values (in mg/kg s.c.,
Lack of Induction of STFs by D1 and α2-AR Antagonists on Induction of STFs by a Low Dose of Dizocilpine. Inasmuch as the dose-response for induction of STFs by dizocilpine is biphasic (Millan, 1991), it might be argued that a loss of dizocilpine-induced STFs in the presence of D1 or α2-AR antagonists RX821,002, L657,743, desfluparoxan, idazoxan, and atipamezole block induction of STFs by dizocilpine (0.08 mg/kg s.c.). Data are means ± S.E. (n = 5 per value). ANOVA results were RX821,002: F_{6,51} = 9.8, P < .001; L657,743: F_{4,34} = 8.6, P < .001; desfluparoxan: F_{3,23} = 8.0, P < .001; idazoxan: F_{3,36} = 7.8, P < .001; atipamezole: F_{3,20} = 6.8, P < .001. *P < .05, significance of drug versus vehicle (VEH). ID_{50} values (95% confidence limits) were RX821,002, 0.02 (0.01–0.04); L657,743, 0.03 (0.01–0.06); atipamezole, 0.05 (0.02–0.10); idazoxan, 0.12 (0.06–0.30); and desfluparoxan, 0.3 (0.2–0.5). (Table 1). Its ED_{50} (95% confidence limits) value was 0.6 (0.3–0.9). Even at doses sufficient to abolish the induction of STFs by dizocilpine, the D1 antagonist SCH23390 and the α2-AR antagonist idazoxan failed to modify the loss of righting reflex provoked by dizocilpine (Table 1). In the mouse, dizocilpine dose dependently decreased the latency to fall in an accelerating Rotarod test with an ID_{50} (95% confidence limits) value of 0.08 (0.06–0.11) mg/kg s.c. Idazoxan and SCH23390 also failed to block the action of dizocilpine in this model (Table 1).

Influence of D1 and α2-AR Antagonists on Induction of STFs by CPP. In analogy to the inhibition of STFs elicited by the open channel blocker dizocilpine, STFs evoked by the NMDA receptor antagonist CPP were potently and dose dependently inhibited by the D1 antagonists SCH23390, SCH39166, and NNC756 (Table 2). Likewise, the α2-AR antagonists atipamezole, RX821,002, and idazoxan all blocked the induction of STFs by CPP (Table 2). Lack of Induction of STFs by D1 and α2-AR Agonists. The selective agonists at D1 receptors, SKF38393 (0.63–10.0 mg/kg s.c.), SKF81297 (0.04–2.5), and dihydrexidine (0.04–2.5), did not elicit STFs over a dose-range corresponding to their...
activity (Deveney and Waddington, 1997) at D₁ receptors in other behavioral models (not shown and Table 3). Similarly, the α₂-AR agonist UK14,304 failed to elicit STFs at doses over which it exerts other actions via α₂-ARs (Millan et al., 1994a; Table 3). Furthermore, the combined administration of UK14304 with SKF81297 or dihydrexidine did not elicit a significant STF response (Table 3).

Fig. 5. The potency of α₁/α₂-AR antagonists in blocking dizocilpine-induced STFs is significantly correlated with their affinity for α₂-ARs and with their activity in functional paradigms of α₂-AR-mediated activity. A, affinity at α₂-ARs. B, blockade of xylazine-induced loss of righting reflex in the rat; C and D, blockade of antinociception elicited in the mouse by the α₂-AR agonist UK14304. AC, abdominal constriction test (C); HP, hot-plate test (D). Affinity values are from Renouard et al., 1994, and in vivo data are from Millan et al., 1994.

### TABLE 1

<table>
<thead>
<tr>
<th>Drug 1</th>
<th>Dose</th>
<th>Drug 2</th>
<th>Loss of Righting Reflex</th>
<th>Latency to Fall</th>
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<td>0.0 ± 0.0</td>
<td>264.3 ± 21.2</td>
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<td>0.0 ± 0.0*</td>
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<td>Dizocilpine</td>
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<tr>
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<td>Dizocilpine</td>
<td>N.T.</td>
<td>0.0 ± 0.0*</td>
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<td>46.3 ± 14.1</td>
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<tr>
<td>SCH23390</td>
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<td>SCH23390</td>
<td>2.4 ± 0.2</td>
<td>7.2 ± 4.6*</td>
</tr>
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</table>

N.T., not tested.

*P < .05 compared with vehicle/vehicle.

### Dizocilpine

Animals treated 1 week earlier with an i.c.v. injection of the serotonergic neurotoxin 5,7-DHT showed a pronounced reduction in levels of 5-HT and the 5-HT metabolite 5-hydroxyindoleacetic acid in the CNS. In the lumbar spinal cord, their levels were reduced by 90 and 96%, respectively (for 5-HT: vehicle, 427.7 ± 42.1 pg/mg tissue; 5,7-DHT, 42.7 ± 9.3, P < .001; for 5-hydroxyindoleacetic acid: vehicle, 564.7 ± 74.3 pg/mg tissue; 5,7-DHT, 24.0 ± 7.4, P < .001). In contrast, there was no significant alterations in levels of NE.
or DA in the spinal cord or other tissues (not shown). Despite the substantial reduction in levels of 5-HT in lesioned rats, the dose-response relationship for induction of STFs by dizocilpine was not significantly modified (Fig. 6). Furthermore, on pretreatment of naive rats with the SSRIs citalopram (2.5 mg/kg s.c.), fluoxetine (2.5 mg/kg s.c.), and paroxetine (2.5 mg/kg s.c.), in no case was the induction of STFs by dizocilpine altered (vehicle/dizocilpine, 48.2 ± 6.1 mg/kg s.c.), fluoxetine (2.5 mg/kg s.c.), and paroxetine (2.5 mg/kg s.c.) failed to modify the action of dizocilpine (vehicle/dizocilpine, 45.8 ± 6.6 mg/kg s.c.), and ritanserin (2.5 mg/kg s.c.) failed to modify the action of dizocilpine [vehicle/dizocilpine (0.08), 49.0 ± 9.8 STFs/5 min, P < .05]. In addition, the 5-HT2 agonist (2,5-dimethoxy-4-iodophenyl)-2-aminopropane (0.04 mg/kg s.c.) did not modify the action of dizocilpine [vehicle/dizocilpine (0.08), 49.8 ± 8.8; (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (0.04 mg/kg s.c.) did not modify the action of dizocilpine [vehicle/dizocilpine (0.08), 49.8 ± 8.8; (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane/dizocilpine, 49.0 ± 9.8 STFs/5 min, P > .05]. Finally, the selective 5-HT3 receptor antagonist ondansetron did not influence the induction of STFs by dizocilpine [vehicle/dizocilpine, 47.0 ± 9.3, ondansetron (2.5 mg/kg s.c.)/dizocilpine, 51.2 ± 7.2, P > .05].

Influence of Antagonists at Multiple Serotonergic Receptors on Induction of STFs by Dizocilpine. The 5-HT1A receptor antagonists BMY7378 (2.5 mg/kg s.c.), NAN 190 (2.5 mg/kg s.c.), and (−)-pindolol (10.0 mg/kg s.c.) all failed to modify the induction of STFs by dizocilpine [vehicle/dizocilpine, 48.6 ± 7.4, BMY7378/dizocilpine, 39.4 ± 8.4, P > .05; vehicle/dizocilpine, 45.1 ± 9.9, NAN190/dizocilpine, 36.7 ± 6.4, P < .05; vehicle/dizocilpine, 42.5 ± 9.1, (−)-pindolol, dizocilpine 45.8 ± 11.1, P > .05]. These doses are higher than their respective ID50 values of 0.4, 0.03, and 0.9 mg/kg s.c. for inhibition of STFs elicited by the 5-HT1A agonist 8-OH-DPAT (8-hydroxy-2-dipropylaminotetralin; Millan et al., 1991). The mixed antagonist at 5-HT2A, 5-HT2B, and 5-HT2C receptors, ritanserin (2.5 mg/kg s.c.), as well as the selective 5-HT2A antagonist MDL100,907 (2.5 mg/kg s.c.), failed to modify the action of dizocilpine (vehicle/dizocilpine, 46.6 ± 7.9, ritanserin/dizocilpine, 31.5 ± 8.8, P > .05; vehicle/dizocilpine, 31.6 ± 4.6, MDL100,907/dizocilpine, 44.3 ± 4.2, P > .05). In addition, the 5-HT2 agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (0.04 mg/kg s.c.) did not modify the action of dizocilpine [vehicle/dizocilpine (0.08), 49.8 ± 8.8; (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane/dizocilpine, 49.0 ± 9.8 STFs/5 min, P > .05]. Finally, the selective 5-HT3 antagonist ondansetron did not influence the induction of STFs by dizocilpine [vehicle/dizocilpine, 47.0 ± 9.3, ondansetron (2.5 mg/kg s.c.)/dizocilpine, 51.2 ± 7.2, P > .05].

Influence of Antipsychotic Agents on Induction of STFs by Dizocilpine. The influence of antipsychotics on dizocilpine-induced STFs may be compared with doses eliciting catalepsy (Millan et al., 1998a,b). Consistent with the lack of a major role of D2 (or D3) receptors in the induction of STFs, the D2/D3 antagonist raclopride only weakly inhibited the induction of STFs by dizocilpine (Table 4). Indeed, its ID50 value was 10-fold higher than that for the induction of catalepsy (0.2 mg/kg s.c.; Millan et al., 1998b). Similarly, the

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### TABLE 2

<table>
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<th>Class</th>
<th>Drug</th>
<th>ID50</th>
<th>95% CL</th>
<th>MOI</th>
<th>Dose</th>
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<td>0.03–0.20</td>
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<td>0.63</td>
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95% CL, 95% confidence limits; MOI, maximal observed inhibition.

### TABLE 3

<table>
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<tr>
<th>Drug 1</th>
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<th>Drug 2</th>
<th>Dose</th>
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<td>SKF81297</td>
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<tr>
<td>UK14304</td>
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<td>Dihydrexidine</td>
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</table>

There were no significant drug effects (P > .05).

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![Fig. 6](image-url) **Fig. 6.** The dose-response relationship for the induction of STFs by dizocilpine is not altered after 5,7-DHT lesions of serotonergic pathways. Data are means ± S.E. (n = 5 per value). ANOVA results were 5,7-DHT × dizocilpine: F4,41 = 0.2, P > .05; dizocilpine: F4,41 = 11.4, P < .001; and 5,7-DHT: F1,41 = 0.1, P > .05. *P < .05, significance of drug versus vehicle (VEH).**
neuroleptic and preferential D₂ antagonist haloperidol blocked dizocilpine-induced STFs (Table 4, Fig. 7) only at doses 6-fold higher than those eliciting catalepsy (0.15 mg/kg s.c.; Millan et al., 1998a). ORG5222 and ocaperidone, which possess potent and prominent D₂ antagonist properties, blocked STFs at doses (Table 4) similar to those eliciting catalepsy: 0.3 and 0.2 mg/kg s.c., respectively. However, risperidone, olanzapine, and ziprasidone, which possess less marked activity at D₂ than other monoaminergic receptor types, all reduced STFs at doses lower than those provoking catalepsy: 1.3, 7.5, and 4.0 mg/kg, respectively. Moreover, sertindole, which shows a similar receptorial profile, blocked STFs without evoking catalepsy (4.0 mg/kg). Similarly, amperozide, clozapine, quetiapine, and S16924, which have modest affinity for D₂ receptors, blocked STFs without eliciting catalepsy at doses up to 40.0 mg/kg s.c.

**Correlation Analysis for Antipsychotics Relative to Affinity at α₂A-ARs.** As indicated earlier, α₂A-ARs are implicated in the induction of STFs by dizocilpine, and the antagonist potency of antipsychotics against dizocilpine-induced STFs correlated significantly with their affinity for rat α₂A-ARs (Millan et al., 1998a; r = 0.65, P < .05). Interestingly,

<table>
<thead>
<tr>
<th>Drug</th>
<th>ID₅₀</th>
<th>95% CL</th>
<th>MOI</th>
<th>Dose</th>
<th>%</th>
<th>mg/kg s.c.</th>
</tr>
</thead>
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<tr>
<td>ORG5222</td>
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<td>0.2–0.5</td>
<td>100</td>
<td>2.5</td>
<td></td>
<td></td>
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<tr>
<td>Ocaperidone</td>
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<td>83</td>
<td>0.63</td>
<td></td>
<td></td>
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<tr>
<td>Risperidone</td>
<td>0.5</td>
<td>0.2–0.9</td>
<td>95</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S16924</td>
<td>0.8</td>
<td>0.5–1.3</td>
<td>98</td>
<td>2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amperozide</td>
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<td>0.3–2.1</td>
<td>95</td>
<td>10.0</td>
<td></td>
<td></td>
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<tr>
<td>Haloperidol</td>
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<td>0.5–1.5</td>
<td>91</td>
<td>2.5</td>
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<td></td>
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<tr>
<td>Raclopride</td>
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<td>1.1–7.6</td>
<td>93</td>
<td>10.0</td>
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<td></td>
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<tr>
<td>Olanzapine</td>
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<td>1.6–5.5</td>
<td>98</td>
<td>10.0</td>
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<tr>
<td>Ziprasidone</td>
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<td>83</td>
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<td>Clozapine</td>
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<td>95</td>
<td>40.0</td>
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<tr>
<td>Sertindole</td>
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<td>2.1–12.8</td>
<td>98</td>
<td>40.0</td>
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<tr>
<td>Quetiapine</td>
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<td>2.8–16.6</td>
<td>89</td>
<td>40.0</td>
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</tr>
</tbody>
</table>

95% CL, 95% confidence limits; MOI, maximal observed inhibition.

**TABLE 4**

Influence of antipsychotic agents on induction of STFs by dizocilpine (0.08 mg/kg s.c.)

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![Image](https://via.placeholder.com/150)

**Fig. 7.** Antipsychotic agents block the induction of STFs by dizocilpine (0.08 mg/kg s.c.). Data are means ± S.E. (n = 5 per value). ANOVA results were haloperidol: F₄,₄₆ = 11.8, P < .001; clozapine: F₃,₃₆ = 6.0, P < .01; olanzapine: F₃,₂₂ = 10.2, P < .001; quetiapine: F₄,₂₄ = 6.6, P < .001; ziprasidone: F₃,₂₉ = 3.0, P < .05; risperidone: F₄,₃₉ = 6.3, P < .001; sertindole: F₄,₄₁ = 7.5, P < .001; and S16924: F₃,₂₉ = 7.3, P < .001. *P < .05, significance of drug versus vehicle (VEH).
when these data were reanalyzed incorporating the α2-AR antagonists indicated in Fig. 5, the correlation coefficient was highly significant ($r = 0.79, P < .001$; Fig. 8).

Discussion

Modulation of Accumbens Levels of NE, DA, and 5-HT. At doses exerting a maximal STF response, dizocilpine and CPP elevated extracellular levels of NE in the nucleus accumbens, whereas the glycineB antagonist L701,324, which fails to elicit STFs, was ineffective (Millan, 1991; Millan et al., 1999a). This elevation in nucleus accumbens levels of NE data suggests that NMDA receptors may exert a tonic, inhibitory influence on extracellular levels of NE in the nucleus accumbens. Inasmuch as glutamatergic input onto adrenergic neurons in the locus ceruleus is excitatory (Jodo and Aston-Jones, 1997), this is an unlikely site of action. Thus, in analogy to local NMDA receptors excitatory to NE release in frontal cortex and hippocampus (Yoshida et al., 1997), NMDA receptors inhibitory to NE release, possibly acting via inhibitory GABAergic interneurons, may be localized in the nucleus accumbens itself (Zhang et al., 1993). Consistent with this possibility, local infusion of dizocilpine into the nucleus accumbens increased extracellular levels of NE therein (Yan et al., 1997). This interaction would also be consistent with induction of STFs by intra-accumbens injection of dizocilpine and CPP (Millan et al., 1999a) and with their blockade of α2-AR antagonists (see later).

The complex pattern of direct and indirect, facilitatory and inhibitory modulation of mesolimbic dopaminergic transmission by various populations of NMDA receptors likely accounts for the variable and dose-dependent influence of systemic dizocilpine on nucleus accumbens levels of DA (Connelly and Shepard, 1997; Meltzer et al., 1997; Morari et al., 1998). Indeed, certain groups reported modest increases (Yan et al., 1997; Mathé et al., 1998), whereas others, using either dizocilpine or selective NMDA receptor antagonists, have seen no increase (Westerink et al., 1996; Pierce et al., 1998). Indeed, certain groups reported modest increases (Yan et al., 1997; Mathé et al., 1998), whereas others, using either dizocilpine or selective NMDA receptor antagonists, have seen no increase (Westerink et al., 1996; Pierce et al., 1998). In line with the latter studies, even a high dose of dizocilpine elicited only a mild and nonsignificant increase in nucleus accumbens levels of DA. Furthermore, at a dose sufficient to elicit a maximal STF response, dizocilpine did not affect DA levels, and CPP likewise did not modify levels of DA in the nucleus accumbens. Thus, an elevation in nucleus accumbens release of DA is not involved in the STF response to open channel blockers and NMDA receptor antagonists.

Dizocilpine elevates dialysate levels of 5-HT in frontal cortex, hippocampus, and striatum and accelerates the turnover of 5-HT in several regions, including the nucleus accumbens (Whittington et al., 1992). Furthermore, systemically administered at 0.3 mg/kg i.p. and locally perfused at 50 μM, dizocilpine augmented extracellular 5-HT levels in the nucleus accumbens (Yan et al., 1997). This suggests that nucleus accumbens-localized NMDA receptors may suppress serotonergic transmission, presumably via GABAergic interneurons (Young and Bradford, 1993; Zhang et al., 1993; Millan et al., 1999a). Indeed, herein, a high (0.63) dose of dizocilpine enhanced levels of 5-HT. However, at the lower dose (0.08), which raised NE levels and elicited a full STF response, dizocilpine did not affect 5-HT levels, and CPP was likewise ineffective. These data suggest that an increase in nucleus accumbens levels of 5-HT is not required for the induction of STFs, which is in line with 5,7-DHT data discussed later.

Role of α2-ARs. A major role of α2-ARs in NMDA receptor-mediated STFs is indicated by their blockade with chemically diverse α2-AR antagonists, the potency of which correlated with their activities in other functional models of α2-AR-mediated activity (Fig. 5). Interestingly, the α2A-AR subtype was previously implicated in these in vivo paradigms (Millan et al., 1994a; Hunter et al., 1997), and antagonist potency for blockade of dizocilpine-induced STFs correlated markedly with affinity at rat α2A-ARs (Fig. 5). Inasmuch as dizocilpine and CPP augment NE levels in nucleus accumbens (vide supra), direct blockade of α2-ARs therein may well be involved in the inhibitions by α2-AR antagonists of STFs. However, adrenergic mechanisms in the frontal cortex also contribute to the control of motor function (Gioanni et al., 1998), and definitive identification of the population of α2-ARs involved in the induction of STFs requires further study.

Inasmuch as the α2A-AR agonist UK14304 did not elicit STFs (Table 3), α2-ARs appear to play a permissive role in their expression. Interestingly, in contrast to the motor-suppressive influence of α1A-AR autoreceptors (Millan et al., 1994a), postsynaptic α2-AR sites may fulfill an excitatory role (Nutt, 1994; Nutt and Nittayekosol, 1997). Notably, dizocilpine and clonidine synergistically enhance motor activity in reserpine-treated mice, a paradigm in which postsynaptic actions of α2-AR agonists are eliminated (Carlsson and Svensson, 1990). Analogous studies of STFs would be of interest to perform.

Role of D1 Receptors. Dizocilpine- and CPP-induced STFs were abolished by the selective D1 antagonists SCH23390, SCH39166, and NNC756 (Josselyn et al., 1997), demonstrating that D1 (or closely related D1) receptors play an essential role in their expression. However, inasmuch as the selective D1 agonists dihydrexidine and SKF81297 did not elicit STFs, D1 receptors may, like α2-ARs, play a permissive role in this response. In contrast to D1 agonists, the D2 antagonist L741,626, the D3 antagonists GR218,231 and S14297, and the D4 antagonists S18126 and L745,870 were ineffective (Audinot et al., 1998; Millan et al., 1998b).

Fig. 8. The potency of antipsychotics in blocking dizocilpine-induced STFs is significantly correlated to their affinity at α2-ARs ($r = 0.65, P < .05$ for antipsychotic agents (▲) and $r = 0.79, P < .001$ for all drugs).
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may synergistically facilitate motor function (see Introduction for citations). Very recently, D1 receptors were shown to enhance NE release in the nucleus accumbens (Vandercruz et al., 1999), suggesting that their activation might intervene in the elevation of dialysate level of NE elicited by dizocilpine and CPP.

As mentioned, neither dizocilpine nor CPP increased DA levels in the nucleus accumbens at doses eliciting a maximal STF response, despite their sensitivity to D1 antagonists. Such observations may be assimilated into an intriguing body of evidence suggesting that certain functional actions of NMDA receptor antagonists, exerted in interaction with dopaminergic mechanisms, are integrated in the nucleus accumbens postsynaptic to dopaminergic pathways, presumably via actions at NMDA/dopaminergic receptors colocalized on individual neurons (Carlsson and Carlsson, 1990; Klockgether and Turski, 1990; Ouagazzal et al., 1994; Svensson et al., 1994; Smith et al., 1997; Snyder et al., 1998). On the other hand, dizocilpine and CPP elevate DA levels in the frontal cortex and, albeit less markedly, the striatum (Nishijima et al., 1994). D1 receptors in these structures also control motor function, and both the frontal cortex (via glutamatergic pathways) and the striatum interact with the nucleus accumbens in the control of motor behavior. Thus, actions at D1 receptors therein could be indirectly involved in the mediation of STFs by open channel blockers and NMDA receptor antagonists (Josselyn et al., 1997; Gioanni et al., 1998).

Independence from Serotonergic Mechanisms. Although activation at postsynaptic 5-HT1A receptors elicits STFs (see Introduction), induction of STFs by dizocilpine and CPP does not reflect serotonergic mechanisms. First, 5-HT1A receptors mediating STFs are localized in the dorsal horn (Bervoets et al., 1992), yet intrathecal administration of dizocilpine or CPP does not elicit STFs (Millan, 1999a). Second, the activity of serotonergic pathways running to the spinal cord from the raphe magnus is not modified by dizocilpine or CPP (Lejeune et al., 1994). Third, in contrast to 5-HT releasers, induction of STFs by dizocilpine was affected by neither the serotonergic neurotoxin 5,7-DHT nor SSRIs. Fourth, in distinction to 5-HT releasers and 8-OH-DPAT (Millan et al., 1991; Bervoets et al., 1993), 5-HT1A receptor antagonists did not attenuate induction of STFs by dizocilpine. Furthermore, although 5-HT2C receptor agonists facilitate induction of STFs by 8-OH-DPAT (Millan et al., 1997), they did not affect the actions of dizocilpine.

Clearly, the modulation of STFs elicited by dizocilpine compared with those evoked by the 5-HT1A agonist 8-OH-DPAT differs markedly. This reflects the involvement of contrasting neuronal circuits and receptorial mechanisms. Notably, although D1 antagonists block dizocilpine-elicited STFs, they do not affect the induction of STFs by 8-OH-DPAT (Millan et al., 1991, 1994b; Bervoets and Millan, 1994). The differential modulation of dizocilpine- compared with 8-OH-DPAT-induced STFs by α1- and α2-AR antagonists is of particular interest. Thus, the ability of α1- and α2-AR antagonists to inhibit and enhance, respectively, 8-OH-DPAT-induced STFs (Millan et al., 1991, 1994b; Bervoets and Millan, 1994) differs to their influence on STFs mediated by inactivation of transmission at NMDA receptors. In the former case, the sensitivity of 8-OH-DPAT-induced STFs to blockade by α2-AR antagonists reflects the engagement of an adrenergic link at motoneurons in the ventral horn (Bervoets and Millan, 1994; Millan et al., 1994b). This connection is clearly not operative for STFs elicited by antagonism of NMDA receptors in the nucleus accumbens (Millan et al., 1999a). Indeed, the pathway or pathways descending to motor centers of the spinal cord via which STFs must ultimately be triggered by NMDA receptor antagonists remain to be elucidated.

Influence of Antipsychotic Agents. In line with the inactivity of L741,626, both the neuroleptic haloperidol and the benzamide raclopride, which are likewise preferential D2 receptor antagonists (Meltzer, 1995; Millan et al., 1998a), weakly blocked the induction of STFs by dizocilpine, being active only at supracataleptic doses (see Results). In distinction, clozapine and several other potentially “atypical” antipsychotic agents possessing modest affinity for D2 receptors, S16924, quetiapine, and amperozide, all blocked STFs despite their lack of cataleptogenic potential (Brunello et al., 1995; Meltzer, 1995; Millan et al., 1998a). Similarly, olanzapine and sertindole (Meltzer, 1995) were active at relatively low doses. As mentioned, the selective blockade of α2-ARs abolished the induction of STFs by dizocilpine and CPP, and there was a significant correlation between antipsychotic potency in blocking STFs elicited by dizocilpine and their affinity for α2-ARs (Fig. 8). However, correlation coefficients were not significant for D1, D2, α1-AR, 5-HT2A, or 5-HT2C receptors (r = 0.14–0.44, P > .05 in each case). This suggests that α2-AR antagonist properties may, at least partially, be involved in inhibition of STFs by antipsychotic agents. There is increasing interest in the potential significance of the α2-AR antagonist properties of clozapine and in the management of schizophrenia (Breier et al., 1994; Nutt, 1994; Brunello et al., 1995). However, rather than selective blockade of α2-ARs per se, it is the association of α2-AR antagonist actions that may improve clinical profiles of antipsychotic agents (Litman et al., 1996). Similarly, although STFs were abolished by D1 antagonists, such agents have not, to date, demonstrated antipsychotic efficacy: rather, balanced D1/D2 blockade may be a more effective strategy to improve efficacy while limiting extrapyramidal side effects (Brunello et al., 1995; Karlsson et al., 1995).

Nevertheless, blockade of dizocilpine-induced STFs by clozapine and other antipsychotics is of considerable interest in light of evidence for a dysfunction of glutamatergic transmission and of NMDA receptors in the pathogenesis of schizophrenia (Tsai et al., 1998). The precise neuronal and receptorial mechanisms subserving the STF response to NMDA receptor blockade thus justify further evaluation. Indeed, although sharing potent blockade by D1 antagonists, dizocilpine-induced STFs may be differentiated from dizocilpine-induced hyperlocomotion, which, in contrast, can be blocked by antagonists at D2, α1-AR, and/or 5-HT2A receptors (Ouagazzal et al., 1993; Carlsson, 1995; Svensson et al., 1995; Narayanan et al., 1996). Moreover, dizocilpine-induced STFs, but not locomotion, are blocked by α2-AR antagonists. The STF paradigm may thus provide novel insights into interactions among glutamatergic and monoaminergic mechanisms involved in the actions of antipsychotics and other classes of drugs that control mood and motor behavior.

Conclusions. STFs elicited by open channel blockers and NMDA receptor antagonists are dependent for their expression on α2-ARs and D1 receptors. Inhibition of STFs by antipsychotics may involve, at least partially, the blockade of
α2-ARs. Although it would be inappropriate to consider blockade of dizocline-induced STFs as predictive of antipsychotic activity per se, this paradigm is of pertinence to schizophrenia in several complementary respects. First, a perturbation of corticolimbic glutamatergic mechanisms may contribute to the pathogenesis of schizophrenia. Second, antagonist properties at D1 and α2-ARs are involved in the actions of clozapine and other antipsychotic agents. Third, a further characterization of the circuitry underlying induction of STFs may provide insights into the interrelationships among glutamatergic transmission, monoaminergic networks, and other transmitters implicated in the functional and emotional deficits accompanying psychiatric and neurological disorders.

References


Carlsson M and Svensson A (1990) The non-competitive NMDA antagonists, MK-801 and PCP, as well as the competitive NMDA antagonist, SDZ EA494 (D-CPPene), interact synergistically with clonidine to promote locomotion in monoamine-depleted mice. Life Sci 47:1729–1736.


