

# SLV313 (1-(2,3-Dihydro-Benzo[1,4]Dioxin-5-yl)-4-[5-(4-Fluoro-Phenyl)-Pyridin-3-ylmethyl]-Piperazine Monohydrochloride): A Novel Dopamine D<sub>2</sub> Receptor Antagonist and 5-HT<sub>1A</sub> Receptor Agonist Potential Antipsychotic Drug

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Combined dopamine D<sub>2</sub> receptor antagonism and serotonin (5-HT)<sub>1A</sub> receptor agonism may improve efficacy and alleviate some side effects associated with classical antipsychotics. The present study describes the *in vitro* and *in vivo* characterization of 1-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-4-[5-(4-fluoro-phenyl)-pyridin-3-ylmethyl]-piperazine monohydrochloride (SLV313), a D<sub>2/3</sub> antagonist and 5-HT<sub>1A</sub> agonist. SLV313 possessed high affinity at human recombinant D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>1A</sub> receptors, moderate affinity at 5-HT<sub>7</sub> and weak affinity at 5-HT<sub>2A</sub> receptors, with little-no affinity at 5-HT<sub>4</sub>, 5-HT<sub>6</sub>,  $\alpha_1$ , and  $\alpha_2$  (rat), H<sub>1</sub> (guinea pig), M<sub>1</sub>, M<sub>4</sub>, 5-HT<sub>3</sub> receptors, and the 5-HT transporter. SLV313 had full agonist activity at cloned h5-HT<sub>1A</sub> receptors (pEC<sub>50</sub> = 9.0) and full antagonist activity at hD<sub>2</sub> (pA<sub>2</sub> = 9.3) and hD<sub>3</sub> (pA<sub>2</sub> = 8.9) receptors. *In vivo*, SLV313 antagonized apomorphine-induced climbing and induced 5-HT<sub>1A</sub> syndrome behaviors and hypothermia, the latter behaviors being antagonized by the 5-HT<sub>1A</sub> antagonist WAY100635. In a drug discrimination procedure SLV313 induced full generalization to the training drug flesinoxan and was also antagonized by WAY100635. In the nucleus accumbens SLV313 reduced extracellular 5-HT and increased dopamine levels in the same dose range. Acetylcholine and dopamine were elevated in the hippocampus and mPFCx, the latter antagonized by WAY100635, suggesting possible 5-HT<sub>1A</sub>-dependent efficacy for the treatment of cognitive and attentional processes. SLV313 did not possess cataleptogenic potential (up to 60 mg/kg p.o.). The number of spontaneously active dopamine cells in the ventral tegmental area was reduced by SLV313 and clozapine, while no such changes were seen in the substantia nigra zona compacta following chronic administration. These results suggest that SLV313 is a full 5-HT<sub>1A</sub> receptor agonist and full D<sub>2/3</sub> receptor antagonist possessing characteristics of an atypical antipsychotic, representing a potential novel treatment for schizophrenia.

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## INTRODUCTION

Schizophrenia is characterized by the expression of a variety of symptomatologies such as negative symptoms (affective flattening, social withdrawal, anhedonia, and poverty of thought and content of speech), positive symptoms (delusions, hallucinations, and conceptual dis-

organization), disruptions of cognitive processing and mood disorders (eg Meltzer, 1999). For many years, the typical antipsychotic agents, for example haloperidol and chlorpromazine, were the most widely used drugs for the treatment of schizophrenia. However, the development of neurological side effects (dystonia, muscle rigidity, tremor and akathisia, and tardive dyskinesia) or extrapyramidal side effects (EPS) are often treatment limiting (Casey, 1996; Seeman *et al*, 1976). Atypical antipsychotics, such as clozapine, olanzapine, ziprasidone, quetiapine, and more recently aripiprazole have been developed for the combination of reduced EPS liability and the ability to treat, for example, negative symptoms.

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Many atypical antipsychotics have interactions with serotonergic receptors and it has been suggested that serotonergic actions are important not only in increasing treatment efficacy but also in reducing EPS. While the serotonin 5-HT<sub>2A</sub> receptor may play a role in mediating the reduction in EPS observed with atypical antipsychotics (Meltzer, 1999) efficacy at 5-HT<sub>1A</sub> receptors might also be beneficial (for reviews see Millan, 2000; Meltzer *et al*, 2003). Interestingly, in the schizophrenic brain 5-HT<sub>1A</sub> receptor binding is altered in the hippocampus, cortex, and amygdala (Burnet *et al*, 1996; Gurevich and Joyce, 1997; Bantick *et al*, 2001; Yasuno *et al*, 2004). Atypical antipsychotics such as clozapine, ziprasidone, aripiprazole, and quetiapine are all 5-HT<sub>1A</sub> (partial) agonists; which may be very relevant for their actions in treating schizophrenia (Newman-Tancredi *et al*, 1996, 2001; Sprouse *et al*, 1999; Rollema *et al*, 2000; Jordan *et al*, 2002). For example, in the conditioned avoidance response (CAR) model of schizophrenia the nonselective 5-HT<sub>1A</sub> agonist ( $\pm$ )-8-hydroxy (di-*n*-aminopropylamino) tetralin (8-OH-DPAT) not only induced CAR disruption but enhanced the effects of the dopamine D<sub>2</sub> receptor antagonists haloperidol and raclopride (Wadenberg and Ahlenius, 1991; Prinssen *et al*, 1996). Rather than increasing EPS the 5-HT<sub>1A</sub> agonists 8-OHDPAT and ipsapirone reduced EPS-like symptoms in rodents and non-human primates (Prinssen *et al*, 1999, 2000; Wadenberg and Ahlenius, 1991; Wadenberg, 1996; Wadenberg *et al*, 1999; Christoffersen and Meltzer, 1998). These preclinical studies are supported by the finding that buspirone and tandospirone attenuated Parkinsonian-like signs and tardive dyskinesia seen in schizophrenics (Goff *et al*, 1991; Yoshida *et al*, 1998). Together these data support the concept that mixed 5-HT<sub>1A</sub> agonistic/dopamine D<sub>2</sub>-like receptor antagonistic effects could be beneficial for the treatment of schizophrenia.

Additional evidence suggests that a variety of atypical antipsychotics produce a 5-HT<sub>1A</sub>-dependent and preferential increase of dopamine levels in the medial prefrontal cortex (mPFCx; an area thought to have reduced dopaminergic tone in schizophrenics (Weinberger and Lipska, 1995)) vs dorsal striatum (Moghaddam and Bunney, 1990; Nomikos *et al*, 1994; Pehek *et al*, 1993; Hertel *et al*, 1996; Ichikawa *et al*, 2001; Rollema *et al*, 1997, 2000). In addition, atypical antipsychotics, also via 5-HT<sub>1A</sub> receptor-dependent mechanisms, augment acetylcholine (ACh) neurotransmission in the mPFCx which should enhance cognitive function (Ichikawa *et al*, 2002a,b). Furthermore, actions on both dopamine and ACh at the level of the hippocampus (important for declarative (storage) memory and pivotally affected in schizophrenia (Eichenbaum *et al*, 1999; Fernandez *et al*, 2002; Seidman *et al*, 2002)) were both augmented by clozapine, olanzapine, risperidone, and ziprasidone (Shirazi-Southall *et al*, 2002; Chung *et al*, 2004). These effects were sensitive to manipulation with 5-HT<sub>1A</sub> receptor antagonists (eg Li *et al*, 2004). Clinical effects of the atypical antipsychotics to improve long-term memory deficits in schizophrenic patients have been demonstrated (Meltzer and McGurk, 1999; Harvey and Keefe, 2001). As such, if the preclinical work can be extrapolated to the human, the effects seen in microdialysis studies suggest, at least in part, that cognitive enhancement with atypical antipsychotics could be due to increases in hippocampal dopamine

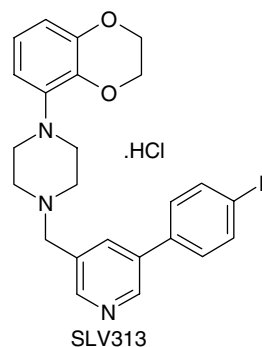
and ACh. Interestingly, the (partial) 5-HT<sub>1A</sub> agonist tandospirone improved cognition in schizophrenic patients (Sumiyoshi *et al*, 2001a,b). It is therefore relevant to further test the hypothesis that novel putative antipsychotics increase mPFCx and hippocampal dopamine and ACh. Compounds that have mixed D<sub>2</sub> and 5-HT<sub>1A</sub> receptor moieties are of special interest.

In summary, it appears that the therapeutic window, side-effect profile and therapeutic efficacy of antipsychotic agents could be improved by the combination of a dopamine D<sub>2</sub> receptor antagonist with 5-HT<sub>1A</sub> receptor agonist properties. Combination approaches with different medications are possible, but not desirable for reasons including issues relating to pharmacokinetics and patient compliance. The obvious choice is to synthesize and develop a single chemical entity. In the present study, we describe the pharmacological characterization of the novel putative antipsychotic agent 1-(2,3-dihydro-benzo[1,4]dioxin-5-yl)-4-[5-(4-fluoro-phenyl)-pyridin-3-ylmethyl]-piperazine monohydrochloride (SLV313; Figure 1), using *in vitro* and *in vivo* assays.

## METHODS

### Receptor Binding

SLV313 was tested for the ability to bind to recombinant receptors expressed in cell systems or receptors sourced from native tissue (rat and guinea pig). The receptor-binding assays were performed at CEREP (Celle l'Evescault, France) using the conditions outlined in Table 1. Following incubation, the receptor preparations were rapidly filtered under vacuum through glass fiber filters which were washed with ice-cold buffer using a harvester. Bound radioactivity was measured by scintillation counting using a liquid scintillation cocktail. Compounds were tested at three logarithm unit concentrations; all determinations were performed as triplicates. IC<sub>50</sub> values were determined by nonlinear regression analysis using Hill equation curve fitting and inhibition constants ( $K_i$ ) calculated using the Cheng-Preushoff equation  $K_i = IC_{50}/(1 + L/K_d)$ , where  $L$  is the concentration of radioligand in the assay and  $K_d$  the affinity of the radioligand for the receptor. Results are expressed as mean  $pK_i \pm SD$  of at least three separate



**Figure 1** The chemical structure of SLV313 (1-(2,3-dihydro-benzo[1,4]-dioxin-5-yl)-4-[5-(4-fluoro-phenyl)-pyridin-3-ylmethyl]-piperazine monohydrochloride).

**Table 1** Details of the Receptor-Binding Assay Methods Used in the Present Studies

Assay	Species	Tissue	Ligand	Non specific binding	Incubation conditions	Bibliography
Adrenergic $\alpha_1$	Rat	Cerebral cortex	[ <sup>3</sup> H]prazosin (0.25 nM)	Prazosin (0.5 $\mu$ M)	60 min/22°C	Greengrass and Bremner (1979)
Adrenergic $\alpha_2$	Rat	Cerebral cortex	[ <sup>3</sup> H]RX-821002 (0.5 nM)	(–) Epinephrine (100 $\mu$ M)	30 min/22°C	Uhlen and Wikberg (1991)
Dopamine D <sub>2S</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]spiperone (0.3 nM)	(+) Butaclamol (10 $\mu$ M)	60 min/22°C	Grandy <i>et al</i> (1989)
Dopamine D <sub>3</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]spiperone (0.3 nM)	(+) Butaclamol (10 $\mu$ M)	60 min/22°C	Mackenzie <i>et al</i> (1994)
Dopamine D <sub>4,4</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]spiperone (0.3 nM)	(+) Butaclamol (10 $\mu$ M)	60 min/22°C	Van Tol <i>et al</i> (1992)
Histamine H <sub>1</sub>	Guinea pig	Cerebellum	[ <sup>3</sup> H]pyrilamine (0.5 nM)	Tripolidine (100 $\mu$ M)	10 min/22°C	Dini <i>et al</i> (1991)
Muscarine M <sub>1</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]pirenzepine (2 nM)	Atropine (1 $\mu$ M)	60 min/22°C	Dorje <i>et al</i> (1991)
Muscarine M <sub>4</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]4-DAMP (0.2 nM)	Atropine (1 $\mu$ M)	60 min/22°C	Dorje <i>et al</i> (1991)
Serotonin 5-HT <sub>1A</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]8-OH-DPAT (0.3 nM)	8-OH-DPAT (10 $\mu$ M)	60 min/22°C	Mulheron <i>et al</i> (1994)
Serotonin 5-HT <sub>2A</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]ketanserin (0.5 nM)	Ketanserin (1 $\mu$ M)	15 min/37°C	Bonhaus <i>et al</i> (1995)
Serotonin 5-HT <sub>2B</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]-LSD (1.2 nM)	Serotonin (10 $\mu$ M)	30 min/37°C	Bonhaus <i>et al</i> (1995)
Serotonin 5-HT <sub>2C</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]mesulergine (0.7 nM)	Mesulergine (1 $\mu$ M)	30 min/37°C	Bonhaus <i>et al</i> (1995)
Serotonin 5-HT <sub>3</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H] BRL43694 (0.5 nM)	MDL-72222 (10 $\mu$ M)	60 min/22°C	Hope <i>et al</i> (1996)
Serotonin 5-HT <sub>4E</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H] GRI 13808 (0.2 nM)	Serotonin (100 $\mu$ M)	30 min/37°C	Mialet <i>et al</i> (2000)
Serotonin 5-HT <sub>6</sub>	Human	Recombinant HEK293 cells	[ <sup>3</sup> H]LSD (2 nM)	Serotonin (100 $\mu$ M)	60 min/37°C	Monsma <i>et al</i> (1993)
Serotonin 5-HT <sub>7</sub>	Human	Recombinant in CHO cells	[ <sup>3</sup> H]LSD (4 nM)	Serotonin (10 $\mu$ M)	120 min/22°C	Shen <i>et al</i> (1993)
Serotonin 5-HT <sub>1P</sub>	Human	Recombinant in HEK 293 cells	[ <sup>3</sup> H]paroxetine (0.1 nM)	Imipramine (10 $\mu$ M)	30 min/22°C	Tatsumi <i>et al</i> (1997)

experiments. The expression of no significant inhibition of radioligand binding at concentrations of 1  $\mu$ M and higher were concluded to be inactive.

### *In Vitro* Functional Activity

Experiments assessing the D<sub>2,L</sub> and 5-HT<sub>1A</sub> receptor functional activity *in vitro* were performed in CHO-K1 cells transfected with either the human D<sub>2,L</sub> receptor (obtained from Dr D Grandy, Vollum Institute Oregon, USA; Bunzow *et al*, 1988) or the human 5-HT<sub>1A</sub> receptor (Allelix Biopharmaceuticals, Mississauga, ON, Canada; Fargin *et al*, 1989). Confluent cultures were grown in 24 well plates in DMEM medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 1 mM pyruvate, 5000 U/ml penicillin, 5000  $\mu$ g/ml streptomycin, and 200  $\mu$ g/ml G418 at 37°C in a 93% air/7% CO<sub>2</sub> mixture. Cells were loaded with 1  $\mu$ Ci [<sup>3</sup>H] adenine in 0.5 ml/well for 120 min and washed with 0.5 ml phosphate-buffered saline (PBS) containing 1 mM IBMX and incubated for 20 min with 0.5 ml PBS containing 1 mM IBMX and forskolin with or without test compound. After aspiration, the reaction was stopped with 1 ml trichloroacetic acid. The [<sup>3</sup>H]ATP and [<sup>3</sup>H]cAMP formed in the cellular extract was assayed as previously reported (Salomon *et al*, 1974; Weiss *et al*, 1985). Briefly, 0.8 ml of extract was passed over Dowex (50WX-4 200–400 mesh) and aluminium oxide columns eluted with water and 0.1 M imidazole (pH = 7.5). Eluates were mixed with 7 ml insta-gel and radioactivity counted with a liquid scintillation counter. The conversion of [<sup>3</sup>H]ATP to [<sup>3</sup>H]cAMP was expressed as the ratio of percentage radioactivity in the cAMP and ATP fractions. Basal activity was subtracted to correct for spontaneous activity. Dopamine D<sub>2,L</sub>-transfected

cells were incubated in the presence of quinpirole (10<sup>–6</sup> M) with the addition of SLV313. Similarly, CHO cells transfected with the 5-HT<sub>1A</sub> receptor were incubated in the presence of SLV313. In addition, the 5-HT<sub>1A</sub> agonist 8-OHDPAT was tested for comparative purposes. All studies were performed in quadruplicate.

Functional activity at the D<sub>3</sub> receptor was assessed using a GTP $\gamma$ S assay (Newman-Tancredi *et al*, 1999) at MDS-PS Laboratories (Taipei, Taiwan). Human recombinant dopamine D<sub>3</sub> receptors were expressed in CHO-K1 cells. SLV313 (dissolved in DMSO) was preincubated in triplicates with the 40  $\mu$ g/ml receptors and 1  $\mu$ M GDP in HEPES buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 1 mM DTT, 1 mM EDTA) for 20 min at 30°C, scintillation proximity assay beads were then added for another 60 min. Ligand displacement was initiated by 0.3 nM [<sup>35</sup>S]GTP $\gamma$ S for an additional 30 min incubation period. Agonistic effects were expressed as percentage of that of control of dopamine (0.3  $\mu$ M) was used as the positive control; antagonistic effects of SLV313 were determined by assessing inhibition of 0.3  $\mu$ M dopamine-induced [<sup>35</sup>S]GTP $\gamma$ S binding and the pA<sub>2</sub> was calculated according to Cheng–Preushoff equation (above). Three independent experiments were performed.

### *In Vivo* Activity

All animals used in the present studies were housed in a temperature (20  $\pm$  2°C) and humidity-controlled environment under a 12-h light-dark cycle (lights on 0700–1900 h). The *a priori* significance levels was set at *p* < 0.05 for all experiments to assess significant differences from control.

All experimental procedures conformed to local animal care and use committee stipulations.

### Antagonism of Apomorphine-Induced Climbing

Male NMRI mice (25–30 g; Charles River, Sulzfeld, Germany  $n = 10$ ) were habituated to a wire-mesh cage 60 min prior to apomorphine (1 mg/kg s.c.; Sigma, St Louis, MO, USA) administration. Vehicle (1% methylcellulose 10 ml/kg; Sigma), SLV313 (0, 0.1, 0.3, 0.6, 1, or 3 mg/kg p.o.), or haloperidol (0.03, 0.1, 0.3, or 1 mg/kg p.o.; Siegfried, Zofingen, Switzerland) were administered 60 min prior to apomorphine (s.c.). In addition, SLV313 (0.3, 1, and 3 mg/kg p.o.) was given 4 h prior to the administration of apomorphine to assess the longevity of the effect. At 10 and 20 min after apomorphine administration the climbing behavior of the mice was scored using an arbitrary rating scale (0: no climbing, 1: standing on hind-paws, and 2: climbing).

Behavioral scores were summed and ED<sub>50</sub> values were calculated from the percentage inhibition of climbing intensity of vehicle-treated mice by logistic regression analysis of the resulting dose–response curve. Fieller's method was used to determine 95% confidence intervals of the ED<sub>50</sub> in comparison to the control. The mean response for each dose is expressed as percentage relative to control together with a 95% confidence interval. A dose was defined to differ significantly from control when the 95% confidence interval did not cover the 100% level of the control group. The lowest dose of a compound that was statistically significant was defined as the minimal effective dose (MED). SAS was used for statistical analyses.

### 5-HT<sub>1A</sub> Receptor-Mediated Syndrome Behavior

Male Wistar rats (180–230 g; Harlan, Zeist, the Netherlands,  $n = 8$  per group) were used in these studies. Vehicle (1% methylcellulose vehicle; 2 ml/kg p.o.), SLV313 (1, 3, and 10 mg/kg p.o.), aripiprazole (Solvay Pharmaceuticals, the Netherlands; 3, 10, and 30 mg/kg p.o.), clozapine (10 mg/kg p.o.), and ziprasidone (Solvay Pharmaceuticals; 10 mg/kg p.o.) were administered and the intensity of flat body posture (FBP), hind limb abduction (HLA), forepaw treading (FPT), and lower lip retraction (LLR) were scored every 10 min 10–60 min after the administration of compound (0 = absent, 1 = present, and 2 = intense) and the intensities were summed across each of the six time epochs (see Berendsen *et al*, 1989; Tricklebank *et al*, 1984). In addition, the ability of SLV313 to induce hypothermia (cf. Bill *et al*, 1991) was assessed by measuring the oral temperature prior to and at the end of the period of behavioral observation. In order to verify that the effects of SLV313 was caused by putative agonist actions at the 5-HT<sub>1A</sub> receptor, the selective 5-HT<sub>1A</sub> receptor antagonist *N*-(2-(4-(2-methoxyphenyl)-1-piperzinyloxy)ethyl)-*N*-(2-pyridinyl)-cyclohexanecarboxamide; WAY100635; 0.03, 0.1, and 0.3 mg/kg s.c.; Solvay Pharmaceuticals) or vehicle were administered 30 min prior to SLV313 (6 mg/kg p.o.) and behavior and hypothermia were assessed as described above.

Temperature data were analyzed by a one-way analysis of variance (1-way ANOVA) with *post hoc* Dunnett's test as appropriate and serotonergic syndrome behaviors were analysed using the nonparametric Kruskal–Wallis with *post hoc* Mann–Whitney test using JMP software (SAS Institute, USA).

### Flesinoxan-Induced Drug Discrimination

Pigeons (Utrecht University, Department of Veterinary Sciences, the Netherlands,  $n = 12$ ) were maintained at approximately 85% free feeding weights by postsessional feeding with standard pigeon grain with water and grit available *ad libitum*, except during behavioral testing.

The apparatus and procedure have been described in detail elsewhere (see Mos *et al*, 1997, for full description). Briefly, experiments were conducted in eight standard Le High Valley three-key pigeon chambers, with only the left and the right key being used. Subjects were trained to discriminate flesinoxan from vehicle on a standard fixed ratio (FR) 30 food reinforced schedule of reinforcement. During training sessions, flesinoxan (Solvay Pharmaceuticals; 0.25 mg/kg p.o., (A)) or vehicle (B) were administered 45 min prior to behavioral sessions. Daily session followed a 10-day repeating (ABAABBABBA) schedule.

**Test sessions.** Test sessions were interspersed between training sessions. The key receiving the first 30 pecks was designated as the 'to-be-rewarded-key' for each individual subject for the rest of that particular test session. A 5 s time out was presented for each peck on the nonselected key after the presentation of the first reinforcement. Test sessions ended after 20 min or 40 reinforcements or if the subject did not respond at all after 5 min, whichever came first. Test sessions were given on Wednesday and Friday, but only if the subject's response to first reinforcement (FRF-value) on three immediately preceding training sessions did not exceed 39. In the latter case, the test session was postponed until accuracy was achieved. Each test dose was given once, unless the FRF-value during the test session exceeded 39.

**Generalization and antagonist tests.** SLV313 (0.01, 0.03, 0.1, and 0.3 mg/kg p.o.) and haloperidol (0.1, 0.3, and 1.0 mg/kg p.o.) were tested for their ability to generalize for the flesinoxan appropriate key. The ability of WAY100635 (0.1, 0.3, and 1 mg/kg p.o.) to attenuate SLV313-induced substitution was also tested. In addition, in an antagonist test the effects of haloperidol on the training dose of flesinoxan were assessed. Flesinoxan (0.25 mg/kg) and compounds used in generalization studies were administered 45 min prior to the experimental session. In antagonism studies, the test drug was administered 15 min prior to administration of the 'agonist,' or 60 min prior to the session. All doses were tested in random order. Drugs were suspended in methylcellulose (1% w/v) and injected orally into the crop in a volume of 1 ml/kg.

**Data analyses.** The percentage of subjects selecting the flesinoxan-appropriate key was taken as a measure of generalization and full generalization was defined as at least 80% of all subjects selected the flesinoxan-associated key, conversely antagonism was defined when <20% of the pigeons failed to respond on the flesinoxan-appropriate key. ED<sub>50</sub> values with 95% confidence limits were calculated using logistic analysis. Response rates were calculated to be the number of keypecks per second until the delivery of the first reinforcement. Control measures were taken from the last three training sessions when flesinoxan was adminis-

tered. Differences between control values and response rates on test sessions were analyzed using Student's *t*-test (two tailed) using the previous training session as the control.

### The Effect of SLV313 on Catalepsy

Male Wistar rats (180–230 g; Harlan, Zeist, the Netherlands,  $n = 8$  per group) were individually housed and habituated to the test room. The rats were treated with vehicle (1% tragacanth; 2 ml/kg p.o.), SLV313 (20 and 60 mg/kg p.o.), or haloperidol (1, 2, 4, or 8 mg/kg p.o.) 4 h before placing their front paws on a 15 cm high bar. The latency to remove their paws was recorded. In addition, an arbitrary definition was used which defined the animals as cataleptic if they maintained their positions for longer than 30 s without the loss of the righting reflex.

**Data analyses.** Latency and incidence (n/N) data were compared using the nonparametric Kruskal–Wallis test with a *post hoc* Wilcoxon test, using JMP statistical software.

### In Vivo Microdialysis Studies

**Effect of SLV313 in the nucleus accumbens.** Male Wistar rats (275–350 g; Harlan, Zeist, the Netherlands;  $n = 3$ –6 per dose) were anesthetized with halothane (1.5% halothane in  $\text{NO}_2/\text{O}_2$  2:1). Baytril (150  $\mu\text{l}$ /rat s.c.; Bayer) and Temgesic (50  $\mu\text{l}$ /rat i.m.; Reckitt and Colman) were injected to prevent infection and induce postoperative analgesia, respectively. Rats were mounted in a stereotaxic instrument (David Kopf Instruments, CA, USA), the scalp incised and a hole was drilled over the anterior part of the nucleus accumbens and guide cannulae (CMA 12, Carnegie Medicine, Sweden) implanted: A: +10.3 (relative to the interaural line); M (relative to midline): –1.4; V (relative to the skull surface): 4.5 mm, (Paxinos and Watson, 1998).

At least 7 days after surgery microdialysis probes (CMA12, 2 mm) were inserted into the guide cannulae the evening before the experiment and perfused with an osmotically balanced Ringers solution containing: 147 mM NaCl, 4 mM KCl, 1.2 mM  $\text{CaCl}_2$ , and 0.7 mM  $\text{MgCl}_2$  at a flow rate of 1.2  $\mu\text{l}$ /min. On the day of the experiment, 20 min samples were collected into a vial containing 50  $\mu\text{l}$  of formic acid/cysteine solution (0.02 M/0.2% w/v) was present in the collecting vial to prevent oxidation of dopamine. After the first five baseline samples, SLV313 (0.1, 0.3, 1, 3, and 10 mg/kg p.o.; 2 ml/kg p.o.) was administered and dialysate samples collected. Resulting samples were frozen on dry ice and stored at  $-80^\circ\text{C}$  until later off-line analysis with high-performance liquid chromatography (HPLC) linked to electrochemical detection.

**HPLC.** Dialysis samples were analyzed with HPLC using electrochemical detection and employing a reversed phase column (Supelcosil LC-8DB,  $25 \times 4.6 \text{ mm}^2$ ,  $\text{dp} = 5 \mu\text{m}$ , Supelco), maintained at  $45^\circ\text{C}$ , a cooled Gilson autoinjector (model 232;  $10^\circ\text{C}$ ) and diluter (model 401) and a Hewlett Packard (model 1050) pump operating at a flow rate of 1 ml/min. The mobile phase consisted of 50 mM acetic acid, sodium acetate (3:1), 1.46 mM HSA, 0.27 mM EDTA, and 16% (v/v) methanol. The final pH was adjusted to 4.9 with 1N NaOH. Dopamine, 3,4-dihydroxyphenyl acetic

acid (DOPAC) and homovanillic acid (HVA), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) were electrochemically detected with an EG&G (model 400, Princeton Applied Research) controller equipped with a glassy carbon working electrode (Antec cell, VT-03, Leiden, the Netherlands). The potential was set at 600 mV vs an Ag/AgCl reference electrode. The output was recorded on a Hewlett Packard (model 3396A) integrator. Calculations (pg/20 min) were made using peak height values of known concentrations of the components previously tested with the apparatus. All reagents used were high standard or HPLC grade.

**Data analyses.** Mean predrug baseline level were designated as 100% and evaluation of the area under the curve (AUC) for 120 min following drug administration, compared to basal dialysate levels (% AUC<sub>120</sub>) was performed to provide a readily visible index for the dose–response relationship. Statistical comparisons were performed with ANOVA and *post hoc* Bonferroni *t*-testing using SAS software.

**Effect of SLV313 in the prefrontal cortex and hippocampus.** Male Sprague–Dawley rats (250–350 g; Zivic-Miller Laboratories, Porterville, PA, USA) as described previously (Ichikawa *et al*, 2001, 2002b) were used in this study. Cannulae were implanted in the mPFCx and hippocampus (coordinates: A: +3.5, L: +1.2 ( $10^\circ$  inclination), V: –5.5 mm, and A: +5.6, L: +5.0, V: –7.0 mm, respectively, relative to bregma; Paxinos and Watson, 1998) and 3–5 days after cannulation, microdialysis probes (polyacrylonitrile/sodium methallylsulfonate polymer, 310  $\mu\text{m}$  o.d., 220  $\mu\text{m}$  i.d., 40 kDa cutoff with 3 mm exposed membrane, AN69HF, Hospal; CGH Medical, Lakewood, CO) were inserted into the mPFCx or hippocampus following light isoflurane-induced anesthesia (induced by placement in an isoflurane-enriched environment and performed during a short 2 min sleep period). Experiments were conducted the following day after an overnight perfusion of Dulbecco's PBS solution (Sigma) including  $\text{Ca}^{2+}$ : (138 mM NaCl, 8.1 mM  $\text{Na}_2\text{HPO}_4$ , 2.7 mM KCl, 1.5 mM  $\text{KH}_2\text{PO}_4$ , 0.5 mM  $\text{MgCl}_2$ , 1.2 mM  $\text{CaCl}_2$  at a pH of 7.4 using a flow rate of 0.4  $\mu\text{l}$ /min). The flow rate was then increased to 1.5  $\mu\text{l}$ /min for the experimental procedures. After stable baseline dialysates were obtained, vehicle or SLV313 (3, 10, and 20 mg/kg p.o.) was administered to the rats and 30 min dialysate samples collected for 210 min from either the hippocampus or mPFCx. In order to assess the role of 5-HT<sub>1A</sub> receptors, following basal dialysate sample collection, as described above, the rats were pretreated with vehicle or WAY100635 (0.2 mg/kg s.c.) and 30 min later with vehicle or SLV313 (10 mg/kg p.o.).

**Determination of dopamine.** Dialysate samples were directly applied onto an HPLC with electrochemical detection and analyzed with a Millennium chromatogram manager (Waters, Milford, MA, USA). Dopamine was separated (BDS Hypersil 3  $\mu\text{m}$  C18,  $1.0 \times 100 \text{ mm}^2$ ; Keystone Scientific, Bellefonte, PA, USA) at  $35^\circ\text{C}$  maintained by column heater (LC-22C Temperature Controller; BAS, West Lafayette, IN, USA). The mobile phase consisted of 48 mM anhydrous citric acid and 24 mM sodium acetate trihydrate containing 0.5 mM EDTA- $\text{Na}_2$ , 10 mM NaCl, 2 mM dodecyl sulfate sodium salt, and 17% (v/v) acetonitrile, adjusted to pH 4.8 with concentrated NaOH and the flow rate was

0.05 ml/min (LC-10AD; Shimadzu, Kyoto, Japan). A unijet working electrode (MF-1003, BAS) was set at +0.58 V (LC-4C, BAS) vs an Ag/AgCl reference electrode. Reagents used were analytical or HPLC grade.

**Determination of ACh.** The method has been described previously in detail (Ichikawa *et al*, 2002b). Briefly, dialysate samples were directly injected onto the liquid chromatography/electrochemistry system assisted by a chromatography manager (Millennium; Waters, Milford, MA, USA), and analyzed for ACh. ACh was separated on a coiled cation exchanger ACh column (analytical column; Sepstik 10 m ID 530°C 1.0 nm; BAS, West Lafayette, IN), followed by the postimmobilized enzyme reactor (IMER; BAS) which consisted of choline oxidase (ChO)/AChesterase. ACh is hydrolyzed by AChesterase to form acetate and choline in the post-IMER, and then choline is oxidized by ChO to produce betaine and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). H<sub>2</sub>O<sub>2</sub> was detected and reduced to H<sub>2</sub>O on a Unijet amperometric detector cell with a peroxidase-redox-coated glassy carbon electrode (MF-9080; BAS), set at +100 mV (LC-4C; BAS) vs Ag/AgCl reference electrode. This reduction was analyzed with the detector (LC-4C; BAS) indicating ACh in the chromatogram.

**Data analyses.** Mean predrug baseline levels were designated as 100% and AUC<sub>120</sub> values were calculated. Following a significant overall repeated measures ANOVA (treatment × time), Fisher's-protected least significant difference *post hoc* pair-wise comparison and one-way ANOVA were used to determine group differences (Stat-View7 4.5. for the Macintosh).

### The Effect of Chronic Administration of SLV313 on the Number of Spontaneously Active Ventral Tegmental Area (VTA) and Substantia Nigra Zona Compacta (SNc) Neurons

Male Sprague-Dawley rats (150–175 g at the start of the study, Taconic Farms, NY, USA) were treated with vehicle (1% methylcellulose, Sigma; 1 ml/kg), clozapine (20 mg/kg p.o., Tocris-Cookson, St Louis, MO, USA) or SLV313 (3 and 10 mg/kg p.o.). All compounds or vehicle were given once daily for 21 days with the experimenter blinded to treatment. The number of spontaneously active SNc and VTA dopamine neurons were determined 2 h after the final administration on day 21.

Animals were anesthetized with chloral hydrate (400 mg/kg i.p., Sigma), mounted in a stereotaxic instrument (David Kopf), placed on a heating pad (Finitronics, CT, USA) to maintain constant body temperature of 37–38°C and the lateral tail vein cannulated with a 25-gauge needle for the administration of supplemental anesthetic. The scalp was incised and a hole drilled over the SNc (anterior 3.0–3.5 mm to the lambda, lateral 1.8–2.5 mm to the midline and 6.0–8.5 mm ventral to the cortical surface) and VTA (anterior 3.0–3.5 mm, lateral 0.5–1.0 mm and ventral 6.0–8.5 mm; Paxinos and Watson, 1998).

Single barrel microelectrodes (glass borosilicate; WPI, FL, USA with a carbon filament and filled with 2 M NaCl and saturated 1% fast green (Sigma Chemical Co., USA)) pulled

with an electrode puller (Narishige PE-2, Japan) and the tip broken back under a light microscope were used for recording single cellular dopamine activity. Impedances were in the range of 0.8–1.2 MΩ measured at 135 Hz *in vitro* and 1.5–2.0 MΩ *in vivo*.

The electrodes were passed through the SNc and VTA areas in a systematic manner 10 times using a hydraulic microdrive (David Kopf, Model 640). The electrode tracks were performed in a preset sequence kept constant from animal to animal. For half of the rats in each group, the order of recording was SNc–VTA and the order was reversed for the other rats. The number of spontaneously active dopamine neurons was determined in 10 stereotaxic electrode descents or tracks as previously described (White and Wang, 1983; Chiodo, 1988). Briefly, recordings from 10 electrode tracks (separated by 200 μm) with a constant sequence from animal to animal were performed. Each electrode descent was made in a slow, uniform speed (1–3 μm/s) using the microdrive. Only those cells whose electrophysiological profile matched those previously established for midbrain dopamine cells were counted. During the recording sessions, neurons encountered were considered dopaminergic if they had a wide action potential, with a distinct initial segment, late positive component and a characteristic low-pitch sound when monitored via an audioamplifier; a slow regular or bursting firing pattern and a spontaneous firing rate of 2–9 Hz (White and Wang, 1983). Data were analyzed using a one-way ANOVA and *post hoc* Student–Newman–Keuls test as necessary.

**Histology.** At the end of each experiments, a 25 μA cathodal current (Finitronics cathodal current source, Model VL-1200D) was passed through the recording electrode for 15 min, to deposit fast green dye in the brain. Animals were overdosed with chloral hydrate (i.v.) and perfused transcardially with 10% buffered formalin (VWR Scientific, Bridgeport, NJ, USA) for 10 min. The brains were removed and serial coronal sections (50 μm) were made using a cryostat at –20°C, stained with cresyl violet and counterstained with neutral red. The dye spot was viewed under a light microscope and served as a reference point for the location of each cell. Only data were used from animals with electrode placements in the SNc and VTA.

## RESULTS

### Receptor Binding Profile

SLV313 binds with high affinity to the serotonin 5-HT<sub>1A</sub> receptor (pK<sub>i</sub> 9.1), the dopamine D<sub>2</sub>-like receptors (D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> receptors (pK<sub>i</sub>s of 8.4, 8.4, and 8.0, respectively) and serotonin 5-HT<sub>2B</sub> receptors (Table 2). Furthermore, SLV313 had moderate affinity for the serotonin 5-HT<sub>7</sub> receptor (pK<sub>i</sub> 7.2), some affinity for serotonin 5-HT<sub>2A</sub> receptors (pK<sub>i</sub> 6.6) and adrenergic α<sub>1</sub> receptors (pK<sub>i</sub> 6.3). SLV313 showed no appreciable affinity for a series of other monoamine sites (pK<sub>i</sub> < 6), including serotonin 5-HT<sub>2C</sub> and 5-HT<sub>6</sub>, adrenergic α<sub>2</sub> receptors, histamine H<sub>1</sub> and the muscarinic M<sub>1</sub> and M<sub>4</sub> receptors. Table 2 shows the pK<sub>i</sub>'s of SLV313 and a series of reference antipsychotics (clozapine, haloperidol, risperidone, ziprasidone, and olanzapine). In addition, a wider

screen demonstrated that there was no receptor binding observed at a range of additional receptors including glutamate, imidazoline, NT<sub>1</sub>, benzodiazepine, H<sub>2</sub>, angiotensin, and neurokinin receptors ( $pK_i < 6$ ; data not shown).

### In Vitro Functional Activity

Quinpirole fully attenuated forskolin-induced accumulation of cAMP in CHO cells with a  $pEC_{50}$  of 6.8. The effect of  $10^{-6}$  M quinpirole was concentration dependently antagonized by SLV313 ( $pA_2$   $9.3 \pm 0.3$ , Figure 2 upper panel). Using similar methodologies, SLV313 was found to be a full and potent agonist at 5-HT<sub>1A</sub> receptors with a  $pEC_{50}$  of  $9.0 \pm 0.2$  (Figure 2, lower panel). In addition, the effects of SLV313 were measured using a GTP $\gamma$ S-binding system in CHO cells transfected with the human dopamine D<sub>3</sub> receptor. SLV313 failed to stimulate the human D<sub>3</sub> receptor at 10  $\mu$ M, yet potently and fully (at 10  $\mu$ M) inhibited dopamine ( $pA_2 = 8.9 \pm 0.2$ , Figure 2, middle panel).

### Apomorphine-Induced Climbing Behavior

SLV313 dose dependently attenuated climbing induced by the dopamine receptor agonist apomorphine (1 mg/kg s.c.) with an  $ED_{50}$  (95% confidence limits) value of 0.41. (0.30–0.56) mg/kg p.o. (Figure 3, upper panel). Similarly, antagonism was observed with the D<sub>2</sub> receptor antagonist and typical antipsychotic haloperidol ( $ED_{50}$  0.10 (0.08–0.15) mg/kg p.o.; Figure 3, lower panel). MEDs were 0.3 and 0.1 mg/kg p.o., respectively. In addition, in a study designed to test the longevity of the effects of SLV313 after administration there was a dose-dependent reduction of apomorphine-induced climbing seen 4 h after administration ( $ED_{50} = 1.21$  ( $< 0.3$  to  $\geq 3$ ) and an MED of 1 mg/kg p.o., data not shown).

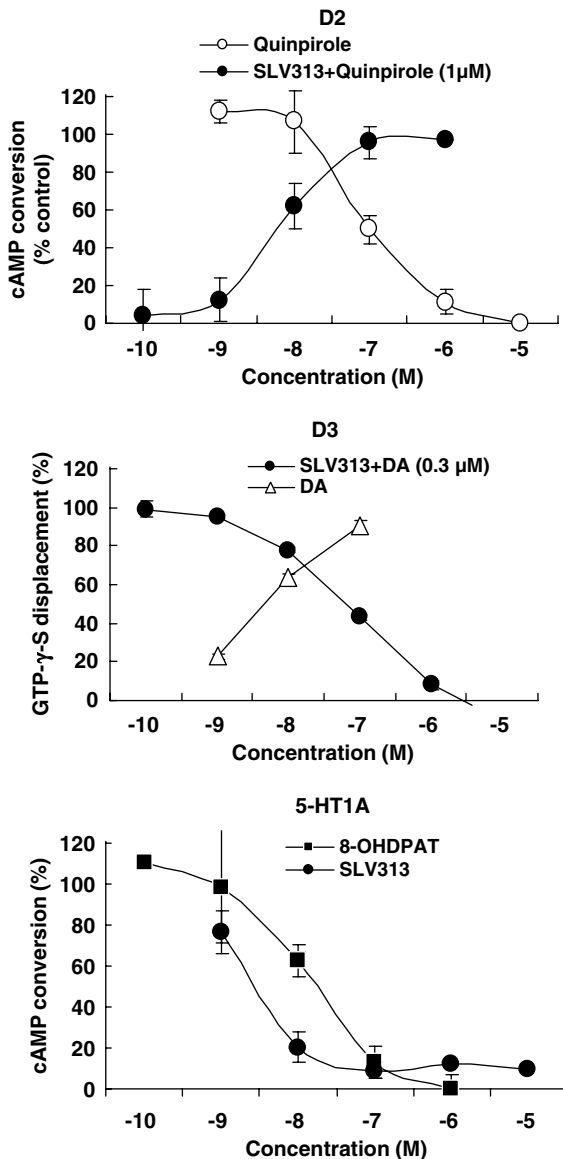
### 5-HT<sub>1A</sub> Receptor Syndrome

The characteristic behaviors associated with 5-HT<sub>1A</sub> receptor stimulation were observed after administration of SLV313. Nonparametric statistics confirmed the significant induction of FBP (MED = 3 mg/kg p.o.), LLR (MED = 1 mg/kg p.o.), and HLA (MED = 10 mg/kg p.o.) but not FPT (Table 3). In addition, temperature measurements showed no difference recorded at the start of the experiment ( $p > 0.05$ ), but significant differences after the completion of the behavioral observations ( $F(3,28) = 6.31$ ,  $p < 0.002$ ) (Table 3). An experiment designed to assess the role of the 5-HT<sub>1A</sub> receptors on the effects of SLV313 demonstrated that WAY100635 antagonized SLV313-induced FBP, HLA, and LLR with MEDs of 0.1, 0.1, and 0.03 mg/kg s.c., respectively (Table 4); no significant effect on FPT was seen. No significant differences in the temperature between treatment groups at the beginning of the experiment ( $p > 0.05$ ), but significant treatment-induced differences in temperature were observed at the end of the experiment ( $F(4,35) = 17.59$ ,  $p < 0.0001$ ) (Table 4). The response in SLV313-treated animals was significantly different from vehicle-treated animals, and animals pretreated with WAY 100635 at 0.3 mg/kg were significantly different from vehicle + SLV313-treated animals. The partial 5-HT<sub>1A</sub> agonists aripiprazole (3, 10, and 30 mg/kg p.o.) failed to elicit any significant serotonergic syndrome behaviors, and no

**Table 2** Receptor-Binding Profiles for SLV313 and the Reference Compounds Aripiprazole, Clozapine, Haloperidol, Risperidone, Ziprasidone, and Olanzapine

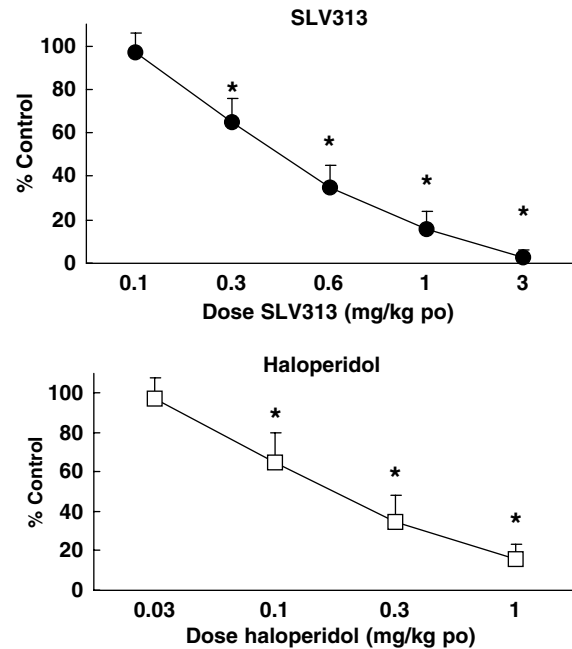
Compound	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	5-HT <sub>1A</sub>	5-HT <sub>2A</sub>	5-HT <sub>2B</sub>	5-HT <sub>2C</sub>	5-HT <sub>4</sub>	5-HT <sub>6</sub>	5-HT <sub>7</sub>	5-HT <sub>3</sub>	5-HT <sub>up</sub>	M <sub>1</sub>	M <sub>4</sub>	$\alpha_1$	$\alpha_2$	H <sub>1</sub>
SLV313	$8.4 \pm 0.2$	$8.4 \pm 0.2$	$8.0 \pm 0.2$	$9.1 \pm 0.1$	$6.6 \pm 0.2$	$7.9 \pm 0.1$	Inactive	Inactive	Inactive	$7.2 \pm 0.1$	Inactive	Inactive	Inactive	Inactive	$6.3 \pm 0.2$	Inactive	Inactive
Aripiprazole	$8.3 \pm 0.2$	$8.4 \pm 0.2$	$7.0 \pm 0.1$	$8.1 \pm 0.1$	$7.8 \pm 0.2$	$8.9 \pm 0.3$	$7.3 \pm 0.2$	Inactive	$6.7 \pm 0.1$	$7.2 \pm 0.2$	$6.3 \pm 0.2$	$7.5 \pm 0.1$	Inactive	Inactive	$7.0 \pm 0.2$	Inactive	$7.0 \pm 0.1$
Clozapine	$6.9 \pm 0.1$	$7.0 \pm 0.2$	$7.3 \pm 0.1$	$7.0 \pm 0.1$	$8.3 \pm 0.2$	$8.5 \pm 0.1$	$8.1 \pm 0.1$	Inactive	$8.1 \pm 0.1$	$7.7 \pm 0.4$	$6.4 \pm 0.1$	Inactive	$8.2 \pm 0.3$	$7.9 \pm 0.1$	$8.0 \pm 0.2$	$7.1 \pm 0.2$	$8.1 \pm 0.2$
Haloperidol	$8.3 \pm 0.2$	$8.8 \pm 0.2$	$8.2 \pm 0.1$	Inactive	$7.0 \pm 0.2$	Inactive	Inactive	Inactive	Inactive	$6.5 \pm 0.1$	Inactive	Inactive	Inactive	Inactive	$7.8 \pm 0.2$	Inactive	$6.4 \pm 0.2$
Olanzapine	$7.7 \pm 0.1$	$7.7 \pm 0.3$	$7.7 \pm 0.3$	Inactive	$8.8 \pm 0.1$	$8.2 \pm 0.0$	$8.3 \pm 0.2$	Inactive	$8.1 \pm 0.1$	$7.1 \pm 0.1$	$6.7 \pm 0.1$	Inactive	$8.0 \pm 0.1$	$7.9 \pm 0.2$	$7.6 \pm 0.3$	$6.5 \pm 0.1$	$8.2 \pm 0.2$
Risperidone	$8.2 \pm 0.1$	$8.0 \pm 0.1$	$8.0 \pm 0.1$	$6.6 \pm 0.1$	$9.0 \pm 0.1$	$7.7 \pm 0.1$	$7.9 \pm 0.1$	Inactive	Inactive	$9.0 \pm 0.2$	Inactive	Inactive	Inactive	Inactive	$8.8 \pm 0.2$	$8.1 \pm 0.1$	$8.1 \pm 0.3$
Ziprasidone	$7.9 \pm 0.2$	$8.2 \pm 0.2$	$7.3 \pm 0.2$	$8.8 \pm 0.1$	$9.2 \pm 0.1$	$8.8 \pm 0.1$	$8.9 \pm 0.2$	Inactive	$7.2 \pm 0.1$	$8.3 \pm 0.1$	$6.4 \pm 0.1$	$7.1 \pm 0.2$	Inactive	Inactive	$8.1 \pm 0.1$	$6.3 \pm 0.1$	$7.8 \pm 0.1$

Results are presented as  $pK_i$  values (mean  $\pm$  SD, at least  $n = 3$ ), ranked by  $pK_i$  for SLV313; inactive denotes the compound had no significant inhibition of radioligand binding at the receptor at a concentration of 1  $\mu$ M ( $pK_i < 6$ ).



**Figure 2** The effect of SLV313 on dopamine D<sub>2</sub>, D<sub>3</sub> and 5-HT<sub>1A</sub> receptors. Upper panel: quinpirole alone (open circles) completely attenuated forskolin-induced accumulation of cAMP in CHO cells transfected with the D<sub>2</sub> receptor with a pEC<sub>50</sub> of 6.8. SLV313 (closed circles) concentration dependently antagonized quinpirole (1 μM) effects (pA<sub>2</sub> of 9.3 ± 0.3). Middle panel: in D<sub>3</sub> receptor-transfected cells SLV313 possessed antagonist properties in the presence of dopamine (0.3 μM), using a GTPγS assay (filled circles; pA<sub>2</sub> of 8.9 ± 0.2; dopamine alone was also tested for control purposes open triangles). Lower panel: in CHO cells transfected with the 5-HT<sub>1A</sub> receptor SLV313 concentration dependently reduced forskolin-stimulated cAMP production in a concentration-dependent manner (filled circles; pEC<sub>50</sub> of 9.0 ± 0.2), similar to the 5-HT<sub>1A</sub> receptor agonist 8-OHDPAT (filled squares). All graphs demonstrate percentage difference from control and means ± SEM from at least triplicate experiments, where SEM are not visible the symbols obscure the SEM.

changes in temperature were seen at the start ( $F(3,28) = 1.71$ ,  $p < 0.84$ ) or at the end of the experiment ( $F(3,28) = 2.45$ ,  $p < 0.08$ ) (Table 3). Ziprasidone (10 mg/kg p.o.) and clozapine (30 mg/kg p.o.) demonstrated some limited ability to increase LLR and FBP together with a hypothermic effect ( $F(2,21) = 17.45$ ,  $p < 0.001$ ; *post hoc*,  $p < 0.05$  Table 3).



**Figure 3** SLV313 and haloperidol dose dependently attenuated climbing behavior induced by the dopamine agonist apomorphine (1 mg/kg s.c.). The ED<sub>50</sub> for SLV313 was 0.41 (0.30–0.56) mg/kg p.o. and for haloperidol 0.10 (0.08–0.15) mg/kg p.o. \* $p < 0.05$  cf. control data are expressed as percent control (100%). Data shown are means ± SEM.

### Flesinoxan Drug Discrimination Paradigm

The dose dependence of flesinoxan-induced generalization to the drug appropriate key has previously been demonstrated in the same subjects (Mos *et al*, 1997; Herremans *et al*, 1999). In the present study, SLV313 fully substituted for the training drug flesinoxan (ED<sub>50</sub> of 0.03 (0.01–0.08)), with no effect on rates of responding ( $F(3,40) = 1.93$ ,  $p < 0.13$ ) (Figure 4, upper and lower panels). However, SLV313 (0.25 mg/kg p.o.) failed to attenuate the training dose of flesinoxan (0.25 mg/kg) in antagonism studies (100% of animals chose the drug key;  $1.91 \pm 0.21$  pecks/s) (data not shown). In antagonism tests, WAY100635 fully antagonized flesinoxan-appropriate responding induced by SLV313 (0.1 mg/kg) with an ED<sub>50</sub> value of 0.32 (0.1–1) (Figure 4, upper panel), with no effect on rates of responding ( $F(2,30) = 0.76$ ,  $p < 0.47$ ) (Figure 4, lower panel). In order to assess whether the dopamine D<sub>2</sub> antagonist properties of SLV313 contributed to the observed generalization, the effects of haloperidol to substitute to flesinoxan were assessed. Haloperidol failed to generalize to the drug key tested at a dose range of 0.1–1 mg/kg and surprisingly failed to reduce rates of responding ( $F(2,31) = 0.75$ ,  $p < 0.47$ ) (Table 5). In an antagonist test haloperidol reduced the effects of flesinoxan, although this was only seen at doses that reduced response rates ( $F(2,30) = 3.83$ ,  $p < 0.05$ ) (Table 5).

### The Effect of SLV313 on Catalepsy

While haloperidol dose dependently increased the proportion of rats defined as cataleptic and the latency to remove their fore-paws from the bar (MED = 4 mg/kg p.o., for both



**Table 3** The Effect of SLV313, Aripiprazole, Ziprasidone (Zipr) and Clozapine (Cloz) on Oral Temperature and Serotonergic Syndrome Behaviors in Rats

Dose (mg/kg p.o.)	Temperature (°C)	FBP	HLA	LLR	FPT
<i>SLV313</i>					
0	38.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	37.6 ± 0.2	1 ± 0.62	0 ± 0	2.25 ± 0.90*	0 ± 0
3	37.3 ± 0.1*	5.63 ± 0.92*	1 ± 0.46	6.75 ± 0.45*	0.3 ± 0.2
10	37.1 ± 0.2*	9.87 ± 0.83*	2.25 ± 0.45*	11.37 ± 0.18*	0 ± 0
<i>Aripiprazole</i>					
0	38.63 ± 0.12	1 ± 0	0 ± 0	0 ± 0	1 ± 0.5
3	38.68 ± 0.11	1 ± 0.62	0 ± 0	0 ± 0	1 ± 0.5
10	38.41 ± 0.12	1 ± 0.92	0 ± 0.46	0 ± 0	1 ± 0.46
30	38.50 ± 0.16	1.1 ± 0.83	0 ± 0.45	0 ± 0	1.13 ± 0.64
<i>Zipr and Cloz</i>					
0	38.53 ± 0.19	0 ± 0	0 ± 0	0 ± 0	0.13 ± 0.13
Zipr 10	37.66 ± 0.14*	1 ± 0.5*	0 ± 0	4.38 ± 0.32*	0 ± 0
Cloz 30	35.31 ± 0.35*	4.13 ± 1.05*	0 ± 0	1.5 ± 0.56*	0 ± 0

FBP = flat body posture; HLA = hind limb abduction; LLR = lower lip retraction; FPT = fore-paw-treading.

The ability of the compounds to induce elements of the 5-HT behavioral syndrome associated with stimulation of the 5-HT<sub>1A</sub> receptor was assessed together with hypothermia. Data represent the mean oral temperature and composite 5-HT syndrome score of eight rats ± SEM and syndrome data represent the mean summed scores over six 10 min time epochs (maximal score = 12 per rat) of eight rats. \**p* < 0.05 compared to vehicle-treated controls.

**Table 4** The Effect of the 5-HT<sub>1A</sub> Receptor Antagonist WAY100635 (s.c.) to Antagonize SLV313 (6 mg/kg p.o.)-Induced Hypothermia and Serotonergic Syndrome Responses

Dose WAY100635/SLV313 (mg/kg)	Temperature (°C)	FBP	HLA	LLR	FPT
0+0	38.8 ± 0.1	0.3 ± 0.2	0 ± 0	0.1 ± 0.1	1.3 ± 0.8
0+6	37.8 ± 0.1 <sup>+</sup>	7.1 ± 1.0 <sup>+</sup>	2.5 ± 0.7 <sup>+</sup>	10.4 ± 0.7 <sup>+</sup>	0.4 ± 0.2
0.03+6	37.9 ± 0.1	4.6 ± 0.7	1.5 ± 0.3	6.5 ± 0.6*	0.8 ± 0.5
0.1+6	38.0 ± 0.1	3.0 ± 0.9*	1.0 ± 0.4*	4.1 ± 0.7*	0.4 ± 0.4
0.3+6	38.2 ± 0.1*	1.1 ± 0.4*	0.3 ± 0.2*	1.3 ± 0.6*	0.2 ± 0.2

<sup>+</sup>*p* < 0.05 compared to vehicle+vehicle group and \**p* < 0.05 compared to vehicle+SLV313 group. Abbreviations as in Table 3 legend.

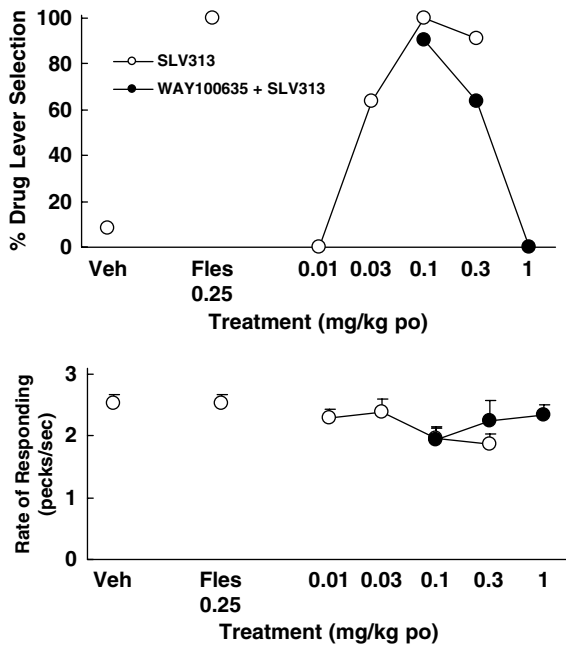
parameters), SLV313 at a dose up to 60 mg/kg p.o. failed to elicit these effects (Table 6).

### In Vivo Microdialysis Studies

**Effect in the nucleus accumbens.** SLV313 induced a dose- and time-dependent increase in extracellular levels of DA, DOPAC, and HVA, a reduction in 5-HT levels and no change in 5-HIAA levels (time course data for 10 mg/kg p.o. are displayed (Figure 5)). Evaluation of the AUC for 120 min following drug administration, compared to basal dialysate levels (% AUC<sub>120</sub>) revealed that SLV313 (0.1–10 mg/kg p.o.) induced a dose-dependent increase in extracellular levels of dopamine (F(4,27) = 4.73, *p* < 0.0001; Figure 5, left upper panel) with an MED of 3 mg/kg p.o. Similar increases in DOPAC (F(4,27) = 21.90, *p* < 0.001, Figure 5, lower left and right panels) and HVA (F(4,27) = 21.41, *p* < 0.0001, Figure 5, lower left and right panels) were also observed. While

SLV313 dose dependently reduced extracellular 5-HT (F(4,13) = 21.82, *p* < 0.0001, Figure 5, upper right panel) no significant effects were observed on the serotonin metabolite 5-HIAA (F(4,27) = 0.74, *p* < 0.57, Figure 5, lower right and left panels).

**Effect in the prefrontal cortex.** SLV313 (3, 10, and 20 mg/kg p.o.) significantly increased dopamine release in the mPFC (F(1,9) = 10.8, *p* < 0.001; F(1,13) = 17.61, *p* < 0.001; F(1,13) = 22.1, *p* < 0.0001, respectively; *n* = 7–8) (Figure 6, upper panel). AUC analyses confirmed that SLV313 (3, 10, and 20 mg/kg p.o.) significantly increased dopamine release in the mPFC (F(1,9) = 10.26, *p* < 0.01; F(1,13) = 33.03, *p* < 0.001; F(1,13) = 35.26, *p* < 0.001, respectively; Figure 8). The increase in cortical dopamine release produced by 10 mg/kg SLV313 was inhibited by WAY100635 (0.2 mg/kg s.c.) (F(1,10) = 24.12, *p* < 0.0001; *n* = 4–8) (Figure 9, upper panel). In addition, SLV313 at 20 mg/kg (p.o.) also significantly increased



**Figure 4** The effect of SLV313 in a flesinoxan drug discrimination procedure. Upper panel: SLV313 (open circles) fully generalized to the flesinoxan-appropriate key in pigeons trained to discriminate flesinoxan from vehicle. In antagonist tests, WAY100635 antagonized the effects of SLV313 (filled circles). Lower panel: in either study the response rates were not affected.

**Table 5** The Effect of Haloperidol on Generalization and Cue Antagonism in Pigeons Trained to Discriminate Flesinoxan from Vehicle

Treatment (mg/kg p.o.)	% Drug key	Response rates (pecks/s)
<i>Haloperidol</i>		
0	8.3	2.54 ± 0.14
0.1	18.2	2.31 ± 0.15
0.3	9.1	2.46 ± 0.16
1	9.1	2.17 ± 0.19
<i>Haloperidol+flesinoxan (0.25)</i>		
0	100	2.54 ± 0.13
0.1	81.8	2.42 ± 0.13
0.3	87.5	2.01 ± 0.15*
1	72.7	1.86 ± 0.17*

Haloperidol failed to induce drug key-appropriate responding in pigeons trained to discriminate flesinoxan from vehicle and caused a mild reduction in flesinoxan-key responding, but at the expense of response rates. \* $p < 0.05$  compared to training day.

ACh release in the mPFCx ( $F(1,11) = 9.03$ ,  $p < 0.0032$ ;  $n = 6-7$ ), but 3 and 10 mg/kg did not ( $F(1,8) = 0.327$ ,  $p < 0.57$ ;  $F(1,11) = 0.147$ ,  $p < 0.7$ , respectively;  $n = 4-6$ ) (Figure 7, upper panel) which was confirmed by analyses of the AUC data ( $F(1,11) = 0.3$ ,  $p < 0.58$ ;  $F(1,8) = 2.22$ ,  $p < 0.11$ ;  $F(1,11) = 9.0$ ,  $p < 0.033$ ; 3, 10, and 20 mg/kg p.o., respectively; Figure 8). AUC analysis for the antagonism experiments was not performed (Figures 6–9).

**Table 6** SLV313 Failed to Induce a Cataleptogenic Profile in Rats at a Dose up to 60 mg/kg p.o., Unlike Haloperidol

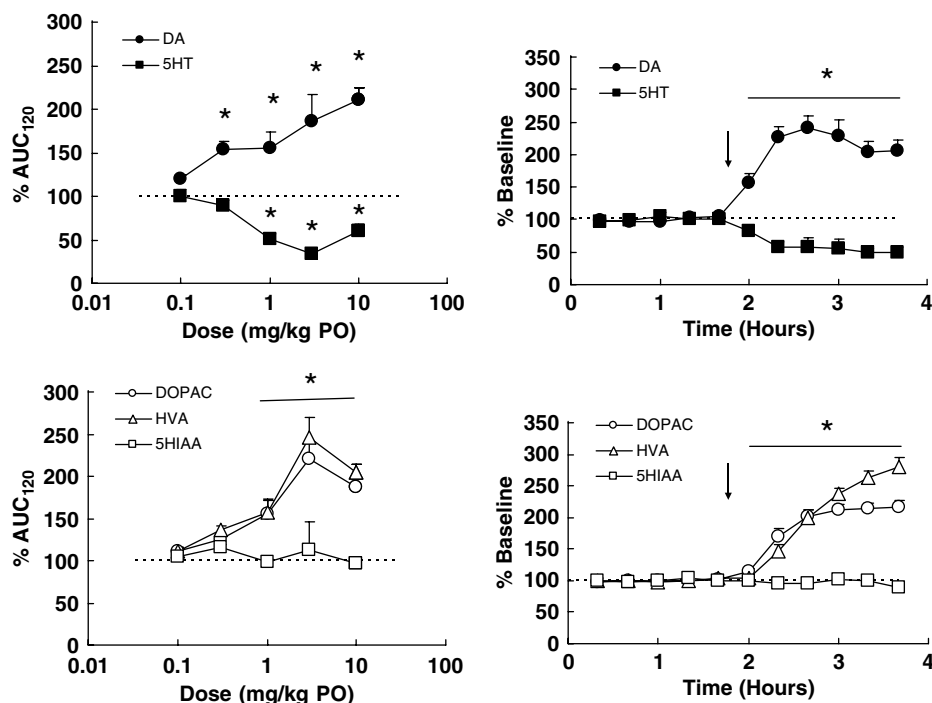
Treatment (mg/kg p.o.)	Latency (s)	Cataleptic rats (n out of eight rats)
<i>Haloperidol</i>		
0	9.37 ± 6.18	1
1	9.12 ± 5.18	1
2	11.00 ± 7.02	1
4	31.87 ± 8.05*	6*
8	36.87 ± 9.66*	5*
<i>SLV313</i>		
0	10.63 ± 7.37	2
20	5.12 ± 1.35	1
60	3.87 ± 0.95	0

The number of rats (out of the eight per group tested) were defined as cataleptic if they failed to remove their fore-paws from the catalepsy bar after 30 s. No loss in the righting reflex was observed. \* $p < 0.05$  vs vehicle control (Kruskal–Wallis followed by *post hoc* Wilcoxon's test).

**Effect in the hippocampus.** Similar to the effect in the mPFC, SLV313 (3, 10, and 20 mg/kg p.o.), significantly increased dopamine release in the hippocampus ( $F(1,7) = 7.22$ ,  $p < 0.0086$ ;  $F(1,10) = 18.89$ ;  $p < 0.0001$ ;  $F(1,11) = 7.99$ ,  $p < 0.006$ , respectively;  $n = 5-8$ ) (Figure 6, lower panel). Analyses of the AUC confirmed these effects ( $F(1,7) = 9.56$ ,  $p < 0.001$ ;  $F(1,10) = 19.36$ ;  $p < 0.001$ ;  $F(1,11) = 13.06$ ,  $p < 0.001$ , respectively) (Figure 8, left panel). WAY100635 (0.2 mg/kg, s.c.) inhibited SLV313 (10 mg/kg, p.o.)-induced dopamine release in the hippocampus ( $F(1,10) = 20.88$ ,  $p < 0.0001$ ;  $n = 4-8$ ) (Figure 7, lower panel). However, SLV313, only at the high dose of 20 mg/kg, transiently increased ACh release in the hippocampus but in the time course analysis this was not confirmed following *post hoc* analysis ( $F(1,9) = 8.61$ ,  $p < 0.004$ ;  $n = 5-6$ ; AUC  $F(1,9) = 13.26$ ,  $p < 0.05$ ) whereas three did not ( $F(1,8) = 0.77$ ,  $p < 0.38$  (AUC  $F(1,8) = 0.772$ ,  $p < 0.38$ );  $n = 4-6$ ) and a significant effect was only seen for the AUC determination at 10 mg/kg ( $F(1,10) = 0.89$ ,  $p < 0.20$  vs AUC  $F(1,10) = 9.08$ ,  $p < 0.05$ ;  $n = 4-6$ ) (Figure 7, lower panel and Figure 8). AUC analyses for the antagonism experiments was not performed.

### The Effect of Chronic Administration of SLV313 on the Number of Spontaneously Active VTA and SNC Neurons

A significant effect of treatment on the number of spontaneously active VTA dopamine neurons ( $F(3,36) = 30.6$ ,  $p < 0.001$ ) but not SNC dopamine neurons ( $F(3,36) = 0.12$ ,  $p < 0.95$ ) was observed. Subsequent *post hoc* analyses of the VTA data indicated that the chronic administration of 3 or 10 mg/kg p.o. of SLV313 or 20 mg/kg p.o. of clozapine significantly decreased the number of spontaneously active VTA dopamine neurons compared to vehicle-treated animals (Figure 10). In addition, the chronic administration of 10 mg/kg of SLV313 produced a significantly greater decrease in the number of spontaneously active VTA dopamine neurons compared to 20 mg/kg of clozapine.



**Figure 5** The effect of SLV313 on dialysate DA, HVA, DOPAC, 5-HT, and 5-HIAA in the nucleus accumbens. SLV313 concomitantly increased extracellular dopamine and decreased extracellular levels of 5-HT in the nucleus accumbens of freely moving rats. No effect was seen on extracellular 5-HIAA, but DOPAC and HVA levels were elevated. Data are expressed as percent of baseline for the time course following treatment with 10 mg/kg p.o. of SLV313 (indicated by the arrow) or with the expression of AUC<sub>120</sub> data. \* $p < 0.05$  for all parameters shown but not for 5-HIAA.

## DISCUSSION

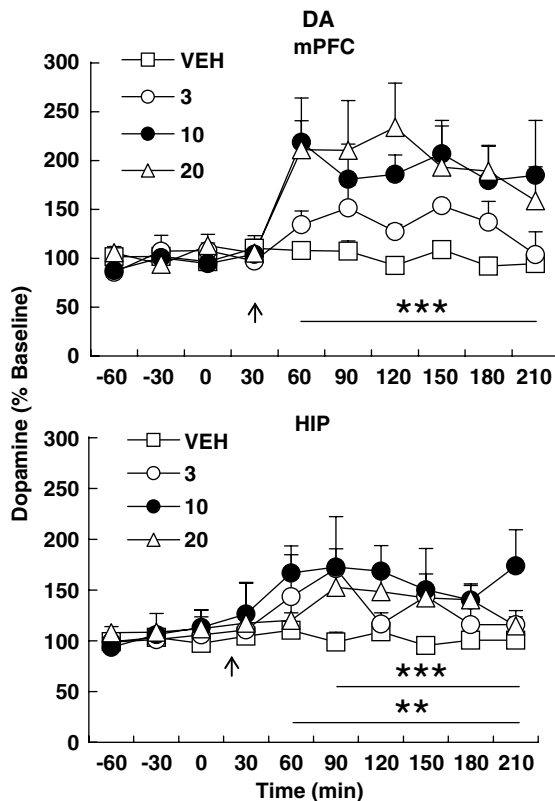
Data from the present studies demonstrate that SLV313 displayed dopamine D<sub>2/3</sub> receptor antagonist and serotonin 5-HT<sub>1A</sub> receptor agonist properties *in vitro* and *in vivo*. Receptor-binding studies demonstrated that SLV313 had appreciable receptor binding to serotonin 5-HT<sub>2B</sub> receptors with moderate affinities at serotonin 5-HT<sub>7</sub>, serotonin 5-HT<sub>2A</sub> and adrenergic  $\alpha_1$  receptors. In addition, SLV313, at  $10^{-6}$  M, and displayed no affinity for a number of other receptors or transporters. *In vivo* SLV313 acted as a serotonin 5-HT<sub>1A</sub> receptor agonist and dopamine D<sub>2</sub> receptor antagonist, did not induce catalepsy, was preferentially active in the meso(cortico)limbic pathway and induced dopamine and ACh increases in the hippocampus and mPFCx.

The pattern of receptor-binding affinity differed notably from that of the other antipsychotic agents tested in that moderate serotonin 5-HT<sub>2A</sub> and noradrenergic  $\alpha_1$  binding was seen and SLV313 was devoid of serotonin 5-HT<sub>6</sub>, adrenergic  $\alpha_2$ , muscarinic, and H<sub>1</sub> activity. Moreover, the dopamine D<sub>2</sub> receptor affinity was greater than that of clozapine and olanzapine and serotonin 5-HT<sub>1A</sub> receptor affinity was similar to ziprasidone. The lack of both H<sub>1</sub> and 5-HT<sub>2C</sub> receptor binding may be beneficial for the actions of SLV313 insofar as 5-HT<sub>2C</sub>, histamine H<sub>1</sub> and muscarinic receptors have all been linked to weight gain induced by some of the novel antipsychotics (Reynolds *et al*, 2002; Hartfield *et al*, 2003).

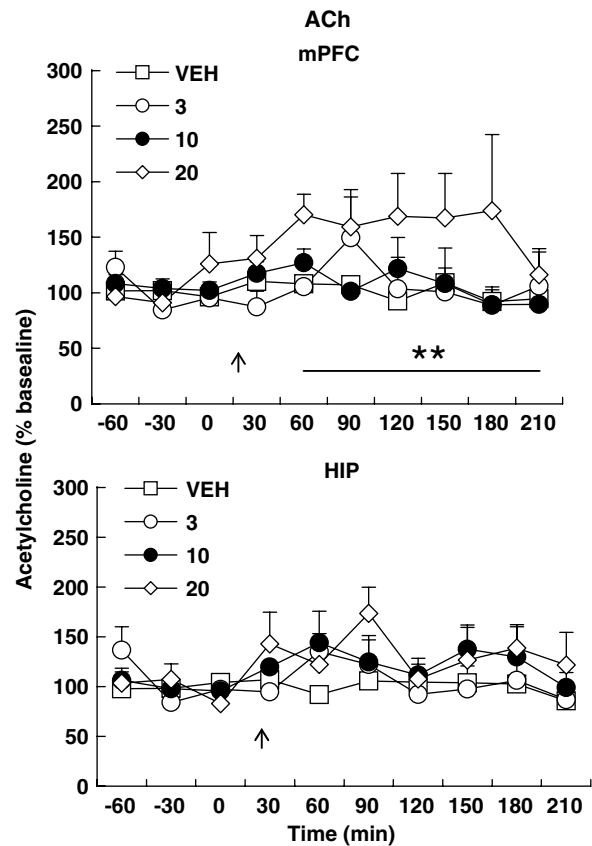
While SLV313 showed potent serotonin 5-HT<sub>2B</sub> receptor binding, further studies demonstrated that these were fully antagonist in nature (data not shown). Cellular functional

assays suggested that SLV313 reversed the effects of the dopamine agonist quinpirole on D<sub>2</sub>-mediated cAMP accumulation. In addition to dopamine D<sub>2</sub> receptor antagonism, full serotonin 5-HT<sub>1A</sub> receptor agonism and dopamine D<sub>3</sub> receptor antagonism was also observed *in vitro*. SLV313 is not unusual in possessing affinity for the dopamine D<sub>4</sub> receptor as many antipsychotics have affinity for this receptor. However, both preclinical (Newman-Tancredi *et al*, 1997; Bristow *et al*, 1997a) and clinical data (Bristow *et al*, 1997b) suggest that dopamine D<sub>4</sub> receptors are probably not a pharmacological target for the treatment of schizophrenia. Conversely, it has been suggested that dopamine D<sub>3</sub> receptor antagonism, with or without dopamine D<sub>2</sub> antagonism may represent a target for antipsychotic strategies (for review see Joyce and Millan, 2005). However, confirmation is needed as novel clinical candidates have not yet cleared Phase II clinical studies. Other antipsychotics demonstrate mixed dopamine D<sub>2</sub> and serotonin 5-HT<sub>1A</sub> effects (at varying ratios), for example ziprasidone, clozapine, SSR181507, and aripiprazole are all serotonin 5-HT<sub>1A</sub> partial agonists (eg Newman-Tancredi *et al*, 1996, 2001; Jordan *et al*, 2002; and see earlier). In support of the present study SLV313 was shown to bind to rat striatal dopamine D<sub>2</sub> and cortical serotonin 5-HT<sub>1A</sub> receptors where it elicits antagonist and agonist effects, respectively (Newman-Tancredi *et al*, 2004).

The *in vitro* efficacy at D<sub>2</sub> and 5-HT<sub>1A</sub> receptors of SLV313 was mirrored using *in vivo* behavioral models insofar as SLV313 attenuated apomorphine-induced climbing, like haloperidol, and attenuated amphetamine- and methylphenidate-stimulated locomotor activity (Kleven *et al*, 2003). Such effects are indicative of putative D<sub>2</sub> receptor



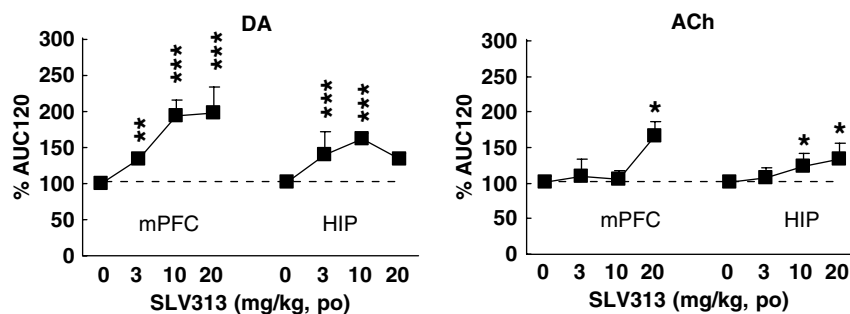
**Figure 6** Time course effects of SLV313 (3–20 mg/kg, p.o.) on extracellular dopamine levels in the medial prefrontal cortex (upper panel) and hippocampus (lower panel). Data are means  $\pm$  SEM of the dialysate dopamine levels, expressed as a percentage of each predrug baseline dopamine value. The arrow indicates drug injection. Upper panel: \*\*\* $p$  < 0.001 from time points 60–210 min for the groups of 10 and 20 mg/kg, compared to vehicle-treated rats. Lower panel: \*\* $p$  < 0.01 from time points 60–210 min for the group of 10 mg/kg, compared to vehicle-treated rats. \*\*\* $p$  < 0.01 from time point 90–210 min for the group 20 mg/kg, compared to vehicle-treated rats.



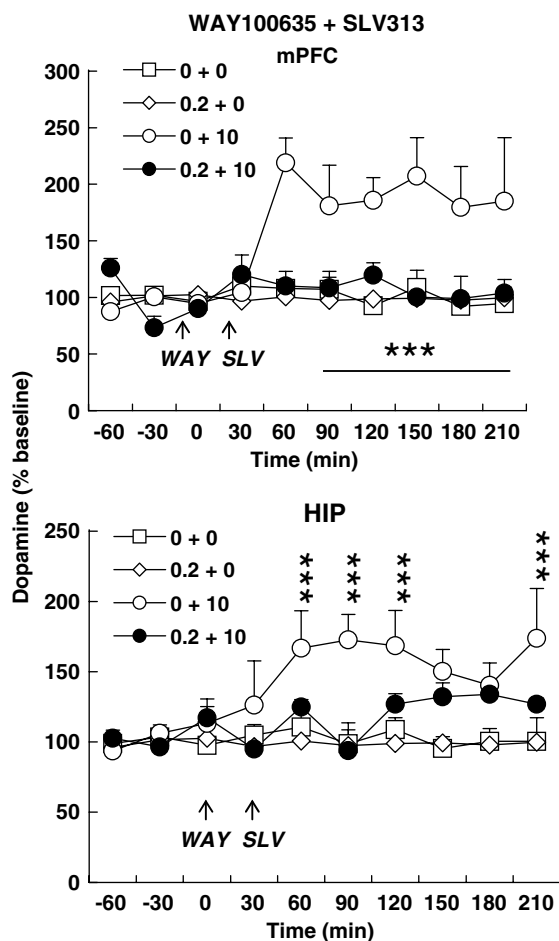
**Figure 7** Time-course effects of SLV313 (3–20 mg/kg, p.o.) on extracellular acetylcholine levels in the medial prefrontal cortex (upper panel) and hippocampus (lower panel). Data are means  $\pm$  SEM of the dialysate acetylcholine levels, expressed as a percentage of each predrug baseline acetylcholine value. The arrow indicates drug injection and \*\* $p$  < 0.01 from time points 60–210 min for the group of 20 mg/kg, compared to vehicle-treated rats with *post hoc* analyses.

antagonism. SLV313 also increased extracellular dopamine levels in the nucleus accumbens of conscious rats, an action consistent with dopamine  $D_2$  receptor antagonism *in vivo*. Consistent with 5-HT<sub>1A</sub> agonist effects SLV313-induced serotonin 5-HT<sub>1A</sub> syndrome behaviors and hypothermia, which was antagonized by pretreatment with the 5-HT<sub>1A</sub> receptor antagonist WAY100635. Unlike the nonselective serotonin 5-HT<sub>1A</sub> agonist 8-OHDPAT (Tricklebank *et al*, 1984; Depoortere *et al*, 2003) SLV313 did not induce fore-paw-treading behaviors. This inability to induce fore-paw-treading behavior was also demonstrated with SSR181507 (Depoortere *et al*, 2003) and the authors proposed that the lack of SSR181507 on fore-paw-treading may indicate a reduced propensity to induce the serotonin syndrome. However, the effects of SSR181507 have not been reported on other serotonergic syndrome behaviors (Depoortere *et al*, 2003) so such a conclusion might prove preliminary. It is unlikely that mixed  $D_2$  antagonists and 5-HT<sub>1A</sub> agonists, like SLV313, will induce the serotonin syndrome in humans to any great extent in a clinical schizophrenia treatment setting, as chronic administration of 8-OH-DPAT results in tolerance to syndrome behaviors (Prinssen *et al*, 2000), but interestingly not in the ability to reduce haloperidol-

induced catalepsy (Prinssen *et al*, 2000). Whether such a direct comparison across preclinical and clinical studies can yet be made is unclear for such mixed  $D_{2/3}$  antagonists with 5-HT<sub>1A</sub> agonist compounds. No components of the serotonergic syndrome were observed following treatment with either ziprasidone or aripiprazole (Wolff *et al*, 2003). This lack of effect of aripiprazole was confirmed in the present study and although ziprasidone and clozapine did show some efficacy the effects were very weak compared to SLV313. Furthermore, neither aripiprazole nor ziprasidone antagonized the actions of 8-OHDPAT (Wolff *et al*, 2003) and aripiprazole failed to reduce hippocampal serotonin 5-HT dialysate levels (Assie *et al*, 2005). Thus, aripiprazole appears devoid of intrinsic agonist actions at serotonin 5-HT<sub>1A</sub> receptors *in vivo* using the paradigms described here. Interestingly, *in vivo* electrophysiological studies have demonstrated that ziprasidone and SSR181507 act as agonists at serotonin 5-HT<sub>1A</sub> receptors and reduce dorsal raphe nucleus serotonergic cell firing (Sprouse *et al*, 1999; Claustre *et al*, 2003). Clozapine and olanzapine, however, appear to mediate this effect via  $\alpha_1$  noradrenergic receptors (Sprouse *et al*, 1999; Lejeune *et al*, 1994). Consistent with such an action *in vivo* microdialysis studies demonstrated that SLV313 reduced extracellular levels of serotonin in the

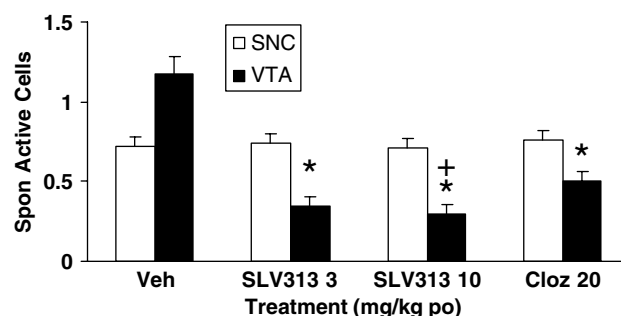


**Figure 8** Time course of the effects of SLV313 (3–20 mg/kg, p.o.) on extracellular dopamine (left panel) and acetylcholine levels (right panel) in the medial prefrontal cortex and hippocampus expressed as AUC<sub>120</sub>. \* $p < 0.05$ , \*\* $p < 0.001$ , and \*\*\* $p < 0.001$  compared with vehicle-treated rats.



**Figure 9** The effect of WAY100635 (0.2 mg/kg, s.c.) on SLV313 (10 mg/kg, p.o.)-induced dopamine release in the medial prefrontal cortex (upper panel) and hippocampus (lower panel). Data are means  $\pm$  SEM of the dialysate dopamine levels, expressed as a percentage of each predrug baseline dopamine value. The arrows indicate drug injection times. Upper panel: \*\*\* $p < 0.001$  from time points 60–210 min for the group of WAY100635 plus SLV313 following *post hoc* tests, compared to the WAY100635 group. Lower panel: \*\*\* $p < 0.001$  from time points 60–120 and 210 min for the group of WAY100635 plus SLV313, compared to the WAY100635 group following *post hoc* tests.

nucleus accumbens suggesting an agonist action at the somatodendritic (5-HT<sub>1A</sub>) autoreceptor (or via feedback inhibition). Similarly SLV313 reduced hippocampal serotonin dialysate levels (Assie *et al*, 2005), which were depen-



**Figure 10** The effect of repeated (21 days) oral administration of vehicle, SLV313, or clozapine on the number of spontaneously active SNC and VTA dopamine neurons in anesthetized male Sprague–Dawley rats. Each value represents the mean number of spontaneously active dopamine neurons per stereotactically defined electrode descent  $\pm$  SEM. A total of 10 animals were examined for each treatment group. Animals received one p.o. administration per day for 21 days. On the test day, animals were given the appropriate treatment and 2 h later, the number of spontaneously active dopamine neurons was determined. \* $p < 0.05$  vs vehicle and + $p < 0.05$  vs clozapine group. Filled bars VTA, open bars SNC.

dent on stimulation of the 5-HT<sub>1A</sub> receptor. SLV313 fully (>80%) substituted for the serotonin 5-HT<sub>1A</sub> agonist flesinoxan in pigeons trained to discriminate flesinoxan from vehicle and was antagonized by WAY100635 consistent with a serotonin 5-HT<sub>1A</sub> agonist profile (Mos *et al*, 1997). Dopamine D<sub>2</sub> receptor antagonism is not involved in the flesinoxan cue as haloperidol did not substitute for flesinoxan and only slightly attenuated the flesinoxan response, but only when response rates were reduced. Taken together, these data convincingly suggest that SLV313 has serotonin 5-HT<sub>1A</sub> agonist and D<sub>2/3</sub> antagonist effects *in vivo* and *in vitro*.

A combination of serotonin 5-HT<sub>1A</sub> agonism and D<sub>2</sub> antagonism may confer positive benefits for the treatment of schizophrenia (see for reviews Millan, 2000; Meltzer *et al*, 2003). One such mechanism would be by the reduction of neurological or EPS side effects seen in preclinical (Prinssen *et al*, 1999, 2000; Wadenberg and Ahlenius, 1991; Wadenberg, 1996; Wadenberg *et al*, 1999; Christoffersen and Meltzer, 1998; and see Depoortere *et al*, 2003) and clinical studies (Goff *et al*, 1991; Yoshida *et al*, 1998). In the rat, the reduction of the cataleptic effect was only evident with moderate to high intrinsic activity 5-HT<sub>1A</sub> receptor agonists (Prinssen *et al*, 1999). At doses up to 60 mg/kg p.o. SLV313 did not induce catalepsy (ie at doses > 20 the CAR activity;

McCreary *et al*, 2002) in contrast to haloperidol. Further, SLV313, like clozapine, significantly reduced the number of spontaneously active cells in the VTA, but not the SNC commensurate with a putative mesolimbic activity and atypical antipsychotic action (Chiodo, 1988; White and Wang, 1983; Chiodo and Bunney, 1983; Freeman and Bunney, 1987). Thus, based on its electrophysiological profile, SLV313 may be classified as an atypical antipsychotic preclinically. It is possible that SLV313-induced agonistic action at serotonin 5-HT<sub>1A</sub> receptors may be important in producing its VTA-selective effects since the *in vivo* electrophysiological results obtained with SLV313 are similar to those reported following the chronic administration of the mixed D<sub>2</sub> antagonist/5-HT<sub>1A</sub> receptor agonists 1192U90 and SSR181507 (Jones-Humble *et al*, 1996; Claustre *et al*, 2003). A role for the D<sub>2</sub> receptor in assisting in this action cannot be excluded (Nakamura *et al*, 2006). Thus, data from the present electrophysiological and catalepsy studies, coupled with the results with 1192U90 and SSR181517 (Jones-Humble *et al*, 1996; Claustre *et al*, 2003), support the hypothesis of limbic selectivity with mixed serotonin 5-HT<sub>1A</sub> agonists and D<sub>2</sub> antagonists in the same molecule.

Besides a potential reduction in EPS liability, combined serotonin 5-HT<sub>1A</sub> agonism and D<sub>2</sub> receptor antagonism could confer additional benefits over typical antipsychotic agents. For example, the potentiation of antipsychotic-like effects in the CAR paradigm (Wadenberg and Ahlenius, 1991; Prinssen *et al*, 1996) at doses far lower than the 'normal' cataleptic dose and the effects seen with SLV313 in the CAR paradigm (McCreary *et al*, 2002) support potential efficacy against the positive symptoms of schizophrenia, but positive symptoms are not the only symptom set in schizophrenia. One of the hypotheses of the abnormalities underlying schizophrenia has been the hypofrontality model which proposes hypoactive mPFCx circuitry in schizophrenia (Weinberger and Lipska, 1995). The mPFCx may have a role in decision making, working memory, and attentional processing (Goldman-Rakic, 1998) and may have an additional role in the treatment of the negative symptoms of schizophrenia (Weinberger and Lipska, 1995). It has also been suggested that ACh may also be of pivotal importance for the cognitive symptoms of schizophrenia at the level of both the mPFCx and hippocampus and that atypical antipsychotics increase dopamine and ACh in both of these brain areas (Eichenbaum *et al*, 1999; Seidman *et al*, 2002; Moghaddam and Bunney, 1990; Kuroki *et al*, 1999; Ichikawa *et al*, 2002a, b; Shirazi-Southall *et al*, 2002; Li *et al*, 2004; Chung *et al*, 2004) via serotonin 5-HT<sub>1A</sub> receptor-dependent mechanisms (Rollema *et al*, 1997; Ichikawa and Meltzer, 1999; Li *et al*, 2004). Recent studies with SSR181507 suggested that combined serotonin 5-HT<sub>1A</sub> and dopamine D<sub>2</sub> antagonism, with the absence of serotonin 5-HT<sub>2A</sub> receptor antagonism, augments dopamine levels in the mPFCx (Claustre *et al*, 2003). This serotonin 5-HT<sub>1A</sub>-dependent increase in hippocampal dopamine was confirmed in the present study with SLV313. The SLV313-induced augmented dopamine efflux was antagonized by pretreatment with the 5-HT<sub>1A</sub> antagonist WAY100635 (see also Assie *et al*, 2005). Such effects have also been noted with other antipsychotics (Ichikawa *et al*, 2002a, b). ACh was also elevated significantly following treatment with SLV313 in

the mPFCx and hippocampus (transiently and modestly), consistent with other antipsychotics (see Li *et al*, 2004). Taken together, the present data, provided they can be extrapolated to humans, suggest that SLV313 may improve the negative symptoms of schizophrenia (Weinberger and Lipska, 1995) as well as cognitive and attentional processing (Goldman-Rakic, 1998; Kuroki *et al*, 1999; Meltzer, 1999; Meltzer and McGurk, 1999; Harvey and Keefe, 2001). Indeed, serotonin 5-HT<sub>1A</sub> receptor agonism in the mPFCx has been shown to improve attentional processing in the rat five-choice serial reaction time task (Winstanley *et al*, 2003) and the serotonin 5-HT<sub>1A</sub> (partial) receptor agonist tandospirone improved cognition in schizophrenic patients (Sumiyoshi *et al*, 2001a, b).

In summary, SLV313 is a novel putative antipsychotic possessing 5-HT<sub>1A</sub> receptor agonist and dopamine D<sub>2/3</sub> receptor antagonist properties both *in vitro* and *in vivo*. It is possible that SLV313 may be a broadly effective antipsychotic with low potential for EPS for the treatment of schizophrenia and other psychoses, for example bipolar disorder. However, verification of this hypothesis is needed in the clinic.

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