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A comparison of the binding of σ opioids and phencyclidine, and the interaction with antipsychotic drugs in rat brain membranes

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1 The present study investigates the relationship between binding at the σ site labelled by the prototypic σ ligand (+)-[^{3}H]-N-allylnormetazocine ((+)-[^{3}H]-SKF10,047) and binding at the phencyclidine (PCP) site labelled by [^{3}H]-phencyclidine in rat whole brain membranes.

2 (+)-[^{3}H]-SKF10,047 bound with a K_D of 251 ± 66 nM. [^{3}H]-PCP bound with a K_D of 180 ± 35 nM (K_D ± asymptotic s.e.).

3 The potencies of a range of compounds to displace these ligands were only poorly correlated ($r = 0.3$). Furthermore selective displacement of (+)-[^{3}H]-SKF10,047 but not of [^{3}H]-PCP was demonstrated using the non-selective dopamine ligand haloperidol and the dopamine$_2$-selective ligand 3-(3-hydroxyphenyl)N-n-propylpiperidine (3PPP). These results indicate that the σ and PCP sites are different entities.

4 The relationship between binding at the σ site and dopamine receptors was investigated in rat whole brain membranes and in striatal membranes.

5 (±)-SKF10,047 displaced [^{3}H]-haloperidol bound to whole brain membranes with a greater potency than it displaced [^{3}H]-haloperidol bound to striatal membranes. The opposite was true for the dopamine antagonist, clozapine, which showed greater potency in striatal membranes.

6 Comparison of [^{3}H]-haloperidol binding in whole brain and striatum gave only a poor correlation ($r = 0.6$). Hence, different binding sites would appear to exist in these brain regions, the binding of [^{3}H]-haloperidol to whole brain being predominantly to σ sites and the binding to striatum being predominantly to dopamine receptors.

Introduction

Behavioural and biochemical studies have provided evidence that the psychotomimetic effects of phencyclidine (PCP) and those of the so-called 'σ opioids' such as N-allylnormetazocine (SKF10,047) and cyclazocine, could be mediated through a common receptor (Martin *et al.*, 1976). These 'σ opioids' have been shown to have phencyclidine-like discriminative stimulus properties in the rat (Holtzman, 1982; Shannon, 1982). They have also been shown to displace [^{3}H]-PCP binding (Quirion *et al.*, 1981; Zukin & Zukin, 1981; Murray & Leid, 1984). These observations have led to the proposition that both σ opioids and PCP exert their behavioural effects via a common receptor mechanism (Zukin *et al.*, 1984). However, we have presented preliminary evidence (Downes *et al.*, 1985) that in the rat brain the σ and PCP sites are not the same, (+)-[^{3}H]-SKF10,047, the selective σ ligand, and [^{3}H]-PCP, binding to more than one site. These findings agree well with those of Tam (1983, 1985) in rat spinal cord and guinea-pig membranes.

It has also been suggested that a common underlying mechanism of action in PCP-induced and SKF10,047-induced psychotomimetic effects is an increase in central dopaminergic activity (Iwamoto, 1980; Martin *et al.*, 1976). Chronic abuse of phencyclidine in man resembles schizophrenia with symptoms including auditory hallucinations, inappropriate affect and agitation. SKF10,047 has been shown to increase spontaneous locomotor activity and cause ipsilateral circling behaviour in rats with a unilateral nigro-striatal lesion. This activity can be significantly diminished by the dopamine receptor blocker,

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spiperone (Iwamoto, 1980). Furthermore, binding studies have demonstrated that the dopamine antagonist haloperidol has a high affinity for the σ site (Tam, 1983).

In the present study we have investigated the binding of the σ opioid (+)-SKF10,047 and PCP in order to show whether they are binding to separate sites. The possible relationship of these sites to the dopamine receptor has also been studied to determine whether the σ site and dopamine receptor are separate entities.

Methods

Male rats (Lister hooded) of 200 to 300 g were killed by decapitation and the whole brain or corpus striata removed. The tissues were homogenized in 10 vol of ice-cold 50 mM Tris HCl, pH 7.4 using a Brinkman polytron (setting 6, 20 s) and centrifuged at 48,000 *g* for 15 min at 4°C. The resulting membrane pellet was resuspended in the same volume of Tris HCl and incubated at 37°C for 45 min to remove bound endogenous ligands. The membranes were recentrifuged at 48,000 *g* for 15 min and resuspended in the same volume of Tris HCl.

Binding assays were carried out using 250 μl of membrane preparation incubated in triplicate at 25°C for 45 min with radiolabelled ligands and unlabelled drugs, made up to a final volume of 1 ml with Tris HCl. Under these binding conditions the radiolabelled ligands have been shown to equilibrate with their binding sites. Fifteen nanomolar (+)-[^{3}H]-SKF10047, 5 nM [^{3}H]-PCP and 5 nM [^{3}H]-haloperidol were used for displacement studies. Specific binding was defined using 100 μM PCP for (+)-[^{3}H]-SKF10047 binding, 100 μM (±)-SKF10,047 for [^{3}H]-PCP binding and 10 μM (±)-butaclamol for [^{3}H]-haloperidol binding; these concentrations were chosen following analysis of displacement curves for each ligand.

The displacing ligands chosen were different from the radiolabelled ligands to increase the probability of obtaining receptor-specific binding. The reaction was terminated by rapid filtration through Whatman GF/C filters. These were pretreated with polyethylenimine to prevent artifactual binding of the radioligands in a manner resembling 'specific' binding (Zukin *et al.*, 1983). The protein content of membrane preparations was determined by the method of Lowry *et al.* (1951). Values for K_D and B_{max} were obtained by analysis of the saturation curves using the mathematical model described by Munson & Rodbard (1980). Curve fitting was performed using the non-linear curve fitting program SAS (Cary, 1982).

(+)-[^{3}H]-N-allylnormetazocine ((+)-[^{3}H]-SKF 10,047) (25.5 Ci mmol^{-1}), [^{3}H]-PCP (49.9 Ci mmol^{-1}) and [^{3}H]-haloperidol (9.4 Ci mmol^{-1}) were obtained from New Engalnd Nuclear. Unlabelled drugs were obtained as follows: butaclamol HCl (Ayerst); phencyclidine HCl (Bio-Ceutic Laboratories); naloxone HCl (Dupont); haloperidol HCl (Janssen), ketamine HCl (Parke-Davis); bremazocine HCl, methysergide hydrogen maleate, tifluadom HCl (Sandoz); atropine sulphate, N-methyl-D-aspartic acid (Sigma), SKF-10,047 HCl (Smith Kline and French), cyclazocine base, ethylketocyclazocine methane sulphonate, ketocyclazocine methane sulphonate, pentazocine base (Sterling Research); U50,488 methane sulphate (Upjohn); clozapine base (Wander).

Results

(+)-[^{3}H]-SKF10,047 and [^{3}H]-phencyclidine binding in whole brain

(+)-[^{3}H]-SKF10,047 and [^{3}H]-PCP showed no significant binding to the traditional opioid sites, μ, δ and κ, as demonstrated by the fact that there was no displacement of either ligand by the opioid antagonsit naloxone up to concentrations of 0.1 mM (Figure 1). The total binding of (+)-[^{3}H]-SKF10,047 displaced by PCP was similar to the total binding of [^{3}H]-PCP displaced by (±)-SKF10,047 in the same brain membrane preparation (Figure 1).

Results showed that both ligands bound to a similar number of sites with a B_{max} of 657 ± 123 fmol mg^{-1} protein and 876 ± 123 fmol mg^{-1} protein, and a K_D of 251 ± 66 nM and 180 ± 35 nM for (+)-[^{3}H]-SKF10,047 and [^{3}H]-PCP, respectively (fitted values ± asymptotic s.e.). These figures were obtained by analysis of saturation curves as described in Methods. Figure 2 shows the same data illustrated in the form of Scatchard plots. Comparison of the potencies of a range of σ opioid compounds to displace the specific binding of (+)-[^{3}H]-SKF10,047 and [^{3}H]-PCP gave only a poor correlation ($r = 0.3$) (Table 1, Figure 3). This observation indicates that (+)-[^{3}H]-SKF10,047 and [^{3}H]-PCP bind to more than one site. In addition, (±)-SKF 10,047 showed Hill coefficients of 0.52 and 0.61 when displacing (+)-[^{3}H]-SKF 10,047 and [^{3}H]-PCP, respectively. PCP showed Hill coefficients of 0.58 and 0.3 when displacing (+)-[^{3}H]-SKF 10,047 and [^{3}H]-PCP, respectively.

Further studies were able to distinguish between the σ and PCP binding sites. Selective ligand displacement was shown by the dopamine ligands haloperidol and 3-(3-hydroxy-phenyl) N-n-propylpiperidine (3PPP) which were found to be potent displacers of (+)-[^{3}H]-SKF10,047, but only displaced approximately 15% of [^{3}H]-PCP, up to concentrations of 0.1 mM (Table 1) (Figure 4). These findings indicate a possible similarity between the σ binding site and dopamine receptors.

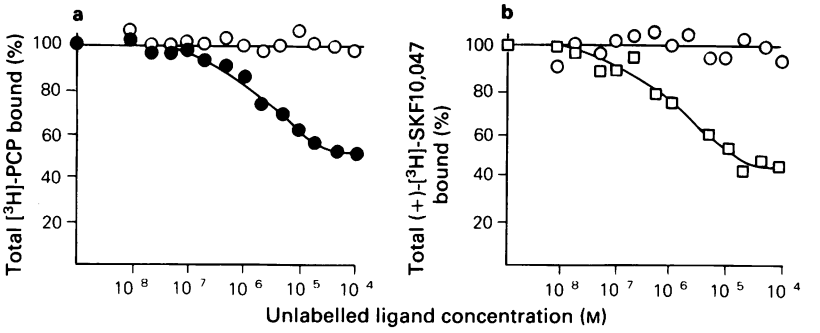

Figure 1 (a) Competitive inhibition of total [^{3}H]-phencyclidine ([^{3}H]-PCP) bound in rat whole brain membranes by (○) naloxone and (●) (±)-SKF10,047. (b) Competitive inhibition of total (+)-[^{3}H]-SKF 10,047 bound in rat whole brain membranes by (○) naloxone and (□) PCP.

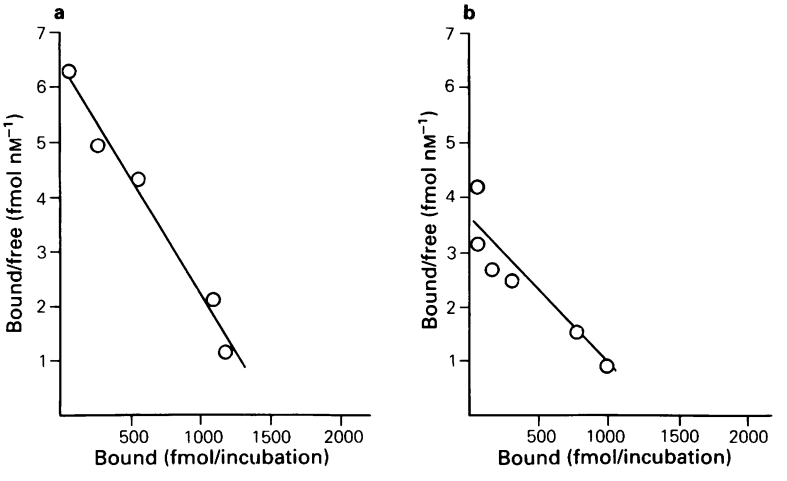

Figure 2 Scatchard plots of the specific binding of (a) [^{3}H]-phencyclidine ([^{3}H]-PCP) and (b) (+)-[^{3}H]-SKF10,047. The concentration ranges of the radiolabelled ligands were achieved by isotopic dilution and covered the range 5 nM to 1 μM. [^{3}H]-PCP was found to bind with a K_D of 180 ± 35 nM and a B_{max} of 876 ± 123 fmol mg^{-1} protein. (+)-[^{3}H]-SKF10,047 was found to bind with a K_D of 251 ± 66 nM and a B_{max} of 657 ± 123 fmol mg^{-1} protein.

[^{3}H]-haloperidol binding in whole brain and striatum

(±)-SKF10,047 was found to displace all of the specifically bound [^{3}H]-haloperidol in whole brain membranes with a K_i of 2.18×10^{-6} M. However, when membranes from the dopamine receptor-rich striatal region of the brain were used in the binding studies, (±)-SKF10,047 displaced specific [^{3}H]-haloperidol binding with a lower affinity ($K_i = 4.52 \times 10^{-5}$ M) (Table 2). This suggests that [^{3}H]-haloperidol may be labelling a predominantly different class of sites in whole brain compared to striatum. [^{3}H]-haloperidol was found to bind to the striatal membrane preparation with a K_D of 5×10^{-9} M which agrees with values previously found for haloperidol binding to dopamine sites (Creese *et al.*, 1975). [^{3}H]-haloperidol bound to whole brain membranes with a lower affinity, $K_D = 1.5 \times 10^{-8}$ M. The greater potency of the σ ligand (±)-SK10,047 in displacing [^{3}H]-haloperidol binding in whole brain suggests that [^{3}H]-haloperidol is labelling predominantly σ sites. In contrast, the dopamine antagonist clozapine displaced [^{3}H]-haloperidol binding in whole brain with a low affinity ($K_i = 2.1 \times 10^{-5}$ M) being a more potent displacer of [^{3}H]-haloperidol in striatum ($K_i = 2.7 \times 10^{-7}$ M). Comparison of the potencies for σ and dopaminergic compounds displacing [^{3}H]-haloperidol in whole brain and striatal membrane

Table 1 Comparison of the inhibitory effects of some 'σ opioids' and other compounds on the binding of (+)-[^{3}H]-SKF 10,047 and [^{3}H]-phencyclidine ([^{3}H]-PCP) in rat whole brain

	(+)-[^{3}H]-SKF 10,047		*[^{3}H]-PCP*	
	pIC_{50} (M)	*IC_{50}* (μM)	*pIC_{50}* (M)	*IC_{50}* (μM)
Bremazocine	7.92 ± 0.07	0.012	5.77 ± 0.14	1.7
Pentazocine	6.80 ± 0.31	0.16	5.40 ± 0.05	4.0
(±)-SKF10,047	6.30 ± 0.18	0.50	5.52 ± 0.09	3.0
Cyclazocine	6.24 ± 0.13	0.58	5.89 ± 0.11	1.3
Ethylketocyclazocine	6.04 ± 0.15	0.92	3.27 ± 0.002	540
PCP	5.96 ± 0.04	1.1	6.70 ± 0.04	0.2
U50,488	5.72 ± 0.35	1.9	2.43 ± 0.006	3700
Ketocyclazocine	4.89 ± 0.16	13	5.07 ± 0.04	8.5
Tifluadom	4.70 ± 0.09	20	<2	>10000
Ketamine	4.59 ± 0.22	26	5.00 ± 0.06	10
Haloperidol	8.30 ± 0.09	0.005	<4	>100
3PPP	6.89 ± 0.01	0.13	<4	>100
NMDA	<4	>100	<4	>100
TEA	<4	>100	<4	>100
Methysergide	<4	>100	<4	>100
Atropine	<4	>100	<4	>100

U50,488 = *trans*-3, 4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl-benzeneacetamide 3-(3-hydroxyphenyl)N-n-propylpiperidine; NMDA = N-methyl-D-aspartic acid; TEA = tetraethylammonium chloride.
IC_{50} values are the geometric mean of at least two separate experiments performed in triplicate. Values were determined from log probit plots. pIC_{50} values are the means of $-\log IC_{50}$ values ± s.e.mean.

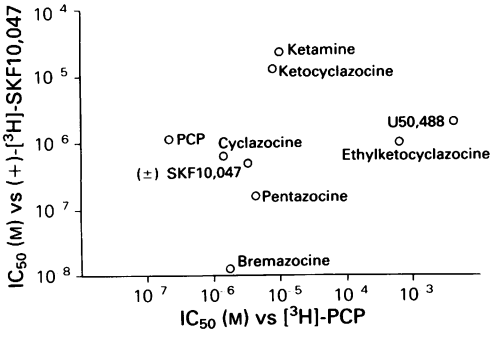

Figure 3 Correlation of potencies of some compounds in displacing (+)-[^{3}H]-SKF10,047 and [^{3}H]-phencyclidine ([^{3}H]-PCP). Correlation coefficient (r) of 0.3 demonstrates only a poor correlation.

preparations gave only a poor correlation ($r = 0.6$ (Table 2).

Discussion

Many opioids of the benzomorphan series are potent analgesics. They have been found to be antinociceptive in dogs and rodents yet neither precipitate withdrawal or suppress abstinence in morphine-dependent dogs or monkeys as do morphine-like analgesics. Three receptor types have been postulated to account for the differences between morphine and benzomorphans (Martin *et al.*, 1976). These are the μ-receptor mediating analgesia and other effects associated with morphine, the κ-receptor mediating analgesic effects associated with benzomorphans and the σ-receptor thought to be responsible for the psychotomimetic effects associated with benzomorphans such as SKF10,047 and cyclazocine. It has been suggested that the (+)-isomer of SKF10,047 is responsible for the psychotomimetic effects whereas the (−)-isomer is thought to mediate analgesia (Aceto & May, 1983). Since (±)-SKF10,047 and cyclazocine have been found to bind to the PCP site, it has been suggested that the σ effects of these opioids may be mediated through the PCP receptor (Zukin & Zukin, 1981). Furthermore, binding studies have shown that the (+)-isomer displays selectivity for the σ and PCP sites (Zukin *et al.*, 1984).

The present study provides evidence for the existence of more than one binding site for the σ ligands (+)-[^{3}H]-SKF10,047 and [^{3}H]-PCP in rat whole brain membranes. It therefore contests the hypothesis that the σ site and PCP site are identical. Although Scatchard analysis of the ligands binding to whole brain membranes failed to demonstrate more than one site, probably due to the similar K_Ds of the ligands, comparison of the rank order of potencies of a number

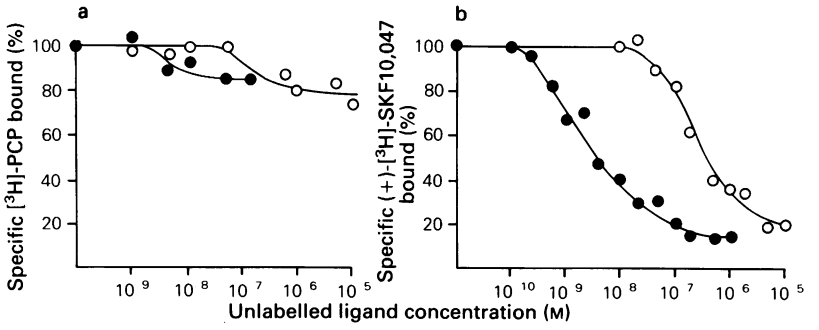

Figure 4 Competitive inhibition of (a) specific [^{3}H]-phencyclidine ([^{3}H]-PCP) binding and (b) specific (+)-[^{3}H]-SKF 10,047 binding in rat whole brain membranes by (○) 3-(3-hydroxyphenyl) N-n-propylpiperidine (3PPP) and (●) haloperidol.

Table 2 Comparison of the inhibitory effect of some compounds on the binding of [^{3}H]-haloperidol in rat whole brain and in corpus striatum

	[^{3}H]-haloperidol			
	Whole brain		*Striatum*	
	pIC_{50} (M)	IC_{50} (μM)	pIC_{50} (M)	IC_{50} (μM)
Haloperidol	7.8 ± 0.03	0.015	8.30 ± 0.05	0.005
(±)-Butaclamol	6.22 ± 0.007	0.59	7.52 ± 0.23	0.03
(±)-SKF10,047	5.66 ± 0.002	2.18	4.34 ± 0.009	45.2
Clozapine	4.68 ± 0.009	21.0	6.57 ± 0.05	0.27

IC_{50} values are the geometric mean of at least two separate experiments performed in triplicate. Values were determined from log probit plots. pIC_{50} values are the means of $-\log IC_{50}$ values ± s.e.mean.

of compounds against (+)-[^{3}H]-SKF10,047 and [^{3}H]-PCP binding gave only a poor correlation ($r = 0.3$) (Figure 3) demonstrating that the σ site and PCP binding site are separate entities. The Hill slopes for (±)-SKF10,047 and PCP displacing (+)-[^{3}H]-SKF 10,047 and [^{3}H]-PCP binding were significantly less than unity, also indicating binding of the radiolabelled ligands to more than one site.

These findings are compatible with those of Tam (1983, 1985) in spinal cord and guinea-pig brain membranes. Further evidence was shown by the selective displacement of (+)-[^{3}H]-SKF10,047, by haloperidol and 3PPP. However, these ligands did displace the small proportion of [^{3}H]-PCP bound to the σ site, and failed to displace the small proportion of (+)-[^{3}H]-SKF10,047, bound to the PCP site.

Given the relative affinities of the tritiated ligands for the two sites, receptor occupancy theory (Ariens, 1964) predicts that 85% of the total binding of (+)-[^{3}H]-SKF10,047 is to the σ site and 15% is to the PCP site, and for [^{3}H]-PCP 85% of the total binding is to the PCP site and 15% is to the σ site. These theoretical percentages for the occupation of the two sites have been demonstrated experimentally using the selective σ ligands haloperidol and 3PPP. Which of these sites is responsible for the spectrum of psychotomimetic effects produced by the σ opiates and PCP is unknown. Activity at both sites may be important for the mediation of dysphoria and hallucinogenic effects.

The fact that the non-selective dopamine receptor ligand, haloperidol, and the dopamine$_2$ selective ligand, 3PPP, showed a high affinity for the σ site prompted us to investigate the relationship between the σ site and the dopamine receptor. Comparison of the binding of [^{3}H]-haloperidol in whole brain and in the dopamine receptor-rich striatal region of brain showed (±)-SK10,047 to be a more potent displacer of [^{3}H]-haloperidol bound to whole brain membranes, whereas the dopamine antagonist clozapine more potently displaced [^{3}H]-haloperidol bound to striatal membranes. The different potencies shown by (±)-SKF10,047 and clozapine displacing [^{3}H]-haloperidol

in whole brain and striatum suggest that [^{3}H]-haloperidol is binding to different sites in these tissues. The K_i for SKF10,047 displacing [^{3}H]-haloperidol in whole brain is similar to the K_i for SKF10,047 at the σ site, suggesting that [^{3}H]-haloperidol binding in whole brain is predominantly to a σ site.

The potency of (±)-SKF10,047 in displacing [^{3}H]-haloperidol binding in striatum was much lower. The ability of clozapine to displace [^{3}H]-haloperidol in striatum is consistent with K_i values found for activity at the dopamine receptor (Creese *et al.*, 1975), whereas the affinity in whole brain is lower. The σ and dopamine sites are therefore different. The displacement curves obtained in the present study for SKF10,047 and clozapine displacing [^{3}H]-haloperidol in whole brain and striatum were not biphasic. Hill slopes were approximately equal to 1.0, representing displacement from a single site. Therefore the whole brain membrane preparation predominantly contains the more ubiquitous σ binding site, whereas striatum contains predominantly dopamine binding sites. These findings suggest that care must be taken in interpreting results using [^{3}H]-haloperidol as a ligand for the dopamine receptor. In some tissues it may be necessary to suppress binding to the σ site in order to demonstrate binding to dopamine receptors only.

Tam (1983) has described reverse stereoselectivity for butaclamol binding to the σ and dopamine sites. The (+)-isomer was found to be more active on the dopamine site and the (−)-isomer, more active on the σ site. Recent binding studies on the guinea-pig brain comparing the potencies of σ and dopamine ligands on (+)-[^{3}H]-SKF10,047 and [^{3}H]-spiperone binding (Tam & Cook, 1984) also support the finding that the σ and dopamine sites are not the same. [^{3}H]-haloperidol binding in guinea-pig striatum appeared to show approximately equal proportions of σ and dopamine sites in contrast to rat striatum which shows almost pure dopamine binding properties.

The interaction of some of the antipsychotic dopamine antagonist drugs with σ sites raises the question of whether antagonism at the σ site is important for mediation of antipsychotic effects. Possibly both σ and dopamine receptor blockade is necessary for effective antipsychotic activity. Similarly the psychotomimetic effects of σ agonists may be mediated partly by direct dopaminergic mechanisms. However, σ opioids appear to have a low affinity for the dopamine receptor and may mediate psychotomimetic effects by indirect activation of the central dopaminergic pathways. Iwamoto (1980) has presented evidence that this may be the case. Data from a 6-hydroxydopamine-induced circling behavioural model suggests that (±)-SKF10,047 indirectly activates the intact dopamine mesostriatal pathway, possibly stimulating at the level of the cell bodies.

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