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Evaluation of 7-OH-DPAT as a DA D3 Receptor Agonist

by

Batool-Faegheh Bahaaldin Bagi Zarandi

A thesis submitted to the faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Doctor of Philosophy

in

Pharmaceutical Sciences

Faculty of Pharmacy and Pharmaceutical Sciences and Faculty of Medicine (Psychiatry)
Neurochemical Research Unit

Edmonton. Alberta

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University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled: Evaluation of 7-OH-DPAT as a DA D3 Receptor Agonist hereby submitted by Batool-Faegheh Bahaaldin Bagi Zarandi in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences.

Dr. A. J. Greenshaw (Supervisor)

Dr. R.T. Coutts (Co-Supervisor)

Dr. G. B. Baker (Committee Member)

Dr. D. Treit (Committee Chair)

Dr. T. B. Wishart (External Examiner)

DATED: December 18, 1997

DEDICATED

to my

Father: Late Jallil Bahaaldin Bagi

Mother: Hamideh Mosavi

&

my sister and brother

For their support and encouragement

to my

Family: Hashem, Alireza and Sina Montaseri

For their endless patience, support and sacrifices

ABSTRACT

The *in vivo* selectivity of 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) for the dopamine (DA) D3 receptor was evaluated in rats. Effects of 7-OH-DPAT on locomotor activity were determined. Test conditions included low, high and nicotine-stimulated baselines of locomotor activity. 7-OH-DPAT induced hypoactivity in rats tested under novel conditions. Under familiar conditions, the lower doses induced hypoactivity and higher doses induced early hypoactivity followed by hyperactivity. The later hyperactivity was antagonized by haloperidol (HAL). 7-OH-DPAT reduced nicotine effects. 7-OH-DPAT, injected directly into the nucleus accumbens or caudate nucleus of the forebrain, dose-dependently decreased locomotor activity without showing site-selectivity. Sc administration of 7-OH-DPAT at high doses induced hyperactivity with continuous sniffing and forward walking. Clozapine reduced hyperactivity, forward walking, and sniffing induced by 7-OH-DPAT but not by APO. SCH 23390 blocked hyperactivity induced by APO but only attenuated the effects of 7-OH-DPAT. HAL antagonized the effect of a high dose of APO but not of 7-OH-DPAT. A low dose of 7-OH-DPAT decreased hyperactivity induced by a high dose of APO in DA-intact rats but not in DA-depleted rats [reserpine + α-methyl-para tyrosine (α-MPT)]. An attempt was made to determine a time at which animals with DA depletion do not exhibit behavioral supersensitivity to a DA agonist. Because behavioral supersensitivity to APO was observed as early as 24 h post medial forebrain bundle (mfb) 6-OHDA-lesion, this DA-lesioned model was not used to test postsynaptic effects of 7-OH-DPAT. A method for extraction and HPLC analysis of 7-OH-DPAT was developed. A level of 0.43 μg/g of 7-OH-DPAT was obtained in the brain

following injection of a low dose of 7-OH-DPAT (0.3 mg/kg, sc). The lack of site-selectivity of 7-OH-DPAT indicates that it may not act selectively on DA D3 receptors. The ability of 7-OH-DPAT to decrease hyperactivity induced by APO supports the hypothesis that effects of low doses of 7-OH-DPAT on locomotor activity may be mediated *via* postsynaptic DA D3 receptors. Effects of high doses of 7-OH-DPAT may not be mediated only by DA D2 receptors, as CLZ, SCH 23390 and HAL had differential effects on the actions of a high dose of 7-OH-DPAT and APO.

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ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine, serotonin
6-OH-DA	6-hydroxydopamine
7-OH-DPAT	7-hydroxy-N,N-di-n-propyl-2-aminotetralin
8-OH-DPAT	8-hydroxy-N,N-di-n-propyl-2-aminotetralin
APO	apomorphine
CLZ	clozapine
CN	caudate nucleus
DA	dopamine
DOPAC	3, 4-dihydroxyphenylacetic acid
g	gram(s)
GABA	γ-aminobutyric acid
h	hour(s)
HAL	haloperidol
HPLC-ECD	high performance liquid chromatography with electrochemical detection
HVA	homovanillic acid
ip	intraperitoneal
iv	intravenous
kg	kilogram(s)
l	litre(s)
mfb	medial forebrain bundle
mg	milligram(s)
min	minute(s)
ml	millilitre(s)
mM	millimolar
mPFC	medial prefrontal cortex
NAS	nucleus accumbens
NE	norepinephrine (noradrenaline)
ng	nanogram(s)
nM	nanomolar
QUIN	quinpirole
sc	subcutaneous
SEM	standard error of the mean
SN	substantia nigra
VTA	ventral tegmental area
α-MPT	alpha-methyl-para-tyrosine
µl	microlitre(s)
µM	micromolar

1. INTRODUCTION

1.1. Dopamine

Dopamine (DA) is an important neurotransmitter in the mammalian central nervous system and is the metabolic precursor of norepinephrine and epinephrine. The DA system has probably been investigated more widely than any other neurotransmitter system. It is involved in many neuronal functions, including learning (Verma, Kulkarni, 1993), reward processes and the control of movement (Zhang et al. 1997). Moreover, CNS DA systems appear to mediate the behavioral effects of drugs of abuse like cocaine by inducing locomotor activity, repetitive (stereotypy) and reward seeking behaviors (Bardo et al. 1996). A wide range of studies has shown the association of the DA receptor system with etiology and treatment of several neuropathological conditions including Parkinson's disease, schizophrenia, Huntington's chorea and drug abuse (Seeman, 1987; Elsworth, Roth, 1997). Detailed information about DA synthesis, metabolism and pathways in the brain are shown in figures 1, 2 and 3, respectively.

1.2. DA receptors

Receptors for DA were originally classified into two categories, D1 and D2 receptors. DA D1 and D2 receptors stimulate or inhibit the activation of adenylate cyclase, respectively (Kebabian, Calne, 1979); their pharmacological and biochemical profiles are also different (Creese et al. 1983; Niznik, 1987). Following the cloning of a DA D2 receptor complementary DNA (Bunzow et al. 1988), two alternatively spliced D2 receptor isoforms

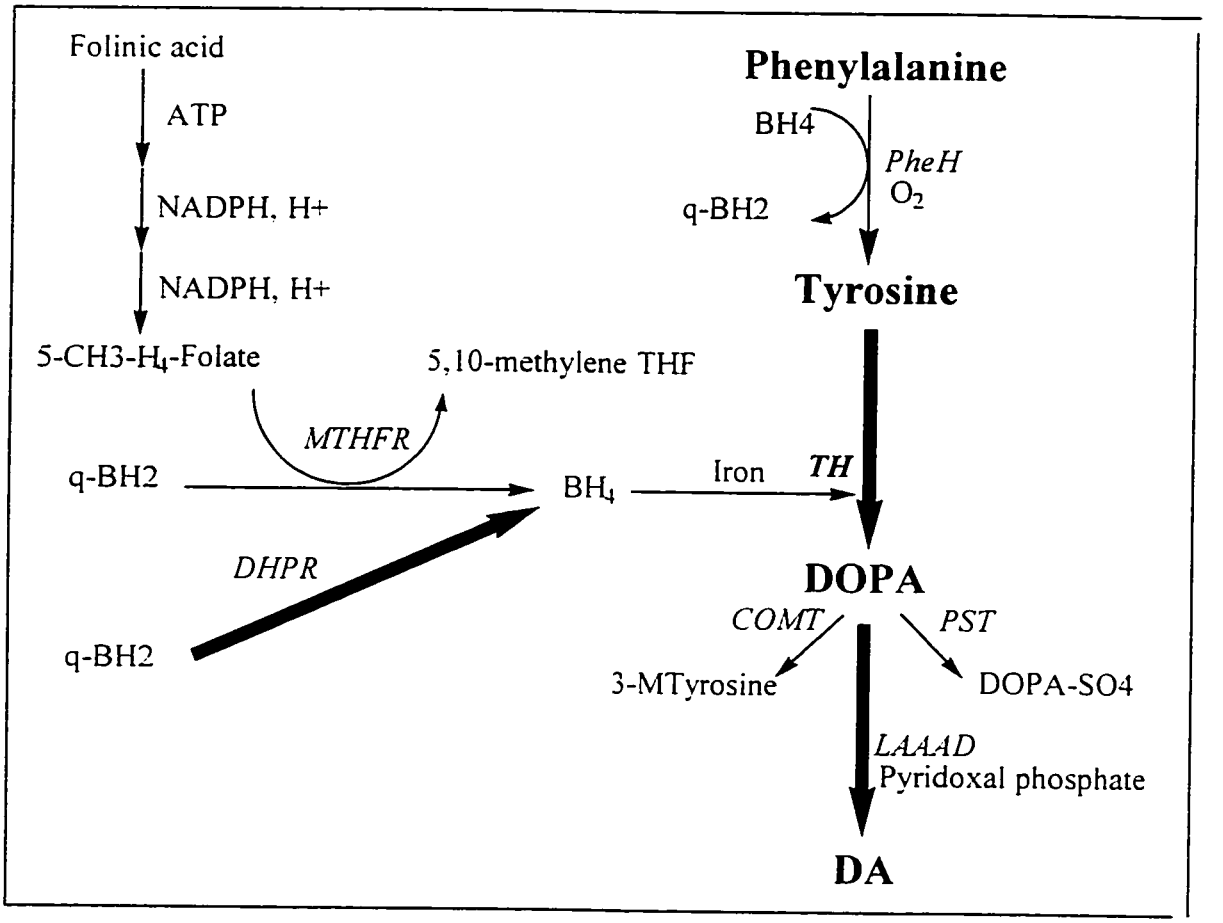

Figure 1: Overview of the dopamine synthetic pathway. Abbreviations used: ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate; 5-CH_3-H_4-folate, 5-methyltetrahydrofolate; THF, tetrahydrofolate; q-BH_2, quinonoid dihydrobiopterin; MTHFR, methyltetrahydrofolate reductase; DHPR, dihydropteridine reductase; BH_4, tetrahydro-biopterin; PheH, phenylalanine hydroxylase; TH, tyrosine hydroxylase; COMT, catechol-O-methyltransferase; PST, phenylsulfotransferase; 3-MTyrosine, 3-methoxytyrosine; LAAAD, L-aromatic amino acid decarboxylase; DOPA-SO_4, L-DOPA sulfate; DA, dopamine. Adapted from Goldstein et al. 1996.

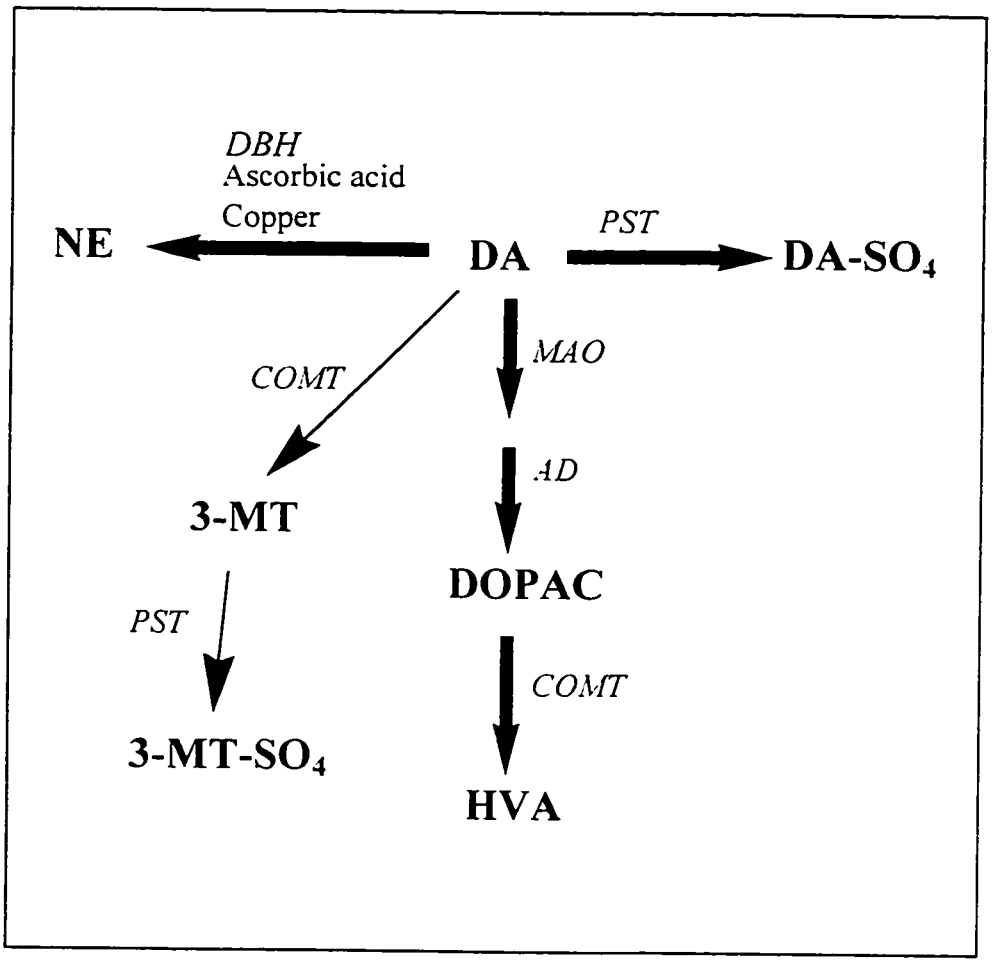

Figure 2: Overview of dopamine catabolic pathways. Abbreviations used: DA, dopamine; PST, phenylsulfotransferase; DA-SO_4, dopamine sulfate; MAO, monoamine oxidase; AD, aldehyde dehydrogenase; DOPAC, 3,4-dihydroxyphenylacetic acid; COMT, catechol-O-methyl transferase; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; 3-MT-SO_4, 3-methoxy- tyramine sulfate; DBH, dopamine-β-hydroxylase; NE, norepinephrine. Adapted from Goldstein et al. 1996.

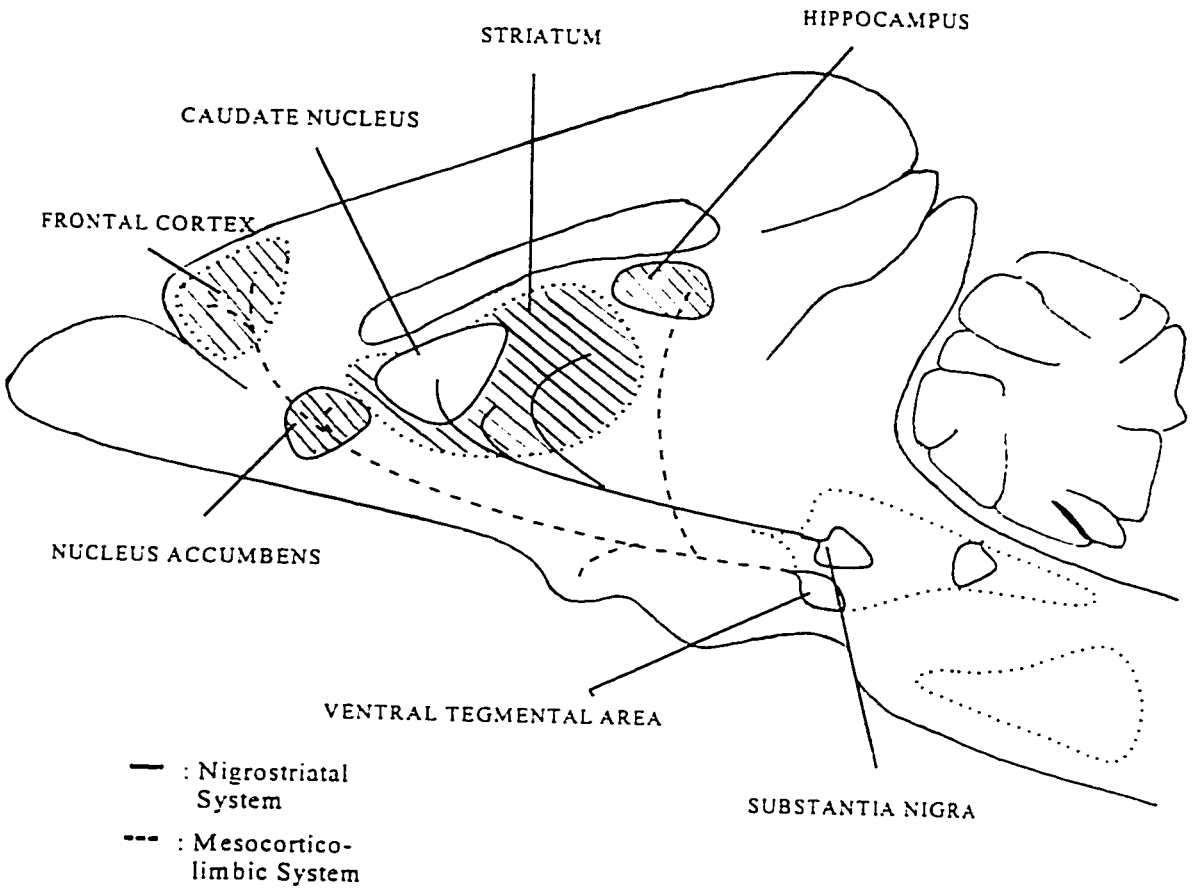

Figure 3: Principal dopaminergic systems in the rat brain (adapted from Waddock, 1997).

of the receptor, termed D2A [D2(444)] and D2B [D2(415)], that display the same pharmacology, were shown to exist on both pre- and postsynaptic DA neurons (Giros et al. 1989; Monsma et al. 1989; Dal Toso et al. 1989; Selbie et al. 1989).

Recently, molecular biology techniques have led to the identification and cloning of the genes corresponding to five DA receptor subtypes, namely D1, D2 (Bunzow et al. 1988; Dearry et al. 1990; Sunahara et al. 1990), D3 (Sokoloff et al. 1990), D4 and D5 (Sunahara et al. 1991; Schwartz, 1992; Sibley, Monsma, 1992; Civelli et al. 1993). These receptors can be classified into two subfamilies, the DA D1- and D2-like receptors, according to their structure, pharmacology and intracellular signalling. Thus, the cloned D1A and D1B/D5 receptors that stimulate adenylate cyclase (Dearry et al. 1990; Sunahara et al. 1991; Tiberi et al. 1991) belong to the DA D1-like family; the D2, D3, and D4 receptors that inhibit adenylate cyclase belong to the DA D2-like family (Bunzow et al. 1988; Sokoloff et al. 1990; Van Tol et al. 1991; Cohen et al. 1992; Griffon et al. 1997). All of these DA receptors belong to the "superfamily" of G-protein-coupled receptors. It has been proposed that the lengths of the third cytoplasmic (i3) loop and the C-terminal tail determine the second messenger characteristics displayed by the DA receptors (Schwartz et al. 1993). The D3 and D4 receptors display DA D2 pharmacology and structural characteristics of a long i3 loop and a short C-terminal tail.

1.3. DA autoreceptors

It is well-known that some endogenous neurotransmitters are able to inhibit their own synthesis and/or release by interacting with presynaptic autoreceptors (Roth, 1984). Activation of autoreceptors on DA neurons reduces dopaminergic neuronal firing and

synthesis and release of DA (Fedele et al. 1993; Carlsson, 1983; Clark et al. 1985b; Clark et al. 1985a; Seeman, 1980). The autoreceptors are present in most central DA pathways on the somatic, dendritic, and axonal parts of the neuron itself (Westerink et al. 1994; Di Chiara et al. 1978; Roth, 1979; Hjorth, 1983). Postsynaptic DA receptors exist on various parts of other (e.g., cholinergic) neurons. Several dopaminergic effects have been correlated with the activation of presynaptic receptors. For example, the inhibition of DA release from tissue slices (Starke et al. 1978), inhibition of DA synthesis in slices (Westfall et al. 1976), reduction of electrical DA cell activity (Skirboll et al. 1979), inhibition of the increased DA synthesis *in vivo* after pretreatment with γ-butyrolactone (GBL) [which reduces impulse flow (Walters, Roth, 1976)] and a decrease in locomotor activity (Elsworth, Roth, 1997), have all been attributed to the DA autoreceptors. Since apomorphine (APO) reduces synaptic release (Farnebo, Hamberger, 1971) and the rate of synthesis of DA (Raiteri et al. 1978), it is thought that APO and other DA autoreceptor agonists suppress locomotion by inhibiting synthesis and/or release of DA. Thus, if a drug acts at certain doses to decrease locomotion via an action on DA autoreceptors then, at these doses, it is expected to inhibit synthesis and/or release of DA. Recently, additional procedures for the study of DA autoreceptors have been developed, including fast cyclic voltammetry (Bull et al. 1991). In this method a time-resolution of DA release as short as 100 milliseconds is possible. Another method is infusion of an antisense oligonucleotide directly into an area containing the midbrain DA neurons (Elsworth, Roth, 1997). In this method a short synthetic oligodeoxynucleotide binds to autoreceptor mRNA and blocks the synthesis of that particular receptor.

Postsynaptic effects of activation of DA receptors are believed to include: the induction of stereotyped behavior (Ungerstedt et al. 1969); the induction of contralateral rotation after unilateral nigrostriatal lesions (Ungerstedt et al. 1969); and the reversal of reserpine-induced hypomotility (Anden et al. 1973). These pre- and postsynaptic model systems have been used in attempts to develop selective pre- and postsynaptic DA receptor agonists. Such selective drugs may be generally useful in the study of dopaminergic mechanisms, while the presynaptic agonists, in particular, might have interesting therapeutic applications (Meltzer, 1980). It has been suggested that DA presynaptic agonists may exhibit clinical antipsychotic activity. As they do not eliminate brain DA neurotransmission, such compounds may lack the extrapyramidal side effects caused by DA receptor antagonists (Carlsson, 1988). Some agents, which have been characterised with these methods, have been identified as putative selective presynaptic DA ligands. For example N,N-dipropyl-2-aminotetralin analogs (Feenstra, et al., 1983); N-n-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP) (Hjorth et al. 1981; Hjorth et al. 1983); B-HT 920 (Anden et al. 1982); EMD-49980 (Seyfried et al. 1989); OPC-4392 (Yasuda et al. 1988b); N-0437 (Van der Weide et al. 1988); SDZ-208-911 and SDZ-208-912 (Coward et al. 1990), have been introduced as DA presynaptic agonists. (+)-UH-232 [cis-(+)-(1S,2R)-5-methoxy-1-methyl-2-(di-n-propylamino)tetralin, (+)-AJ-76 [cis-(+)-(1S,2R)-5-methoxy-1-methyl-2-(n-propylamino)tetralin and CGP-24454A are examples of putative presynaptic DA receptor antagonists (Elsworth, Roth, 1997).

1.4. DA and locomotor activity

The role of DA systems in behavioral phenomena including locomotor activity is the topic of a large number of scientific investigations. The effects of manipulations of DA systems on unconditioned or spontaneous behavior have been reviewed by several authors (Ungerstedt et al. 1969; Pijnenburg et al. 1976; Pijnenburg et al. 1975a). Following the discovery of new DA receptor subtypes, the involvement of these receptors in different components of locomotor activity has been studied using the antisense approach (Zhang et al. 1997). These authors found that DA D2 and D4 antisense treatment decreased spontaneous locomotor activity. In contrast, D3 antisense treatment increased locomotor activity. The latter finding contrasts markedly with traditional views of the DA system. It has been generally accepted for some time that decreased activity in DA systems of adult animals results in hypoactivity. Thus, locomotor activity is generally reduced by administration of DA receptor blocking drugs (Anden et al. 1970; Zhang et al. 1997), bilateral injection of the neurotoxin 6-hydroxydopamine (6-OH-DA) or electrolytic lesions of ascending DA systems, drugs such as reserpine and α-MPT that deplete catecholamines (Ungerstedt, 1971) or presynaptic DA agonists that decrease DA release (Elsworth, Roth, 1997). Conversely, drugs that enhance transmission at DA synapses generally increase locomotor activity or induce stereotyped behavior (Fray et al. 1980); these stimulant effects are produced by direct acting DA agonists [e.g., APO, DA, quinpirole (QUIN)] and indirect acting DA agonists [e.g., nicotine, (+)-amphetamine, cocaine, L-3,4-dihydroxyphenylalanine (L-DOPA)] (Costall et al. 1979; Isaacson et al. 1978). Direct DA receptor agonists induce hyperactivity by stimulation of postsynaptic DA D2 receptors (Protais et al. 1986). Indirect

acting DA agonists induce hyperactivity by increasing DA release and/or synthesis and by blocking DA uptake (Zhang et al. 1997). Nicotine receptors are involved in regulation of DA release. Nicotine induces hyperactivity and increases DA release in rat striatum and more selectively in the mesolimbic system (Lapin et al. 1987; Imperato et al. 1986).

Studies also have been conducted to characterize individual DA nuclei or terminal areas involved in locomotor activity. It has been suggested that the nigrostriatal DA pathway is an important pathway in this regard. Thus, bilateral 6-OH-DA lesions of the substantia nigra (SN) that produce extensive (> 90%) depletion of striatal DA result in severe hypokinesia (Marshall et al. 1974). The mesolimbic-mesocortical DA neurons also have been implicated in the regulation of locomotor activity. Nevertheless, variable results have been reported with bilateral 6-OH-DA lesions at the origin of these neurons, i.e. in the ventral tegmental area (VTA): no change in locomotion, decreased locomotion or an increase in locomotor activity (Koob et al. 1981; Le Moal et al. 1975) have been reported. It has been concluded that these discrepancies may be due to partial damage to VTA DA neurons in some studies, as large 6-OH-DA lesions of VTA may induce hypoactivity whereas smaller lesions may result in hyperactivity (Koob et al. 1981). The DA cells originating in the VTA are apparently not uniformly involved in locomotion. Whereas bilateral 6-OH-DA lesions of the limbic terminal regions, i.e., nucleus accumbens (NAS) and olfactory tubercle, result in decreased locomotor activity (Iversen, Koob, 1977; Koob et al. 1978), similar lesions of the frontal cortical DA terminal area produce hyperactivity (Carter, Pycock, 1980). Also, electrocoagulation of the VTA results in a decrease of frontal cortical DA and increased locomotor activity (Tassin et al. 1978). Studies employing direct injections of DA or DA

agonists [e.g., DA, APO, (+)-amphetamine] into the NAS result in hyperactivity (Arnt, 1981; Costall, Naylor, 1975; Costall et al. 1977; Dill et al. 1979; Pijnenburg et al. 1976; Wachtel et al. 1979). In summary, it has been suggested that the DA neurons projecting to basal forebrain areas, caudate putamen, NAS, and olfactory tubercle enhance locomotor activity whereas mesocortical DA neurons normally inhibit locomotion.

Results of a number of recent studies suggest different roles for mesolimbic and nigrostriatal DA pathways in behaviors. The mesolimbic DA neurons may be involved primarily in an increased locomotion induced by DA agonists such as (+)-amphetamine and APO. Nigrostriatal DA neurons, on the other hand, may be more involved in stereotyped licking, biting and gnawing produced by high doses of these compounds (Fray et al. 1980). In support of this hypothesis, it has been reported that disruption of DA function in the caudate nucleus (CN) decreases (+)-amphetamine-stimulated stereotyped behavior without affecting locomotion induced by this drug (Creese, Iversen, 1974; Kelly et al. 1975; Pijnenburg et al. 1975b), whereas similar manipulations of the NAS disrupt locomotion stimulated by DA agonists (Pijnenburg et al. 1975b). Costall and Naylor have shown that this distinction may not be complete; it is possible to induce hyperactivity with intrastriatal injections of DA and, conversely, it is possible to induce stereotypy with intraaccumbens injections of DA agonists (Costall, Naylor, 1976). Furthermore, it is now clear that some VTA DA neurons terminate in striatal areas, including the anteromedial part of the CN (Simon et al. 1979), and some nigral DA neurons may project to non-neostriatal regions (Fallon et al., 1978).

Regarding the mechanism of hypoactivity, some researchers have mentioned that damage to the nigrostriatal system may result in sensory rather than motor impairments (Beninger,

1983). Rats undergoing a unilateral 6-OH-DA lesion of the SN were tested for orientation responses to mild somatosensory stimulation of specific areas of the body surface. Although they showed well-localized orientation responses to touch stimuli ipsilateral to the lesion, there was a marked lack of orientation to somatosensory stimuli presented to the contralateral side of the body (Marshall, 1979). Following unilateral intrastriatal injections of DA, these rats showed enhanced responses to stimuli presented to the side contralateral to the injections (Joyce et al. 1981). These results suggest that DA may normally play a role in mediating an animal's level of responsiveness to sensory stimulation. It has been concluded that the apparent lack of response to somatosensory stimulation (sensory neglect) probably does not result from a motor deficit, i.e., a simple inability to respond. This was shown by a study on the ability of a light stimulus to produce conditioned suppression of licking in SN-lesioned cats. The onset of a cue light produced lick suppression comparable to that seen in control animals, if the cue light was located in the visual field ipsilateral to the lesion. But the same stimulus presented on the contralateral side failed to produce the effect. As the lesioned cats clearly were capable of performing the conditioned response, it has been suggested that sensory input to the side contralateral to the lesion failed to induce normal responses (Feeney, Wier, 1979). In a similar way, it has been argued that unilateral 6-OH-DA lesions of the SN do not result in sensory neglect through a sensory deficit (i.e., a simple inability to perceive the stimulus). This suggestion was based on the observation that unilateral SN-lesioned and sham-operated rats re-learned a visual discrimination at similar rates even though the eye ipsilateral to the lesion was occluded. The authors concluded that the contralateral (neglected) eye does see and sensory neglect is observed because stimuli

contralateral to DA denervation fail to arouse the animal (Siegfried, Bures, 1979). Therefore, the apparent contralateral sensory neglect seen in animals with unilateral damage to the SN is not the result of impaired processing of sensory input; these animals appear to be deficient in their ability to interface sensory input with response systems.

1.5. DA D3 receptors: A literature review

1.5.1. Identification of DA D3 receptors

DA receptors have been identified by techniques that include binding studies and molecular biology techniques. Additional DA receptors, e.g. D3, D4 and D5 receptors, recently identified by molecular biological techniques may possibly be used to discover more selective antipsychotic drugs. In 1990, Sokoloff and colleagues cloned and identified the gene for the DA D3 receptor subtype (Sokoloff et al. 1990), and 7-[^{3}H]hydroxy-2-(N,N-di-n-propyl- amino)tetralin ([^{3}H]7-OH-DPAT) was identified as a selective ligand for this receptor (Levesque et al. 1992). In 1991, Snyder and co-workers also isolated a cDNA for the rat and human DA D3 receptor (Snyder et al. 1991). Seeman and Schaus reported that the guanine nucleotide-insensitive component of [^{3}H]QUIN binding (about 30%) may be related to DA D3 receptors (Seeman, Schaus, 1991). A truncated DA D3-receptor-like mRNA, named D3nf, predicting a protein that differs from the DA D3 receptor only in the carboxyl terminal, has been reported (Liu et al. 1994b). It has also been shown that human peripheral blood lymphocytes express the DA D3 receptor (Ricci, Amenta, 1994). The DA D3 receptor shows a high degree of homology with the DA D2 receptor. It differs from the DA D2 receptor with regard to *in vitro* binding pharmacology and anatomical distribution (Sokoloff et al. 1990).

1.5.2. Distribution of DA D3 receptors

The anatomical distribution of mRNA for DA D3 receptors and the receptor protein has been studied by *in situ* hybridization and radioligand binding techniques in human and rat brain. By using *in situ* hybridization, DA D3 receptor mRNA has been detected mainly in the ventral striatum, i.e., NAS, islands of Calleja, bed nucleus of the stria terminalis and other limbic areas such as the hippocampus and the mammillary nuclei receiving dopaminergic inputs from the A10 cell group. DA D3 receptor mRNA has also been detected at the level of the SN and VTA. Therefore, these receptors may function as both autoreceptor and postsynaptic receptors (Sokoloff et al. 1990; Diaz et al. 1995). By using radioligand binding techniques, Lahti and co-workers have reported the highest level of the DA D3 receptor protein in the NAS (Lahti et al., 1995). The DA D3 receptor has also been detected in the rat cerebellar cortex using radioligand binding techniques (Ricci et al. 1995). A recent study suggests that the DA D2 receptor is present in greater density than the DA D3 receptor in rat brain, even in limbic regions (Parsons et al. 1993), using QUIN and domperidone as ligands for DA D3 and D2 receptors, respectively. However, in another study using 7-OH-DPAT and (-)-sulpiride as a DA D3 or D2 ligand, respectively, a greater density of DA D2 (two-fold) compared to DA D3 receptors has been found in the striatum, but a 1:1 ratio in the NAS was found (Booze et al. 1992). Regarding the existence of DA D3 receptors in the shell or core subdivisions of the NAS, different results have been reported. Diaz and colleagues have reported that the DA D3 receptor mainly exists in the medium-sized neurons of the rostral pole and ventromedial shell subdivisions, but not of the core or septal pole (Murray et al. 1994). However, another study reported highest levels of D3 binding in

both the core and shell regions of the NAS and in the islands of Calleja (Booze et al. 1992). Herroelen and colleagues reported a much more widespread pattern of distribution in human brain. They reported the highest densities of DA D3 receptors in the ventral striatum and NAS, the remainder of the neostriatum and the cerebellar cortex and moderate amounts in the SN. Low densities of DA D3 receptors were reported in the pituitary gland (posterior lobe > anterior lobe), amygdala, and hippocampus. The globus pallidus and thalamus contained lower densities (Herroelen et al. 1993). It has been reported that the distributions of D3 sites in human and rat brain may be similar (Landwehrmeyer et al., 1993).

1.5.3. Pharmacological effects of 7-OH-DPAT

Recently, the DA agonist 7-OH-DPAT was identified as having > 100-, >1000- and >10,000-fold selectivity for DA D3 over D2, D4 and D1 receptors, respectively. It binds to DA D3 receptors with an affinity of <1 nM (Levesque et al. 1992). Studies of *in vivo* effects of 7-OH-DPAT suggest the involvement of DA D3 receptors in regulation of locomotor activity, reinforcement, DA turnover, yawning, emesis, hormone secretion, body temperature and blood pressure. Also, studies conducted in cocaine substitution tests, diabetic mice and a spontaneously hypertensive strain of rat show involvement of DA D3 receptors in these conditions. Some details of these studies are reviewed below.

1.5.3.1. Reward properties

Several studies have investigated the rewarding properties of 7-OH-DPAT using different models of positive reinforcement in rats. These models include intracranial self-stimulation (ICSS), conditioned place preference, drug self-administration and substitution for cocaine. R-(+)-7-OH-DPAT inhibits ICSS behavior over a wide dose range. At higher doses, it

induces a facilitation of ICSS (Kling-Petersen et al. 1995; Gilbert et al. 1995). Rats discriminate 7-OH-DPAT from a drug vehicle with an ED_{50} value of 0.038 mg/kg (McElroy, 1994; Nader, Mach, 1996). Results from conditioned place preference tests are not consistent. Some investigators have reported that 7-OH-DPAT does not induce significant place preference conditioning. It has been reported that a 0.03 mg/kg dose of 7-OH-DPAT induced a conditioned place aversion (Khroyan et al. 1995), and administration of either 0.25 or 5.0 mg/kg of 7-OH-DPAT prevented the acquisition of a morphine-induced conditioned place preference (Rodriguez De Fonseca et al. 1995). However, in other studies, 7-OH-DPAT (0.5-5 mg/kg) was effective in inducing a conditioned place preference (Mallet, Beninger, 1994; Kling-Petersen et al. 1995). 7-OH-DPAT and another DA D3 receptor ligand, (+)-PD-128907 [R-(+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol], may be effective substitutes for the discriminative stimulus effects of cocaine in rats (Acri et al. 1995). At a dose (1-4 μg, iv) that is not self-administered, 7-OH-DPAT decreased cocaine self-administration, indicating that 7-OH-DPAT may increase reinforcing effects of cocaine (Caine and Koob, 1993). All DA agonists induce cocaine-like stimulus effects in squirrel monkeys that have been trained to discriminate cocaine from a vehicle in a two-lever choice procedure. The reported order of potency of drugs for cocaine-like stimulus effects [(+)-4-propyl-9-hydroxynaphthoxazine > (R)-(-)-propylnorapomorphine > (±)-7-OH-DPAT ≥ PD-128907 ≥ QUIN > bromocriptine] correlated with their reported order of affinity for binding to D3 rather than to DA D2 receptors. The cocaine-like stimulant effects of the DA D3 receptor agonist, PD-128907, were attenuated by DA D2-like receptor antagonists with

an order of potency [nemonapride > eticlopride > YM-43611] that again corresponds more closely to their reported order of affinity at cloned human DA D3 than D2 receptors. The effects of PD-128907 were not attenuated by the D1-like receptor antagonist SCH 39166 (Spealman, 1996; Lamas et al. 1996). These results may indicate that the positive reinforcing effects and cocaine-like stimulus effects of 7-OH-DPAT are mediated by DA D3 receptors. Very recently, Parsons and co-workers have shown that DA D3 receptor agonists decrease cocaine-induced elevations in extracellular DA concentrations in NAS and appear to increase the reinforcing effects of cocaine, simultaneously. Therefore, they have suggested that postsynaptic DA D3 receptors are involved in enhancing the effects of cocaine (Parsons et al. 1996).

1.5.3.2. Y-maze test

It has been shown that whereas 7-OH-DPAT decreases locomotor activity of rats exploring a Y-maze, a brief electric shock applied to the grid floor during exploration converts this suppression effect into stimulation. This locomotor stimulation was completely antagonized by pretreatment with sulpiride (Franklin, Tang, 1995). These authors have concluded that a brief electric shock (acute stress) that increases the release of DA in brain (Abercrombie et al. 1989) is sufficient to compensate the effect of DA agonists on DA autoreceptors, i.e., decrease in DA release.

1.5.3.3. Locomotor activity

7-OH-DPAT induces biphasic effects on spontaneous locomotor activity, i.e., suppression of locomotor activity at low doses (0.02-0.5 mg/kg), followed by hyperactivity and sniffing behavior at higher doses (0.5-10 mg/kg) in rats (Daly, Waddington, 1993; Van den Buuse,

1993; Kurashima et al. 1995; Ferrari, Giuliani, 1995). Licking and gnawing behaviors have not been reported for even very high doses of 7-OH-DPAT. Different mechanisms for these effects have been suggested. DA D2 receptor agonists may decrease locomotor activity via stimulation of DA D2 autoreceptors (Raiteri et al. 1978; Farnebo, Hamberger, 1971) and DA D3 receptor agonists may act *via* stimulation of DA D3 autoreceptors (Daly, Waddington, 1993) or postsynaptic inhibitory DA D3 receptors (Svensson et al. 1994b). 7-OH-DPAT (0.3-3 μg total dose) injected bilaterally in the NAS resulted in hypolocomotion (Gilbert, Cooper, 1995). In non-habituated mice, 7-OH-DPAT (0.04-10 mg/kg, sc) suppressed behaviors and induced frozen postures, with only occasional evidence of weak behavioral stimulation occurring at 5-10 mg/kg. Also, 7-OH-DPAT (3-10 mg/kg) did not reinstate locomotion in 4 h habituated mice (Starr, Starr, 1995). Khroyan and co-workers (Khroyan et al. 1995) reported that administration of 7-OH-DPAT (5 mg/kg) results in an increased locomotor response (i.e., behavioral sensitization). Also, in contrast to QUIN, subchronic treatment with 7-OH-DPAT did not result in cross-sensitization to either APO or cocaine. Subsensitivity of DA autoreceptors may be important for the development of behavioral sensitization to cocaine. For example, in rats behaviorally sensitized to cocaine, a challenge injection of cocaine shows less inhibitory effect on striatal DOPA accumulation than in control rats (Elsworth, Roth, 1997). Taken together, these investigators have suggested that locomotor inhibition produced by low doses of 7-OH-DPAT may not be related to DA autoreceptor stimulation. Also the development of behavioral sensitization to high doses of 7-OH-DPAT may not be due to the development of DA autoreceptor subsensitivity (Khroyan et al. 1995).

7-OH-DPAT (0.2-10 mg/kg) dose-dependently reversed the akinesia of mice and rats treated 24 h previously with reserpine. This response was blocked by the DA D2 receptor antagonist raclopride (10 mg/kg ip), but not by the DA D1 receptor antagonist SCH 23390 (0.05 mg/kg, ip) (Starr, Starr, 1995; Ahlenius, Salmi, 1994). With unilateral lesions of the nigrostriatal system, sc injection of 0.01-1 mg/kg of 7-OH-DPAT induced dose-dependent contralateral turning behavior (Van den Buuse, 1993). Spontaneous locomotor activity is increased in diabetic mice, and 7-OH-DPAT (0.1-30 mg/kg, sc) dose-dependently decreased this spontaneous hyperactivity. 7-OH-DPAT (0.1 mg/kg) decreased morphine-induced hyperactivity in nondiabetic mice, but not in diabetic mice. Furthermore, 7-OH-DPAT (0.1 mg/kg) significantly attenuated the morphine-induced increase in DA turnover in both nondiabetic and diabetic mice. However, basal DA turnover was significantly greater in diabetic mice than in nondiabetic mice (Kamei, Saitoh, 1996). These authors have concluded that it is likely the enhanced spontaneous locomotor activity in diabetic mice is partially related to the down-regulation of D3-receptor-mediated modulation of DA release in the limbic area. Treatment with low doses of 7-OH-DPAT (0.1 and 0.3 mg/kg, sc) attenuated morphine (10 and 20 mg/kg, sc)-induced hyperlocomotion and morphine-induced increases in levels of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the limbic forebrain i.e., NAS and olfactory tubercle (Suzuki et al. 1995).

1.5.3.4. Yawning

Reports on effects of 7-OH-DPAT on yawning behavior are not consistent. Several studies have reported that 7-OH-DPAT failed to induce yawning (Levesque, 1996; Gilbert, Cooper,

1995). However, in other studies 7-OH-DPAT at low doses induced yawning (Kurashima et al. 1995; Van den Buuse, 1993; Ferrari, Giuliani, 1995; Damsma et al. 1993). Additionally, repeated administration of 0.1 mg/kg resulted in a sensitized yawning response (Khroyan et al. 1995). 7-OH-DPAT injected bilaterally in the NAS (0.3-3 μg total dose) resulted in hypolocomotion but did not induce yawning (Gilbert, Cooper, 1995). It appears that the effect of 7-OH-DPAT on yawning may depend on the route of drug administration. Gilbert and Cooper used ip injections and did not observe yawning behavior (Gilbert, Cooper, 1995), whereas other studies that used the sc route with a range of doses observed this behavior. This effect of 7-OH-DPAT may not be mediated *via* an action at the NAS as intraaccumbal injections of this drug did not induce yawning.

1.5.3.5. Emesis

It is not clear which DA receptor subtype is involved in emesis induced by DA agonists. Low doses of the DA agonists R-(+)-7-OH-DPAT, APO and QUIN dose-dependently cause emesis, while SKF-38393, a selective D1 receptor agonist, failed to induce emesis in dogs. The emesis induced by R-(+)-7-OH-DPAT could be inhibited by S-(-)-eticlopride, a potent DA D2 and D3 receptor antagonist, but not by SCH 23390 (1 mg/kg, sc), a selective D1 receptor antagonist, or by clozapine (CLZ) (1 mg/kg, sc), a D1/D4 receptor antagonist. It has been proposed that DA D3 receptors play an important role in the genesis of emesis in dogs (Yoshida et al. 1995).

1.5.3.6. Body temperature

7-OH-DPAT, 0.3-1.3 mg/kg, induced a decrease in body temperature, and this effect was inhibited by pretreatment with haloperidol (HAL) (Van den Buuse, 1993; Ahlenius, Salmi,

1994; Kurashima et al. 1995). Across DA agonists tested for inducing hypothermia, potency correlated significantly with affinity at DA D3, but not D2, sites. Across antagonists, the potency for inhibition of (+)-7-OH-DPAT-induced hypothermia also correlated more strongly with affinity at DA D3 than at D2 sites. Based on these results, an involvement of DA D3 receptors in body temperature control has been suggested (Millan et al. 1995a).

1.5.3.7. Blood pressure

7-OH-DPAT (0.1 or 1 mg/kg, iv) increased blood pressure in rats. Pretreatment with the DA receptor antagonist HAL partially prevented this pressor response (Van den Buuse, 1993). This effect may be mediated by peripheral DA D3 receptors (Ricci et al. 1995).

1.5.3.8. Hormone secretion

It has been suggested that DA D3 receptors are involved in mechanisms regulating secretion of oxytocin but not prolactin in the rat (Uvnas-Moberg et al. 1995). It has been reported that the DA D2/D3 receptor agonists QUIN (0.5-8.0 mg/kg, sc) and 7-OH-DPAT (0.2-3.2 mg/kg, sc), but not the preferential DA D2 receptor agonist bromocriptine (2.0-32.0 mg/kg, sc), increased plasma oxytocin and decreased plasma prolactin levels in rats. HAL, but not the D3 antagonists (+)-S-14297 and (±)-S-11566, increased prolactin secretion. Across all antagonists, potency for eliciting prolactin secretion and catalepsy correlated better with affinity at DA D2 ($r = 0.95$ and 0.96, respectively) than at D3 ($r = 0.76$ and 0.91, respectively) sites (Millan et al. 1995b).

1.5.4. The effects of 7-OH-DPAT on DA release

DA receptor ligands affect synthesis, release and metabolism of DA *in vivo* and *in vitro*. DA agonists, at low doses, decrease the turnover of DA *via* an action at autoreceptors; DA

receptor antagonists increase DA turnover by indirect feedback mechanisms (Elsworth, Roth, 1997; Gozlan et al. 1987; Nakazawa et al. 1991; Erwin et al. 1986). Several studies have shown the involvement of DA D3 receptors in DA release, synthesis and metabolism. 7-OH-DPAT inhibited electrically stimulated endogenous DA release in slices of rat NAS (Patel et al. 1995). Using brain microdialysis, R-(+)-7-OH-DPAT and other 2-aminotetralin derivatives, e.g. S-(-)-N-0437, R-(+)-N-0438, and R-(+)-PHNO, were shown to decrease DA release by 45-60% (Timmerman et al. 1991; Damsma et al. 1993; Waters et al. 1993; Rayevsky et al. 1995). (+)-7-OH-DPAT (0.16 mg/kg, sc) decreased dialysate levels of DA in the NAS and this effect was antagonized by (+)-S-14297 [(+)-[7-(N,N-dipropylamino)-5,6,7,8-tetra-hydronaphth(2,3b)dihydro,2,3-furane]] (1.25 mg/kg, sc) a preferential DA D3 receptor antagonist (Rivet et al. 1994). 7-OH-DPAT decreased extracellular DA and DOPAC levels in the NAS and dopaminergic cell firing (Devoto et al. 1995). Atypical neuroleptic drugs (CLZ and thioridazine) and the preferential DA autoreceptor antagonists (+)-UH-232 and (+)-AJ-76 increased DA release and DOPAC levels (Gainetdinov et al. 1994). The effects of DA agonists for reversal of GBL-induced elevation of striatal L-DOPA levels correlated with the affinities (Ki) of the agonists for the DA D3 but not the DA D2 receptor. GBL-induced elevation of striatal L-DOPA levels is an index of inhibition of DA synthesis. These data suggest that striatal synthesis-inhibiting autoreceptors are of the DA D3 rather than the D2 subtype (Meller et al. 1993; Aretha et al. 1995). The ED_{50} of 7-OH-DPAT in this model was about 0.03 mg/kg for NAS and CN and 0.01 mg/kg for olfactory tubercles in rats (Aretha et al. 1995). In another study comparing 8 agonists, the potency for inhibition of DA synthesis correlated more highly with affinity at DA D3 than

D2 receptors. However, across nine antagonists, potency in facilitating DA synthesis correlated more highly with affinity at DA D2 than D3 sites (Gobert et al. 1995). Acute, but not chronic, treatment with 0.007-1 mg/kg of 7-OH-DPAT decreased DA synthesis in both striatal and mesolimbic regions (Mattingly et al. 1996; Ahlenius, Salmi, 1994). Booth and co-workers have reported that 7-OH-DPAT at a high dose (ID_{50} = 4.8-6.4 mg/kg) inhibited tyrosine hydroxylase (IC_{50} = 0.6-0.7 μM) and inhibited DOPA accumulation in two autoreceptor models (i.e., *in vitro* tyrosine hydroxylase activity or *ex vivo* DOPA accumulation after NSD-1015 plus GBL pretreatment). This effect of 7-OH-DPAT was not site selective with extrapyramidal or limbic tissue of rat forebrain. However, they showed some limbic selectivity (ID_{50} = 10 *vs* 29 mg/kg) in an *in vivo* model of postsynaptic DA D3 and D2 receptors activity (i.e., NSD-1015 pretreatment without GBL). The effects were partially blocked by S-(-)-eticlopride alone, and fully blocked after reserpine pretreatment (Booth et al. 1994). Intracerebroventricular infusion of an all-phosphorothioate antisense oligodeoxynucleotide targeted at the rat DA D3 receptor mRNA (10 μg/h, 5 days) resulted in an increase in DA synthesis in NAS but not in caudate-putamen; in contrast, antisense treatment did not counteract the effect of APO on DA synthesis. These results strongly suggest that DA D3 receptors may influence DA synthesis (Nissbrandt et al. 1995).

1.5.5. The effects of 7-OH-DPAT on firing rate of DA neurons

(+)-7-OH-DPAT inhibited the firing rate of dopaminergic neurons in the VTA of anaesthetised rats dose-dependently (0.31-5.0 μg/kg, iv), and similar effects have been observed in the SN pars compacta and VTA of rat brain slices (Bowery et al. 1994). This effect was dose-dependently (16-125 μg/kg, iv) antagonized by the selective DA D3

receptor antagonist, (+)-S-14297 (Lejeune, Millan, 1995). Glutamate-induced firing was also inhibited by 7-OH-DPAT. These findings suggest that DA D3 receptors are also involved in the postsynaptic inhibition of NAS neurons by DA (Amano et al. 1994).

1.5.6. Regional selectivity of 7-OH-DPAT in brain

7-OH-DPAT has been examined in several electrophysiological assays to determine whether it exhibits preferential effects in the mesolimbic (A10) versus nigrostriatal (A8/A9) DA systems. Intravenous 7-OH-DPAT potently and completely inhibited the firing of both A9 and A10 DA neurons (ED_{50}s: 3.5-0.7 μg/kg and 3.9-0.9 μg/kg, respectively). Also no site-selectivity was observed in response to iontophoretically applied (+)-7-OH-DPAT or in cell-attached patch-clamp recordings (Liu et al. 1994). However, studies on the effect of 7-OH-DPAT on DA release showed site-selectivity. Thus, local application of 7-OH-DPAT *via* reverse-dialysis decreased DA release in the NAS but not in the ipsilateral striatum of the same animals (Parsons et al. 1996). 7-OH-DPAT also had differential effects on evoked DA release from slices of striatum and NAS (Yamada et al., 1992).

1.5.7. DA D3 receptor affinity

Several investigators have studied the affinity and selectivity of DA receptor ligands for the DA D3 receptor using cloned DA D3 receptors in different cell lines. Based on these studies, (+)-butaclamol and domperidone showed 5-fold DA D3 receptor selectivity compared to D2 receptors. APO and bromocriptine failed to demonstrate selectivity for DA D3 receptors. DA, QUIN, (+)-4-propyl-9-hydroxynapthoxazine (PHNO), 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (6,7-ADTN), 7-OH-DPAT and N-0434 showed marked human DA D3 (hD3) receptor selectivity (Freedman et al. 1994). Another selective

DA D3 receptor ligand is PD-128907, which exhibits about a 1000-fold selectivity for human DA D3 receptors (Ki<1 nM) versus human DA D2 receptors (Ki<1183 nM) and therefore should be useful for further characterizing DA D3 receptors (Akunne et al. 1995; Pugsley et al. 1995). Pramipexole (SND 919; 2-amino-4,5,6,7-tetrahydro-6-propylamino benzthiazole dihydrochloride) exhibits a 5-fold selectivity for DA D3 receptors (Mierau et al. 1995; Camacho-Ochoa et al. 1995). (±)-7-OH-DPAT showed 64-fold greater selectivity for DA D3 than for D2 receptors (Levesque et al. 1992). Its enantiomer, R-(+)-7-OH-DPAT, is reported to have a 2-fold greater D3 affinity than the racemate (Baldessarini et al. 1993). Certain substituted hexahydrobenzo[α]phenanthridines also have been reported as high affinity ligands selective for the DA D3 receptor (Watts et al. 1993). Several compounds have been reported as selective DA D3 antagonists; these include U-99194A, which has 20 fold D3 *vs* D2 DA receptor preference (Waters et al. 1993), (+)-S-14297 (Newman-Tancredi et al. 1995) and nafadotride which have 10 times higher affinity at DA D3 than DA D2 receptors (Sautel et al. 1995). These compounds bound with higher affinity and selectivity to recombinant, human DA D3 versus D2 receptors (Millan et al. 1994). Figure 3 shows chemical structures of some putative DA D3 receptor agonists.

1.5.8. Function of DA D3 receptors

The DA D3 receptor subtype has been associated with cognitive, emotional, and endocrine functions as a result of its localization in limbic areas of the brain. It binds a range of neuroleptics with high affinity, suggesting that it may be an important target for antipsychotics. Previously, it was thought that antipsychotics interact only with DA D2 receptors (Sokoloff et al. 1990; Sokoloff et al. 1992; Malmberg et al. 1993). Several lines of studies

indicate possible functional differences between the DA D2 and D3 receptors.

1. Doses of R-(+)-7-OH-DPAT leading to a reduction of locomotion failed to affect DA release or synthesis rate. These data suggest that the DA D3 receptor is a postsynaptic receptor with an inhibitory influence on rat locomotor activity (Svensson et al. 1994a, 1994b).

2. Preferential D3 antagonists increase spontaneous locomotor activity, while DA D2 receptor antagonists decrease locomotor activity, supporting the hypothesis that the DA D3 receptor is functionally relevant at the postsynaptic level (Waters et al. 1994).

3. DA D2 and D4 antisense treatment decreased spontaneous locomotor activity, while, in contrast, D3 antisense treatment increased locomotor activity (Zhang et al. 1997).

4. HAL and sulpiride, two DA D2-like receptor antagonists, but not SCH 23390, a D1-like receptor antagonist, increased proneurotensin mRNA in the DA D2 receptor mRNA-rich areas but decreased this substrate in the DA D3 receptor mRNA-rich areas. These findings suggest that the DA D2 and D3 receptors control neurotensin mRNA expression negatively and positively, respectively (Diaz et al. 1994).

5. Parsons and co-workers have reported that 7-OH-DPAT and quinelorane decreased cocaine intake in a manner indicating an enhancement of cocaine reinforcement and simultaneously decreased the cocaine-induced elevations in DA levels in NAS by >50%. Subsequent to self-administration of either

7-OH-DPAT or quinelorane, two putative DA D3 receptor agonists, drug intake was increased and DA levels in NAS were decreased to approximately 50% below drug-naive baseline levels. These authors have concluded that stimulation of postsynaptic DA D3 receptors in the NAS enhances the reinforcing properties of cocaine. Furthermore, local application of 7-OH-DPAT *via* reverse-dialysis dose-dependently decreased DA efflux in the NAS, whereas there was no effect of this agonist on DA efflux in the ipsilateral striatum of the same animals. Nafadotride, a putative DA D3 antagonist, dose-dependently blocked this effect of 7-OH-DPAT on DA efflux in NAS (Parsons et al. 1996).

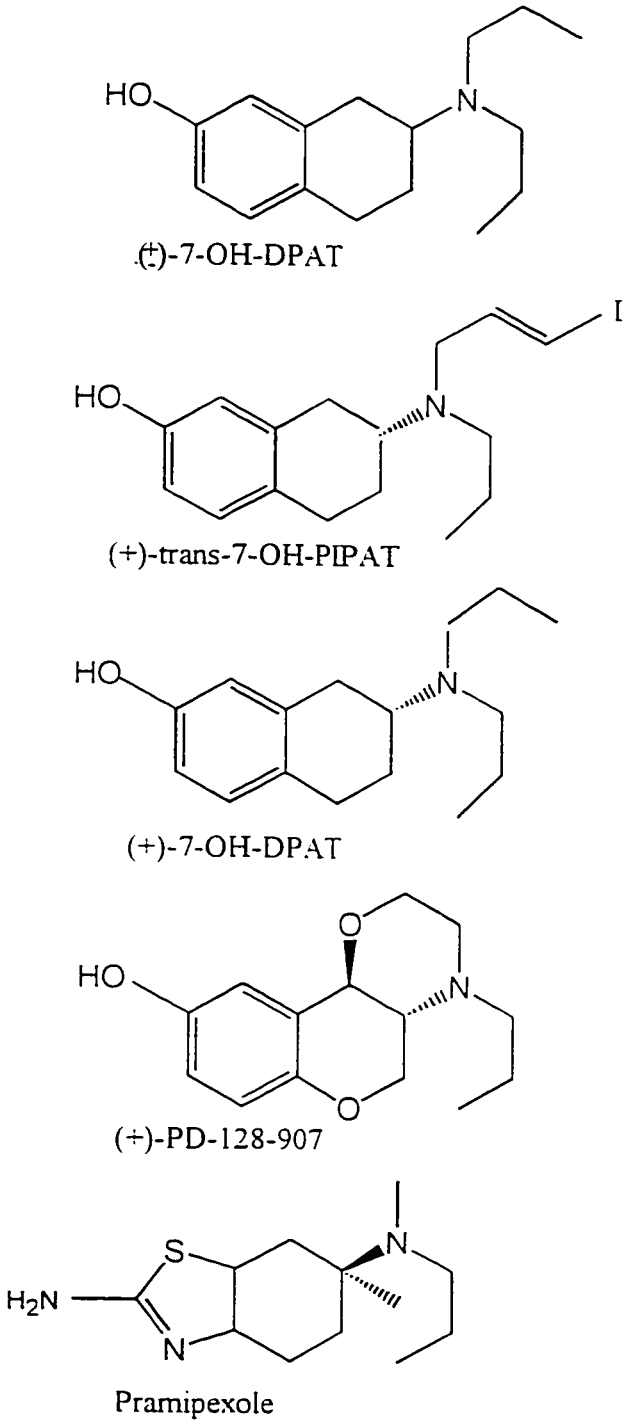

Figure 4: Chemical structures of some DA D3 receptor agonists

1.6. Schizophrenia: DA hypothesis

According to the DA hypothesis of schizophrenia certain DA pathways are overactive in schizophrenia (Matthysse, 1973). Although this hypothesis has been reviewed elsewhere (Snyder, 1976; Meltzer, Stahl, 1976; Seeman, 1980; Seeman et al. 1987; Seeman, Niznik, 1990; Davis et al. 1991), the recent cloning of various DA receptors opens up the possibility for new insights into the DA hypothesis, particularly on the dopaminergic basis of antipsychotic therapy of schizophrenia. The presence of DA D3 receptors in the mesolimbic DA system and the high affinity of most antipsychotics for DA D3 receptors (Sokoloff et al. 1990) suggest the involvement of the DA D3 receptor in therapeutic effects of neuroleptic drugs. The DA hypothesis of schizophrenia along with other alternative hypotheses such as those involving glutamate and serotonin are reviewed in this section.

The DA hypothesis of schizophrenia stems from the 1952 discovery of neuroleptic drugs. In seeking a possible biologic basis for schizophrenia, investigators searched for the primary target of neuroleptic action (Matthysse, 1973; Seeman, Lee, 1975; Seeman et al. 1975) followed by a search for abnormalities in these target sites in schizophrenia (Lee, Seeman, 1980). Much of the evidence for the hypothesis of DA overactivity in schizophrenia is based on the fact that neuroleptic drugs block DA receptors *in vivo* and *in vitro* (Anden et al. 1970; Seeman et al. 1975; Creese et al. 1976). The clinical antipsychotic potencies of various neuroleptic agents are directly related to their potencies at DA receptors, particularly the DA D2 receptor (Seeman et al. 1975; Creese et al. 1976). The neuroleptic K_d values at the DA D2 receptor site are reported to match closely the free neuroleptic concentrations in

the patients' plasma water for all neuroleptics except CLZ (Farde et al. 1988; Farde et al. 1989; Nordstrom et al. 1993; Farde, von Bahr, 1990; Farde et al. 1990). The DA D2 receptor-blocking action of neuroleptic drugs also accounts for: neuroleptic-induced Parkinsonism and hyperprolactinemia; a blockade of the action of DA-mimetic drugs (APO, L-DOPA, amphetamine, methamphetamine, methylphenidate); and acceleration of catecholamine turnover (Seeman et al. 1987). Additional evidence for a dopaminergic basis of schizophrenia comes from the fact that DA-mimetic drugs may induce and exacerbate the symptoms of schizophrenia (Lieberman et al. 1987a, 1987b) and also the elevation of DA D2 receptors in the post-mortem brains of schizophrenic patients. The density of DA D1 receptors is normal in schizophrenia (Seeman et al. 1987; Seeman, Niznik, 1990), while the density of DA D2 receptors is elevated in post-mortem schizophrenic brain tissues (Lee et al. 1978; Lee, Seeman, 1980; Cross et al. 1983; Seeman et al. 1984; Joyce et al. 1988; Toru et al. 1988; Mita et al. 1986; Seeman, Niznik, 1990). However, several authors have concluded that this DA D2 site elevation is a result of neuroleptic treatment (Kornhuber et al. 1989; Wyatt, 1986; Reynolds, 1981) since these investigators observed that the DA D2 density in samples from drug-naive patients is in the control range. Also, several studies have shown that long-term neuroleptic treatment increased DA D2 receptors density without elevation of DA D1 receptors in laboratory animals (Watanabe, 1993).

Positron emission tomography studies resulted in opposite findings depending on the radioligand that they used. Using [^{11}C]methylspiperone, a DA D2 receptor elevation has been reported in the CN of neuroleptic-naive living schizophrenic patients (Wong et al. 1986). However, using [^{11}C]raclopride, no elevation of DA D2 sites in the putamen of such

patients was found (Farde et al. 1987). It has been hypothesized that this DA D2 elevation is specifically related to DA D4 receptor subtype (Seeman et al. 1993), since spiperone shows a higher affinity for the DA D4 subtype and occupies DA D2, D3 and D4 receptors while raclopride selectively binds to DA D2 and D3 receptors. A recent study does not support this hypothesis; Reynolds and Mason showed that all the specific binding by [^{3}H]emonapride, a DA D2, D3 and D4 ligand, is displaced by a DA D2/D3 ligand raclopride (Reynolds, Mason, 1994). Another theory proposes that there are two active sites at the DA D2 receptor for the raclopride molecule, indicating an underestimation of DA receptors in those studies which have used raclopride (Seeman et al. 1989a, 1990; Seeman, 1992a). Nevertheless, there is no clear-cut evidence for an intrinsic DA D2 receptor elevation in schizophrenia. After the discovery of other DA D2-like receptor subtypes such as the DA D3 receptor, attention has been paid to these new DA receptor subtypes as targets for treatment purposes. Thus, the DA D3 receptor is positively associated with the efficacy of neuroleptics as a result of its preferential expression distribution in limbic areas and its high affinity for neuroleptics. In contrast, a blockade of DA D2 receptors in the striatum has been associated with the extrapyramidal side effects of antipsychotic drugs.

1.6.1. Atypical properties of CLZ

The CLZ K_d value at the DA D2 receptor does not match the free CLZ concentrations in the patients' plasma water corresponding to a clinically effective dose (Farde et al. 1988, 1989, 1990; Nordstrom et al. 1993; Farde, von Bahr, 1990). However, the CLZ K_d values at the DA D4 receptor do appear to match the free CLZ concentration in the patients' plasma water. In addition to blocking DA D4 receptors at 10 nM, it is important to note that CLZ

also blocks both muscarinic cholinergic receptors and 5-HT2 receptors at about this same concentration (Seeman, 1990). Although the atypical nature of CLZ in not causing extrapyramidal signs in patients may possibly be attributed to its muscarinic receptor blockade, the combination of HAL and a muscarinic blocker was not as satisfactory as CLZ in avoiding extrapyramidal effects (Kane et al. 1988). Therefore, it has been concluded that the atypical nature of CLZ may not be explained by its anticholinergic action. The blockade of 5-HT2 receptors by CLZ, thioridazine, and risperidone (Janssen et al. 1988) possibly accounts for their atypical lack of Parkinsonian side effects, as has discussed elsewhere (Seeman, 1992b). This is consistent with the fact that neuroleptic-induced catalepsy may be antagonized by 5-HT2 blockers such as cyproheptadine or mianserin (Seeman, 1990). However, chlorpromazine, which blocks both DA D2 and 5-HT2 receptors, elicits considerable Parkinsonism. It has been suggested that the neuroleptic dissociation constant for the 5-HT2 receptor must be about one-tenth of that for DA D2 receptor, as in risperidone (Janssen et al. 1988), in order to result in an atypical neuroleptic action (Seeman, 1992b). This is not the case, however, for such atypical neuroleptics as remoxipride, raclopride, and thioridazine (Seeman et al. 1989b). Recently the involvement of other neurotransmitters such as glutamate has been hypothesized in mediating the effects of CLZ.

1.6.2. The glutamate system and schizophrenia

Degeneration or dysfunction of the glutamatergic system has been hypothesized in the etiology of schizophrenia (Kim et al. 1980). Kim and co-workers found that glutamate concentrations were lower in the cerebrospinal fluid of schizophrenic patients than in controls. Using proton magnetic resonance spectroscopy, Stanley and co-workers also

showed lower prefrontal glutamate in patients never treated for schizophrenia. In addition, it has been shown that chronic amphetamine lowers glutamate content in rat brain (Kim et al., 1981). Glutamate is involved in cognitive functions such as learning and memory which are known to be dysfunctional in schizophrenia (Collingridge, 1987). There is increasing evidence indicating interactions between DA and glutamate systems. A prefrontal glutamatergic cortical efferent projects to areas containing DA cell bodies such as the VTA and to DA terminal areas such as the dorsal and ventral regions of the medial striatum (Beckstead, 1979; Christie et al. 1987; Sesack et al. 1989; Herkenham, Nauta, 1979; Gonzales, Chesselet, 1990). These descending pathways of the prefrontal cortex are thought to modulate the release of DA in subcortical areas such as the striatum. This modulation has been suggested as a part of the pathophysiology of disorders associated with subcortical DA dysfunction such as schizophrenia and Parkinson's disease (Weinberger et al. 1986; Grace, 1991; Lange, Riederer, 1994). For example, chemical and electrical stimulations of the prefrontal cortex increased DA release in the striatum (Murase et al. 1993). *In vivo* and *in vitro* reports have indicated that excitatory amino acids (glutamate and aspartate), the primary neurotransmitters of cortical efferents (Spencer, 1976; McGeer et al. 1977; Carter, 1982; Christie et al. 1985; Girault et al. 1986; Young, Bradford, 1986), increase striatal DA release (Roberts, Anderson, 1979; Cheramy et al. 1986; Carter et al. 1988; Clow, Jhamandas, 1989; Barbeito et al. 1990; Imperato et al. 1990a, 1990b; Leviel et al. 1990; Moghaddam et al. 1990; Krebs et al. 1991; Keefe et al. 1992; Westerink et al. 1992; Youngren et al. 1993). These studies have proposed a presynaptic modulation of DA release by cortical excitatory projections. Although, morphological studies have failed to

demonstrate synaptic contacts between striatal excitatory and dopaminergic terminals (Bouyer et al. 1984; Fuller et al. 1996). recent ultrastructural studies have demonstrated synaptic contacts between prefrontal cortical efferents and DA containing neurons in the VTA (Sesack, Pickel, 1992). Furthermore, electrophysiological studies have demonstrated that local application of exogenous excitatory amino acids and cooling or chemical stimulation of prefrontal cortical efferent modulates the rate and pattern of DA neuron firing (Gariano, Groves, 1988; Grenhoff et al. 1988; Svensson, Tung, 1989; Tung et al. 1991; Suaud-Chagny et al. 1992; Chergui et al. 1993; Murase et al. 1993; Wang, French, 1993). Also, chemical lesions of the prefrontal cortex have been shown to alter DA turnover in lateral or medial striatum (Pycock et al. 1980b; Pycock et al. 1980a; Rosin et al. 1992; Bubser, 1994), to decrease the cataleptogenic effect of HAL (Scatton et al. 1982; Worms et al. 1985; Jaskiw et al. 1993), and to reduce the responsiveness of subcortical DA systems to pharmacological challenges (Rosin et al. 1992; Roberts et al. 1994). A reduction in DA turnover in the striatum has been reported after pharmacological manipulation of the prefrontal cortex with amphetamine (Louilot et al. 1989; Kolachana et al. 1995) or APO (Jaskiw et al. 1991). It has been suggested that a deficit in glutamate input would lead to a dopaminergic supersensitivity (Pettegrew et al. 1991). These investigators also suggested that a degenerative process affecting frontal glutamatergic projections could lead to structural and functional changes seen in temporal and striatal regions.

However, L-glutamate receptor antagonists do not decrease basal dopamine release in NAS (Taber and Fibiger, 1995). Activation of N-methyl-D-aspartate (NMDA) receptors inhibits dopaminergic transmission in rat medial frontal cortex. Thus, administration of D,L-2-

amino-5-phosphanovalerate (DL-APV), a NMDA antagonist, into the medial frontal cortex increased the amount of DOPAC and the DOPAC/DA ratio in the cortical area (Hata N et al. 1990). There is a reciprocal effect of NMDA receptors in the subcortical area. Thus, agonists for excitatory amino acid receptors increase DA release from the striatum (Carter et al. 1988; Clow, Jhamandas, 1989; Roberts, Anderson, 1979; Snell, Johnson, 1986), NAS (Jones et al. 1987) and SN (Araneda, Bustos, 1989) *in vitro* and *in vivo*. NMDA antagonists also increase DA release in the NAS and CN, and induce stereotypy behavior (Imperato et al. 1990b) and hyperactivity (O'Neill, Liebman, 1987). The involvement of NMDA receptors in the action of CLZ has been indicated (McCoy, Richfield, 1996). CLZ antagonized hyperlocomotion and stereotypy induced by MK-801, a NMDA antagonist (Lang et al. 1992; Tiedtke et al. 1990).

1.6.3. The serotonin system and schizophrenia

Increasing evidence also suggests the involvement of the 5-HT system in the etiology of schizophrenia (Breier, 1995). This system alone or its interaction with the DA system may be a suitable target for superior therapeutic agents in schizophrenia. Different lines of evidence, including the therapeutic success of CLZ and risperidone, interactions between DA and 5-HT systems, and elevated 5-HT2A/2C receptors in schizophrenic patients support this view (Bleich et al. 1988). However, studies on 5-HT and 5HIAA levels in schizophrenic patients have not yielded consistent results. The earliest convincing evidence for a serotonin-DA interaction in humans came from Ceulemans and co-workers (Ceulemans et al. 1985), who treated schizophrenic patients with setoperone, a 5-HT2 antagonist, in an open trial and demonstrated a beneficial effect on extrapyramidal symp-

toms. It was unclear in this study whether the benefit resulted from the discontinuation of the typical neuroleptic or from the initiation of setoperone. In subsequent studies, other investigators used ritanserin, a more specific 5-HT2A/2C antagonist, in double-blind, placebo-controlled, add-on trials and showed a significant improvement in extrapyramidal symptoms (Gelders et al. 1990; Kapur, Remington, 1996). Silver and co-workers (Silver et al. 1989) have reported a beneficial trend for cyproheptadine in ameliorating extrapyramidal symptoms in patients receiving neuroleptics. In contrast, using mianserin in a double-blind crossover trial in patients with neuroleptic-induced Parkinsonism, failed to support a beneficial effect of 5-HT2 antagonism (Korsgaard, Friis, 1986). CLZ, which is thought to improve negative symptoms, induces an increased turnover of DA in the prefrontal cortex of rodents, an effect not seen with typical antipsychotics (Moghaddam, Bunney, 1990; Moghaddam, 1994). More recent studies suggest that this property of CLZ can be explained by its 5-HT2 antagonism (Nomikos et al. 1994; Schmidt, Fadayel, 1995). Thus, if current speculations regarding the role of the prefrontal cortex in negative symptoms are correct, 5-HT2A/2C antagonists could ameliorate negative symptoms by means of their effects on the dopaminergic system. Serotonergic projections also have a direct inhibitory effect on the prefrontal neurons, separate from their effect on the dopaminergic projections. Therefore, some of the effects of 5-HT2 blockers on negative symptoms in animal models and humans may reflect a direct effect rather than a DA-mediated effect on prefrontal neurons (Mantz et al. 1990; Ashby et al. 1990). Generally the serotonin system inhibits dopaminergic function at the level of the origin of the DA system in the midbrain as well as at the terminal dopaminergic fields in the forebrain, but it may also stimulate DA activity depending on the

site and receptor (Gerlach, Peacock, 1995). Thus, 5-HT inhibits the firing of the DA cells projecting from the SN, and decreases the synaptic release of DA in the striatum and cortex and probably the synthesis of DA. Therefore, serotonergic agonists, serotonin precursors, and selective serotonin re-uptake inhibitors (SSRIs) enhance the inhibition of the DA system. Conversely, lesions of the raphé nuclei, or administration of 5-HT1A agonists (through their action on autoreceptors) or 5-HT2 antagonists disinhibit the DA system. Understanding the interaction between serotonin and DA and its therapeutic implications is valuable because a number of new antipsychotic medications (e.g., olanzapine, seroquel, sertindole, ziprasidone) with serotonin-DA interaction profiles are being tested in clinical trials (Gerlach, Peacock, 1995). Neuroleptic-induced extrapyramidal symptoms in humans may result from occupancy of DA D2 receptors in the striatum (Farde et al. 1992). Manipulations that inhibit the serotonin system (e.g., midbrain raphé lesions, 5-HT1A agonists, and 5-HT2A/2C antagonists) generally disinhibit the DA system. This increase in DA may account for the alleviation of neuroleptic-induced extrapyramidal symptoms (Costall et al. 1975). A similar disinhibition in the prefrontal cortex may ameliorate negative symptoms (Moghaddam, Bunney, 1990; Moghaddam, 1994). 5-HT1A agonists inhibit the firing of serotonergic neurons *via* stimulation of their somatodendritic autoreceptors. Several studies (Yasuda et al. 1988a) reported a beneficial effect of 5-HT1A receptor agonists in reversing and preventing the development of catalepsy in rodents, and this effect has now been confirmed in primate models of extrapyramidal symptoms (Casey, 1993). With respect to 5-HT2A/2C antagonism and its effect on catalepsy in rodents, specific 5-HT2A/2C antagonists have confirmed a role for 5-HT2A/2C receptors in

alleviating catalepsy (Kapur, Remington, 1996). It has been concluded that the benefits of a combined serotonergic-dopaminergic blockade may be observed when DA D2 receptor antagonism is partial but not if DA D2 receptor blockade is complete (Kapur, Remington, 1996).

1.7. Aims of this thesis

1.7.1. Importance of exploring DA D3 receptors

The discovery of the DA D3 receptor opened new avenues for research into the DA hypothesis of schizophrenia. DA D3 receptors are most abundant in the mesolimbic DA system. Consequently, the DA D3 receptor may play a role as a target for antipsychotic drugs, since the efficacy of these drugs has been related to DA receptors in the mesolimbic system and their extrapyramidal side effects have been related to the stimulation of the DA nigrostriatal system. Therefore, drugs acting at DA D3 receptors in the mesolimbic system may be free from extrapyramidal side effects.

The introduction of 7-OH-DPAT as a putative DA D3 receptor agonist by Levesque and colleagues (1992) opened the path for *in vivo* studies of DA D3 receptor functions. *In vivo* selectivity of 7-OH-DPAT was unclear at that time. In the present thesis, the *in vivo* selectivity of 7-OH-DPAT for DA D3 receptors was assessed. The aims of this thesis were as follows:

1. To determine dose-dependent effects of 7-OH-DPAT under different behavioral conditions.
2. To investigate possible forebrain site-selectivity of 7-OH-DPAT based on distribution of DA D3 receptors.
3. To test whether there would be differential responses of 7-OH-DPAT to CLZ, SCH-23390 and HAL in comparison to APO.

4. To attempt to demonstrate DA D3 receptor mediated blockade of postsynaptic stimulation effects of DA agonists in DA-intact rats following administration of 7-OH-DPAT.

5. To investigate possible blockade of postsynaptic stimulation effects of APO following administration of 7-OH-DPAT in DA-depleted and DA-lesioned rats.

6. To demonstrate entry of 7-OH-DPAT to brain and measure its concentration in brain following peripheral administration of this drug.

1.8. Summary of the approach and hypotheses of this thesis

The depressant and stimulant effects of DA agonists are more detectable under novel and familiar conditions, respectively, as rats show a high baseline of activity in a novel environment and a low baseline of activity in a familiar environment. The effects of increasing doses of 7-OH-DPAT on locomotor activity were assessed in these different conditions.

The effects of nicotine on the dopaminergic system have been attributed to the mesolimbic dopaminergic system. Also, it has been shown that DA D3 receptors are more abundant in the NAS than in the CN. Therefore, it was hypothesized that 7-OH-DPAT may decrease hyperactivity induced by nicotine *via* stimulation of DA D3 receptors in limbic areas.

As it has been shown that DA D3 receptors are more abundant in limbic areas than striatum, it was hypothesized that 7-OH-DPAT injected directly into the NAS is more potent than that in the CN. Also, it was hypothesized that the effects of 7-OH-DPAT on DA receptors are specific and in this regard it was expected that 8-OH-DPAT would not show the effects of 7-OH-DPAT. 8-OH-DPAT is a structural analog of 7-OH-DPAT with a low affinity for DA receptors.

Effects of CLZ have been attributed to preferential action on the mesolimbic dopaminergic system. Also, it has been shown that CLZ increases DA D3 receptor mRNA in brain. It is well known that CLZ does not antagonize stereotypic behavior induced by APO, a DA D1/D2 agonist. It was of interest to study the interaction between CLZ and 7-OH-DPAT in comparison with APO.

SCH 23390 has a low affinity for DA D2 receptors but it antagonizes the effects of DA D2 agonists; this effect has been related to the interaction between DA D1 and D2 receptors. It was of interest to study if SCH 23390 differentiates the effects of 7-OH-DPAT and of APO. HAL is a DA D2 receptor antagonist; HAL antagonizes the effects of a high dose of APO and also increases DA D2 mRNA. It was hypothesized that if the effect of a high dose of 7-OH-DPAT is mediated predominantly *via* DA D2 receptors stimulation, HAL will not differentiate between the effects of a high dose of 7-OH-DPAT and of APO.

It has been hypothesized that 7-OH-DPAT induces hypoactivity *via* stimulation of postsynaptic inhibitory DA D3 receptors as it induces hypoactivity at low doses that do not decrease DA release. Based on this, it was hypothesized that this postsynaptic inhibition of locomotor activity by a low dose of 7-OH-DPAT may be able to decrease the stimulatory effects of APO at postsynaptic DA D2 receptors in DA-intact rats. Also it was of interest to further study the interaction between 7-OH-DPAT and APO in the absence of the presynaptic effect (i.e., in DA-depleted rats). As behavioral supersensitivity to APO in DA-depleted rats could mask this interaction, it was attempted to determine a time at which DA-lesioned rats did not develop behavioral supersensitivity to APO.

In order to relate the effects of systemic administration of 7-OH-DPAT to central DA receptors, an assay of 7-OH-DPAT in brain was carried out following systemic administration of increasing doses of 7-OH-DPAT.

2. MATERIALS AND METHODS

2.1. Subjects

Male Sprague Dawley rats (Bioscience Animal Services, Ellerslie, Alberta, Canada) were used as subjects for all experiments. On arrival, the rats, weighing 200-250, were housed in groups of two with water and food freely available. Animals were fed Lab-standard lab chow (Wayne Food Division, Continental Grain Co., Chicago, IL, USA), composed of 4.0% crude fat (min.), 4.5% crude fibre (max.), and 24% crude protein (min.). Rats were maintained on a 12 h light/dark cycle with lights on at 6:00 a.m. at a room temperature of 20 $\pm 1^{\circ}$ C. Animals subjected to surgery were operated on within 1-2 weeks of arrival in the laboratory. Immediately after recovery from the procedure these animals were housed in individual cages under the conditions described above. Implanted animals were handled daily and their implants were checked for visible signs of infection.

2.2. Locomotor activity measurements

Locomotor activity of each rat was measured using an activity monitoring system (41.5 cm x 41.5 cm x 30 cm, width x depth x height; Acadia, Infra-Red Grid Model 17-12 with vertical sensors, Acadia Instruments Ltd., Saskatoon, SK, Canada). Locomotor activity boxes were used to measure total motor activity (total number of beam breaks, indicating all locomotor activities), consecutive locomotion (consecutive breaking of the same beam, indication of stereotyped behaviors), and vertical activity (breaking of the upper beam array, rearing). These boxes were equipped with a row of 12 photocells on each side, sensitive to infrared light, placed 2.5 cm above the floor. These test cage sensors were interfaced with a microcomputer system for data logging and temporal analysis of activity counts. Rats are

nocturnal animals and thus quite sensitive to light, so locomotor activity testing was conducted under red light illumination. At the end of each test period, the rats were returned to either their respective housing cages to complete their drug regimen or they were killed for monoamine assays or histology as described in sections 2.8. and 2.9., respectively. The length of test session was 30 or 60 min.

2.3. Recording of stereotyped behavior

Two observers recorded the behaviors of rats during locomotor activity measurement. A sheet including ten compartments of behavior (forward walking, sniffing, licking, grooming, chewing, gnawing, padding, yawning, head movement, and backward walking) was prepared for each rat, and the observers recorded independently the presence or absence of behaviors. Each rat was observed at one minute episodes for every 6 min over a period of 30 min, yielding a total observation time of 5 min for each rat. Total occurrence of each behavior in each group was used in data analysis.

2.4. Cannula implantation

Guide cannulae 22G (C313G, Plastics One Inc.), 11.0 mm long, were implanted using a Kopf stereotaxic frame instrument (David Kopf instruments, Tujunga, California) into the CN or NAS. First the cannula was positioned at intraural zero and the zero coordinates were taken. Coordinates for different target sites were based on the atlas of Paxinos and Watson (1986) and adjusted for an 8° angle (Greenshaw, 1985). The coordinates for NAS were AP: +1.09, L: +0.11, and V: +0.37 and for the CN were AP: +0.1, L: +0.33, and V: -0.52, at a lateral angle of 8°. Animals anaesthetized under Somnotol (sodium pentobarbital, 60-65 mg/kg, ip) were positioned in the frame with the incisor bar set at 2.4 mm below the

interaural zero according to the atlas of Paxinos and Watson (1986). Small burr holes were drilled in the skull over the target sites with a Dremel modelling drill (Racine, Wisconsin, USA) and a dental carbide drill bit (size 3, Buffalo Dental Co., New York) until the dura was exposed. Four screw holes, two rostral and two caudal to the implantation sites, were also drilled (drill bit # K96, Kodex Waledent Instruments, New York) and stainless steel jewellers' screws (Lomat Watch Co. Montreal, Quebec) inserted as anchor points for the implant. The dura was then carefully split with a needle, each cannula lowered to the target site and anchored to the screws with dental acrylic (Caulk Dentsply, orthodontic resin, The L.D. Caulk Co., Milford, Delaware, USA). The wound was then cleaned and sutured, the animal removed from the frame and kept warm until fully recovered from the anaesthetic. A minimum recovery period of 7 days was allowed before animals were subjected to behavioral testing.

2.5. Intracerebral microinjection

Unilateral or bilateral microinjections were given with a 1µl Hamilton syringe (7001KH 26GA PT3, Hamilton Co., Reno, Nevada) connected to a 28G needle (C313I, Plastics one INC.) through a PVC manifold tube (Fisherbrand Accu-Rated, Fisher scientific limited, Edmonton, CA). A syringe pump (model 341A, SAGE Instruments, Cambridge, USA) was used to deliver drug solutions. The PVC tube was filled with the vehicle followed by a small air bubble. This bubble could be observed during drug injection to check the delivery of fluid. One ml of drug solution was then drawn into the tip of the needle and the needle positioned in the guide cannula. A volume of 0.5 µl was delivered to each site over a 3 min

period. The needle was then left in place for a further one min to allow diffusion of the drug away from the cannula tip. Rats were held manually during injections.

2.6. Preparation of drug solutions

7-OH-DPAT HBr was dissolved in saline for systemic injections and in an artificial CSF (table 1) for intracranial microinjections. A stock solution of artificial CSF was prepared and kept at a temperature of 5° C between drug injections. D-Glucose was added to the artificial CSF and the pH was adjusted to 7.1-7.3 with sodium carbonate solution before making drug solutions. APO was dissolved in 0.1% ascorbic acid solution and kept in a container surrounded by an aluminium foil and ice to prevent oxidation. CLZ, HAL and reserpine were dissolved in distilled water after adding a drop of glacial acetic acid and then pH was checked (pH = 6.5). SCH 23390 HCl, QUIN HCl, α-MPT methyl ester and desmethylimipramine HCl were dissolved in distilled water. Drugs were administered in a volume of 1 ml/kg. 6-OH-DA HBr was dissolved in a solution of ascorbic acid 0.1% in 0.9% saline and kept in a container surrounded by an aluminum foil and ice to prevent oxidation. This solution was prepared immediately before starting microinjections.

2.7. The HPLC-ECD system

A reverse phase HPLC system (Waters, Milford, MA, USA) equipped with an electrochemical detector (ECD, model M460, Waters) and a WISP 710B automatic injector was used. The mobile phase consisted of acetonitrile and phosphate buffer (table 1). The oxidation potential was set at 0.75 v and the flow rate of the mobile phase was 1 ml/min. The volume of injection was 15 µl for the monoamine assay and 50 µl for the 7-OH-DPAT assay. A µbondapak C18 precolumn (Waters), connected to a Spherisorb 5 ODS 2 column

(250 x 4.6 mm; 5 µm particle size; Phenomenex, Torrence, CA, USA) was used to separate the compounds of interest. An integrator system (model 740 Data Module, Waters) was used to record and integrate the peaks.

2.8. Sample preparation for HPLC assay

Samples of CN, NAS and whole brain (minus striatum) tissues were taken from subjects treated with reserpine and α-MPT, 6-OH-DA or 7-OH-DPAT and were prepared for an assay with a HPLC-ECD system. The tissues were homogenized in 5 volumes for whole brain, 10 volumes for striatum and 20 volumes for NAS of ice-cold homogenization solution (table 1) using a Tri-R Stir-R homogenizer (model S63C, Tri-R Instruments, INC., Rockville, N.Y., USA) equipped with a Teflon glass pestle. The rotor shaft had a maximum speed of 12,000 rpm with a ten-speed setting. A speed setting of 3 or 7 was used routinely depending on the volume of the homogenate. Homogenates were then centrifuged for 10 min at 10,000 x g. A Fisher microcentrifuge (MSE, Baxter corp, Edmonton, Alberta, Canada) and a Beckman Model J-21B refrigerated preparative centrifuge (Palo Alto, CA, USA) were used for small and larger volumes, respectively. The supernatant was used for injection into the HPLC or further extraction of 7-OH-DPAT.

Extraction of 7-OH-DPAT was carried by a back-extraction procedure using 2.5% di-(2-ethylhexyl)phosphate (DEHPA, the liquid ion pairing agent) in chloroform and 0.5 N HCl, see figure 5. A low-speed centrifuge, up to 1500 g, (Sorvall GLC-2b, Dupont Instruments, Wilmington, DE, USA) was used for centrifugation to separate phases and a Savant Speed Vac SS1 (Savant Instruments, Inc., Farmington, NY, USA) was used for evaporating samples to dryness.

Figure 5: The procedure for extraction of 7-OH-DPAT

- 5 ml of the supernatant (see section 2.8) or standard solution
- Basify by addition of potassium carbonate (2.5%) until a pH of 9.5 is attained
- Extract with 3 ml chloroform containing 2.5 % of DEHPA
- Back extract the chloroform layer with 1 ml HCl (0.5 N)
- Take the HCl layer to dryness
- Dissolve the residue in 100 µl of 0.1 N perchloric acid ($HClO_4$)
- Injection on the HPLC

2.9. Histology

After completion of behavioral testing the positions of the tips of needles were verified by histological analysis. Rats, deeply anaesthetised with Somnotol (Sodium pentobarbital), were infused with 5 ml of 0.9% saline solution followed by 5 ml of 10% buffered formaldehyde solution (Table 1) directly into the left ventricle of the heart, while the femoral artery was cut. After several min they were decapitated and the cannulae were removed at the same angle at which they were implanted to minimise tissue damage. The fixed brain was removed from the skull and kept in the fresh 0.5% buffered formaldehyde solution (Table 1) saline for a minimum of seven days until sectioning. A cryostat (Microtome model 880, American Optical Corp, Buffalo, New York, USA) was used to cut 50 µm coronal sections of brain tissue. The slices were placed on microscope slides and the site of injection was checked microscopically with reference to the atlas of Paxinos and Watson (1986).

2.10. DA depletion by reserpine and α-MPT

A treatment with reserpine (2.5 mg/kg, sc) 24 h before locomotor activity recording, followed by α-MPT (50 mg/kg, sc) 4 h before locomotor activity recording was used to deplete DA in rats. DA depletion was verified by measuring DA concentrations in the striatum in a separate group of rats by HPLC-ECD.

2.11. Lesion of DA neurons by 6-OH-DA

Lesion of DA neurons were made in Somnotol (Sodium Pentobarbital, 60-65 mg/kg, ip) anaesthetized rats by bilateral injection of a solution of 6-OH-DA (8 μg/4μl/4min) into the medial forebrain bundle rostral to the SN. In order to protect noradrenergic neurons, rats were pretreated with 25 mg/kg, sc of desmethylimipramine 20 min before injection of Somnotol. Desmethylimipramine is a noradrenaline re-uptake inhibitor that prevents uptake of 6-OH-DA into the noradrenaline neurons. Stereotaxic coordinates were AP: +0.48, L: +0.11, and V: +0.18 (Paxinos and Watson, 1986).

2.12. Experimental design

2.12.1. Systemic dose-dependent effects of 7-OH-DPAT in habituated rats

A counter-balanced repeated-measures design was used as shown in Table 2. Twelve rats were exposed to the activity cages for 30 min per day during 15 consecutive days at approximately the same time of day. On probe days the animals were randomly treated with 0.9% saline, or 0.003, 0.01, 0.03, 0.1 or 0.3 mg/kg of 7-OH-DPAT, sc, immediately before the test session. One half of the animals in each treatment dose received 7-OH-DPAT on the first day of probe days and the others received the 0.9% saline vehicle. This order of

Table 1: Contents of solutions

Solution	Contents
Artificial CSF	NaCl (98 mM), Na_2CO_3 (21 mM), KCl (4 mM), Na_2HPO_4 (1 mM), $CaCl_2$ (5 mM), $MgCl_2$ (1 mM), dextrose (11 mM)
Mobile phase for monoamine assay	NaCl (9.99 mM), sodium octyl sulfate (0.73 mM), EDTA (0.37 mM), NaH_2PO_4 (0.048 M), CH_3CN (7%); pH=3
Mobile phase for 7-OH-DPAT assay	NaCl (9.99 mM), sodium octyl sulfate (0.73 mM), EDTA (0.37 mM), NaH_2PO_4 (0.048 M), CH_3CN (30%); pH=3.65
Homogenization solution	$HClO_4$ (0.1 M), EDTA (37 mM), ascorbic acid (0.05 mM)
10% Buffered formaldehyde solution	NaCl (0.154 M), NaH_2PO_4 (29 mM), Na_2HPO_4 (46 mM), formaldehyde 40%, sucrose 5%
0.5% Buffered formaldehyde solution	NaCl (0.154 M), NaH_2PO_4 (29 mM), Na_2HPO_4 (46 mM), formaldehyde 2%, sucrose 5%

drug/vehicle administration was reversed on the second probe day to counterbalance injection order. In this way each animal served as its own control for within-groups comparisons. Consequently, each rat was used as control repeatedly during 6 probes. These repeated control measures were not significantly different. Therefore, the overall average of these control performances for each rat was used in data analysis and in graphs for clarity. This procedure has previously been used in antidepressant drug testing (Greenshaw et al., 1988). There was a 5 day wash-out between probe days. This procedure continued for 6 probes until there were 12 rats treated for each dose of 7-OH-DPAT, i.e, each rat received all doses of 7-OH-DPAT.

2.12.2. Systemic effects of 7-OH-DPAT in non-habituated rats

Thirty two rats were randomly divided to 4 groups and were treated with 0.9% saline, or 0.003, 0.03 or 0.3 mg/kg of 7-OH-DPAT, sc, immediately before the test session. Each animal received only one drug trial.

Table 2: Repeated-measures design

	Habituation period	Probe days		Wash-out period	Next probe days	
Day	1-15	16	17	18-22	23	24
Treatment	_	Drug or Vehicle	Vehicle or Drug	_	Drug or Vehicle	Vehicle or Drug

2.12.3. Systemic effects of 7-OH-DPAT in chronically nicotine-treated rats

A counter-balanced repeated-measures design was used; see Table 2. Eighteen rats were divided randomly into 2 groups. The 2 groups were treated daily with saline or nicotine (0.6 mg/kg/day), respectively, followed by a second saline injection 20 min later, as vehicle for drug treatment on probe day. Rats were then placed into the locomotor activity boxes for 30 min immediately after the second injection to record locomotor activity. This procedure was repeated during 15 consecutive days at approximately the same time of day. On the first day of the probe days, animals were treated with saline or nicotine as usual and 20 min later they received 0.9% saline,or 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg of 7-OH-DPAT randomly, immediately before the test session. One half of the animals in each treatment dose received 7-OH-DPAT on the first day of the probe days and the others received the 0.9% saline

vehicle. This order of drug/vehicle administration was reversed on the second day of probe days to counter-balance injection order. In this way each animal served as its own control for within-groups comparisons. There was a 5 day wash-out between probes. This procedure continued for 6 probes until there were 9 rats treated for each dose of 7-OH-DPAT in each saline- or nicotine-treated group.

2.12.4. Drug interaction study

For each drug CLZ, SCH 23390 or HAL, animals were randomly divided into groups of 8-12 and pretreated with 0.9% saline or drug. Three doses of CLZ, (0.625, 1.25 or 5 mg/kg), two doses of HAL, (0.03 and 0.1 mg/kg) and one dose of SCH 23390, (0.01 mg/kg) were used. The second injection, (i.e., vehicle, 7-OH-DPAT (0.1, 2.5, or 5 mg/kg) or APO (0.5 mg/kg), was done 20 min after CLZ, 2 h after HAL, and 10 min after SCH 23390. Locomotor activity was recorded immediately after vehicle or 7-OH-DPAT and 15 min after APO injection; see section 2.2. Stereotypy behaviors were recorded in the CLZ study, see section 2.3. Each animal received one drug trial in the novel condition except in the study of the interaction between HAL (0.03, mg/kg) and 7-OH-DPAT (0.1 mg/kg) which was conducted under familiar conditions and for which each rat was used twice (see table 2). One group of rats was used for injection of HAL and saline on probes one and two, respectively; the second group was used for injection of 7-OH-DPAT and HAL+7-OH-DPAT on probes one and two, respectively.

2.12.5. Interaction between 7-OH-DPAT or QUIN and APO in DA-intact rats

Animals, randomly divided into nine groups of 8, were injected with (±)-7-OH-DPAT (0.03

mg/kg), (-)-QUIN (0.05 mg/kg) or the distilled water vehicle followed by APO (0.25 or 0.5 mg/kg) or the 0.1% ascorbic acid vehicle, immediately. Locomotor activity was recorded 15 min after the second injection, see section 2.2.

2.12.6.Interaction between 7-OH-DPAT and APO in DA-depleted rats

Animals were randomly divided into groups of 8 and were treated with reserpine (2.5 mg/kg, sc) followed by α-MPT (50 mg/kg, sc) 24 and 4 h prior to the test, respectively. On test sessions a 5 minute baseline of activity was recorded for each rat. They then received 7-OH-DPAT (0.01 mg/kg, sc) or the distilled water vehicle followed immediately by APO (0.05 or 0.1 mg/kg, sc) or the 0.1% ascorbic acid vehicle. Locomotor activity was recorded 15 min after second injection; see section 2.2. DA depletion was verified by *ex vivo* measurements of DA by HPLC-ECD in striatum of rats treated with reserpine + α-MPT or vehicle in a separate group of rats, see section 2.8.

2.12.7. Supersensitivity to APO following 6-OH-DA-induced lesion of medial forebrain bundle DA neurons

Animals were randomly divided into groups of 8 and guide cannulae were implanted as described in section 2.4. 6-OH-DA, 8 μg/4μl/4min, was injected bilaterally. Sham-operated rats received 0.1% ascorbic acid vehicle. After 1, 2 or 3 post-lesioning days, rats received APO, 0.1 mg/kg, sc, and locomotor activity was recorded 15 min later, see section 2.2. At day 8 of post-lesioning animals were decapitated. The CN and NAS were dissected out and kept on dry ice immediately. Then all tissues were frozen (-80° C) for analysis of monoamines (DA, NE, and 5-HT) by HPLC-ECD; see section 2.8.

2.13. Assay of 7-OH-DPAT in the rat brain

Rats were injected with vehicle or different doses of 7-OH-DPAT (0.3, 1 and 5 mg/kg, sc) 15 min before decapitation. Sample preparation was performed as described in section 2.8., standard solutions were extracted and run parallel to samples in order to adjust the data for any loss of sample due to extraction.

2.14. Statistics

Analysis of the data for repeated-measures experimental designs was performed using 1-, 2- or 3-way repeated measures ANOVA (RMANOVA). Data for independent group designs were analyzed by 1- or 2-way ANOVA (SPSS program, version 7.5). These tests were followed by a Newman-Keuls or Dunnett's test for multiple comparisons. Observational data were analysed by the Kruskal-Wallis two-way ANOVA followed by the Mann-Whitney U test. Total scores were used to describe the observational data. Biochemical comparisons were made by use of the two-tailed Student's t-test. Statistical significance was based on the probability value of $P<0.05$.

3. RESULTS

3.1. Effects of 7-OH-DPAT on locomotor activity in habituated rats

7-OH-DPAT was described as a DA D3 receptor agonist based on *in vitro* binding studies (Levesque et al., 1992). The aim of the present experiment was to examine the *in vivo* dose-dependent effects of 7-OH-DPAT on spontaneous motor activity in habituated rats. Animals were familiarized to the environment by daily exposure to the locomotor activity boxes through 15 days until they showed stable baselines of activity. In the counter-balanced repeated-measures design (See section 2.13.1) each animal served as its own control. These repeated control measures of activities were not significantly different by RMANOVA (Figure 6) so the overall average of control performance for each animal was used in data analysis and graphs for clarity. The effects of increasing doses of 7-OH-DPAT on total, consecutive and vertical activities are displayed in Figure 7 (A-C), respectively. Each data point is expressed as the mean ± SEM. Considering total counts of activity for 30 min, the effect of 7-OH-DPAT on locomotor activity was significant as shown by one way RMANOVA: [total, 7-OH-DPAT: $F(5, 100) = 2.95$, $P<0.05$], Figure 7A; [consecutive, 7-OH-DPAT: $F(5, 100) = 10.21$, $P<0.05$], Figure 7B; [vertical: 7-OH-DPAT: $F(5, 100) = 1.76$, $P>0.05$], Figure 7C. An analysis of time-course activity revealed that 7-OH-DPAT decreased activity significantly at times 1 and 2 and the two higher doses increased it at times 4-6 as shown by 2-way RMANOVA followed by *post hoc* tests: [total, 7-OH-DPAT: $F(5, 100) = 2.95$, $P<0.05$; Time: $F(5, 100) = 100.81$, $P<0.05$; 7-OH-DPAT x Time: $F(25, 275) = 15.83$, $P<0.05$], Figure 8; [consecutive, 7-OH-DPAT: $F(5, 100) = 3.46$, $P<0.05$;

Time: $F(5, 100) = 38.94$, $P<0.05$; 7-OH-DPAT x Time $F(25, 275) = 4.74$, $P<0.05$], Figure 9; [vertical: 7-OH-DPAT: $F(5, 100) = 1.54$, $P>0.05$; Time: $F(5, 100) = 49.17$, $P<0.05$; 7-OH-DPAT x time: $F(25, 275) = 9.69$, $P<0.05$], Figure 10.

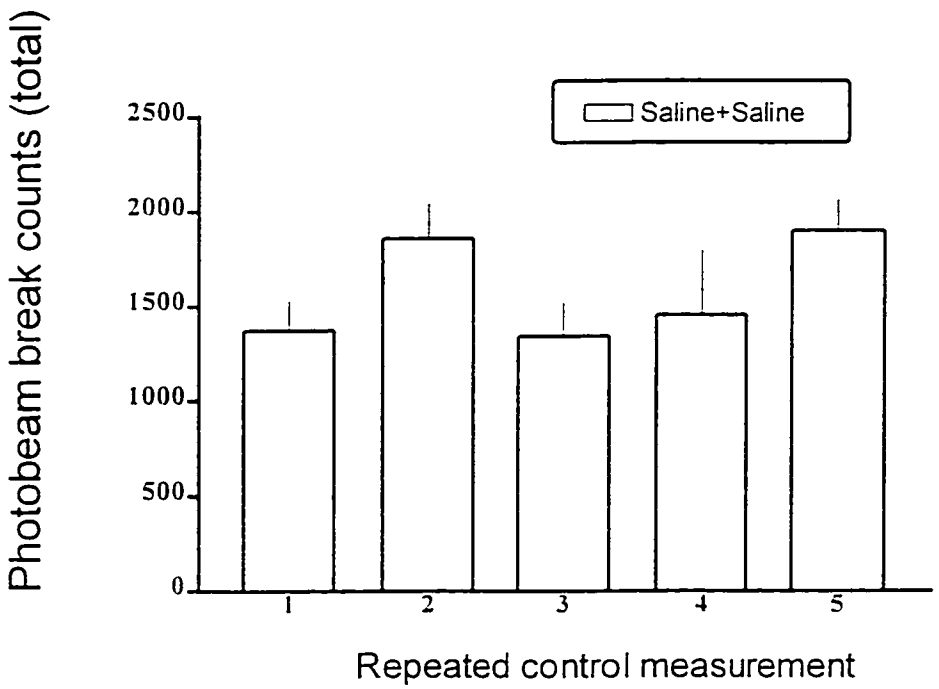

Figure 6: Repeated control measures of total locomotor activity in a 30 min test session in habituated rats. Rats received 2 injections of saline with 20 min interval as control treatment on one day of probe. | : SEM for each mean, n=12.

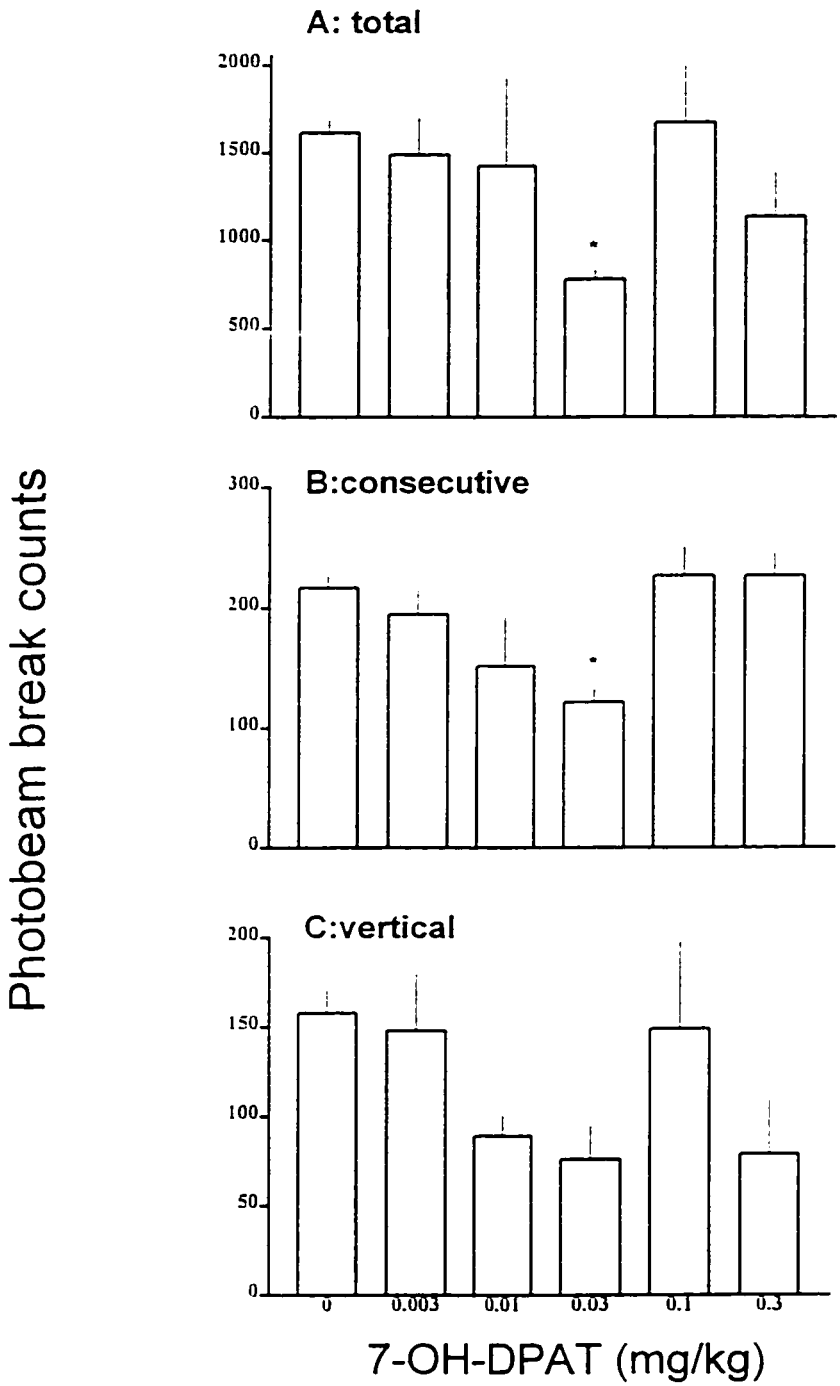

Figure 7: Eeffects of 7-OH-DPAT (0.003-0.3 mg/kg, sc, immediately before test) on A: total, B: consecutive and C: vertical activity in a 30 min test session in habituated rats. | : SEM for each mean. *: Significant vs other doses, $P<0.05$, n=12.

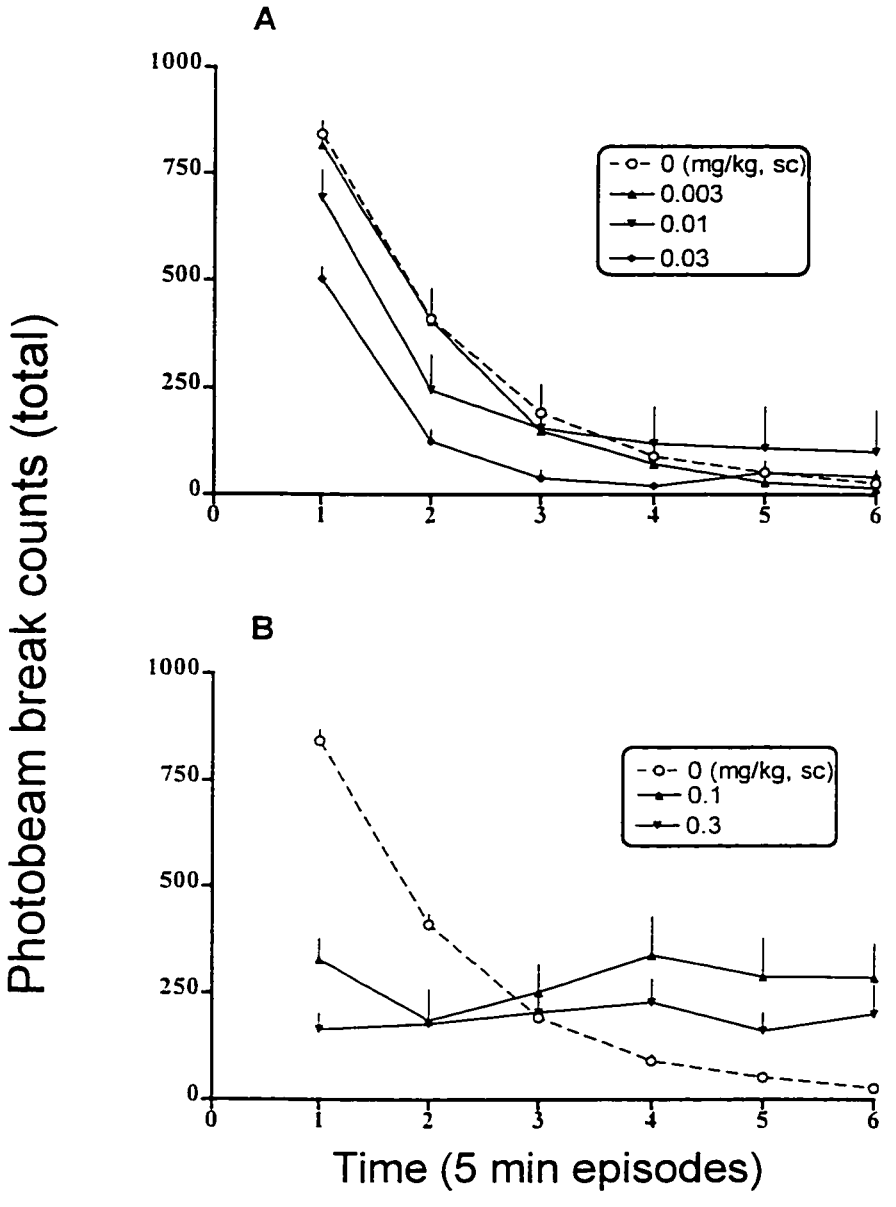

Figure 8: Time-course of effects of 7-OH-DPAT (0.003- 0.3 mg/kg, sc, immediately before test) on total activity in a 30 min test session in habituated rats. |: SEM for each mean. Effect of 7-OH-DPAT is significant at times 1, 2, 4-6, $P<0.05$, $n=12$. This is one experiment shown in two graphs for clarity. A: Three lower doses and B: Two higher doses.

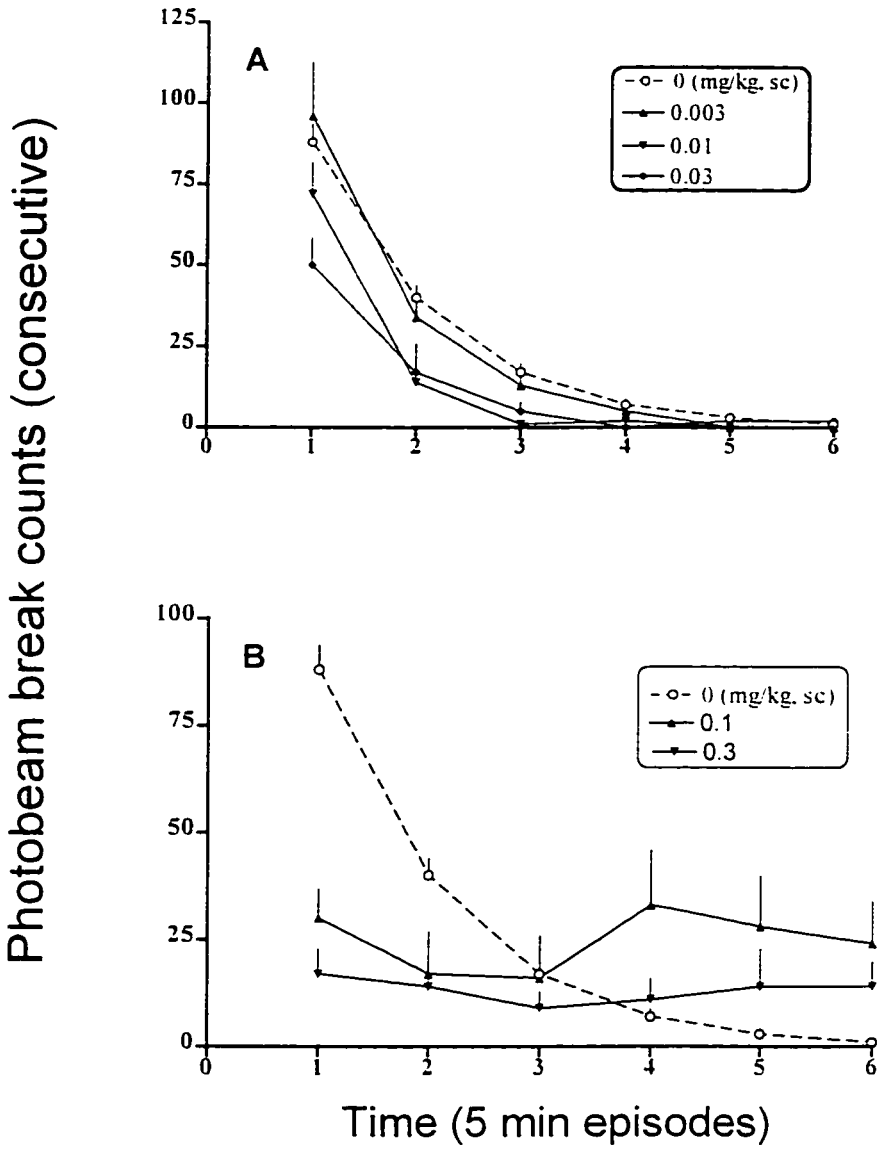

Figure 9: Time-course of effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc. immediately before test) on consecutive activity in a 30 min test session in habituated rats. | : SEM for each mean. Effect of 7-OH-DPAT is significant at times 1-3, 4 and 6, $P<0.05$, $n=12$. This is one experiment shown in two graphs for clarity. A: Three lower doses and B: Two higher doses.

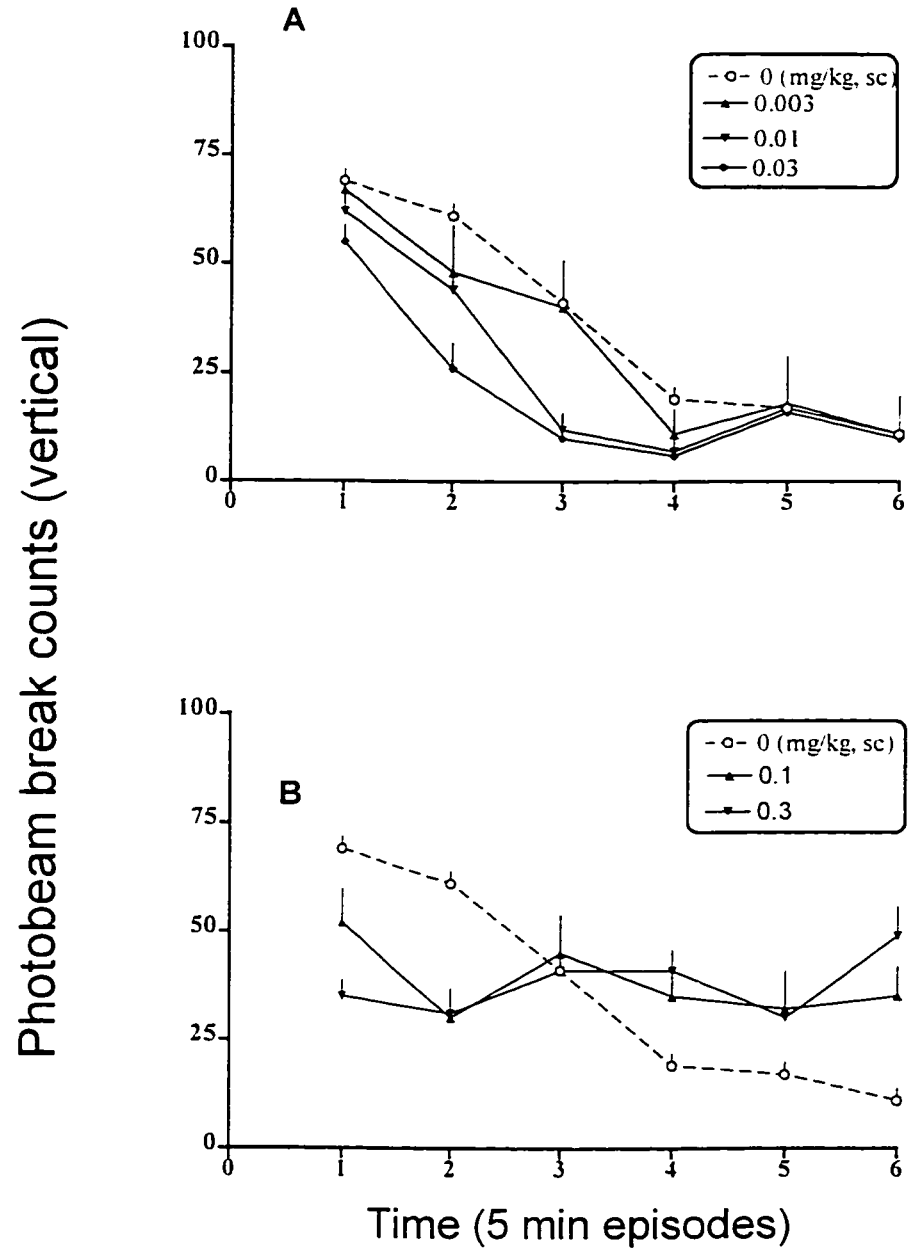

Figure 10: Time-course of effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc, immediately before test) on vertical activity in a 30 min test session in habituated rats. |: SEM for each mean. Effect of 7-OH-DPAT is significant at times 1, 3, 4-6, $P<0.05$, $n=12$. This is one experiment shown in two graphs for clarity. A: Three lower doses and B: Two higher doses.

3.2. Effects of 7-OH-DPAT on locomotor activity in non-habituated rats

The aim of this experiment was to examine the effects of several doses of 7-OH-DPAT on spontaneous motor activity in non-habituated rats. In the previous experiment with habituated rats the doses of 0.1 and 0.3 of 7-OH-DPAT showed a tendency to increase locomotor activity after a period of 5-10 min of locomotor activity suppression. This second experiment was designed to further investigate the dose-dependent effects of 7-OH-DPAT on a high baseline of activity. The baseline of activity in non-habituated rats was about twice as high as that in habituated rats. The effects of increasing doses of 7-OH-DPAT on total, consecutive and vertical activities are displayed in Figure 11 (A-C), respectively. Each data point is expressed as the mean ± SEM. Total activity was decreased by 7-OH-DPAT. This effect on the total activity was significant with doses of 0.03 and 0.3 mg/kg as shown by one-way ANOVA followed by the Dunnett t test [$F(3, 31) = 61.99$, $P<0.05$]. Similar results were observed with consecutive [$F(3, 31) = 14.78$, $P<0.05$] and vertical [$F(3, 31) = 10.12$, $P<0.05$] activities. Figure 12 (A-C) show the effects of 7-OH-DPAT on the time-course of activity for total, consecutive and vertical activity, respectively. 7-OH-DPAT significantly reduced activity during all 6 episodes of time in the 30 min test session, as shown by 2-way RMANOVA followed by one-way ANOVA and *post hoc* Dunnett t (2-sided) tests: [total, Time: $F(5, 140) = 41.73$, $P<0.05$; 7-OH-DPAT: $F(3, 28) = 61.99$, $P<0.05$; Time x 7-OH-DPAT: $F(15, 140) = 6.72$, $P<0.05$], Figure 12A; [consecutive, Time: $F(5, 140) = 3.82$, $P<0.05$; 7-OH-DPAT: $F(3, 28) = 13.96$, $P<0.05$; Time x 7-OH-DPAT: $F(15, 140) = 0.744$, $P>0.05$], Figure 12B; [vertical, Time: $F(5, 140) = 33.32$, $P<0.05$; 7-OH-

DPAT: $F(3, 28) = 20.16$, $P<0.05$; Time x 7-OH-DPAT: $F(15, 140) = 5.26$, $P>0.05$], Figure 12C.

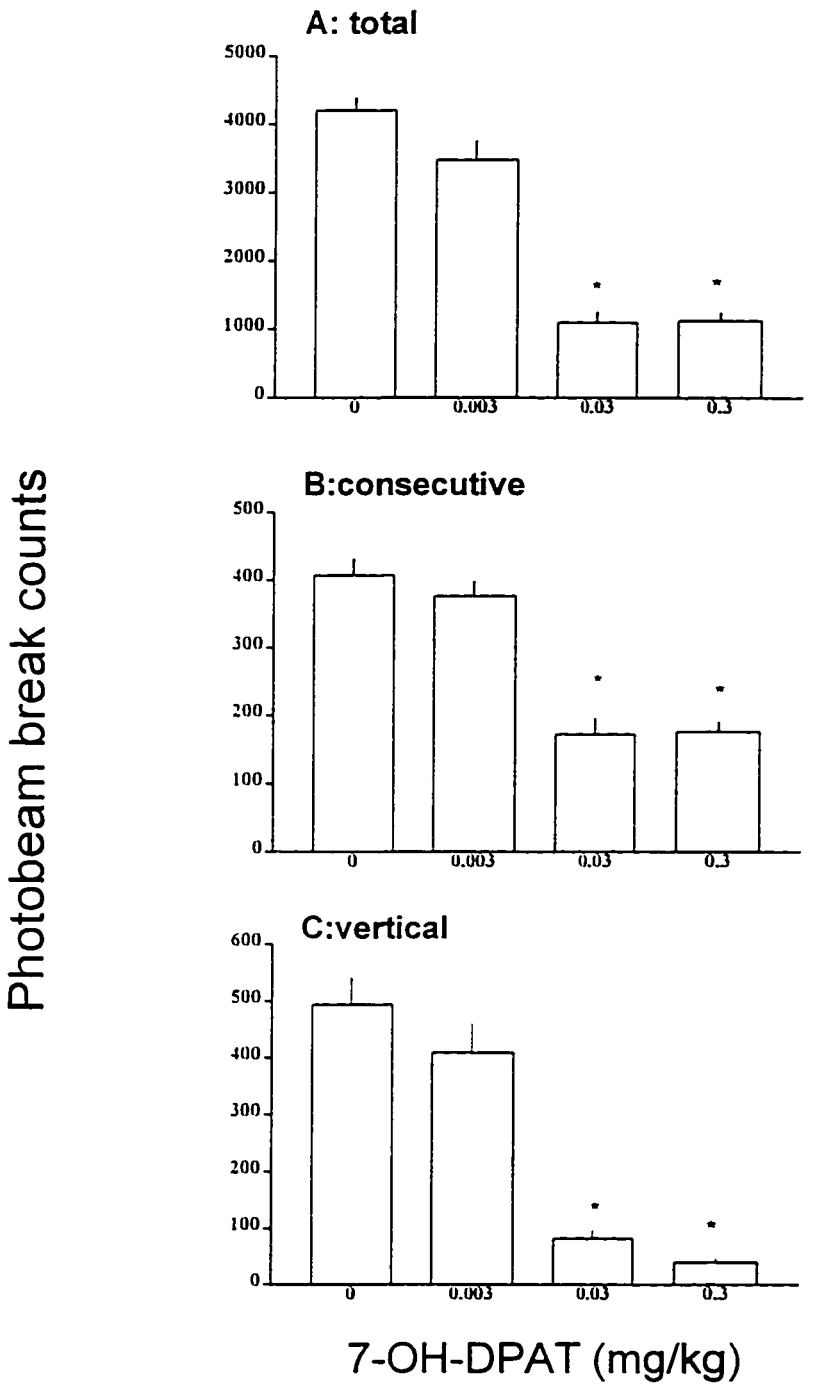

Figure 11: Effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc, immediately before test) on A: total, B: consecutive and C: vertical activity in a 30 min test session in non-habituated rats. | : SEM for each mean. *: Significant vs control. $P<0.05$, n=8.

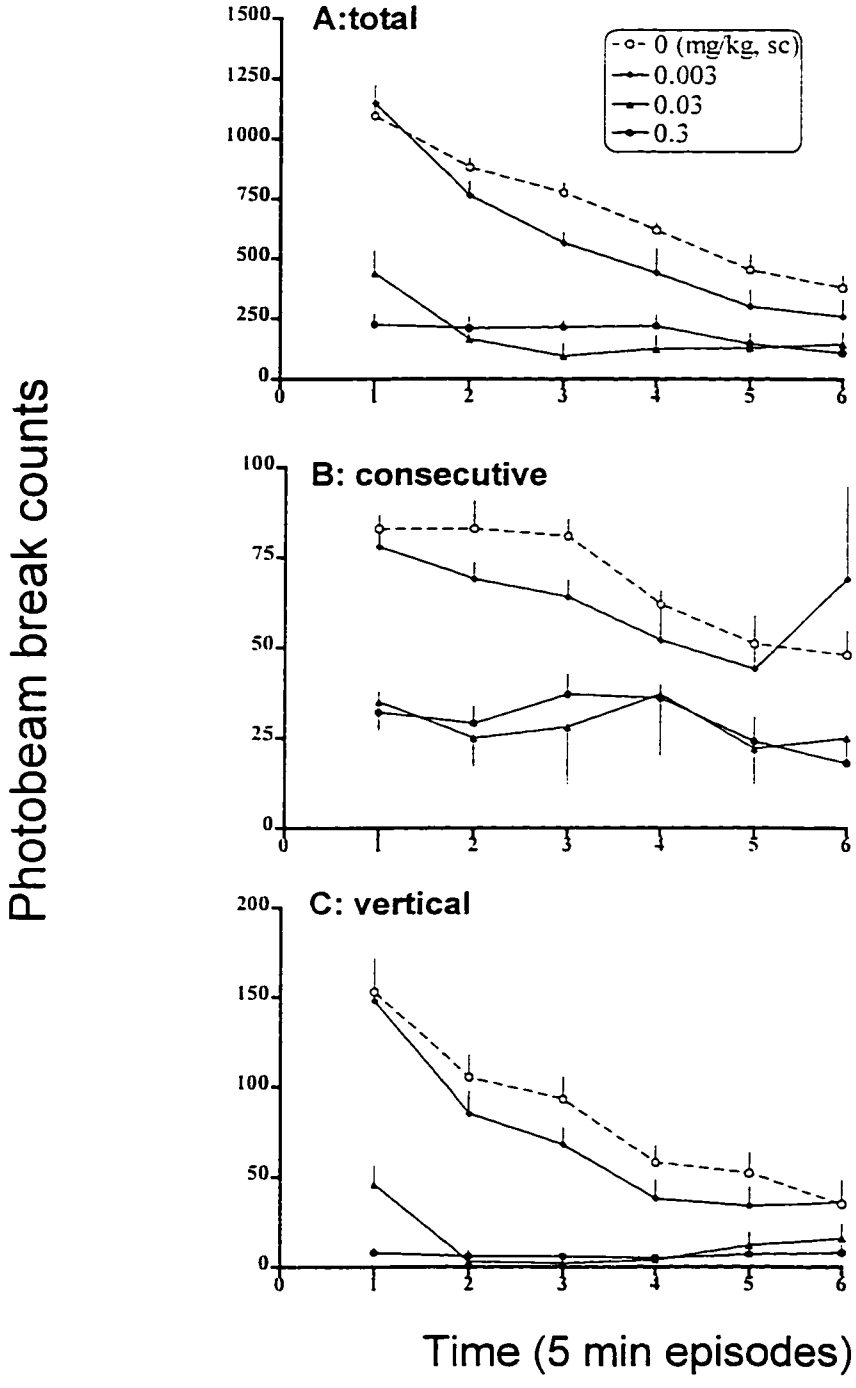

Figure 12: Time-course of effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc. immediately before test) on the locomotor activity in a 30 min test session in non-habituated rats. | : SEM for each mean. Effects of the 2 last doses are significant vs control at times 1-6, $P<0.05$, n=8.

3.3. The interaction of HAL with a low dose of 7-OH-DPAT in habituated rats

The two previous experiments showed that doses of 0.1 and 0.3 mg/kg of 7-OH-DPAT induced different effects in habituated and non-habituated rats. In habituated rats they increased locomotor activity after a period of suppression, while in non-habituated rats they decreased locomotor activity. To further investigate the two-phase effect of 7-OH-DPAT in habituated rats, the effect of HAL on the later hyperactivity was studied. In a similar way, the overall average of control performance for each animal was used in data analysis and graphs for clarity (see section 2.12.4 on page 51). HAL, 0.03 mg/kg, did not decrease significantly any categories of locomotor activity for 30 min [Figure 13 (A-C) (open bars)]. 7-OH-DPAT reduced locomotor activity at 0.1 mg/kg during the first 10 min but increased it during the last 20 min of the test session in habituated rats [Figure 14 (A-C)]. HAL at 0.03 mg/kg antagonized the later hyperactivity induced by 7-OH-DPAT [total, HAL: $F(1, 44) = 0.5$, $P>0.05$; 7-OH-DPAT: $(1, 22) = 0.13$, $P>0.05$; HAL x 7-OH-DPAT: $F(1, 44) = 19.87$, $P<0.05$] [Figure 13 (A-C)]. An analysis of time-course shows that HAL significantly antagonized the early hypoactivity and the later hyperactivity induced by 7-OH-DPAT in habituated rats, as shown by 2-way RMANOVA [total, Time: $F(5, 110) = 111.95$, $P<0.05$; HAL: $F(1, 22) = 30.11$, $P<0.05$; 7-OH-DPAT: $F(1, 22) = 0.01$, $P>0.05$; HAL x 7-OH-DPAT: $F(1, 22) = 5.68$, $P>0.05$; Time x HAL: $F(5, 110) = 10.59$, $P<0.05$; 7-OH-DPAT x Time: $F(5, 110) = 25.86$, $P<0.05$; 7-OH-DPAT x Time x HAL: $F(5, 110) = 13.78$, $P<0.05$]. Similar ANOVA performed on consecutive and vertical activity showed significant effects of HAL on the late hyperactivity induced by 7-OH-DPAT [consecutive, Time: $F(5, 110) =$

52.31, P<0.05; HAL: F(1, 22) = 18.69, P<0.05; 7-OH-DPAT: F(1, 22) = 1.57, P>0.05; 7-OH-DPAT x HAL: F(1, 22) = 12.38, P>0.05; Time x HAL: F(5, 110) = 5.32, P<0.05; 7-OH-DPAT x Time: F(5, 110) = 15.02, P<0.05; 7-OH-DPAT x Time x HAL: F(5, 110) = 5.25, P<0.05]; [vertical, Time: F(5, 110) = 57.93, P<0.05; HAL: F(1, 22) = 11.82, P<0.05; 7-OH-DPAT: F(1, 22) = 0.03, P>0.05; 7-OH-DPAT x HAL: F(1, 22) = 1.76, P>0.05; Time x HAL: F(5, 110) = 5.69, P<0.05; 7-OH-DPAT x Time: F(5, 110) = 12.99, P<0.05; 7-OH-DPAT x Time x HAL: F(5, 110) = 7.04, P<0.05].

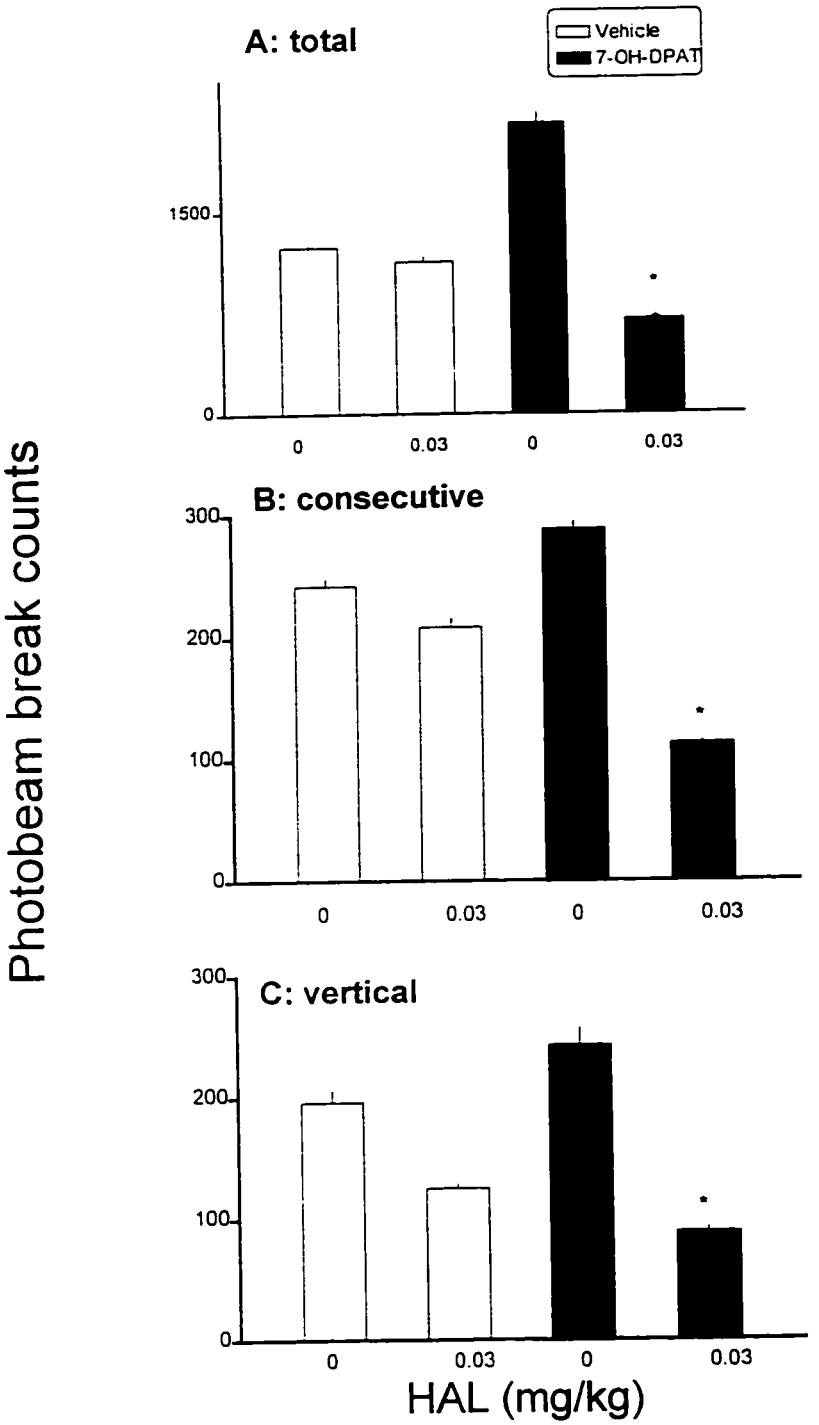

Figure 13: The effects of haloperidol (HAL), 0.03 mg/kg, sc on hyperactivity induced by 7-OH-DPAT (0.1 mg/kg, sc) on A: total B: consecutive and C: vertical activity in habituated rats. | : SEM for each mean. *: Significant drug interaction, $P<0.05$, n=12.

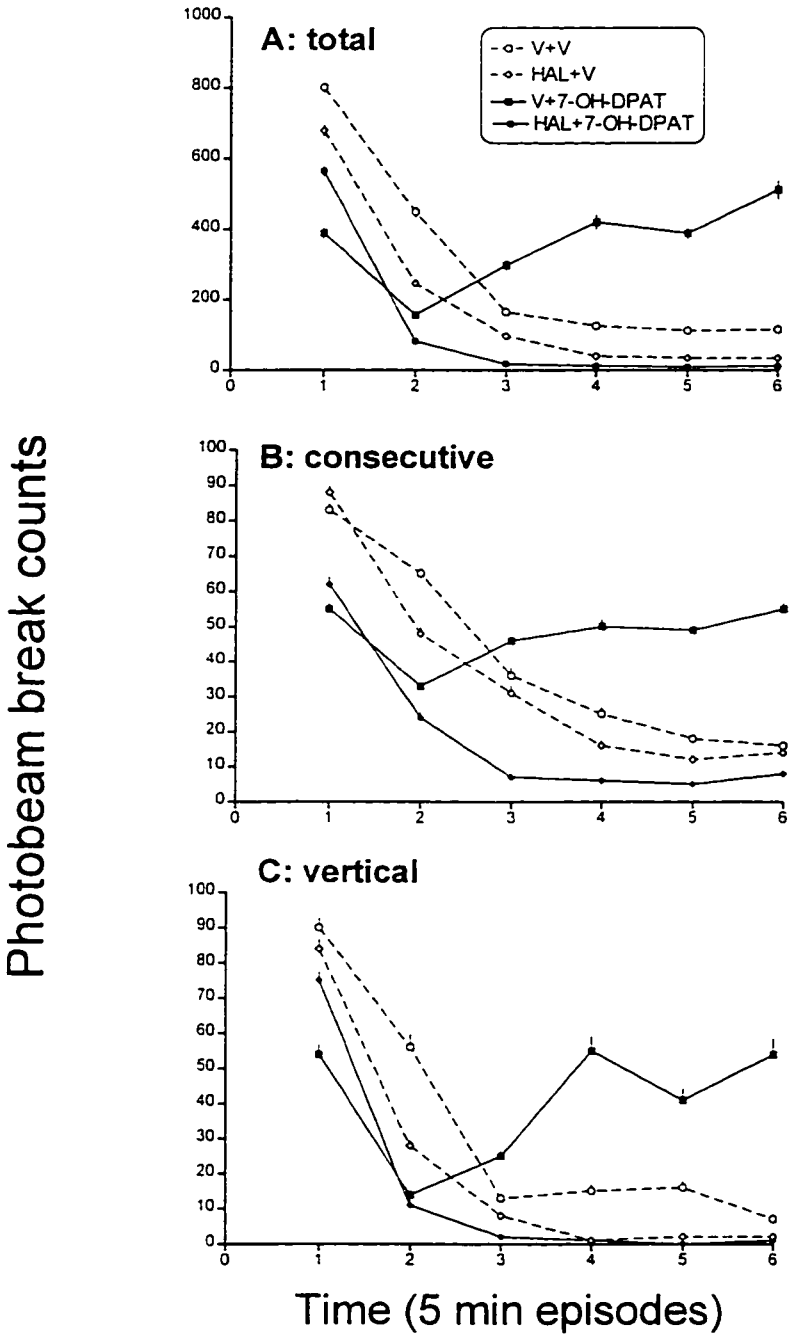

Figure 14: Time-course effects of haloperidol (HAL), 0.03 mg/kg, sc, on later hyperactivity induced by 7-OH-DPAT (0.1 mg/kg, sc) on A: total B: consecutive and C: vertical activity in habituated rats. | : SEM for each mean. Effect of HAL on 7-OH-DPAT is significant at times 4-6, $P<0.05$. n=12. V= vehicle.

3.4. Effects of 7-OH-DPAT on locomotor activity in chronically nicotine-treated rats

It has been reported that DA D3 receptors are more abundant in the NAS rather than in the CN (Sokoloff et al. 1990). Also, the involvement of the mesolimbic dopaminergic system in the locomotor stimulating effects of nicotine has been shown (Museo, Wise, 1990). The aim of the current experiment was to examine the effects of several doses of 7-OH-DPAT on hyperactivity induced by chronically nicotine administration (as a model of mesolimbic dopaminergic stimulation). In a similar way (see section 2.13.1 on page 48), the overall average of control performance for each animal was used in data analysis and graphs for clarity. Nicotine increased total [nicotine: $F(1, 17) = 15.54$, $P<0.05$] and consecutive [nicotine: $F(1, 17) = 17.01$, $P<0.05$] activities without changing vertical activity, as shown by one-way RMANOVA, [Figure 15 (A-C) (first black bar)]. The effects of increasing doses of 7-OH-DPAT on total, consecutive and vertical activities in chronic saline- or nicotine- treated rats are displayed in Figure 15 (A-C). Each data point is expressed as the mean ± SEM. 7-OH-DPAT changed total, consecutive and vertical activity in both chronic saline- and nicotine-treated rats significantly as shown by 2-way RMANOVA [total: 7-OH-DPAT: $F(5, 80) = 6.01$; Nicotine: $F(1, 16) = 18.33$, 7-OH-DPAT x Nicotine: $F(5, 80) = 7.21$ $P<0.05$]; [consecutive, 7-OH-DPAT: $F(5, 80) = 9.44$; Nicotine: $F(1, 16) = 27.39$; 7-OH-DPAT x Nicotine: $F(5, 80) = 6.82$, $P<0.05$]; [rearing: 7-OH-DPAT: $F(5, 80) = 1.80$; Nicotine: $F(1, 16) = 3.94$, $P>0.05$; 7-OH-DPAT x Nicotine: $F(5, 80) = 6.15$, $P<0.05$].

Figures 16-18 show the effects of 7-OH-DPAT on the time-course of activity in saline- (panel A) and nicotine-treated rats (panel B). Consistent with results from the habituated

rats, see 3.1, the higher doses of 7-OH-DPAT (0.1 and 0.3 mg/kg) increased total and vertical activities after a period of suppression in chronic saline- (Figures 16A-18A) rats, significantly, but the effect of 7-OH-DPAT in nicotine treated rats was not time dependent as shown by 3-way RMANOVA [total, 7-OH-DPAT: $F(5, 80) = 6.01$, $P<0.05$; Time: $F(5, 80) = 119.91$; 7-OH-DPAT x Time: $F(25, 400) = 10.43$, $P<0.05$; Nicotine: $F(1, 16) = 18.33$; Time x Nicotine: $F(5, 80) = 4.30$, $P<0.05$; 7-OH-DPAT x Time x Nicotine: $F(25, 400) = 1.44$, $P>0.05$] (Figures 16-18). ANOVA on vertical activity revealed time dependent effect of 7-OH-DPAT on nicotine [vertical, 7-OH-DPAT: $F(5, 80) = 1.80$, $P>0.05$; Time: $F(5, 80) = 31.91$; 7-OH-DPAT x Time: $F(25, 400) = 6.45$, $P<0.05$; Nicotine: $F(1, 16) = 3.93$, $P>0.05$; Time x Nicotine: $F(5, 80) = 5.98$, $P<0.05$; 7-OH-DPAT x Time x Nicotine: $F(25, 400) = 3.45$, $P<0.05$]. With consecutive activity, the effect of 7-OH-DPAT on nicotine did not change over time, significantly [consecutive, 7-OH-DPAT: $F(5, 80) = 9.44$, $P<0.05$; Time: $F(5, 80) = 30.79$; 7-OH-DPAT x Time: $F(25, 400) = 6.51$, $P<0.05$; Nicotine: $F(1, 16) = 27.39$; Time x Nicotine: $F(5, 80) = 6.24$, $P<0.05$; 7-OH-DPAT x Time x Nicotine: $F(25, 400) = 1.29$, $P>0.05$].

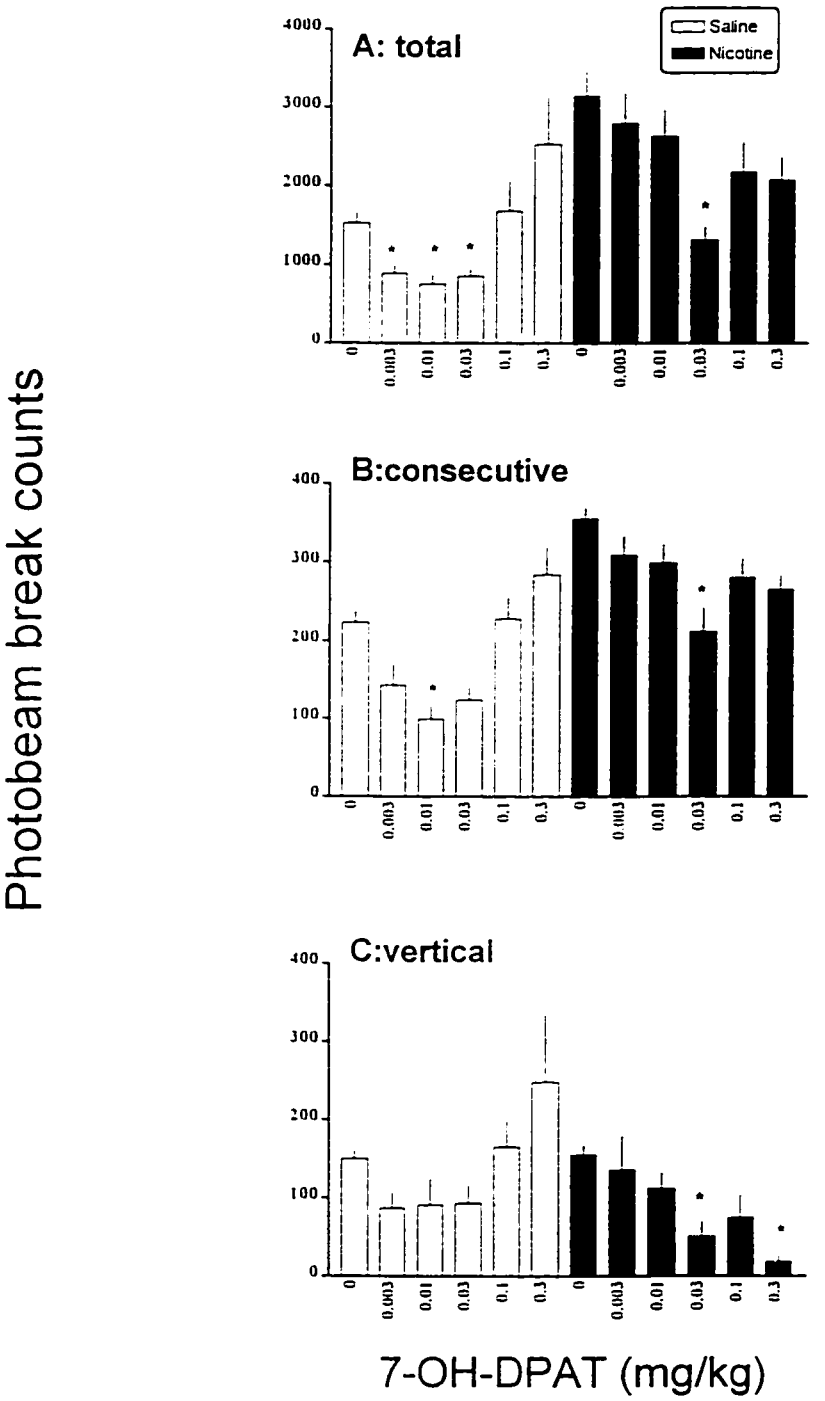

Figure 15: Effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc, immediately before test) on A: total, B: consecutive and C: vertical activity in a 30 min test session in repeated saline- (open bar) or nicotine (0.6 mg/kg/day, sc, 20 min before the second injection)- (black bar) treated rats. | : SEM for each mean. *: Significant vs other doses within each treatment group, $P<0.05$, n=9.

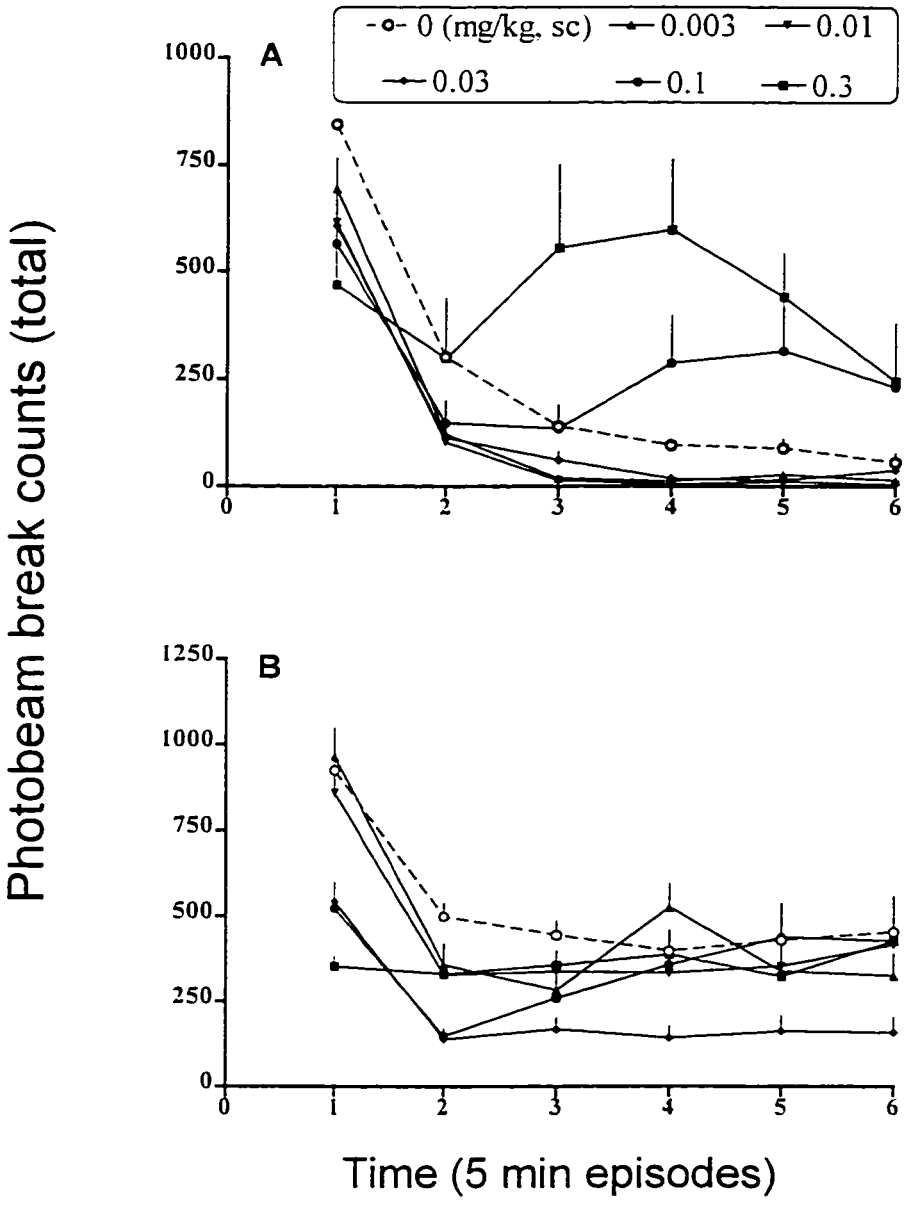

Figure 16: Time-course of effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc, immediately before test) on total activity in a 30 min test session in repeated A: saline- and B:nicotine (0.6 mg/kg, sc, 20 min before the second injection)-treated rats. |: SEM for each mean. Effect of 7-OH-DPAT is significant in panel A at times 1, 4 and 5 and in panel B at times 1, 2, 3, and 6, $P<0.05$, n=9.

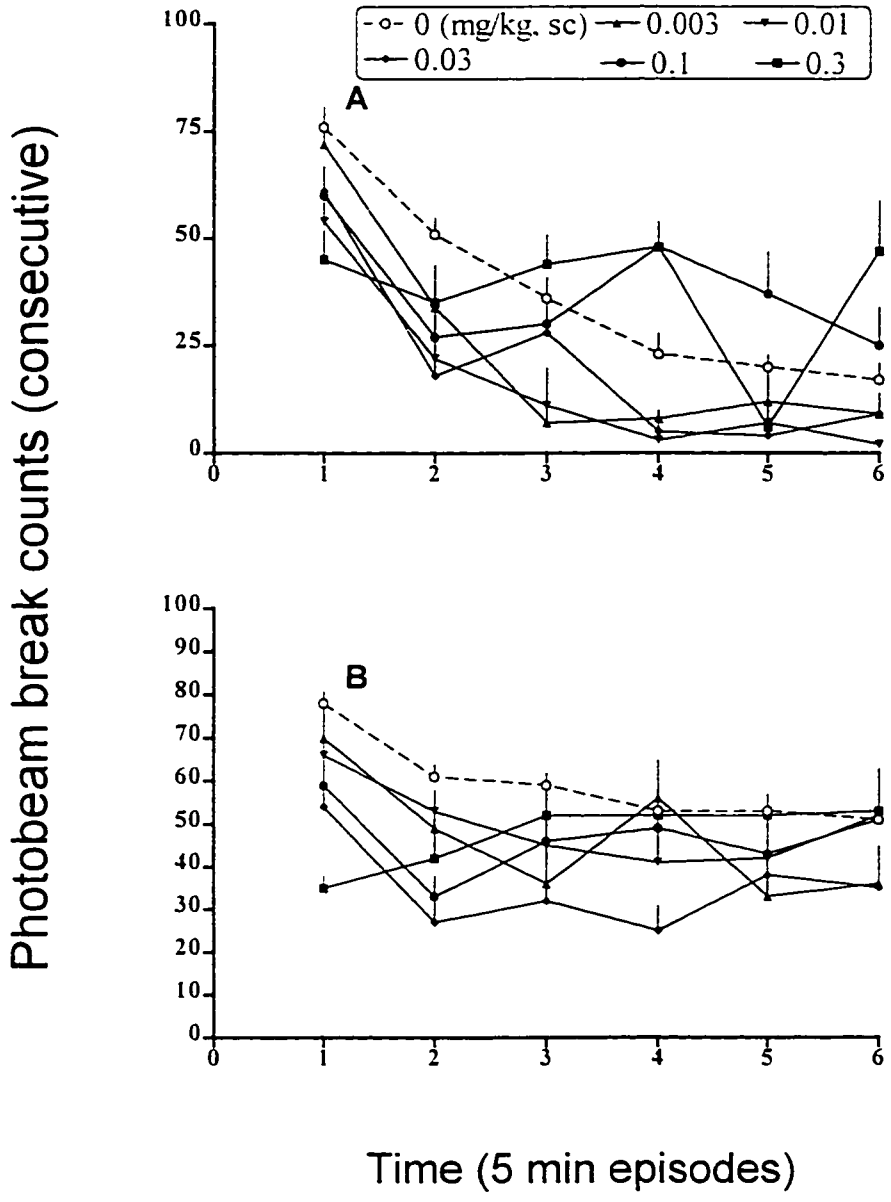

Figure 17: Time-course of effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc, immediately before test) on consecutive activity in a 30 min test session in repeated A: saline- and B:nicotine (0.6 mg/kg, sc, 20 min before the second injection)-treated rats. | : SEM for each mean. Effect of 7-OH-DPAT is significant in panel A at times 1 and 2 and in panel B at times 1, 2 and 3, $P<0.05$, n=9.

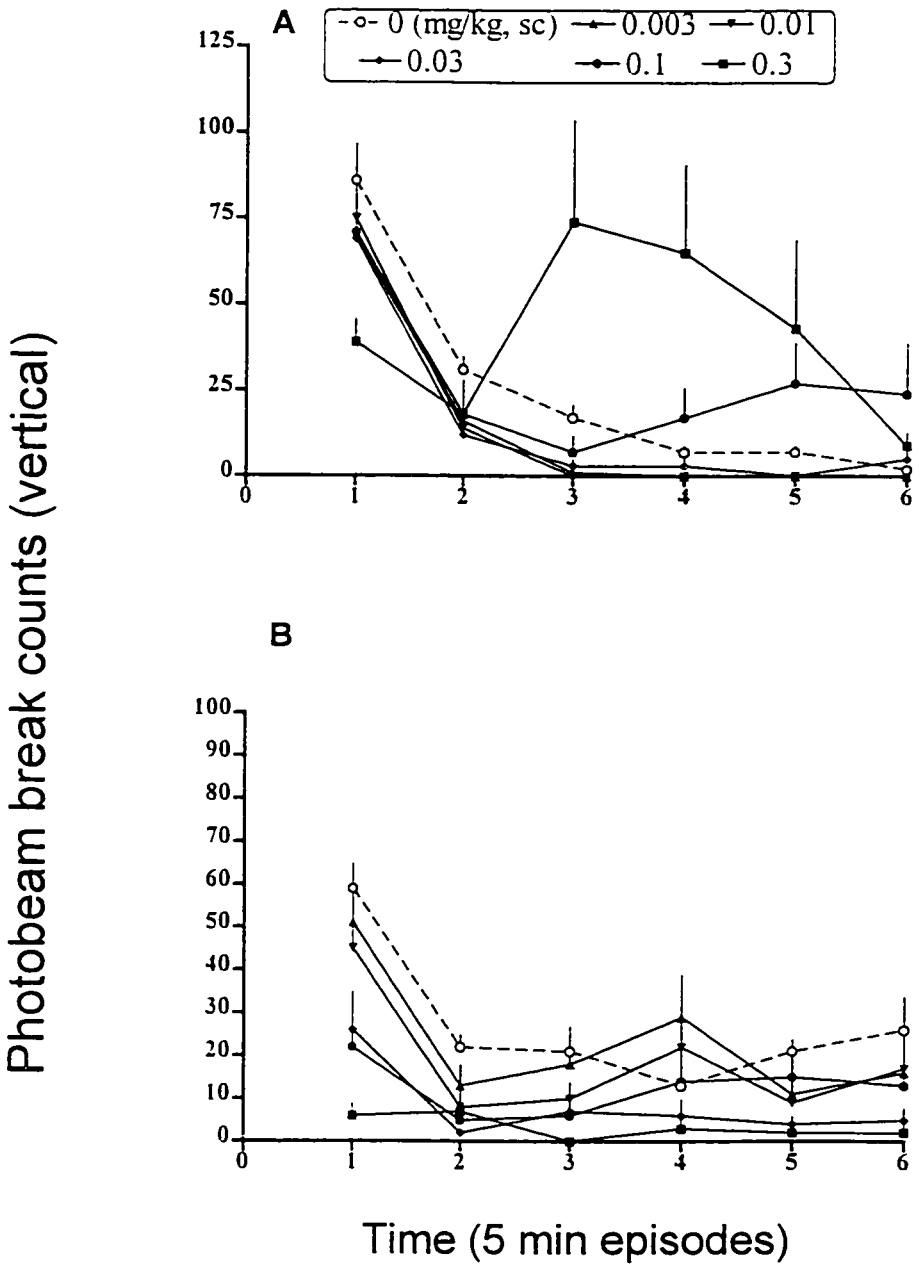

Figure 18: Time-course of effects of 7-OH-DPAT (0.003-0.3 mg/kg, sc, immediately before test) on vertical activity in a 30 min test session in repeated A: saline- and B:nicotine (0.6 mg/kg, sc, 20 min before the second injection)-treated rats. | : SEM for each mean. Effect of 7-OH-DPAT is significant in panel A at times 1, 3 and 4 and in panel B at times 1 and 2, $P<0.05$, $n=9$.

3.5. Effects of unilateral microinjection of 7-OH-DPAT into the NAS on locomotor activity

In the previous experiment 7-OH-DPAT decreased hyperactivity induced by nicotine. It has been shown that nicotine increases release of DA in the NAS more efficiently than in the CN (Imperato et al., 1986), indicating that hyperactivity induced by nicotine may be preferentially mediated through the mesolimbic dopaminergic system. It has also been shown that the DA D3 receptors are more abundant in the limbic area. Taken together, it was expected that some low doses of 7-OH-DPAT may show more efficacy in the NAS than in the CN. Therefore, this experiment was designed to investigate the site-selectivity of the effects of 7-OH-DPAT in these brain regions. In order to determine the specificity of the effects of 7-OH-DPAT, the effects of 8-OH-DPAT, an analog of 7-OH-DPAT with low affinity for DA receptors, were also investigated. 7-OH-DPAT, 0.5-5 μg/0.5μl/rat, unilaterally injected into the NAS decreased locomotor activity as shown by one-way ANOVA [total, 7-OH-DPAT: $F(4, 62) = 4.27$, $P<0.05$]; [consecutive, 7-OH-DPAT: $F(4, 62) = 3.17$, $P<0.05$]; [vertical, 7-OH-DPAT: $F(4, 62) = 2.97$, $P<0.05$], see Figure 19. The effects of 7-OH-DPAT on the time-course of total, consecutive and vertical activity, are shown in Figure 20 (A-C), respectively.

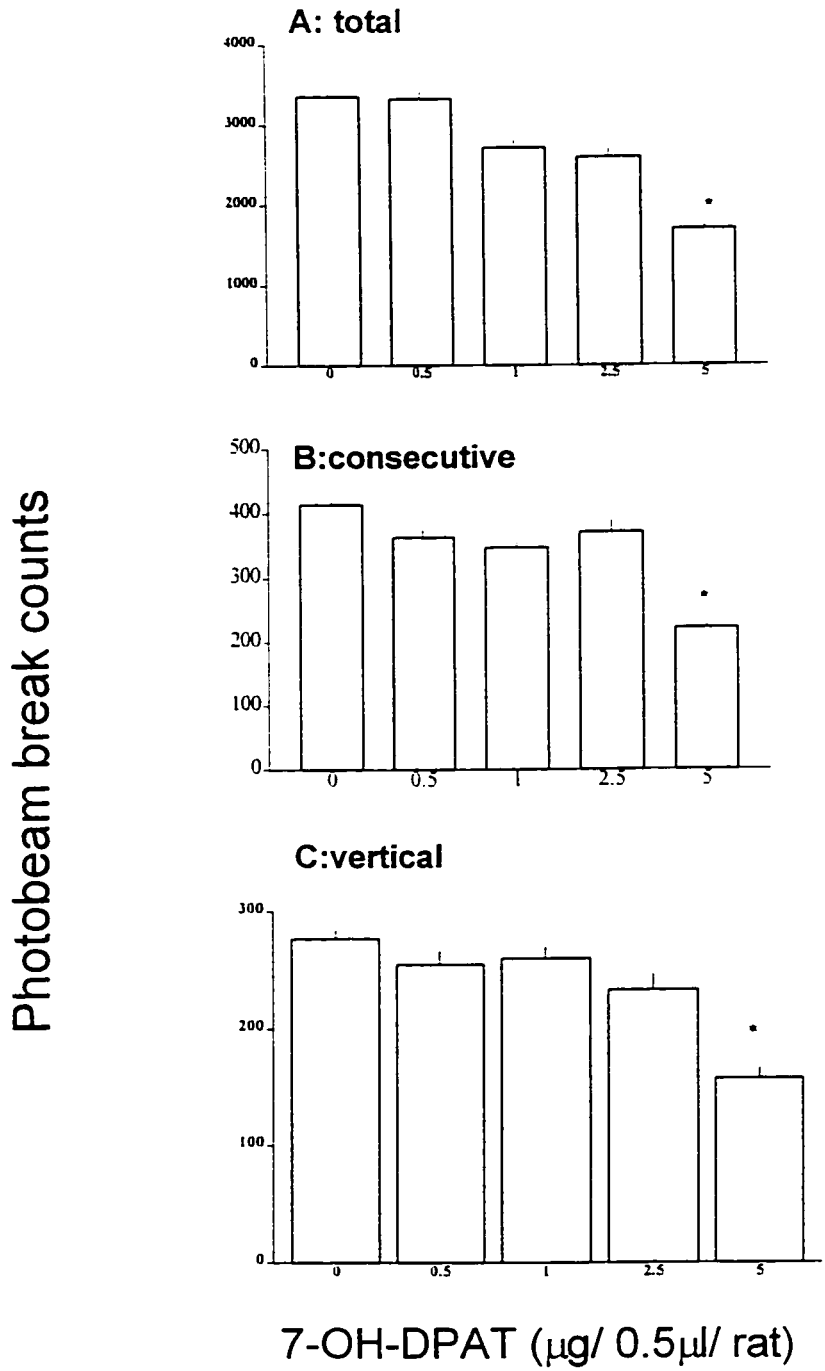

Figure 19: Effects of 7-OH-DPAT (0.5-5.0 μg/0.5μl, immediately before test) by unilateral microinjection into the NAS on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. *: Significant vs control, $P<0.05$, n=10-12.

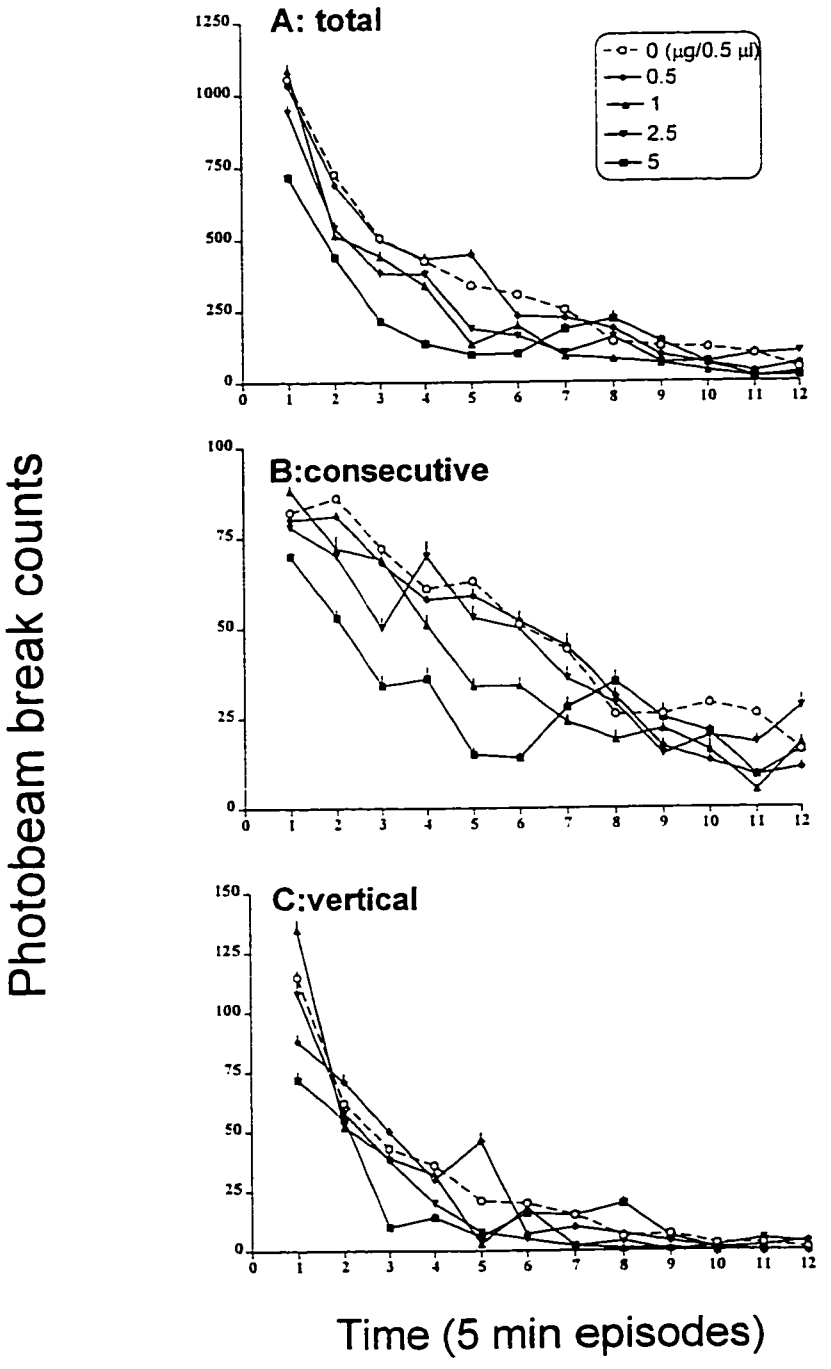

Figure 20: Time-course of effects of 7-OH-DPAT (0.5-5.0 µg/ 0.5µl, immediately before test) by unilateral microinjection into the NAS on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. Effect of 7-OH-DPAT is significant in panel A at times 1-7, in panel B at times 1-8 and in panel C at time 1, $P<0.05$, n=10-12.

3.6. The effect of unilateral microinjection of 7-OH-DPAT into the CN on locomotor activity

The highest dose from the previous experiment (5 μg/0.5μl/rat) was used in this experiment as it showed significant effects when injected into the NAS. It decreased total, consecutive and vertical locomotor activity. Figure 22 (A-C) shows the effects of 7-OH-DPAT on the time-course of total, consecutive and vertical activity, respectively.

ANOVA on the effects of dose of 5 μg of 7-OH-DPAT in both NAS and CN showed a lack of site-selectivity for 30 min counts of activity [total, Site: $F(1, 55) = 0.28$, $P>0.05$; 7-OH-DPAT: $F(1, 55) = 39.56$, $P<0.05$; Site x 7-OH-DPAT: $F(1, 55) = 0.001$, $P>0.05$]; [consecutive, Site: $F(1, 55) = 0.001$, $P>0.05$; 7-OH-DPAT: $F(1, 55) = 46.98$, $P<0.05$; Site x 7-OH-DPAT: $F(1, 55) = 0.15$, $P>0.05$]; [vertical, Site: $F(1, 55) = 0.61$, $P>0.05$; 7-OH-DPAT: $F(1, 55) = 25.23$, $P<0.05$; Site x 7-OH-DPAT: $F(1, 55) = 1.14$, $P>0.05$]; see Figures 20A-20C and 22A-22C. ANOVA on the time-course of total and consecutive, but not vertical, activity showed no significant site-selectivity [total, Time: $F(5, 275) = 111.88$, $P<0.05$; Time x Site: $F(5, 275) = 1$, $P>0.05$; 7-OH-DPAT x Time: $F(5, 275) = 0.18$, $P>0.05$; Time x Site x 7-OH-DPAT: $F(5, 275) = 0.73$, $P>0.05$]; [consecutive, Time: $F(5, 275) = 20.34$, $P<0.05$; Time x Site: $F(5, 275) = 1.03$, $P>0.05$; 7-OH-DPAT x Time: $F(5, 275) = 3.30$, $P<0.05$; Time x Site x 7-OH-DPAT: $F(5, 275) = 1.70$, $P>0.05$]; [vertical, Time: $F(5, 275) = 64.45$, $P<0.05$; Time x Site: $F(5, 275) = 1.18$, $P>0.05$; 7-OH-DPAT x Time: $F(5, 275) = 5.36$, $P<0.05$; Time x Site x 7-OH-DPAT: $F(5, 275) = 2.55$, $P<0.05$].

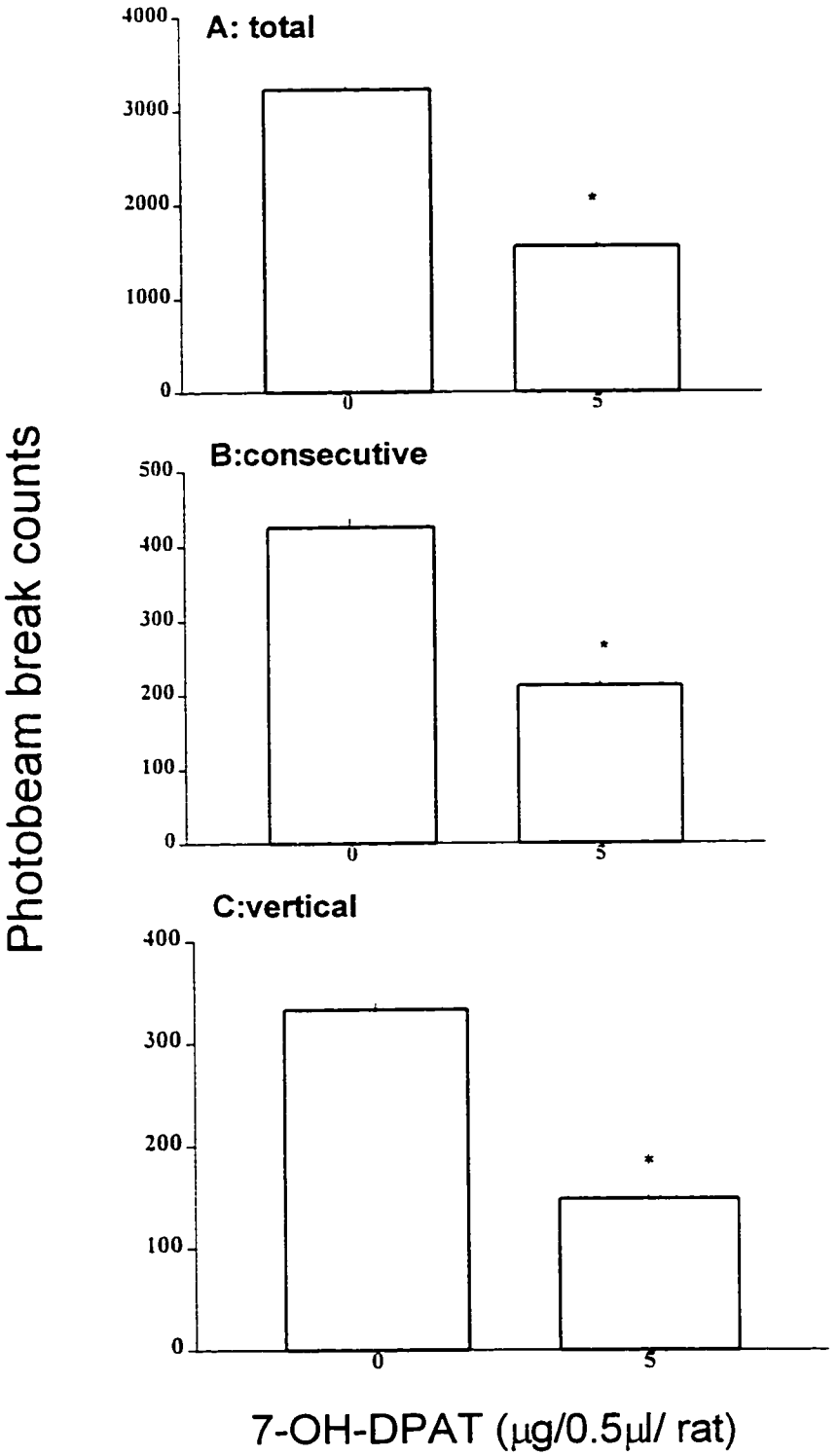

Figure 21: The effects of 7-OH-DPAT (5.0 μg/0.5μl, immediately before test) by unilateral microinjection into the CN on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. *: Significant vs control, $P<0.05$, n=10-12.

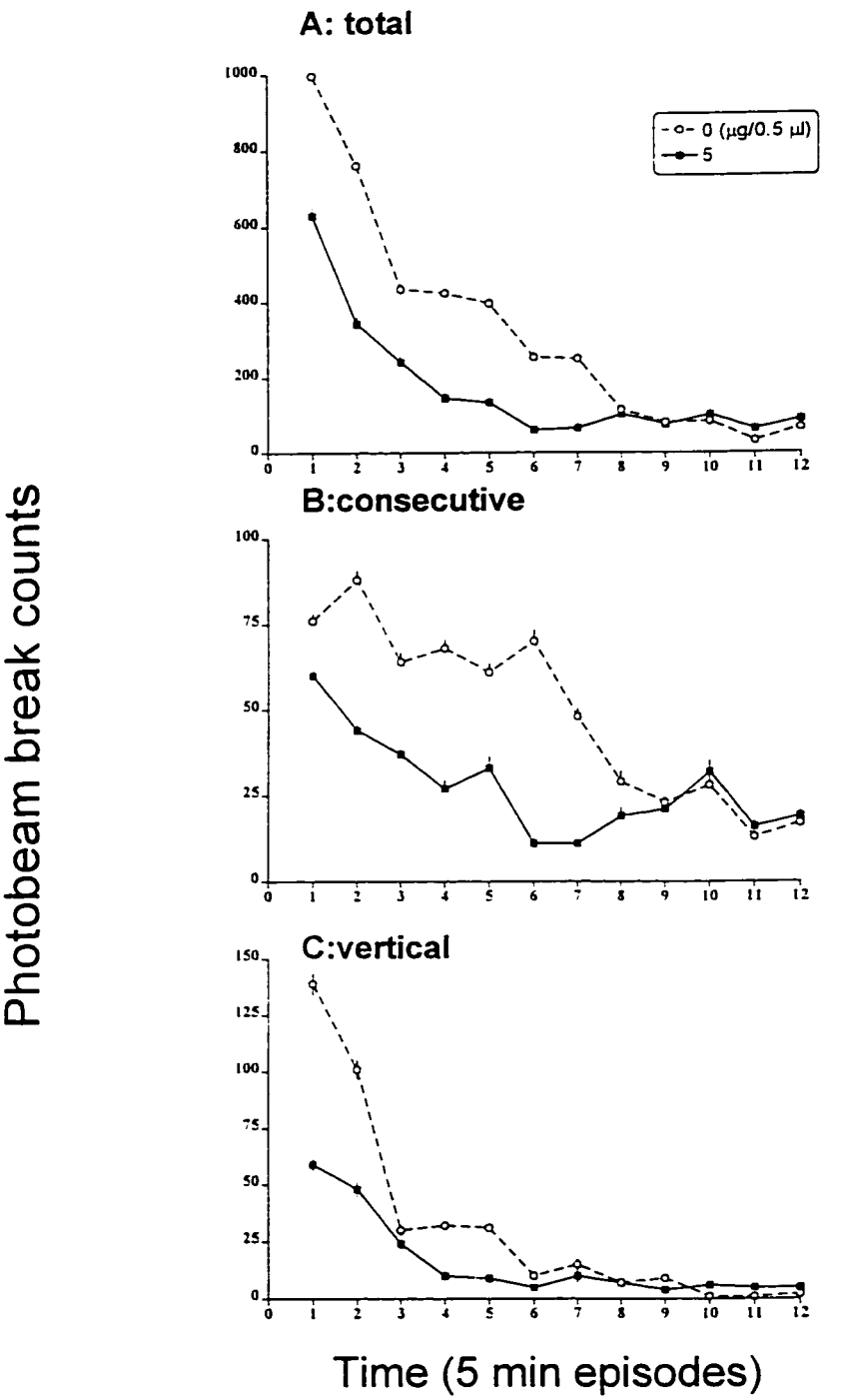

Figure 22: Time-course of effects of 7-OH-DPAT (5.0 µg/0.5µl, immediately before test) by unilateral microinjection into the CN on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. Effect of 7-OH-DPAT is significant in panel A at times 1-7, in panel B at times 1-7 and in panel C at times 1 and 2, $P<0.05$, n=10-12.

3.7. Effects of bilateral microinjection of 7-OH-DPAT into the NAS on locomotor activity

7-OH-DPAT at doses 0.5, 1, 2.5 and 5 μg/0.5 μl/rat, injected bilaterally into the NAS, decreased total, consecutive and vertical locomotor activity. The lower doses of 7-OH-DPAT that were inactive unilaterally, when injected bilaterally decreased locomotor activity, significantly as shown by one-way ANOVA [total, 7-OH-DPAT: $F(4, 99) = 27.06$, $P<0.05$]; [consecutive, 7-OH-DPAT: $F(4, 99) = 4.69$, $P<0.05$]; [vertical, 7-OH-DPAT: $F(4, 99) = 15.58$, $P<0.05$]; see Figure 23 (A-C). These effects on the time-course of total, consecutive and vertical activities are shown in Figure 24 (A-C), respectively.

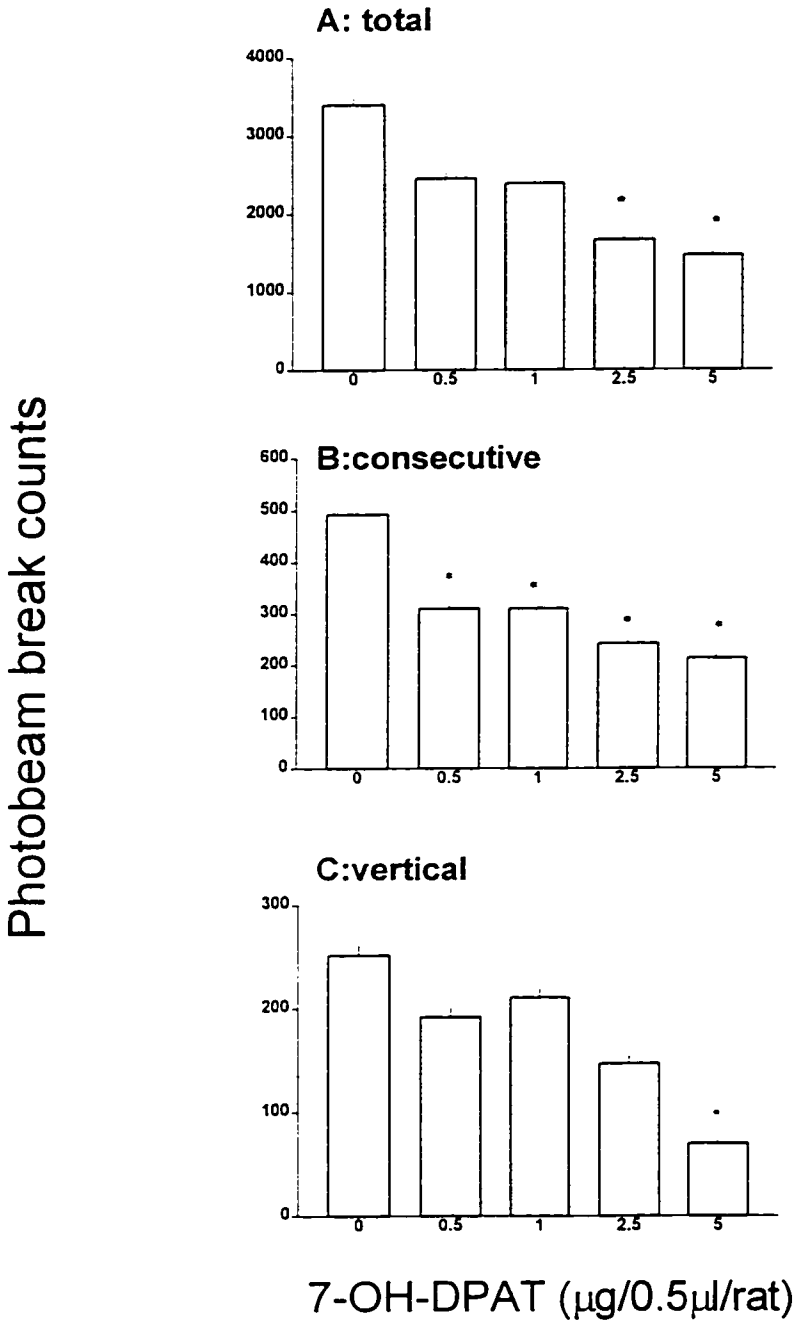

Figure 23: Effects of 7-OH-DPAT (0.5-5.0 µg/0.5µl, immediately before test) by bilateral microinjection into the NAS on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. | : SEM for each mean. *: Significant vs control, $P<0.05$, n=10-12.

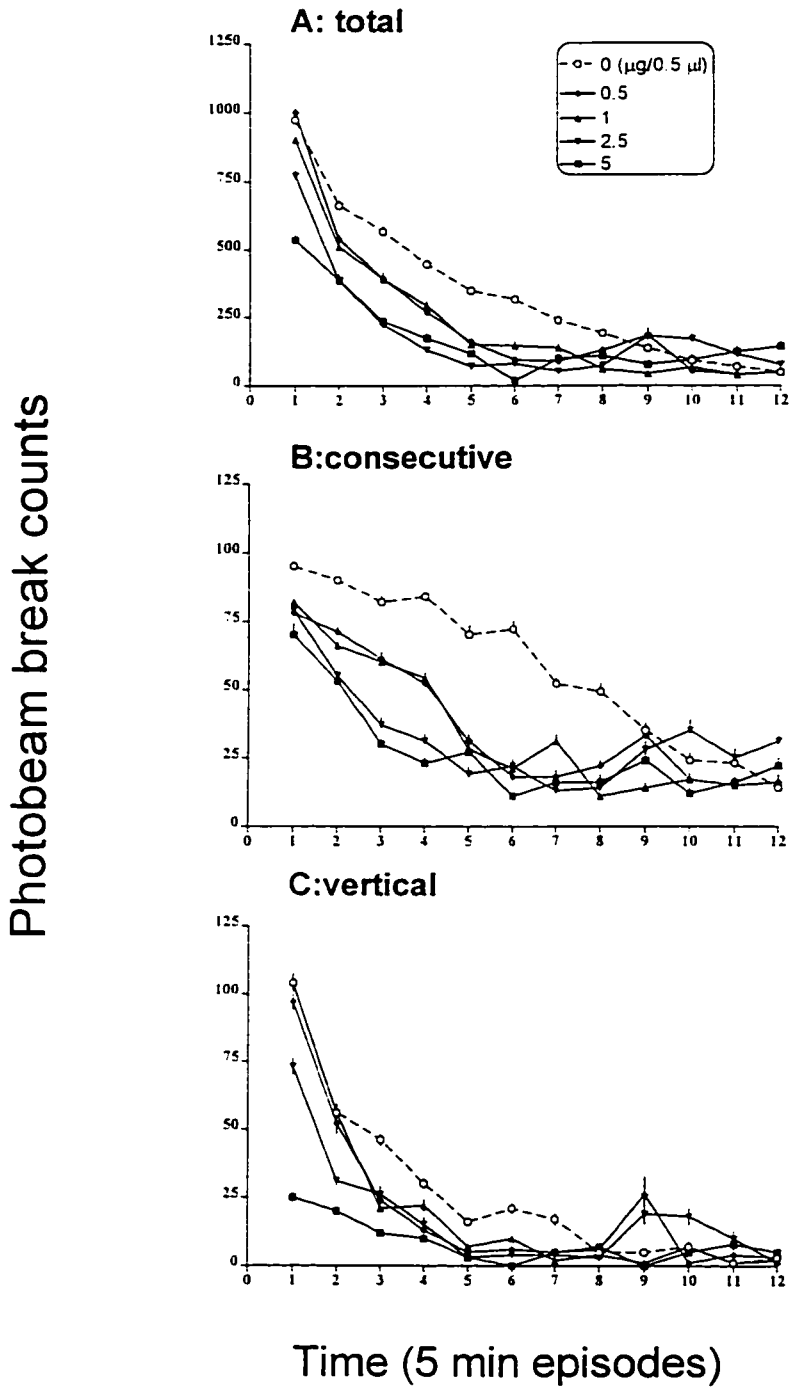

Figure 24: Time-course of dose-dependent effects of 7-OH-DPAT (0.5-5.0 µg/0.5µl, immediately before test) by bilateral microinjection into the NAS on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. Effect of 7-OH-DPAT is significant at times 1-7 on all activities, $P<0.05$, n=10-12.

3.8. Effects of bilateral microinjection of 7-OH-DPAT into the CN on locomotor activity

7-OH-DPAT at doses of 0.5, 1, 2.5 and 5 μg/0.5μl/rat injected bilaterally into the CN decreased total, consecutive and vertical locomotor activity [Figure 25 (A-C)]. 7-OH-DPAT decreased total [7-OH-DPAT: $F(4, 99) = 27.06$, $P<0.05$], consecutive [7-OH-DPAT: $F(4, 99) = 4.69$, $P<0.05$], and vertical [7-OH-DPAT: $F(4, 99) = 15.58$, $P<0.05$] activities [Figure 25 (A-C)]. These effects on the time-course of total, consecutive and vertical activities are shown in figure 26 (A-C), respectively.

ANOVA on the time-course of vertical but not total and consecutive activity in both NAS and CN showed significant site-selectivity [total, Time: $F(5, 495) = 282.49$, $P<0.05$; Site: $F(1, 99) = 0.003$, $P>0.05$; 7-OH-DPAT: $F(4, 99) = 26.74$, $P<0.05$; Site x 7-OH-DPAT: $F(4, 99) = 0.98$, $P>0.05$; Time x Site: $F(5, 495) = 1.10$, $P>0.05$; Time x 7-OH-DPAT: $F(5, 495) = 5.08$, $P<0.05$; Time x Site x 7-OH-DPAT: $F(5, 275) = 1.25$, $P>0.05$]; [consecutive, Time: $F(5, 495) = 28.00$, $P<0.05$; Site: $F(1, 99) = 1.47$, $P>0.05$; 7-OH-DPAT: $F(4, 99) = 4.65$, $P<0.05$; Site x 7-OH-DPAT: $F(4, 99) = 0.77$, $P>0.05$; Time x Site: $F(5, 495) = 2.63$, $P<0.05$; Time x 7-OH-DPAT: $F(5, 495) = 1.37$, $P>0.05$; Time x Site x 7-OH-DPAT: $F(5, 275) = 0.62$, $P>0.05$]; [vertical, Time: $F(5, 495) = 144.17$, $P<0.05$; Site: $F(1, 99) = 0.35$, $P>0.05$; 7-OH-DPAT: $F(4, 99) = 15.37$, $P<0.05$; Site x 7-OH-DPAT: $F(4, 99) = 1.93$, $P>0.05$; Time x Site: $F(5, 495) = 1.10$, $P>0.05$; Time x 7-OH-DPAT: $F(5, 495) = 7.08$, $P<0.05$; Time x Site x 7-OH-DPAT: $F(5, 275) = 1.71$, $P>0.05$].

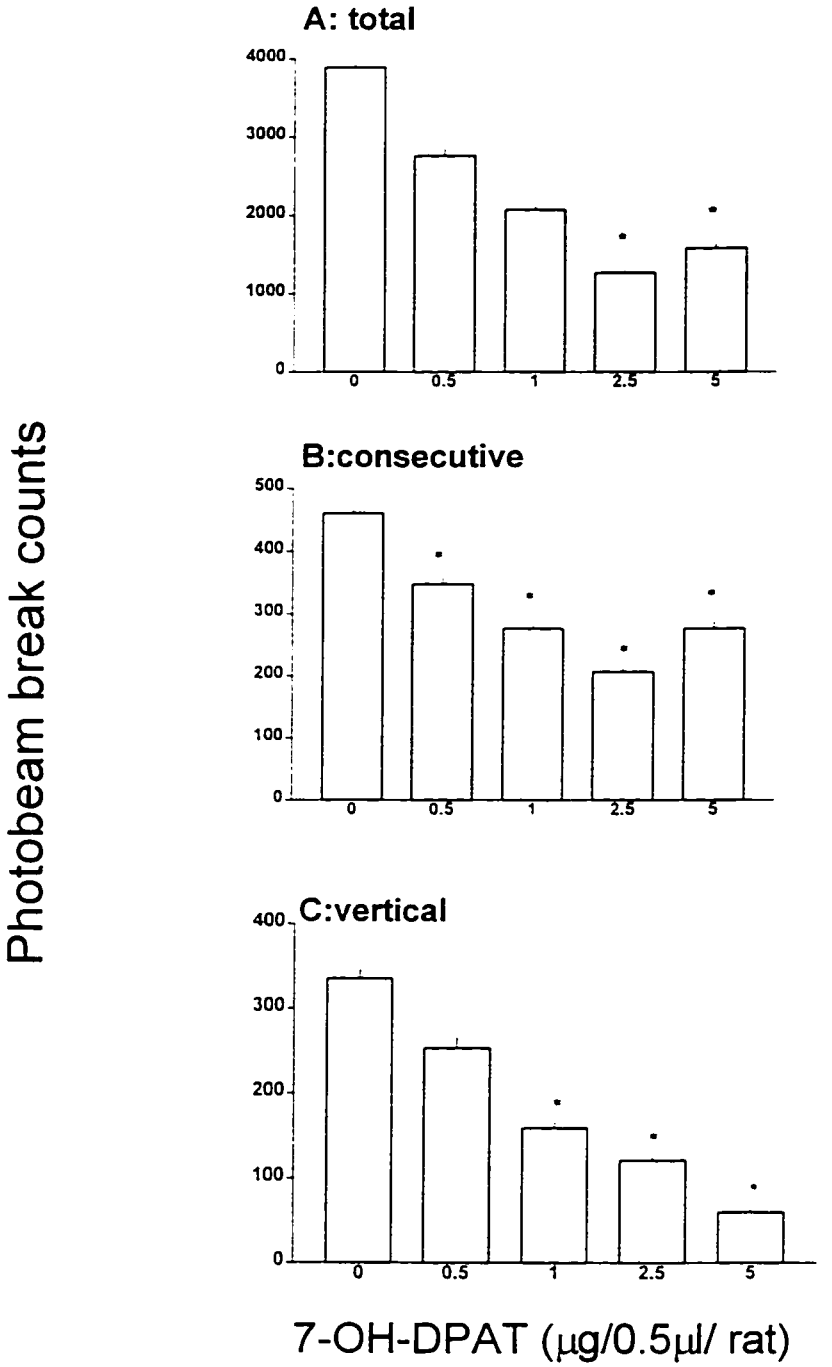

Figure 25: Dose-dependent effects of 7-OH-DPAT (0.5-5.0 μg/0.5μl. immediately before test) by bilateral microinjection into the CN on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. *: Significant vs control. $P<0.05$. n=12.

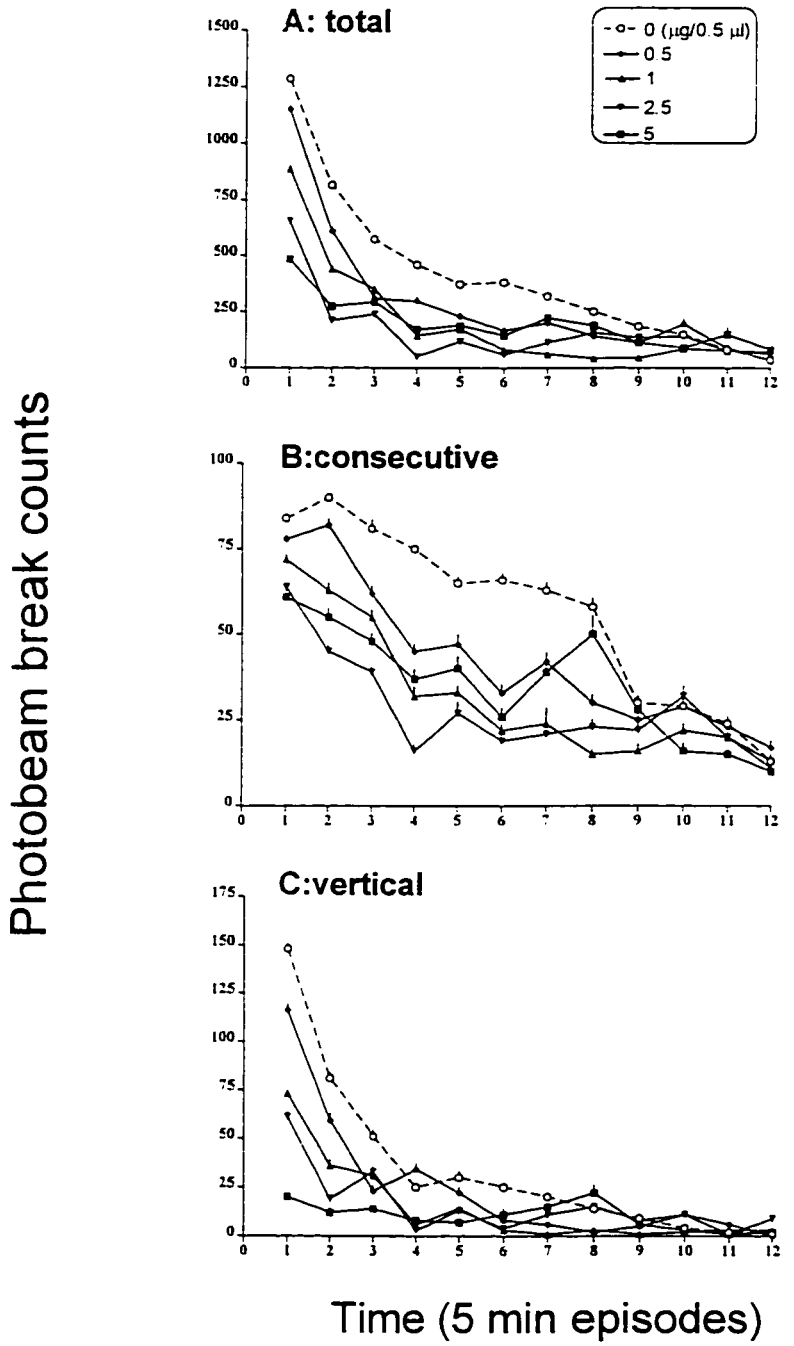

Figure 26: Time-course of dose-dependent effects of 7-OH-DPAT (0.5-5.0 µg/0.5µl, immediately before test) by bilateral microinjection into the CN on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. Effect of 7-OH-DPAT is significant at times 1-7 on all activities, $P<0.05$, n=12.

3.9. Effects of bilateral microinjection of 8-OH-DPAT into the NAS on locomotor activity

8-OH-DPAT at doses of 0.5, 1, 2.5 and 5 μg/ 0.5 μl/ rat, injected bilaterally into the NAS did not decrease the total and consecutive locomotor activity significantly [Figure 27 (A-B). Vertical activity was reduced significantly by this drug treatment as shown by one-way ANOVA followed by the Dunnett t (2-sided) test [vertical, 8-OH-DPAT: $F(4, 25) = 8.57$, $P<0.05$]; see Figure 27C. These effects on the time-course of total, consecutive and vertical activities are shown in Figure 28 (A-C). The effect of 8-OH-DPAT was not time-dependent.

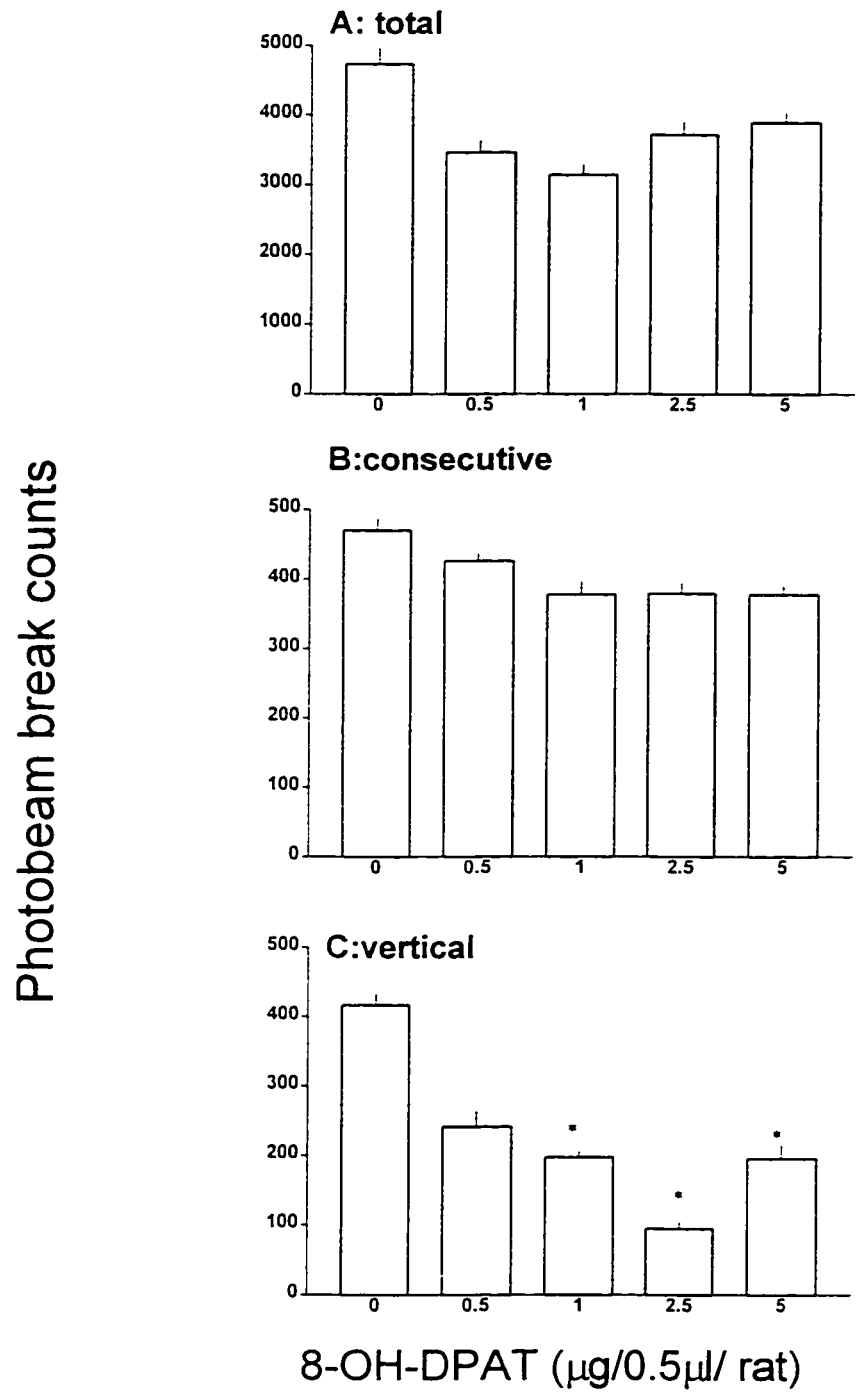

Figure 27: Dose-dependent effects of 8-OH-DPAT (0.5-5.0 µg/0.5µl, immediately before test) by bilateral microinjection into the NAS on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. |: SEM for each mean. *: Significant vs control, $P<0.05$, n=5-6.

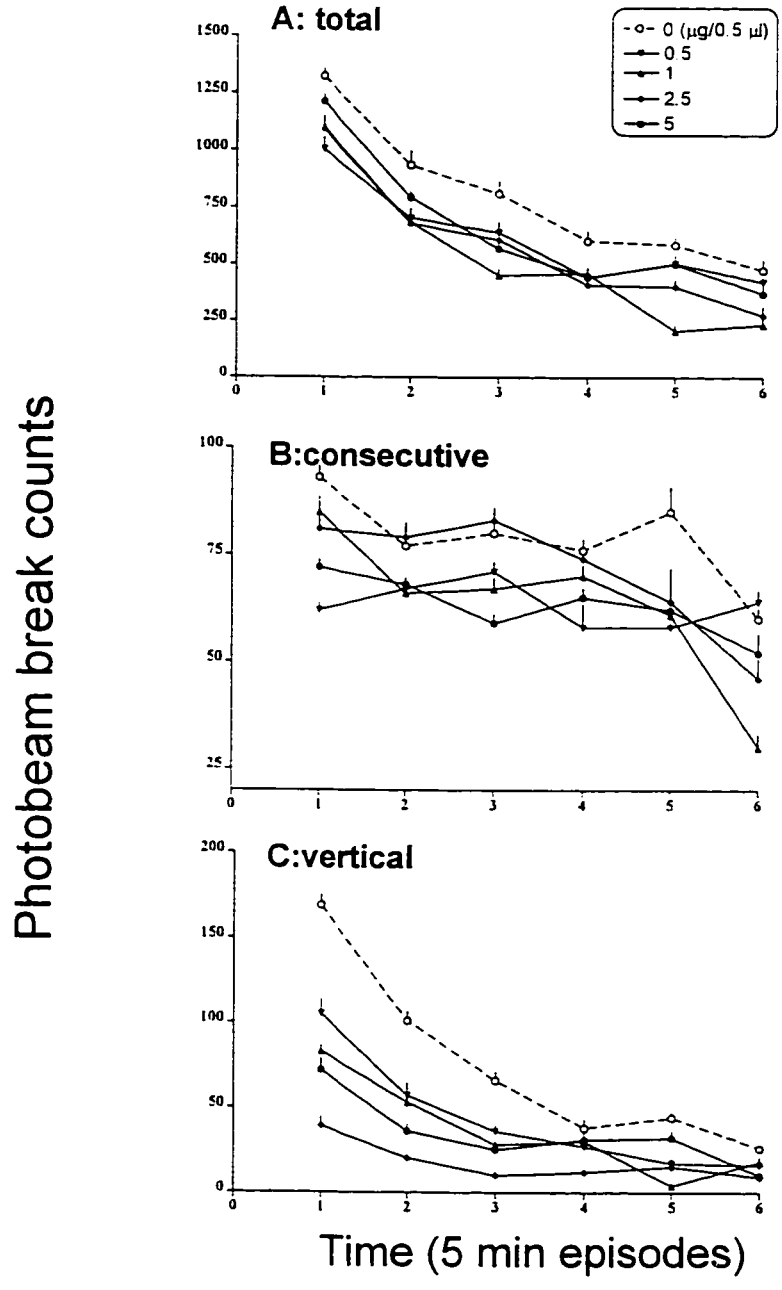

Figure 28: Time-course of effects of 8-OH-DPAT (0.5-5.0 µg/ 0.5µl, immediately before test) by bilateral microinjection into the NAS on A: total. B: consecutive and C: vertical activity in a 30 min test session in rats. | : SEM for each mean. Effect of 8-OH-DPAT is significant at times 1-3 on vertical activity, $P<0.05$, n=5-6.

3.10. Effects of bilateral microinjection of 8-OH-DPAT into the CN on locomotor activity

8-OH-DPAT at doses of 0.5, 1, 2.5 and 5 μg/0.5 μl/rat, injected bilaterally into the CN did not decrease the total and consecutive activity [Figure 29 (A-B)]. In contrast to the NAS region, injections of 8-OH-DPAT into the CN did not reduce vertical activity (Figure 29C). The time-courses of total, consecutive and vertical locomotor activities are shown in Figure 30 (A-C).

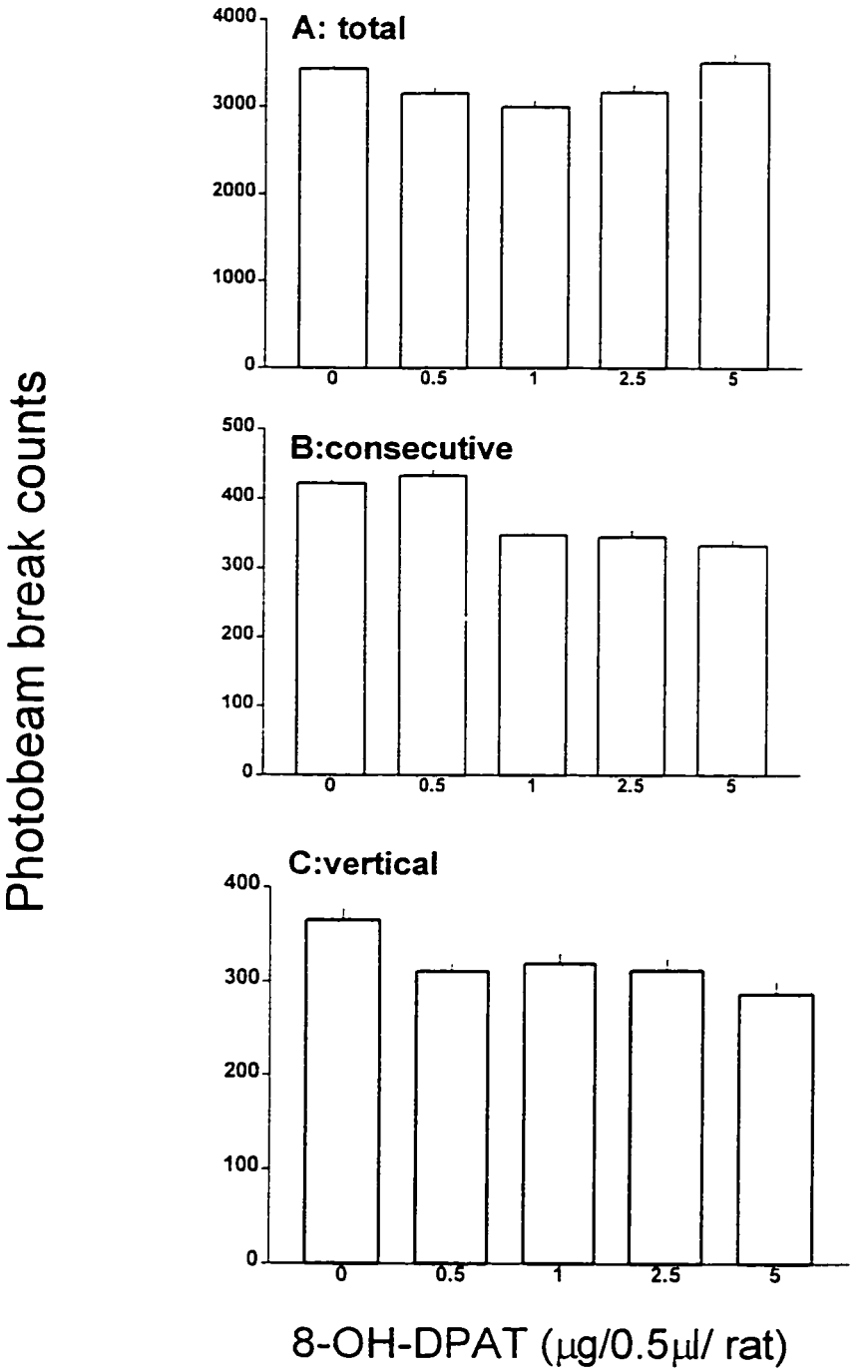

Figure 29: The effect of 8-OH-DPAT (0.5-5.0 µg/0.5µl, immediately before test) by bilateral microinjection into the CN on A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. | : SEM for each mean, n=6. ANOVA revealed no statistically significant effects.

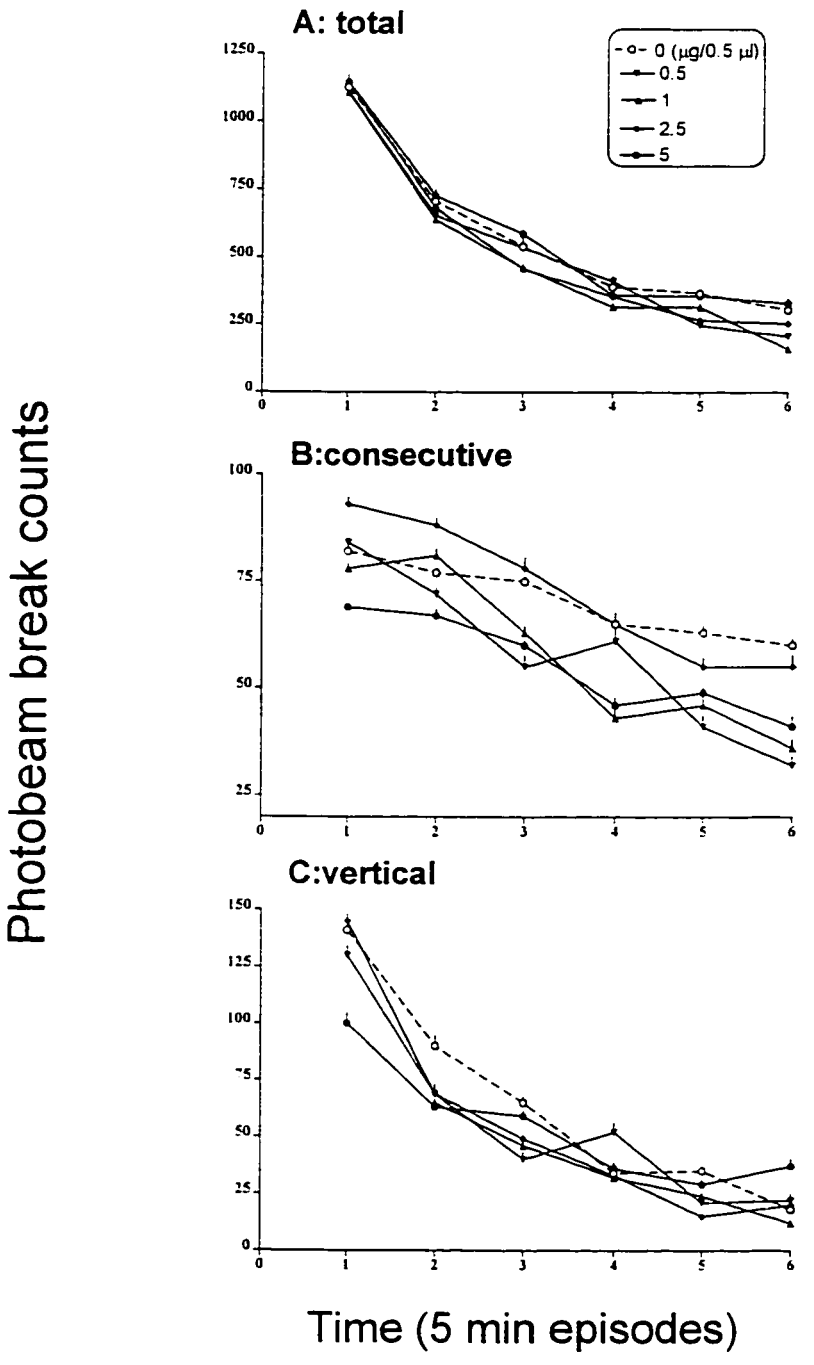

Figure 30: Effects of 8-OH-DPAT (0.5-5.0 µg/ 0.5µl, immediately before test) by bilateral microinjection into the CN on time-course of A: total, B: consecutive and C: vertical activity in a 30 min test session in rats. | : SEM for each mean, n=5-6. ANOVA revealed no statistically significant effects.

3.11. **Histological examination**

Figures 31-34 show placements for the centre of the tips of needles in experiments including unilateral or bilateral microinjections of 7-OH-DPAT or 8-OH-DPAT into the NAS or CN. Rats with needle placement in areas other than those shown in these pictures were excluded from the data analysis. Overlapping sites are shown only by one indicator.

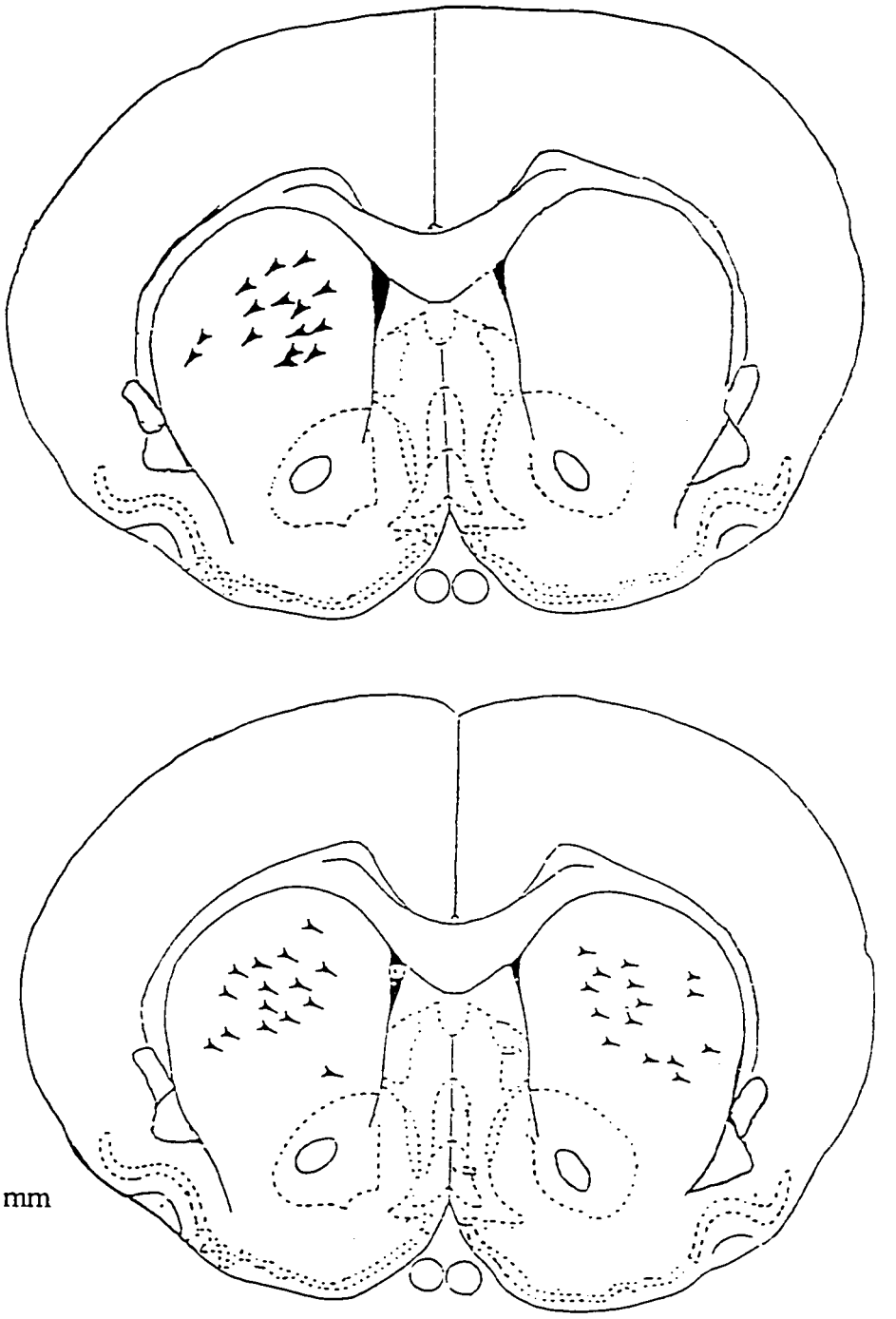

Figure 31: The positions of the centres of the tips of needles (indicated by ►) in A: (unilateral) and B: (bilateral) caudate nucleus for 7-OH-DPAT microinjections. The number refers to coronal section according to the atlas of Paxinos and Watson (1986).

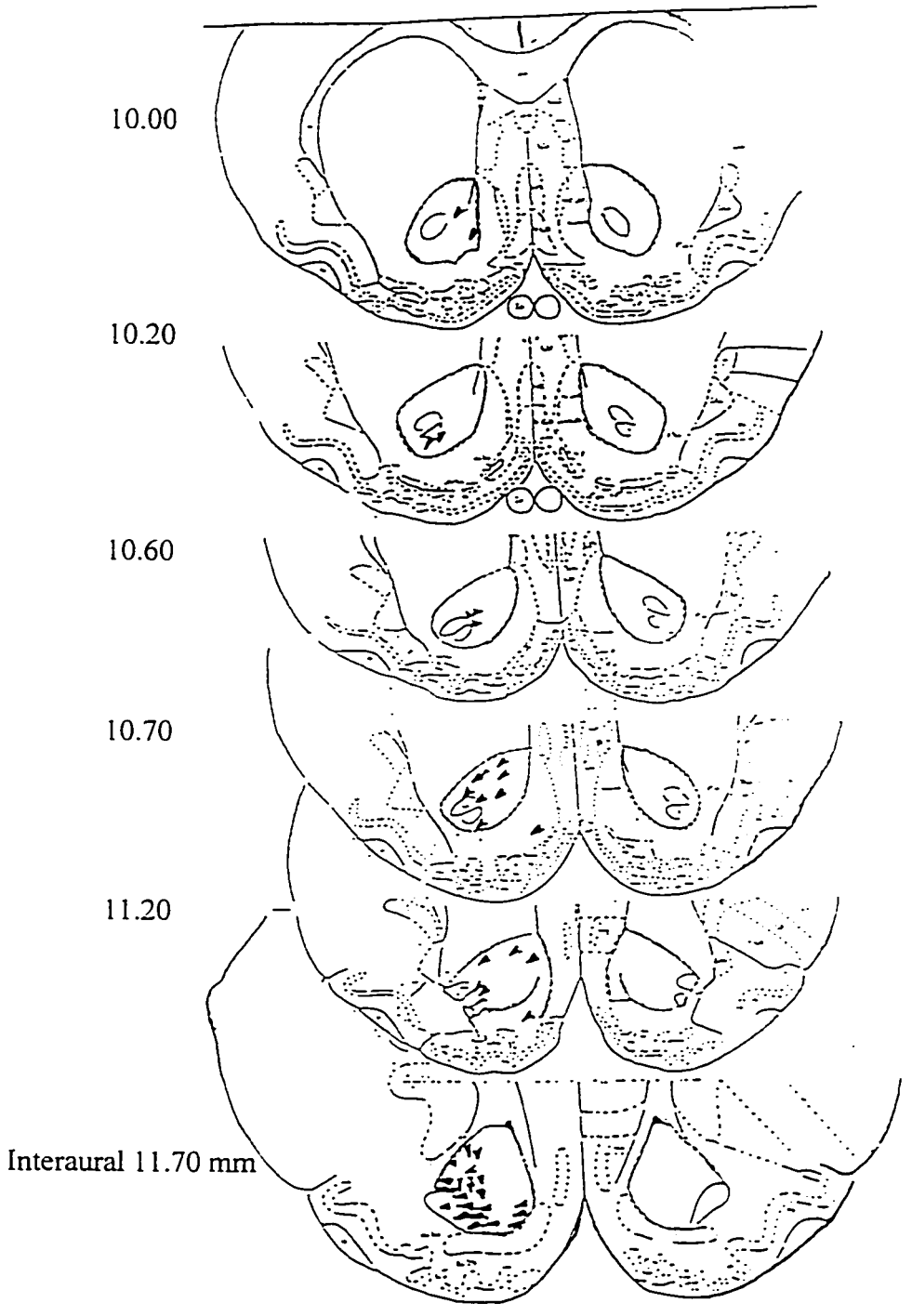

Figure 32: The positions of the centres of the tips of needles (indicated by ►) in unilateral nucleus accumbens microinjections for 7-OH-DPAT. The numbers refer to coronal sections according to the atlas of Paxinos and Watson (1986).

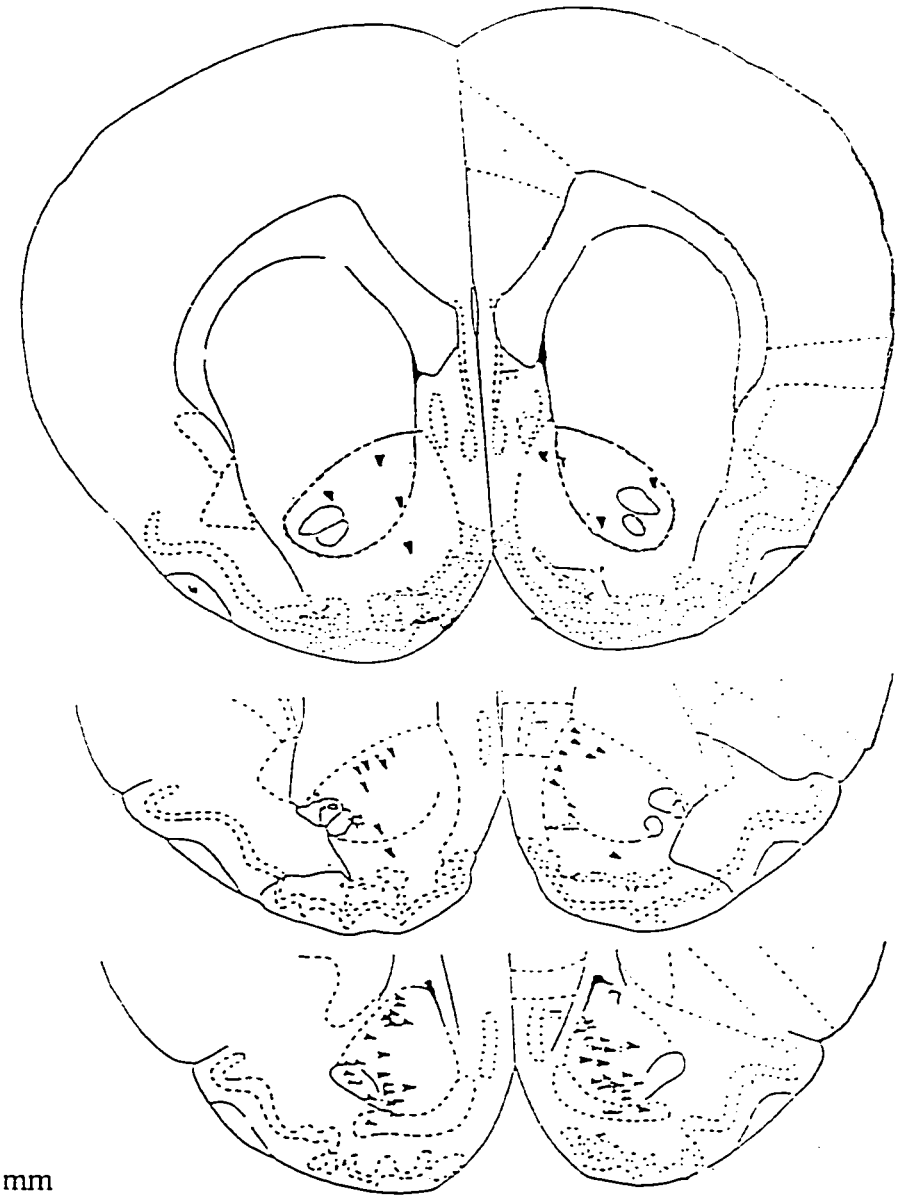

Figure 33: The positions of the centres of the tips of needles (indicated by) in bilateral nucleus accumbens microinjections for 7-OH-DPAT. The numbers refer to coronal sections according to the atlas of Paxinos and Watson (1986).

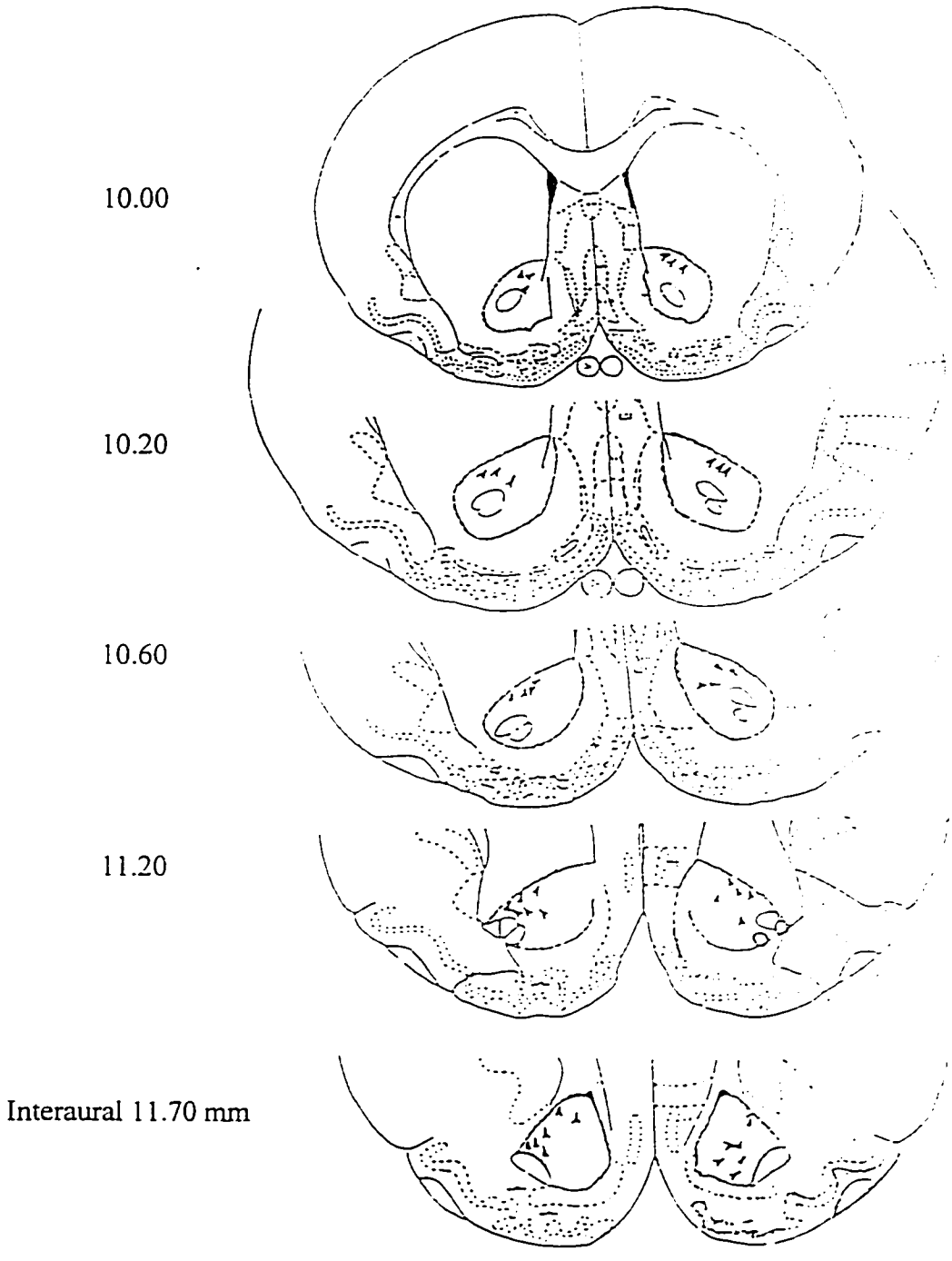

Figure 34: The positions of the centres of the tips of needles (indicated by ►) in bilateral nucleus accumbens microinjections for 8-OH-DPAT. The numbers refer to coronal sections according to the atlas of Paxinos and Watson (1986).

3.12. The interaction of CLZ with high dose of 7-OH-DPAT or APO

The atypical properties of CLZ have been proposed to be related to the preferential effects of CLZ in the mesolimbic system. The distribution of the DA D3 receptors also has been shown in this area in rats. Also, it has been reported that CLZ increases levels of mRNA for DA D3 receptors. Therefore, the present experiment was designed to investigate a possible interaction between CLZ (0.625, 1.25 and 5 mg/kg) and 7-OH-DPAT (2.5 and 5 mg/kg), a DA D3 receptor agonist, in comparison with APO (0.5 mg/kg), a DA D1/D2 receptor agonist. One way ANOVA followed by a Newman-Keuls test showed that CLZ decreased locomotor activity. APO (0.5 mg/kg) and 7-OH-DPAT (5 mg/kg) induced significant hyperactivity [CLZ: $F(2, 32) = 8.28$, $P<0.05$]; [APO/7-OH-DPAT: $F(2, 47) = 24.16$, $P<0.05$]. 7-OH-DPAT induced a higher level of hyperactivity than APO (Figure 35). The effects of CLZ on total hyperactivity induced by APO and 7-OH-DPAT were significant as shown by two-way ANOVA followed by a Newman-Keuls test; [total, CLZ: $F(2, 87) = 39.34$; APO/7-OH-DPAT: $F(2, 87) = 26.16$; CLZ x APO/7-OH-DPAT: $F(4, 87) = 5.19$, $P<0.05$]; [consecutive, CLZ: $F(2, 87) = 11.86$; APO/7-OH-DPAT: $F(2, 87) = 8.57$, $P<0.05$; CLZ x APO/7-OH-DPAT: $F(4, 87) = 1.54$, $P>0.05$]; [vertical, CLZ: $F(2, 87) = 3.64$; APO/7-OH-DPAT: $F(2, 87) = 4.26$, $P<0.05$; CLZ x APO/7-OH-DPAT: $F(4, 87) = 0.65$, $P>0.05$] [Figure 35 (A-C)]. The time-course effects of CLZ on total hyperactivity induced by APO and 7-OH-DPAT are displayed in Figure 36. As ANOVA shows, the effect of CLZ is not time-dependent [Time: $F(5,435) = 20.04$, $P<0.05$; CLZ: $F(2, 87) = 39.34$; Time x CLZ: $F(10,435) = 0.36$, $P>0.05$] but the effects of APO, 7-OH-DPAT and their interaction

with CLZ are time-dependent [Time x APO/7-OH-DPAT: $F(10, 435) = 10.03$; Time x CLZ x APO/7-OH-DPAT: $F(20,435) = 3.46$, $P<0.05$]. *Post hoc* tests revealed that the lower dose of CLZ, 1.25 mg/kg, decreased the effect of 7-OH-DPAT but not of APO at episodes 3, 4, 5 and 6 of time in the 30 min test session. Figures 37 and 38 show these effects on the consecutive and vertical activities, respectively; there were not significant drug interactions with these activities. CLZ (5 mg/kg) also significantly blocked forward walking (Figure 39A) and sniffing (Figure 39B) induced by 7-OH-DPAT but not by APO, as shown by the Kruskal-Wallis test followed by the Mann-Whitney U test [sniffing: $Q = 3.71$; forward walking: $Q = 3.04$, $P<0.05$]. In the second experiment, the interactions between several doses of CLZ (0.625, 1.25, 5 mg/kg) and a lower dose of 7-OH-DPAT (2.5 mg/kg) were studied. In the previous experiment APO induced a lower level of hyperactivity compared to 7-OH-DPAT (Figure 35, first black and grey bars), so the lower dose of 7-OH-DPAT was used in present experiment in an attempt to determine whether this difference in the level of hyperactivity contributed to the differential effects of CLZ. Similar to the first experiment, CLZ decreased total activity during 30 min, dose-dependently [CLZ: $F(3, 46) = 18.22$], and 7-OH-DPAT induced hyperactivity [7-OH-DPAT x time: $F(5, 203) = 51.94$], (Figure 40). The interactions among all doses of CLZ and 7-OH-DPAT (2.5 mg/kg) on the time-course of total activity were significant as shown by 3-way RMANOVA [total, Time: $F(5,230) = 57.01$; CLZ: $F(3, 46) = 17.74$; 7-OH-DPAT: $F(1, 46) = 2.92$, $P>0.05$; CLZ x 7-OH-DPAT: $F(3, 46) = 0.95$, $P>0.05$; Time x CLZ: $F(15, 230) = 3.83$; Time x 7-OH-DPAT: $F(5, 230) = 51.94$; Time x CLZ x 7-OH-DPAT: $F(15, 230) = 2.24$, $P<0.05$]; [vertical, Time: $F(5, 230) = 65.40$; CLZ: $F(3, 46) = 21.56$; 7-OH-DPAT: $F(1, 46) = 108.38$, $P<0.05$; CLZ x 7-OH-

DPAT: $F(3, 46) = 16.91$, $P<0.05$; Time x CLZ: $F(15, 230) = 8.05$; Time x 7-OH-DPAT: $F(5, 230) = 55.04$; Time x CLZ x 7-OH-DPAT: $F(15, 230) = 6.54$, $P<0.05$]; see Figures 41-43.

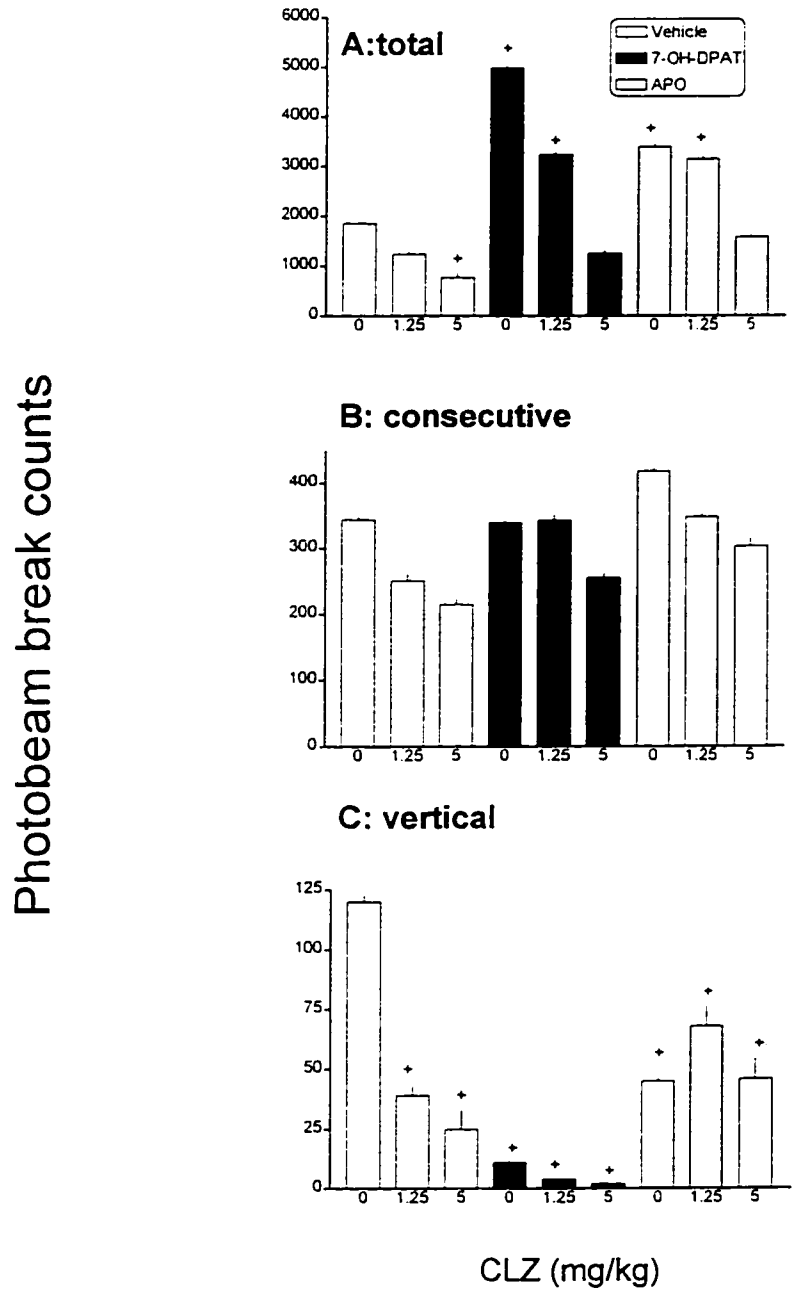

Figure 35: The effects of CLZ (1.25 or 5 mg/kg, sc) on A: total, B: consecutive and C: vertical activity for 30 min in vehicle-, 7-OH-DPAT (5 mg/kg, sc)- or APO (0.5 mg/kg, sc)-treated rats. |: SEM for each mean. +: significant vs vehicle, $P<0.05$, n=8.

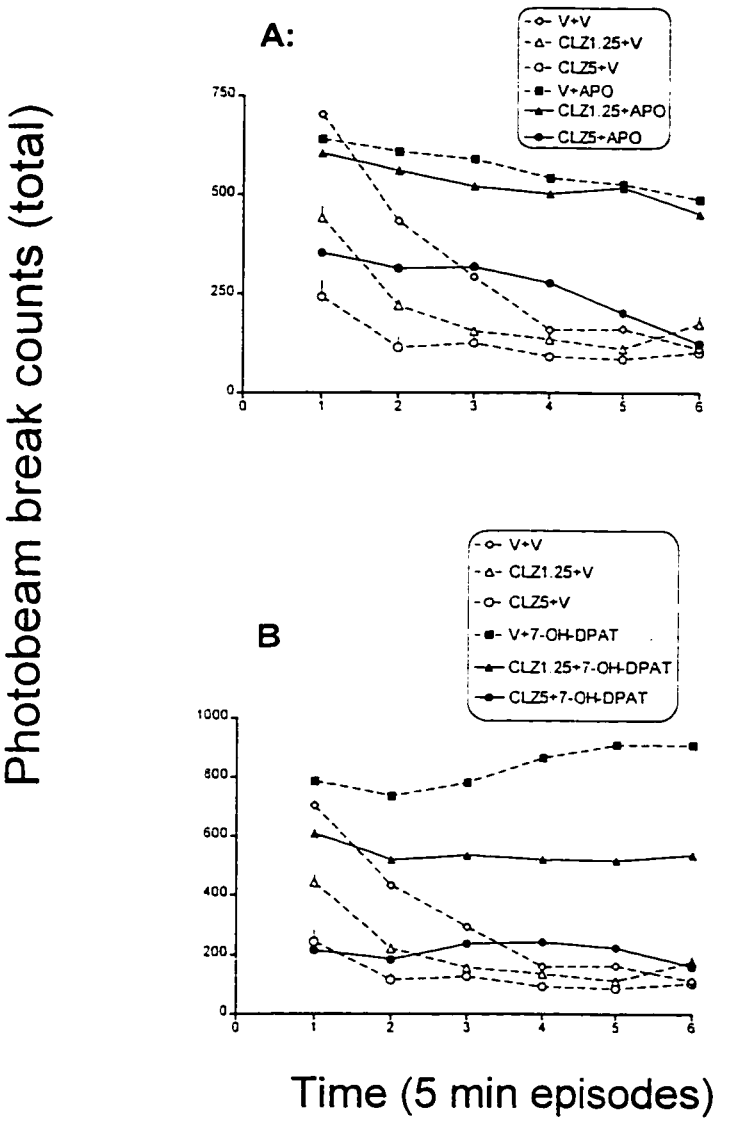

Figure 36: The effects of CLZ (1.25 or 5 mg/kg, sc) on time-course of total activity for 30 min in A: APO (0.5 mg/kg, sc)- or B: 7-OH-DPAT (5 mg/kg, sc)-treated rats. |: SEM for each mean. Effect of CLZ on APO and 7-OH-DPAT is significant at times 3-6, $P<0.05$, n=8. V= vehicle.

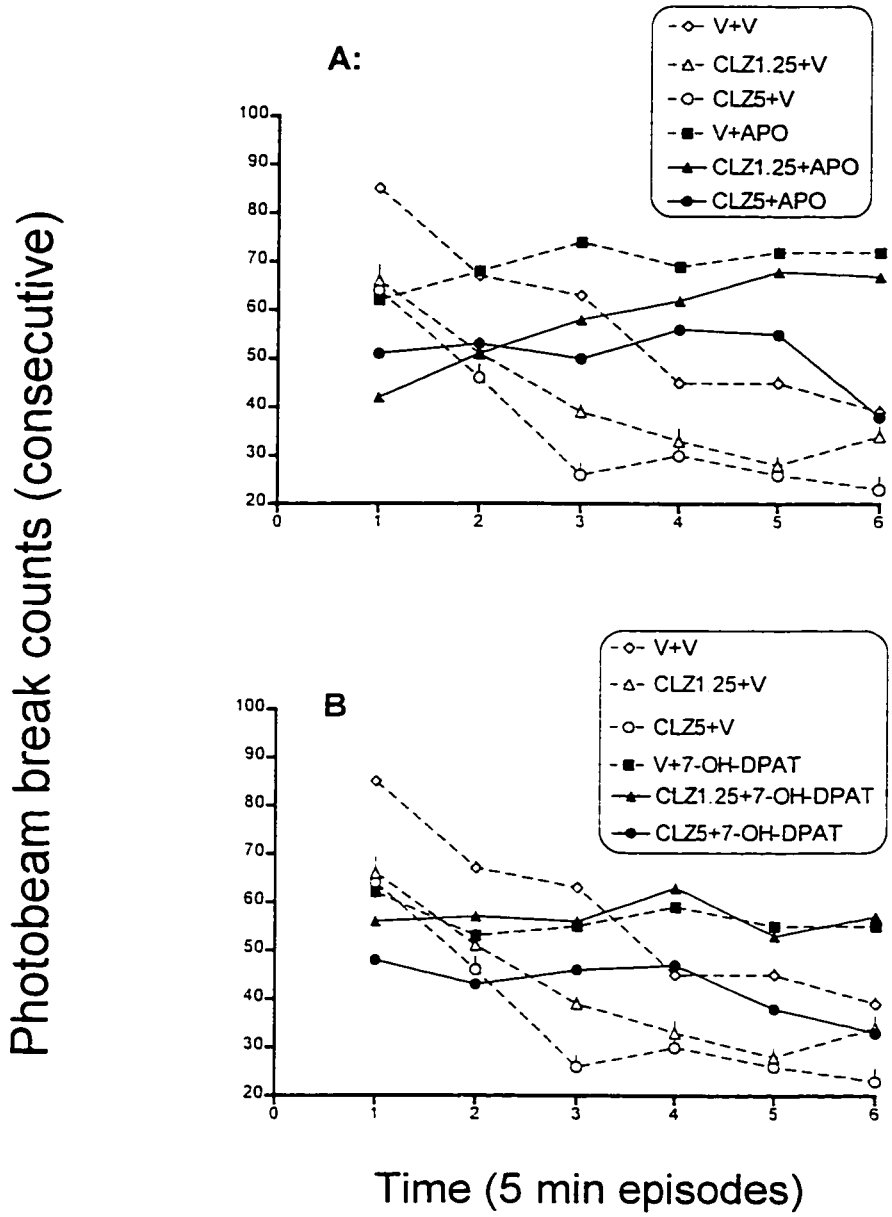

Figure 37: The effects of CLZ (1.25 or 5 mg/kg, sc) on time-course of consecutive activity for 30 min in A: APO (0.5 mg/kg, sc)- or B: 7-OH-DPAT (5 mg/kg, sc)-treated rats. | : SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

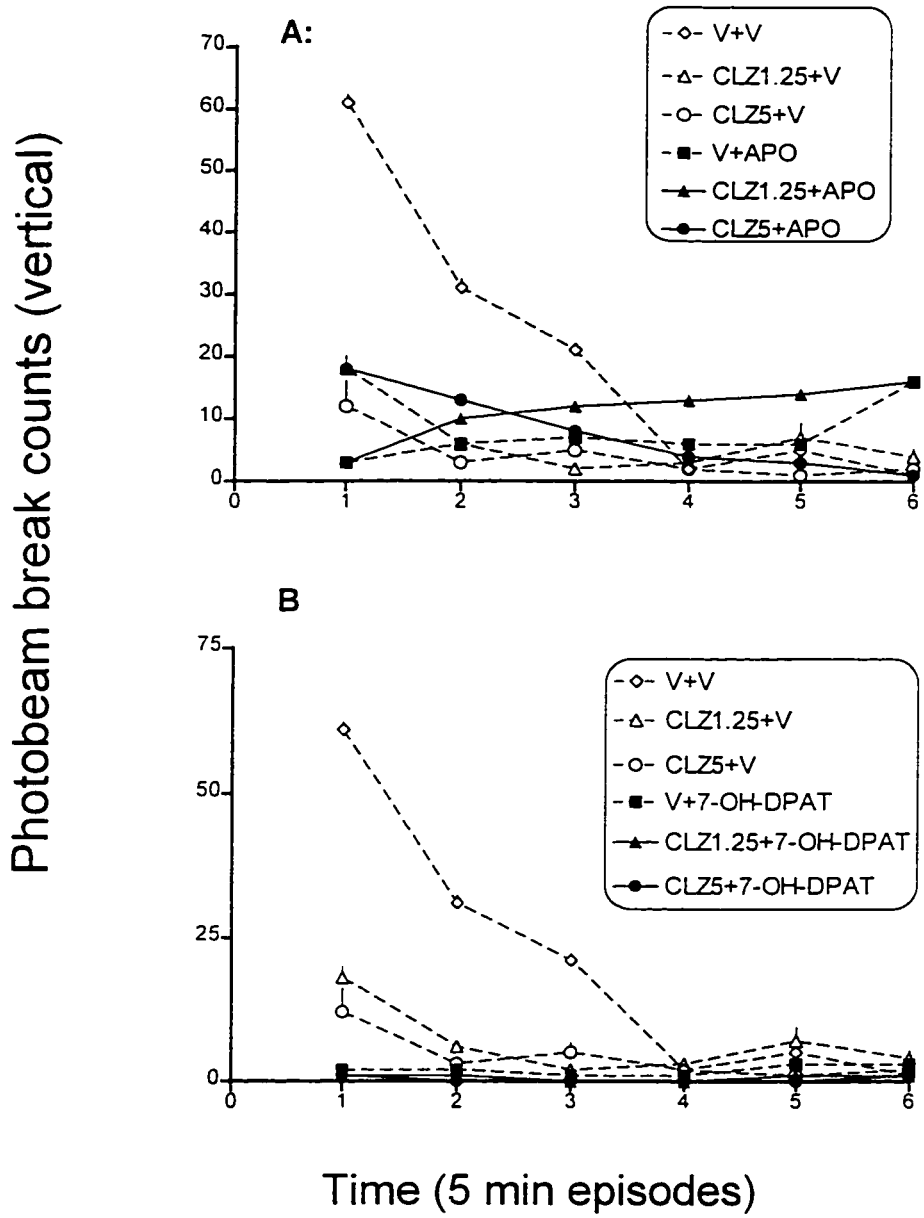

Figure 38: The effects of CLZ (1.25 or 5 mg/kg, sc) on time-course of vertical activity for 30 min in A: APO (0.5 mg/kg, sc)- or B: 7-OH-DPAT (5 mg/kg, sc)-treated rats. |: SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

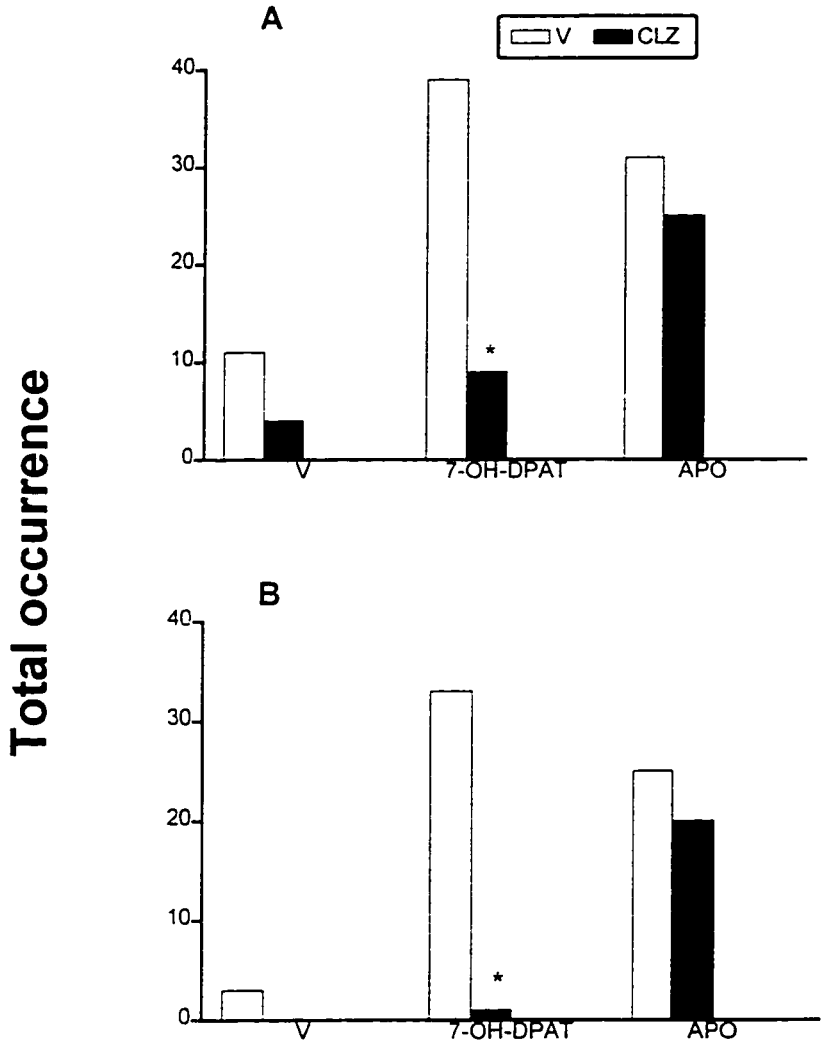

Figure 39: The effects of CLZ (5 mg/kg, sc) on A: forward walking and B: sniffing behaviors induced by APO (0.5 mg/kg, sc) or 7-OH-DPAT (5 mg/kg, sc). *: Significant effect of CLZ on 7-OH-DPAT, $P<0.05$, $n=8$. V= vehicle.

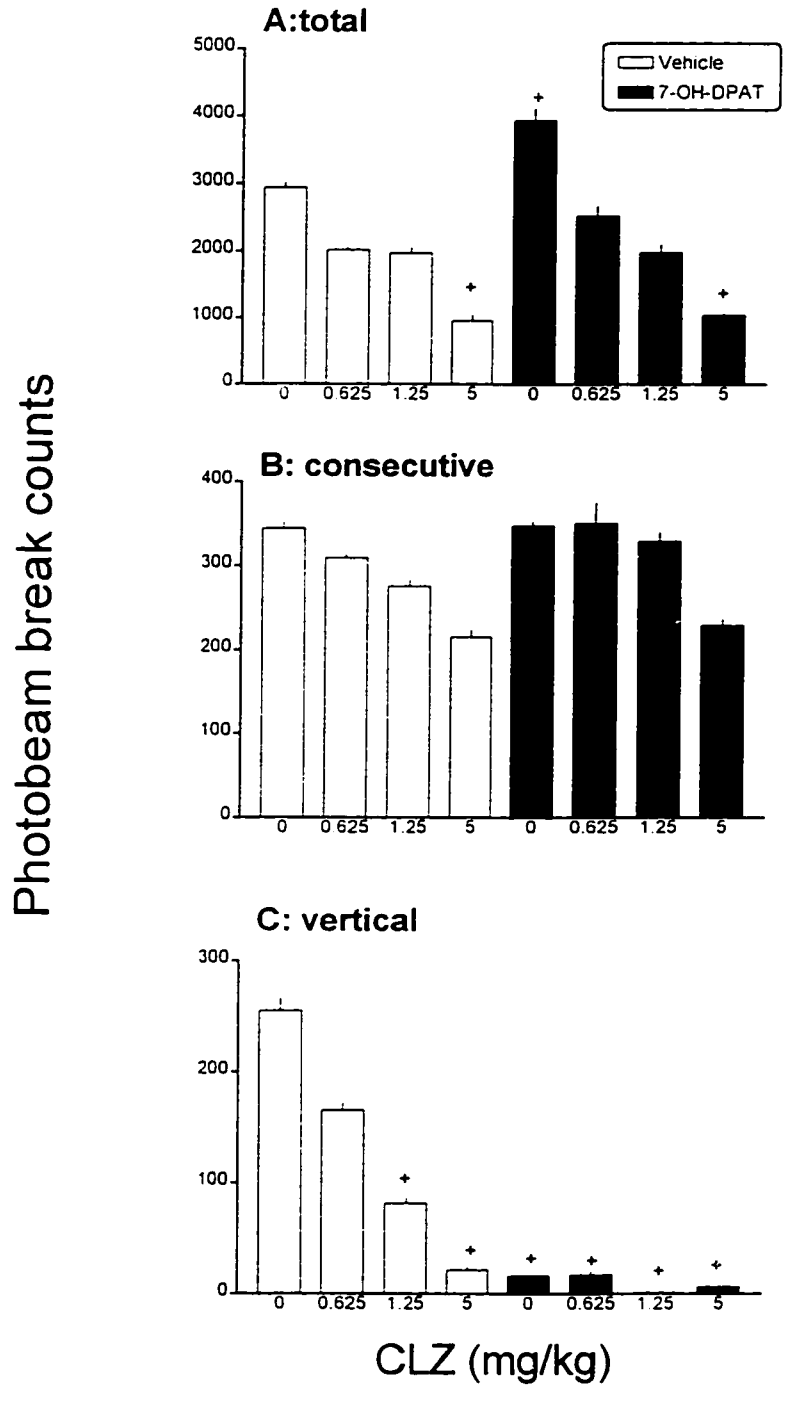

Figure 40: The effects of CLZ (0.625, 1.25 or 5 mg/kg, sc) on locomotor activity for 30 min in vehicle- or 7-OH-DPAT (2.5 mg/kg, sc)-treated rats. |: SEM for each mean. +: significant vs vehicle, $P<0.05$, n=8.

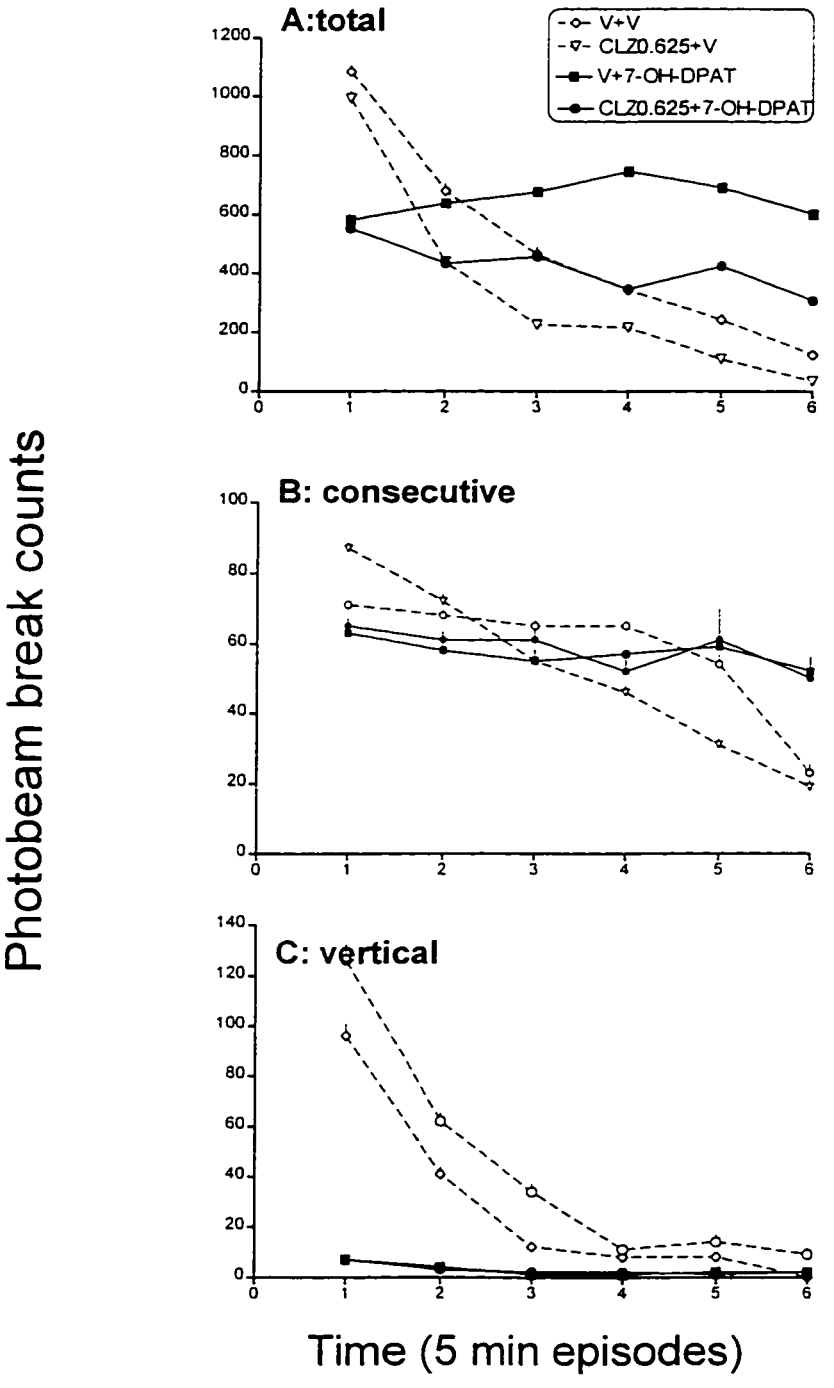

Figure 41: The effects of CLZ (0.625 mg/kg, sc) on time-course of locomotor activity for 30 min in vehicle- or 7-OH-DPAT (2.5 mg/kg, sc)-treated rats. |: SEM for each mean. Effect of CLZ on 7-OH-DPAT is significant at times 4-6 in panel A and at times 1-2 in panel B, $P<0.05$, n=8. V= vehicle.

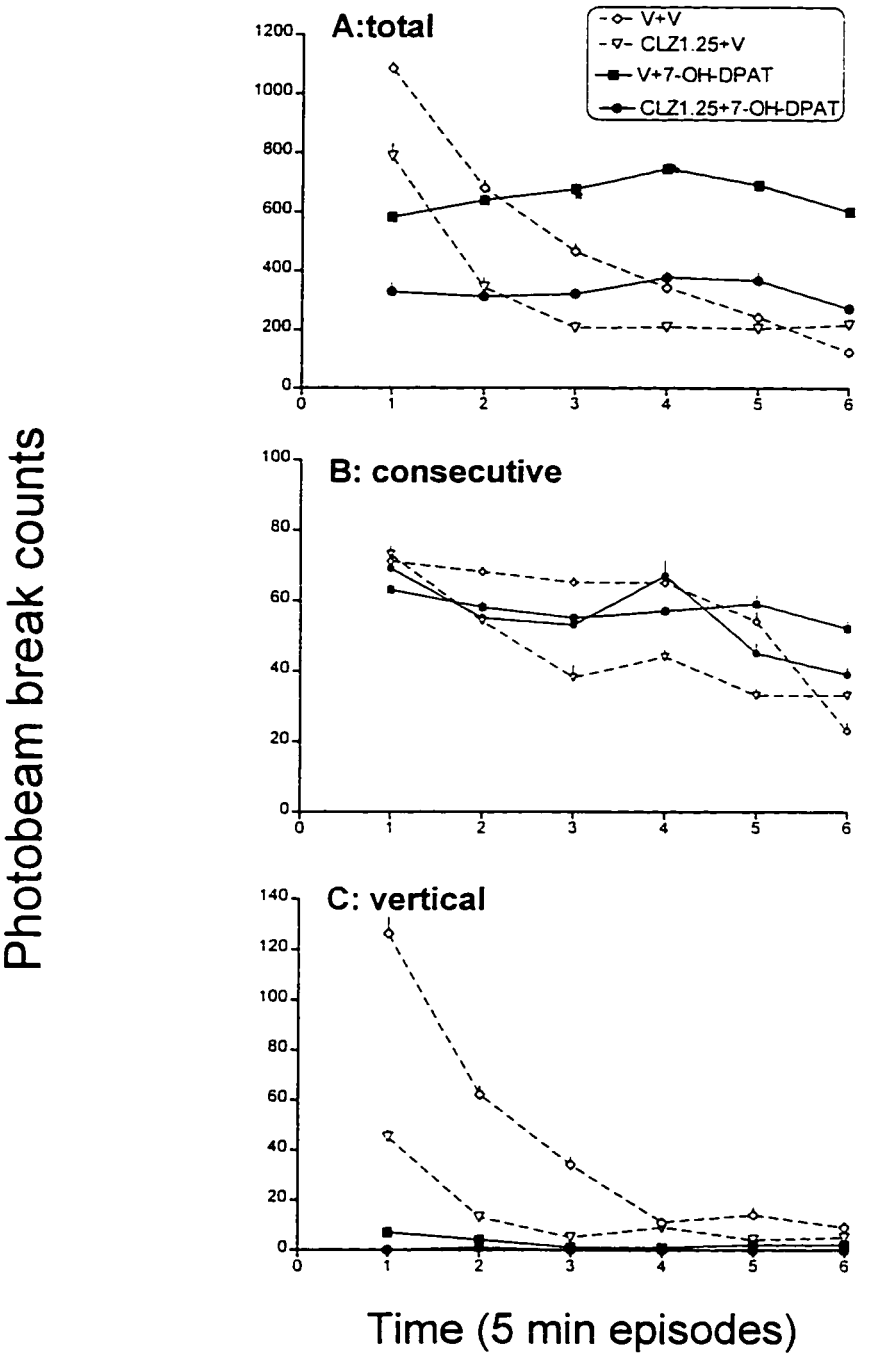

Figure 42: The effects of CLZ (1.25 mg/kg, sc) on time-course of locomotor activity for 30 min in vehicle- or 7-OH-DPAT (2.5 mg/kg, sc)-treated rats. |: SEM for each mean. Effect of CLZ on 7-OH-DPAT is significant at times 4-6 in panel A and at times 1-2 in panel B, $P<0.05$, n=8. V= vehicle.

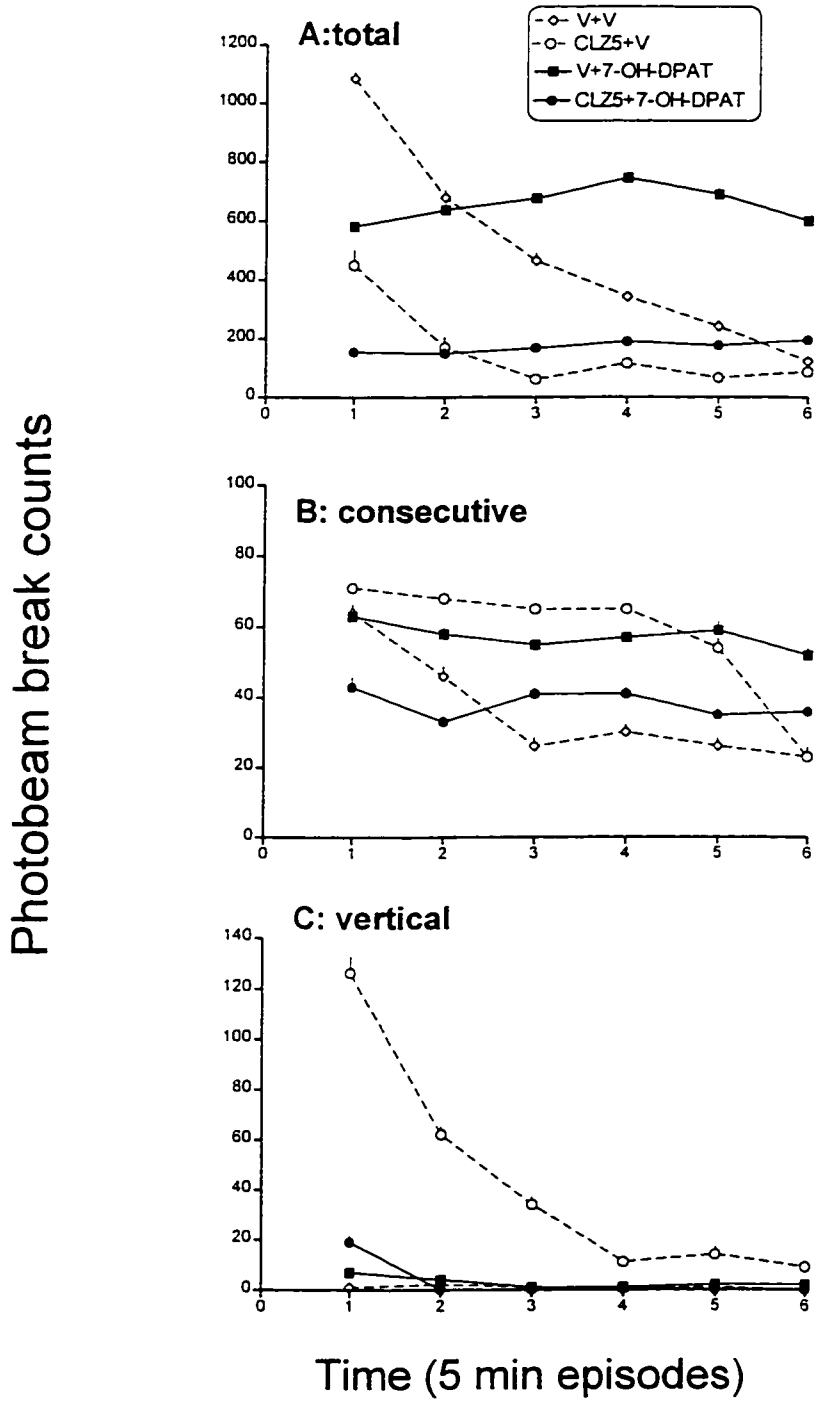

Figure 43: The effects of CLZ (5 mg/kg, sc) on time-course of locomotor activity for 30 min in vehicle- or 7-OH-DPAT (2.5 mg/kg, sc)-treated rats. |: SEM for each mean. Effect of CLZ on 7-OH-DPAT is significant at times 4-6 in panel A. and at times 1-2 in panel B, $P<0.05$, n=8. V= vehicle.

3.13. The interaction of SCH 23390 with high dose of 7-OH-DPAT or APO

In the present experiment the effects of SCH 23390, a DA D1 antagonist, on the actions of 7-OH-DPAT (a DA D3 receptor agonist) and APO (a prototypical DA D1/D2 agonist) were investigated. SCH 23390 displays a high affinity for DA D1 and a low affinity for DA D2 receptors (Iorio et al., 1983). However, it shows similar properties as DA D2 receptor antagonists. Thus, it blocks hyperactivity and stereotypic behavior induced by APO. An interaction between DA D1 and D2 receptors has been hypothesized to explain these effects of SCH 23390 (Pugh et al., 1985). A study on interactions of this drug with 7-OH-DPAT was, therefore, of interest. As Figure 44 shows, SCH 23390 decreased locomotor activity, APO and 7-OH-DPAT induced significant hyperactivity; the effect of SCH 23390 on 30 min measures of activity in rats treated with APO or 7-OH-DPAT was significant as shown by two-way ANOVA followed by Newman-Keuls tests [SCH: $F(1, 82) = 118.63$; APO/7-OH-DPAT: $F(2, 82) = 22.66$; SCH x APO/7-OH-DPAT: $F(1, 82) = 12.87$, $P<0.05$]. However, some differential effects of SCH 23390 on APO and 7-OH-DPAT were observed on the time-course of changes in activity [total, Time: $F(5, 410) = 113.93$, $P<0.05$; Time x SCH: $F(5, 410) = 0.17$, $P>0.05$; Time x APO/7-OH-DPAT: $F(10, 410) = 21.11$; Time x SCH x APO/7-OH-DPAT: $F(10, 410) = 5.21$, $P<0.05$]; [consecutive, Time: $F(5, 410) = 34.31$, $P<0.05$; Time x SCH: $F(5, 410) = 8.181$, $P<0.05$; Time x APO/7-OH-DPAT: $F(10, 410) = 11.11$; Time x SCH x APO/7-OH-DPAT: $F(10, 410) = 6.95$, $P<0.05$]; [vertical, Time: $F(5, 410) = 29.62$, $P<0.05$; Time x SCH: $F(5, 410) = 2.74$, $P>0.05$; Time x APO/7-OH-DPAT: $F(10, 410) = 32.69$; Time x SCH x APO/7-OH-DPAT: $F(10, 410) = 4.63$,

P<0.05]. SCH 23390 returned the pattern of constant activity induced by APO (Figures 45A and 46A), but not by 7-OH-DPAT (Figures 45B and 46B), to a descending pattern of activity, a pattern that is seen in control rats (habituation to the locomotor activity box during a 30 min test session, see vehicle group in Figure 45). These results may indicate that SCH 23390 blocks stereotyped behaviors induced by APO not by 7-OH-DPAT while decreasing hyperactivity induced by both APO and 7-OH-DPAT. All treatments, i.e. SCH 23390, APO, 7-OH-DPAT and their combination decreased vertical activity (Figure 47).

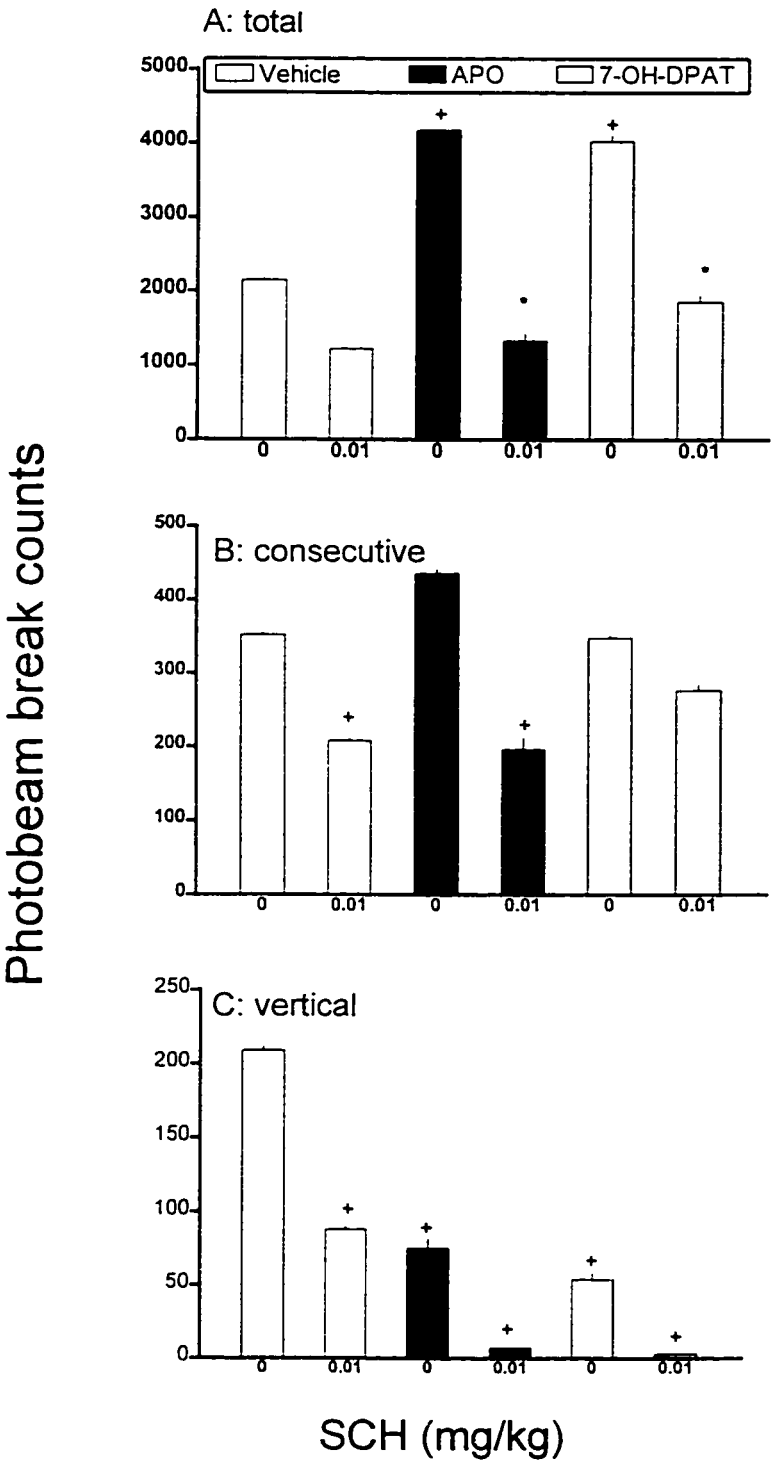

Figure 44: The effects of SCH 23390 (SCH), 0.01 mg/kg, sc on locomotor activity for 30 min in vehicle-, APO- or 7-OH-DPAT- treated rats. | : SEM for each mean. +: significant vs vehicle, *: significant drug interaction, $P<0.05$, n=8.

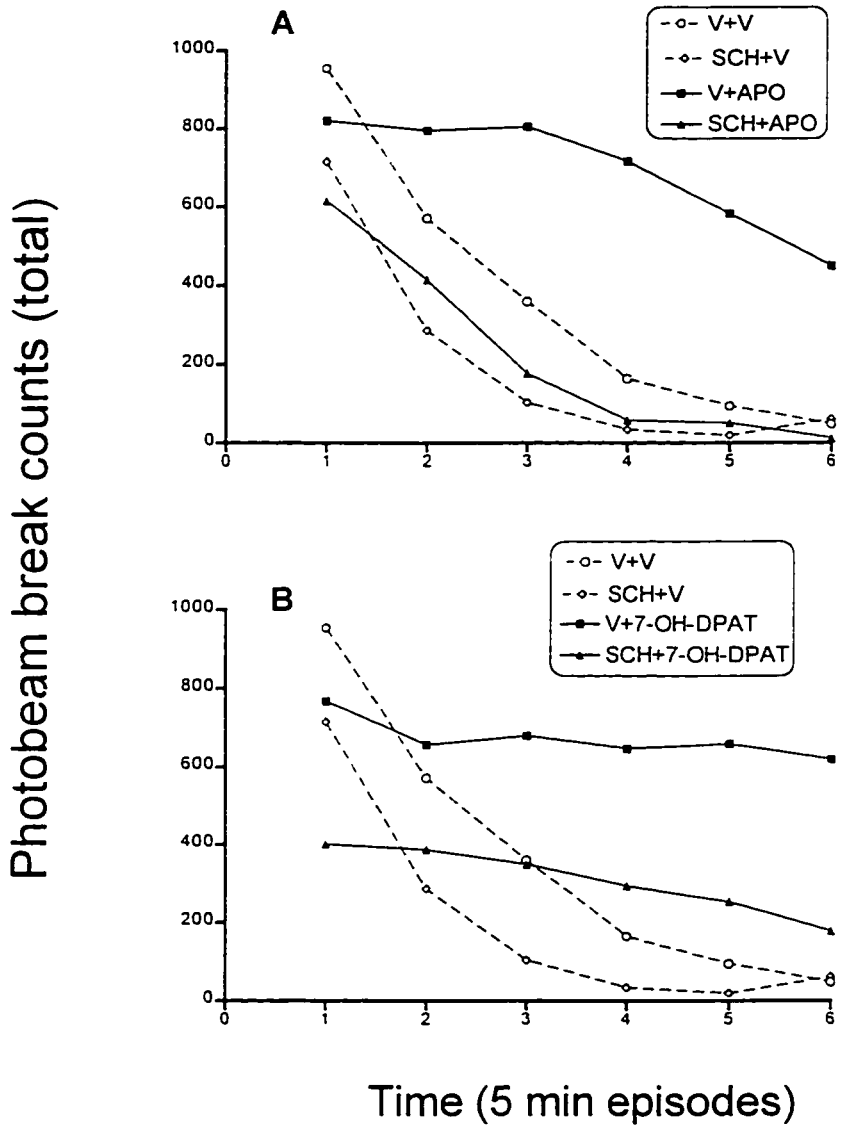

Figure 45: The effects of SCH 23390 (SCH), 0.01 mg/kg, sc on time-course of total activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)- treated rats. | : SEM for each mean. Effect of SCH on APO and 7-OH-DPAT was significant at times 3-6, $P<0.05$, n=8. V= vehicle.

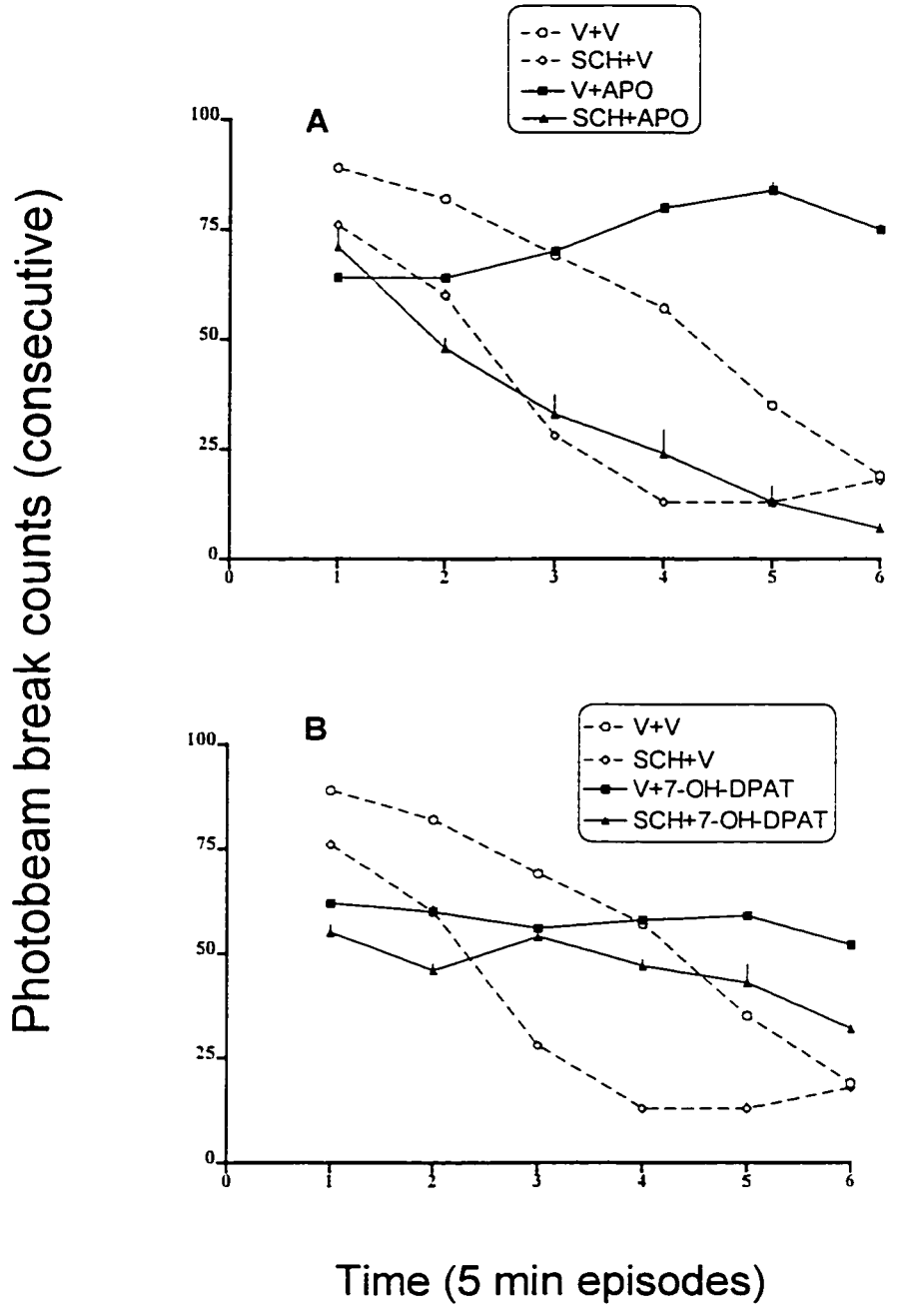

Figure 46: The effects of SCH 23390 (SCH), 0.01 mg/kg, sc on time-course of consecutive activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. | : SEM for each mean. Effect of SCH on APO was significant at times 3-6, $P<0.05$, n=8. V= vehicle.

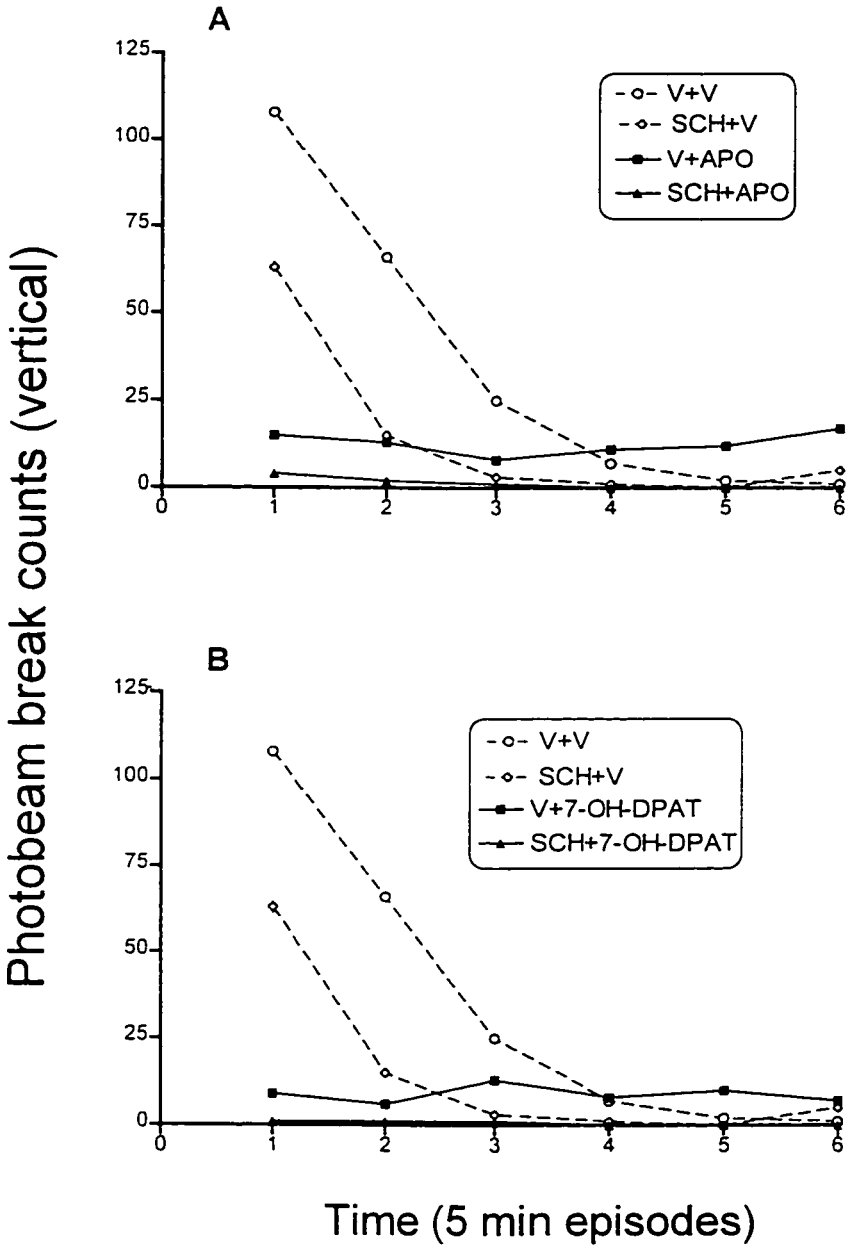

Figure 47: The effects of SCH 23390 (SCH), 0.01 mg/kg, sc on time-course of vertical activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. | : SEM for each mean, n=8. V= vehicle.

3.14. The interaction of HAL with high dose 7-OH-DPAT or APO

HAL is a prototypical DA D2 antagonist. We were interested in investigating the interaction of HAL with 7-OH-DPAT (a DA D3 receptor agonist) and APO (a prototypical DA D1/D2 receptor) agonist. HAL, 0.03 mg/kg sc, did not change locomotor activity significantly, but both APO and 7-OH-DPAT increased activity as shown by one-way ANOVA followed by the Newman-Keuls tests [total, HAL: $F(2, 103) = 4.52$; APO/7-OH-DPAT: $F(2, 103) = 49.77$, $P<0.05$; HAL x APO/7-OH-DPAT: $F(4, 103) = 0.40$, $P>0.05$; consecutive, HAL: $F(2, 103) = 2.44$; APO/7-OH-DPAT: $F(2, 103) = 6.45$, $P<0.05$; HAL x APO/7-OH-DPAT: $F(4, 103) = 0.32$, $P>0.05$; vertical, HAL: $F(2, 103) = 3.59$; APO/7-OH-DPAT: $F(2, 103) = 19.69$, $P<0.05$; HAL x APO/7-OH-DPAT: $F(4, 103) = 2.05$, $P>0.05$], see Figure 48. Although HAL did not change total activity for 30 min in APO- or 7-OH-DPAT-treated rats (Figures 48 and 52), the time-course analysis of activity shows that HAL returned the pattern of the constant activity induced by APO (Figures 49A and 53A) but not by 7-OH-DPAT (Figures 49B and 53B) to a descending pattern of locomotor activity (see page 109). ANOVA on the time-course data showed a significant effect of HAL [total, Time: $F(5, 515) = 120.62$; Time x HAL: $F(10, 515) = 3.77$; Time x APO/7-OH-DPAT: $F(10, 515) = 21.61$; Time x HAL x APO/7-OH-DPAT: $F(20, 515) = 5.75$, $P<0.05$]; [consecutive, Time: $F(5, 515) = 26.18$, $P<0.05$; Time x HAL: $F(10, 515) = 1.19$, $P>0.05$; Time x APO/7-OH-DPAT: $F(10, 515) = 12.05$; Time x HAL x APO/7-OH-DPAT: $F(20, 515) = 2.05$, $P<0.05$]; [vertical, Time: $F(5, 515) = 33.07$, $P<0.05$; Time x HAL: $F(10, 515) = 1.75$, $P>0.05$; Time x APO/7-OH-DPAT: $F(10, 515) = 30.82$; Time x HAL x APO/7-OH-DPAT: $F(20, 515) =$

2.34, $P<0.05$]. *Post hoc* analysis showed that the effects of HAL on APO were significant at the second, third, fifth and sixth episodes of time (Figures 49 and 53).

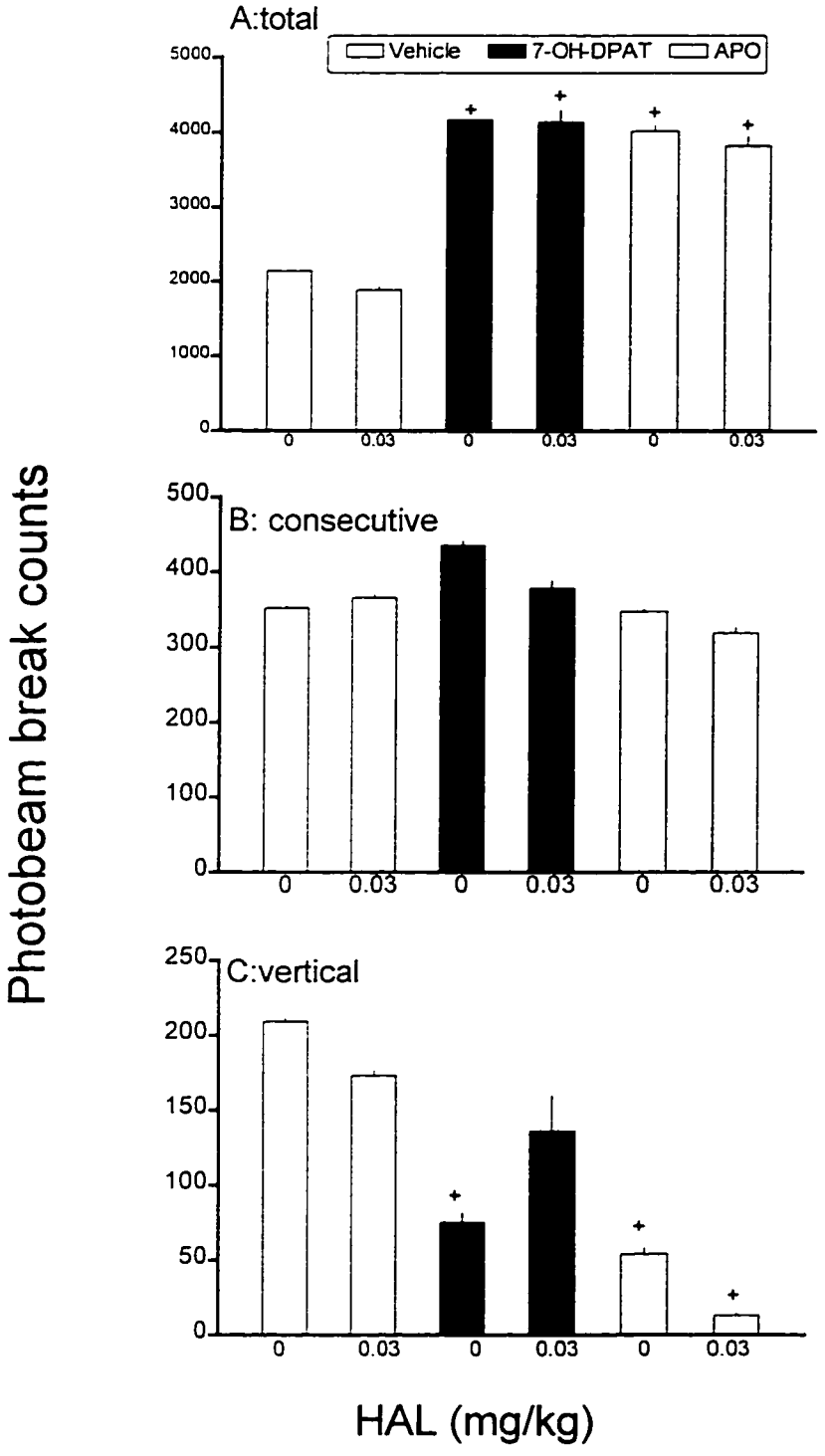

Figure 48: The effects of haloperidol (HAL), 0.03 mg/kg, sc on locomotor activity for 30 min in vehicle-, APO (0.5 mg/kg, sc)- or 7-OH-DPAT (2.5 mg/kg, sc)-treated rats. | : SEM for each mean. +: significant vs vehicle, $P<0.05$, n=8.

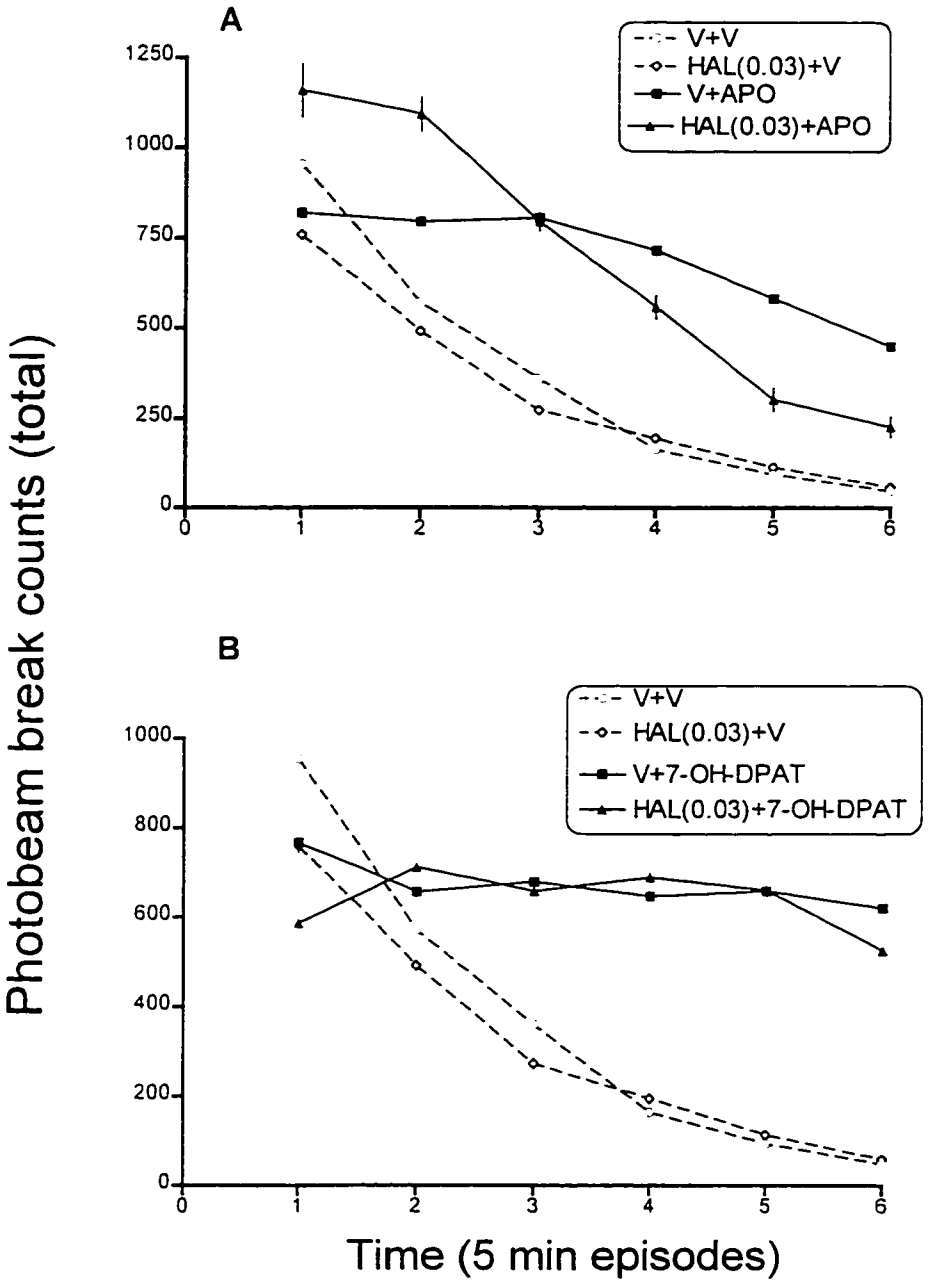

Figure 49: The effects of haloperidol (HAL), 0.03 mg/kg, sc on time-course of total activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. | : SEM for each mean. Effect of HAL on APO was significant at times 1, 5 and 6, $P<0.05$, n=8. V= vehicle.

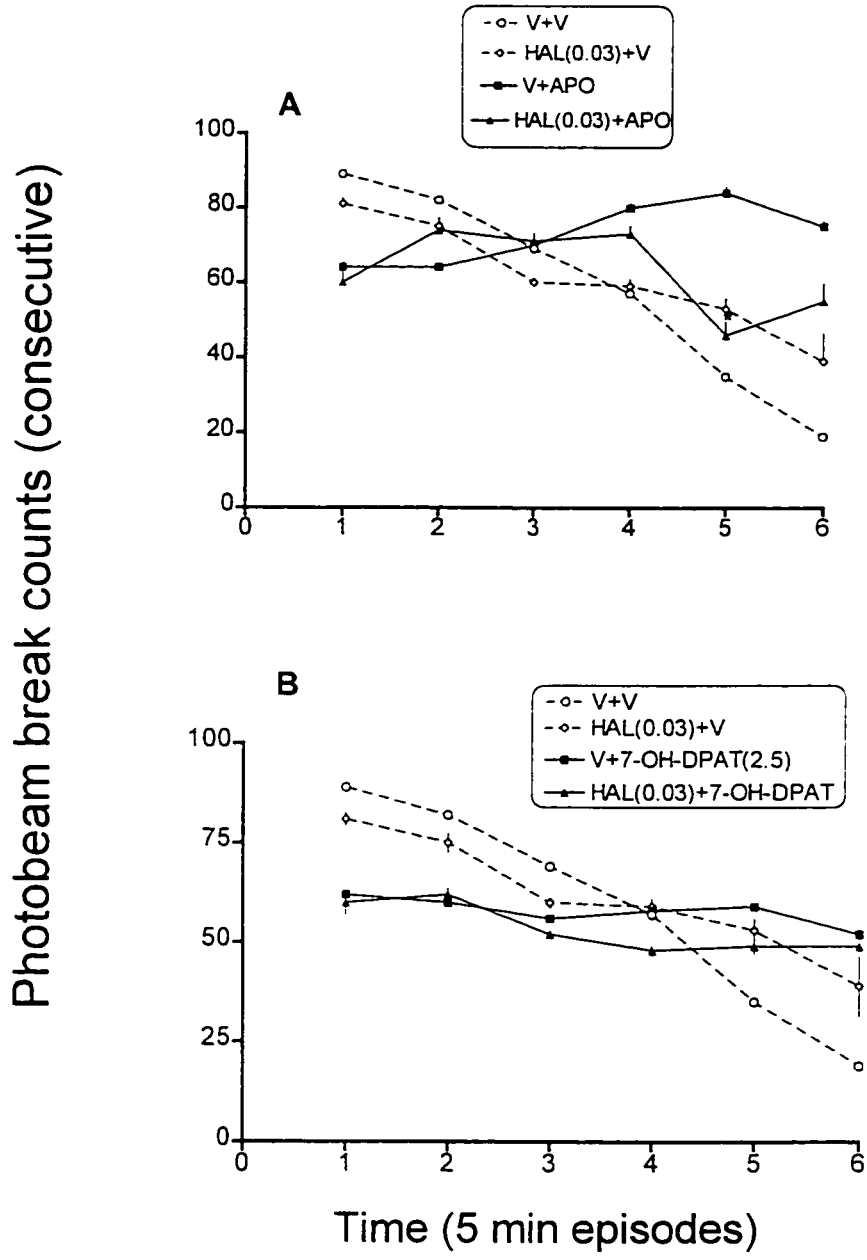

Figure 50: The effects of haloperidol (HAL), 0.03 mg/kg, sc on time-course of consecutive activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. | : SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

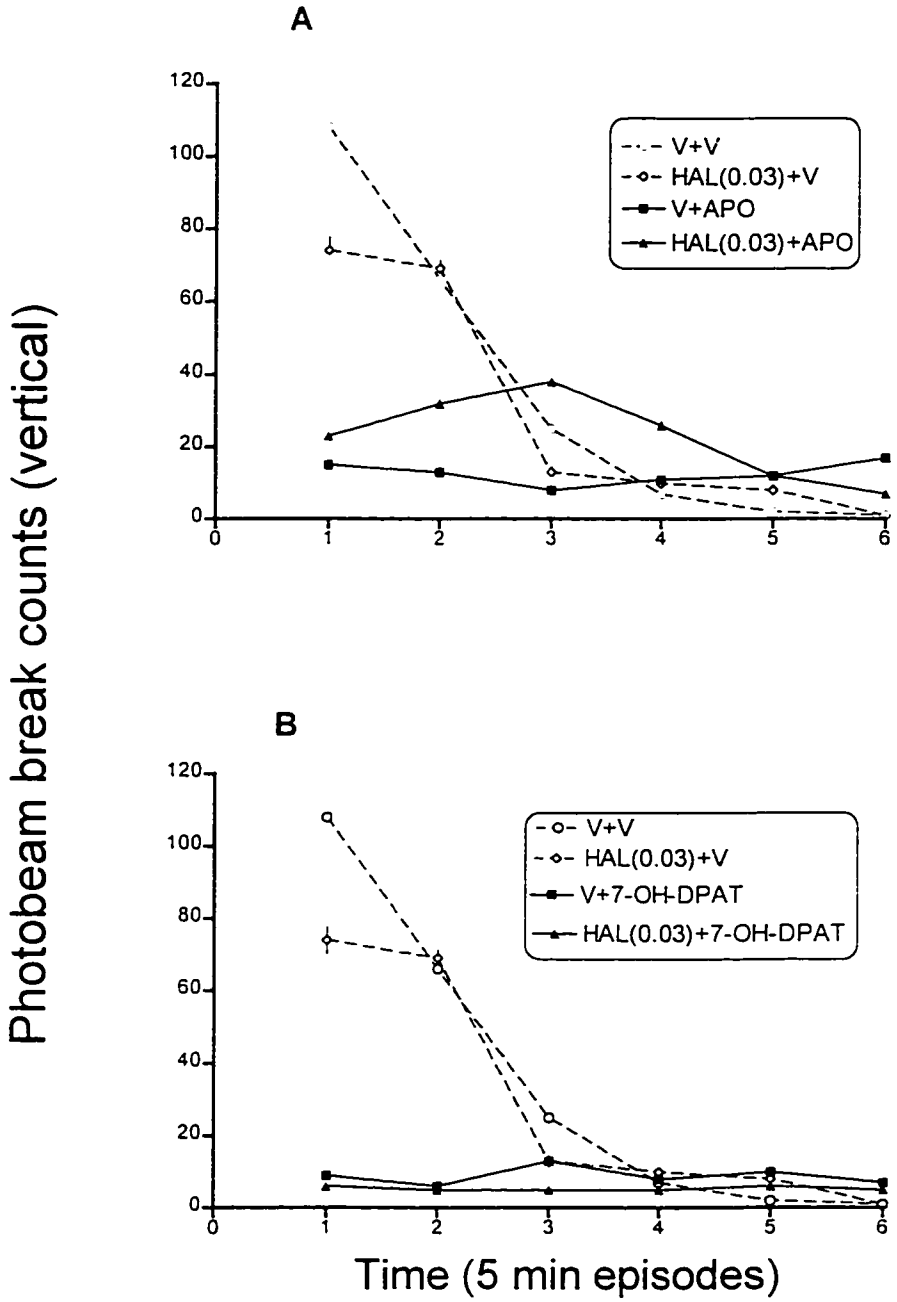

Figure 51: The effects of haloperidol (HAL), 0.03 mg/kg, sc on time-course of vertical activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. | : SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

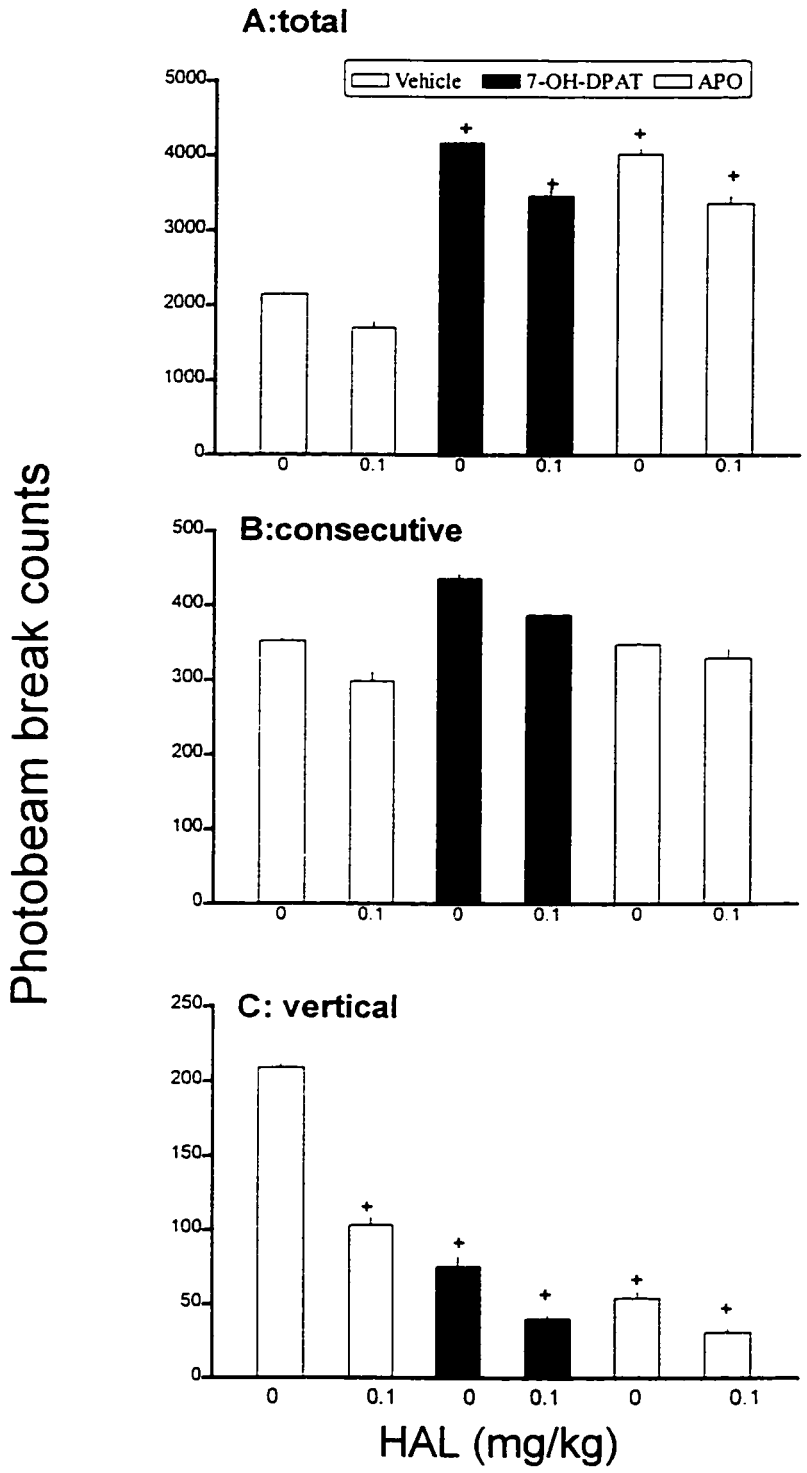

Figure 52: The effects of haloperidol (HAL), 0.1 mg/kg, sc on locomotor activity for 30 min in vehicle-, APO (0.5 mg/kg, sc)- or 7-OH-DPAT (2.5 mg/kg, sc)-treated rats. | : SEM for each mean. +: significant vs vehicle, $P<0.05$, n=8.

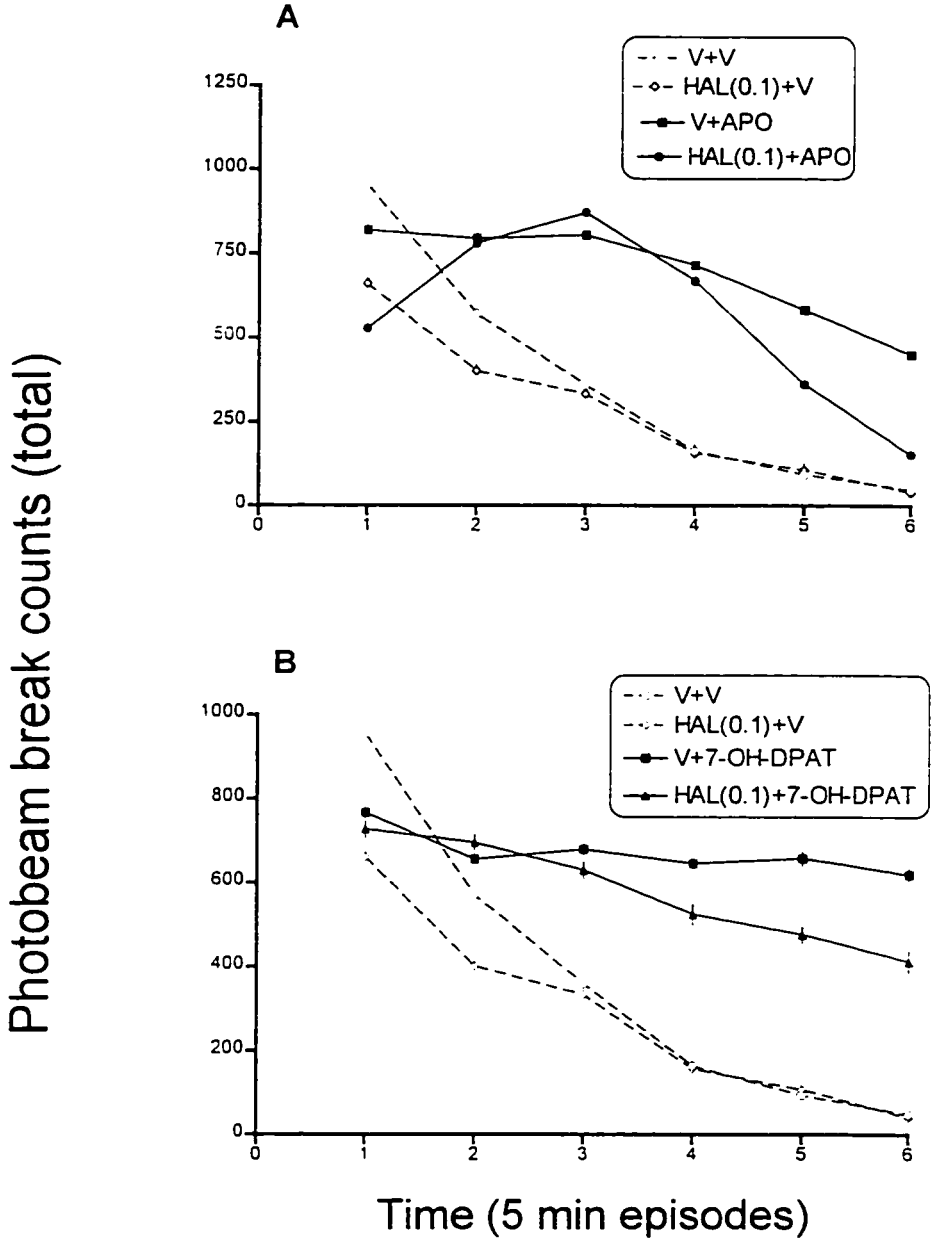

Figure 53: The effects of haloperidol (HAL), 0.1 mg/kg, sc on time-course of total activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. |: SEM for each mean. Effect of HAL on APO and 7-OH-DPAT was significant at times 1, 5 and 6, $P<0.05$, n=8. V= vehicle.

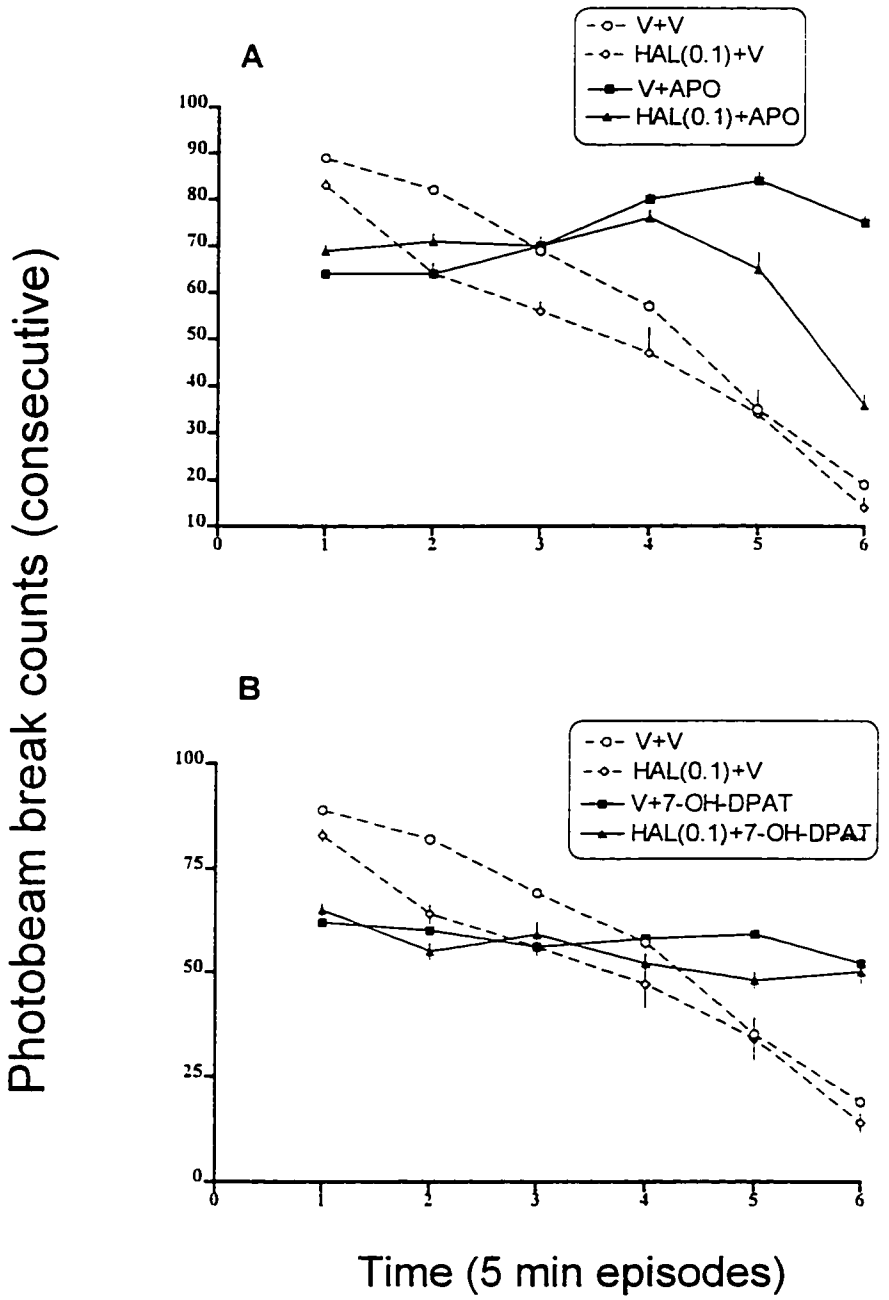

Figure 54: The effects of haloperidol (HAL), 0.1 mg/kg, sc on time-course of consecutive activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. |: SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

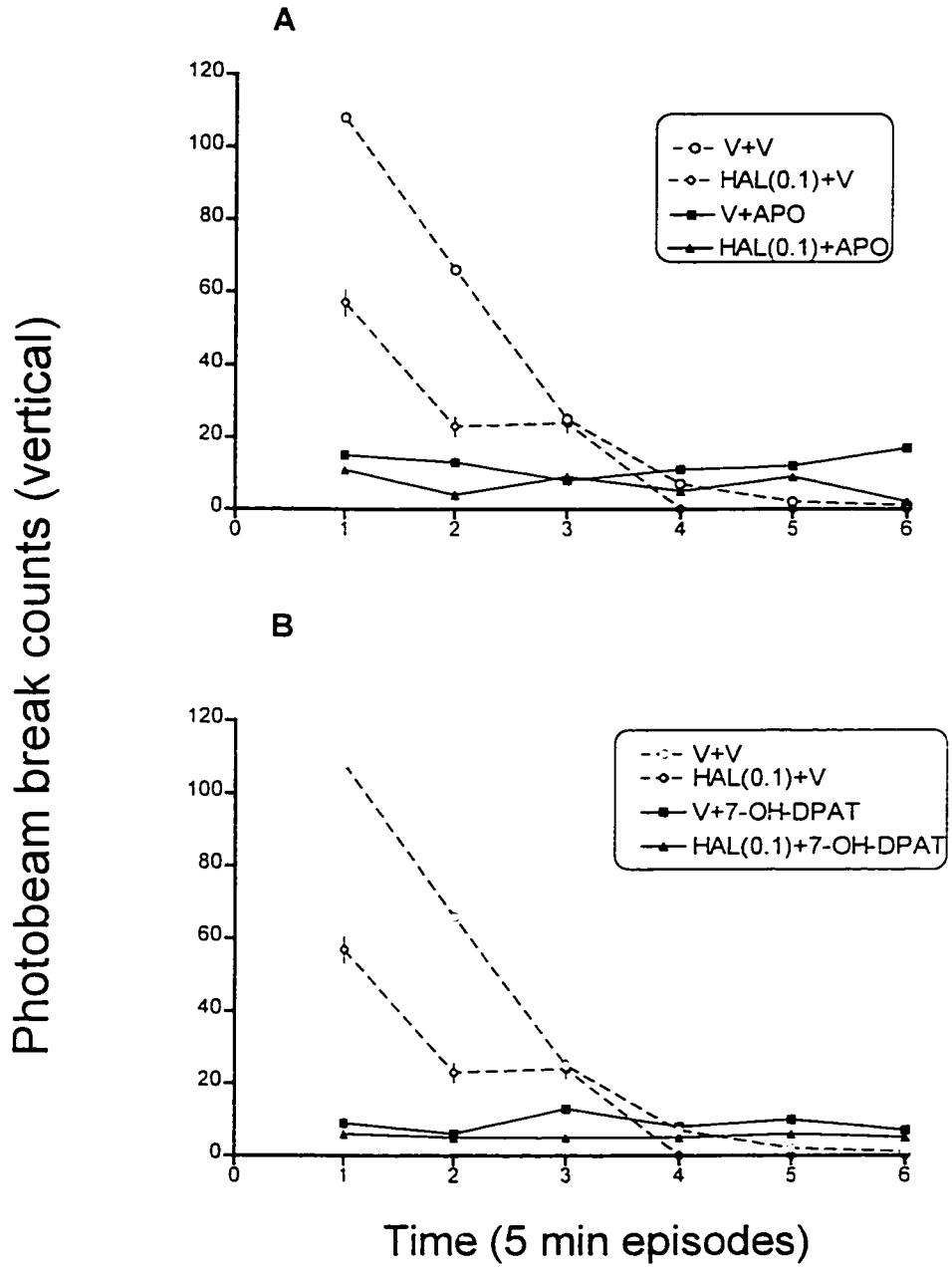

Figure 55: The effects of haloperidol (HAL), 0.1 mg/kg, sc on time-course of consecutive activity for 30 min in A: APO (0.5 mg/kg)- or B: 7-OH-DPAT (2.5 mg/kg)-treated rats. | : SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

3.15. The interaction of a low dose of 7-OH-DPAT or QUIN with a high dose of APO in DA-intact rats

Recently it has been hypothesized that the effects of low doses of 7-OH-DPAT are relevant to the inhibitory postsynaptic action of DA D3 receptors (Waters et al., 1994). In the present experiment it was proposed that this postsynaptic effect of 7-OH-DPAT may be able to decrease the stimulatory effects of APO at postsynaptic DA D2 receptors on locomotor activity. As figure 56 shows, low doses of both 7-OH-DPAT and QUIN decreased locomotor activity and APO induced hyperactivity significantly; neither 7-OH-DPAT nor QUIN decreased the effects of the high dose of APO, 0.5 mg/kg (Figure 56). Also no interaction on the time-course of activities except total activity was observed as shown by 3-way ANOVA followed by a Newman-Keuls test [total, QUIN/7-OH-DPAT: $F(2, 42) = 0.2$, $P<0.05$; APO: $F(1, 42) = 60.39$, $P<0.05$; QUIN/7-OH-DPAT x APO: $F(2, 42) = 2.78$, $P>0.05$; TIME: $F(5, 210) = 3.76$, $P<0.05$; Time x APO: $F(5, 210) = 9.46$; Time x QUIN/7-OH-DPAT x APO: $F(10, 210) = 7.65$, $P<0.05$], see Figures 57 and 58. However, 7-OH-DPAT, but not QUIN, attenuated the stimulatory effects of a lower dose of APO (0.25 mg/kg) on total activity (Figures 59 and 60). The interaction between 7-OH-DPAT and APO, 0.25 mg/kg, was significant as shown by two-way ANOVA followed by Newman Keuls tests [total, QUIN/7-OH-DPAT: $F(2, 54) = 4.10$; APO: $F(2, 54) = 31.52$; QUIN/7-OH-DPAT x APO: $F(4, 54) = 3.64$, $P<0.05$], [consecutive, QUIN/7-OH-DPAT: $F(2, 54) = 3.29$; APO: $F(2, 54) = 15.14$; QUIN/7-OH-DPAT x APO: $F(4, 54) = 2.46$, $P<0.05$]; [vertical, QUIN/7-OH-DPAT: $F(2, 54) = 6.41$; APO: $F(2, 54) = 11.00$; QUIN/7-OH-DPAT x APO: $F(4, 54) = 8.13$, $P<0.05$]. APO, 0.125 mg/kg induced hypoactivity (Figure 63), and

neither QUIN nor 7-OH-DPAT changed the effects of this dose of APO significantly (Figures 63-65).

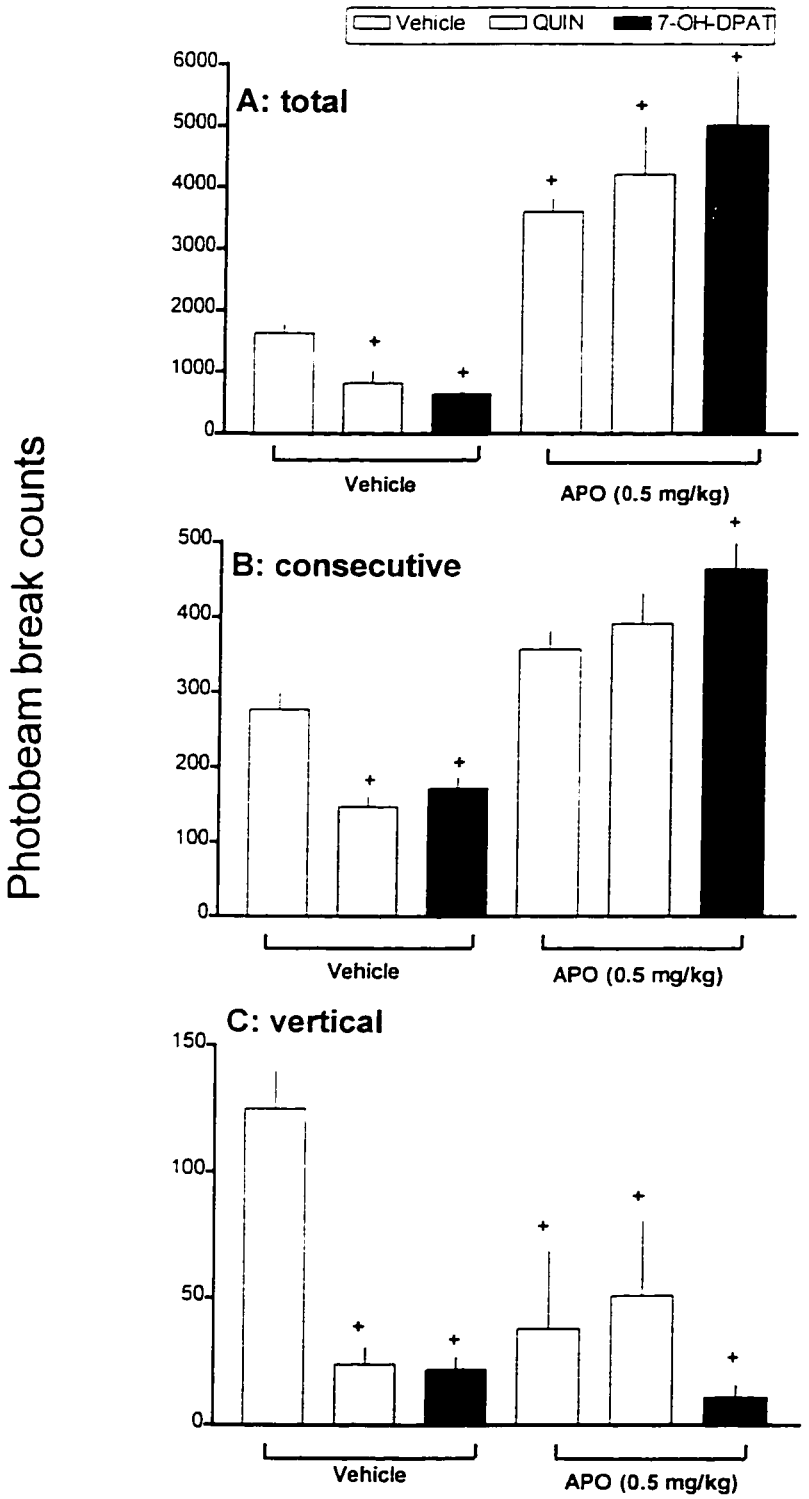

Figure 56: The effects of low doses of quinpirole (QUIN), 0.05 mg/kg, sc or 7-OH-DPAT (0.03 mg/kg, sc) on locomotor activity for 30 min in vehicle- or APO (0.5 mg/kg, sc)-treated rats. | : SEM for each mean. +: significant vs vehicle, $P<0.05$, n=8.

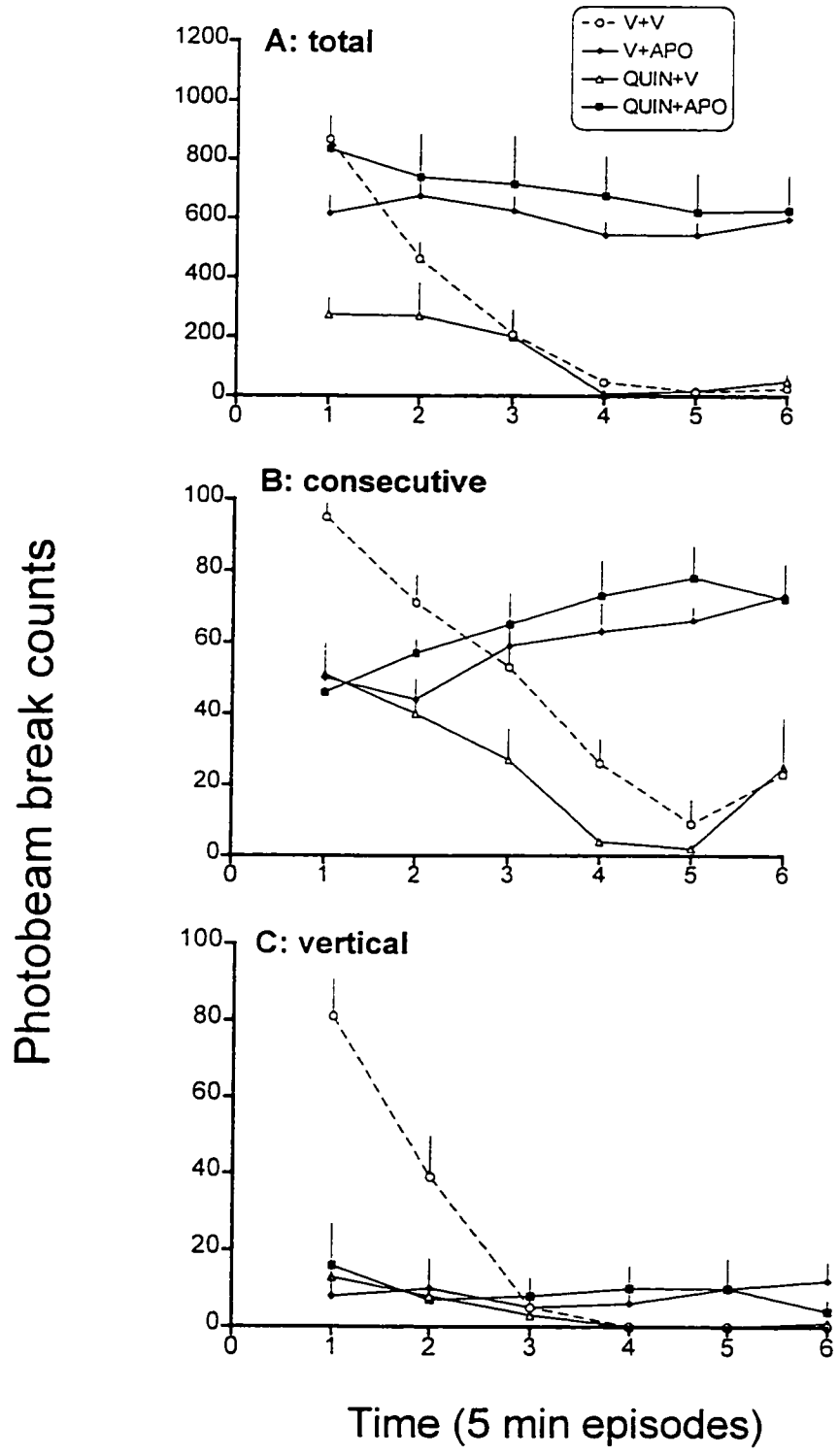

Figure 57: The effects of low doses of quinpirole (QUIN), 0.05 mg/kg, sc on time-course of locomotor activity for 30 min in vehicle- or APO (0.5 mg/kg, sc)-treated rats. | : SEM for each mean, n=8. V= vehicle.

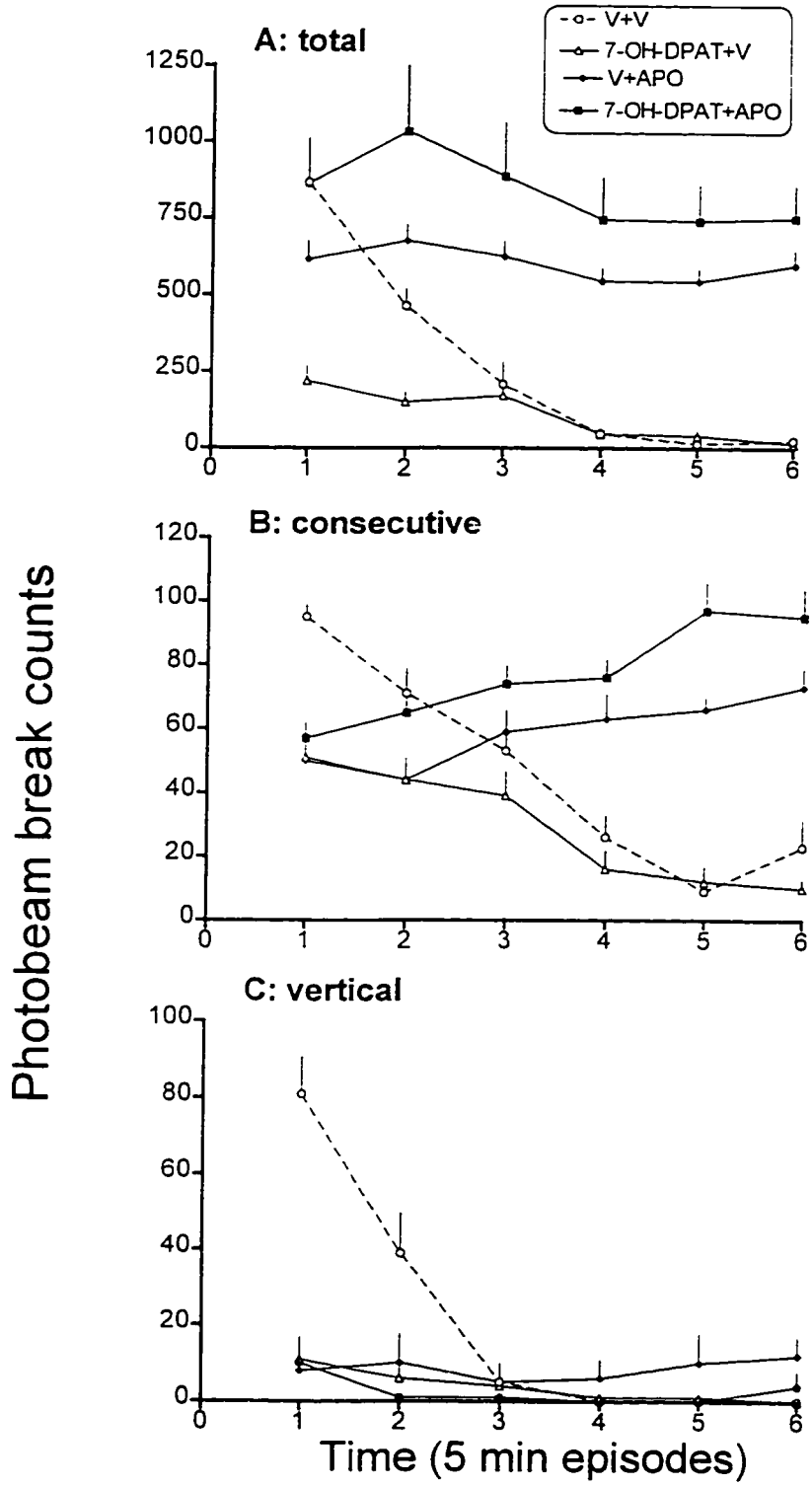

Figure 58: The effects of low dose of 7-OH-DPAT (0.03 mg/kg, sc) on time-course of locomotor activity for 30 min in vehicle- or APO (0.5 mg/kg, sc)- treated rats. | : SEM for each mean, n=8. V= vehicle.

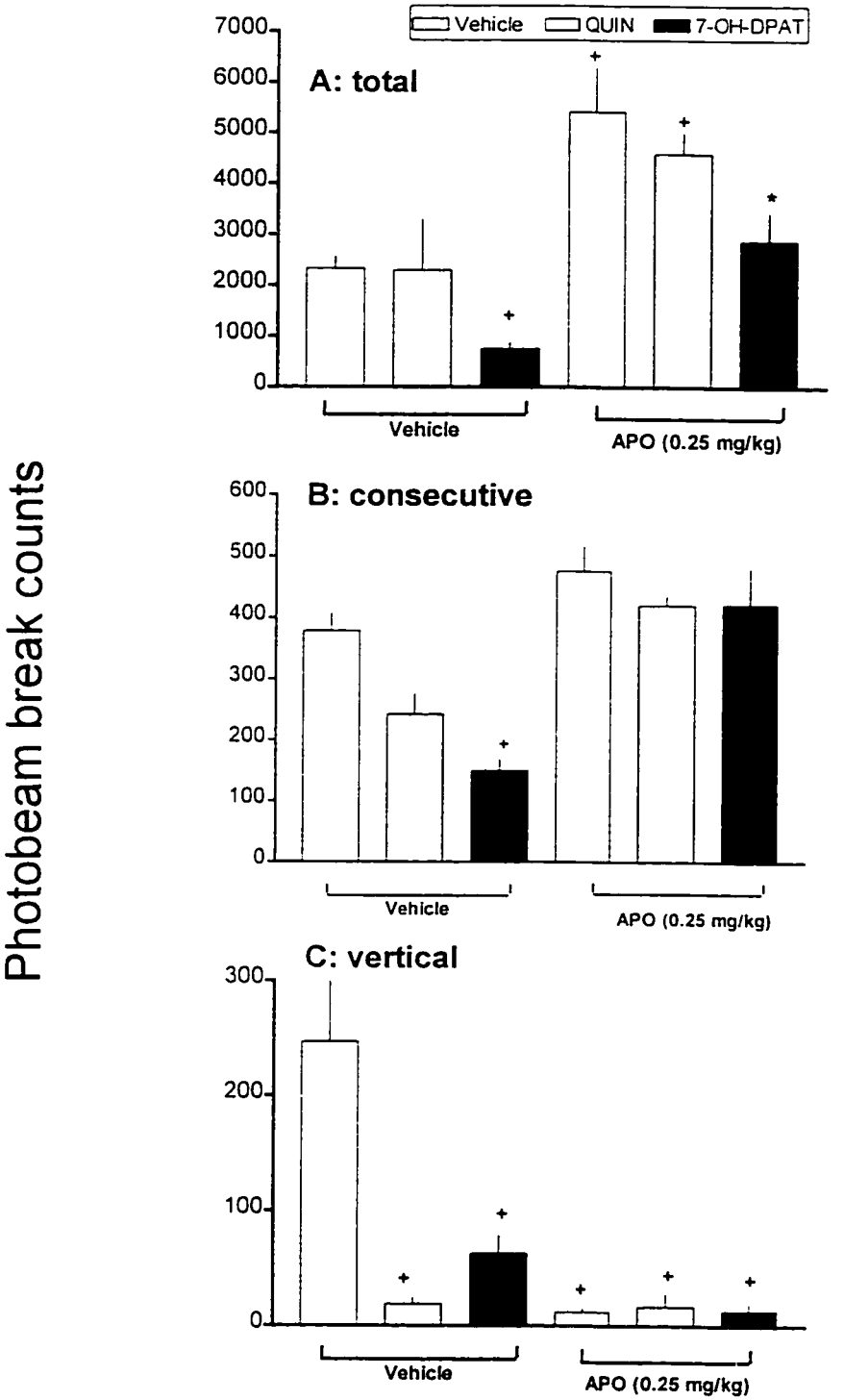

Figure 59: The effects of low doses of quinpirole (QUIN), 0.05 mg/kg, sc or 7-OH-DPAT (0.03 mg/kg, sc) on locomotor activity for 30 min in vehicle- or APO (0.25 mg/kg, sc)-treated rats. |: SEM for each mean. +: significant vs vehicle, *: significant effect of 7-OH-DPAT on APO, $P<0.05$, n=8.

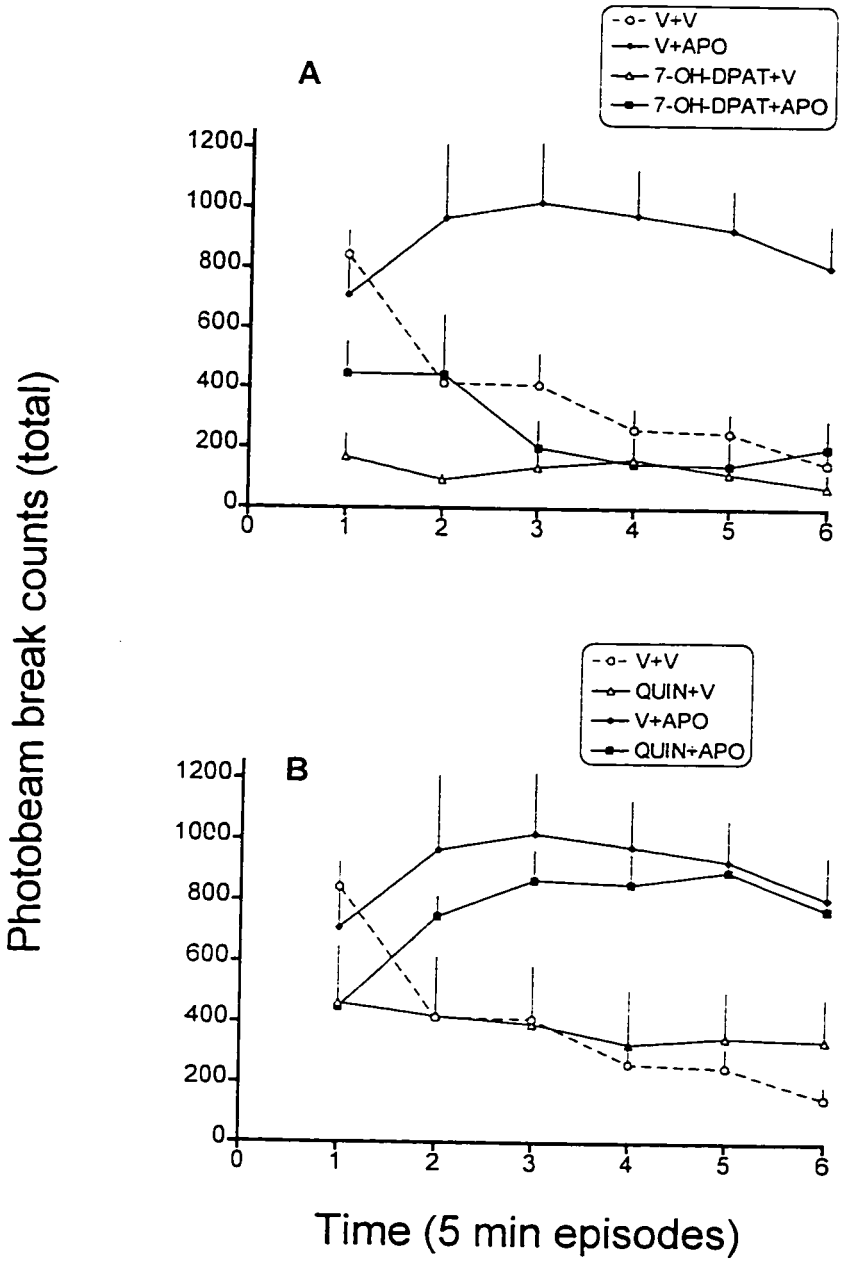

Figure 60: The effects of low dose of A: 7-OH-DPAT (0.03 mg/kg, sc) or B: quinpirole (QUIN), 0.05 mg/kg, sc on time-course of total activity for 30 min in vehicle- or APO (0.25 mg/kg, sc)-treated rats. |: SEM for each mean. Effect of 7-OH-DPAT on APO was significant at times 3-6, $P<0.05$, n=8. V= vehicle.

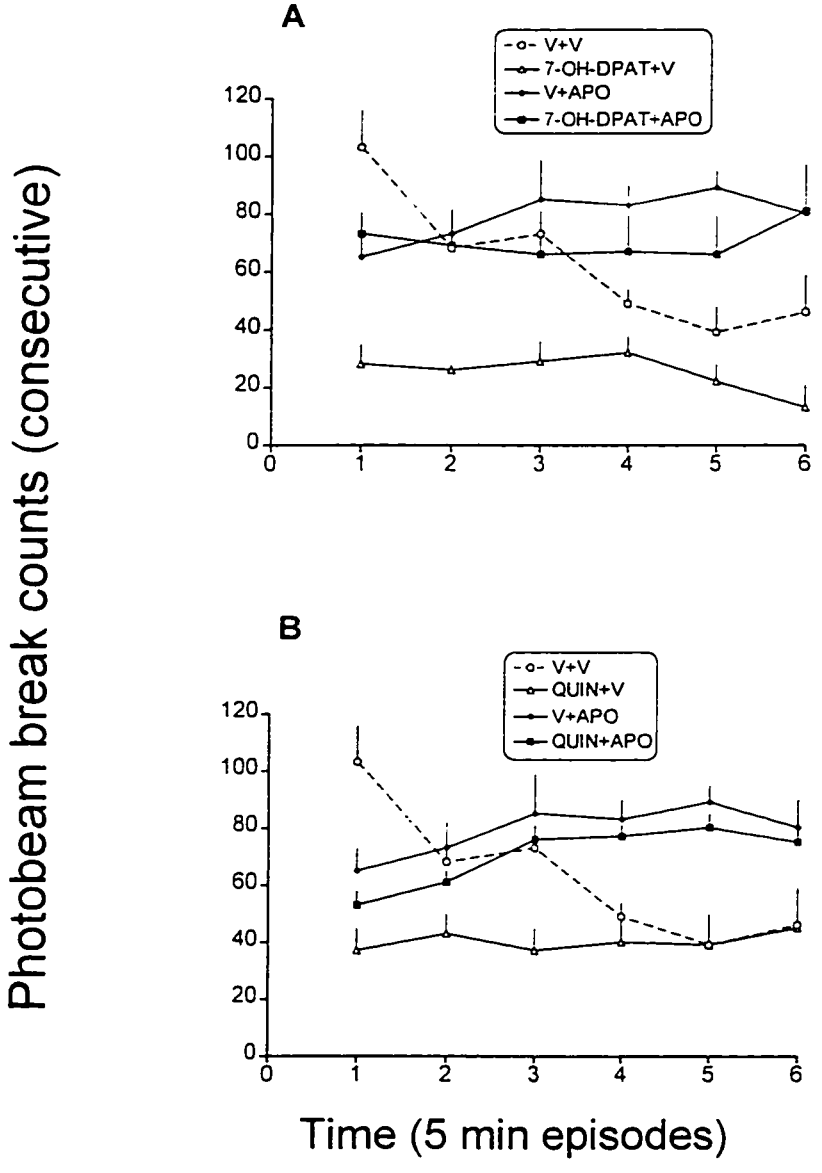

Figure 61: The effects of low dose of A: 7-OH-DPAT (0.03 mg/kg, sc) or B: quinpirole (QUIN), 0.05 mg/kg, sc on time-course of consecutive activity for 30 min in vehicle- or APO (0.25 mg/kg, sc)-treated rats. |: SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

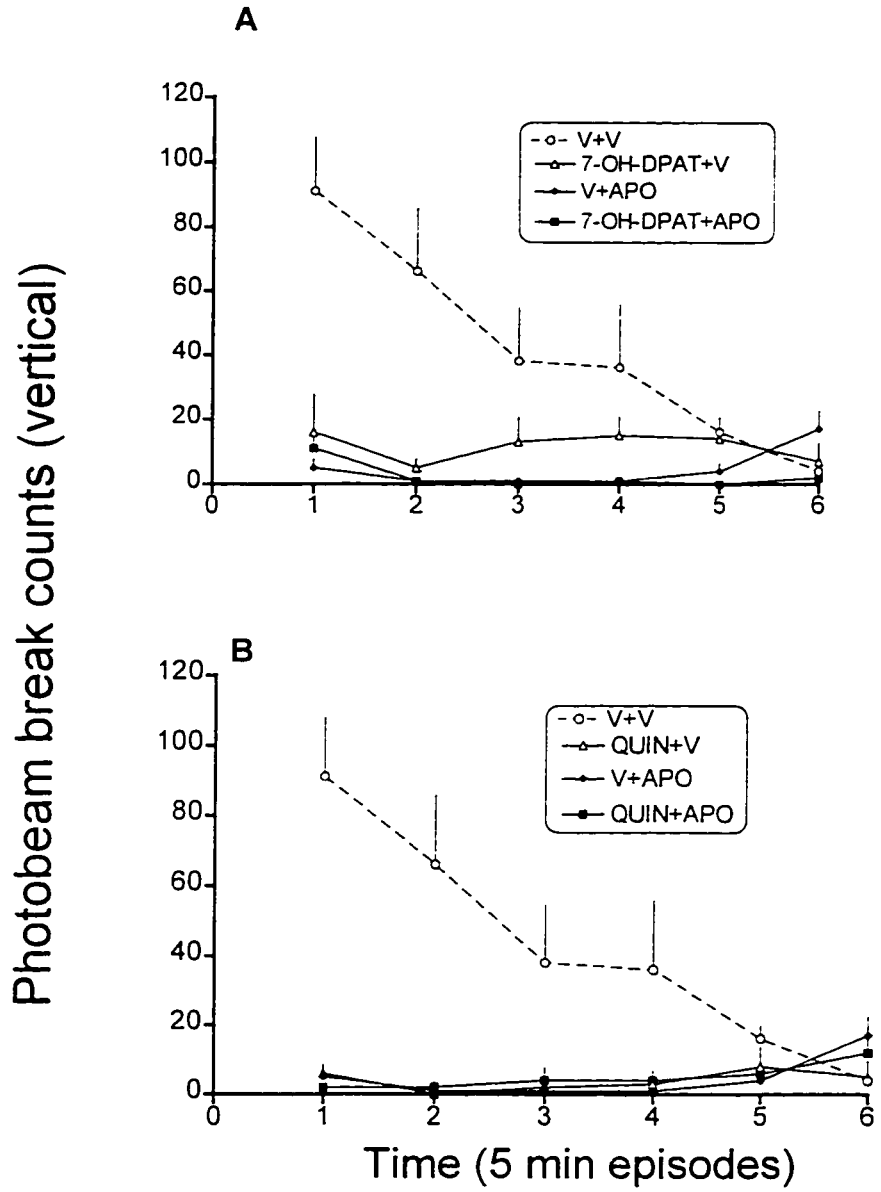

Figure 62: The effects of low dose of A: 7-OH-DPAT (0.03 mg/kg, sc) or B: quinpirole (QUIN), 0.05 mg/kg, sc on time-course of vertical activity for 30 min in vehicle- or APO (0.25 mg/kg, sc)-treated rats. | : SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

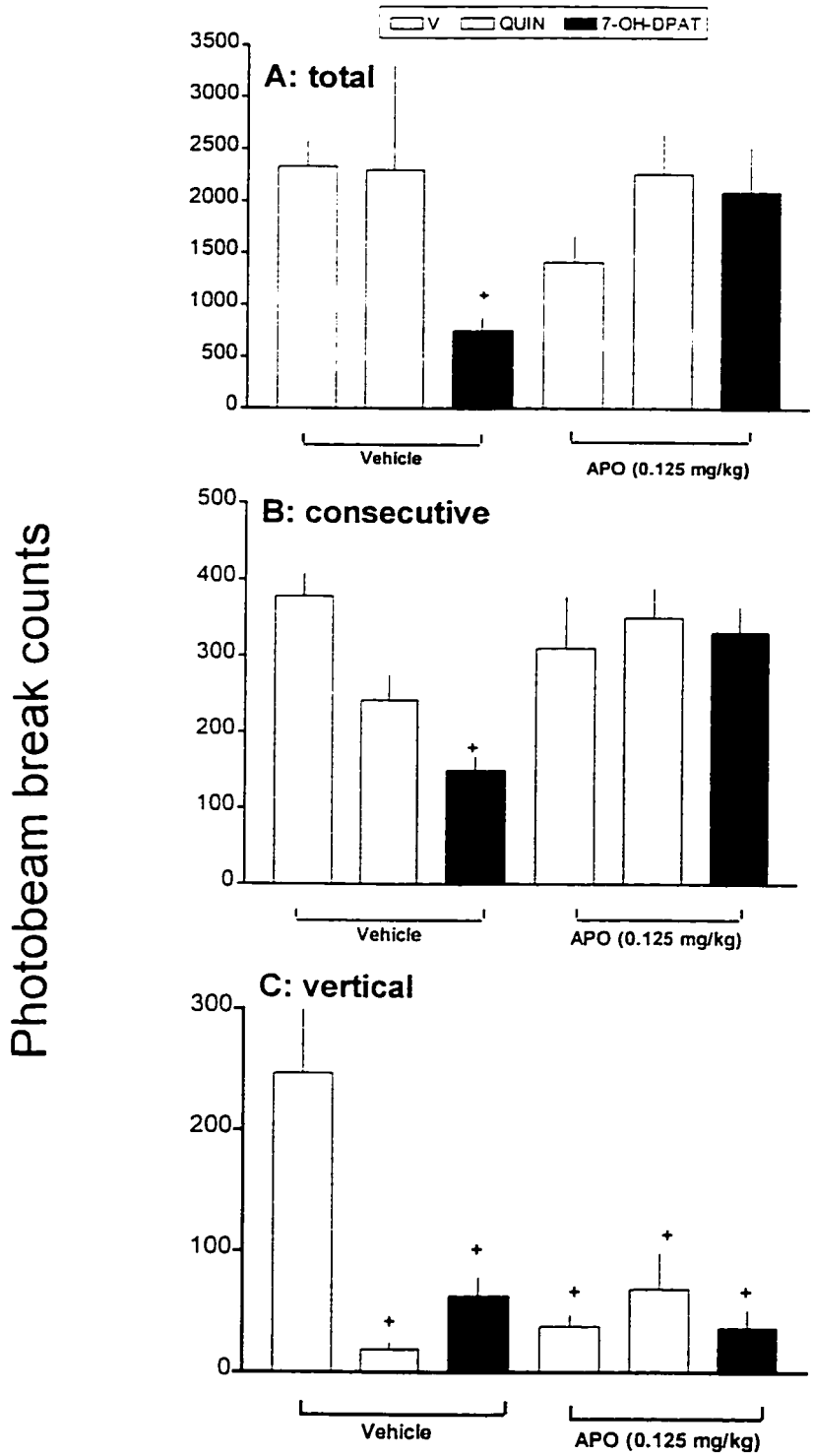

Figure 63: The effects of low dose of quinpirole (QUIN), 0.05 mg/kg, sc or 7-OH-DPAT (0.03 mg/kg, sc) on locomotor activity for 30 min in vehicle- or APO (0.125 mg/kg, sc)-treated rats. |: SEM for each mean. +: significant vs vehicle, $P<0.05$, n=8. V= vehicle.

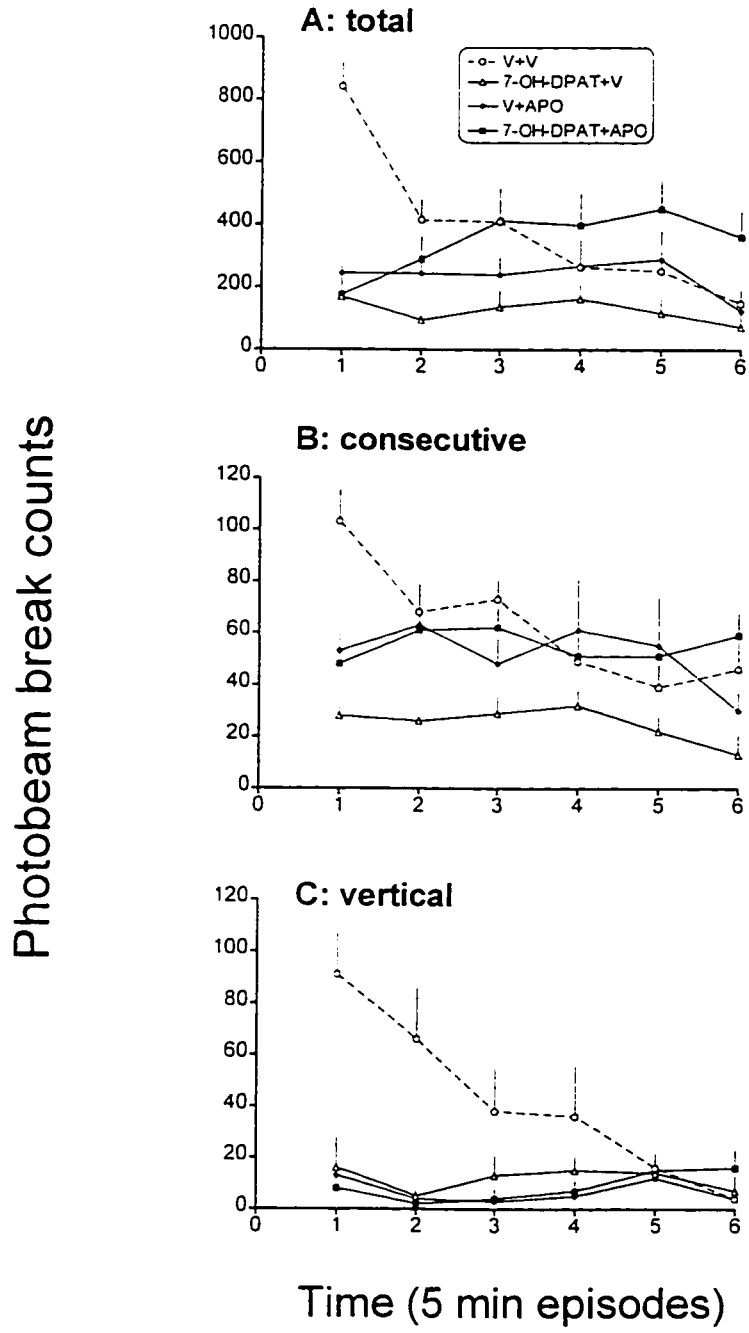

Figure 64: The effects of a low dose of 7-OH-DPAT (0.03 mg/kg, sc) on time-course of activity for 30 min in vehicle- or APO (0.125 mg/kg, sc)-treated rats. | : SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects.

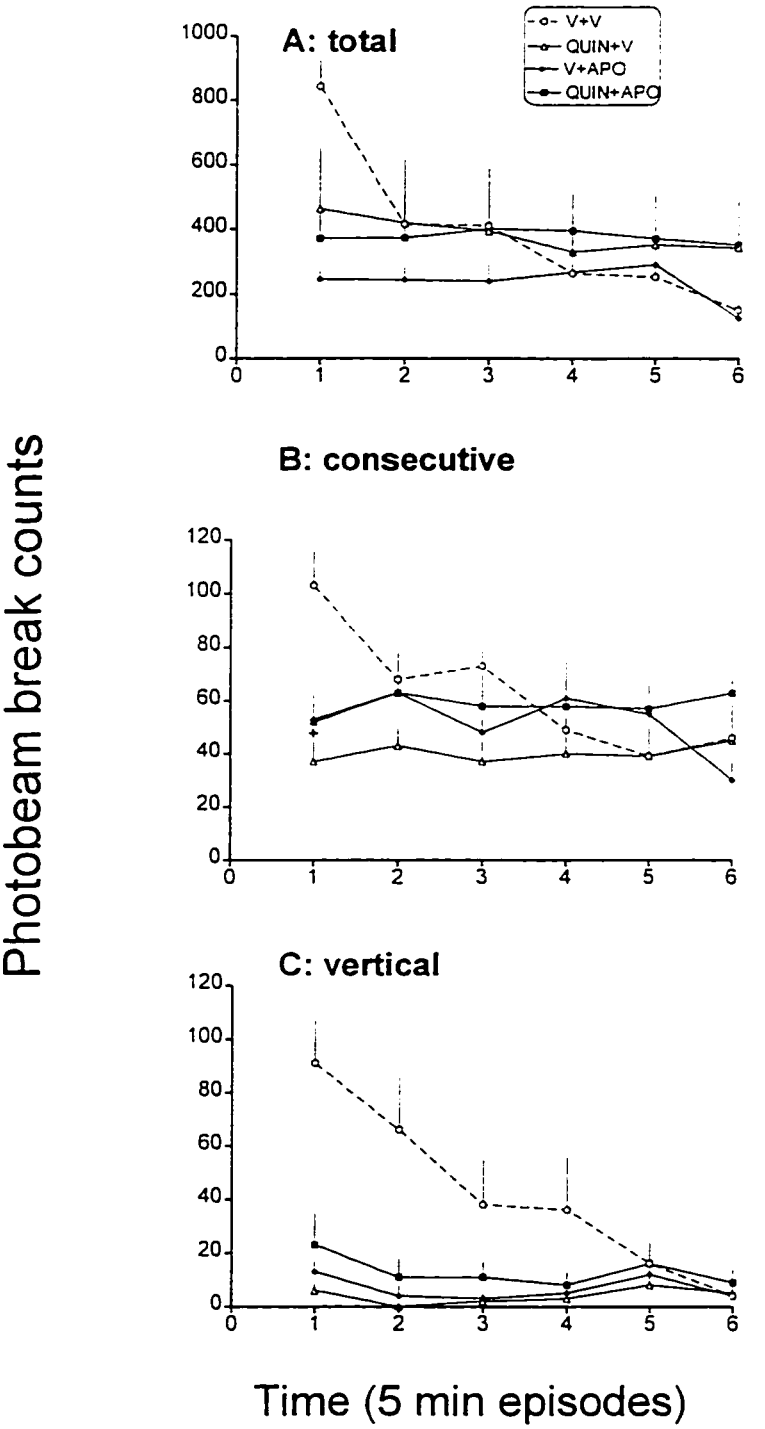

Figure 65: The effects of a low dose of quinpirole (0.05 mg/kg, sc) on time-course of activity for 30 min in vehicle- or APO (0.125 mg/kg, sc)-treated rats. | : SEM for each mean, n=8. V= vehicle. ANOVA revealed no statistically significant effects. ANOVA revealed no statistically significant effects.

3.16. The interaction of a low dose of 7-OH-DPAT with a high dose of APO in (reserpine + α-MPT)-treated rats

In order to further study the effects of 7-OH-DPAT at hypothesized inhibitory postsynaptic DA D3 receptors, the interaction between 7-OH-DPAT and APO in a model of postsynaptic DA receptors was assessed. Reserpine + α-MPT treatment caused approximately a 90% reduction in DA levels in the CN. APO (0.05 and 0.1 mg/kg, sc) increased spontaneous locomotor activity in DA-depleted rats. 7-OH-DPAT (0.01 mg/kg, sc) potentiated the effect of APO (0.05 mg/kg) but not of APO (0.1 mg/kg) at the fifth and sixth episodes of time-course as shown by 2-way RMANOVA followed by *post hoc* tests [total, Time: $F(5, 160) = 10.10$, $P<0.05$; 7-OH-DPAT: $F(1, 32) = 3.10$, $P>0.05$; APO: $F(1, 32) = 33.13$, $P<0.05$; 7-OH-DPAT x APO: $F(1, 32) = 6.84$, $P<0.05$; Time x 7-OH-DPAT: $F(5, 160) = 3.14$, $P<0.05$; Time x APO: $F(5, 160) = 8.46$, $P<0.05$; Time x 7-OH-DPAT x APO: $F(5, 160) = 2.54$, $P<0.05$], see Figures 67A and 67B.

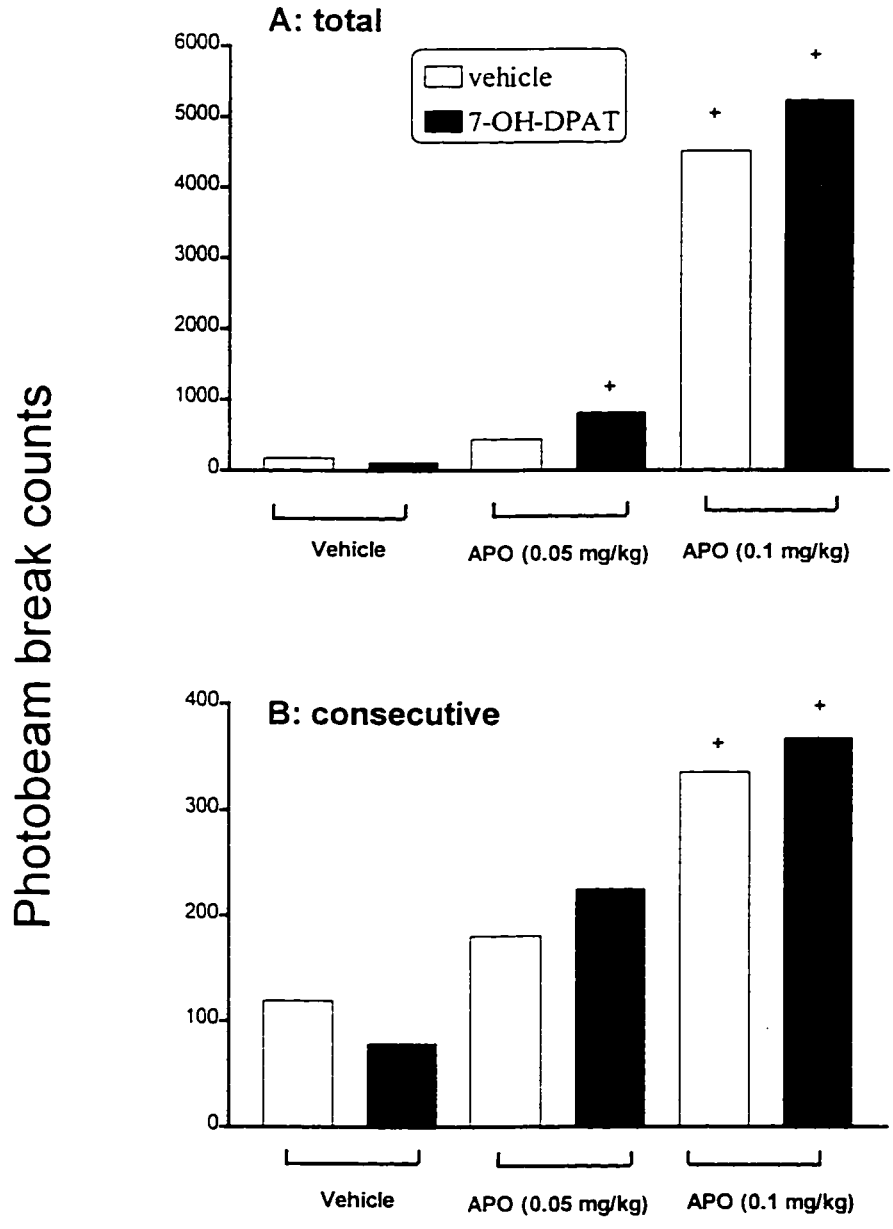

Figure 66: The effects of a low dose of 7-OH-DPAT (0.01 mg/kg, sc) on hyperactivity, A: total and B: consecutive, induced by APO (0.05 or 0.1 mg/kg, sc) in reserpine+α-MPT treated rats. | : SEM for each mean. +: significant vs vehicle, $P<0.05$, n=8.

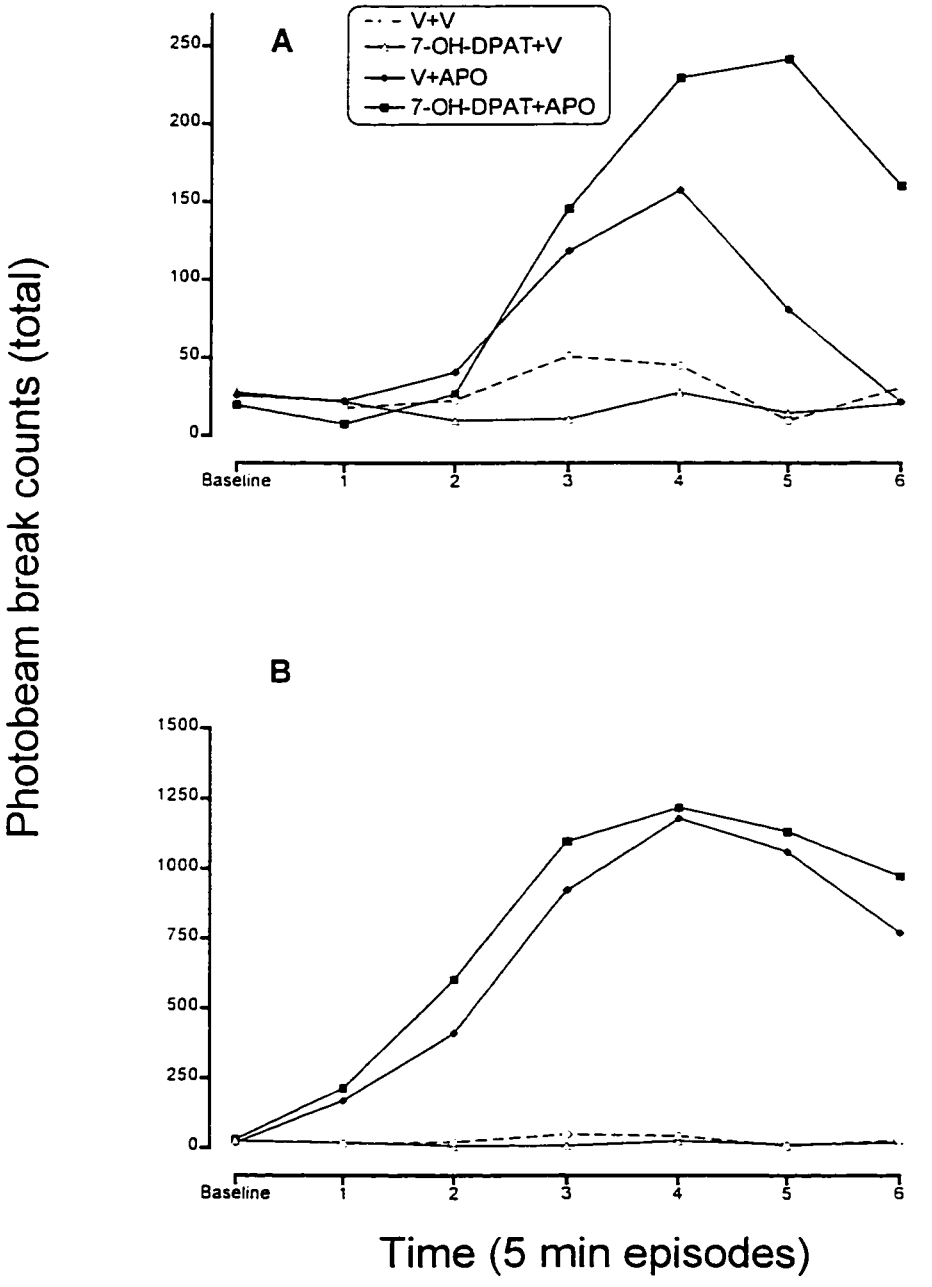

Figure 67: The effects of a low dose of 7-OH-DPAT (0.01 mg/kg, sc) on time-course of hyperactivity (total) induced by APO (A: 0.05 or B:0.1 mg/kg, sc) in reserpine+α-MPT treated rats. | : SEM for each mean. In panel A, the effect of 7-OH-DPAT on APO was significant at times 4-6. $P<0.05$, n=8. V= vehicle.

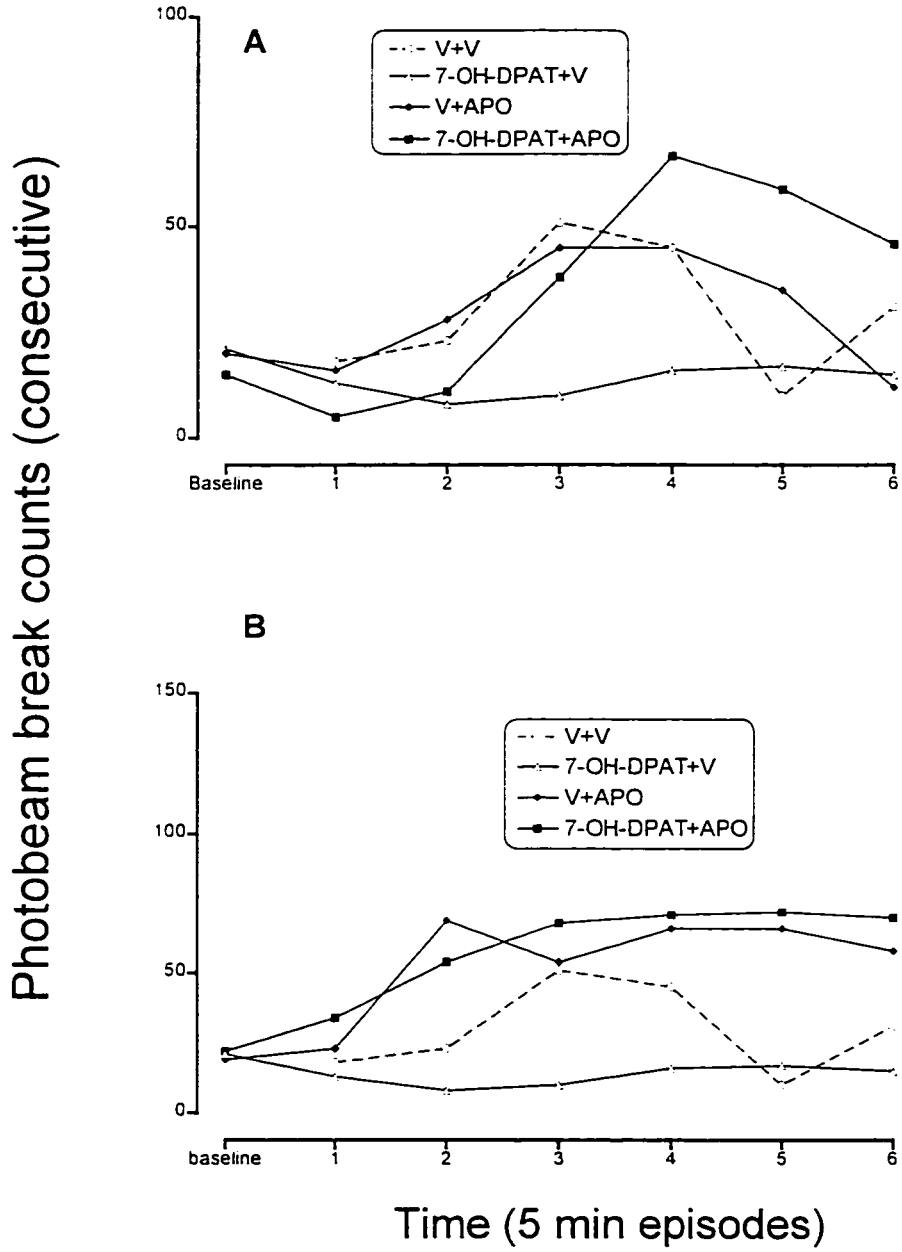

Figure 68: The effects of a low dose of 7-OH-DPAT (0.01 mg/kg, sc) on time-course of hyperactivity (consecutive) induced by APO (A: 0.05 or B:0.1 mg/kg, sc) in reserpine+α-MPT treated rats. |: SEM for each mean, n=8. V= vehicle.

3.17. Time-course of supersensitive response to APO in the 6-OH-DA-lesioned rats

The lack of a suppressant effect of 7-OH-DPAT on hyperactivity induced by APO in the DA-depleted rats in the previous experiment was related to a supersensitive response of DA D2 receptors. Therefore, in the present experiment the time-course of development of supersensitivity to APO in 6-OH-DA-lesioned rats was studied. The aim of the study was to determine a specific post-lesioning time at which the lesion develops without inducing functional supersensitivity of DA D2 receptors. Upon recovery from the lesions, rats showed the acute effects of aphagia, and soaked ground food was made available to them. The loss of body weight was less than 20% under these conditions. 6-OH-DA treatment decreased DA levels about 60%, 80% and 70% at 1, 2 or 3 days of post-lesion, respectively in both CN and NAS (Figure 69A). The 5-HT level was unchanged in the CN, but increased about 20% in 1 or 2 day post-lesioned rats and 80% in 3 day post-lesioned rats in the NAS (Figure 69B). NE concentrations were decreased about 40% and 30% in 2 and 3 day post-lesioned rats, respectively in the CN, and were unchanged in the NAS (Figure 69C). Intact rats showed a low baseline of activity and APO (0.1 mg/kg, sc) increased locomotor activity, probably because of the low baseline of activity. However, in sham-operated rats APO did not increase locomotor activity (Figure 70). This low dose of APO increased spontaneous locomotor activity in 1, 2 and 3 day post-lesioned rats but not in sham-operated rats (Figures 70 and 71), [day 1, Time: $F(4, 48) = 6.73$; sham/lesion: $F(1, 12) = 15.05$; Time x sham/lesion: $F(4, 48) = 5.66$]; [day 2, Time: $F(4, 48) = 8.40$; sham/lesion: $F(1, 12) = 9.00$; Time x sham/lesion: $F(4, 48) = 4.53$]; [day 3, Time: $F(4, 48) = 2.18$,

P>0.05; sham/lesion: F(1, 12) = 1.93, P>0.05; Time x sham/lesion: F(4, 48) = 41.58, P<0.05] .

Because of the observed supersensitive response to APO this DA-lesioned model was not used to test postsynaptic effects of 7-OH-DPAT.

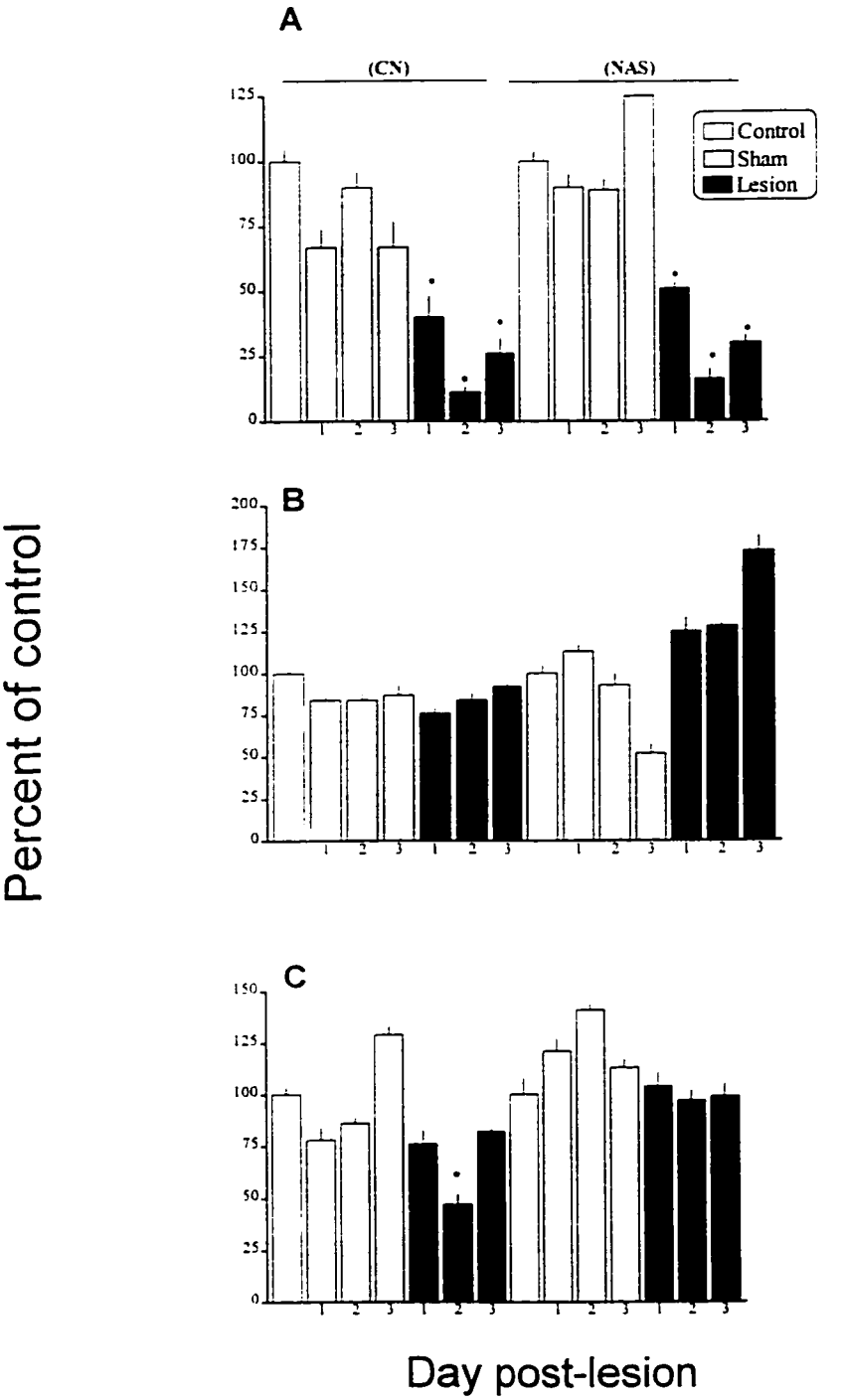

Figure 69: Levels of A: DA , B: 5-HT and C: NE in the CN or NAS in control, sham-operated and lesioned (6-OH-DA, 8 µg/0.5µl) rats. | : SEM for each mean. *: significant vs sham-operated rats, $P<0.05$, n=8. The absolute levels in a control group in CN: DA = 4520 ± 38 ng/g, 5-HT = 743 ± 30 ng/g, NE = 277 ± 25 ng/g; in NAS: DA = 4010 ± 34 ng/g, 5-HT = 739 ± 96 ng/g, NE = 658 ± 81 ng/g.

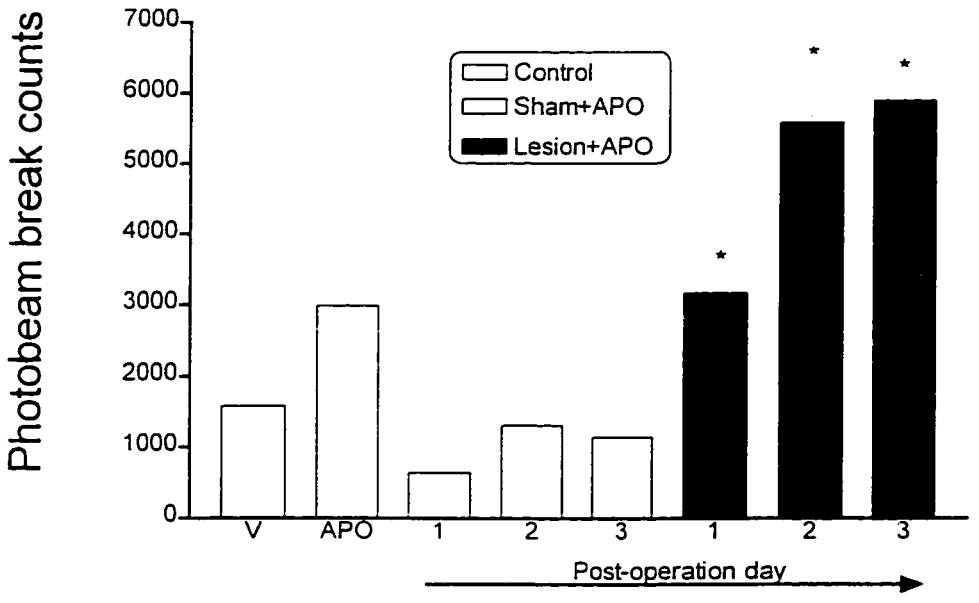

Figure 70: The effect of APO, 0.1 mg/kg, sc on total locomotor activity for 30 minutes in intact, 1-3 day post-sham-operated and post-6-OH-DA-lesioned rats. | : SEM for each mean. *: significant vs the same day sham-operated, $P<0.05$, n=8. V= vehicle.

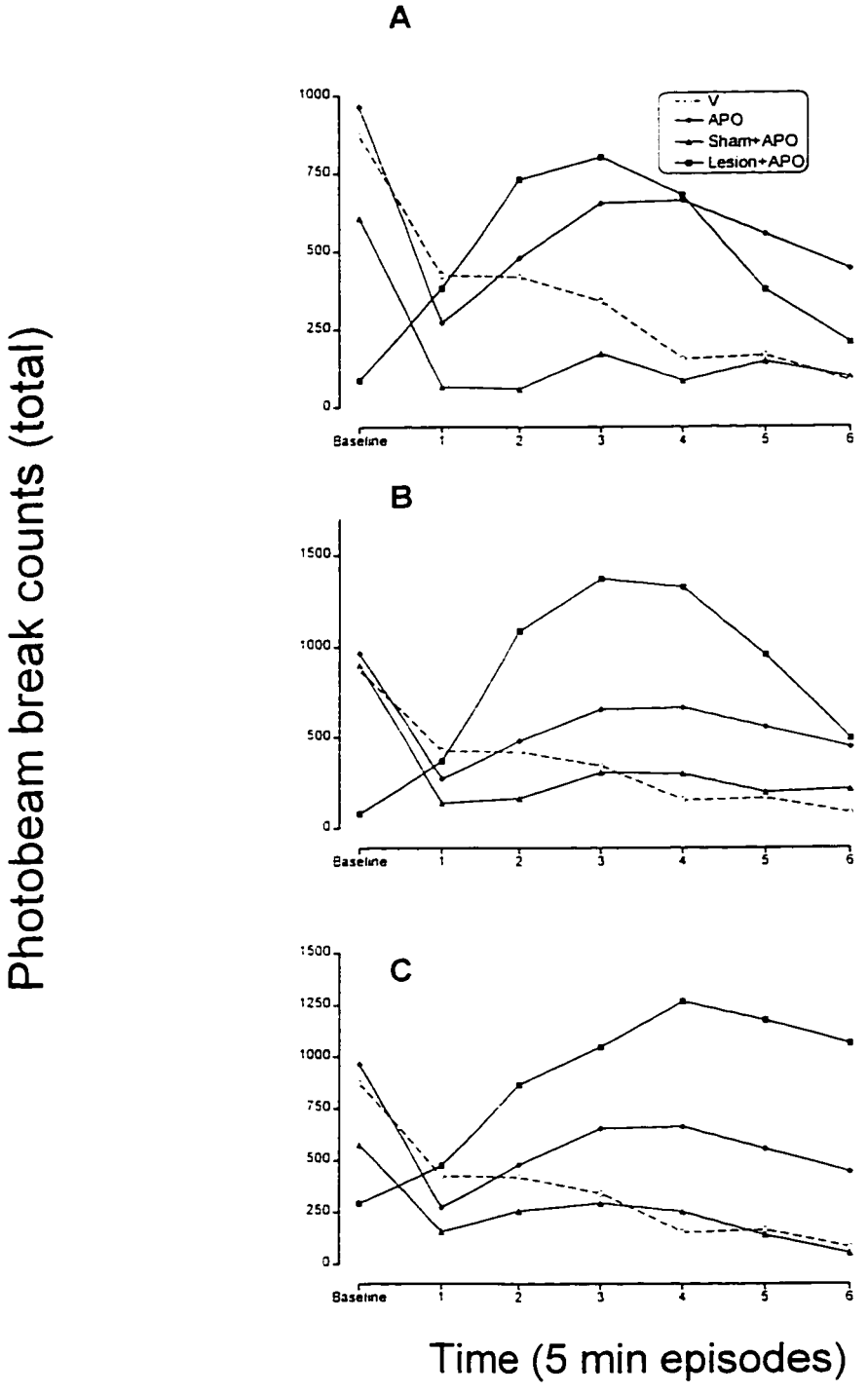

Figure 71: The effect of APO, 0.1 mg/kg, sc on time-course of total activity for 30 min in A: 1-day, B:2-day and C: 3-day post-lesioned rats. | : SEM for each mean. Effect of APO was significant at times 1-6, $P<0.05$, n=6. V= vehicle.

3.18. Analysis of 7-OH-DPAT in the brain

Typical chromatograms of 7-OH-DPAT from a standard solution and from an extract of homogenate of brain spiked with 7-OH-DPAT are shown in Figures 72A and 72B, respectively. Electrochemically active compounds in this region of the chromatogram from brain extracts of rats treated with the vehicle were negligible (Figure 73). The retention times of 7-OH-DPAT on the chromatogram in an extract from rats treated with 7-OH-DPAT (Figure 74B) and that of 7-OH-DPAT added to a brain extract from control rats (Figure 73B) were the same. The standard curves for 7-OH-DPAT dissolved in perchloric acid or extracted from spiked brain homogenates showed linearity (see Figure 75). The concentrations of 7-OH-DPAT in whole brain (minus striatum) following subcutaneous injection of the drug are shown in Figure 75.

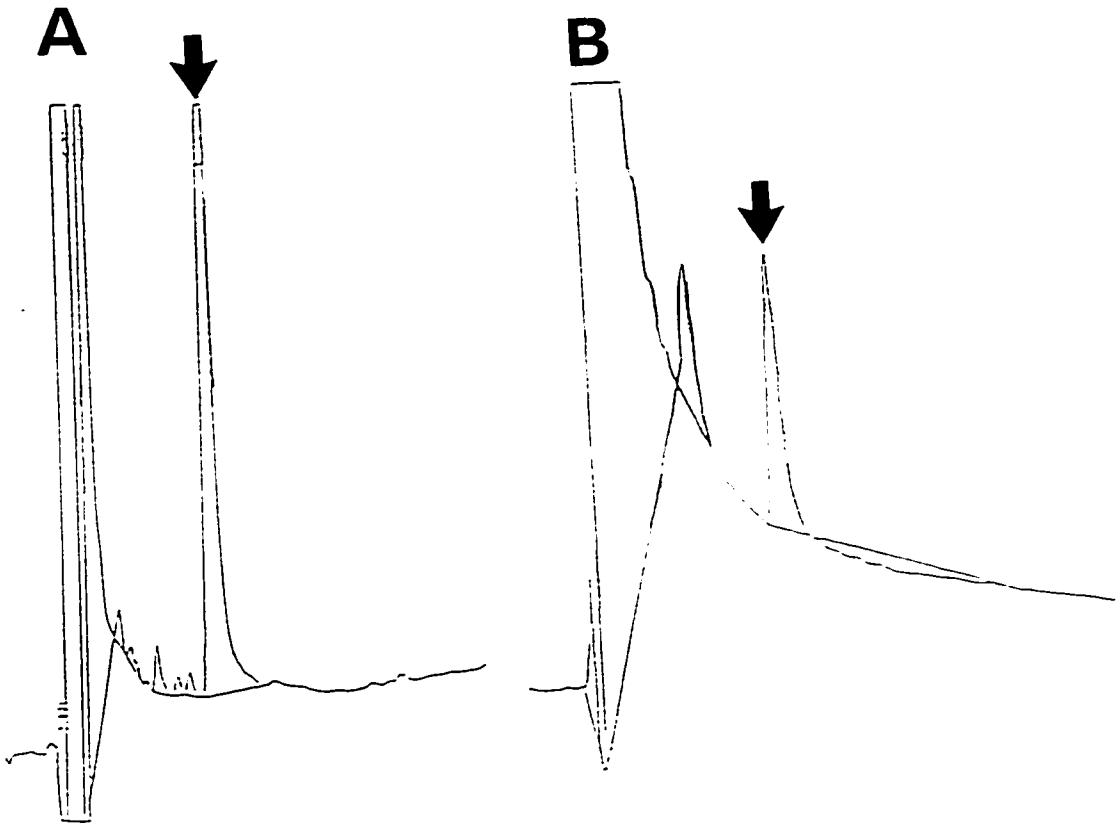

Figure 72: A typical HPLC chromatogram of 7-OH-DPAT in A: standard solution and B: spiked into brain homogenate and extracted as shown in Figure 5. The arrow indicates the position of 7-OH-DPAT.

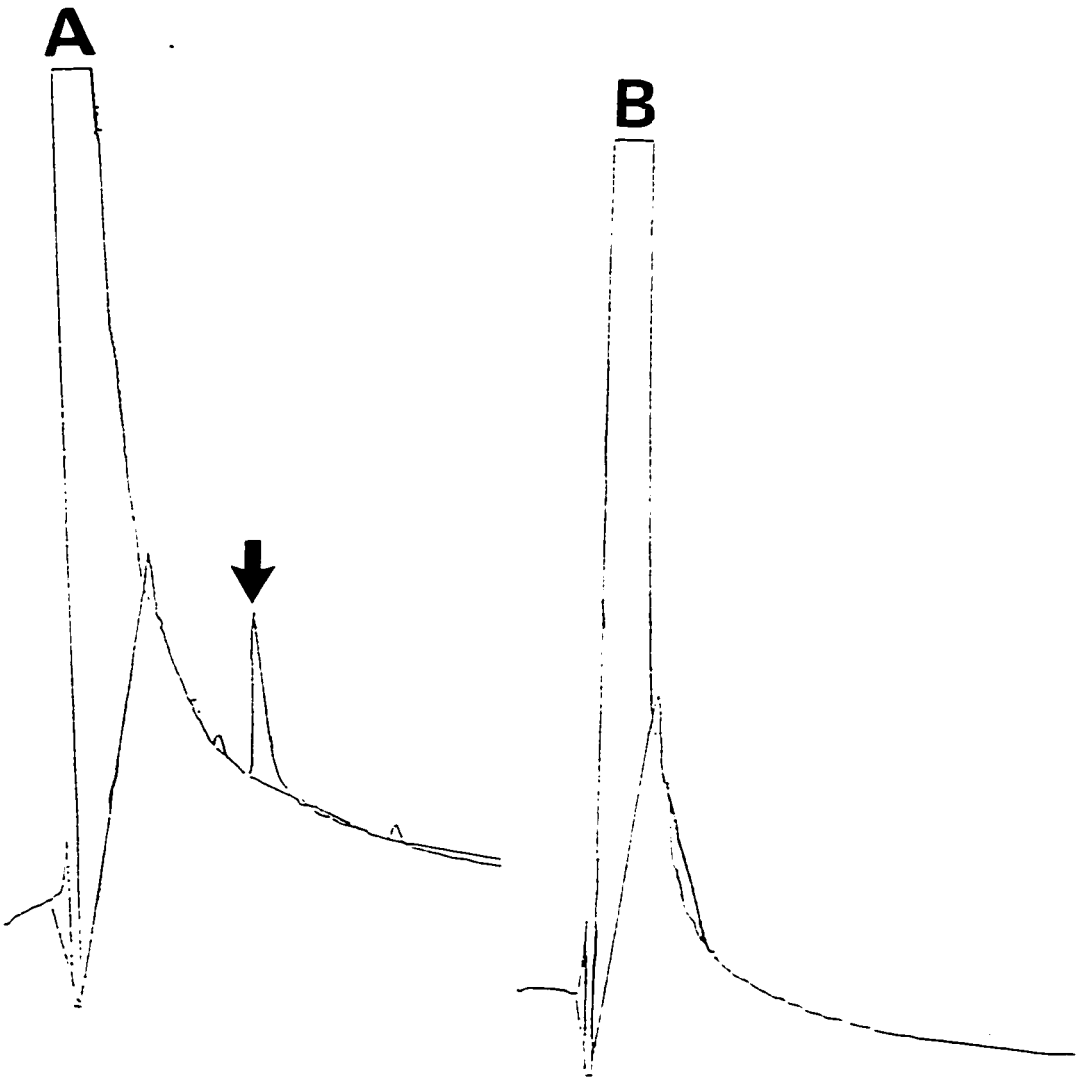

Figure 73: A typical HPLC chromatogram of 7-OH-DPAT extracted from brain of A: 7-OH-DPAT-treated rats and B: vehicle-treated rats

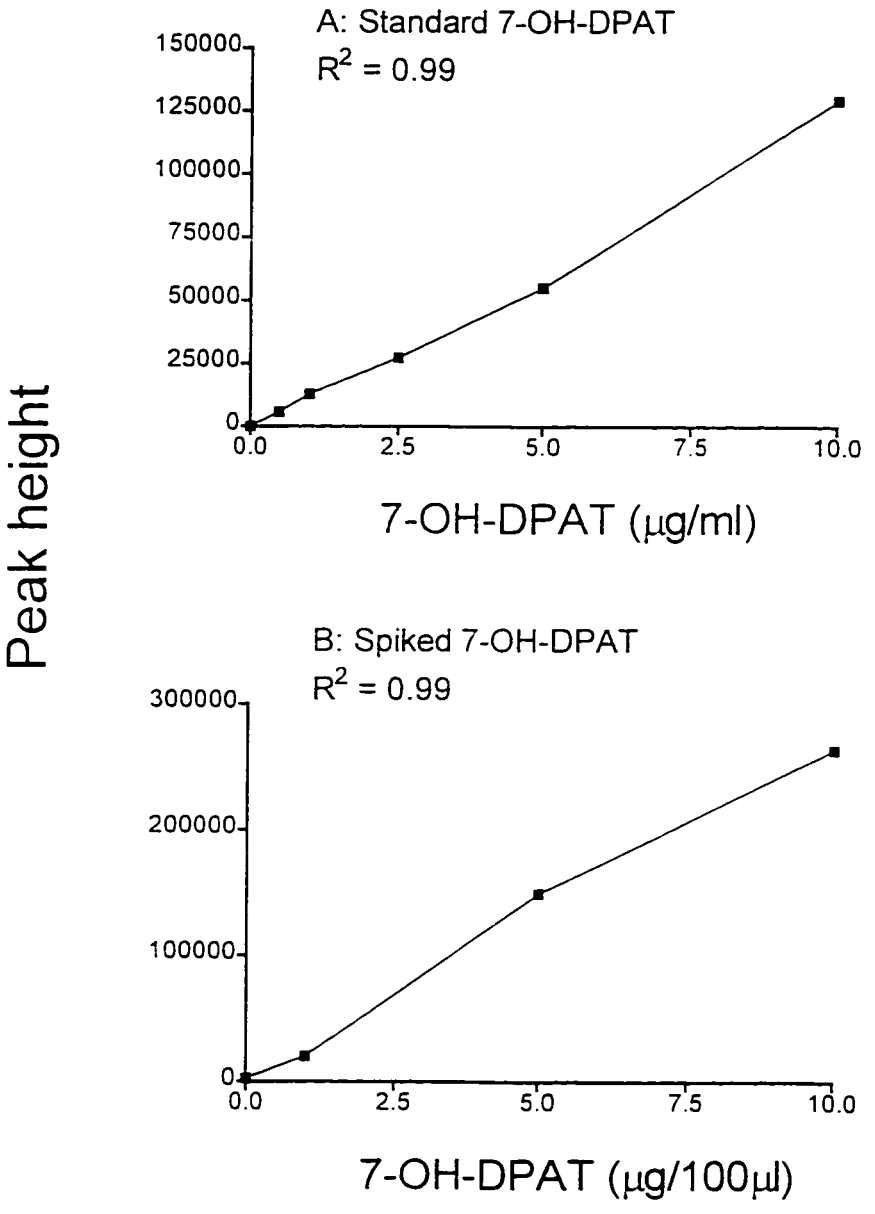

Figure 74: Standard curve for A: 7-OH-DPAT in perchloric acid and B: 7-OH-DPAT spiked into the brain homogenate and extracted as shown in Figure 5.

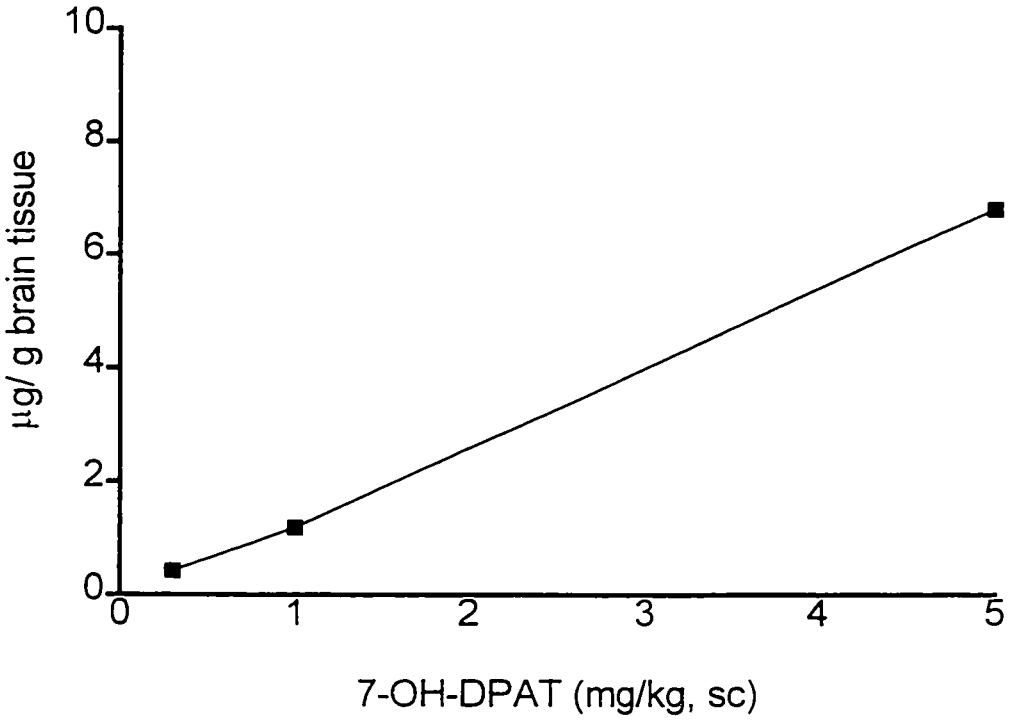

Figure 75: Brain levels of 7-OH-DPAT following sc administration of various doses (0.3, 1, 5 mg/kg, sc) of the drug to rat. Concentrations of 7-OH-DPAT in rat whole brain (minus striatum) 15 min after injection are shown. Two sets of samples were run separately, with the resultant values being within 10% of each other.

4. Discussion

4.1. Dose-dependent effects of 7-OH-DPAT on locomotor activity in habituated rats

The results from this experiment show that systemic administration of low doses of 7-OH-DPAT in habituated rats may decrease or increase locomotor activity, dependent on the dose and post injection time. 7-OH-DPAT at doses of 0.003-0.03 mg/kg decreased locomotor activity during a 30 min test session. but doses of 0.1 and 0.3 mg/kg suppressed locomotor activity for the first 10 min then increased it for the last 20 min of the test session. The latter hyperactivity may be due to the onset of stereotypic behavior. This is the first reported study of the effects of 7-OH-DPAT in long term (15 days) habituated rats. Daly and Waddington (1993) have reported that 7-OH-DPAT decreased locomotor activity over a 1 h observation session at doses of 0.01 and 0.1 mg/kg and induced sniffing, chewing and locomotion at a dose of 1 mg/kg; the rats were habituated to the observation cages for a period of 2.5 h before the test session. Exploration and habituation to a novel environment can be explained as survival-related activities, since animals explore an environment for localization of food, sexual partners and possible predators (Cheal, 1982). The baseline of activity depends on the degree of novelty and habituation to an environment. The baseline of activity is an important factor when measuring the effect of a drug on locomotor activity. The stimulant effect of a drug may be masked by a high baseline of activity. On the other hand, detection of a depressant effect of a drug is more likely with a high baseline of activity and a stimulant effect of a drug is more likely to be detected with a low baseline of

activity (Robbins, 1997). It has been postulated that DA and NE are involved in the mechanisms of exploration and habituation (Cheal, 1980). A study showed that the firing activity of locus coeruleus neurons was increased by exposure to novelty in freely exploring rats and that these responses of neurons were absent after habituation (Vankov et al. 1995), indicating that habituation induces some changes in catecholamine systems.

It has been shown that a number of direct DA agonists (e.g., APO, QUIN, and 2-aminotetralins) reduce locomotor activity at low doses, probably because of a decrease in DA release or synthesis subsequent to stimulation of DA autoreceptors on cell bodies and/or terminals of dopaminergic neurons (Kehr et al. 1975; Langer et al. 1981). This hypothesis is based on the observation that low doses of APO, a DA D1/D2 receptor agonist, decrease DA release (Van Ree et al. 1981; Svensson et al. 1994b). Since the DA D3 receptor mRNA is present in the SN and VTA (Sokoloff et al. 1990), this receptor, as well as the DA D2 receptor, may play an autoreceptor role. Recent reports suggest that 7-OH-DPAT may bind to the DA D3 receptors with an affinity of <1 nM (Kd = 0.67 nM) that is 100 times higher than its affinity for DA D2 receptors (Levesque et al. 1992). Also, Parsons and colleagues (Parsons et al. 1996) have shown *in vivo* selectivity of 7-OH-DPAT for DA D3 receptors *vs* DA D2 receptors. These investigators have reported that local administration of 7-OH-DPAT into the NAS decreased dialysate levels of DA but had no effect on DA release when injected into the ipsilateral striatum, a region containing DA D2 receptors but few DA D3 receptors (Diaz et al., 1995), of the same animal. Moreover, this effect was reversed by the putative DA D3 receptor antagonist nafadotride (Sautel et al., 1995). The effects of low doses of 7-OH-DPAT may thus be mediated by presynaptic D3 DA receptors. Several

studies show an attenuating effect of 7-OH-DPAT on DA release and synthesis (Ahlenius, Salmi, 1994; Gobert et al. 1995; Aretha et al. 1995; Rivet et al. 1994; Booth et al. 1994; Parsons et al. 1996; Yamada et al. 1995; Gainetdinov et al. 1994). Whether this stimulation of DA D3 autoreceptors is the cause of hypoactivity induced by a low dose of 7-OH-DPAT is controversial. It has been shown that 7-OH-DPAT decreased locomotor activity at low doses which did not decrease the brain DA synthesis rate (DOPA accumulation) measured one h after systemic administration of the drug and DA release measured by microdialysis (Svensson et al. 1994b). These investigators hypothesized that 7-OH-DPAT may reduce locomotor activity by stimulating a subgroup of postsynaptic inhibitory receptors. Further support for this hypothesis is provided by a study on the simultaneous effects of 7-OH-DPAT and quinelorane, two DA D3 receptor agonists, on cocaine self-administration and DA release (Caine et al., 1996). This study shows that 7-OH-DPAT decreased cocaine intake in a manner indicating an enhancement of cocaine reinforcement and simultaneously decreased the cocaine-induced increase in NAS DA levels by more than 50%. These authors have concluded that the effect of 7-OH-DPAT is related to postsynaptic DA D3 receptors.

4.2. Effects of 7-OH-DPAT on locomotor activity in non-habituated rats

7-OH-DPAT reduced locomotor activity in non-habituated rats. In contrast to the situation in habituated rats, 7-OH-DPAT (0.3 mg/kg) did not induce any hyperactivity in the last 20 min of the test session. These findings indicate that the effects of doses of 0.1 and 0.3 mg/kg on locomotor activity are different in habituated and non-habituated rats. Thus, 7-

OH-DPAT decreases locomotor activity at doses of 0.003-0.03 mg/kg in habituated rats and at doses of 0.003-0.3 in non-habituated rats. The reason for the tendency of 7-OH-DPAT to increase locomotor activity after 10 min of suppression in habituated rats remains unclear.

4.3. The interaction of HAL with a low dose of 7-OH-DPAT in habituated rats

Similar to the previous experiment (see section 3.1.), 7-OH-DPAT at 0.1 mg/kg induced a biphasic response, i.e., early hypoactivity followed by a later hyperactivity in habituated rats. HAL at 0.03 mg/kg antagonized the later hyperactivity induced by 7-OH-DPAT (0.1 mg/kg) in habituated rats. A study by Protais et al. (1983) shows that HAL (0.05 mg/kg, ip) antagonized the effect of a medium dose of APO (0.075 mg/kg, sc). This dose of APO induced hypoactivity followed by hyperactivity in habituated rats, and has been referred to as a restoring dose of APO. But it is referred to as a late hyperactivity, rather than restoring the activity, in this thesis because it represents an increase in activity compared to controls at a matching time (Figure 8B). Taken together, these data indicate that DA D2 receptors are involved in the effects of both APO and 7-OH-DPAT at these specific doses in habituated rats. Several studies have indicated that the rates of responding prior to drug administration can influence the behavioral effects of drugs (Witkin, Katz, 1990). Therefore, the late hyperactivity induced by 7-OH-DPAT may be related to the low level of activity in habituated rats, as these phenomena were not observed in non-habituated rats whose level of activity was about twice that of habituated rats.

4.4. Effects of 7-OH-DPAT on locomotor activity in nicotine-stimulated rats

In the present experiment 7-OH-DPAT reduced hyperactivity induced by nicotine. Nicotine induces hyperactivity in rats without producing stereotyped behavior (Lapin et al. 1987). Hyperactivity and stereotyped behaviors are thought to be mediated by the mesolimbic and nigrostriatal DA systems, respectively (Kelly et al. 1975).

The effects of nicotine on locomotor activity may be mediated preferentially *via* the mesolimbic DA system. Acute nicotine increases the striatal DA turnover rate (Andersson et al. 1981) and the release of DA from the striatum and mesolimbic system (Imperato et al. 1986; Rowell et al. 1987; Rapier et al. 1990). This effect of nicotine is several times greater in the NAS than in the CN (Imperato et al. 1986; Robinson et al. 1989; Rowell et al. 1987; Lapin et al. 1989). Also, administration of nicotine into the NAS or VTA increases release of DA and locomotor activity (Mifsud et al. 1989; Museo, Wise, 1990). Clarke and co-workers have found that systemic injection of nicotine (Opielka et al. 1985; Andersson et al. 1985) stimulated locomotor activity and increased the ratio of the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) to that of DA in the olfactory tubercle, but did not alter 5-HT utilization (Clarke et al. 1988). These workers found that the stimulant effect of nicotine was abolished by 6-OH-DA-lesioning of the NAS.

Two mechanisms of action involving the mesolimbic DA system have been suggested for the effects of nicotine on locomotor activity. Nicotine may bind to receptors on DA cell bodies in the VTA and increase cell firing; this probably increases the release of DA at terminal sites and thereby facilitates locomotion. Support for this comes from studies in

electrophysiology (Grenhoff et al. 1986; Mereu et al. 1987) and autoradiography (Clarke, Pert, 1985). Reports of increase in locomotor activity following intra-VTA injections of cytisine, a potent nicotinic agonist, also support the hypothesis that nicotine can act at the level of the cell body to influence DA activity (Museo, Wise, 1990a). Nicotine may also act directly on receptors at DA terminals, as bilateral injection of nicotine or cytisine into the NAS induced hyperactivity in rats (Reavill, Stolerman, 1990; Fung, 1990; Museo, Wise, 1990b). Following chronically administration of nicotine (0.8 mg/kg, 14 days), a nicotine challenge still increased DA turnover in the NAS, although the response was less than in animals not previously treated with nicotine (Lapin et al. 1989). A lack of tolerance to the effect of intermittent administration of nicotine on DA release in the NAS has been reported in rats (Damsma et al. 1989).

DA receptors are apparently involved in the hyperactivity induced by nicotine. It has been reported that SCH 23390, a DA D1 receptor antagonist, HAL (a DA D1/D2 receptor antagonist) and raclopride (a DA D2 receptor antagonist) reduced hyperactivity induced by nicotine in rats (O'Neill et al. 1991; Damaj, Martin, 1993). In the present experiment, 7-OH-DPAT may decrease effects of nicotine through DA D3 receptors that are abundant in the mesolimbic dopaminergic system.

Regarding the pre- and postsynaptic effects of 7-OH-DPAT, there are two possible explanations. 7-OH-DPAT may decrease the effects of nicotine as a consequence of decreasing the release of DA through its effects on presynaptic DA D3 receptors. However, chronic nicotine treatment may influence the pre- or postsynaptic DA receptor sensitivity. Generally, repeated stimulation of DA receptors by an agonist induces down-regulation of

the receptor, while a blockade of receptors or depletion of neurotransmitter induces up-regulation of receptors. After chronic nicotine treatment, postsynaptic DA D2 receptor binding activity is not changed (Wiener et al. 1989) or increased (Fung, Lau, 1988; Fung, Lau, 1989) and the response of rats to APO is potentiated (Suemaru et al. 1993). It may be concluded that release of DA by nicotine may leave DA receptors deprived of DA between injections, causing up-regulation of DA receptors. Measurement of DA levels at different times between injections may provide more information in this regard. DA autoreceptors are also susceptible to regulation after chronic nicotine treatment. One study shows that chronic nicotine treatment may cause development of DA D2 autoreceptor subsensitivity. Thus, chronic nicotine treatment abolished the inhibitory effect of QUIN, in addition to the stimulatory effect of (±)-sulpiride on electrically stimulated DA release from superfused striatum preloaded with [^{3}H]DA, suggesting that subsensitivity for DA autoreceptors develops in the striatum (Harsing et al. 1992). A decrease in sensitivity of DA autoreceptors is not unique for nicotine treatment. It also has been reported that chronic cocaine treatment may decrease the inhibitory effect of N-0437, a DA agonist, on DA release (Yi and Johnson, 1990). Also, the inhibitory effects of APO on extracellular single unit recording of A10 DA neurons were abolished with chronic cocaine treatment (Kalivas, Duffy, 1988; Henry et al., 1989). Similarly, the ability of APO to inhibit ventral tegmental neuron activity was decreased by chronic amphetamine treatment (Kamata and Rebec, 1984) and repeated administration of nicotine attenuated the ability of APO to induce hypoactivity in two strains of mice (Sershen et al. 1991). If it is assumed that D3 DA autoreceptors may develop subsensitivity subsequent to repeated nicotine treatment, then presynaptic DA D3 receptors

may not mediate the effects of 7-OH-DPAT on nicotine-induced hyperactivity. A study that measures the effects of 7-OH-DPAT on DA release and locomotor activity simultaneously after repeated treatment of nicotine may provide some more information about the involvement of presynaptic mechanisms of 7-OH-DPAT. A postsynaptic effect of 7-OH-DPAT has been suggested, as 7-OH-DPAT induced hypoactivity at doses that did not affect DA synthesis or release in the striatum or NAS (Svensson et al. 1994a). Therefore, 7-OH-DPAT may reduce the stimulant effects of nicotine *via* inhibitory postsynaptic D3 DA receptors in mesolimbic dopaminergic system.

4.5. Effects of intracranial microinjection of 7-OH-DPAT into the NAS or CN on locomotor activity

The ability of 7-OH-DPAT to decrease hyperactivity induced by nicotine (an effect preferentially mediated *via* the mesolimbic dopaminergic system) in the previous experiment revealed the possibility that the effect of 7-OH-DPAT on locomotor activity may be mediated preferentially *via* the mesolimbic system. However, results from intracranial microinjections of 7-OH-DPAT did not show NAS selectivity in the locomotor activity tests in rats. Intracranial injections of 7-OH-DPAT into two regions of dopaminergic terminals (i.e., CN or NAS) decreased locomotor activity. The effects of 7-OH-DPAT were specific, since 8-OH-DPAT, a structural analog of 7-OH-DPAT with a lack of significant DA activity (Arvidsson et al. 1981), did not induce hypoactivity when injected into the CN or NAS; it only reduced vertical activity when injected into the NAS. 8-OH-DPAT at high doses may have affinity for DA D2 receptors; it induced forward walking and decreased total and vertical activities at a dose of 0.4 mg/kg, sc (Millan et al. 1995c; Hillegaart et al.

1996). The present results are consistent with another report on the lack of mesolimbic selectivity of 7-OH-DPAT shown by *in vitro* and *in vivo* electrophysiological tests (Liu *et al.*, 1994), but are in contrast to the differential effects of this drug on evoked DA release from slices of striatum and NAS in rats (Yamada et al. 1992). Some other studies also show the preferential effect of 7-OH-DPAT in NAS or olfactory tubercle *vs* striatum on DA synthesis (Booth et al. 1994; Nissbrandt et al. 1995).

Several pieces of evidence indicate the involvement of DA D3 receptors in DA turnover. 7-OH-DPAT, a DA D3 receptor agonist, decreases DA release and synthesis (Patel et al. 1995; Timmerman et al. 1991; Damsma et al. 1993; Waters et al. 1993; Rayevsky et al. 1995; Devoto et al. 1995; Meller et al. 1993; Aretha et al. 1995; Mattingly et al. 1996; Ahlenius, Salmi, 1994) in striatum, NAS and olfactory tubercle, in a concentration dependent manner (1-150 μmol/kg, or 0.01-10 μM) measured *in vitro*, *ex vivo* or by microdialysis. EEDQ (N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline) treatment that alkylates DA D1 and D2 receptors does not alkylate DA D3 receptors. 7-OH-DPAT binding was not changed after EEDQ treatment (Hamblin, Creese, 1983; Levant, 1995; Aretha, Galloway, 1996), while binding of DA D1 and D2 receptor ligands was decreased. The effect of 7-OH-DPAT on DA synthesis exists after EEDQ pretreatment. Moreover, (+)-S-14297, a preferential DA D3 receptor antagonist, blocks the effect of 7-OH-DPAT on DA release (Rivet et al. 1994). The lack of NAS selectivity of 7-OH-DPAT in locomotor activity tests may indicate a lack of *in vivo* selectivity of this drug for DA D2 and D3 receptors. Also it is possible that in the striatum 7-OH-DPAT binds to the low density population of DA D3 receptors to induce hypoactivity; the density of DA D2 receptors is twice that of DA D3 receptors in the CN. In

the NAS. 7-OH-DPAT more likely binds to DA D3 receptors to induce hypoactivity as there is a relatively high density of this receptor subtype in this region of brain.

These results also suggest that the effects of 7-OH-DPAT on DA release and locomotor activity may not be correlated to each other, since 7-OH-DPAT shows regional selectivity on DA release but not on locomotor activity. If reduction of DA release by 7-OH-DPAT results in hypoactivity, then the effects of 7-OH-DPAT on DA release and locomotor activity should show parallel results. However, results from the present study and studies on the effect of 7-OH-DPAT on DA release do not confirm such parallel effects of 7-OH-DPAT on DA release and locomotor activity. 7-OH-DPAT shows more efficacy in the NAS in reducing DA release, but it is not more potent in decreasing locomotor activity when injected into the NAS.

4.6. The interaction of CLZ with high doses of 7-OH-DPAT or APO

The results of this experiment show that the effects of CLZ on high doses of 7-OH-DPAT (5 mg/kg) and APO (0.5 mg/kg) are different. APO is a non-selective DA D1/D2 receptor agonist. It has been reported that CLZ does not block stereotypy induced by APO (Murray, Waddington, 1990). The present data show that CLZ reduced forward walking and sniffing induced by 7-OH-DPAT but not those induced by APO. So far several mechanisms have been suggested for the effects of CLZ. These include DA D1 receptor antagonism, a benzodiazepine-like effect, 5HT2A and 5HT2C receptor antagonism, anticholinergic and antiadrenergic properties. The DA D1 receptor antagonistic effect of CLZ (Amenta et al. 1995; Daly, Waddington, 1994) is not likely to mediate the effects of CLZ on sniffing and

forward walking induced by 7-OH-DPAT, since even a high dose of CLZ (5 mg/kg) was not able to attenuate sniffing and forward walking induced by APO. However, SCH 23390, a DA D1 receptor antagonist, blocks the stimulant effects of APO (Iorio et al. 1983). In a recent study Buckland and co-workers (1993) reported that CLZ (30 mg/kg/day) increased DA D3 receptor mRNA density by 350% after 4 days of treatment. CLZ also increased DA D1B receptor mRNA by 50%, but it had no significant effect on the mRNA levels of DA D1A or D2 receptors or of enzymes involved in DA synthesis (tyrosine hydroxylase and aromatic L-amino acid decarboxylase). HAL also increased the mRNA level of DA D3 receptors and these authors concluded that up-regulation of DA D3 receptor mRNA may be concomitant with the therapeutic action of antipsychotic drugs. Although *in vitro* binding studies show that CLZ's affinity for DA D2 and D3 receptors is low, it may bind to the DA D3 receptor *in vivo* or affect this receptor beyond the recognition site.

Present data also show that 7-OH-DPAT and APO may induce locomotion, forward walking and sniffing behaviors by different mechanisms, as CLZ can differentially affect them. In the literature, effects of a high dose of 7-OH-DPAT on locomotor activity have been related to DA D2 receptors (Van den Buuse, 1993; Kurashima et al. 1995). The present results from locomotor activity experiments in this thesis show that DA D2 receptors may not mediate all the effects of the high dose of 7-OH-DPAT on locomotor activity since CLZ blocks some effects induced by 7-OH-DPAT but not by APO. It has been concluded that stereotyped behavior and hyperactivity are mediated by different mechanisms (Ljungberg, Ungerstedt, 1978) since HAL at doses of 0.1 and 0.2 mg/kg reduced gnawing without reducing locomotion and HAL (1.0 mg/kg) blocked all stimulant effects of APO (5 mg/kg);

in contrast CLZ (5 mg/kg) reduced locomotion induced by APO without reduction of gnawing. This is consistent with our findings that CLZ (5 mg/kg) decreased locomotor activity induced by APO without reduction of sniffing and forward walking. The present findings may provide an animal model for screening of atypical neuroleptics. Since CLZ, an atypical neuroleptic that does not block APO-induced stereotypy, is able to block hyperactivity as well as sniffing and forward walking behaviors induced by 7-OH-DPAT. Mechanisms involved in the interaction between CLZ and 7-OH-DPAT are unknown. A direct effect of CLZ on DA D3 receptors has been suggested by other investigators (Anssari et al. 1997). Imaging studies may provide more information regarding *in vivo* affinity of CLZ for DA D3 receptors.

4.7. The interaction of SCH 23390 with high dose 7-OH-DPAT or APO

In the present study SCH 23390 blocked hyperactivity induced by APO completely and that of 7-OH-DPAT partially. High doses of APO or 7-OH-DPAT induced constant total and consecutive activity during 30 min. SCH 23390 changed this pattern of constant activity induced by APO but not that induced by 7-OH-DPAT. The interaction between SCH 23390 and APO in this study is consistent with other studies. SCH 23390 is a DA D1 antagonist (Iorio et al. 1983) and its *in vitro* affinity for DA D2 and D3 receptors is very low (Tice et al. 1994). It reduces some effects of DA D2 agonists due to a probable interaction between DA D1 and D2 receptors (Pugh et al. 1985; Molloy, Waddington, 1985). Mechanisms involved in the effects of high doses of 7-OH-DPAT are unknown. Some effects of 7-OH-DPAT at

this dose may be mediated by DA D1/D2 receptors, as SCH 23390 blocked its effects partially.

4.8. The interaction of HAL with high dose 7-OH-DPAT or APO

In this study, HAL (0.03 or 0.1 mg/kg, sc) changed hyperactivity induced by APO (0.5 mg/kg) but not by 7-OH-DPAT (2.5 mg/kg). HAL at doses of 0.05-0.2 mg/kg reduced the stimulant effects of DA agonists as shown in different studies. It has been reported that HAL (0.05 mg/kg, ip. 1h prior to APO) blocked the effect of APO (0.3 mg/kg, sc) on consecutive activity (Vaccheri et al. 1986). Also, HAL (0.4 but not 0.2 mg/kg, 30 min prior to APO) reduced hyperactivity induced by APO (5 mg/kg). Schremmer and co-workers (1990) have demonstrated that HAL (0.03-0.25 mg/kg, ip. 30 min prior to APO) antagonized hyperactivity induced by APO, but only the effect of 0.125 mg/kg was significant. Another study (Ljungberg, Ungerstedt, 1978) showed that HAL at doses of 0.1 and 0.2 mg/kg reduced gnawing without reducing locomotion; at 1.0 mg/kg HAL blocked all stimulant effects of APO (5 mg/kg). In the present study, HAL changed the pattern of constant locomotor activity induced by APO to a descending pattern of activity. Although the animals were not observed during these test sessions, the constant pattern of activity in APO- and 7-OH-DPAT-treated rats may indicate stereotyped behavior since this constant pattern of activity is due to constant forward walking and continuous sniffing during the 30 min test session based on the observations from a previous experiment, 3.11. Therefore, it can be concluded that HAL at 0.03 mg/kg may decrease sniffing and forward walking behaviors induced by APO but not by 7-OH-DPAT.

4.9. The interaction of a low dose of 7-OH-DPAT with a high dose of APO in rats

In this experiment, 7-OH-DPAT, but not QUIN, at a low dose was able to decrease the effect of a high dose of APO (0.25 but not 0.5 mg/kg), significantly. The blockade of effects of high doses of APO has been used as an animal model for the screening of antipsychotic drugs. 7-OH-DPAT shows a 100-fold greater affinity for DA D3 receptors than DA D2 receptors. Also, it has been suggested that DA D3 receptors may function as inhibitory postsynaptic receptors. Therefore, the stimulation of these inhibitory postsynaptic DA D3 receptors by 7-OH-DPAT may be able to decrease the effect of APO at postsynaptic stimulatory DA D2 receptors. The differential effects of QUIN and 7-OH-DPAT on APO may be related to the greater selectivity of 7-OH-DPAT for the D3 DA receptor. The general hypotheses that DA agonists induce hypoactivity *via* activation of DA autoreceptors is based on the observation that low doses of these compounds decrease DA release and induce hypoactivity at the same range of doses. Application of this hypothesis to the effects of 7-OH-DPAT is controversial. Several studies show the inhibitory postsynaptic action of DA D3 receptors. A recent study demonstrated that doses of R-(+)-7-OH-DPAT producing reduction of locomotion failed to affect DA release or synthesis rate (Svensson et al. 1994b). Also, preferential D3 antagonists increased spontaneous locomotor activity while DA DA D2 antagonists decreased locomotor activity, supporting the hypothesis that the DA D3 receptor is a postsynaptic receptor with an inhibitory effect on locomotor activity (Waters et al. 1994). However, Gainetdinov and co-workers have reported that 7-OH-DPAT (0.025 mg/kg, ip) decreases DA release in striatum by 30% and in NAS by 50%. 7-OH-DPAT at a

dose of 0.005 mg/kg, ip also decreased locomotor activity by 50% (Gainetdinov et al., 1996). These researchers concluded that the presynaptic effect of 7-OH-DPAT on DA release may account for hypoactivity induced by 7-OH-DPAT. The effects of monohydroxy-2-aminotetralins administered ip may differ from those of the drugs administered sc because of two reasons:

1. 7-OH-DPAT may undergo metabolism following ip administration, resulting in an active hydroxylated metabolite (McDermed et al. 1975; Cannon et al. 1977) with a higher affinity for DA D2 receptors.
2. Rapid absorption of the drug after ip administration compared to the sc route may result in a higher concentration in brain that increases the possibility of binding to DA D2 receptors.

Svensson and colleagues have used the enantiomer R-(+)-7-OH-DPAT, which is more potent than the racemate, and reported that it reduced locomotor activity 50% at 4 nmol/kg (0.0015 mg/kg) by sc administration without reducing DOPA accumulation measured *ex vivo* or DA release measured by microdialysis (Svensson et al. 1994b). Also, it is evident that 7-OH-DPAT decreases locomotor activity as early as 5 min after injection, while the maximum effect on DA release (50%) is observed 1 h after injection. In conclusion, the results from present study provide some support for a possible behavioral action of 7-OH-DPAT at inhibitory postsynaptic DA D3 receptors.

4.10. The interaction of a low dose of 7-OH-DPAT with a high dose of APO in (reserpine + α-MPT)-treated rats

In the present study the interaction between 7-OH-DPAT (0.01mg/kg, sc) and APO (0.05 or 0.1mg/kg, sc) was investigated in DA-depleted rats (using reserpine + α-MPT) to assess the possible postsynaptic effects of 7-OH-DPAT. Classically, DA-depleted rats are used to assess the effects of activation of postsynaptic DA receptors. Reserpine treatment releases DA from storage vesicles and α-MPT blocks DA synthesis. This combination treatment depletes DA and there is no DA to be released from presynaptic neurons subsequent to DA autoreceptor stimulation. Therefore, any effect of DA agonists can be related to postsynaptic DA receptors. It was expected that 7-OH-DPAT would decrease hyperactivity induced by APO *via* probable postsynaptic DA D3 receptor-mediated actions.

Under these conditions there was no significant interaction between 7-OH-DPAT and APO. The doses of APO that resulted in increased activity in DA-depleted rats usually decrease locomotor activity in control animals. This indicates that the DA-depleted rats show a supersensitive response to APO. This supersensitive response of DA D2 receptors may increase the binding of 7-OH-DPAT to DA D2 receptors. A previous experiment (4.11) showed that 7-OH-DPAT was able to decrease hyperactivity induced by a dose of 0.25 (but not of 0.5) mg/kg of APO, suggesting that interactions between 7-OH-DPAT and APO depend on the degree of DA D2 receptor occupancy. Therefore, 7-OH-DPAT may not be able to decrease the effect of APO in the presence of supersensitive DA D2 receptors. A study showed that 7-OH-DPAT up to a dose of 100 μmol/kg did not reverse the reserpine-induced immobility of mice (van Oene et al. 1984). But in rats, 7-OH-DPAT reversed

reserpine-induced immobility at a dose of 1μmol/kg (0.25 mg/kg) and higher (Ahlenius, Salmi, 1994). This dose of 7-OH-DPAT decreased locomotor activity in DA-intact rats in previous experiments (see 3.3.). Therefore, the DA-depleted rat prepared in this way may not provide a suitable postsynaptic model for assessing the interaction between APO and 7-OH-DPAT.

4.11. Time-course of supersensitive response to APO in 6-OH-DA-lesioned rats

In present study the bilateral 6-OH-DA-lesion caused behavioral changes including aphagia and akinesia, indicating an effective lesion (Ungerstedt, 1971). Measurement of DA levels verified an extensive degeneration of DA neurons in both CN and NAS tissues. APO at a low dose which decreased locomotor activity in normal rats induced hyperactivity in lesioned rats. This is consistent with previous studies (Schultz, 1982). The supersensitive response to APO developed as early as 1 day post-lesioning. It has been shown that selectivity of DA autoreceptor agonists is lost in 6-OH-DA-lesioned rats or in rats repeatedly treated with reserpine; autoreceptor agonists (i.e., 2-aminotetralins and 3-PP) induced hyperactivity in these conditions. It has been suggested that in the presence of supersensitive DA D2 receptors and in the absence of endogenous DA the intrinsic activity of autoreceptor agonists may change (Arnt, Hyttel, 1984). In the present experiments, a specific time at which the lesion has been produced but at which DA D2 receptors have not developed supersensitivity was not determined. Since this supersensitive response of DA D2 receptors may decrease the binding of 7-OH-DPAT to DA D3 receptors, this is not a suitable model for assessing the interaction of APO and 7-OH-DPAT in a postsynaptic

model. Therefore, we did not study further the interaction between 7-OH-DPAT and APO in this model.

4.12. Analysis of 7-OH-DPAT in the brain

The method developed in this assay is a convenient means of measuring 7-OH-DPAT concentrations in brain, although it needs some refinement to increase sensitivity. These data show that 7-OH-DPAT passes the blood-brain barrier and that the brain level of this drug is dependent on the dose. In the present assay, the concentration of 7-OH-DPAT in brain tissue increased linearly with the dose (0.3-5 mg/kg). Following systemic injection of a low dose (0.3 mg/kg, sc), the level of 7-OH-DPAT was 0.43 μg/g in whole brain (minus striatum). This level is in agreement with the level of 8-OH-DPAT (following a dose 0.33 mg/kg) reported by Yu and Lewander (1996). Using a microdialysis method, a value of 1.6 μM has been reported for the concentration of 7-OH-DPAT in the striatum following systemic injection of a high dose (6 mg/kg, ip) of the drug (Gainetdinov et al. 1995).

The quantitative study of the brain concentrations of 7-OH-DPAT may prove to be valuable for interpreting the effects of 7-OH-DPAT injected *via* a systemic route.

5. CONCLUSION

The evaluation of 7-OH-DPAT as a DA D3 receptor agonist revealed some unique effects that may be attributed to the higher affinity of this compound for DA D3 receptors.

7-OH-DPAT showed different dose-response curves in habituated and non-habituated rats. In habituated rats doses of 0.01-0.03 mg/kg, sc decreased locomotor activity during a 30 min test session and doses of 0.1-0.3 increased activity after a period of 5-10 min suppression during a 30 min test session. In non-habituated rats, with a baseline of activity about twice that of habituated rats, 7-OH-DPAT in a wide range of doses (0.01-0.3 mg/kg, sc) decreased activity during the test session. This difference may be attributed to the different baseline of activity (i.e., rate-dependent effects).

The dose-response curves for effects of 7-OH-DPAT were equivalent in both CN and NAS, indicating no site-selectivity for the effects of this compound on locomotor activity. Effects of 7-OH-DPAT were specifically mediated by DA receptors, as parallel experiments with 8-OH-DPAT did not show similar effects.

Results from interactions of 7-OH-DPAT or APO with CLZ, SCH 23390 and HAL indicate that the effects of high doses of APO and 7-OH-DPAT may not be mediated *via* similar mechanisms i.e., postsynaptic DA D2 receptor stimulation. The effects of high doses of 7-OH-DPAT and APO were differentially antagonized by CLZ, SCH 23390 and HAL.

7-OH-PAT decreased hyperactivity induced by a high dose of APO in DA-intact rats, which supports the hypothesis that 7-OH-DPAT decreases locomotor activity *via* stimulation of

inhibitory postsynaptic DA D3 receptors. However, 7-OH-DPAT potentiated the effects of APO on locomotor activity in DA-depleted rats; this potentiation effect may be attributed to the development of a supersensitive response of DA D2 receptors.

Systemically administered 7-OH-DPAT passes the blood-brain barrier and the brain level of the drug is dose-dependent. A high concentration of 7-OH-DPAT exists in whole brain (striatum not included) following systemic injection of a high dose of the drug.

6. POSSIBLE FUTURE RESEARCH

Experiments and results from this thesis created several questions and ideas for further research. The reasons for biphasic effects of 7-OH-DPAT in habituated rats are unknown. Parallel measurements of DA release, DA D2 and D3 receptor affinity and locomotor activity may provide more information in this regard.

Differential effects of CLZ on the effects of high doses of APO and 7-OH-DPAT deserve more study. Assessment of interactions of local microinjections of CLZ into the DA related areas such as VTA, SN, NAS, CN and hippocampus, with 7-OH-DPAT may provide greater understanding of mechanisms involved in the effects of high doses of 7-OH-DPAT.

Although 7-OH-DPAT *in vivo* may not show selective DA D3 receptor agonist properties, there are some approaches to study the more DA D3 selective effects of 7-OH-DPAT. EEDQ has been claimed to destroy DA D1 and D2 receptors with less effect on DA D3 receptors. Also, DA D3 receptor antisense oligodeoxynucleotides have been used successfully. Assessment of the effects of DA D3 receptor agonists in the absence of DA D1 and D2 receptors may help to clarify the functional role of DA D3 receptors.

A positron emission topography study may show preferential *in vivo* binding of 7-OH-DPAT or other selective DA D3 receptor agonists in areas of brain in which DA D3 receptors are abundant.

Low doses of APO as a DA autoreceptor agonist has been claimed to show benefits in schizophrenia (Tamminga et al. 1978). 7-OH-DPAT that has been defined as one of the

most selective DA autoreceptor agonist and a putative DA D3 receptor agonist may be used in a clinical study to assess its value in treatment of schizophrenia. Obviously, Further preclinical tests are necessary to prove the safety of this drug prior to clinical applications.

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IMAGE EVALUATION
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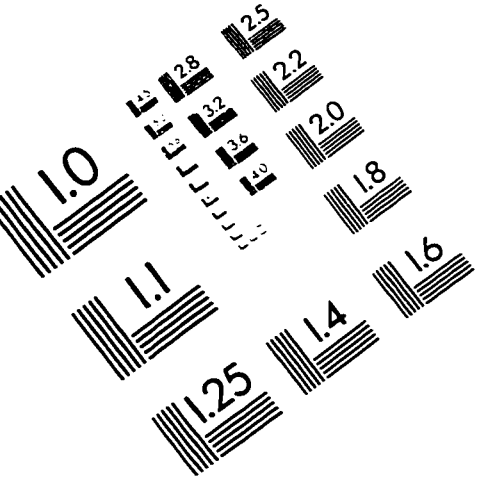

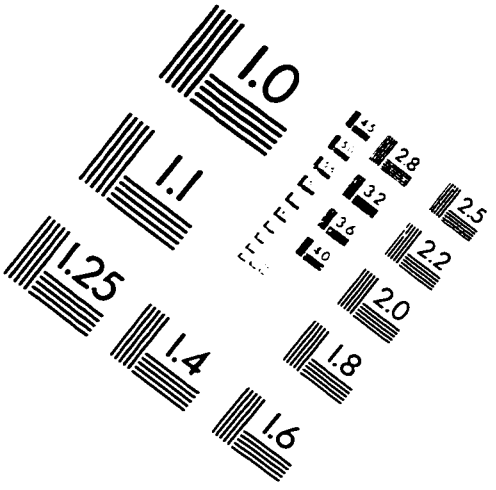

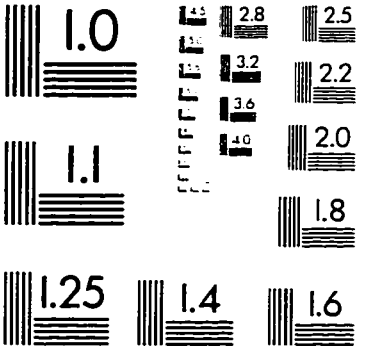

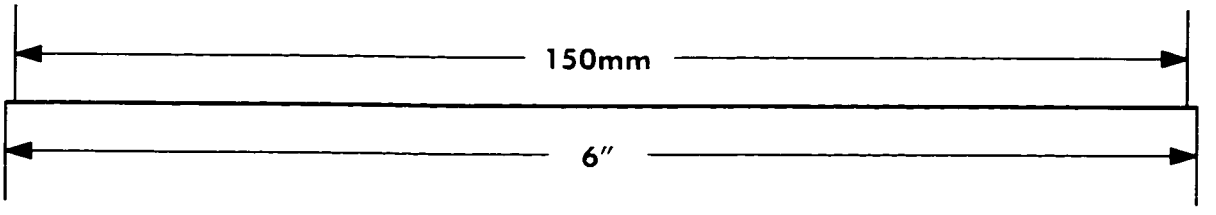

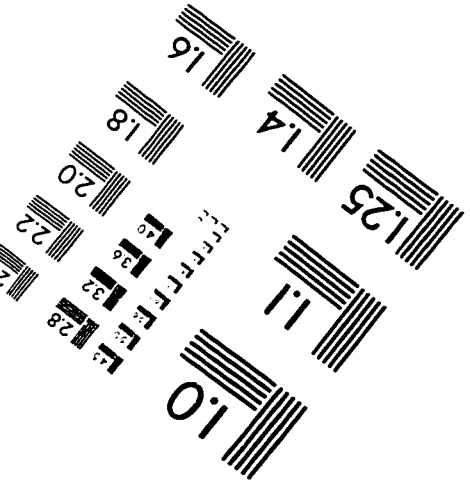

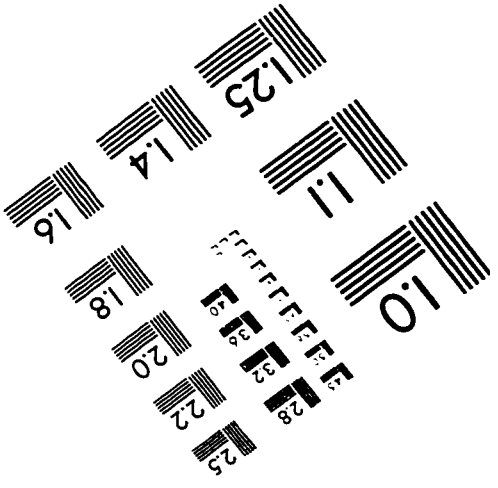

APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989