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EFFECTS OF DOPAMINE D1 AND D2 RECEPTOR AGONISTS ON COGNITIVE FUNCTIONS IN RATS

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Abstract

The dopamine system modulates many functions through its 5 G-protein-coupled receptors. Many studies have identified a synergy in gene expression, neuronal firing and stereotyped behaviours when the D1 and D2 dopamine receptors are concomitantly activated. This project aims to study the effects of co-administrating D1 and D2 agonists on various cognitive functions.

Well established models were selected to evaluate cognitive functions: the Social Interaction Test of olfactory memory, the Delayed Non-Match-to-Sample T-Maze Test of working memory and the Active Avoidance Test of associative memory. We demonstrate that co-administration of D1 and D2 dopamine receptor agonists is deleterious to performance in olfactory and prefrontal-dependent working memory, but has no effects in striatal-dependent associative memory.

The results of this study suggest a role, though detrimental, for the D1 and D2 dopamine receptor synergy in cognitive functions. Additional work will be necessary to properly characterize this role and its mechanism of action.

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List of Abbreviations

D1R	D1 dopamine receptor
D2R	D2 dopamine receptor
DNMSP T-Maze	Delayed Non-Match-to-Sample Paradigm for the T-Maze
GABA	γ-aminobutyric acid
i.p.	Intraperitonneal
i.v.	Intravenous
NAcc	Nucleus accumbens
PFC	Prefrontal cortex
VTA	Ventral tegmental area

1 Introduction

1.1 Dopamine

Dopamine is a major catecholamine present in the mammalian central nervous system. It is synthesized from tyrosine by tyrosine hydroxylase. Its receptors are widely distributed in the brain and it mediates a variety of behaviours, from motor function to motivation, memory and many others (Jaber et al., 1996). Deregulations in various parts of the dopamine system have been attributed to the development of neurological disorders and diseases such as depression, Parkinson's disease and schizophrenia, which has made the study of the dopamine system and its receptors very important to the management and treatment of these diseases.

1.2 Dopamine Synthesis and Neurotransmission

The conversion of tyrosine into dopamine occurs in two steps. Tyrosine is first converted to L-Dopa by tyrosine hydroxylase, which is then converted to dopamine by the aromatic amino acid decarboxylase. Dopamine is then stored in vesicles by the vesicular monoamine transporter 2 (Lawlor & During, 2004).

Upon action potential mediated calcium influx into the neuron terminal, dopamine vesicles dock onto the pre-synaptic membrane and release their contents into the synaptic cleft. The released dopamine diffuses across the synaptic space to the post-synaptic membrane, upon which are located its receptors. Dopamine release is regulated in part by the pre-synaptic re-uptake of dopamine through the dopamine transporter (Giros & Caron, 1993), at which point it is broken down by the monoamine oxidase.

1.3 Neuroanatomy of the Dopamine System

Much of the understanding about the functional role of dopamine has come to light based on research examining neurodegenerative diseases and disorders. More specifically, lesion studies and genetic manipulations in animals as well as pharmacological studies in animals and humans have shed light on the functional role of dopamine. Three major dopaminergic pathways have been identified in the brain, which emanate from the ventral tegmental area (VTA) and the substantia nigra. The breadth of reach of these pathways and the variety of dopamine receptors expressed within them have resulted in dopamine playing a role in mediating a wide variety of behaviours. Though they are often described as distinct pathways, there is often cooperation between the dopamine pathways and thus, multiple pathways may work together to modulate certain behaviours.

1.3.1 The Mesolimbic Dopamine Pathway

The mesolimbic pathway runs from the VTA, in the midbrain, to the nucleus accumbens (NAcc), the medial prefrontal cortex (PFC), the amygdala and other areas of the limbic system (Thomas, Kalivas, Shaham, 2008). Extensive study of the mesolimbic dopamine pathway has found that it is a key player in the mediation of feelings of pleasure, which are linked to reward, motivation and addiction.

Dopamine release in the NAcc is understood to be necessary for reward (Di Chiara & Imperato, 1988). Current theories of addiction generally include alterations dopamine neurotransmission in the NAcc as the mediating factor of the reinforcing effects of addictive drugs. These alterations are seen as an increase in dopamine transmission, either by inhibiting re-uptake or by enhancing release from presynaptic terminals (Di Chiara & Imperato, 1988; Woolverton and Johnson, 1992; Seiden et al., 1993).

The self-administration animal models of drug addiction have led to a much better understanding of addiction. Briefly, animals are trained to press a lever, or to perform any other operant behaviour for which they then receive a drug reinforcement. Animals are generally eager to self administer drugs of abuse, and by using intra-cranial self administration much has been understood about the structures that modulate addiction. Confirming the implication of the mesolimbic dopamine system in the formation of addiction are a series of studies that have demonstrated that 6-Hydroxydopamine (which selectively destroys dopamine neurons; Simola et al., 2007) induced lesions to the NAcc, the VTA or the ventral pallidum almost completely attenuate self-administration of cocaine and heroin, two

of the most addictive drugs (Hubner & Koob, 1990; Roberts et al., 1980; Roberts & Koob, 1982)

More recently, evidence has suggested that heightened activity in the meso-limbic dopamine pathway mediates the positive symptoms of schizophrenia such as hallucinations and delusions (Carlsson, Carlsson & Nilsson, 2004), which has led to one of the current theories of schizophrenia.

Though the mesolimbic dopamine system is known to be highly involved in the addiction process, other brain pathways, including the mesocortical dopamine system (Volkow et al., 1993), have been identified as playing some role in addiction. It appears its role is in modulating the cognitive and executive functions related to addiction.

1.3.2 The Mesocortical Dopamine Pathway

The mesocortical pathway connects the VTA to the cerebral cortex, particularly the frontal lobes. Primarily, this pathway is critical to proper cognitive function, including mainly working memory and executive processes (Sawaguchi & Goldman-Rakic, 1994; Goldman-Rakic, 1996). The effects of dopamine on spontaneous activity in the PFC are inhibitory (Bunney & Aghajanian, 1976; Ferron et al., 1984; Mantz et al., 1988).

Dysfunction in this pathway, more specifically a reduction in activity, is thought to mediate in part the negative symptoms of schizophrenia, such as anhedonia and avolition and impaired judgment (Carlsson, Carlsson & Nilsson, 2004). For example, it was observed that schizophrenic patients have less blood flow in the PFC than controls during the Wisconsin

Card Sort Test and that this is correlated with a decrease in dopamine metabolites that are indicative of pre-synaptic function (Weinberger et al., 1988). Various pharmacological studies of antipsychotic drugs have found that the function of these drugs partially relies on their ability to regulate the function of dopamine receptors (Scatton et al., 1976; Scatton et al, 1977; Matsumoto et al., 1983), suggesting that dopamine helps maintain typical cognitive functions.

1.3.3 The Nigrostriatal Dopamine Pathway

The nigrostriatal pathway joins the substantia nigra and the striatum. This pathway is mostly involved in motor control and movement and is part of the basal ganglia motor loop system. Current theory stipulates that the striatum controls goal-oriented movements through two main pathways, the direct and the indirect, which differently contribute to the basal ganglia output nuclei (the internal segment of the globus pallidus and the substantia nigra pars reticulata) that inhibit cortical projections to the prefrontal and motor cortices though inhibition and excitation respectively (Gerfen, 1992; Gerfen, 2000). The direct projection pathway provides inhibitory input directly to the output nuclei while the indirect pathway projects from the striatum through the external segment of the globus pallidus to project to and inhibit the output nuclei of the basal ganglia (Gerfen 2000).

Confirming that the nigrostriatal dopamine pathway plays a key role in mediating proper motor function is the fact that degeneration of the nigrostriatal dopamine pathway leads to dysfunction of the direct and indirect output pathways from the striatum, and

ultimately results in the pathophysiology of Parkinson's Disease (Obeso et al., 2000; Smith & Kieval, 2000; Onn, West & Grace; 2000) which is largely characterized by impairments of motor control. Moreover, the most effective option for managing the motor impairments associated with Parkinson's disease is treatment with L-Dopa, the precursor to dopamine synthesis (Cools, 2006). Though this treatment does not undo the degeneration of the nigrostriatal dopamine pathway, it does temporarily allow the reinstatement of dopamine signaling, thus lessening the motor impairments.

1.3.4 Dopamine Receptors

Five dopamine receptors, i.e. D1, D2, D3, D4 and D5, have been identified, all of which belong to the super-family of G-protein coupled receptors (GPCRs). Like all GPCRs, the dopamine receptors have a common structure consisting of seven transmembrane domains, an extracellular N-terminal, three extracellular loops, three intracellular loops and an intracellular C-terminal. Similarly to other GPCRs, dopamine receptors are particularly important pharmacological targets. Based on their coupling to adenylyl cyclase, dopamine receptors can be divided into two family subgroups, the D1-like and the D2-like (Jaber et al., 1996). The dopamine receptors are widely distributed in the brain, on dopaminergic neurons and various neurons co-expressing other neurotransmitters.

1.3.4.1 The D1 Family of Dopamine Receptors

The D1-like family of receptors, comprising the D1 and the D5, couples to G_s and activates adenylyl cyclase, thus increasing cyclic AMP levels. D1dopamine receptor (D1R) mRNA has been located in the striatum, NAcc, olfactory tubercle, hypothalamus and thalamus. D1R are also present on the projections of γ-aminobutyric acid (GABA) neurons in the striatum to various areas in the mid-brain (Jaber et al, 1996). D5 receptor mRNA has been located in the olfactory tubercle and the hippocampus.

1.3.4.2 The D2 Family of Dopamine Receptors

The D2-like family of receptors, comprising the D2, D3, and D4, couple to G_i and inhibit adenylyl cyclase thus decreasing cyclic AMP levels. D2 dopamine receptors (D2R) have been located in the striatum, olfactory tubercle, NAcc, substantia nigra pars compacta and in the midbrain. D3 dopamine receptors have been located mainly in the limbic system as well as in the NAcc. D4 dopamine receptors have a higher expression in the frontal cortex, the hypothalamus and the amygdala, but their expression is relatively low when compared to other dopamine receptors.

There are splice variants for some of the D2-like dopamine receptors. One variation of general interest exists in the D2R, where there is a long (D2L) and a short (D2S) form. The D2L can be found pre-synaptically as an auto-receptor or post-synaptically, which differs from all other dopamine receptors that are strictly post-synaptic.

In summary, the relative abundance of the dopamine receptors throughout the mammalian brain, from highest to lowest, appears to be as follows: D1, D2, D3, D5 and D4 (Sealfon and Olanow, 2000; Jaber et al., 1996).

1.4 Dopamine Receptor Synergy

There are numerous accounts of a synergy between the D1R and D2R *in vitro* and *in vivo*. This synergy is observed following treatment with non-specific and specific D1 and D2-like agonists, and can be seen from gene expression to behaviour. The mechanism underlying the observed synergy between D1R and D2R is not well understood, but there are some interpretations relating to protein:protein interactions.

1.4.1 Synergy in Immediate Early Gene Expression

The immediate gene c-*fos* has been found to be a marker of neuronal activation in many brain regions. The expression of these types of genes precedes the transcription of

other genes, thus they are crucial to neuronal function. Fos-like immunoreactivity was quantified by immunohistochemistry in the striatum of rats following acute treatment with SKF 38393 and quipirole (respective D1R and D2R agonists), together or alone, and it was observed that the concomitant stimulation of the D1R and D2R is required for an increase in Fos expression (Lahoste et al., 1993; Svenningsson et al., 2000). Moreover, combined treatment with the D1R and D2R agonists SKF82598 and quinelorane (respective D1R and D2R agonists) also induces *c-fos* mRNA in striatal cholinergic interneurons (Svenningsson et al., 2000). Similar results involving increases in Fos expression were observed by immunohistochemistry following concomitant treatment with SKF 38393 and quinpirole in the frontal and parietal cortices under normal conditions (LaHoste, Ruskin, Marshall, 1996). However, they also noted that treatment with either agonist independently could increase levels of Fos in abnormal conditions, such as following 6-hydroxydopamine induced depletion of dopamine neurons.

1.4.2 Synergy in Neuronal Firing Rates

In vivo neuronal activity can also be evaluated by electrophysiological recordings of neuronal firing rates. The basal ganglia receive neuronal inputs from the substantia nigra pars compacta, an area rich in dopamine expression; thus, recordings from its output neurons reflect changes mediated by dopamine receptor agonists. Recordings from the globus pallidus, a major element of the basal ganglia, showed a potentiated increase in neuronal firing rate when D1R and D2R agonists (SKF 38393 and quinpirole) were intravenously

(i.v.) administered together. Similarly, a potentiated increase in neuronal firing was observed in the globus pallidus following the i.v. administration of the non-specific dopamine receptor agonist apomorphine, which was reversed by treatment with haloperidol, a D2R antagonist (Walters et al., 1987). Similarly, neurons of the substatia nigra pars compacta were found to display a synergistic change in firing rate following bilateral infusions of apomorphine to the ventral-lateral striatum (Waszczak et al., 2002).

1.4.3 Synergy in Stereotyped Behaviour

As previously discussed, many types of behaviour are mediated at least in part by dopamine. Following treatment with D1R and D2R agonists as well as with non-specific dopamine agonists, synergistic increases have been observed in several behaviours. Behavioural stereotypies, described as repetitive oral movements, tongue protrusions, chewing and sniffing, were significantly increased for 20 minutes following bilateral infusions of combined SKF 82958 and quinpirole, or apomorphine, to the striatum. Also, both these treatments led to increases in locomotion over a 45 minute trial (Waszczak et al., 2002). Similarly, treatment with apomorphine to the dorso-lateral striatum was effective in increasing locomotion and grooming, a result that was not obtained by treating with SKF 81297 (a D1R agonist) or quinpirole alone (Presti et al., 2004).

Repetitive and stereotyped behaviours are effects commonly caused by psychomotor stimulants. Consequently, it was found that D1R and D2R activation was required to evoke

cocaine-type stereotypies, the behavioural phenotype of cocaine sensitization (Capper-Loup et al., 2002).

1.4.4 Synergy Occurs Between the D1R and the D2R

Dopamine agonists can be specific to the D1 or the D2 class of dopamine receptor, however, there are no specific agonists that can distinguish between receptors within one class. This is due to the vast sequence homology between receptors of the same class. Therefore, to further study the mechanism behind the D1R and D2R synergy, a group set out to explore whether or not the synergy was mediated by one specific D2-like receptor. Knowing that amphetamine acts as a non-specific dopamine receptors agonist and that it increases Fos expression in the striatum which can be reversed by D1R or D2R class antagonists, LaHoste et al. (2000) found that only the D2R specific antagonist and not the D3 or D4 receptor antagonists reversed the amphetamine induced increase in striatal Fos expression and sniffing behaviour. This suggests that the synergistic activity upon co-administration of the D1 and D2 dopamine class of receptors occurs through the D1R and the D2R.

1.5 Other Areas Under Dopaminergic Control

Though the functional roles of dopamine are often studied in relation to the dopamine pathways, dopamine is also released in other areas of the brain. One such example is the olfactory system, where dopamine plays a role in mediating the GABAergic and glutamatergic neurotransmission that results in olfactory function. Similarly, dopamine is released in the centers that are thought to play a role in learning and memory.

1.5.1 The Olfactory System

The olfactory system is a fundamental part of maternal functions, emotional responses, food selection, the detection of predators and prey, etc. Depending on species, its importance varies greatly. In rats, olfactory receptor neurons within the nasal cavity form the olfactory nerve that projects to the olfactory bulb. The olfactory bulb in turn has projections to higher order olfactory structures and cortices, and other parts of the brain such as the amygdala. Higher order olfactory structures include the piriform cortex (often considered the primary olfactory cortex), the olfactory tubercle and the entorhinal cortex (Shipley & Ennis, 1995; LeBel, Grossman & Barkai, 2001; Brosh & Barkai, 2004, Wilson et al., 2006). There is bidirectional control in much of the olfactory system, and this is particularly observed between the olfactory bulb and the piriform cortex (Wilson et al., 2006).

1.5.1.1 Dopamine Receptor Expression in the Olfactory System

Dopamine receptor mRNA has been located in various parts of the olfactory system, which has one of the biggest populations of dopamine containing neurons in the brain (McLean and Shipley, 1988). The olfactory bulb's glomerular layer, which receives direct input from the olfactory sensory nerves, has intrinsic dopaminergic neurons that contain D1, D2 and to a much lesser extent D3 dopamine receptors (Halasz et al., 1977). More specifically, these dopamine neurons make up 75% of the olfactory glomeruli, which surround the olfactory sensory cell axons. Glomerular dopamine neurons are thought to modulate neurotrasmitter release in glutamate and GABAergic synapses (Gutièrrez-Mecinas et al., 2005; Brünig et al., 1999). The mitral cell layer of the olfactory bulb, whose dendrites permeate into the glomeruli and project out to olfactory cortices also has high levels of postsynaptic dopamine receptor expression (Gutièrrez-Mecinas et al., 2005). Other dopamine-containing areas of the olfactory bulb include the granular interneurons and the plexiform layer. The majority of dopamine receptors expressed in the olfactory bulb are within GABAergic neurons.

There is also much evidence supporting the role of dopamine in the function of the piriform cortex. Dopamine fibers have a heterogeneous distribution in the piriform cortex, as observed by dopamine B-hydroxylase visualization and tyrosine hydroxylase immunohistochemistry (Datiche & Cattarelli, 1996). The piriform cortex receives extrinsic dopamine innervations from the neocortex and limbic regions (Wilson et al., 2006). Also, dopamine has been found to have a variable influence on synaptic transmission within the piriform cortex. Excitatory influence has been suggested to be modulated by indirectly by

noradrenalin release while inhibitory influence appears to be regulated directly by dopamine receptor action (Collins et al., 1985)

1.5.1.2 The functional role of dopamine in olfaction

There is some indication that dopamine action mediates performance in odor discrimination tasks. Briefly, these tasks involve training the animal to associate an odorant with a food-reward. Two bowls are then presented to the animal, one with the food reward hidden below bedding that has been scented with the proper odorant, and the other scented with a similar odorant (difficult task) or a different odorant (easy task). A correct response involves the animal digging in the correct bowl to retrieve the reward without digging into the other bowl (Cleland et al., 2002; Linster & Hasselmo, 1999). Intra-peritonneal injections of L-Dopa, the precursor to dopamine synthesis, have been shown to greatly increased performance in an odor discrimination task when compared to rats injected with saline (Pavlis et al., 2006). Upon investigating the effects of specific dopamine receptor agonists, it was found that the D1R and the D2R have inverse effects on performance in odor discrimination tasks. More specifically, when a D1R agonist or a D2R antagonist was intraperitonneally administered (i.p.), the discrimination of odorants was facilitated. However, when a D1R antagonist or a D2R agonist was administered (i.p.), performance in odorant discrimination was significantly impaired (Yue et al., 2004).

These reports indicate that dopamine modulates olfaction in some way; however, because the drugs were administered intra-peritoneally, they not only affected the dopamine

receptors in the olfactory bulb and cortices, but also throughout the brain. Processes like locomotion and motivation, which are also modulated by dopamine, play a role in the reward-driven olfactory discrimination task and thus, any changes in these functions may confound results.

Interestingly, functional alterations in the piriform cortex have been observed following odour learning tasks. Long-term potentiation and long-term depression are synaptic modifications that are considered to be indicative of learning. Electrophysiological analyses of piriform cortex synapses indicate that learning of reward-paired odours induces modifications in synaptic transmission, which in turn entail physiological changes (Saar et al., 1999; Brosh & Barkai, 2004; Lebel et al., 2001). These reports confirm that olfactory learning occurs at least in part in the piriform cortex.

1.5.2 Learning and Memory

Cognitive functions such as learning and memory have been the focus of much research. Various centers of the brain are thought to play an active part in the acquisition of learning and the formation of memories, and many neurotransmitter systems appear to work in the regulation of these processes. It has been found that dopamine has a modulatory effect on learning and memory. Many neurodegenerative diseases and disorders in which dopamine signaling is impaired are often accompanied by cognitive disorders (El-Ghundi et al., 2007). Also, treatment of Parkinson's disease with L-Dopa, which increases levels of dopamine in the brain, has been found to cause both positive and negative effects on cognitive functions

(Cools, 2006). Also supporting the role of dopamine in modulating learning and memory are pharmacological studies with animals that have shown that the manipulation of dopamine neurotransmission alters performance in behavioural tests.

1.5.2.1 Dopamine Receptor Expression in Learning and Memory Centers

Dopamine innervates many of the brain areas responsible for learning and memory: the hippocampus, the PFC, the amygdala and the ventral and dorsal striatum. Certain features of learning and memory have been ascribed to particular structures, such as the PFC which is highly involved in working memory (the ability to actively maintain information available for further processing - Goldman-Rakic, 1996; Baddeley, 1992), and the hippocampus which is highly involved in spatial and contextual information (Jarrad, 1993; Eichenbaum et al., 1999). For example, the entire forebrain contains dopamine innervations, and the proper function of dopamine in the PFC has been shown to be essential to working memory processes. Prefrontal depletions of dopamine in rats and monkeys are deleterious to working memory performance in a delayed alterations task, and this can be reversed by treatment with L-Dopa or apomorphine (Brozoski et al., 1979; Bubser, Schmidt, 1990). However, the discovery of various integrative pathways and loops that link the key areas of cognitive functions to one another have led researchers to understand that structures involved in learning and memory may cooperatively work together rather than work independently to achieve proper cognitive performance.

1.5.2.2 Effect of Dopamine in Mediating Integrative Memory Pathways

Much evidence supports that prefrontal dopamine transmission functionally interacts with the ventral hippocampus and vice versa, which is thought to be a major element to the integration of learning processes (Peleg-Raibstein et al., 2005). Also, cortical dopamine is thought to play a role in mediating the activity of the integrative cortico-subcortico-cortical loops (Nieoullon & Coquerel, 2003), which are essential to proper memory function. Likewise, long-term potentiation and long-term desensitization in the cortico-striatal memory pathways have been shown to be dependent on cortical and striatal dopamine activity (Calabresi et al., 1996; Chudasama & Robbins, 2006).

The breadth of dopamine expression and innervation in the structures and pathways of cognitive function highly support the role dopamine plays in mediating learning and memory functions.

1.6 Project Outline

With this project, we intend to further study the synergy between the D1R and D2R. More specifically, we aim to study whether the concomitant activation of these receptors will induce synergistic effects on memory functions, as it has in a variety of other functions. Based on the existing data regarding the synergy between the D1R and the D2R, we

hypothesize their concomitant activation by treatment with SKF 38393 and quinpirole will be synergistically beneficial to performance in memory oriented tasks. Specifically, the following objectives have been elaborated and will be addressed by this project:

1. To evaluate the effects of D1R and D2R agonist co-administration on the acquisition and retention of olfactory memory using the Social Interaction Test.
2. To evaluate the effects of D1R and D2R agonist co-administration on the retention of PFC-dependent working memory using the Delayed Non-Match-to-Sample Paradigm in the T-Maze.
3. To evaluate the effects of D1R and D2R agonist co-administration on the acquisition of striatal-associated associative memory using the Active Avoidance Test.
4. To evaluate the effects of D1R and D2R antagonist administration on olfactory memory using the Social Interaction Test.

2 Methods

2.1 Animals

Adult male Fisher-344 weighing between 225-250g, purchased from Charles-River laboratories Canada, were used for all tests. Adult male Long-Evans rats, weighing between 225-250g, purchased from Charles River laboratories, were used as the stimulus rat in the Social Recognition Test of olfactory memory. Animals were individually housed upon arrival and were given at least 5 days to habituate to the vivarium before testing or surgeries began. Standard conditions, including a 12 h light/dark cycle (lights on at 7:00 am and off at 7:00 pm), a temperature between 22 and 23 degrees Celsius and humidity levels between 35 and 50 %, were maintained in the vivarium at all times. Cages were lined with woodchips and free access to food and water was granted, unless otherwise noted. All procedures were in accordance with the guidelines established by the Canadian Council on Animal Care as approved by the uOttawa Institute of Mental Health Research Animal Care Committee.

2.2 Behaviour Room

All tests were conducted in an area surrounded by black curtains from ceiling to floor. A radio insured that constant noise was maintained in the room in order to diminish the

effects of any unpredictable external noise. The apparatus surfaces were cleaned with a 70% alcohol solution and allowed a chance to fully dry between trials with different animals.

2.3 Social Recognition Test: a measure of spontaneous olfactory memory

The procedure was generally the same as previously described (Millan et al. 2007). Adult Long-Evans rats were used as the stimulus rats. On the first testing day, adult Fischer-344 rats, in their home-cages, were placed in the behaviour room for a 5-minute habituation period. Immediately following the habituation, a stimulus rat was introduced into the home-cage for a 5-minute trial (Trial 1), followed 20-24 hours later by a second trial (Trial 2) with the same stimulus rat. The habituation and each trial was recorded by video and analyzed by blind observers. The time spent sniffing the stimulus rat, from head to tip of tail, was recorded. Sniffing was characterized by the proximity of the rat of interest's nose to the stimulus rat (touching or very near touching), and a faint but visible wiggle of the rat of interest's nose.

The Social Recognition Test was used to test the effects of the agonist administration on the acquisition of olfactory memory (by administering the agonist only before Trial 1; intraperitonneally or through cannulae injections to the piriform cortex) and the retention of olfactory memory (by administering the agonists i.p. only before trial 2). Also, the effects of pre-treating with D1 or D2 receptor antagonists (i.p. or through cannulaes to the piriform cortex) before the i.p. agonist co-administeration on the acquisition of olfactory memory was assed.

2.4 Modified Home-Cage Olfactory Discrimination Test: a spontaneous assessment of olfactory function

A modified home-cage was built to allow the separation of used and new bedding. A clear plexi-glass insert (1.5 inches in height) was slid into the centre of a cage and held into place by a plexiglass track mounted on either side of the cage, thus separating the cage into 2 areas. The procedure was essentially the same as previously described (Prediger et al., 2005). One side of the modified home-cage was filled with new bedding up to the level of the insert, and the other side was filled with the bedding from the animal's home-cage that had not been changed for at least 4 days prior to testing, and thus was soiled. A 5-minute trial was recorded onto video, during which rats had unrestricted access to either side of the modified home-cage. The time spent on either side was the measure of interested. The starting position for each rat was randomly assigned.

2.5 Delayed Non-Match-to-Sample Performance (DNMSP) in the T-Maze: a measure of working memory

The apparatus for this test consists of an Elevated Plus Maze with a door blocking access to one of the enclosed arms, as previously described (Canal et al., 2005). Two of the three remaining arms were open, while one was enclosed in walls that were 24 inches high. The enclosed arm was always used as the start position.

Pre-test training lasted 2 or 3 days, and required rats to roam freely and traverse the entire maze in less than 2 minutes. The acquisition training lasted 6 days. Each animal had 10 trials per acquisition day. Each trial included a simple run and one choice run. During the simple run, one arm was blocked off and a food reward was placed at the end of the opened arm. The rat was then returned to his home-cage for 0.5 minutes before the choice run, during which both arms were opened and the food reward (approx. 0.2 g of sweet powdered food) was placed in the newly opened arm. The testing phase lasted 4 days, during which the rats did 9 trials per day. The simple run and choice run were exactly the same as during the acquisition phase, however the interval between them was of 0.5, 1 or 2 minutes.

Rats were placed on a food deprivation diet one week before the testing (Canal et al., 2005). Rats received approximately 15 g of a food and reward mixture, as long as their weight did not drop below 80 % of their baseline weight. If their weight did drop to 80 % of the baseline weight, the amount of food given was adjusted as to prevent further weight loss.

2.6 Active Avoidance Test: a measure of associative learning

The apparatus consists of a two-way shuttle box, complete with stimulus light in the lid of each of the two compartments, electrified steel bars controlled by a programming/recording unit as a floor and a base equipped with transmitting/receiving infrared signals to record animal position in one dimension (TSE systems). The procedure was fundamentally the same as previously described (Montero-Pastor et al., 2004; Ulloor and Datta, 2005). The testing took place over 6 days, and one testing session was administered

each day. The testing session began with 10 minutes habituation, followed by 30 trials. Each trial was separated by a variable inter-trial interval of 30 seconds ± 15 seconds. Each trial consisted of a conditioned stimulus (light) presented in the target compartment (i.e. the compartment not occupied by the animal) for a duration of 5 seconds, followed by an unconditioned stimulus (electrical foot-shock; 0.5 mA) that was administered for 10 seconds in the presence of the continued conditioned stimulus. If the animal moved to the target compartment during the unconditioned stimulus, the shock was stopped. A response was evaluated as correct if the animal moved to the target compartment during the phase of conditioned stimulus, which was the only way to avoid the unconditioned stimulus. The lights in the behaviour room were kept off during this test to ensure visibility of the conditioned stimulus (light) in the target compartment. All data was automatically recorded by the software provided by the manufacturer.

2.7 Open Field: a measure of hyperactivity and anxiety

The apparatus, made of opaque plexiglass, had a floor of 36 inches x 3 inches (divided by black lines into 36 squares of 6 inches x 6 inches) and walls were 6 inches high. Each rat was randomly placed in a corner of the Open Field, and the number of total squares crossed by the hind legs and the total time spent rearing were evaluated over a 6 minute trial. Also evaluated was the proportion of time spent in center squares versus the time spent at the perimeter as a measure of anxiety.

2.8 Cannulae implantations

Under anaesthesia with a mixture of isofluorane and oxygen, rat was placed on the stereotaxic frame. The skull was exposed and the coordinates were located from Bregma (1.70 mm forward, +/- 3.90mm lateral). A drill was used to expose the brain in the desired locations and two cannulae were lowered to the proper depth (7.60 mm) in the bilateral piriform cortices. The cannulae were secured to the skull with dental cement. After surgery, animals were placed under a heat lamp (250 watts) until they recovered from the anaesthesia and received temgesic (0.03 mg/kg) twice daily for 3 days as an analgesic. Rats were allowed a full recovery of 10 days before testing began.

All drugs were freshly prepared each day. The total volume administered through each cannulae was of 1 μl, and the cannulae was held in place for 30 seconds following agonist administration.

2.9 Histology

After the behavioural testing was complete, cannulae locations were verified by cresyl violet staining. Animals were anesthetised with 40% chloral hydrate and perfused intracardially with 200 ml of 0.1 M phosphate buffered saline, followed by 400 ml of 4%

buffered Formalin. After decapitation, brains were extracted and left in 4% buffered Formalin for 18-24 hours, then in 40% sucrose solution for 48 hours. Brains were cut on a sliding microtome (Leica) at a thickness of 40 μm. The sections were mounted onto glass slides and then stained with cresyl violet. Briefly, slides were sequentially incubated in xylene, decreased concentrations of alcohol and water, followed by incubation in 0.1% cresyl violet for 3-10 minutes. After a brief washing with water, slides were dehydrated with increased concentration of alcohol and then cleared with xylene before cover-slipped. Cannulae locations were deemed correct if the tips of the cannulae were within the piriform cortex.

2.10 Data Analysis

Results were reported as mean ± SE. Statistical analysis of the data was performed using a one-way ANOVA, or a one-way ANOVA for repeated measures, followed by LSD *post hoc* analysis. Statistical significance was set at $p < .05$.

3 Results

3.1 Effects of Dopamine Receptor Agonist Co-Administration on Olfactory Memory

In order to investigate the effects of D1R and D2R agonists on the acquisition of olfactory memory, SKF 38393 (3 mg/kg) and quinpirole (2 mg/kg) were i.p. administered alone or in combination 30 minutes before Trial 1 of the social recognition test (n = 8 per group). As shown in Figure 1, one-way ANOVA revealed significant group effects in both Trial 1 ($F_{(3, 28)} = 7.929$, $p < .001$) and Trial 2 ($F_{(3, 28)} = 12.119$, $p < .001$). The LSD post hoc test further revealed that, in Trial 1, rats receiving the co-administration of D1R and D2R agonists displayed a significant decrease in the time to sniff the stimulus rat when compared to rats receiving vehicle, D1R agonist, or D2R agonist treatment ($p < .05$). In trial 2, both D2R and D1R+D2R agonist treated groups, but not D1R agonist-treated group, have significantly longer times to sniff the stimulus rat than control animals ($p < .005$). Taken together, these data suggest that co-administration of D1R and D2R agonists impairs the acquisition of olfactory memory.

However, it is also possible that decreased sniffing time by co-administration of D1R and D2R agonists resulted from an impairment of olfactory function by both agonists together. To examine this possibility, we conducted the modified home-cage version of an olfactory discrimination task following i.p. injections of vehicle (n = 7), SKF 38393

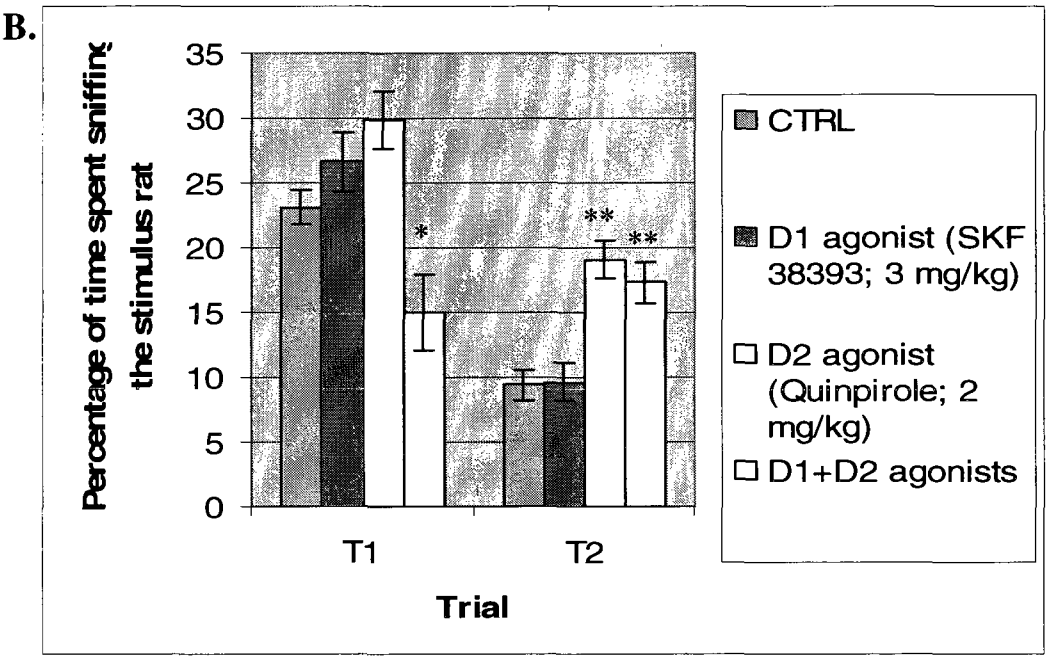


Figure 1: The effects of D1 and D2 dopamine receptor agonist administration on the acquisition of olfactory memory. Systemic administration of D1R and D2R agonists before trial 1 of the Social Recognition Task (as described by figure **A**) significantly decreases the time spent sniffing the stimulus rat during trial 1 (**B**) (one-way ANOVA; $p < .05$). Rats in the D2R and combined D1R+D2R agonist groups have

(3 mg/kg; n = 8), quinpirole (2 mg/kg; n = 8) or SKF 38393 and quinpirole together (n = 8). As shown in Figure 2, one-way ANOVA did not show significant group effects ($F_{(3, 27)} = 1.671$, $p = .197$), suggesting that olfaction itself was not affected by the co-administration of D1R+D2R agonists. Therefore, our above findings that the co-administration of D1R and D2R agonists significantly decreased sniffing time in Trial 1 suggest an impairment in the acquisition of olfactory memory by D1R and D2R agonists.

The above experiments were conducted by an i.p. injection of D1R and D2R agonists, which were not able to provide any clues for answering the important question of through which brain region the co-administered D1R and D2R agonists impaired the acquisition of olfactory memory. Because the piriform cortex has been shown to play an important role in olfactory memory, we explored the possible involvement of piriform cortex in the impaired aquisition of olfactory memory following a combined treatment of D1R and D2R agonist. Rats were bilaterally implanted with cannulaes targeting the piriform cortex, the primary olfactory cortex (Roesch et al., 2007), and received either vehicle (n=6), SKF 38393 (30 μg per side, n=8), quinpirole (20 μg per side, n=7) or both agonists together (n=7). As depicted by Figure 3, there was a significant group effects with one-way ANOVA analysis ($F_{(3, 24)} = 3.235$, $p < .05$). The LSD post hoc test further show that the combined treatment of SKF 38393 and quinpirole to the piriform cotex prior to trial 1 significantly decreased the time spent sniffing the stimulus rat when compared to the vehicle group or the groups that received either agonist alone ($p < .05$). Thus, it appears that either i.p. or intra-piriform injection of D1R and D2R agonists together significantly impairs the acquisition of olfactory memory.

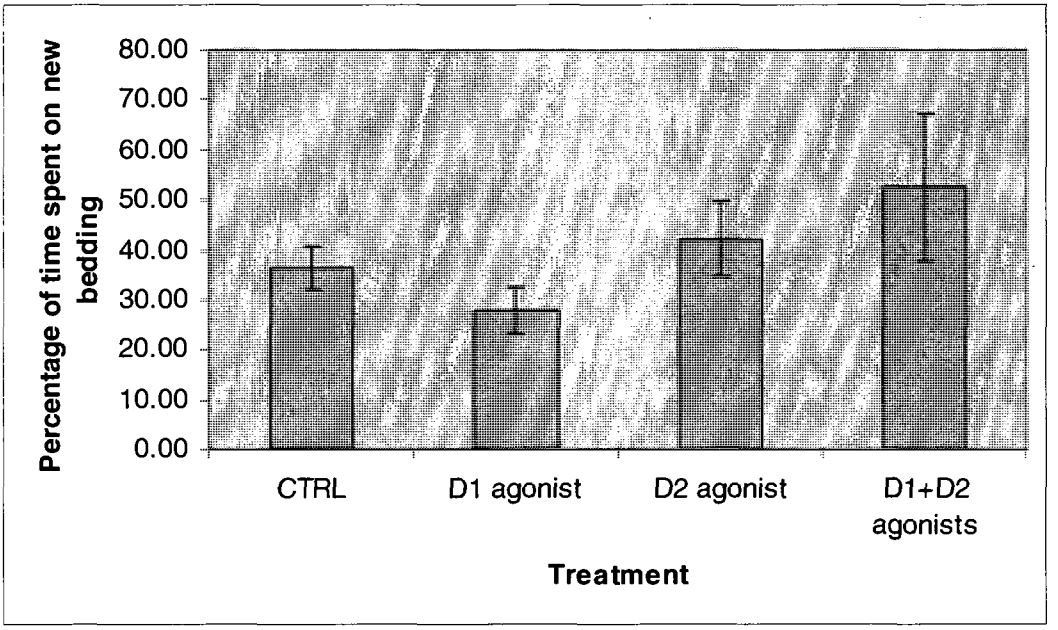


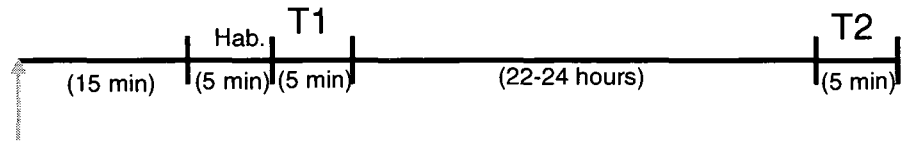
Figure 2: The effects of a systemic co-administration of dopamine receptor agonists prior to the modified home-cage version of an olfactory discrimination task. Systemic treatment with SCH 38393 (D1R agonist; 3 mg/kg) and/or quinpirole (D2R agonist; 2mg/kg) does not significantly alter olfactory discrimination functions when compared to control animals, as assessed by the modified home-cage version of an olfactory discrimination task (one-way ANOVA). Control: n = 7; D1: n =8; D2: n =8; D1+D2: n = 8

In order to investigate the effects of administrating D1R and D2R agonists on the recall of olfactory memory, SKF 38393 (3 mg/kg) and quinpirole (2 mg/kg) were administered alone or in combination 30 minutes before Trial 2 of the social recognition test (n = 8 per group). One-way ANOVA did not show significant group effects ($F_{(3, 28)} = 1.062$, p = .381; Figure 4), suggesting that the administration of D1R agonist, D2R agonist or both did not significantly affect the recall of olfactory memory.

3.2 Effects of Pre-Treatment with Dopamine Receptor Antagonists on Agonist Co-Administration in Olfactory Memory

The above results are not conclusive, considering that the D1R agonist SKF 38393 and the D2R agonist quinpirole may non-selectively act on other receptors. To explore this possibility, we conducted another experiment, in which rats received a systemic or bilateral intra-piriform injection of vehicle, SCH 23390 (a D1R antagonist) or raclopride (a D2R antagonist), followed by an i.p. co-administration of SKF 38393 and quinpirole, 30 minutes before trial 1 of the Social Recognition Test. When the antagonists were systemically administered 30 minutes before the co-administration of agonists (vehicle: n = 10; SCH 23390: n = 10; raclopride: n = 11), there was no significant group effect with one-way ANOVA analysis ($F_{(2, 28)} = 0.933$, p = .405), suggesting that SCH23390 (0.1 mg/kg) or raclopride (0.2 mg/kg) did not significantly alter the effects produced by co-administration of D1R and D2R agonists (Figure 5). However, when the antagonists were administered

A.



B.

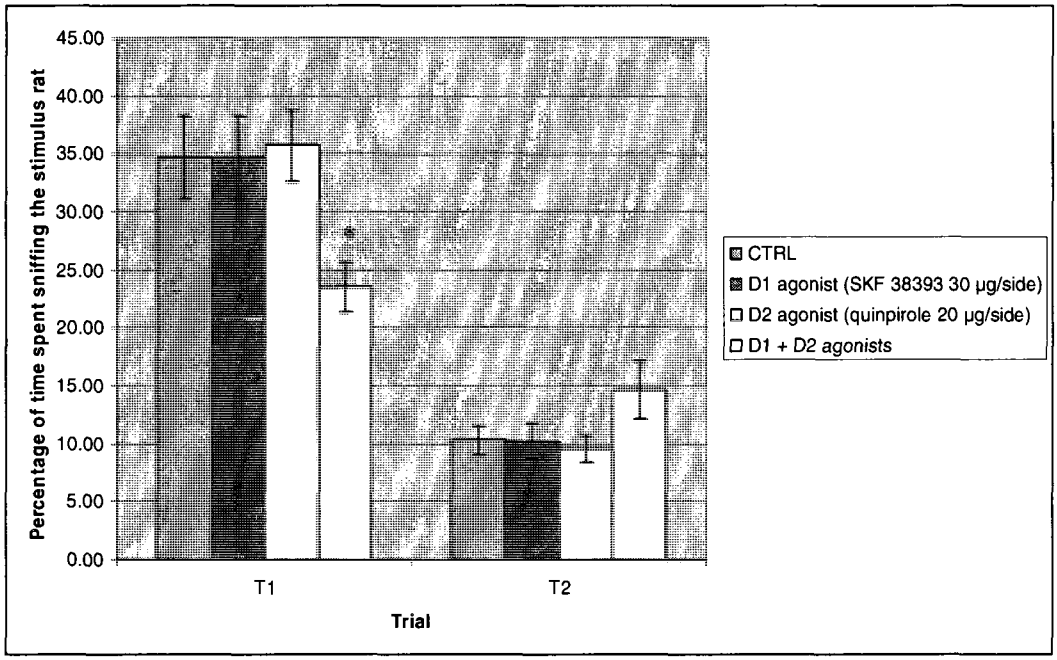
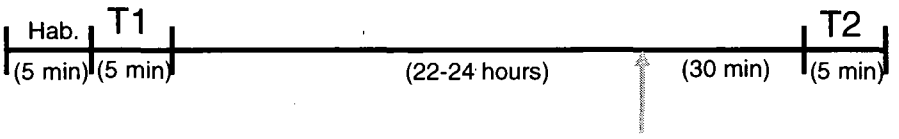


Figure 3: The effects of co-administration of D1R and D2R agonists to the piriform cortex on olfactory memory acquisition as measured by the Social Recognition test. Agonist co-administration before trial 1 of the Social Recognition Task (as described by figure **A**) significantly decreases the amount of time spent sniffing the stimulus rat in the first trial of the social recognition task (**B**) (one-way ANOVA; $p < .05$). Control: n = 6; D1: n =8; D2: n =7; D1+D2: n =7

A.



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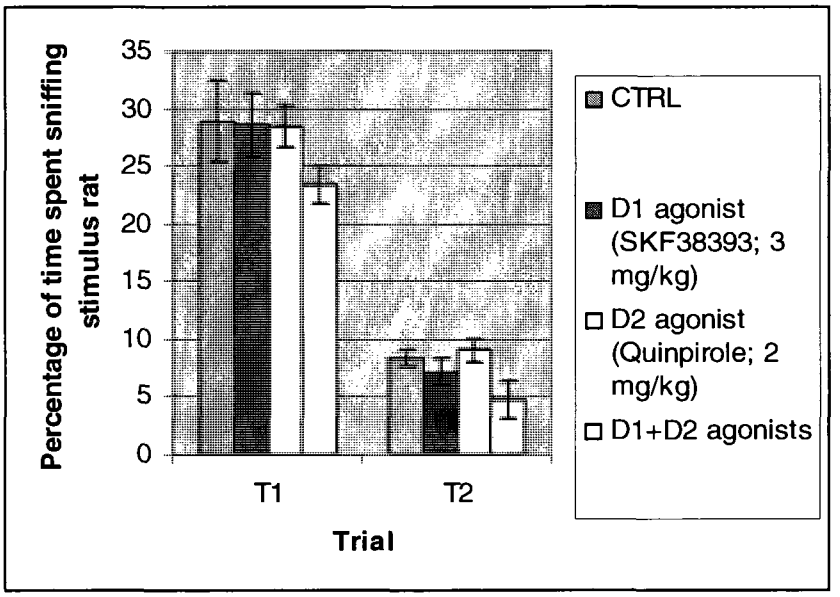

Figure 4: The effects of the systemic co-administration of D1R and D2 dopamine receptor agonists prior the second trial of the Social Recognition Test of olfactory memory (as described by figure **A**) does not alter the amount of time spent sniffing the stimulus rat (**B**). Control: n = 8; D1: n =8; D2: n =8; D1+D2: n = 8

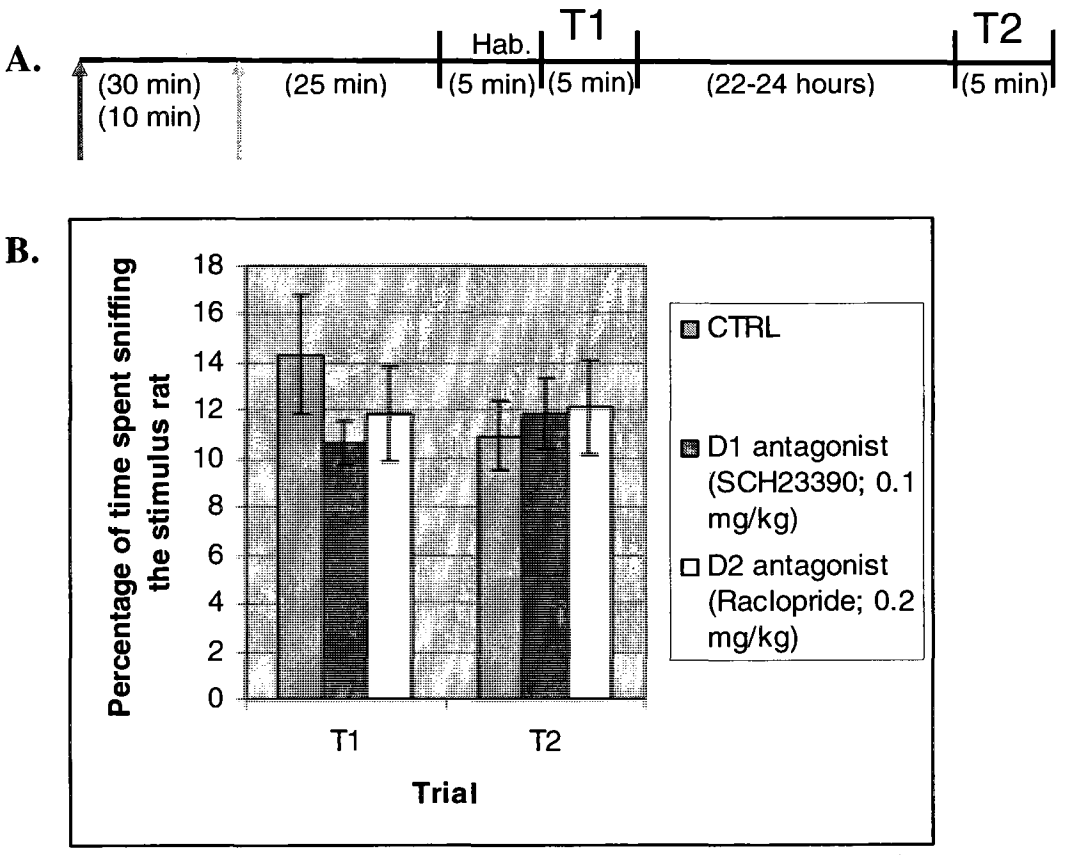

Figure 5: The effects of pre-treatment with antagonists on the co-administration of agonists before the first trial of the Social Recognition Task (as described in figure **A**). Systemic pre-treatment with D1R or D2R antagonist does not alter the effect of i.p. co-administration of D1R and D2R agonists before the acquisition phase of the Social Recognition Test (**B**) (one-way ANOVA). Control: n = 8; D1: n =8; D2: n =8; D1+D2: n=8.

through bilateral cannulaes implanted in the piriform cortex 10 minutes before the co-administration of agonists (n = 8 per group), there was a significant group difference (one-way ANOVA: $F_{(2, 21)} = 5.521$, $p < .05$). Specifically, the post hoc analysis showed that pre-treatment with the D2R antagonist raclopride (5 μg per side) significantly decreased the time spent sniffing when compared to the vehicle pre-treated group ($p < .005$; Figure 6). Rats pre-treated with the D1R antagonist SCH 23390 (5 μg per side) appeared to sniff less than vehicle pre-treated rats, but the difference was not statistically significant.

3.3 Effects of Dopamine Receptor Agonist Co-Administration on Working Memory

The results obtained in the Social Recognition Test suggest an impairment of olfactory memory induced by the co-administration of D1R and D2R agonists, but it is unknown whether co-administration of D1R and D2R agonists affects other forms of memory. To answer this question we first conducted the DNMSP T-Maze Test, which has widely been used to examine the PFC-dependent working memory (Ramos et al, 2003). SKF 38393 (3 mg/kg) and quinpirole (2 mg/kg) were i.p. administered alone or in combination 30 minutes before each testing phase session of the DNMSP T-maze (n = 6 per group). Prior to the testing phase, all groups had successfully completed the acquisition phase of the test at the same rate (one-way ANOVA: $F_{(3, 20)} = 0.048$, $p = .986$), during which no drugs were administered (Figure 7A). Pretreatment with D1R and D2R agonists before the testing phase produced a significant group effect on performance in the DNMSP T-maze (one-way

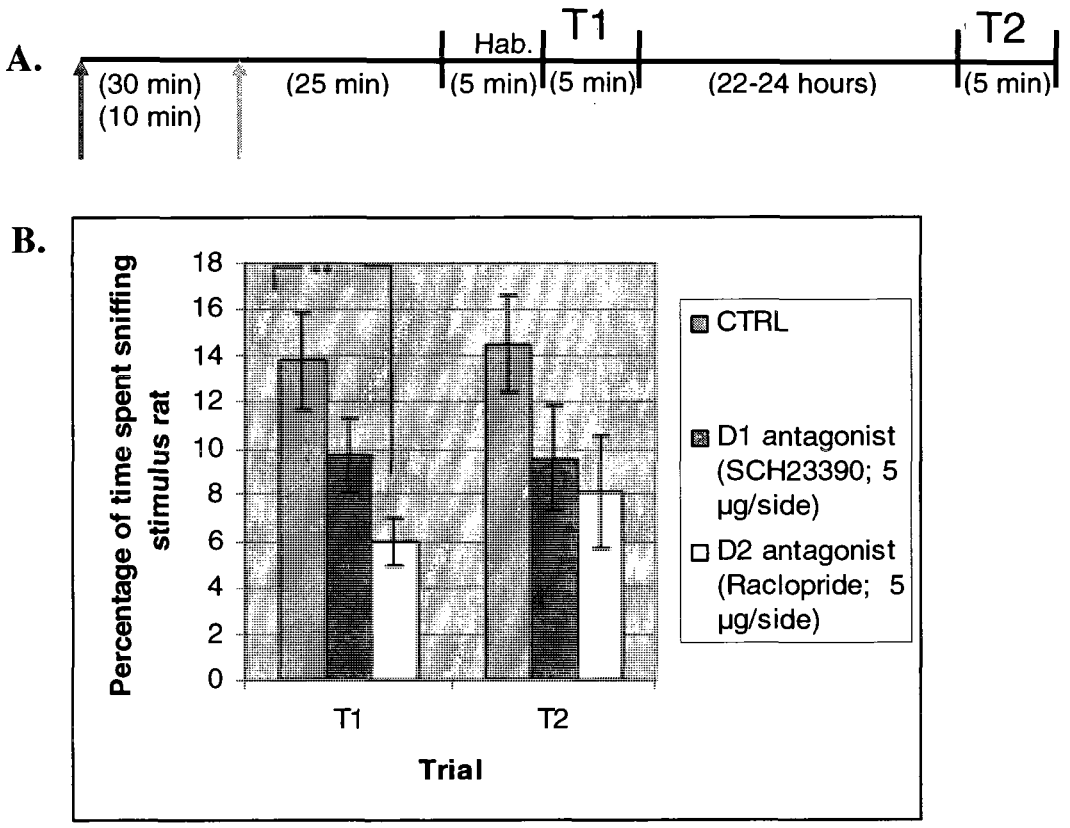

Figure 6: The effects of pre-treatment with antagonists to the piriform cortex on the co-administration of agonists in the acquisition phase of the Social Recognition Test (as described in figure **A**). Pretreatment with a D2R antagonist to the piriform cortex significantly alters the effect of D1R and D2R agonist co-administration on sniffing the stimulus rat in the Social Recognition Test (**B**) (one-way ANOVA). Control: n = 6; D1: n =6; D2: n =7;

ANOVA: $F_{(3, 20)} = 6.276$, $p<.005$). Specifically, as demonstrated by Figure 7B, animals receiving the combined treatment of D1R and D2R agonists during the testing phase had a significantly lower rate of correct responses at each inter-trial interval when compared to vehicle treated and individual agonist treated groups (LSD post hoc test, $p<.005$). No significant differences were observed upon single administration of either agonist, similarly to the effects observed in the test for olfactory memory.

3.4 Effects of Dopamine Receptor Agonist Co-Administration on Associative Memory

To further answer the question of whether the co-administration of D1R and D2R agonists affects other forms of memory, we conducted the Active Avoidance Test to examine associative memory. SKF 38393 (1.5 mg/kg) and quinpirole (0.5 mg/kg) were i.p. administered alone or in combination 10 minutes before each session in the Active Avoidance Test ($n = 8$ per group). There was a significant group effect (ANOVA for repeated measures: $F_{(3, 20)} = 20.607$, $p < .001$), mediated by the treatment with the D2R agonist as determined by post hoc analysis ($p < .001$). Although an increased rate of correct responses was seen in the combined treatment group, this appears to have been mediated by the D2R agonist and not the combined treatment as there was no significant difference between the performances of these two groups (Figure 8).

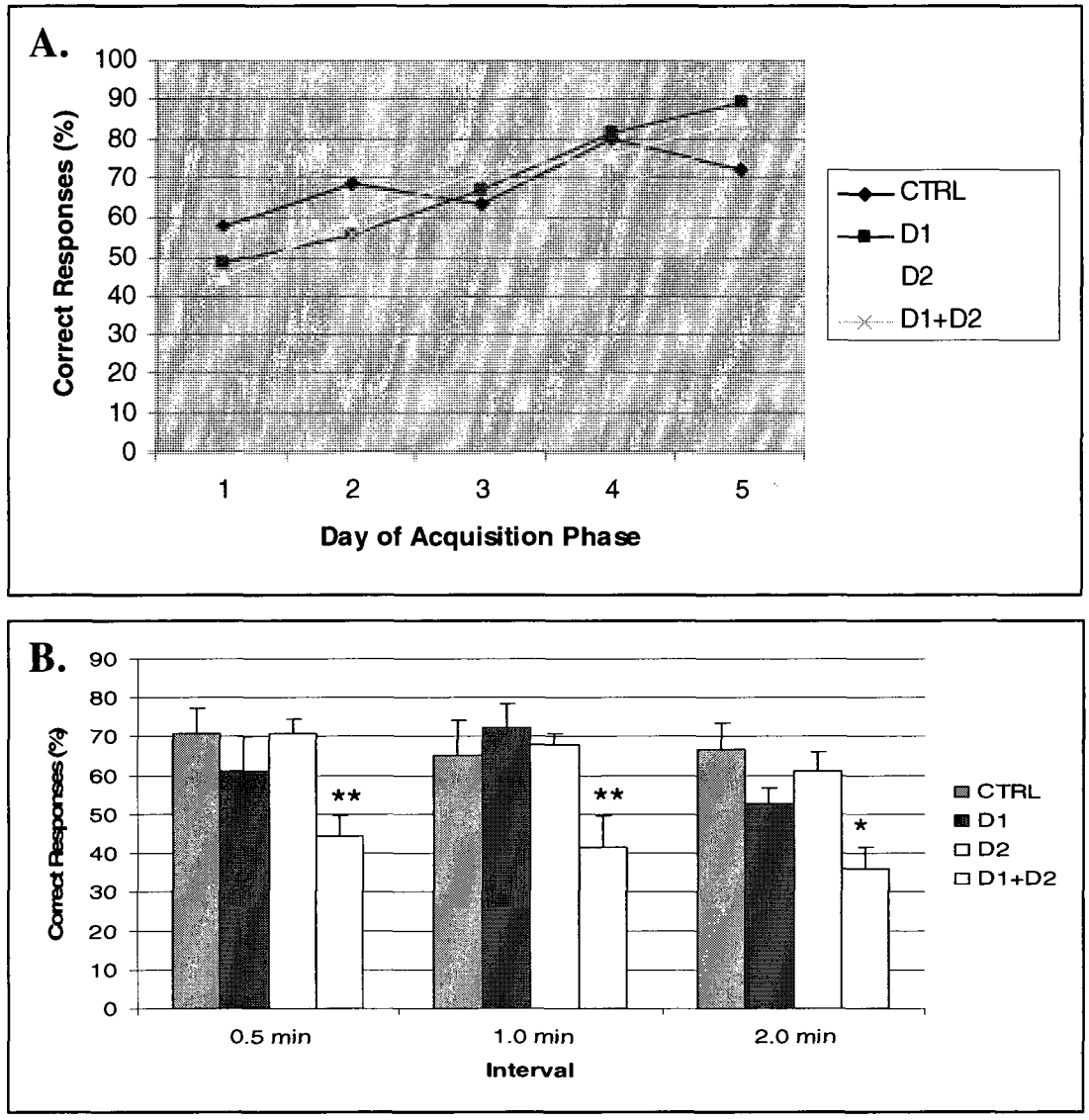

Figure 7: The effects of dopamine agonist administration on working memory. **(A)** All rats perform at the same rate during the acquisition phase of the DNMSP T-Maze Test, when no treatment is administered (one-way ANOVA). **(B)** Systemic combined treatment with D1R and D2R agonists (SKF38393 3 mg/kg and quinpirole 2 mg/kg respectively) is deleterious to performance of working memory at all interval lengths tested by the testing phase of the DNMSP T-Maze Test (one way ANOVA; $p < .05$). Control: n=6; D1: n=6; D2: n=6; D1+D2: n=6.

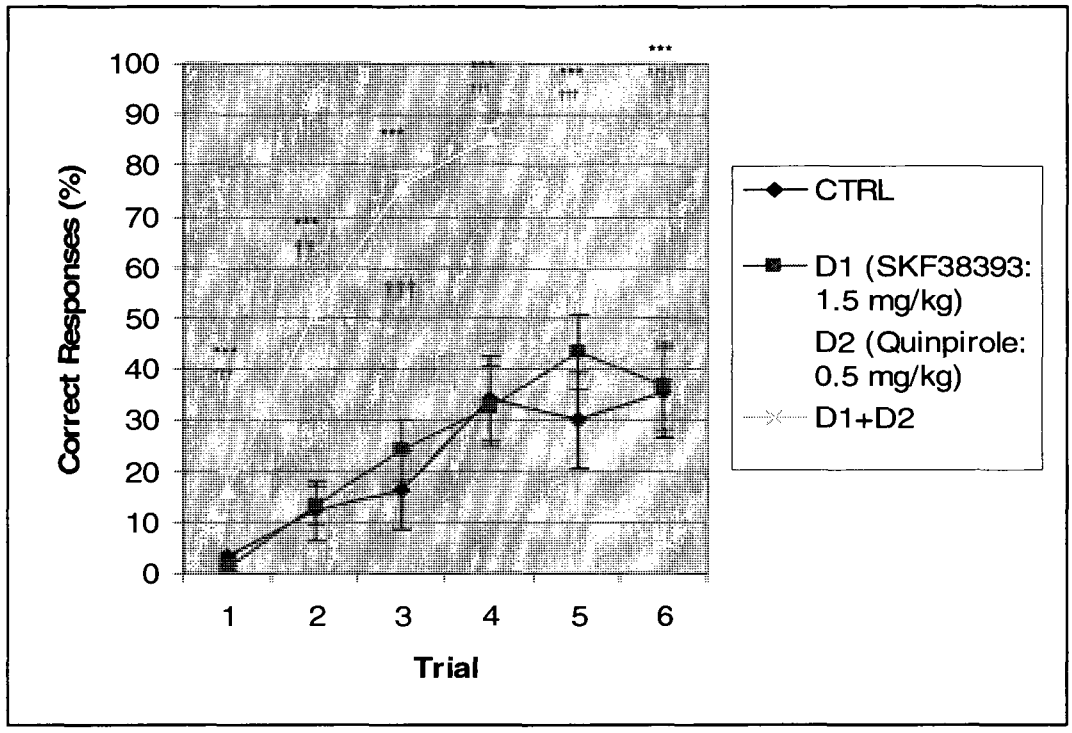

Figure 8: Systemic treatment with quinpirole (0.5 mg/kg, ***: $p < .001$) and the combined administered quinpirole and SKF 38393 (1.5 mg/kg, †††: $p < .001$) enhanced performance in associative memory, as tested by the Active Avoidance Test. n=8 per group.

3.5 Effects of Dopamine Receptor Agonist Co-Administration on Locomotion and Anxiety

In summary, we have used the Social Recognition Test, the DNMSP T-Maze Test and the Active Avoidance Test and found that the combined treatment of D1R and D2R agonist has deleterious effects on olfactory memory and working memory, while the treatment with a D2R agonist increases performance in associative memory. These results are not conclusive in themselves, given that the deleterious effects on different forms of memory may be produced by a possible suppression of motor activity or anxiety instead of actual decreased memory performance.

To investigate this possibility, we examined the effects of D1R and D2R agonists on locomotion and anxiety 30 minutes after agonist administration. Rats were systemically injected with vehicle, SKF 38393 (3 mg/kg), quinpirole (2 mg/kg) or SKF 38393 and quinpirole 30 minutes prior to the Open Field test (n = 8 per group). One-way ANOVA revealed no significant group effects in terms of locomotion at ($F_{(3, 28)} = 0.473$, $p = .704$; Figure 9). This indicates that changes in locomotion are not responsible for the changes observed in cognitive performance following the co-administration of D1R and D2R agonists. However, one-way ANOVA revealed a group effect in rearing ($F_{(3, 28)} = 3.053$, $p < .05$). Post hoc analysis indicated that only the D2R agonist treated group was significantly different than the control group and the agonist co-administered group, in that they spent less time rearing (data not shown).

In terms of anxiety, a significant effect of group was revealed by one-way ANOVA ($F_{(3, 28)} = 4.077$, $p < .05$). Post hoc analysis illustrated that the D2R agonist and the combined

D1R and D2R agonist treated groups displayed significantly less anxiety than control by spending more time at the center of the Open Field ($p < .05$; Figure 10). However, because they were not different from each other it is assumed that the D2R agonist administration is mediating this effect and not the co-administration of agonists.

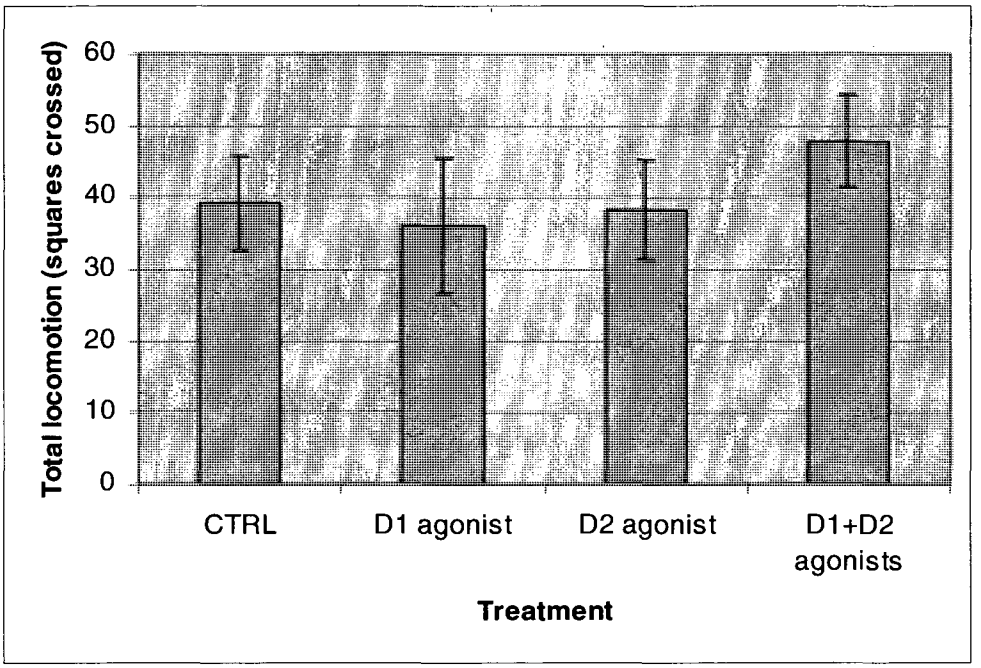

Figure 9: Systemic treatment with D1R and/or D2 agonists (SKF38393 3 mg/kg; quinpirole 2 mg/kg respectively) 30 minutes before test has no effect on total locomotion as evaluated by the Open Field (one-way ANOVA). n=8 per group

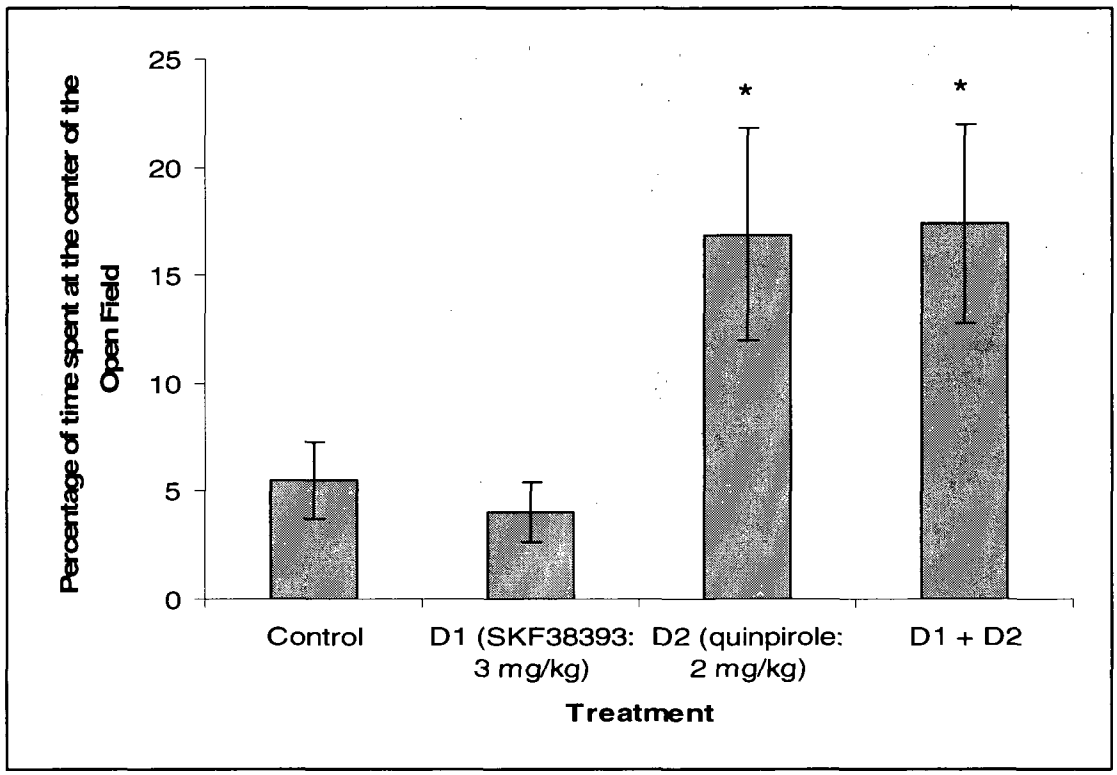

Figure 10: Systemic treatment with quinpirole (2 mg/kg) 30 minutes before testing has anxiolytic effects on rats as evaluated by an increase in the percentage of time spent at the center of the Open Field (one way ANOVA; $p < .05$; n=8 pre group).

4 Discussion

The dopamine receptors are important pharmaceutical targets because of their diverse expression and their role in mediating a variety of functions. Understanding how dopamine receptors function is of utmost importance, since they are the targets of a variety of treatments for neurological diseases and disorders. The present study is focused on the functional interaction of the D1R and D2R, based on the consideration that if well understood, a cooperative synergy between the these receptors could lead to a better understanding of a variety of neurological disorders and diseases, including depression, schizophrenia and Parkinson's disease.

4.1 General Considerations for the Study

Inherent flaws exist in the study of specific dopamine receptors using systemic administration of dopamine receptor agonists. One such problem is caused by the fact that these agonists are specific in terms of receptor family only. For example, there is no specific agonist or antagonist that can distinguish between the D1 and D5 dopamine receptors, and the D2R agonist we used also activates the D3 receptor. Therefore, it is difficult to tell by agonist administration alone whether the effects observed, in our case, are a result of the stimulation of the D1R and D2R specifically. In order to overcome, at least partially, these

potential problems, we used selective D1R and D2R antagonist pretreatments in an attempt to counteract the effect of the agonist treatment, which can be useful in determining whether specific receptors are responsible for the observed effects. Another potential problem is that a systemic administration of D1R and D2R agonists activates receptors from various brain regions, not just those whose functions are evaluated by the chosen behavioural paradigms. To minimize the impact of this problem, we administered the agonists through cannulaes to specific brain regions so as to allow a better inference in the source of the effect.

Another point to consider is that the affinity dopamine receptors have for dopamine is different than the affinity they have for agonists. Consequently, an effect observed following agonist administration is not necessarily indicative of regular neuronal function, although it can still be relevant. Dopamine agonists are currently used to treat various diseases and disorders of neurological nature, thus an understanding of how they affect the brain is of utmost importance.

4.2 Social Recognition Model of Olfactory Memory

There are various olfactory memory tests used with rats. The most commonly used Odour-Reward Association Test consists of an odour-reward association, in which the animal must first learn to which odour is paired a reward and must then distinguish between two similar odours to find the reward. Though this test has proven to be particularly effective at characterising olfactory memory, the result of correctly discriminating between both odours confounds with the animal's motivation to find the reward. Since dopamine is known to

modulate motivational and reward systems, we chose to work with a model of olfactory memory that was independent of motivation. Therefore, the Social Recognition Test was selected based on its ability to provide a spontaneous measure of olfactory memory. Though its use is not as widespread as other memory tests, its accuracy in assessing olfactory memory has been validated by previous studies (Millan et al. 2007; Steckler et al., 1998).

The results we obtained with this test were interesting for two main reasons. First of all, we expected to see an effect on olfactory memory following the independent administration of agonists, based on the results of a previous study examining the role of dopamine on olfactory memory. The effects of dopamine agonists in the odour-reward association model of olfactory memory showed that independently administered D1R and D2R agonists differently modulated olfactory memory. More specifically, D1R-like agonists increased while the D2R-like agonists impaired the ability to discriminate between similar odours (Yue et al., 2004). However, these results are not in agreement with our findings that the independent administration of dopamine agonists had no effect of olfactory memory as measured by the Social Recognition Test. This discrepancy can be explained by two possibilities. The results in the Odour-Reward Association Test could have been modulated by motivation, or the Social Recognition Test may not be as sensitive to the modulation of olfactory memory by dopamine agonist administration as the odour-reward association test is.

Our second interesting result with this test is that the co-administration of D1R and D2R agonists was deleterious to olfactory memory. We had expected that the co-administration of agonists would induce a facilitation in memory performance due to the previous findings regarding the synergy between the D1R and D2R. This result, opposed to

our expectations, suggested that the synergy between the D1R and D2R may have a negative effect on cognitive performances while having an augmentative effect in other areas (stereotyped behaviour, early gene expression and neuronal firing rates). A direct inhibitory modulation of the piriform cortex by dopamine receptors has previously been noted (Collins et al., 1985), which may partly account for our observations.

4.2.1 Olfactory Discrimination

The Modified Home-Cage version of an Olfactory Discrimination Task was selected because motivation is generally excluded from the factors that could confound with the results, similarly to the Social Recognition Test. The results we obtained in this test showed that none of the treatments administered had an impact on olfactory discrimination abilities (no groups differed significantly from control), which suggests that our previous results in the Social Recognition Test were dependent on learning and memory rather than alterations in olfactory function. However, it was expected that the animals would spend the majority of their time exploring one of the two types of bedding, the familiar or the novel. This was not the case; rats of all groups spent approximately the same amount of time in both the new and used bedding, which suggests a lack of test validity. This may be due to the fact that the new bedding is not a novel or interesting stimulus (though it has been effective in previous experiments; Prediger et al., 2005), even when presented next to used bedding. This may be the case as the animals had previously been presented with new bedding every time their home-cages were cleaned.

Based on the existing literature exploring the role of the piriform cortex and olfactory learning and memory, we further explored the effects of co-administering the D1R and D2R agonists to the piriform cortex, suspecting that the effect we had observed in the Social Recognition Task were in fact due to alterations in olfactory memory rather than olfaction.

4.2.2 The Piriform Cortex: a centre for olfactory learning

Much work has been done to investigate the role of the piriform cortex in olfactory learning and memory. It was found that following olfactory learning, the piriform cortex displays synaptic changes associated with long-term potentiation and long-term depression (Saar et al., 1999; Brosh & Barkai, 2004; Lebel et al., 2001). The decreased olfactory memory performance following the co-administration of D1R and D2R agonists into the piriform cortex suggests that piriform cortex dopamine plays a role in olfactory memory.

Only the D2R antagonist administered to the piriform cortex induced significantly inferior levels of sniffing when compared to the vehicle pre-treated group in the first trial of the Social Recognition Task, and this result was opposed to our expectations. Since we observed that the co-treatment with agonists induces a decrease in sniffing, we expected the pre-treatment with antagonists to undo the effects by resulting in increased sniffing, restoring near-control levels. This was not the case, as what we observed was a further decrease in function. As there is bidirectional input between the olfactory bulb and the piriform cortex (Roesch et al., 2007), it is possible that the antagonists were exerting their effects on the olfactory bulb so as to alter olfaction. To further investigate this, it would be interesting to

consider the effects of antagonist treatment to the piriform cortex on an olfactory discrimination tasks. This would help elucidate whether or not olfaction is altered by the antagonist treatments in the piriform cortex or whether something else may be implicated in the results we observed.

4.3 The DNMSP of Working Memory

A unique characteristic of working memory is the requirement of manipulating previously acquired information for the achievement of the cognitive task at hand. Since its creation in the late 1940s, the DNMSP T Maze Test has proven to be a very reliable measure of working memory (Jones, 2002; Canal et al., 2005). The animal must remember which arm the reward was in during the simple run (first trial) in order to successfully complete the choice run by choosing the opposite arm (second trial), even though both runs are separated by a variable delay. Similarly to the Social Recognition Test of olfactory memory, the co-administration of D1R and D2R agonists was deleterious to the performance of animals in the DNMSP T Maze Test. Interestingly, once again, the administration of either agonist independently had no effect on performance.

As the testing procedure begins with food deprivation, we had little concerns about whether or not the treatment with dopamine receptor agonists would result in varied motivational states between our groups, much to the contrary of the olfactory memory test. Though the mesolimbic dopamine system is known to modulate motivation and reward (Di Chiara & Imperato, 1988; Wise, 1996), the food deprivation ensures that all animals are

hungry, and thus that they are motivated in some way to retrieve the morsel of food that is the reward.

Because the animal must recall the spatial location of simple run arm, the results obtained from the DNMSP T-Maze Test are considered a joint indication of pre-frontal-based working memory and hippocampal-based spatial memory (Jones, 2002). There were visible spatial cues in the behavioural room where the DNMSP T-Maze was tested so it is likely that the rats would have incorporated this information into their successful completion of the task. Perhaps the results we obtained in this test indicate that the co-administration of D1R and D2R agonists would be deleterious to spatial memory as well. To confirm this, a more precise test of spatial learning and memory, such as the Morris Water Maze, should be used.

4.4 The Active Avoidance Model of Associative Memory

The Active Avoidance Test was selected as a measure of striatal-mediated memory. Our results demonstrated that the co-administration of D1R and D2R agonists had no effect on performance in this test, however the D2R agonist did. Our results suggest that treatment with a D2R agonist augmented performance abilities in the Active Avoidance Test. However, an interesting consideration in the validity of the data collected during this test is the fact that, after 8 days, the control group had not yet reached the learning objective. This is quite different from the results obtained by other groups (Fetsko et al., 2005; Montero-Pastor et al., 2004; Olloor & Datta, 2005), whose control animals achieved a 75-80% success rate within 5 days. There is a difference between the procedure we used and theirs, which could account

for the results we obtained. The conditioned stimulus in our test was a light, while some other groups used an auditory tone that was used either in place of the light, or paired with the light. Including a tone was impossible for us as our behaviour testing room is not soundproof, and ambient noises would have interfered with the effectiveness of the tone as a conditioned stimulus.

4.5 The Open Field: an evaluation of locomotion and anxiety

When working with behavioural paradigms, it is important to consider the effect of confounding variables. Dopamine receptor agonists can have an impact on more than just the behaviours tested, and this can unknowingly interfere with results. The seemingly decreased ability of a rat to perform in memory tasks may in reality be decreased mobility, hyper- or hypo-activity, or altered states of anxiety, which can all be modulated to some extent by dopamine receptor agonist (Jaber et al, 1996). For this reason, it is important for us to consider the effects of our treatment on locomotion and anxiety.

In an attempt to shed some light on whether or not the effects we observed were modulated by alterations in locomotion, we tested rats in the Open Field for a 5 minute trial, 30 minutes after administration of single or combined D1R and D2R agonists. This timeframe perfectly mimics the timeframe used throughout our testing, and thus allowed us to determine that during the time the testing took place, there were no differences between groups when it came to total locomotion in the Open Field. Although there may be differences in locomotion at an earlier or later point in time after the administration of the

treatments, our results in the Open Field support that our findings that the co-administration of D1R and D2R agonists is deleterious to performance in olfactory and working memory and that this was not mediated by alterations in locomotor functions. Although a difference was detected in the D2R agonist treated group in terms of rearing, it is doubtful that this indication of altered activity alone is enough to alter performance in memory tasks. The D2R treatment was not beneficial or deleterious to olfactory and working memory, though it was in associative memory. However, it is doubtful that alterations in rearing would have accounted for the better performance observed in the groups treated with the D2R agonist in the Active Avoidance Test. Adding to this doubt is the fact that D2R and the co-administered agonist group performed equally better than control and D1R agonist group in the Active Avoidance Test, while only the D2R agonist treated group was affected by a decrease in rearing in the Open Field. Consequently, it is unlikely that this effect altered performance in the memory tests.

The Open Field is a common test of anxiety, and examining the percentage of time spent in the perimeter versus the middle of the apparatus can give a reliable account of changes in anxiety. Our results suggest that the D2R agonist treatment decreased anxiety (as observed in both the independently administered D2R agonist group and the combined agonist group), which is similar to various results in the existing literature. Specifically, it has been found that quinpirole decreases anxiety, and that haloperidol and sulpiride (D2R antagonists) increase anxiety as measured by the Open Field (Siemiatkowski et al., 2001).

Only in the Active Avoidance test did we observe an effect of independent D2R agonist administration (in both the D2R and combined agonist treated groups). One possible explanation for the results we observed with that model is that it could be sensitive to

anxiety. Our control and D1R agonist groups may have endured higher levels of anxiety following the foot-shock unconditioned stimulus (especially if the light alone was not a good enough conditioned stimulus), which could have impaired their performance in the test. The two other groups, having been treated with quinpirole would have been less susceptible to developing anxiety during the test, thus allowing them to achieve the success rate of correct responses indicating associative learning.

4.6 Theories Behind the Dopamine Synergy

In the past, there have been many investigations in the dopamine D1R and D2R synergy. Though it is not clear what mechanism underlies the synergy, there are two main possibilities. Dimerization of GPCRs is a current topic in molecular biology that has become very popular since the demonstration of dimerization between μ- and δ-opioid receptors. Since then, it has been found that various receptors form homo- and hetero-dimers. It has been demonstrated that the D1, D2 and D3 dopamine receptors can form homo-dimers, and that some even form hetero-dimers, such as the coupling of the D2 dopamine receptor to the A_2 adenosine receptor (George et al., 2002). A hetero-dimerization between the D1R and D2R could explain the functional synergistic effects observed upon co-stimulation of these receptors, as hetero-oligomers have been found to have ligand-binding and signalling properties that differ from those associated to their monomeric constituents (George et al., 2002).

Much work has been done to investigate the possible dimerization between the D1R and D2R. One group found that the co-activation of both receptors when co-expressed *in vitro* resulted in an increase in intracellular calcium (Lee et al., 2004). This signal was not observed when either receptor was expressed alone or when the co-expressed receptors were stimulated by just one agonist. Using inhibitors of various calcium increasing pathways, they found that phospolipase C was responsible for the increase they observed. Also, using co-immunoprecipitation (Co-IP) assay they were able to suggest that the receptors form a complex. Though the co-IP result is interesting and would support dimerization between the receptors, their controls are not sufficiently compelling. Also of interest, a study using Förster resonance energy transfer demonstrated that the D1R and D2R exist as dimers at the cell membrane of human embryonic kidney 293 cells over-expressing the fluorescently tagged D1R and D2R (Dziedzicka-Wasylewska et al., 2006).

While results such as these are appealing, the validity of information gathered from over-expressed cells is limited when it comes to accurately describing what goes on in the brain. For this reason, we have decided to pursue the Co-IP of the D1R and D2R from rat brain tissues collected immediately following decapitation. Our preliminary results support the suggested idea of the formation of a D1R-D2R complex in several brain regions including the piriform cortex, and co-administration of D1R and D2R agonists appears to significantly enhanced the Co-IP of the D1R and D2R (data not published). This suggests that our behavioural results (an impairment in olfactory and working memory following co-administration of D1R and D2R agonists) could result, at least partially, from the enhanced formation of a D1R-D2R complex in the piriform and prefrontal cortices.

Though it is possible that the observed effects of co-administrating D1R and D2R agonists are mediated by a neuronal pathway interaction, virtually no work has of yet been dedicated to the investigation of this possibility.

4.7 Future Direction

Taken together, the data from this project suggest that synergistic effects are observed in cognitive functions following D1R and D2R agonist co-administration, but that the effect hinders performance. Much work remains to be done to better understand how the interaction between the D1R and D2R modulates cognitive functions. Continued work with various memory paradigms will help clarify the results we obtained in this project. This has already begun; other members of our laboratory have continued the exploration of the D1R and D2R synergy using the Morris Water Maze test for hippocampal-based cognitive function. Thus far, our preliminary data conforms to the results we observed with the measures of olfactory and working memory: systemic co-administration of D1R and D2R agonists appears to be deleterious to spatial learning and memory, which was not affected by treatment with a D5 antisense oligonucleotide or a D3 selective antagonist (unpublished data). This supports the idea that the deleterious effects of co-administered D1R and D2R agonists are produced by the activation of D1R and D2R receptors specifically. However, further experiments need to be done in order to assess whether the underlying mechanism of these effects is a protein-protein interaction between the D1R and D2R.

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