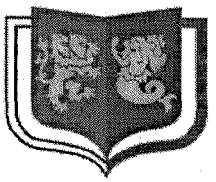

THE UNIVERSITY
OF BIRMINGHAM

DOPAMINE RECEPTOR SUBTYPE INVOLVEMENT IN THE BEHAVIOURAL EFFECTS OF COCAINE

BY

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ABSTRACT

The relationship between the behavioural effects of cocaine and the increase in dopamine caused by its blockade of dopamine re-uptake has been a major focus of research interest. However, little is known regarding the involvement of recently-cloned dopamine D2-like receptor subtypes (D2, D3 and D4) in different cocaine-induced behaviours. The purpose of the work described in this thesis was to use a series of behavioural tests to assess dopamine receptor subtype involvement in cocaine's effects. In the first series of experiments, we tested the effects of antagonists selective for receptors within the D2-like subfamily on the discriminative stimulus effects of cocaine (10 mg/kg), and compared them with the effects of a D1-like receptor antagonist. A separate group of rats were trained to discriminate a low dose of cocaine (3 mg/kg). Neither U-99194A (a D3 antagonist) nor L-745,870 (a D4 antagonist) substituted for cocaine, and neither drug shifted the dose-response function for cocaine at the higher training dose. On the other hand, pre-treatment with SCH 39166 (a selective D1-like antagonist) produced significant dose-related rightward shifts in the cocaine generalisation curve, indicating effective antagonism. Three other centrally-acting D2-like antagonists (L-741,626; haloperidol and raclopride) produced rightward shifts in the dose-response function for cocaine at both training doses. The D2-like antagonists, however, produced dissimilar effects on cocaine-induced hypophagia and hyperactivity in the rat. The D3 and D4 antagonists (which produced minimal effects on feeding and motor behaviours on their own) failed to alter any of the behavioural effects induced by cocaine. The D2/D3 antagonist, raclopride, produced only a marginal attenuation of cocaine-induced hyperactivity and rearing, but a marked attenuation of cocaine-induced decreases in grooming. On the other-hand, a D1-like antagonist potently reversed cocaine-induced hypophagia, hyperactivity and rearing, but failed to affect grooming behaviour. While drug discrimination studies suggests negligible involvement of D3 and D4 receptors in cocaine's effects, an important role for D1-like and D2 receptors was observed. In contrast, it seems that the D1-like subfamily may play a more prominent role than the D2-like subfamily in cocaine-induced hypophagia and motor hyperactivity, although cocaine-induced inhibition of grooming appears to be specifically a D2-mediated effect.

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ABBREVIATIONS

ANOVA	=	Analysis Of Variance
cAMP	=	Cyclic adenosine monophosphate
CL	=	Confidence Limits
CNS	=	Central Nervous System
DA	=	Dopamine
D1	=	Dopamine D1 receptor subtype
D2	=	Dopamine D2 receptor subtype
D3	=	Dopamine D3 receptor subtype
D4	=	Dopamine D4 receptor subtype
DD	=	Drug Discrimination
DS	=	Discriminative stimulus
ED_{50}	=	Effective Dose (50% response)
FR10	=	Fixed ratio of 10 responses
5-HT	=	5-Hydroxytryptamine (serotonin)
i.c.v.	=	Intracerebroventricular
i.p.	=	Intraperitoneal
i.v.	=	Intraventricular
Ki	=	Inhibitory Constant value
ML	=	Mesolimbic
mRNA	=	Messenger Ribonucleic acid
nM	=	Nanomolar
NE	=	Noradrenaline
NAC	=	Nucleus accumbens
RP	=	Relative Potency
s.c.	=	Subcutaneous
SEM	=	Standard Error of the Mean
SN	=	Substantia Nigra
VTA	=	Ventral Tegmental Area

CHAPTER 1:

'GENERAL INTRODUCTION'

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1.1 History of Cocaine Use

According to archaeological evidence, the Inca people of what are now Peru and Bolivia learned the practice of chewing the Coca leaf from the Aymara Indians of Bolivia, where records of cocaine use go back at least to 300 BC (Maisto, Galizio and Connors; 1995). The Coca shrub (Erythroxylan Coca) in whose leaves cocaine (the alkaloid form) could be found, grew in the eastern highlands of the Andes mountains in Bolivia, Peru, Ecuador and Colombia. Coca chewers would take some lime or ash with the leaves to make the pH of the saliva more alkaline, thereby decreasing ionisation of the cocaine and promoting absorption across the mucous membranes of the oral cavity. Because the Inca people thought of Coca as a gift from the Sun God, use of the drug was initially restricted to ceremonial or religious occasions. However, Coca chewing later became widespread until the practice was banned by the Spanish Conquerors in the 1500's. The Spaniards subsequently lifted the ban when they discovered that the Incan slaves worked harder and longer when allowed to chew Coca.

1.1.2 Cocaine Use in the 19th Century

Until the 1800's, use of the Coca plant was relatively unknown in Europe. Although Coca leaves were brought back to Europe from South America, Coca chewing never caught on in Europe, probably due to degradation of the active ingredient during the long sea voyage. The situation changed, however, when Niemann isolated and characterised the active alkaloid in 1859. Over the next 30 years, cocaine became tremendously popular as many notable scientists and physicians lauded its properties. A chemist named Mariani concocted an infamous mixture of cocaine and wine ('Vin Mariani'), while the Italian neurologist Mantegazza wrote, "*I would rather have a life span of ten years with coca than one of a million centuries without coca*" (Gold, 1990).

With regard to cocaine specifically, perhaps the most famous user was Sigmund Freud. Freud obtained a sample of cocaine in 1884 and, after taking it a few times, felt he

had come across a miracle drug that could cure many ailments. He advocated cocaine as a local anaesthetic and as a treatment for depression, indigestion, asthma and drug addiction (morphine). Only one of these therapeutic uses has turned out to be valid, namely the use of cocaine as a local anaesthetic. In 1884 Freud also performed the first recorded psychopharmacological experiments on cocaine and published the results in a paper (1885) entitled "A contribution to the knowledge of the effect of cocaine". The euphoria of the cocaine experience was described vividly:

"*The psychic effect of cocainum muriatiaum in doses of 0.05 - 0.10 g consists of exhileration and lasting euphoria, which does not differ in any way from the normal euphoria of a healthy person. The feeling of excitement which accompanies stimulus by alcohol is completely lacking; the characteristic urge for immediate activity which alcohol produces is also absent*" (Freud, Cited In: Warburton, 1990).

Freud was mistaken in many of his early claims about cocaine, and he helped launch a period of widespread cocaine abuse. One of the first documented cases of cocaine addiction concerned one of Freud's friends, Ernst Von-Fleischl. Fleischl suffered from chronic pain and had become a morphine addict. Freud prescribed cocaine, and Fleischl began to consume larger and larger doses. Although doing quite well at abstaining from morphine, Fleischl was eventually consuming a gram of cocaine daily. Not only had Fleischl become one of the first European cocaine addicts, but he also began to show bizarre symptoms that we now recognise as characteristics of cocaine overdose. These symptoms included paranoid delusions, of the kind that are often seen in schizophrenia, and a feeling of itching called the 'formication syndrome', which is described as like having insects or snakes crawling on or under the skin. Surprised by the disastrous effects of cocaine on Fleischl, Freud in his later writings on cocaine was no longer enthusiastic, but the damage had already been done (Musto, 1992).

The Parke-Davis Pharmaceutical Company declared that cocaine was potentially 'the most important therapeutic discovery of the age' and began selling cocaine-containing products such as coca cigarettes to treat throat infections. Cocaine was promoted as a cure for practically everything, from seasickness to haemorrhoids. In 1886,

John Pemberton, a pharmacist from Atlanta, created a drink, 'Coca-Cola' made from a formula of Coca leaves, Kola nuts and a small amount of cocaine in a sugary, carbonated syrup. With such marketing and availability, it is not difficult to understand why cocaine became so popular, and with so many people using the drug, casualties soon began to emerge. With the increasing prevalence of cocaine-induced psychosis, deaths by overdose, and severe dependence on the drug, popular sentiment against cocaine began to rise.

1.1.3 The Decline and Re-emergence of Cocaine Use

Thus the enthusiasm that surrounded cocaine use in the 1880's soon dissipated as evidence mounted against the early claims of cocaine's safety. People realised that the subjective feelings of power and euphoria that initially accompany cocaine use deteriorated with repeated use into feelings of powerlessness and profound depression. In an attempt to curtail its abuse, the drug's availability was placed directly under a physician's control. However, even though cocaine could now be legally obtained only from a physician, a 'black market' for the sale of cocaine emerged.

While cocaine use declined dramatically, users turned to amphetamines and other central nervous system stimulants that were first developed in the 1930's. Users reported that the effects of cocaine and amphetamines were virtually indistinguishable, with the possible exception that the 'high' from amphetamines lasts longer. From the 1920's to the 1960's, cocaine use continued primarily among a relatively small group of 'avant garde' artists, musicians and other performers. It had become difficult and expensive to obtain, and being glamorised as the drug of 'movie stars' it thus acquired a reputation as the 'champagne' of the stimulants.

So why did cocaine re-emerge as a stimulant of choice? The ready commercial availability of cocaine was a key issue in its re-emergence from the 1970's onwards. A new method of cocaine administration called 'free-basing' allowed users to smoke the drug and absorb much higher doses than before. Freebase is smokable cocaine obtained by dissolving the white crystalline powder, cocaine hydrochloride, in a strong base. Smoking 'crack' gives a quicker, more intense 'high' than inhaling cocaine powder

because the drug passes quickly and unhindered from the lungs to the bloodstream. Peak plasma levels after intranasal (crystalline) cocaine administration occur approximately 30 minutes subsequent to inhalation, and cocaine has a half-life of about 40-60 minutes via this route (Johanson and Fischman, 1989). When cocaine is taken by the intranasal route, it limits its own absorption by causing constriction of the nasal mucous membranes (Johanson and Fischman, 1989). On the other hand, smoked cocaine has a pharmacokinetic profile and produces effects similar to those of intravenous administration.

The arrival of the 'crack' form of cocaine in the mid-1980's signalled a major escalation in the problems associated with cocaine abuse. Freebase cocaine was mass-produced, and the low cost of crack made it available to younger users. Within two decades, what was considered a typical 'dose' of cocaine changed significantly. Higher doses of the more potent forms of cocaine had transformed people's experiences of the drug and its impact on society. Concern with cocaine abuse was so great that an increased awareness developed of the need to understand the behavioural effects of cocaine and the determinants of those effects.

1.2 Behavioural Effects of Cocaine

1.2.1 Physiological and Psychological Effects In Humans

Psychostimulants such as cocaine are typically sympathomimetic drugs. That is, they act to stimulate or mimic activity in the sympathetic branch of the autonomic nervous system. Thus, many of their physiological effects are similar to those seen during emotional arousal: heart rate increases, blood pressure and respiratory rate both rise, sweating increases; blood flow to the viscera and extremities decreases, blood flow increases in the large muscle groups and the brain, body temperature is elevated and pupils are dilated (Kiritsy-Roy et al., 1990; Tella et al., 1992, 1993).

Common subjective reports of cocaine's acute behavioural effects are presented in Table 1.1. The table lists the most prominent effects of a low-to-average dose of cocaine, approximately 10-100 mg when inhaled. These effects occur from all methods of using cocaine, but may be more rapid and intense with smoking or intravenous use. Subjective

effects, such as measures of 'high', typically peak between 3 and 5 min after use and usually disappear within 30-40 min (Johanson and Fischman, 1989).

1. Euphoria (seldom dysphoria)
2. Increased sense of energy
3. Feelings of enhanced mental acuity
4. Increased sensory awareness (sexual, auditory, tactile, visual)
5. Decreased appetite (anorexia)
6. Increased anxiety and suspiciousness
7. Decreased need for sleep
8. Physical symptoms of a generalised sympathetic discharge

Table 1.1. Acute effects of cocaine (From: Gold, 1993).

The enjoyable psychological effects of low-to-average doses of cocaine begin as soon as cocaine reaches the brain; the precise nature of the effects varies depending on the dose, the level of drug tolerance and the route of administration. Typical features of the cocaine 'high' are feelings of exhilaration and euphoria, a sense of well-being, and greater self-confidence (Wallach and Gerson, 1971; Grinspoon and Bakalar, 1985; Spotts and Shontz, 1986). A moderate dose of cocaine markedly suppresses appetite, with consequent rebounds later (Jonas and Gold, 1986). Sleep is delayed and fatigue postponed (Gawin and Ellinwood, 1988; Shere et al., 1988; Brady et al., 1991); individuals show increased talkativeness and sociability (Spotts and Shontz, 1986).

Clearly, one of the most important behavioural effects of cocaine is its mood-altering property, particularly since it is generally believed that these effects are related to its abuse. The lack of studies on the subjective effects of cocaine within a treatment research context may be due, at least in part, to the risks of administering cocaine to patients seeking treatment. Measuring the subjective effects as indicators of dependence potential can only be possible in humans via their verbal abilities. Hence, subjective effects are assessed with questionnaires such as the Addiction Research Centre Inventory (ARCI; Haertzen, 1960) and the Profile of Mood States (POMS; McNair et al., 1971), which measure the presence and intensity of drug-induced perceptual changes (e.g. detachment, paranoia, cognition) and mood states (e.g. anger, vigour, fatigue, elation).

The ARCI is a 550-item true-false questionnaire grouped into scales labelled by drug category, withdrawal state and personality assessment. Each of the scales purportedly measures mood effects characteristic of specific drugs or drug groups. The POMS is a list of adjectives describing different moods, and the participant indicates how he or she feels at a given moment in relation to a series of adjectives on a numerical scale from 'not at all' to 'extremely'. The adjectives have been grouped using factor analysis into separate scales that are constructed to measure unique mood states, such as anxiety or elation. Many studies have shown that this instrument is a reliable and sensitive indicator of changes in mood after administration of drugs (Fischman et al., 1985). Fischman and colleagues demonstrated that cocaine produces typical psychomotor stimulant mood effects (Fischman and Scuster, 1982). For example, intravenous cocaine at doses ranging from 4-32 mg increases scores on the Benzedrine Group (BG) and Amphetamine scales of the ARCI in a dose-dependent manner. As the names of these scales imply, the changes produced by cocaine were similar to those produced by amphetamine. Cocaine and amphetamine also increased scores on the Morphine-Benzedrine Group scale, which is considered a measure of 'euphoria', and decreased scores on the Pentobarbitol-Chlorpromazine-Alcohol Group scale (sedative-like effects); these changes were also dose-dependent (Fischman, 1984).

1.2.2 Unconditioned Behavioural Effects In Animals

In laboratory animals such as rodents, one of the defining behavioural characteristics of cocaine as a psychomotor stimulant is its ability to elicit increases in motor activity. Cocaine produces an alert response of increased exploration, locomotion, grooming and rearing (Scheel-Kruger et al., 1977; Snoddy and Tessel, 1985). As the dose is increased, locomotor activity decreases and the behavioural patterns become stereotyped, i.e. there is a continuous repetition of one or a few items of behaviour (Scheel-Kruger, 1971; Scheel-Kruger et al., 1977). In rats, the repetitive behaviour includes head bobbing, gnawing, sniffing and licking (Scheel-Kruger et al., 1977). At still higher doses, cocaine produces convulsions and death (Woolverton and Johnson, 1992). Cocaine is also an anorectic in animals and humans (Bedford et al., 1980; Blavet and De

Feudis, 1982; Foltin et al., 1983; Cooper and Van der Hoek, 1993); see section 1.6.1 for further discussion.

1.3 Cocaine's Mechanisms of Action

1.3.1 Neurochemical Effects

Cocaine has long been known to be an inhibitor of the synaptosomal uptake of the monoamines such as dopamine (DA), noradrenaline (NE) and serotonin (5-Hydroxytryptamine, 5-HT). It does so by interacting with the plasma membrane transporters for these neurotransmitters, thereby blocking cellular uptake of all three monoamines (Harris and Baldessarini, 1973; Taylor and Ho, 1978). These experiments demonstrated that cocaine binds to the three uptake sites with a similar, though not identical, potency. Cocaine-like analogues have been used to study structure-activity relationships for cocaine binding at the DA, NE and 5-HT transporter sites (Ritz et al., 1990). Measuring the transporter binding potencies of cocaine derivatives that involved N-substitution and/or C2 and C3 substituent modifications revealed differences in structure-activity relationships between the cocaine analogues. Removal of the N-methyl group produced little change in binding potency at the DA transporter but produced increases in binding potencies at the NE and 5-HT transporter sites. Modifications to the C2 and C3 substituents, especially substitution of a hydroxyl moiety, produced changes in affinity at NE and 5-HT transporters which were much greater than occurred at DA transporters (Ritz et al., 1990). In general, the study indicated the unique structural requirements which exists for each transporter site, but it also showed that cocaine binding at DA and NE transporters can be described by more similar structure-activity relationships in comparison with binding at the 5-HT transporter.

Consistently, microdialysis studies have demonstrated that acute cocaine treatment causes increases in extracellular DA, NE and 5-HT levels (Reith et al., 1986; Ritz et al., 1987; Carboni et al., 1989; Nomikos et al., 1990) and that the time course of such increases is correlated with changes in extracellular cocaine concentration (Hurd, Kehr and Ungerstedt, 1988). Overall, these findings confirmed that the blockade of the monoamine transporters observed in vitro likewise occurs in vivo.

Although there are several binding sites for cocaine, the binding site that has been most clearly related to the drug's reinforcing properties is the DA transporter (Ritz et al., 1987; Bergman et al., 1989). Thus, the DA transporter appears to be the critical site of action where events relevant to the reinforcing effects of cocaine are initiated. Indeed, many of cocaine's behavioural effects have been attributed to its ability to elevate DA levels specifically. For example, cocaine produces stereotyped behaviour and locomotor activity in rodents, behaviours that are associated with activation of the nigrostriatal and mesolimbic DA systems, respectively (Scheel-Kruger et al., 1977; Reith et al., 1986). These motor effects are blocked by antagonists of post-synaptic DA receptors (Cabib et al., 1991) and attenuated by other treatments that disrupt DA neurotransmission, such as lesions of DA fibers in the nucleus accumbens (Calcagnetti and Schuchter, 1992). In addition, structural analogs of cocaine produce behavioural effects similar to those of cocaine with potencies that correlate with their binding affinities at the DA transporter, both for locomotor activity (Cline et al., 1992) and for the induction of stereotyped sniffing and biting (Reith et al., 1986). Similar to its other behavioural effects, the only binding site that has been directly related to cocaine's reinforcing properties is the DA transporter (Ritz et al., 1987; Bergman et al., 1989). The DA hypothesis of the reinforcing properties of cocaine suggests that cocaine binds to the DA transporter of mesocorticolimbic neurons, which results in the inhibition of DA reuptake, the potentiation of dopaminergic transmission, and ultimately the behavioural phenomenon of reinforcement (Figure 1.1). In animal studies, electrical self-stimulation of these pathways produces reward-seeking behaviour that mirrors cocaine self-administration (Gawin, 1991). Both electrical self-stimulation of these pathways and administration of cocaine increase extracellular DA concentrations in brain nuclei that control reward behaviour; lesions of these pathways or treatment with DA receptor antagonists attenuate these effects of cocaine (e.g. Gawin, 1991).

It should also be noted that at high concentrations, cocaine interacts with several membrane proteins in addition to the monoamine transporters. These proteins include σ (sigma) sites (Sharkey, Glen et al., 1988); 5-HT3 receptors (Kilpatrick et al., 1987); muscarinic cholinergic receptors (Sharkey et al., 1988b) and most importantly, voltage-

gated Na^+ channels (Matthews and Collins, 1983). Because of its ability to block axonal Na^+ channels, cocaine is a potent local anaesthetic. However, these additional properties of cocaine are generally not considered to be important to its reinforcing effects.

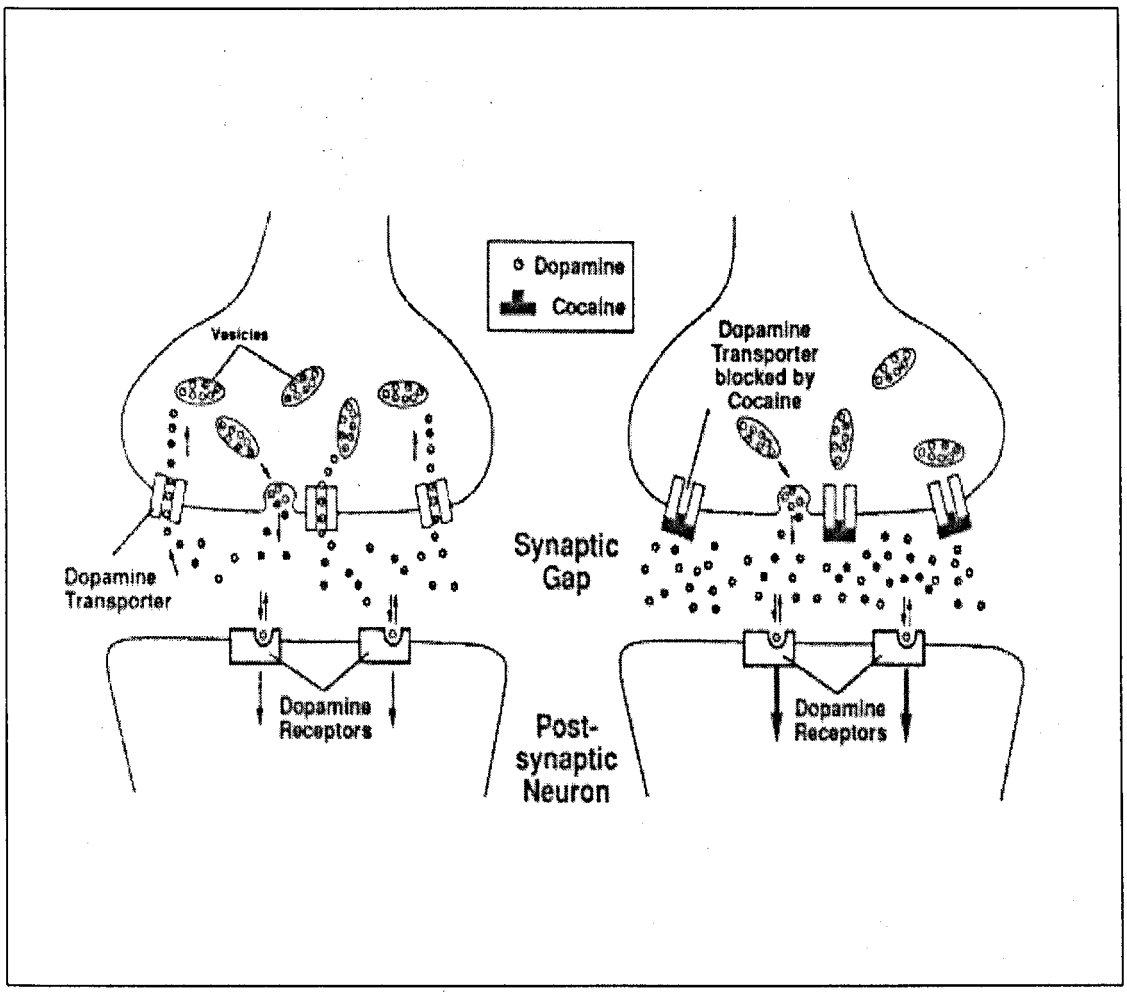

Figure 1.1. The DA hypothesis of the reinforcing properties of cocaine. Cocaine binds to the DA transporter and blocks the reuptake of DA in neurons of the mesocorticolimbic pathway. This potentiates dopaminergic neurotransmission and initiates the sequence of events that ultimately cause the rewarding effects of the drug. (From Kuhar, 1992; In: Cocaine, Scientific and Social Dimensions, Wiley-Interscience publication).

1.3.2 Neural Systems Relevant to Reward

Extensive behavioural and neuropharmacological evidence suggests that the mesolimbic (ML) DA neurons are critical for the development and regulation of goal-directed behaviour established and maintained by various natural and drug reinforcers

(for reviews, see Kiyatkin, 1995; Robbins and Everitt, 1996). It has long been accepted that the psychomotor stimulants increase DA transmission and that such drug-induced changes in the activity of the ML DA system are of special importance in the initiation and maintenance of cocaine self-administration (Altman et al., 1996). Generally, the effects of abused drugs are correlated with increased extracellular DA in the nucleus accumbens in the following rank order of effectiveness: stimulants (such as amphetamines and cocaine) > nicotine > opiates > ethanol > caffeine > benzodiazepines/barbiturates (Wise, 1993; Di Chiara, 1995).

There is also evidence for a more general link between reward and the ML DA system. For example, it has been shown that dopaminergic activity can be increased by presentation of a broad range of natural reinforcers: food (Phillips et al., 1991; Damsma et al., 1991) water (Phillips et al., 1991) sex (Damsma et al., 1992) and by electrical stimulation of the lateral hypothalamus (Hernandez and Hoebel, 1988b) associated with conditioned stimuli (Apicella et al., 1991; Schultz et al., 1992). Using in vivo voltammetry, Phillips et al. (1991) showed that DA levels increase in both the nucleus accumbens and neostriatum in response to a continuous stimulus for food. The elevation in DA levels persisted until and throughout the meal, finally decreasing to baseline 10-30 min after the meal (Phillips et al., 1991).

The importance of ML DA function to reward has been supported by hundreds of studies, and is perhaps captured most vividly in Wise's famous "anhedonia hypothesis" (Wise, 1982). Wise elegantly mustered several lines of evidence to argue that moderate suppression of dopaminergic function reduces the hedonic value of various rewarding stimuli, such as provided by intracranial self-stimulation, drugs of abuse, and palatable foods. These effects are reportedly independent from any alterations of motor control or general arousal (Wise et al., 1978). The anhedonia hypothesis implies a reduction in incentive motivation after suppression of dopamine function. In studies of the effects of DA antagonists on consumption of food or water, for example, the drugs appear to specifically target appetitive rather than consummatory behaviour (see section 4.2). Prominent among these effects is the ability of DA antagonists (including D1-like and D2-like antagonists) to produce changes in intake patterns that mimic a natural dilution of

reward value. Such observations have typically been interpreted in terms of the anhedonia hypothesis and have provided support for the hypothesis that DA is an important mediator of food reward (Berridge, 1996).

The ML DA neurons synthesise and release DA from their terminals and project to various limbic structures. The system originates in the Ventral Tegmental Area (VTA, also known as the A10 region) and projects densely to the ventral striatum, which includes the nucleus accumbens, and to other limbic sites such as the amygdala and septal nuclei (Figure 1.2). A mesocortical DA system that also originates in the VTA and projects mainly to the prefrontal and cingulate cortices is sometimes viewed as a separate system, but is often grouped with the system innervating the ventral striatum and collectively is called the 'mesocorticolimbic DA system' (e.g. Feldman et al., 1997). In the rat there are rich connections between 'limbic' forebrain structures and the ventral striatum. The basolateral amygdala, the hippocampal formation and specific regions of the medial pre-frontal cortex all project to the nucleus accumbens and ventromedial caudate-putamen, collectively known as the ventral striatum. The ventral striatum projects in large part to the ventral pallidum, which in turn projects via the medial dorsal nucleus of the thalamus to the prefrontal cortex, so providing the key element of re-entrant cortico-striatal circuitry (Groenewegen at al., 1990).

By analogy, limbic input to the primate striatum is not restricted to the nucleus accumbens. In the monkey, the amygdala projects to ventral parts of the head of the caudate nucleus, ventromedial anterior putamen, and nucleus accumbens, and some fibers from this structure even reach the rostral dorsal putamen and ventral parts of the body and tail of caudate (Seleman and Goldman-Rakic, 1985). Orbitofrontal cortex and anterior cingulate cortex project to the very rostral parts of caudate, to ventral parts of anterior caudate and putamen, to ventral parts of the body and tail of the caudate (Seleman and Goldman-Rakic, 1985). Thus, the ventral striatum is strongly innervated by these cortical and subcortical limbic areas, although limbic inputs are to a lesser extent also directed to more dorsal and posterior striatal territories. This makes the ventral striatum the predominant, albeit not exclusive, striatal territory with limbic input, thereby refuting a dichotomy of "associational" dorsal and "motivational" ventral striatum.

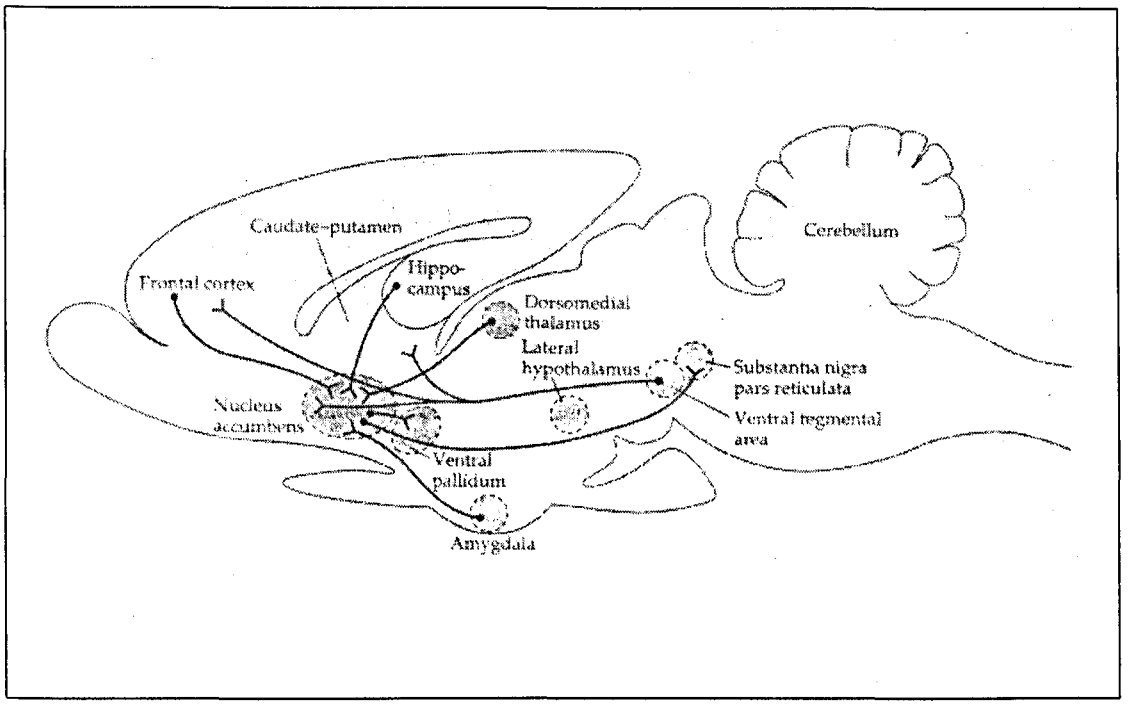

Figure 1.2. Neural circuits presumed to mediate the reinforcing effects of psychostimulant drugs. A critical part of this circuit involves DA pathways from the VTA to the nucleus accumbens, ventral caudate-putamen and frontal cortex. The accumbens is additionally regulated by limbic system afferents from the frontal cortex, amygdala, hippocampus and dorsomedial thalamus. The behavioural output of this circuit is thought to be mediated by efferent pathways from the accumbens to the ventral pallidum and substantia nigra pars reticulata, which are components of the extrapyramidal motor system (From Feldman et al., 1997). Complex processing at a cognitive level, such as memory processes, subjective attribution and craving are assumed to depend on neocortical mechanisms. The hippocampus and amygdala are responsive to conditioned aspects of the environment such as contextual or specific cues associated with drug taking. These limbic structures interface via their anatomical connections with DA-dependent processes of the ventral striatum (nucleus accumbens, core and shell compartments) to effect the control of instrumental actions and their outcomes. Descending pathways, including such regions as the central grey, may mediate the aversive aspects of drug dependence while outputs of the striatum via the globus pallidus to the brainstem are assumed to control drug-seeking behaviour. A further possible output from the nucleus accumbens includes the cholinergic neurons of the basal forebrain, which may play an important role in mediating cortical arousal and the subjective sequelae of drug reinforcement (Altman et al., 1996).

In addition, the ventral pallidum projects directly to the brainstem motor areas, especially to the subthalamic nucleus, substantia nigra pars reticulata, as well as to midbrain DA neurons and the cholinergic neurons of the nucleus basalis magnocellularis, which project diffusely to the neocortex. Thus, the limbic cortex, via its projections through the ventral striatum, has access to motor output domains of the brainstem, as well as specific projections (via the thalamus) to the prefrontal cortex, and access to a major diffuse cholinergic cortical arousal system.

It has been mentioned above that specific cortical areas and limbic structures send excitatory projections to selected portions of the striatum (comprising the caudate nucleus, putamen and ventral striatum), which is generally thought to represent the 'input' stage of the basal ganglia. However, it is also important to note the striatal outputs of the above circuits. The basal ganglia output nuclei exert a tonic GABA-mediated inhibitory effect on their target nuclei in the thalamus (Alexander and Crutcher, 1990). Within each circuit, this inhibitory outflow appears to be differentially modulated by two opposing but parallel pathways that pass from the striatum to the basal ganglia output nuclei. Each circuit includes a 'direct' pathway to the output nuclei, which arises from inhibitory striatal efferents (Albin et al., 1989). Activation of this pathway tends to disinhibit the thalamic stage of the circuit. Each circuit also includes an 'indirect' pathway, which passes first to the external segment of the globus pallidus via striatal projection neurons, then from the globus pallidus to the subthalamic nucleus, and finally to the output nuclei via an excitatory projection from the subthalamic nucleus (Smith and Parent, 1988). The role of DA within the basal ganglia appears to be complex, and many issues remain unresolved. However, there is recent evidence that the nigrostriatal DA projections exert contrasting effects on the direct and indirect striatofugal pathways. Dopaminergic inputs appear to have a net excitatory effect on striatal neurons that send GABA/substance P projections to the basal ganglia output nuclei (via the direct pathway), and a net inhibitory effect on those that send GABA/enkephalin projections to the globus pallidus (via the indirect pathway) (Bouras et al., 1986). Thus, in effect, the overall influence of DA within the striatum may be to reinforce any cortically initiated activation of a particular basal ganglia-thalamocortical circuit by both facilitatory conduction

through that circuit's direct pathway (which has a net excitatory effect on the thalamus) and suppressing conduction through the indirect pathway (which has a net inhibitory effect on the thalamus) (Alexander and Crutcher, 1990).

The nucleus accumbens has recently been described as comprising anatomically (and perhaps functionally) two distinct domains, the 'core' and 'shell' regions, further indicating its complexity (Heimer et al., 1995). Each of these domains receives distinct patterns of cortical afferents and has distinct projections to medial and lateral pallidal sites. Mogenson et al. (1980) first proposed that the nucleus accumbens serves as a critical interface between the limbic and motor systems, thus linking motivation with action. In this model, the VTA-accumbens dopaminergic pathway is seen as part of a gating mechanism that governs the translation of motive states into overt motor responses. For example, electrical stimulation of the amygdala or hippocampus elicits alterations in the firing of ventral striatal and ventral pallidal neurons that are subject to modulation by direct manipulations of DA within the nucleus accumbens (Robbins et al., 1989; Mogenson and Yang, 1991). Therefore certain limbic structures, such as the amygdala and the hippocampus, and cortical structures such as the frontal cortex, may also be important to drug reward via modulation of nucleus accumbens activity. It is not clear why activation of this system is reinforcing; one hypothesis put forward is that the ML system has a critical role in the species-specific motor arousal associated with anticipation of reward (Robbins et al., 1989; Koob, 1992; Dickinson and Balleine, 1994; Robbins and Everitt, 1996).

An alternative interpretation was provided by Robinson and Berridge (1993) who proposed a theory of drug craving based on the concept of 'incentive-sensitisation'. This theory distinguishes between the motivational process of 'liking' (i.e. broadly synonymous with "pleasure") and the process of 'wanting' (which Robinson and Berridge formally refer to as the attribution of incentive salience to environmental stimuli). The latter process, which is not consciously experienced, causes the perception or psychological representation of an object to become desirable and to be pursued. Most importantly, repeated exposure to an addictive drug in the presence of various drug-

related stimuli is hypothesised to produce conditioned sensitisation of the neural substrates (the ML DA system) that mediate attribution of incentive salience. Consequently, the user can experience an increasing desire for the drug, even though the person may actually 'like' the drug less and less. Therefore, the theory claims that ML DA subserves the 'wanting' aspect of drug abuse, but it fails to identify the brain mechanisms or receptors that mediate the subjective responses to such drugs.

It is generally agreed that only a limited number of brain structures are likely to process the appetitive aspects of rewarding events. Neural activity related to reward occurs in several distinctive forms, many of which are not seen with DA neurons. It is no longer adequate to consider the ML DA system in isolation when exploring the neural bases underlying the effects of addictive drugs. There are clearly important afferent systems that regulate the firing frequency and pattern of VTA DA neurons (Schultz et al., 1992). The anatomical substrate underlying these functions may comprise the conjunction of different afferents from limbic structures and ML DA neurons. Major limbic structures in primates, such as the anterior cingulate gyrus, orbitofrontal cortex and amygdala, project to the ventral striatum, including the nucleus accumbens, in a dense and interdigitating fashion, whereas their projections to the dorsal striatum are more sparse and scattered (Seleman and Goldman-Rakic, 1985). Interactions in the ventral striatum between afferents from the amygdala and from DA neurons appear to be necessary for mediating the effects of stimulus-reward associations on behaviour (Cador et al., 1989). Investigations have also shown that the dorsal and ventral caudate neurons are activated when different kinds of food are shown to the animal (Nishino et al., 1984) and that ventral striatal neurons respond to external stimuli associated with reward through prior conditioning (Cador et al., 1989). The dorsal part of the striatum, or caudate putamen in the rat, is composed of at least two distinct compartments: the 'matrix' and the 'striosomes' or patches (Gerfen, 1992). The matrix receives afferents from the superficial layers of the medial prefrontal cortex and motor, somatosensory, and visual cortices (Gerfen, 1992). In contrast, the striosome/patch compartment receives projections from the deep layers of the medial prefrontal and limbic cortices (Gerfen, 1992). Neurons in

both the ventral and dorsal parts of the striatum seem also to be involved during reinforcement (Apicella et al., 1991). This notion has been supported by the recent finding that electrodes contacting the striosomes/patches of the rat caudoputamen sustain electrical self-stimulation more reliably than electrodes in the matrix that surrounds them (White and Hiroi, 1998). The idea that self-stimulation results from activation of cell bodies in the striosome/patch compartment suggests that strionigral neurons terminating in pars compacta may also be involved in the reinforcement process. Thus, the striatum may receive information about predictable environmental events from associative cortex, information about reward reception from subcortical limbic structures, and information about the presence of salient, incentive stimuli from DA neurons. Neurons on the dorsal striatum may have access to representations of several task events, such as instruction stimuli, hedonic responses and reward (Schultz et al., 1992; White and Hiroi, 1998).

It should be apparent from the above that it is now widely accepted that CNS DA is an important mediator of reward-related behaviour, and plays a primary role in the behavioural effects of cocaine. Consequently, there has been substantial interest in determining the roles of the different types of DA receptor in cocaine's effects. In order to approach this issue, it is necessary to describe the molecular biology, distribution and pharmacology of the various DA receptor subtypes and to consider the behavioural effects of receptor-selective compounds.

1.4 Dopamine Receptors: Molecular Biology and Behavioural Aspects

Modern DA receptor research began in the early 1970's with the discovery of DA-stimulated adenylyl cyclase activity (Kebabian, Petzold and Greengard, 1972). Biochemical evidence for two distinct DA receptors was followed shortly by the designation of these sites as "D1" and "D2" receptor subtypes (Kebabian and Calne, 1979). D1 receptors were shown to activate the enzyme adenylyl cyclase and to increase intracellular levels of cAMP, whereas D2 receptors exerted an inhibitory influence on this enzyme (Andersen, et al., 1990). D2 receptors were also linked to additional second messenger systems, including activation of K^+ channels and inhibition of Ca^{2+} channels and phospotidylinositol turnover (Valler and Meldolesi, 1989).

Gradually, however, pharmacological and molecular biological evidence began accumulating to suggest that further receptor subtypes probably existed. A major breakthrough occurred with the cloning and characterisation of three novel DA receptor species; these are called D3 (Giros et al., 1990; Sokoloff et al., 1990), D4 (O'Malley et al., 1992; Van Tol et al., 1991) and D5 receptors (Grandy et al., 1991; Sunahara et al., 1991; Weinshank et al., 1991) (for review see, Jackson and Westlind-Danielsson, 1994).

All of the known DA receptors belong to the G-protein-coupled receptor superfamily. The five subtypes have been well characterised, designated D1 through D5, and a sixth member of the family arises from the fact that the D2 receptor exists in two isoforms (termed D_{2S} and D_{2L}). Many different names have been proposed for the different molecular forms of DA receptor. Sibley and Monsma Jr (1992) proposed a hierarchical nomenclature based on the pharmacology and molecular structure of the different receptors. This classification scheme recognises two families of DA receptors that correspond to the classically-recognised D1 and D2 receptors, now termed 'D1-like' and 'D2-like' receptor subfamilies. There are important similarities between the D1 and D5 subtypes and between the D2, D3 and D4 subtypes, hence their categorisation into D1-like and D2-like families, respectively (Table 1.2). The discussion below is organised on the basis of 'D1-like' and 'D2-like' receptor subfamilies.

1.4.1 The 'D1-Like' Receptor Subfamily

A. D1 Receptors

The D1 receptor was first distinguished by virtue of its ability to stimulate adenylyl cyclase activity, and later by means of binding studies that differentiated it from the D2 subtype. The gene for the D1 receptor was cloned by four different groups working independently (Dearry et al., 1990; Monsma et al., 1990; Sunahara et al., 1990; Zhou et al., 1990). As with other G-protein-coupled receptors, the structure of the D1 receptor suggests the presence of seven membrane-spanning domains.

A number of DA agonists have been developed that discriminate between D1 receptors and members of the D2-like subfamily. Among the most widely used D1-selective agonists are members of a family of 1-phenyl-tetrahydrobenzazepines including

	D1	D5	D2S/D2L	D3	D4
Alternative Nomenclature	D_{1A}	D_{1B}	D_{2AS} / D_{2AL}	D_{2B}	D_{2C}
Amino Acids:					
Human	446	477	414 / 443	400	387
Rat	446	475	415 / 444	446	383
Introns in Gene	No	No	Yes	Yes	Yes
Human Chromosome	5	4	11	3	11
Effector Pathways	↑ cAMP	↑ cAMP	↓ cAMP ↑ K^+ channels ↓ Ca^{2+} channels	↓ cAMP ?	↓ cAMP ?
mRNA Distribution	caudate putamen nucleus accumbens olfactory tubercle amygdala frontal cortex	hippocampus hypothalamus	caudate putamen nucleus accumbens olfactory tubercle limbic areas	olfactory tubercles hypothalamus nucleus accumbens island of calleja	frontal cortex medulla amygdala midbrain
Agonists	SKF 7560 SKF 77434 SKF 38393 A-68930 Dihydrexidine		N-0437 Bromocriptine	Quinpirole 7-OH-DPAT PD 128907	PD 168077
Antagonists	SCH 23390 SK&F 83566	SCH 23390 SK&F 83566	Remoxipride Raclopride (-) Sulpiride L-741,626	(+) AJ 76 (+) UH 232 U-99194A GR 103691	Clozapine L-741,742 L-745,870

Table 1.2. DA receptor subtypes defined from physiological, pharmacological and biochemical studies. (Source from: Andersen et al., 1990; Sibley et al., 1992; 1993; Strange, 1996). Drugs are listed as commonly used for the receptors given.

the prototypical compound SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-7,8-dihydroxy-(1H)-3-benzazepine) (Setler et al., 1978; Stoof and Kebabian, 1984) along with a variety of congeners such as SKF 81297, SKF 82958 and SKF 82526 (fenoldopam) (Andersen and Jansen, 1990). Some of the benzazepine compounds, such as SKF 38393 and SKF 82526, are only partial D1 agonists, as assessed by the stimulation of D1-sensitive adenylyl cyclase (Andersen and Jansen, 1990). Hence the maximal efficacy of these drugs is less than that of DA itself. Dihydrexidine is an example of a non-benzazepine DA agonist that exhibits moderate selectivity between D1-like and D2-like receptors and near-full efficacy (Brewster et al., 1990). Recently, a potent and selective D1-like receptor agonist has been the isochroman compound A-68930 (DeNinno et al., 1990; Kebabian et al., 1990). A-68930 has a high affinity for the D1-like receptor and stimulates carp retinal adenylate cyclase (DeNinno et al., 1991); it also stimulates DA-sensitive adenylate cyclase in the rat caudate putamen, and acts as a full agonist in this assay (DeNinno et al., 1991).

Several substituted benzazepines act as D1-like receptor antagonists rather than agonists. The best known of these compounds are SCH 23390 (3-methyl-1-phenyl-2,3,4,5-tetrahydro-7-chloro-8-hydroxy-(1H)-3-benzazepine) and its brominated analog SKF 83566 (Hyttel, 1983). Other commonly used D1-like antagonists include the benzonapthazepine, SCH 39166 (Chipkin et al., 1988).

The distribution of D1 receptors in the rat brain has been mapped by means of in vitro autoradiography using several different agonists and antagonists. The highest receptor densities have been found in the terminal regions of the mesostriatal system, namely the caudate-putamen, nucleus accumbens and olfactory tubercle, and in the substantia nigra (Monsma Jr et al., 1990; Sunahara et al., 1990; Zhou et al., 1990; Table 1.2). Intermediate levels of receptor binding occur in the ventral pallidum, entopeduncular nucleus, and in some of the nuclei of the amygdala. Low D1 receptor levels have been found in the neocortex, thalamus, cerebellum, hippocampus, septum and most areas of the hypothalamus (Monsma Jr et al., 1990; Sunahara et al., 1990; Zhou et al., 1990).

In most instances, DA D1 receptor mRNA distribution, carried out using Northern blot analysis, agrees well with the distribution of the corresponding binding sites identified by autoradiography (Monsma et al., 1990; Sunahara et al., 1990; Zhou et al., 1990). An exception is the substantia nigra (SN), where considerable DA D1 receptor binding is evident, whereas DA D1 receptor mRNA expression is generally not detectable. This may imply that DA D1 receptors are not synthesised in the SN, but are transported to this site, presumably from the caudate putamen.

B. D5 Receptors

The only other known member of the D1-like receptor subfamily is the D5 receptor (Sunahara et al., 1991; Weinshank et al., 1991). Some investigators have used the terminology D1a and D1b to refer to the D1 and D5 receptors, respectively (e.g. Jackson and Westlind-Danielsson, 1994). The two receptors have significant amino acid sequence homology, very similar pharmacological profiles, and both stimulate the formation of cAMP. It is interesting to note that in humans, but not in rats, the D5 receptor exhibits a tenfold higher affinity for DA than the D1 receptor (Sunahara et al., 1991; Weinshank et al., 1991).

Because of the pharmacological similarity of the D1 and D5 receptors, the so-called D1-selective agonists and antagonists available at this time affect both D1 and D5 receptors. However, D5 receptors appear to be expressed at very low levels in the brain, mainly in the hippocampus, the hypothalamus and the parafascicular nucleus of the thalamus (Meador-Woodruff et al., 1992; Tiberi et al., 1991). This might suggest that the behavioural effects caused by D1-like agonists or antagonists may be better attributed to activation or blockade of the D1 subtype rather than the D5 subtype.

1.4.2. The 'D2-Like' Receptor Subfamily

A. D2 Receptors

D2-like receptors were first identified on the basis of their high affinity for antipsychotic (neuroleptic) drugs and by the fact that, unlike D1-like receptors, they either inhibited adenylyl cyclase or had no effect on its activity (Kebabian and Calne,

1979). The DA D2 receptor was first cloned in the rat by Bunzow et al. (1988) followed by the human D2 receptor which was found to possess 96 % sequence homology with the rat receptor (Dal Toso et al., 1989). Other studies demonstrated that two isoforms of the D2 receptor exist in both rats (Giros et al., 1989) and humans (Dal Toso et al., 1989). The DA D2 receptor isoform differs from the initially cloned DA D2 receptor by an additional 29 amino acids. Hence, the longer and shorter forms are designated D2L and D2S respectively.

Bromocriptine (which is an ergot derivative), LY-171555 (also called quinpirole) and apomorphine are some well-known D2-like receptor agonists with limited selectivity for D2 receptors within the D2-like subfamily. The classic antipsychotic drugs are DA receptor antagonists that possess varying degrees of selectivity for D2-like as compared with D1-like receptors. Members of this group with D2-like selectivity include haloperidol, pimozide, raclopride and sulpiride (Meltzer et al., 1989; Schwartz et al., 1993). Only recently have truly D2-selective drugs been developed; one such example is the compound L-741,626 (Kulagowski et al., 1996; Bowery et al., 1996).

D2 receptors display a widespread but heterogeneous distribution in the brain. In rats, the highest levels of D2 receptor binding have been detected in the caudate-putamen, nucleus accumbens, olfactory tubercle, substantia nigra pars compacta and the glomerular layer of the olfactory bulbs (Boyson et al., 1986; Charuchinda et al., 1987). Intermediate receptor levels have been found in the central nucleus of the amygdala, lateral septum, molecular layer of the hippocampus and the entorhinal cortex. It is interesting to note that in virtually all areas in which D1 and D2 receptor densities were directly compared, levels of D1 receptors were distinctly greater (Boyson et al., 1986).

B. D3 Receptors

The D3 receptor was first identified by Sokoloff et al. (1990). Although D3 receptor pharmacology is not unlike that of D2 receptors, some important distinctions should be noted. A few of the previously mentioned D2-like agonists, particularly quinpirole and pergolide, actually have a greater affinity for the D3 subtype. The compound 7-OH-DPAT (7-hydroxy-N-N-di-N-propyl-2-aminotetralin) is more D3-selective in receptor binding studies (Levesque et al., 1992) and this drug has been used

to investigate the possible behavioural and physiological effects of D3 receptor activation (Levesque et al., 1992). The novel naphthofurane compound, (+)-S-14297 ((+)-[7-(N,N-dipropylamino)-5,6,7,8-tetrahdronaphtho(2,2b)dihydro, 2,3-furane]) was the first reported selective D3 antagonist, with a 200-fold greater affinity for cloned human D3 than D2 receptors (Millan et al., 1994). Although ligands with high selectivity for the DA D3 receptor are not available at present, data on compounds with some preferential affinity for D3 receptors, such as (+) AJ 76, (+) UH 232, and the phenylpiperidine, (-) DS121, suggest that these compounds do differ in their pharmacological profiles from "traditional" D2-like receptor agonists and antagonists (e.g. Svensson et al., 1986; Sonesson et al., 1993). More recently, the putative DA D3 receptor antagonist U-99194A has been shown to have a 20-30 fold preference for the D3 vs. D2 receptor subtype (Waters et al., 1993).

Highest densities of D3 receptor binding and mRNA are found in the islands of calleja, dense clusters of small granular neurons located mainly within the olfactory tubercle (Levesque et al., 1992). Other areas of high D3 receptor concentration include the anterior nucleus accumbens, the olfactory tubercles, the bed nucleus of the stria terminalis, and the molecular layer of lobules 9 and 10 of the cerebellum (Giros et al., 1990; Sokoloff et al., 1990; Bouthenet et al., 1991).

C. D4 Receptors

Another D2-like receptor cloned from human and rat tissues is referred to as the D4 receptor (O'Malley et al., 1992; Van Tol et al., 1991). There appear to be multiple genetic variants of this receptor in the human population, although the functional significance of such variation remains to be established (Van Tol et al., 1992). In monkeys, rats and/or mice, D4 receptor mRNA has been identified in the frontal cortex, hypothalamus, thalamus, midbrain, medulla, amygdala, olfactory bulbs and the retina (Cohen et al., 1992; Van Tol et al., 1991). Only low levels have been found in the striatum. However, rat heart was shown to possess more D4 mRNA than the nervous system, suggesting that this receptor subtype may at least partially mediate the positive inotropic effects of D2-like agonists on heart muscle (Zhao, Ferrell and Abel, 1990). As yet, the functional roles of the D4 receptor subtype still need to be determined.

The most conspicuous feature of the human DA D4 receptor is its high affinity for clozapine (Van Tol et al., 1991). However, other groups (e.g. Malmberg et al., 1993) have reported an affinity of clozapine for the DA D2 receptor subtype comparable to its originally-reported affinity for DA D4 receptors (Van Tol et al., 1991). However, binding data from the rat receptor species has only demonstrated a Ki_{D2}/ Ki_{D4} quotient of 2-3 for clozapine (O'Malley et al., 1992) which limits the usefulness of this compound for the study of DA D4 receptor function. Despite considerable interest in the physiological functions of the D4 receptor, pharmacological research has been hampered by the paucity of agonists and antagonists with selectivity for the receptor. Recently, the newly-developed D4 receptor antagonists YM-5001 (Ki = 5.62nM; Hidaki et al., 1996), U-101307 (Ki = 10nM; Merchant et al., 1996), and the most selective of them all, L-745,870 (Ki = 0.42nM; Kulagowski et al., 1996) enable one to characterise the functional role of this receptor subtype for the first time.

1.5 Behavioural Effects of Selective Dopamine Receptor Subtype Agonists and Antagonists

1.5.1 'D1-Like' Receptor Subfamily

During the early 1980's, when the simple D1/D2 classification still dominated DA receptor research, it is interesting to note that virtually all behavioural effects of DA were ascribed to its action at D2 receptors (Seeman, 1980). This was due primarily to the fact that the behavioural potencies of most DA agonists and antagonists then available correlated more strongly with their affinities for D2 than for D1 receptors.

DA has been implicated in many different behavioural processes, and various selective DA receptor subtype agonists and antagonists have been used to elucidate the receptors underlying these behavioural functions. For example, the most pronounced effect of D1-like agonists (e.g. SKF 38393) and D1-like antagonists at low doses (e.g. SCH 23390) in rats and mice is to stimulate grooming (Molloy and Waddington, 1984; Murray and Waddington, 1989; Starr and Starr, 1986a) an effect which D2-like agonists or antagonists are unable to produce. SKF 38393-induced grooming in mice can be

inhibited by the i.c.v. injection of antisense oligonucleotides to D1 receptor mRNA (Zhang, Zhou and Weiss, 1994).

With regard to the effects of D1-like agonists on locomotor activity, the findings are ambivalent. For example, Molloy and Waddington (1984, 1985) first described a syndrome of diffuse behavioural activation, including mild locomotor stimulation, in rats treated with SKF 38393, but this profile was most robustly observed only after habituation for 2-5 hr to the test environment. This result has since been replicated (Molloy et al., 1986; Molloy and Waddington, 1987) and has been observed with other D1-like agonists (e.g. Murray and Waddington, 1989). Others reporting significant locomotor stimulation by SKF 38393 in habituated rats include Mazurski et al. (1991), Shannon et al. (1991) and Zarrindast and Elliasi (1991).

For non-habituated rats and mice, there have been few reports of locomotor hyperactivity following SKF 39393. However, Bruhwyler et al. (1991) demonstrated a significant increase in rat open-field activity after SKF 38393, and Tirelli and Terry (1993) showed a dose-related stimulation of activity in mice where the time-course effect was biphasic at high doses (at doses of 100-300 mg/kg SC there was a dose-related locomotor depression followed by long-term hyperlocomotion). Direct infusion of D1-like agonists into the striatum or nucleus accumbens elicits various patterns of locomotor hyperactivity and stereotyped behaviour (Bordi and Meller, 1989; Meyer et al., 1993), convincingly demonstrating the functional relevance of central D1-like receptors in such behaviours.

The typical effect of administering a DA receptor antagonist is a suppression of spontaneous exploratory and locomotor behaviour. D1-like antagonists, such as SCH 23390, have been reported to inhibit motor activity in mice (Starr and Starr, 1986b) and to reduce horizontal activity (Gessa et al., 1985, Hoffman and Beninger, 1985; Meyer et al., 1993) and rearing in a dose-dependent manner in rats (Hoffman and Beninger, 1985; Meyer et al., 1993). D1-like antagonists can also produce catalepsy in rats and mice, an effect which appears to be due to blockade of DA receptors in the striatum (Hoffman and Beninger, 1985; Chandler et al., 1990).

1.5.2 'D2-Like' Receptor Subfamily

D2-like receptor activation by D2-like agonists has been reported to produce hyperlocomotion and sniffing in rats (Molloy and Waddington, 1985). However, although D2-like receptor agonists are often assumed to stimulate activity especially in rats (Eilam et al., 1991; 1992) they have been shown to produce hypoactivity in mice over wide dose ranges (Jackson et al., 1989a 1989b; Tirelli et al., 1997), although a stimulant effect may emerge over long periods (Jackson et al., 1990). Evidence from several sources suggests that the full expression of D2-like receptor-mediated effects requires concurrent occupancy of D1 receptors (D1/D2 synergism, e.g. Waddington and Daley, 1993). For example, moderate to high doses of D2-like agonists such as quinpirole or RU 24213 have been reported to produce increased locomotion, sniffing, repetitive scratching, gnawing and biting (Eilam et al., 1992; Molloy et al., 1986; Walters et al., 1987) which can be blocked not only by administration of a D2-like antagonist, but also by a D1-like antagonist or by depletion of endogenous DA (Waddington, 1986; Walters et al., 1987). Moreover, combined administration of D1-like and D2-like agonists, either systemically or directly into the striatum, causes much more intense stereotyped behaviour than does either agonist alone (Bordi and Mellor, 1989; Walters et al., 1987). Such results have given rise to the hypothesis of D1/D2 synergism, which states that D1-like receptor occupancy is necessary for the full expression of D2-like receptor-mediated effects (Waddington and Daly, 1993).

As with D1-like antagonists, D2-like receptor antagonists can also elicit catalepsy (Wanibuchi and Usuda, 1990). Moreover, receptors in the striatum appear to play an important role in both cases (Sanberg, 1980; Fletcher and Starr, 1988). These results are not inconsistent with the prevailing hypothesis of D1/D2 receptor synergism and indicate that normal motor functioning requires continued activity at both subtypes in the striatum.

The identification of the DA D3 receptor by Sokoloff et al. (1990) enabled a number of selective DA D3 receptor agonists and antagonists to be developed, so allowing investigation of the behavioural and physiological effects of D3 receptor activation or blockade. One such compound is the D3 agonist, 7-OH-DPAT (7-hydroxy-N-N-di-n-propyl-2-aminotetralin) which has been shown to be among the most D3-

selective in receptor binding studies (Levesque et al., 1992). However, several reports suggest that this compound may be controversial as a selective D3-receptor ligand (e.g. Tirelli et al., 1997). It has been shown that, given systemically, 7-OH-DPAT will produce biphasic effects on spontaneous sniffing and locomotor activity in rats: behaviours were inhibited at low doses but stimulated at higher doses (Daly and Waddington, 1993; McElroy et al., 1993). These findings may suggest that D3 receptor-mediated effects are apparent at low doses, but that higher doses causes the additional stimulation of D2 receptors, so changing the behavioural profile.

A number of studies have looked at the effects of putative DA D3 receptor antagonists on locomotor activity (Svensson et al., 1986). These compounds include (+) AJ 76 and (+) UH 232 (which display 3-5 times higher affinity for D3 than D2 receptors); it has also recently been suggested that these drugs may exert a preference for DA autoreceptors (Arethra et al., 1995; 1996). It is possible that D3 receptor activation reduces locomotor activity due to an autoreceptor-mediated effect; however, various findings conflict with this hypothesis (e.g. Svensson et al., 1994b). The lack of autoreceptor involvement is also supported by work using the antagonist U-99194A, a compound 30-fold more selective for D3 than D2 receptors (Waters et al., 1993, 1994). Waters et al. (1993) demonstrated that U-99194A can increase locomotor activity without any concomitant effects on DA release. Kling-Petersen et al. (1995) also showed that U-99194A produced an increase in locomotor activity when injected into the nucleus accumbens at doses that did not affect DA release in the striatum or nucleus accumbens, areas which are established to be important to the behaviour. These results have led to the suggestion that the D3 receptor is functionally relevant primarily at the post-synaptic level, and that its blockade leads to stimulation of locomotor activity. Consistent with the effects of D3 antagonism on locomotor activity, the D3-preferring antagonist nafadotride produces a biphasic effect on locomotor activity in rats, stimulating locomotor activity at lower doses and inhibiting it at higher doses (Sautel et al., 1995). Doses of nafadotride which increased locomotor activity were shown to produce negligible occupancy of D2 receptors, while those which inhibited locomotor activity produced significant D2 occupancy (Levant and Vansell, 1997).

Looking at other behaviours, Millan and co-workers (1995) recently reported that 7-OH-DPAT induced hypothermia that could be completely blocked by the D3 antagonist S-14297. 7-OH-DPAT was recently found to decrease the rate of cocaine self-administration (interpreted as an increase in reinforcing efficacy) and was also self-administered when substituted for cocaine (Caine and Koob, 1995). D3 agonists also decrease alcohol consumption (Ahlenius and Larsson, 1995). Acri (1995) also reported that 7-OH-DPAT substituted for cocaine's discriminative stimulus effect in rats. Therefore recent evidence has indicated that postsynaptic D3 receptors may be involved in the regulation of locomotor behaviour, body temperature and reward. Based on the somewhat limited in vivo and in vitro pharmacological data currently available, it is possible that the D3 receptor may play a role in several other behavioural and physiological effects, such as yawning and hypothermia (Damsma et al., 1993; Ahlenius and Salmi, 1994; Ferrari and Guiliani, 1995; Khroyan et al., 1995), penile erection and ejaculatory behaviour (Ferrari and Guiliani, 1995), inhibition of climbing in mice (Sautel et al., 1995), and emesis in the dog (Yoshida et al., 1995).

By contrast, the functional role of the D4 receptor subtype still needs to be determined. The receptor has been little studied in the past, in part due to lack of availability of receptor-selective compounds. The recent emergence of a number of compounds acting specifically at the DA D4 receptor now make investigation possible. For example, the antagonists L-745,870 (Kulagowski et al., 1996) and U-101387 (Merchant et al., 1996) have been shown to have negligible effects in behavioural tests of antipsychotic activity (Bristow et al., 1996; Merchant et al., 1996; Millan et al., 1998) and anxiety tests in mice (Cao and Rodgers, 1997). As yet, there are no compounds available that act as agonists at the D4 receptor site, so impeding our understanding of this receptor.

1.6 Methods to Study Dopamine Involvement in Cocaine's Behavioural Effects

Animal models are recognised as indispensable tools for certain types of investigation, including mechanistic studies of brain substrates, the identification of sites of drug action, and the preclinical evaluation of novel agents. The usefulness of animal

models often depends on their validity as simulations of human behaviour. This can often be difficult to determine, in part because of the considerable differences in the methodologies used to study animal behaviour on one hand and human psychopathology on the other. However, in the case of animal models of addiction, these difficulties appear less acute. In the context of drug abuse research, the self-administration model is often used to analyse the neurochemical systems involved in drug reinforcement, and as a screening test for potential drugs of abuse. Similarly, the drug discrimination procedure is used both as a bioassay to examine drug actions on receptors and as a screening test for drugs with potential therapeutic applications. However, the drug discrimination procedure is sometimes used as an animal simulation of human 'subjective' responses to drugs. Such 'subjective' responses are often thought to be closely associated with abuse liability, and the drug discrimination procedure therefore represents a potential tool for studying this aspect of drug abuse.

In order for a model to be accepted as a useful tool to investigate drug abuse, it ought to meet certain criteria. In this regard, three main criteria are often applied, although it is generally unrealistic for any one model to meet all of these. These criteria have been outlined in reviews by Willner (1991; 1997). The model must have **'predictive'** validity, i.e. performance in the test must predict performance in the condition being modelled. **'Face'** validity suggests that animal models of behavioural disorders should, ideally, resemble clinical syndromes in terms of aetiology, biochemistry, symptomatology and treatment. Finally, **'construct'** validity presupposes that the model(s) used should have sound theoretical bases.

1.6.1 Self-administration

Many drugs of abuse are readily self-administered intravenously by animals, and in general, drugs that are self-administered have high abuse potential. Indeed, this relationship is so reliable that drug self-administration is widely considered to be an animal model that is predictive of abuse potential; hence it has been suggested that the procedure should be used for the preclinical assessment of the abuse liability of new

agents (Johanson, Balster and Bonese, 1976; Bozarth and Wise, 1985; Nader and Woolverton, 1992).

Cocaine functions as a positive reinforcer to maintain self-administration in a variety of species, including humans, by several routes of administration (Johanson and Fischman, 1989). In most experiments, cocaine is delivered through a chronically indwelling intravenous catheter implanted during brief surgical anaesthesia. Rates and patterns of responding maintained by cocaine are determined for the schedule of reinforcement under which the drug is available (Woolverton and Johnson, 1992). Although cocaine is a highly efficacious positive reinforcer, environmental manipulations such as punishment, increasing the effort required to obtain the drug, or offering alternative reinforcers, are effective in decreasing cocaine self-administration (Johanson and Fischman, 1989; Carroll et al., 1989; Nader and Woolverton, 1991). Pharmacological manipulations can also increase the self-administration rate on a simple fixed ratio schedule resembling decreases in the unit dose, hence causing a shift to the right of the dose effect function and a decrease in the reinforcing potency of cocaine (Wilson and Schuster, 1972; Roberts and Vickers, 1984; Bergman et al., 1990; Caine and Koob, 1994). For example, low to moderate doses of DA receptor antagonists can increase cocaine self-administration maintained on such a schedule in a manner similar to decreasing the unit dose of cocaine (Wilson and Schuster, 1972; Roberts and Vickers, 1984; Bergman et al., 1990; Caine and Koob, 1994). This suggests that partial blockade of DA receptors by competitive antagonists can reduce the reinforcing potency of cocaine (Koob, 1995). Conversely, DA agonists decrease cocaine self-administration in a manner similar to increasing the unit dose of cocaine (Yorkel and Wise, 1978; Woolverton et al., 1981; Self and Stein, 1992; Weed et al., 1993), suggesting that the effects of administering DA agonists together with cocaine can be additive, perhaps due to their mutual activation of the same neural substrates.

The use of different schedules of reinforcement in drug self-administration can provide important controls for non-specific motor and motivational factors. For example, fixed-interval schedules of self-administration can be designed to measure response rate independently of frequency of reinforcement (Altman et al., 1996). Progressive ratio

schedules have been used to evaluate the reinforcing efficacy of cocaine by increasing the response requirements for successive reinforcements and determining the breaking point, i.e. the point at which the animal will no longer respond. A variety of evidence supports the hypothesis that this schedule is effective in determining the rank order of reinforcing efficacy for different reinforcers, including cocaine (Griffiths et al., 1978; Roberts et al., 1989).

Intravenous drug self-administration in animals is reliable and has predictive validity. The dependent variable is reliable as a measure of the motivation to obtain drugs (the amount of work an animal will perform to obtain the drug). It also has predictive validity because doses of drugs that are highly reinforcing in animals are reported to have reinforcing effects in humans, as measured by both operant tests and subjective reports. Construct validity refers to the extent to which the model has a sound theoretical rationale; its evaluation requires a good theoretical understanding of both the model and the condition modelled. Unfortunately, both sides of this equation are rarely met; in the present case, drug intake by animals is now well understood (at least under steady-state conditions), but a coherent theoretical understanding of substance abuse by people remains elusive. It is difficult to assess the construct validity of the self-administration model because of the tenuous nature of the constructs of abuse and dependence. However, some of the findings from self-administration studies potentially have profound implications for the construct of dependence (e.g. with reference to the importance of withdrawal in dependence).

1.6.2 Conditioned Place Preference

The reinforcing effects of cocaine have also been investigated using place-conditioning procedures (e.g. Spyraki et al., 1982; Schenk et al., 1986; Morency and Benninger, 1986). In such studies, a distinctive environment is paired repeatedly with administration of drug, and a different environment is associated with the undrugged state. Typically, the environments differ with respect to visual cues (e.g. brightness), tactile cues (e.g. textured floors) and/or odour. Reinforcement is measured as an increase in the time spent in the compartment previously paired with drug. It is assumed that the

environmental cues take on secondary (conditioned) reinforcing effects due to their previous temporal association with the effects of the drug itself, and that the animals are displaying approach behaviour directed towards these conditioned reinforcers (Stolerman, 1992). Evidence that this effect is related to classical conditioning was provided by Bardo et al. (1986) since partial reinforcement (i.e. sometimes exposing the animal to the chamber, or CS, in the absence of drug) attenuated the place preference. The advantages of place conditioning as a model for evaluating cocaine's effects are similar to those of drug self-administration, but also include (a) a high sensitivity to low doses of cocaine; (b) potential utility in studying both sides of hedonic valence (e.g. both positive and negative reinforcement); (c) testing for drug reinforcement is under drug-free conditions and (d) it allows for precise control over the interaction of environmental cues with drug administration (Carr et al., 1989). The major disadvantages of place conditioning are the enormous cost, effort and time required to generate meaningful results.

To what extent does the conditioned place preference procedure meet the various criteria by which an animal model of drug dependence should be assessed? The model has a reasonable degree of predictive validity. The procedure has as not yet been used to compare drugs in terms of their relative abuse potential: it therefore is less useful in this respect than the self-administration model. Indeed, it is often difficult to generate graded dose effect curves with any one drug (Carr et al., 1989) so it is difficult even to compare the relative efficacies of different doses of the same drug. As far as face validity is concerned, the procedure makes no attempt to model the symptomatology of drug abuse, although people may well seek out drug-associated stimuli and environments for their conditioned incentive effects. Thus the model may possess face validity to an extent that remains to be seen. The conditioned place preference procedure gains most of its validation from the fact that it is a measure of drug-induced reward. As this concept is central to most conceptualisations of drug abuse, the model clearly has some degree of construct validity. However, conditioned place preference models are not 'pure' measures of reward, but they do provide a useful alternative to the self-administration procedure.

1.6.3 Drug Discrimination

Individual's feelings are said to be private events that can only be revealed by verbal, written, or other modes of communication unique to humans. Because of this, the development of animal models of subjective drug effects poses a substantial challenge. A methodology holding considerable promise in this regard is drug discrimination (Holtzman, 1990). In a typical drug discrimination experiment, animals are injected with drug or placebo on separate test sessions. Depending upon which solution is administered, responding on one of two levers results in the delivery of a reinforcer (usually as food) according to a particular schedule of reinforcement. Stimuli that are uniquely associated with the availability of a reinforcer are called 'discriminative stimuli' when they acquire control over the frequency of a response that is reinforced in their presence. The accuracy of the discrimination can be tested by administering the training drug or placebo during test sessions. Sensitivity and specificity of the discrimination are evaluated by administering different doses of the training drug and other test drugs. Test drugs may include those that are likely to be discriminated as the training drug (usually drugs from the same pharmacological class) or positive controls (usually compounds from different pharmacological classes).

Animals readily learn to discriminate cocaine from vehicle injections. Discriminative control by cocaine in rats was first shown by Colpaert, Niemegeers and Janssen (1976), and has been demonstrated subsequently in other species, such as pigeons, rhesus monkeys and squirrel monkeys (de la Garza and Johanson, 1983; 1985; Woolverton and Trost, 1978). Discrimination of cocaine from saline can be achieved under a variety of schedule conditions and different routes of administration. Wood et al. (1987) demonstrated that the DS properties of cocaine were mediated centrally by showing that intracerebroventricular (i.c.v.) injections resulted in cocaine-appropriate responding in rats trained to discriminate intraperitoneal (i.p.) cocaine from saline. Evaluation of the DS effects of cocaine has shown the drug to be similar to other psychomotor stimulants. For instance, in studies with animals trained with either cocaine or amphetamine, generalisation tests have shown that the DS effects of these drugs fully cross-generalise (Emmett-Oglesby et al., 1983). A number of good reviews can be found

on drug discrimination methodology (e.g. Jarbe, 1989; Holtzman, 1990; Goudie, 1991; Goudie and Leathley, 1993), of which a summary is given below.

1.6.4 Methods of Training and Testing For Drug Discrimination Procedures

A. Species

Drug discrimination studies have been conducted using a variety of animal species, including rats, mice, gerbils, pigeons, rhesus and squirrel monkeys. However, there have been no indications of species differences between these studies. Therefore, the choice of species can be based upon cost factors, research facilities available and personal experience and preference.

B. Paradigm

Virtually all drug discrimination studies today are carried out using operant behavioural procedures. Most commonly, differential responding for drug and vehicle is maintained by food reinforcement. For example, a rat is placed in a standard operant chamber with two levers as the response manipulanda (Figure 1.3). The rat is trained to press one lever during sessions that follow injection of the training drug, and to press the other lever during sessions that follow injection of drug vehicle. The rat receives a food pellet for emitting a particular number of responses (e.g. FR10 or FR20, fixed ratio of 10 or 20 responses, respectively) on the injection-appropriate lever. Various schedules of reinforcement are commonly used. For example, in fixed interval schedules (FI), responding is reinforced for the first response x seconds after the last reinforcement, and in fixed ratio schedules (FRn), responding is reinforced on the last response of a fixed number (n) of responses. Responding may also be maintained by variable interval (VI) or variable ratio (VR) schedules where the response requirements are expressed as an average for a range of possible values.

When animals are responding reliably (both levers) on the appropriate schedule, discrimination training begins with the drug and its vehicle being administered in a pseudorandom sequence prior to testing. Drug and vehicle sessions are held on different days according to a single alternation schedule (e.g. Drug, Vehicle, Drug, Vehicle, Drug, Vehicle, etc.), or by double alteration schedules (e.g. drug, drug, vehicle, vehicle, etc.), or

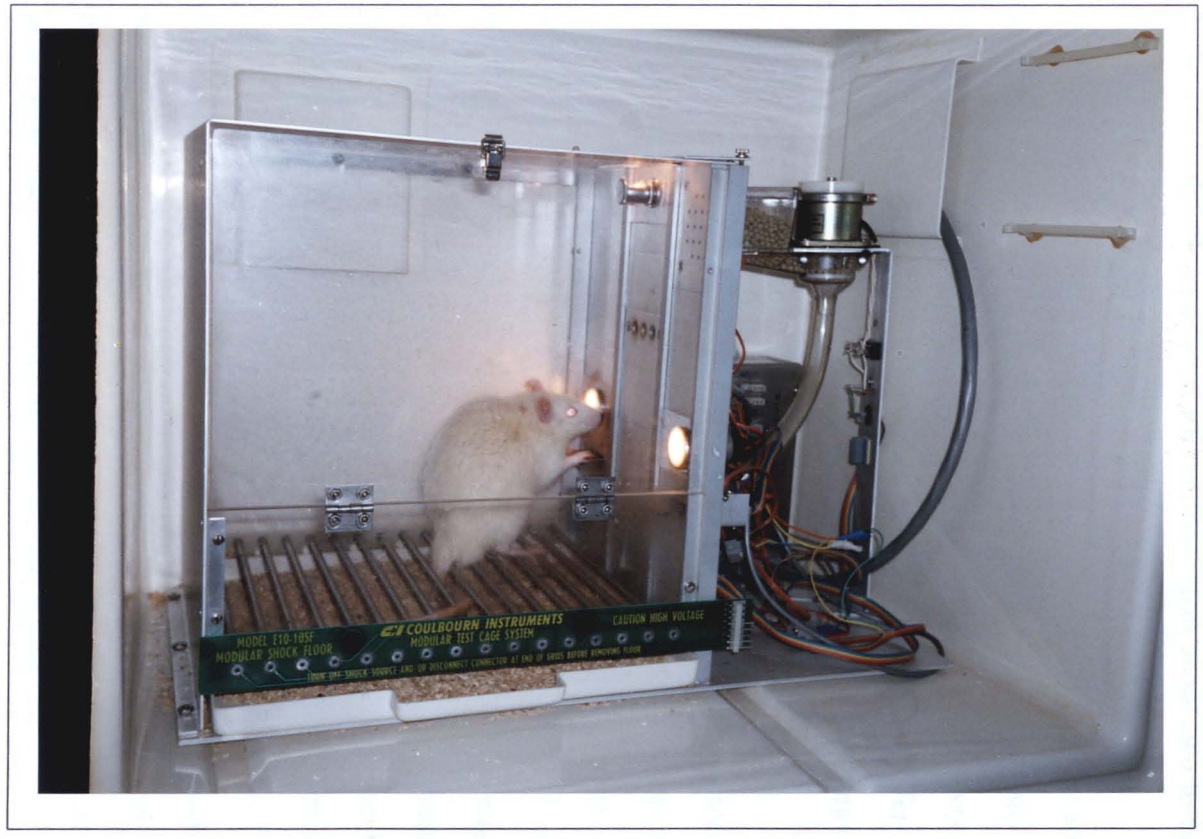

Figure 1.3. **A rat in one of the operant chambers. The rat is trained to press the levers (response) to activate a food delivery mechanism (reinforcement).**

some other balanced schedule of treatment. Numerous variations of this sort of sequence can be devised, but the alternation schedules have numerous advantages, such as (Goudie and Leathley, 1993):

1. To avoid lever bias, there are equal numbers of drug and vehicle sessions.
2. To facilitate learning there are not more than about two to three consecutive sessions of administration of either drug or vehicle.

Operant sessions usually run for 15-30 min, and the animals are typically maintained at approximately 80% of their ad-lib body weights. To avoid any effects of inherent lever bias, for half of the group of animals the drug-appropriate lever should be different from that of the other half. Even though studies often differ in methodology, these methodological differences do not seem to make much difference to the results.

C. Assessment of Discriminative Responding

During training, the development of discriminative control over lever selection can be measured by recording the total number of responses accumulated by each individual animal on both levers prior to presentation of the first reinforcer or over a series of reinforcers. Results are usually derived in one of two ways. With the **'quantitative'** method, the amount of responding on the lever appropriate to the training drug is expressed as a percentage of the total number of responses emitted during a test epoch (i.e. drug-appropriate plus vehicle-appropriate responses). The **'quantal'** method derives from the view that discrimination of drug-induced stimuli is an all-or-none event rather than occurring along a stepwise continuum of intensity. The subject either does or does not discriminate the test drug as being similar to the training drug. With the quantal method, the percentage of animals receiving the first reinforcement of the session on the drug-appropriate lever is the principal dependent measure (rather than percentage of drug-appropriate responses). Quantal assays are necessarily only used with large groups (typically, N>8) since each subject on test will only generate one data point (that is, the subject will make only one of the two possible response options). In contrast, with quantitative assays, it is possible to obtain from a single subject a dose-response curve since, in repeated tests with different doses, the subject will emit quantitatively different

degrees of responding. Thus, if one is working with a restricted number of animals, it is more appropriate to use a quantitative assay (Goudie and Leathley, 1993).

D. Training Dose

The rate at which a discrimination is learned is a key factor in choosing a training dose. Therefore, the optimal procedure is to use a large dose so that the discrimination can be learned relatively rapidly. In studies requiring low training doses, it is possible to train animals initially to discriminate a higher dose and then to 'fade' the dose down to a level that animals would not have been able to discriminate if they had been trained on it initially. However, such 'fading' techniques take a considerable amount of time and therefore have practical limitations (Holtzman, 1990). Most studies assessing cocaine's DS effects in rats have used a training dose of 10 mg/kg (e.g. Barrett and Appel, 1989; Broadbent et al., 1989; Callahan et al., 1991; Callahan and Cunningham, 1991; Baker et al., 1993). However, lower training doses of cocaine (e.g. 3 mg/kg) have also been used, and such studies have sometimes demonstrated qualitative as well as quantitative differences in the drug substitution profiles of compounds (e.g. Terry et al., 1994). Recently, a novel discrimination procedure has been devised, whereby rats are trained to discriminate between two doses of cocaine (2.5 vs. 10 mg/kg; Kleven and Koek, 1998). The authors claimed an advantages of the dose versus dose discrimination procedure for interaction studies, since sensitivity to pre-treatments may be enhanced as a consequence of the steeper dose-response function in dose-dose trained animals. Compounds that partially substitute in drug versus saline trained animals are therefore expected to engender primarily low-dose lever selection, so minimising the influence of baseline, high-dose-lever selection following pre-treatments that may have partial cocaine-like effects when given alone.

E. Pharmacological Characteristics of Discriminative Stimulus Control

An important attraction of the drug discrimination methodology is that for many classes of drugs the discriminative stimulus effects satisfy the criteria for receptor-mediated events. For example:

1. Drugs that interact with the same defined population of receptors on other assay systems have qualitatively similar discriminative stimulus properties, as reflected by their ability to generalise with one another.
2. Drugs do so with an order of potency that parallels their potencies in those other assay systems.
3. Antagonists that bind reversibly to the receptor block the discriminative effects of drugs of the same class in a competitive (i.e. surmountable) manner (Holtzman, 1990).

These features enable the investigator to determine dose-response curves; slopes can then be compared and differences in the potencies of drugs can be established. Hence, the drug discrimination procedure provides a discrete and objectively quantifiable behavioural endpoint that reflects the actions of a drug at the cellular level (Slangen, 1991). In summary, the drug discrimination procedure has relatively low predictive validity as a model of abuse potential, since many drugs (some of which are not abused by humans) act as discriminative stimuli. The model does have some predictive value in that it has a high degree of pharmacological specificity, and allows researchers to compare novel drugs with known drugs of abuse, and to determine whether they have similar mechanisms of action. However, the model does not attempt to reflect aspects of the symptomatology of drug abuse (face validity). But the model implies that human 'subjective' responses to drugs of abuse, which are important determinants of abuse potential, can be measured in animals (Goudie, 1991).

1.6.5 Reasons for Using The Drug Discrimination Model

The advantages of intravenous self-administration and drug discrimination as preclinical models for studying the psychological effects of drugs are several. Drug self-administration has potential utility in studying both the positive and negative reinforcing effects of drugs. Both procedures have predictive validity and are reliable. They also lend themselves to within-subjects designs, limiting the number of subjects required. Indeed,

once an animal is trained, full dose-effect functions can be generated for different drugs, and the animal can be tested for weeks or months.

The disadvantages of intravenous self-administration are largely technical. Special skills and procedures are required to implement and maintain a chronic catheter preparation, and success in maintaining viable catheter preparations in rodent studies can be difficult to achieve. Disadvantages of drug discrimination studies are that subjects receive numerous doses of the training drug and any neuropharmacological changes caused by such phenomena as sensitisation or tolerance cannot be measured easily. However, tolerance and sensitisation to cocaine's effects do not usually seem to occur as indicated by test-retest similarities of dose-response curves (Emmett-Oglesby et al., 1983). The major disadvantage of place conditioning is the enormous cost, effort, and time required to generate results. Each drug dose requires testing 8-12 animals in an independent (between subjects) design, and although each animal is tested numerous times it yields only one independent data point. Also, there is some concern, as mentioned previously, as to what precisely is being measured in the model.

The importance of animal models used to measure the psychological effects of cocaine lies in the assumption that the findings obtained in the animal models will have relevance to the problems of drug abuse and addiction in man. Certainly, some animal models can predict abuse potential, but the value of these animal models goes far beyond just the capacity to predict abuse potential. Animal models can provide a means of studying both the behavioural and biological bases of drug addiction. The drug-discrimination method will therefore be used as a model to examine DA receptor subtype involvement in cocaine's effects.

CHAPTER 2:

'DOPAMINE RECEPTOR SUBTYPE INVOLVEMENT IN THE DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE'

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2.1 Introduction

Drug discrimination procedures have been particularly useful in characterising the neuronal mechanism(s) underlying the in vivo effects of cocaine. Cocaine has been known for a long time to serve as a discriminative stimulus and has been extensively studied in this regard. For example, in 1976 Colpaert and colleagues were first to demonstrate that 10 mg/kg of cocaine could function as a DS in rats (Colpaert et al., 1976). Many subsequent studies have shown that cocaine is capable of controlling differential responding in a variety of other species including pigeons (e.g. de la Garza and Johanson, 1985; Jarbe, 1981, 1984) squirrel monkeys (e.g. Woolverton and Trost, 1978) and rhesus monkeys (e.g. de la Garza and Johanson, 1983). Furthermore, the cocaine/saline discrimination can be trained under a variety of schedule conditions and using different routes of administration. The purpose of the present chapter is to provide a brief review of the scientific evidence that supports dopaminergic involvement in the DS effects of cocaine and to identify research questions that need to be answered in order to further elucidate these neurochemical processes.

2.2 Effects of Monoamine Re-uptake Inhibitors:

DA appears to play a prominent role in the DS effects of cocaine. DA re-uptake inhibitors such as GBR 12909, mazindol, nomifensine and bupropion readily substitute for cocaine in rats (Broadbent et al., 1991; Cunningham and Callahan, 1991; Witkin et al., 1991; Baker et al., 1993), monkeys (Kleven et al., 1990; Spealman, 1995), pigeons (Johanson and Barrett, 1993), and mice (Middaugh et al., 1998) trained to discriminate cocaine from saline (Table 2.1). These findings consistently indicate that the DS properties of cocaine are reproduced by compounds that block DA re-uptake. In contrast, selective noradrenergic (NE) and serotonergic (5-HT) uptake inhibitors do not produce responding on the cocaine-appropriate lever. However, there have been reports that NE may play a more important role when low training doses of cocaine are used (Terry et al., 1994), or in species

TABLE 2.1

Summary of substitution tests with monoamine re-uptake inhibitors assessed in species trained to discriminate cocaine from saline.

Study	Species	Dose (mg/kg)	Drug Used	Comment
Kleven et al. (1990)	Rhesus Monkeys	0.2 - 0.4	GBR 12909 (DA)	Generalisation to cocaine
			Mazindol	
			Nomifensine	
			Bupropion	
			Tomoxetine (NE)	No effect
			Nisoxetine	
			Fluoxetine (5-HT)	No effect
Broadbent et al. (1991)	Rat	10.0	Nomifensine (DA)	Complete substitution. Order of potency : Nomifensine > GBR 12909 > Bupropion
			GBR 12909	
			Bupropion	
			Nisoxetine (NE)	Partial effect
			Desipramine	
			Imipramine	
Cunningham and Callahan (1991)	Rat	10.0	GBR 12909 (DA)	Complete substitution
			Desipramine (NE) &	No effect by itself - coadministration with low doses of cocaine enhanced discriminative stimulus effects of cocaine
			Fluoxetine (5-HT)	
Witkin et al. (1991)	Rat	10.0	GBR 12909 (DA)	Full substitution
			WIN 35,428	
			Mazindol	
Baker et al. (1993)	Rat	10.0	Mazindol (DA)	Complete substitution. Order of potency: Mazindol > Nomifensine > GBR 12909 > Bupropion
			Nomifensine	
			GBR 12909	
			Bupropion	
			Desipramine (NE)	Partial effect
			Citalopram	No effect
			Fluoxetine (5-HT)	

Johanson and Barrett (1993)	Pigeons	1.0 - 1.7	L-deprenyl(DA) Imipramine (NE) Tomoxetine Bupropion	Substitution
			Fluoxetine (5-HT) GBR 12909 (DA)	Partial substitution
Terry et al. (1994)	Rat	3.0	WIN 35,428 (DA) GBR 12909	Fully substituted for cocaine
			Tomoxetine (NE) Nisoxetine (NE)	Fully substituted for cocaine
			Fluoxetine (5-HT)	No effect
Spealman (1995a)	Squirrel Monkeys	0.18 - 1.0	GBR 12909 (DA)	Substituted for all conditions
			Talsupram (NE) Tomoxetine Nisoxetine Desipramine	Substituted for cocaine for low dose training conditions
Schama et al. (1997)	Squirrel Monkeys	0.3 - 1.0	Fluoxetine (5-HT)	No effect on its own - In combination with cocaine enhanced discriminative stimulus effects of cocaine
Middaugh et al. (1998)	Mice	10.0	Mazindol (DA) Nomifensine	Substituted completely for cocaine
			Nisoxetine (NE) Fluoxetine (5-HT)	No effect No effect
Kleven and Koek (1998)	Rat	2.5 vs. 10.0	Paroxetine Citalopram (5-HT) Fluoxetine Alaproclate	Dose-related increases in high dose lever selection when injected with combination of low training dose of cocaine
			Desipramine Talsupram (NE) Nortriptyline	Same as above
			Nisoxetine (NE)	Intermediate levels of responding

Key: (DA) - Dopaminergic, (NE) - Noradrenergic, (5-HT) - Serotonergic mediated events.

other than rodents, since Johanson and Barrett (1993) and Spealman (1995) showed that NE re-uptake inhibitors substitute for cocaine in pigeons and squirrel monkeys, respectively. Nevertheless, drug discrimination studies suggests that DA plays a more important role in the behavioural effects of cocaine than NE: compounds that act primarily through noradrenergic (NE) mechanisms (e.g. desipramine, imipramine, nisoxetine) substitute fully for cocaine in squirrel monkeys (Spealman, 1995a) at best only partially in rats (Broadbent et al., 1991; Baker et al., 1993) or not at all in mice (Middaugh et al., 1998). By contrast, the highly selective DA re-uptake inhibitor, GBR 12909, substitutes completely for cocaine in both monkeys (Kleven et al., 1990; Spealman, 1995a) and rats (Broadbent et al., 1991; Cunningham and Callahan, 1991; Witkin et al., 1991; Baker et al., 1993) regardless of training dose. It could be that species and/or training conditions are important determinants of the extent to which NE is involved in the DS effects of cocaine. Even so, taken together with findings that selective NE inhibitors enhance the DS effects of cocaine in rats and non-human primates (Kleven et al., 1990), it is conceivable that NE may exert a modulatory function in both species.

Likewise, the involvement of 5-HT in the DS effects of cocaine should not be discounted. 5-HT re-uptake blockade reliably enhances the DS effects of cocaine when combined with cocaine (Cunningham and Callahan, 1991; Schama et al., 1997; Kleven and Koek, 1998), but has negligible effects when tested alone for substitution in a variety of species (Kleven et al., 1990; Baker et al., 1993; Terry et al., 1994, Middaugh et al., 1998). Also, Schama et al. (1997) showed that fluoxetine, a 5-HT re-uptake inhibitor, did not substitute for cocaine in squirrel monkeys trained to discriminate a range of cocaine doses (0.3 - 10 mg/kg), but in combination with a low dose of cocaine it enhanced the DS effects of cocaine. In a different discrimination procedure, where rats were trained to discriminate between two doses of cocaine (2.5 vs. 10 mg/kg; Kleven and Koek, 1998) it was shown that paroxetine, citalopram, fluoxetine, and alaproclate (all 5-HT re-uptake inhibitors), as well as desipramine, talsupram and nortriptyline (all NE re-uptake inhibitors) caused dose-related increases in high-dose lever selection when injected in combination with the low training dose of cocaine. It has been suggested that, like NE re-uptake inhibition, 5-HT re-uptake blockade may play a modulatory function in the DS effects of cocaine (see Walsh and

Cunningham, 1997, for review). In conclusion, the evidence so far points to DA as being paramount to the DS effects of cocaine. If this is the case, then it is important to evaluate the effects of DA receptor-selective compounds in rats trained to discriminate cocaine from saline in order to determine whether specific DA receptors are critical to cocaine's DS effects.

2.3 Effects of D1-like and D2-like Agonists:

Over recent years, extensive work has been carried out to identify the relative contributions of different DA receptor subtypes to the DS effects of cocaine. Several studies have addressed this question by examining the effects of selective DA receptor agonists in subjects trained to discriminate cocaine from saline injections. For example, Barrett and Appel (1989) found that the D2-like agonist, quinpirole, fully substituted for cocaine in rats trained to discriminate 10 mg/kg cocaine from saline. In contrast, the D1-like agonist, SKF 38393, produced a maximum of only 40 % drug-appropriate responding in the same subjects. Other studies with rats have found qualitatively similar effects, although the maximal effects of the agonists have varied. For example, quinpirole has been reported to produce approximately 85 % (Callahan, Appel and Cunningham, 1991; Callahan et al., 1991; Callahan and Cunningham, 1993) or 40 % (Witkin et al., 1991; Callahan, Appel and Cunningham, 1991) cocaine-appropriate responding; SKF 38393 has been reported to produce approximately 75 % (Callahan et al., 1991) or 60 % (Witkin et al., 1991) cocaine-appropriate responding (Table 2.2). However, it is clear that both quinpirole and SKF 38393 substitute to some extent for cocaine. These findings have led to the conclusion that effects mediated by both D1-like and D2-like receptors contribute to the effects of cocaine, but that stimulation of either receptor subtype alone is generally not sufficient to fully reproduce the DS effects of cocaine (Witkin et al., 1991).

In contrast to the data from rats, neither SKF 38393 nor quinpirole produced any cocaine-like responding in rhesus monkeys trained to discriminate cocaine from saline (Kleven et al., 1990), although both D1-like and D2-like agonists have been shown to substitute near-fully in squirrel monkeys (Spealman et al., 1991). Katz and Witkin (1992) showed that when SKF 38393 and quinpirole were combined in squirrel monkeys trained to discriminate cocaine from saline, the combinations was no more effective than quinpirole alone. In pigeons, these two drugs only produce partial

TABLE 2.2

Summary of substitution tests with DA receptor agonists assessed in species trained to discriminate cocaine from saline.

Study	Species	Dose (mg/kg)	Drug Used	Comment
Barrett and Appel (1989)	Rat	10.0	SKF 38393 (D1)	No effect
			Quinpirole (D2)	Complete generalisation
Kleven et al. (1990)	Rhesus Monkeys	0.2 - 0.4	SKF 38393 (D1) Quinpirole (D2)	No effect
Spealman et al. (1991)	Squirrel Monkeys	0.3	SKF 82958 (D1) SKF 82917 [(+) - PHNO] (D2) Quinpirole Quinelorane CY 208-248 (-) - apomorphine (D1/D2)	Substitution on average of only 54 - 77% responding
Callahan and Cunningham (1991)	Rat	10.0	SKF 38393 (D1) Quinpirole (D2)	Partial substitution Full substitution
Callahan, Appel and Cunningham (1991)	Rat	10.0	SKF 38393 (D1) Quinpirole (D2)	Partial substitution Full substitution
Witkin et al. (1991)	Rat	10.0	(-) - apomorphine (D1/D2)	Full substitution
			SKF 38393 SKF 75670 (D1) CY 208-243 Pergolide (D2) (-) - NPA RU 24213 N-0434 N-0437	Partial substitution (40-80%)
Katz and Witkin (1992)	Squirrel Monkeys	0.3	SKF 38393 (D1)	No effect
			Quinpirole (D2)	Partial generalisation

			(SKF 38393 and Quinpirole combined)	Not effective when compared to quinpirole alone
Johanson and Barrett (1993)	Pigeons	1.0 - 1.7	SKF 75670 (D1) Quinpirole (D2)	Partial substitution
Callahan and Cunningham (1993)	Rat	10.0	Bromocriptine Quinpirole (Full D2 agonists)	Complete substitution
			Preclamol Terguride (Partial D2 agonists)	Less than 50% responding
Terry et al. (1994)	Rat	3.0	SKF 38393 SKF 77434 (D1) SKF 75670	Fully substituted for cocaine
			Quinpirole (D3/D2)	Fully substituted for cocaine
			N-0434 (-) - NPA (D2) SDZ 208-912	< 32% responding
Spealman (1995b)	Squirrel Monkeys	0.3	Terguride SDZ 208-911 SDZ 208-912 (Partial D2 agonists)	No effect
Vanover and Woolverton (1994)	Rhesus Monkeys	0.2 - 0.4	PD 128483 (High affinity partial D2 agonist - DA autoreceptors)	Substituted for cocaine - did not work as an antagonist
Acri et al. (1995)	Rat	10.0	(+) - PD 128907 (D3/D2) (±) - 7-OH-DPAT	Substituted for cocaine
Lamas et al. (1996)	Rhesus Monkeys	0.4	Quinpirole (D3/D2) (±) - 7-OH-DPAT	Completely generalised to cocaine Complete generalisation in 2/4 monkeys
Spealman (1996)	Squirrel Monkeys	0.3	PD 128907 (D3/D2) (±) - 7-OH-DPAT Quinpirole	Partially reproduce the discriminative stimulus effects of cocaine. Additive when combined with cocaine

Key: (DA) - Dopaminergic, (D1) - DA D1 receptor, (D2) - DA D2 receptor mediated events.

substitution (Johanson and Barrett, 1993).

Together, these results suggest that species difference may be important in evaluating the DS effects of cocaine, especially with regard to D1 receptor involvement. For example, recent findings have shown that the efficacies of the D1-like agonists SKF 38393, SKF 81297 and R(+)-6-Br-APB in stimulating adenylate cyclase activity in caudate membranes of squirrel monkeys is limited compared to rats (Izenwasser and Katz, 1993). This implies that the behavioural effects of DA D1-like agonists in monkeys will not always correspond to their effects on the activity of adenylate cyclase in rats. In addition, the apparently different interoceptive effects of SKF 38393, on the one hand, and SKF 81297 / SKF 82958 on the other, may reflect differences in the intrinsic activity of these drugs at DA D1-like receptors. Whereas SKF 38393 is less effective than DA in stimulating adenylate cyclase in vitro, SKF 81297 and SKF 82958 have efficacies approaching or exceeding that of DA itself (Andersen and Jansen, 1990).

Studies of DA receptor involvement in the DS effects of psychostimulants other than cocaine, such as amphetamine, and other indirect agents such as GBR 12909 and bupropion, have yielded both similar and dissimilar results. For example, neither the full DA D1-like agonist SKF 81297 nor the partial DA D1-like agonist SKF 38393 generalise to amphetamine (Arnt, 1988; Nielsen et al., 1989; Callahan et al., 1991; Vangroll and Appel, 1992) suggesting that DA D1-like receptors are not critically involved in mediating the amphetamine cue. However, agonists of the DA D2-like receptor family, such as quinpirole, pergolide and piribedil, do generalise to amphetamine in various species (Arnt, 1988; Nielsen et al., 1989; Sasaki et al., 1995), although negative results have been reported by Vangroll and Appel (1992) using quinpirole. A similar lack of substitution by SKF 38393 has also been observed in monkeys trained to discriminate GBR 12909 from saline (Kamien and Woolverton, 1989; Melia and Spealman, 1991). Partial substitution was also only found for D1-like and D2-like drugs in rats trained to discriminate bupropion from saline (Terry and Katz, 1997). In conclusion, these studies involving DA receptor subtype agonists suggest that amphetamine and GBR 12909 produces DS effects which are different from those of cocaine. However, more work still needs to be carried out with GBR

12909. The results for bupropion, on the other hand, indicate that it may well share similar actions to cocaine.

Although differences in training parameters or species may explain the partial substitution of D1-like and D2-like agonists for cocaine (Kamien and Woolverton, 1989; Kleven et al., 1990; Witkin et al., 1991; Spealman et al., 1991) it might also be explained by the choice of dose and/or pre-treatment intervals at which these agonists preferentially stimulate post synaptic receptors (Callahan and Cunningham, 1993). For example, by extending the pre-treatment time for bromocriptine and quinpirole (D2-like agonists), Callahan and Cunningham (1993) obtained full substitution of these drugs for cocaine, whereas they obtained only partial substitution at shorter pre-treatment times.

Partial D2-like receptor agonists, such as preclamol and terguride, have been shown to antagonise the cocaine DS (Callahan and Cunningham, 1993). The authors' explanation of this effect was that partial D2-like agonists may act as 'buffers' to reduce excessive dopaminergic activity, while their weak agonist activity maintains sufficient postsynaptic dopaminergic tone. Thus, the pharmacological profile of a compound with low intrinsic activity would shift from that of an agonist to an antagonist in the presence of an endogenous ligand competing for the same receptor. However, in another study the putative partial DA D2-like agonists SDZ 208-911 and SDZ 208-912 had no such effects in squirrel monkeys (Spealman, 1995).

Most studies therefore have not indicated a preferential involvement of one class of receptor over the other; however, DA D1-like receptors may be more important at low training doses of cocaine in rats (Terry et al., 1994; Kantak et al., 1995). Training rats to discriminate a low dose of cocaine from saline can alter the profile of DA receptor subtype agonist substitution, with D1-like agonists substituting fully and D2-like agonists showing either full (quinpirole) or minimal substitution ((-)-NPA, N-0434, SDZ 208-912). Given that quinpirole has a higher affinity for the D3 than the D2 receptor (Sokoloff et al., 1990), differential selectivities at subtypes of D2-like receptors may explain some of the different outcomes described above. However, in no previous experiments using a training dose of 10 mg/kg cocaine have D1-like agonists fully substituted or substituted for cocaine more completely than D2-like agonists (Barrett and Appel, 1989; Witkin et al., 1991; Callahan et al., 1991). The

findings therefore suggest a preferential effect of relatively high doses of cocaine (10 mg/kg) at the D2-like receptor subtype, with increasing D1-like receptor involvement at lower training doses.

Little is known concerning the effects of agonists at specific DA receptor subtypes within each of the two receptor sub-families (i.e. the DA D1, D5, and D2, D3, D4 receptors). Acri et al. (1995) demonstrated full substitution of a relatively selective DA D3 receptor agonist (PD 128907) for the DS effects of cocaine, albeit at doses where response rates were very low. This has been supported in other species using the D3/D2 agonists quinpirole and (±) 7-OH-DPAT (Lamas et al., 1996). Spealman (1996) showed that these same compounds, together with PD 128907, only partially reproduced the DS effects of cocaine in squirrel monkeys, but there was an additive effect in combination with a low dose of cocaine. Since these compounds are not completely selective for the DA D3 receptor subtype or may be functionally more active at D2 sites, the possibility that substitution for cocaine occurs via the DA D2-receptor subtype cannot be ruled out. In conclusion, it can be said that D1-like and D2-like agonists substitute at least partially for cocaine, with D2-like agonists being more efficacious at high training doses.

2.4 Effects of D1-like and D2-like Antagonists:

Antagonist experiments further support a role for DA receptors in the DS effects of cocaine. The non-selective DA antagonist cis-flupenthixol produces rightward shifts in the cocaine-dose response function in squirrel monkeys (Spealman et al., 1991). Studies in rats, monkeys and pigeons have shown that responding on the cocaine-trained lever is reduced by treatment with either a DA D1-like or a D2-like receptor antagonist (Kleven et al., 1988; Barrett and Appel, 1989; Kleven et al., 1990; Witkin et al., 1991; Spealman et al., 1991; Callahan et al., 1991; Baker et al., 1993 and Johanson and Barrett, 1993) (Table 2.3). However, studies in rodents have not always demonstrated antagonism of the DS effects of cocaine by DA D2-like receptor antagonists (e.g. Barrett and Appel, 1989; Witkin et al., 1991). Antagonism, especially by the D2-like antagonist haloperidol, has often been less pronounced in rats than in primates (Kleven et al., 1990; Spealman et al., 1991) even when comparable doses, routes of injection and pre-treatment times have been used across species. In an effort to resolve these inconsistencies for haloperidol, Callahan and Cunningham (1993)

TABLE 2.3

Summary of antagonism tests with DA receptor antagonists assessed in species trained to discriminate cocaine from saline; plus miscellaneous substitution/antagonist tests.

Study	Species	Dose (mg/kg)	Drug Used	Comment
Kleven et al. (1988)	Rhesus Monkeys	0.2	SCH 23390 (D1)	Blockade of cue
Barrett and Appel (1989)	Rat	10.0	SCH 23390 (D1) Spiperone (D2) Haloperidol (D2)	Blockade of cue Partial blockade of cue No effect
Kleven et al. (1990)	Rhesus Monkeys	0.2 - 0.4	SCH 23390 (D1) Haloperidol (D2)	Blockade of cue Blockade of cue
Spealman et al. (1991)	Squirrel monkeys	0.3	SCH 39166 (D1) YM 09151-2 (D2) Cis-flupenthixol (D1/D2)	Blockade of cue - rightward shifts indicative of surmountable antagonism
Witkin et al. (1991)	Rat	10.0	Haloperidol (D2) SCH 23390 (D1)	No effect 50% blockade of cue
Callahan et al. (1991)	Rat	10.0	SCH 23390 (D1) Haloperidol (D2)	Complete attenuation of cue
Baker et al. (1993)	Rat	10.0	SCH 23390 (D1) Cis-flupenthixol (D1/D2) (±) Sulpiride (D2)	Cis-flupenthixol and SCH 23390 showed greater attenuation than Haloperidol or (±) Sulpiride
Johanson and Barrett (1993)	Pigeons	1.0 - 1.7	SCH 23390 (D1) Raclopride (D2)	Blockade of cue Blockade of cue
Callahan and Cunningham (1993)	Rat	10.0	Bromuride (D2) Haloperidol (D2)	Blockade of cue Blockade of cue
Callahan et al. (1994)	Rat	10.0	Microinjection of Cocaine	Into the nucleus accumbens engendered full substitution

			Microinjection of SCH 23390 (D1)	Completely antagonised when administered into nucleus accumbens, a dose of cocaine that produced > 90% responding
Callahan et al. (1995)	Rat	10.0	Systemic administration of cocaine	Produced dose-related increase in cocaine-lever responding
			Bilateral injections of cocaine into central amygdala Bilateral injection of SCH 23390 (D1) into central amygdala	Partial substitution (< 60% drug-lever responding) Blockade of systemic dose of cocaine
Clark et al. (1995)	Rat	5.0	(-) - DS 121 (autoreceptor antagonist)	Partially substituted - but not dose-dependent. Small reduction in responding when combined with cocaine
Geter-Douglass and Riley (1996)	Rat	7.5 - 13.0	SCH 23390 (D1) and Haloperidol (D2) combination	Complete blockade of cue
Meert et al. (1996)	Rat	10.0	SCH 23390 (D1) and Haloperidol (D2) combination	Complete antagonism of cueing properties of cocaine
Baker et al. (1997)	Rat	10.0	PNU-99194A (D3)	Did not substitute for cocaine nor block the cocaine cue, or potentiated a low dose of cocaine
MISCELLANEOUS COMPOUNDS:				
Johanson and Barrett (1993)	Pigeons	1.0 - 1.7	8-OH-DPAT (5-HT_{1a} agonist) NAN-190 (5-HT_{1a} antagonist) Prazosin (α_1 antagonist)	Blockade of cue
Spealman (1995a)	Squirrel Monkeys	0.18 - 1.0	ST 587 & SDZ NV1 085 (α_1 agonist) Clonidine & UK 14,304 (α_2 agonist) Clenbuterol ($\beta_{1/2}$ agonist)	No Effect

			Prazosin (α_1 antagonist)	Blockade of cocaine cue for both training conditions
			Efaroxon (α_2 antagonist) Phentolamine (non-selective α blocker) Propranolol (β blocker)	No effect
Schama et al. (1997)	Squirrel Monkeys	0.3 - 1.0	Quipazine (non-selective 5-HT agonist)	Generalised to cocaine - additive when combined with cocaine
			Ketanserin & Ritanserin (5-HT_2 antagonists)	Attenuation of discriminative stimulus effects of cocaine
Kleven and Koek (1997)	Rat	2.5 vs. 10.0	(-) - Propranolol (β blocker) Tertatolol ($\beta_{1/2}$ blocker) ICI 118,551 (β_2 blocker)	Discriminative stimulus effects of low dose of cocaine were enhanced by pre-treatment with centrally acting β - adrenergic antagonists
			Nadolol (peripheral β antagonist) Betaxolol (centrally active β_1 antagonist)	No effect, therefore, β_2 effect
Kleven and Koek (1998)	Rat	2.5 vs. 10.0	Prazosin (α_1 antagonist)	Did not decrease cocaine-enhancing effects of Desipramine - suggesting negligible NE activity. However it did reverse enhancing effects of (-) - Propranolol suggesting that NE mechanisms may mediate the DS effects of cocaine

Key: (DA) - Dopaminergic, (D1) - DA D1 receptor, (D2) - DA D2 receptor, (5-HT) - Serotenergic, (α and β) - Noradrenergic mediated events

demonstrated that by increasing the time interval from 30 min to 120 min between injection of haloperidol (0.5 mg/kg) and testing for recognition of the cocaine cue in rats the percentage of cocaine antagonism increased from 20% to 85%. Thus, antagonism of the DS effects of cocaine in rats by haloperidol appears to require optimal DA D2-like receptor occupancy.

The finding that the selective D1-like antagonist SCH 23390 blocks the cocaine cue more effectively than haloperidol is consistent across species (e.g. in rats: Barrett and Appel, 1989; Callahan et al., 1991; Witkin et al., 1991; Baker et al., 1993; in monkeys: Kleven et al., 1990), and supports the view that D1-like receptors play a particularly important role in cocaine's DS effect. However, the significance of cocaine's indirect effects at D2-like receptors should not be downplayed since in addition to reports of antagonism by haloperidol, both sulpiride and YM-0915-2 (D2-like antagonists) reliably suppress cocaine-lever responding (Baker et al., 1993; Spealman et al., 1991). In addition to the influence of pre-treatment time, the occasional inconsistencies with D2-like receptor antagonists may be related to factors such as the age of the animals, their previous drug history, animal species (monkeys versus rats), doses and routes of cocaine administration (0.3 - 0.5 mg/kg i.v. in monkeys versus 10 mg/kg i.p. in rats) or the method of testing. For example, many studies in rats have used independent groups, and animals have usually been tested with antagonists only against the training dose of cocaine (Barrett and Appel, 1989; Witkin et al., 1991; Callahan et al., 1991; Baker et al., 1993; Callahan and Cunningham, 1993). On the other hand, studies using monkeys have tested antagonists against the full cocaine dose-response curve (Kleven et al., 1988, 1990; Spealman et al., 1991), an approach that as yet, has not been carried out in rats. This method has the advantage of allowing tests for changes in efficacy, potency and parallelism (non-parallelism indicating different mechanisms of action). Pharmacological antagonism can therefore be shown when the antagonist shifts the dose-response curve of the training drug to the right and the observed antagonism is surmountable by increasing the dose of the training drug. Despite these findings from antagonist experiments, little is known concerning the extent to which the D3 and D4 receptors (within the D2-like subfamily) contribute to the DS effects of cocaine, and this issue therefore warrants further investigation.

CHAPTER 3:

'PHARMACOLOGICAL CHARACTERISATION OF THE STIMULUS EFFECTS OF COCAINE: ASSESSMENT BY THE USE OF DOPAMINE 'D2-LIKE' AND 'D1-LIKE' ANTAGONISTS'

CHAPTER 3:

PHARMACOLOGICAL CHARACTERISATION OF THE STIMULUS EFFECTS OF COCAINE: ASSESSMENT BY THE USE OF DOPAMINE 'D2-LIKE' AND 'D1-LIKE' ANTAGONISTS

3.1 Aims Of The Drug Discrimination Experiments:

The results to date suggest that indirect stimulation of either DA D1-like or D2-like receptors by cocaine is necessary but not always sufficient for the full expression of the DS effects of cocaine. However, it remains unclear which of the recently-cloned DA receptors (D2, D3 and D4 receptors; Sibley and Monsma, 1992) within the D2-like receptor subfamily may be involved in cocaine's effects. The further examination of specific DA receptor subtype involvement in the DS effects of cocaine has been hampered by the limited availability of selective drugs. However, two compounds currently available are the selective DA D3 and D4 receptor subtype antagonists, U-99194A (Waters et al., 1993) and L-745,870 (Kulagowski et al., 1996) respectively. U-99194A [5,6-di-methoxy-2-(dipropylamino)indan-hydrochloride] is reported to have 25-40 fold preference for the DA D3 receptor over the DA D2 receptor subtype. It is also reported to increase locomotor activity in rats at doses (1-10 mg/kg) that do not significantly increase DA release in the striatum or nucleus accumbens (Waters et al., 1993), suggesting that U-99194A might be producing its behavioural effects via post synaptic D3 receptors. In contrast, L-745,870 [(3-([4-(4-chlororphenyl) piperazin-1-yl] methyl)-1H-pyrrolo [2,3-b] pyridine)] has been shown to have >2000- and >5000-fold binding selectivity for the D4 receptor relative to D2 and D3 receptors respectively, and > 20 000-fold selectivity over DA D1-like receptors (Kulagowski et al., 1996; Patel et al., 1996). L-745,870 does not have any agonist activity at doses of 0.1 - 10 mg/kg p.o. but inhibits in vivo binding of [^{3}H] SKF 10,047 in mouse brain - a model for in vivo sigma binding used to determine brain penetration and to estimate occupancy of central D4 receptors (Patel et al., 1996b). At doses up to 1 mg/kg p.o. in rats, any effects observed with this compound are likely to be mediated via D4 receptor antagonism, since at this dose L-745,870 will occupy > 90% of D4 receptors in the CNS but not other receptors. At higher doses, L-745,870 begins to occupy other receptors; for example, at 3 mg/kg L-

745,870 occupies 50 % of CNS sigma binding sites, and at > 10 mg/kg any antagonism may be attributed to blockade of D2 receptors (Patel, personal communication). At this higher dose of 10 mg/kg the drug does not effect DA metabolism in mouse striatum or nucleus accumbens, indicating that the drug acts on postsynaptic sites up to relatively high doses (Patel et al., 1996b).

The purpose of the present experiments was to:

(1) Investigate for the first time the roles of D3 and D4 receptor subtypes in the DS effect of cocaine. Many of the inconsistent results reported to date with antagonists may be due to methodological differences, such as repeated measures vs. independent groups of animals, or the testing of rats with antagonists only against the training dose of cocaine vs. use of full dose-response curves in monkeys. The present study therefore adapted a within-subject design testing against the full cocaine dose response curve; no studies to date have used this approach in rats. For comparison, the effects of a DA D1-like receptor antagonist, SCH 39166 ((-)-trans-6,7,7a,8,9, 13b-hexahydro-3-chloro-N-methyl-5H-benzop[d]napthop[2,1-b]azepine; Chipkin et al., 1988) were also tested.

(2) Terry et al. (1994) showed that training dose may also be an important determinant of the extent to which DA receptor subtypes are involved in the DS effects of cocaine. Therefore in another series of experiments, rats were trained to discriminate cocaine from saline using two different training doses. One group of rats was trained to discriminate a low dose of cocaine, 3 mg/kg i.p., and a second group was trained at the conventional dose, 10 mg/kg i.p. Since the study by Terry et al. (1994) used agonist compounds that were non-selective for D2-like receptors within the subfamily we followed up the earlier study by testing three compounds that display different receptor affinities within the D2-like receptor subtype family. The three centrally acting D2-like receptor antagonists were haloperidol, raclopride and L-741,626; the effects of these were then compared with those of SCH 39166, a selective D1-like receptor antagonist.

Haloperidol has been used as the prototypical antagonist for DA D2 receptors (Table 2.3, Section 2.4). However, it binds to all receptors within the D2-like receptor subfamily: it is roughly equipotent across all three receptor subtypes: Ki = 2.6 nM for D2 versus 1.5 nM for D3 and 5.0 nM for D4 human DA receptor subtypes (Freedman

et al., 1994; Van Tol et al., 1991). It is also a non-selective antagonist for NMDA and sigma sites (Lynch and Gallagher, 1996). Raclopride, on the other hand, has been shown to have equal affinity for rat DA D3 and D2 receptors, but much lower affinity for DA D4 receptors: Ki = 1.8 nM and 3.5 nM for D2 and D3 receptors, compared to 237 nM for D4 receptors (Van Tol, 1991). In contrast, L-741,626 (3-{[4-(4-chlorophenyl)-4-hydroxy]piperidin-1-yl}methyl-1H-indole HCl) is 40-fold selective for the human D2 receptor over the D3 receptor, although its selectivity is reduced to 10-fold in the rat: Ki = 12 nM versus 120 nM for rat D2 and D3 receptors, respectively (Bowery et al., 1996; Bristow et al., 1998). L-741,626 penetrates the brain well and is functionally active in vivo at a dose range of 1-5 mg/kg (Bristow, personal communication); it is one of the most selective DA D2 receptor antagonists identified to date.

A number of positive control drugs were also tested including for the first time the selective NE/5-HT re-uptake inhibitor venlafaxine (Holliday and Benfield, 1995), a drug with no affinity for the DA transporter.

3.2 Materials and methods

3.2.1 Subjects:

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 320-400 g, were housed with free access to water under a 12 h light/dark cycle (lights on 07:00). All testing was between 09:00 and 11:00. Rats were fed 15 g standard diet daily, 30 min after testing. Two groups of eight were trained at the 'high' dose of cocaine, 10mg/kg, and another two groups of eight were trained at the low 'dose' of cocaine, 3mg/kg. These groups were not tested at the same time but over a 2 year period.

3.2.2 Apparatus:

Two-lever operant chambers (Coulbourn and Med-Associates, all controlled by Med-PC software), housed within fan-ventilated, light- and sound-attenuating shells. Ambient illumination was by a lamp set centrally above the two levers (10 cm above the cage floor). The levers, were set 17 cm apart and 7 cm from the cage floor and required a force of 0.4N through 1mm to register a lever press. Reinforced presses activated an audible click, and dispensed one 45 mg pellet (Noyes Precision) into a centrally located food tray.

3.2.3 Procedure:

Rats were trained initially to press both levers separately under a fixed-ratio 1 (FR1) schedule of food reinforcement. They were then trained after FR10 to discriminate i.p. injections of cocaine (10 mg/kg) from i.p. injections of saline. After cocaine, responses on only one lever were reinforced; after saline, responses on the other lever were reinforced. The assignment of cocaine and saline-appropriate levers was counterbalanced across rats. Rats were placed in the chambers directly after injection, and a 5-min time-out period was initiated, during which lamps were off and responding was not reinforced. Lamps were then illuminated, and responses on the appropriate lever were reinforced; each reinforcement was followed by a 20 s time-out. The FR value was increased to FR20 over several sessions, and responses on the inappropriate lever reset the FR requirement on the appropriate lever. Sessions ended after 20 food presentations or 20 min, whichever occurred first. As the FR value reached 20, training sessions with cocaine and saline were scheduled in a double-alternating sequence. The criteria for successful completion of a session were: at least 85% injection-appropriate responding overall, and during the first FR of the session.

In the second two subsets of eight rats, a fading procedure was used to establish discriminative control by a low dose of cocaine. Initially, the rats were trained to discriminate 10 mg/kg of cocaine from saline (at FR10), as described. As the discrimination was acquired, the training dose was first reduced to 5.6 mg/kg at FR15 and then finally to 3 mg/kg at FR20.

Substitution tests began after 6 consecutive successful training sessions at FR20: tests were identical to training sessions, except that 20 consecutive responses on either lever were reinforced. Thereafter, tests were conducted when a given rat met criterion on both preceding saline and cocaine training sessions. Cocaine was injected immediately before chamber entry; injections of the antagonists (random order within drug) were 30 min beforehand.

3.2.4 Data Analysis:

Data from any rat that pressed fewer than 20 times were not included in the calculation of mean cocaine-appropriate responding on that test, and if fewer than three rats met the requirement, no mean value was derived on that test. Mean response rates were calculated for all rats on each test. Standard analysis of variance (ANOVA)

and regression were used for difference testing and to calculate ED_{50} values, their 95% confidence limits (CL), and relative potency estimates (the dose of standard drug (mg/kg) equal to 1 mg/kg of comparison drug; a significant difference was assumed if the 95% CL did not include 1.0). pA2 values were calculated by 'Schild' analysis using a specially-written programme.

3.2.5 Drugs:

(-)-Cocaine HCl, Chlordiazepoxide HCl, d-amphetamine sulphate (Sigma Chemicals, Poole, UK); Pentobarbitone sodium (Segatal) (RMB Animal Health Ltd, Dagenham, UK); S(-)-Raclopride L-tartrate (RBI, St. Albans, Herts, UK); Venlafaxine HCl (Wyeth-Ayerst Research, Princeton NJ, USA) and SCH 39166 HCl (Schering Corporation, Bloomfield NJ, USA) were dissolved in 0.9% saline; L-745,870 HCl (Tocris Cookson, Bristol, UK) was dissolved in distilled water. L-741,626 (Tocris Cookson, Bristol, UK) was dissolved in distilled water and suspended in solution with Tween 80; while Haloperidol (Tocris Cookson, Bristol, UK) was dissolved in distilled water with a few drops of acetic acid and neutralised to a pH of 7.0 with 1M NaOH solution. All drugs were injected i.p. at 1 ml/kg.

3.3 Results

All eight rats in each subset learned to discriminate 10 mg/kg cocaine from drug vehicle (0.9 % saline) requiring 50.25 ± 5.29 and 68.63 ± 2.66 (mean ± S.E.M.) training sessions. The 10 mg/kg training dose of cocaine reliably maintained cocaine-appropriate responding (> 95%) throughout the period during which substitution tests were conducted. There was no apparent difference between the two subsets of rats trained at 10 mg/kg of cocaine in terms of the potency of cocaine's DS effects; ED_{50} values for the two subsets were 2.37 mg/kg (95% CL: 1.55-3.61 mg/kg) and 2.49 mg/kg (95% CL: 1.86-3.29 mg/kg) (Table 3.1).

For the 3 mg/kg training dose of cocaine, two groups of eight rats were initially trained. However, only a total of eight rats (6 from one group and 2 from the other) reliably learned to discriminate this dose, requiring 76.13 ± 2.30 training sessions. The ED_{50} value for the 3 mg/kg cocaine training dose was 1.00 mg/kg (95% CL: 0.71-1.40 mg/kg). This corresponds to a significant difference in potency when compared with the 10 mg/kg training dose of cocaine (estimated relative potency: 2.70; 95% CL: 1.83-3.90) showing that the two training conditions are not

overlapping and that the DS effects are quantitatively different from each other. Response rates did not differ significantly between cocaine and vehicle training sessions in either of the two training dose conditions.

ED_{50} values for cocaine from two separate determinations on all three groups, spanning more than a year for each, are presented in Table 3.1. All test-retest values are similar: there were no significant differences in the potency of the drug between these replications.

Figure 3.1 shows the dose-response curve for the DS effects of cocaine in rats trained to discriminate 10 mg/kg cocaine from vehicle. Cocaine produced a dose-dependent increase in cocaine lever responses. Response rates did not differ significantly between cocaine and vehicle test sessions across the doses (Figure 3.1, lower panel). A series of compounds were tested to establish the pharmacological specificity of the discrimination. First, the effects of d-amphetamine in substitution tests are shown. D-amphetamine (1-3 mg/kg) substituted fully for cocaine (> 80 % drug lever responses) with an ED_{50} value of 0.36 mg/kg (95% CL: 0.13-1.02 mg/kg), being approximately 6 times more potent than cocaine (Table 3.1). Response rates were reduced to < 30 % vehicle rates at the highest dose of d-amphetamine. However, neither the benzodiazepine, chlordiazepoxide (CDP), nor the barbiturate, pentobarbitone, elicited reliable cocaine-appropriate responding (Figure 3.2 and 3.3, respectively), with only partial substitution (35 % drug lever responses) at the highest dose of CDP (10 mg/kg), and negligible substitution with pentobarbitone. Each of these drugs was tested across a range of doses, from those that had no effect on response rates to those that decreased response rates to less than 20 % of control rates. This was also the case when pentobarbitone was tested in the low training dose group: pentobarbitone failed to produce cocaine-appropriate responding (Figure 3.4 and Table 3.1).

TABLE 3.1

DRUGS	ED_{50} (95 % CL)	RELATIVE POTENCY
TRAINING DOSE (10 mg/kg)		(1st DETERMINATION RELATIVE TO 2nd DETERMINATION)
COCAINE (1ST GROUP)		
1ST DETERMINATION	2.37 (1.55 - 3.61)	1.36 (0.83 - 2.29)
2ND DETERMINATION	1.74 (1.39 - 2.17)	
COCAINE (2ND GROUP)		
1ST DETERMINATION	2.49 (1.86 - 3.29)	0.89 (0.62 - 1.29)
2ND DETERMINATION	2.77 (2.07 - 3.71)	
TRAINING DOSE (3 mg/kg)		
1ST DETERMINATION	1.00 (0.71 - 1.40)	0.94 (0.61 - 1.43)
2ND DETERMINATION	1.08 (0.84 - 1.39)	
		(POTENCY RELATIVE TO COCAINE)
TRAINING DOSE (10 mg/kg)		
COCAINE (1ST GROUP)	2.37 (1.55 - 3.61)	1.00
AMPHETAMINE	0.36 (0.13 - 1.02)	5.80 (2.78 - 14.89)
COCAINE (2ND GROUP)	2.49 (1.86 - 3.29)	1.00
CDP	a	-
PENTOBARBITONE	a	-
TRAINING DOSE (3 mg/kg)		
COCAINE	1.00 (0.71 - 1.40)	1.00
PENTOBARBITONE	a	-

a = No value could be calculated due to lack of substitution

ED_{50} values (mg/kg) and relative potencies for compounds tested for substitution for discriminative stimulus effects of 10 mg/kg and 3 mg/kg cocaine. N=8 for each group.

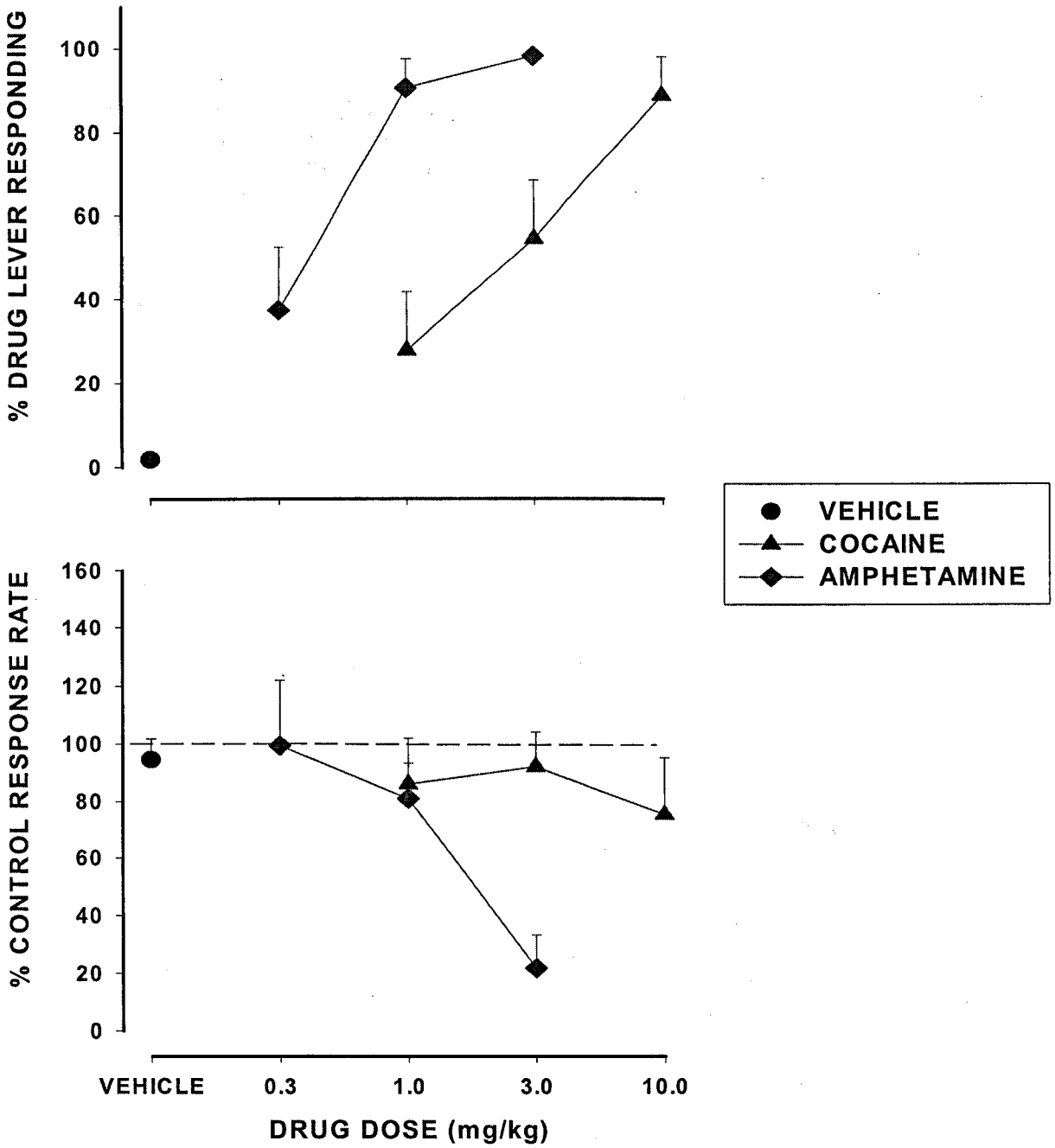

Figure 3.1 Upper panel: Effects of cocaine and d-amphetamine on the percentages of responding on the cocaine-correlated lever for rats trained at 10 mg/kg. Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents mean performance of at least three out of eight rats tested at each dose (upper panel)

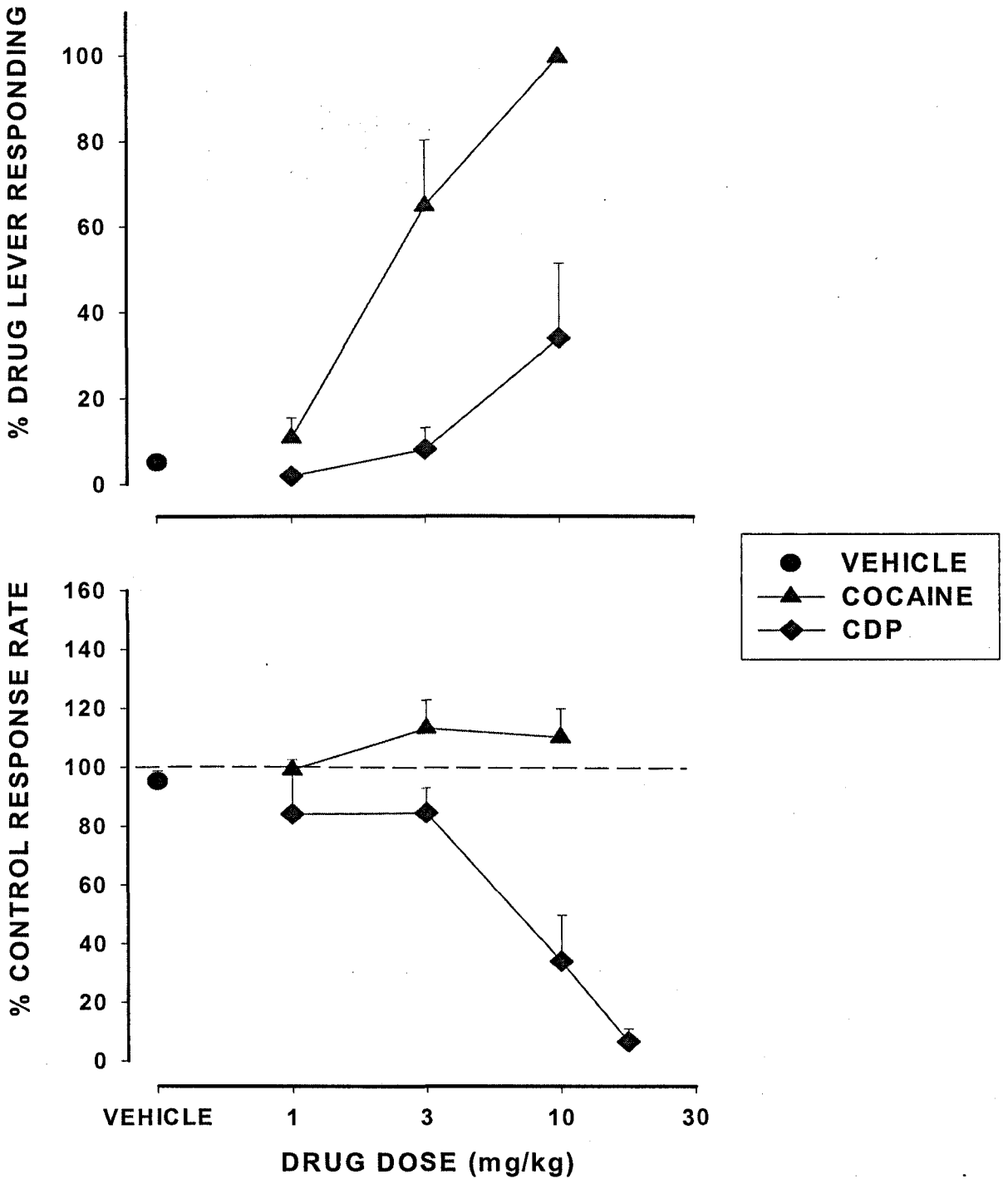

Figure 3.2 Upper panel: Effects of cocaine and chlordiazepoxide (administered 30 min pre-test) on the percentages of responding on the cocaine-correlated lever for rats trained at 10 mg/kg. Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents mean performance of at least three out of eight rats tested at each dose (upper panel)

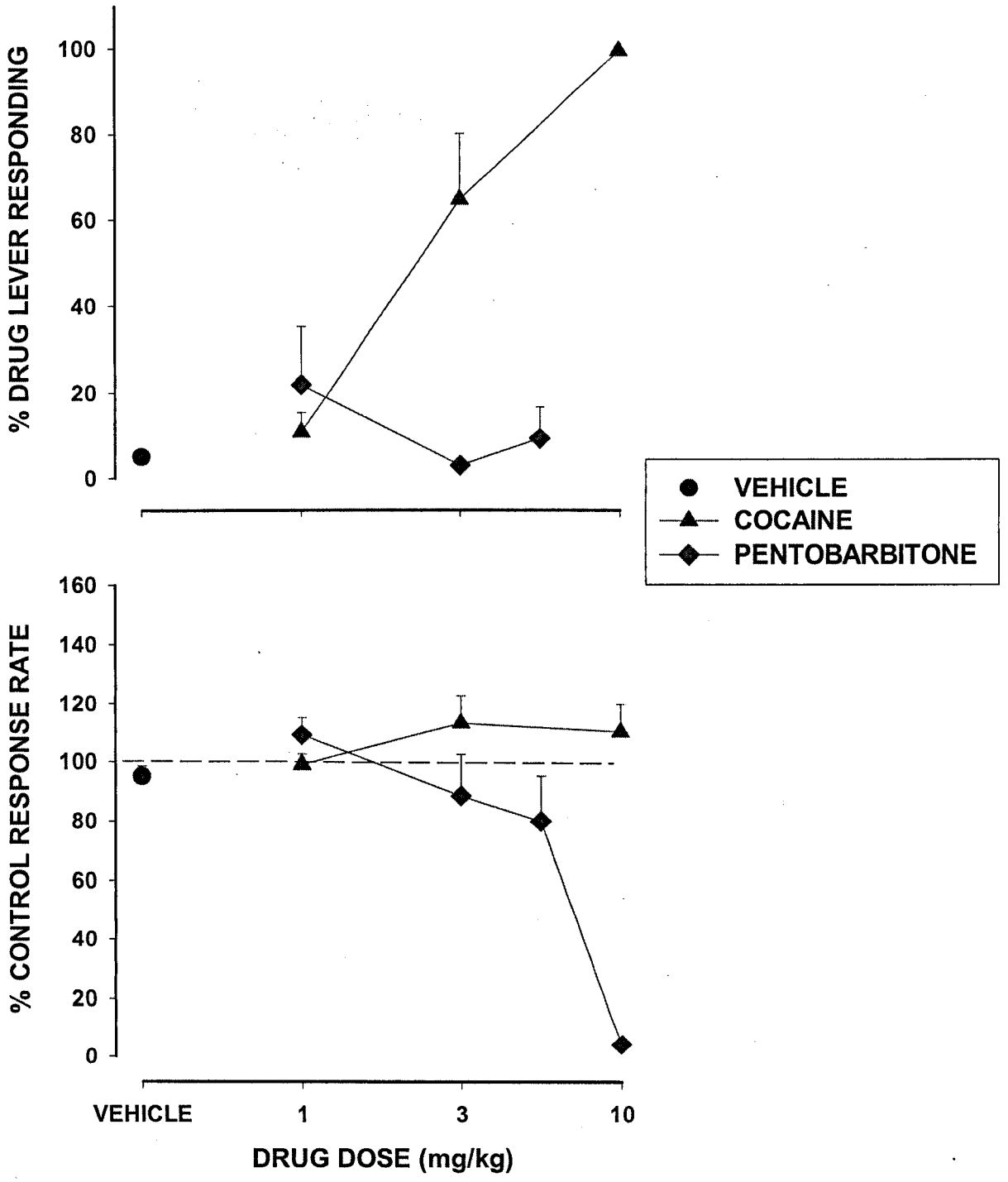

Figure 3.3 Upper panel: Effects of cocaine and pentobarbitone (administered 30 min pre-test) on the percentages of responding on the cocaine-correlated lever for rats trained at 10 mg/kg. Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents mean performance of at least three out of eight rats tested at each dose (upper panel)

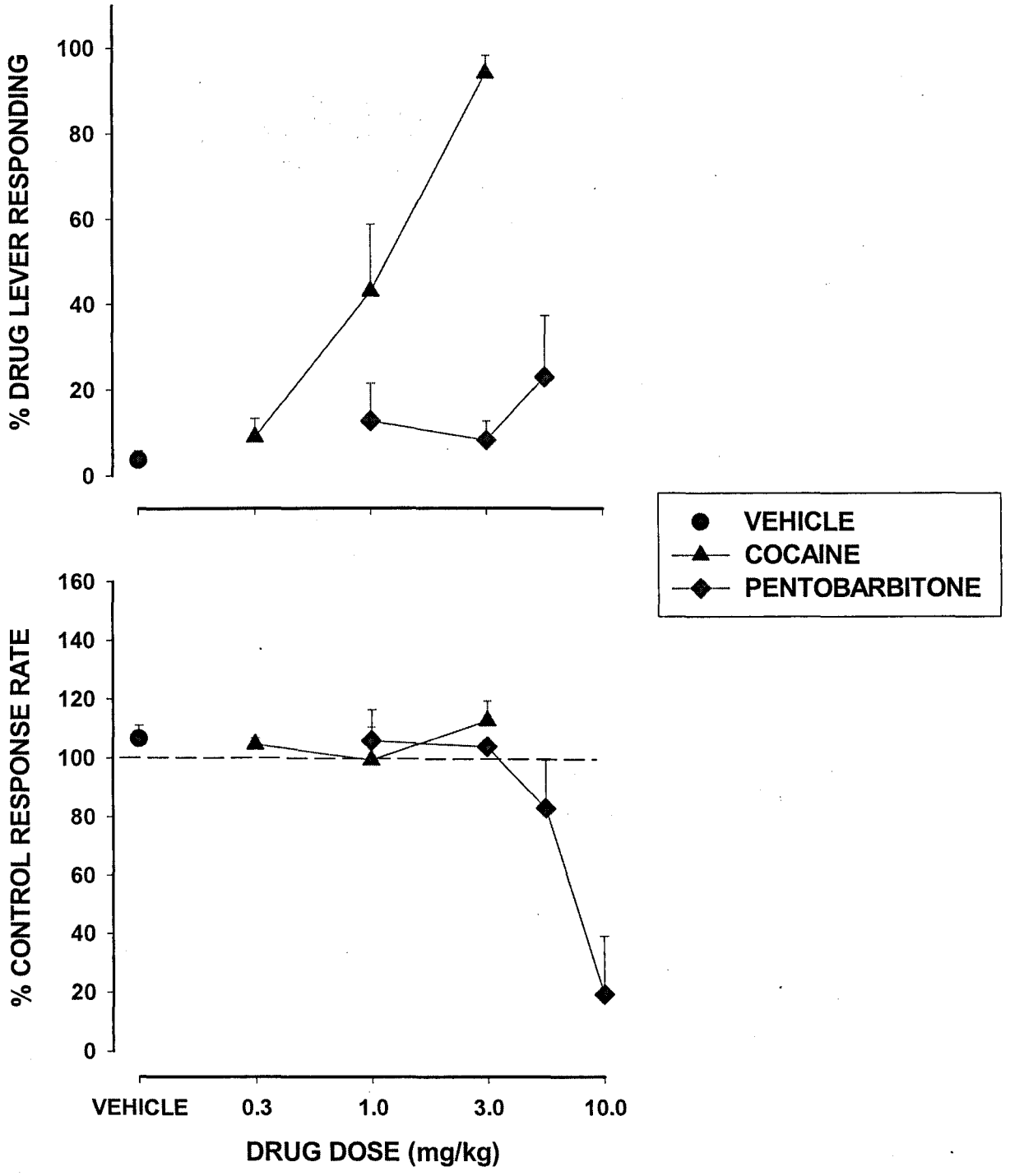

Figure 3.4 Upper panel: Effects of cocaine and pentobarbitone (administered 30 min pre-test) on the percentages of responding on the cocaine-correlated lever for rats trained at 3 mg/kg. Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents mean performance of at least three out of eight rats tested at each dose (upper panel)

3.3.1 The effects of DA D3 and D4 receptor subtype antagonists, and a D1-Like antagonist, on the DS effects of 10 mg/kg cocaine.

Neither of two doses of the DA D3 receptor antagonist U-99194A (3 and 10 mg/kg) substituted for cocaine, and neither dose shifted cocaine's dose-effect function (Table 3.2 and Figure 3.5). However, at the highest dose of U-99194A, there was some indication that the effects of the lowest dose of cocaine might be potentiated (Figure 3.5), but this effect was not significant [$F(2,12) = 3.61$, $P = 0.059$]. The DA D3 receptor antagonist produced no significant effect on response rates [$F(1,6) = 3.0$, NS], and no interaction with cocaine [$F(3,18) = 0.85$, NS] (Figure 3.5, lower panel), although at 10 mg/kg alone it reduced responding to approximately 50 % of control levels. Figure 3.5 clearly shows no evidence that U-99194A attenuated cocaine's DS effects.

At 1, 3 and 10 mg/kg, the DA D4 receptor antagonist L-745,870 produced no reliable cocaine-appropriate responding in the absence of cocaine, and was without effect when administered as a pre-treatment to a series of cocaine doses (Figure 3.6, upper panel). The ED_{50} values for the DS effects of cocaine were similar whether preceded by 0, 1 or 3 mg/kg of L-745,870 (Table 3.2). Although ED_{50} values could not be calculated for the combination with 10 mg/kg of L-745,870, the graph shows no indication of blockade at either 1, 3 or 10 mg/kg cocaine. Despite the lack of effect in terms of lever-choice behaviour, the antagonist produced a significant dose-related decrease in response rates [$F(3,12) = 18.60$, $P<0.001$] but no interaction with cocaine dose [$F(9,36) = 0.57$, NS] (Figure 3.6, lower panel).

In clear contrast, pre-treatment with the DA D1-like receptor antagonist SCH 39166 (0.03 and 0.3 mg/kg) produced a significant rightward shift in the dose-response function for cocaine's effects, indicating antagonism (Figure 3.7). There was a significant reduction in relative potency for the DS effects of cocaine when preceded by injection with 0.03 and 0.3 mg/kg SCH 39166 (Table 3.2). The DA D1-like receptor antagonist also produced a dose-related decrease in response rates [$F(2,12) = 26.10$, $P<0.001$] but no interaction with cocaine dose [$F(6,36) = 1.66$, NS] (Figure 3.7, lower panel).

TABLE 3.2

DRUGS	ED_{50} (95 % CL)	POTENCY RELATIVE TO COCAINE
TRAINING DOSE (10 mg/kg) 1ST GROUP		
DOPAMINE D3 RECEPTOR ANTAGONIST		
COCAINE ALONE (N=7)	1.74 (1.39 - 2.17)	1.00
+ 3 mg/kg U-99194A	1.10 (0.68 - 1.79)	1.28 (0.93 - 1.80)
+ 10 mg/kg U-99194A	a	-
DOPAMINE D4 RECEPTOR ANTAGONIST		
COCAINE ALONE (N=5)	1.89 (1.52 - 2.35)	1.00
+ 1 mg/kg L-745,870	1.67 (1.20 - 2.32)	1.07 (0.79 - 1.46)
+ 3 mg/kg L-745,870	2.29 (1.63 - 3.23)	0.84 (0.59 - 1.19)
+ 10 mg/kg L-745,870	a	a
DOPAMINE D1-LIKE RECEPTOR ANTAGONIST		
COCAINE ALONE (N=7)	1.31 (0.51 - 3.39)	1.00
+ 0.03 mg/kg SCH 39166	5.01 (2.99 - 8.39)	0.46 (0.16 - 0.97)
+ 0.3 mg/kg SCH 39166	8.47 (3.71 - 19.33)	0.29 (0.10 - 0.58)

a = insufficient data to calculate ED_{50} values

ED_{50} values (mg/kg) and relative potencies for the DS effects of cocaine in combination with a DA D3 (U-99194A), D4 (L-745,870) and D1-like (SCH 39166) receptor antagonist.

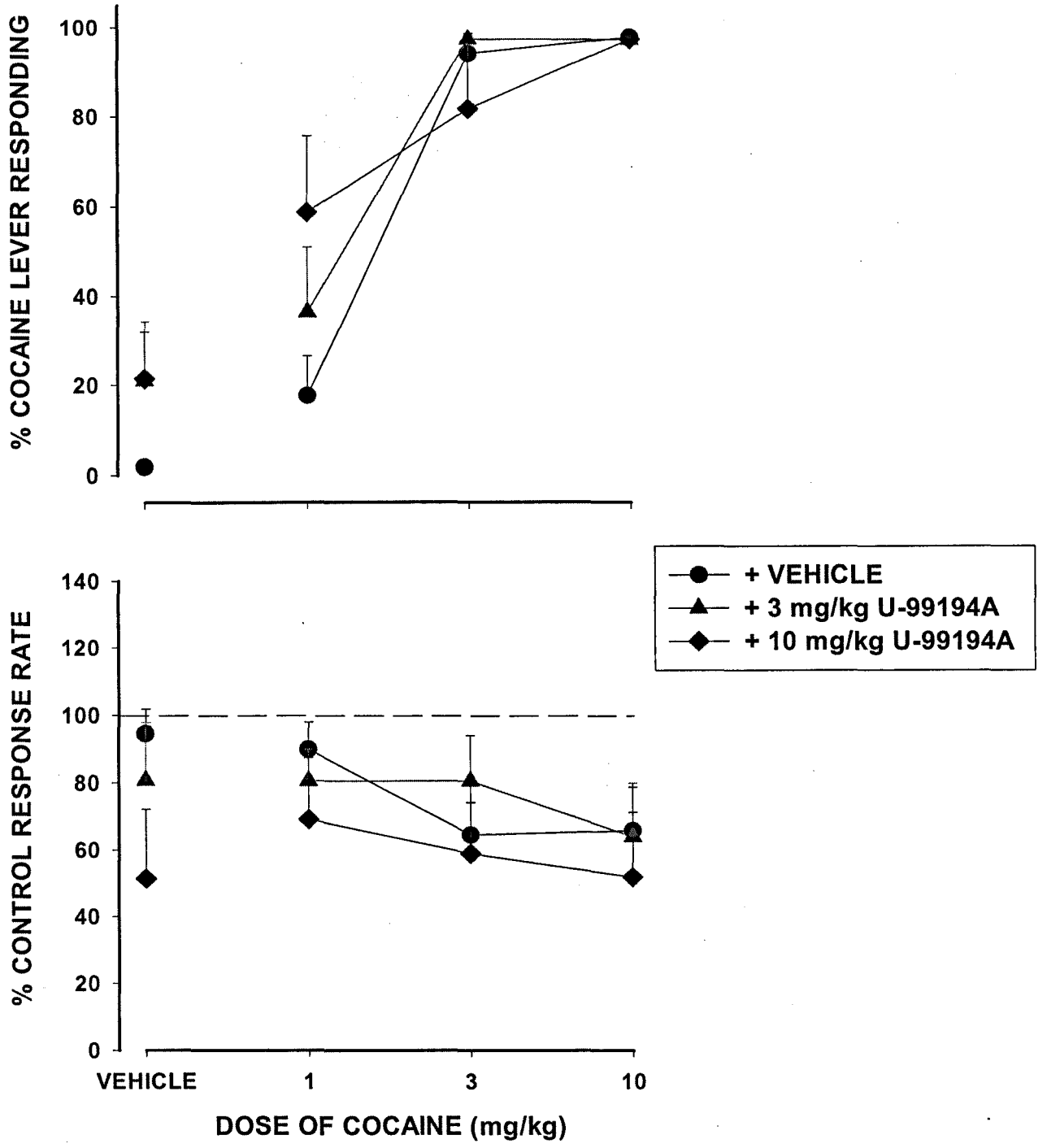

Figure 3.5 Upper panel: Effects of the DA D3 receptor antagonist U-99194A alone and in combination with 1, 3 and 10 mg/kg of cocaine in rats trained at 10 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to saline response rates. Each point represents the mean of at least three out of seven rats tested at each dose (upper panel)

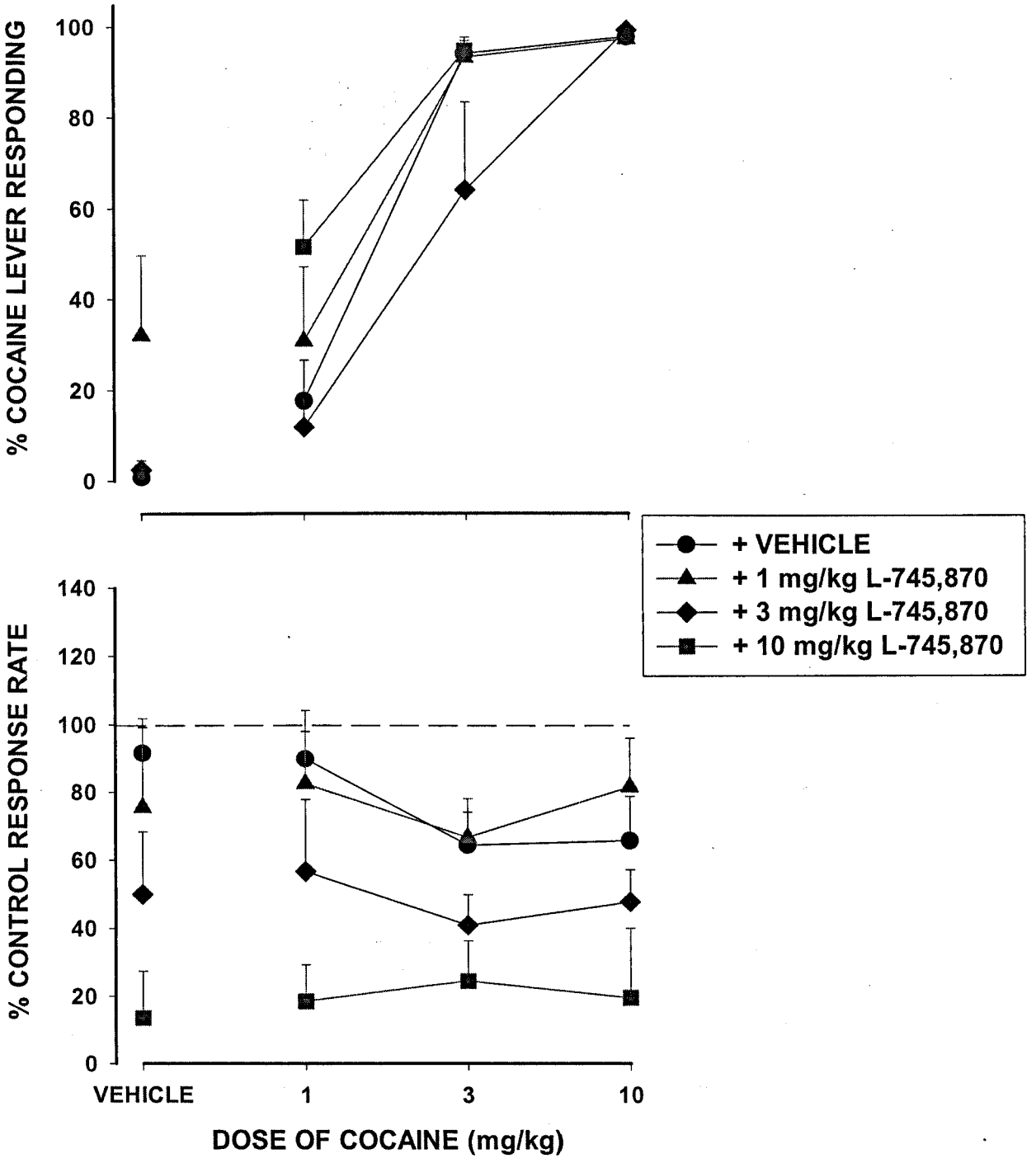

Figure 3.6 Upper panel: Effects of the DA D4 receptor antagonist L-745,870 alone and in combination with 1, 3 and 10 mg/kg of cocaine in rats trained at 10 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to saline response rates. Each point represents the mean of at least three out of five rats tested at each dose (upper panel)

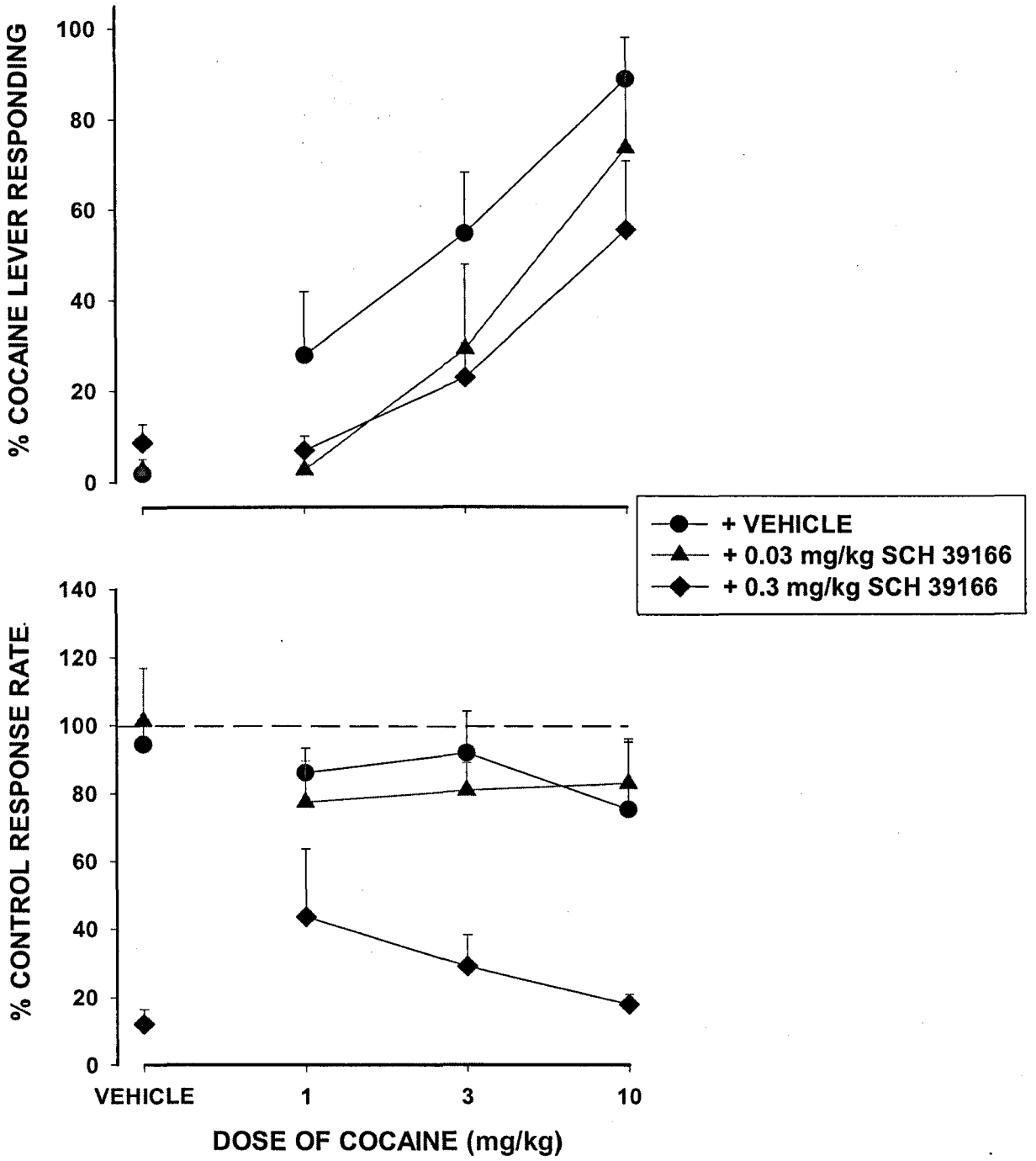

Figure 3.7 Upper panel: Effects of the DA D1-like receptor antagonist SCH 39166 alone and in combination with 1, 3 and 10 mg/kg of cocaine in rats trained at 10 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to saline response rates. Each point represents the mean of at least three out of seven rats tested at each dose (upper panel)

3.3.2 The effects of D2-Like (L-741,626, haloperidol, raclopride) and D1-Like (SCH 39166) receptor antagonists on the discriminative stimulus effects of cocaine in rats trained at 10 mg/kg or 3 mg/kg.

(i) Rats Trained At The Higher Dose (10 mg/kg):

When given 30 min before administration of 10 mg/kg cocaine, all three centrally acting D2-like antagonists, L-741,626 (3, 6 mg/kg) haloperidol (0.3 mg/kg) and raclopride (0.3 mg/kg) produced rightward shifts in the dose-response function for cocaine's DS effects (Figures 3.8-3.10, upper panels). All dose-response functions were parallel to the cocaine curve (Table 3.3). By comparing the minimum dose of each antagonist to produce a similar change in potency, the rank order of potencies was shown to be haloperidol > = raclopride > L-741,626. When these D2-like antagonists were administered alone, they all produced DS effects that were not significantly different from saline (Figures 3.8-3.10, upper panels), but they all produced significant decreases in response rates when compared with saline (Figures 3.8-3.10, lower panels). Dose-effect curves for response rates also revealed a dose-related decrease in response rates for all of the D2-like receptor antagonists tested in the presence of cocaine [L-741,626: $F(2,14) = 10.44$, $P<0.01$; haloperidol: $F(1,7) = 53.28$, $P<0.01$; and raclopride: $F(1,7) = 42.07$, $P<0.01$]. Only haloperidol produced an interaction with cocaine dose [$F(3,21) = 3.52$, $P<0.05$] (Figures 3.8-3.10, lower panels). This interaction between haloperidol and cocaine for response rates suggests that haloperidol is attenuating the rate-suppressant effects of cocaine. Although this interaction was not statistically supported for L-741,626 and raclopride, Figures 3.8 and 3.10 clearly show a similar trend.

(ii) Rats Trained At The Lower Dose (3 mg/kg):

At 1 mg/kg, the D2 receptor antagonist L-741,626, had no effect when administered as a pre-treatment to cocaine in rats trained at the low dose (3 mg/kg) (Figure 3.11, upper panel). However, there was evidence of antagonism when it was administered in combination with 1 mg/kg of cocaine. An increase in ED_{50} value occurred, together with a decrease in cocaine's potency; however, this was accompanied by a significant deviation from linearity which prevented a reliable relative potency estimate (Table 3.3). On the other hand, the higher dose of 5 mg/kg L-741,626 decreased cocaine-appropriate responding to 58 % at the training dose, an

effect that was accompanied by a significant decrease in response rates [$F(2,14) = 32.58$, $P<0.01$] (Figure 3.11, lower panel). An ED_{50} value could not be calculated due to insufficient data, but a relative potency value could still be calculated; the higher dose of L-741,626 reduced cocaine's potency approximately three-fold (Table 3.3).

As at the high training dose, the D2-like receptor antagonists haloperidol and raclopride produced rightward shifts of the dose-response curves for rats trained at the low dose (Figure 3.12 and 3.13, upper panel). The ED_{50} value for cocaine in combination with the highest dose of each D2-like antagonist was significantly different from that of cocaine alone (3.91 mg/kg for haloperidol and 2.54 mg/kg for raclopride). The order of potencies for these drugs paralleled that in the high dose-training conditions, with raclopride marginally more potent than haloperidol, and both more potent than L-741,626 (Table 3.3). Again, as for the high dose trained rats, when the D2-like antagonists were administered alone they all produced DS effects that were not significantly different from saline (Figures 3.11-3.13, upper panels). Response rates also decreased when the D2-like antagonists were combined with cocaine: [haloperidol: $F(2,14) = 10.24$, $P<0.05$; raclopride: $F(1,7) = 22.20$, $P<0.05$] but there was no interaction with cocaine dose [$F(6,42) = 0.65$, NS; $F(3,18) = 0.60$, NS; respectively) (Figures 3.12 and 3.13, lower panels).

Pre-treatment with the DA D1-like receptor antagonist SCH 39166 (0.1 mg/kg) also produced significant rightward shift in the percentage of drug lever responding observed after cocaine (Figure 3.14, upper panel). The ED_{50} value of cocaine with SCH 39166 increased significantly, its potency reduced approximately three times (Table 3.3). The DA D1-like receptor antagonist also produced a decrease in response rates [$F(1,6) = 15.87$, $P<0.01$] but there was no interaction with cocaine (Figure 3.14, lower panel).

pA2 values calculated by 'Schild analysis' characterising the receptor subtypes at which these antagonists are acting for the two different training doses of cocaine are given in Table 3.4. These represent the -log values of the concentration of the antagonist that necessitates an x-fold increase in agonist concentration (i.e. cocaine) applied in order to obtain a standard response. This is based on selective antagonism and is quantitated by determining a derivative of Kd for antagonist binding, since pA2 $\equiv pK_B \equiv$ -Log Kd. This information tells us, by comparing the pA2 values obtained

for each compound, whether they are acting at the same receptor subtype. From, table 3.4, it is difficult to conclude that the DA receptor antagonists employed in the present study are acting across different receptors, although the values for L-741,626 seem to distinguish it from the other compounds.

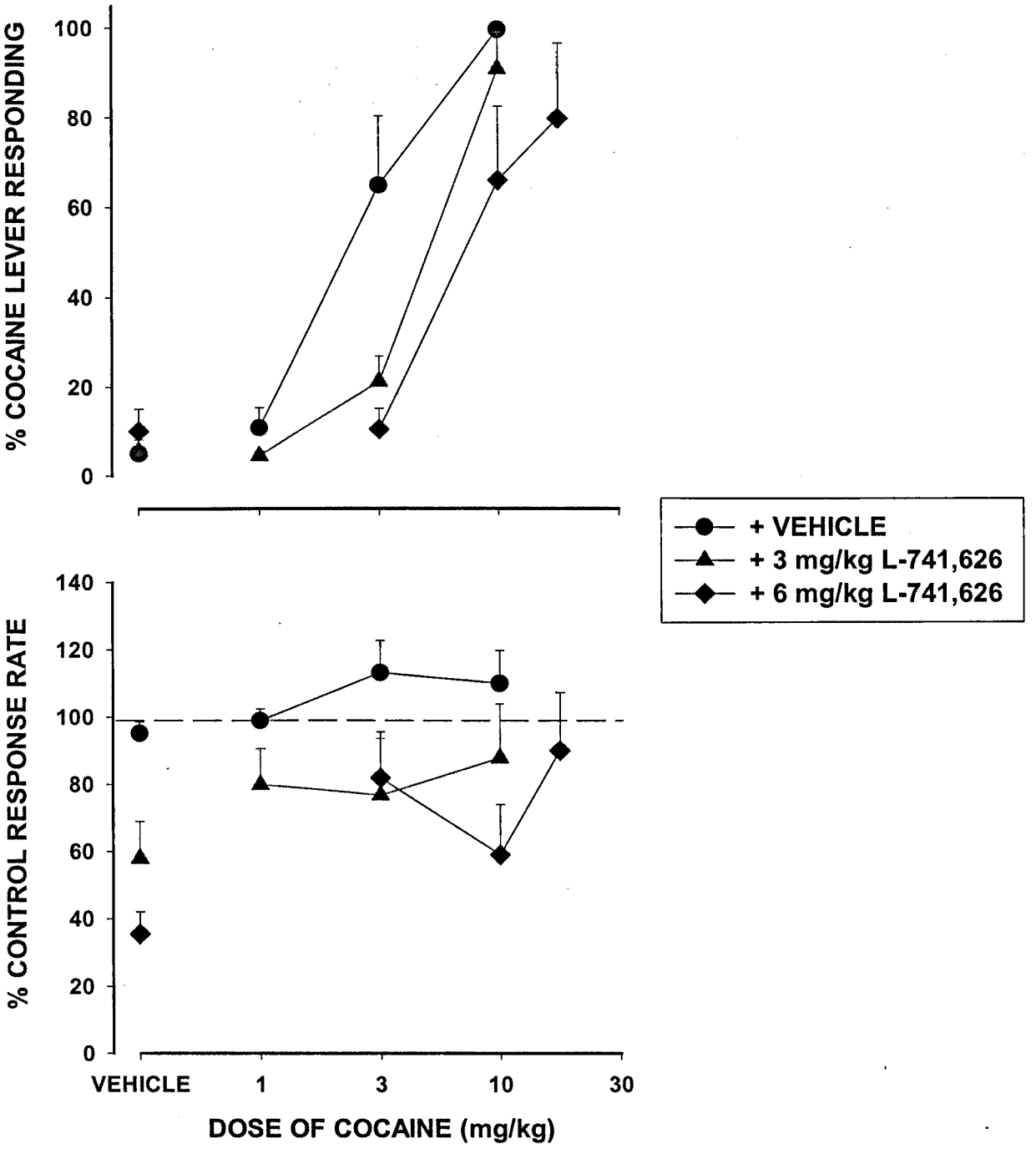

Figure 3.8 Upper panel: Effects of the DA D2 receptor antagonist L-741,626 alone and in combination with 1, 3, 10 and 17 mg/kg of cocaine in rats trained at 10 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents the mean of at least three out of six rats tested at each dose (upper panel)

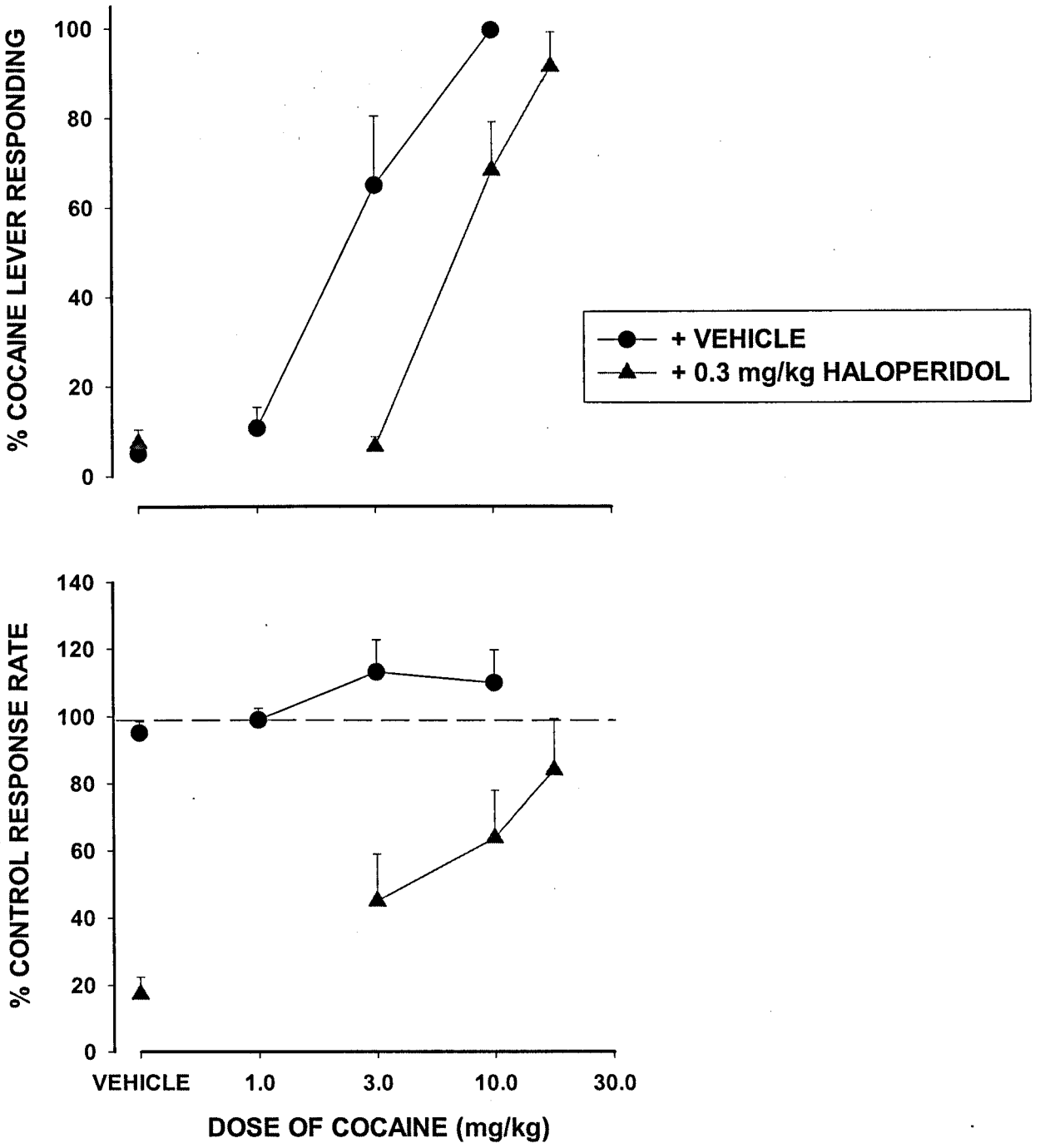

Figure 3.9 Upper panel: Effects of the DA D2-like receptor antagonist haloperidol alone and in combination with 3, 10 and 17 mg/kg of cocaine in rats trained at 10 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents the mean of at least three out of eight rats tested at each dose (upper panel)

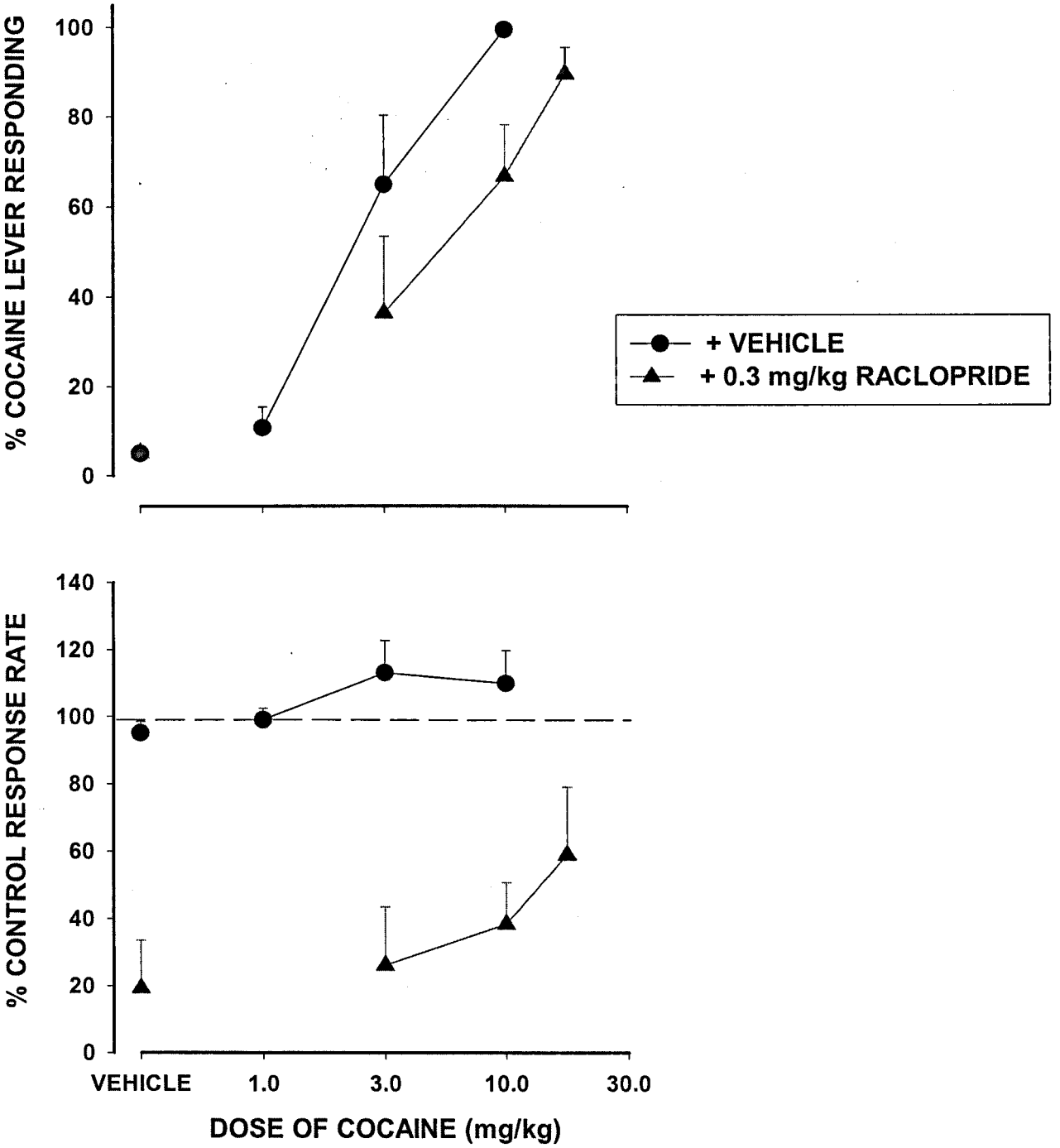

Figure 3.10 Upper panel: Effects of the DA D2-like receptor antagonist raclopride alone and in combination with 3, 10 and 17 mg/kg of cocaine in rats trained at 10 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents the mean of at least three out of eight rats tested at each dose (upper panel)

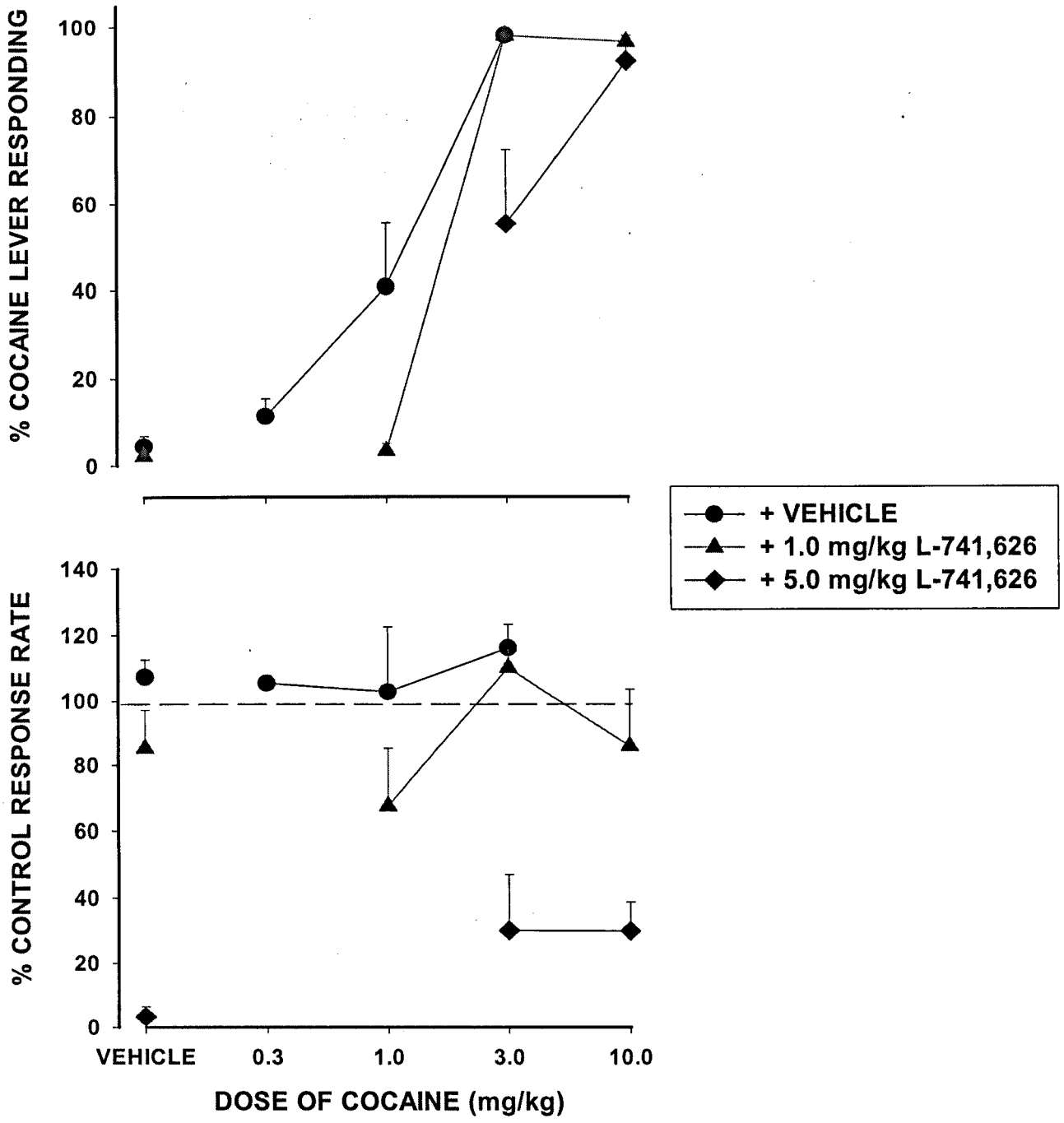

Figure 3.11 Upper panel: Effects of the DA D2-like receptor antagonist L-741,626 alone and in combination with 1, 3 and 10 mg/kg of cocaine in rats trained at 3 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents the mean of at least three out of six rats tested at each dose (upper panel)

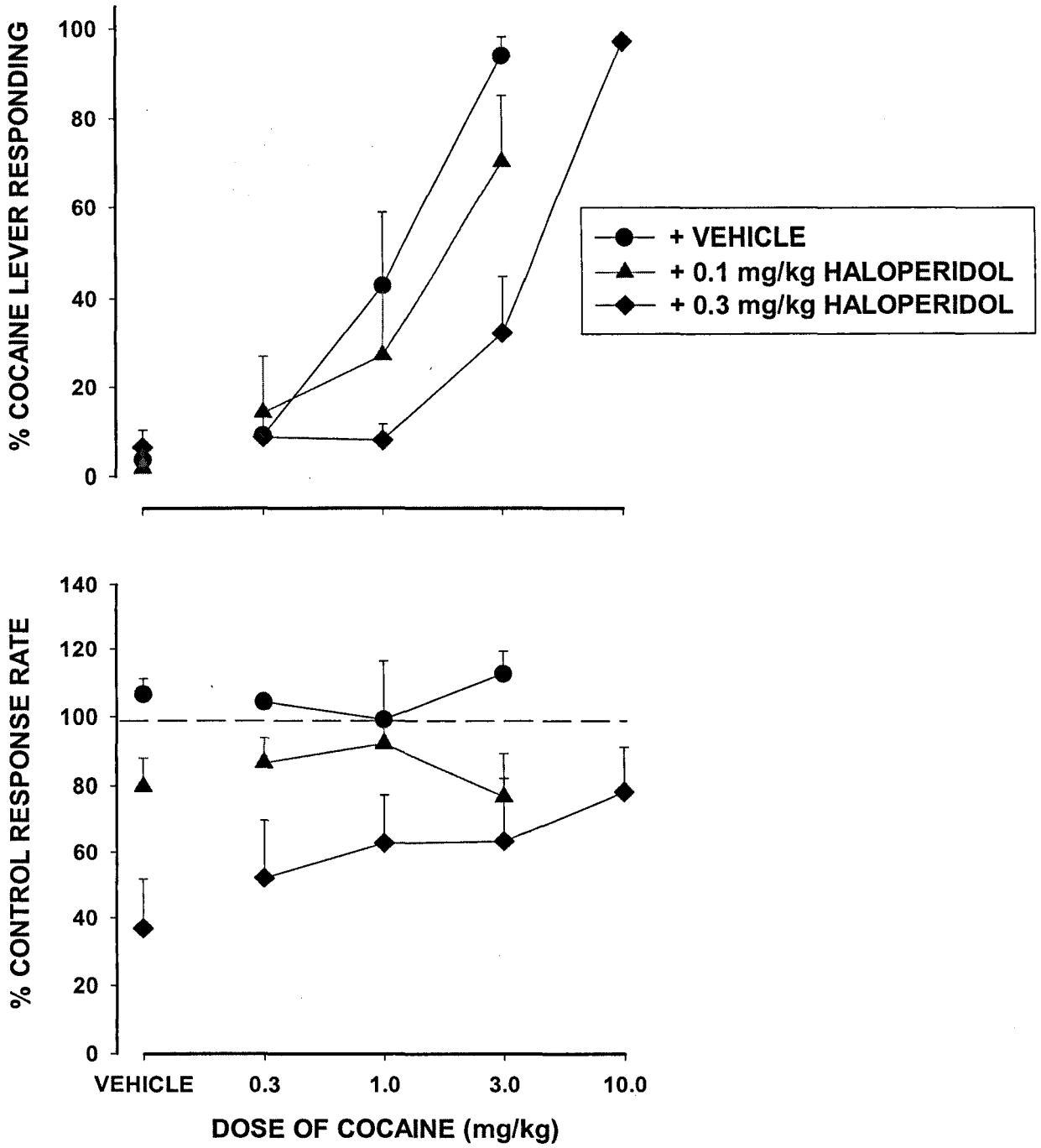

Figure 3.12 Upper panel: Effects of the DA D2-like receptor antagonist haloperidol alone and in combination with 0.3, 1, 3 and 10 mg/kg of cocaine in rats trained at 3 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents the mean of at least three out of eight rats tested at each dose (upper panel)

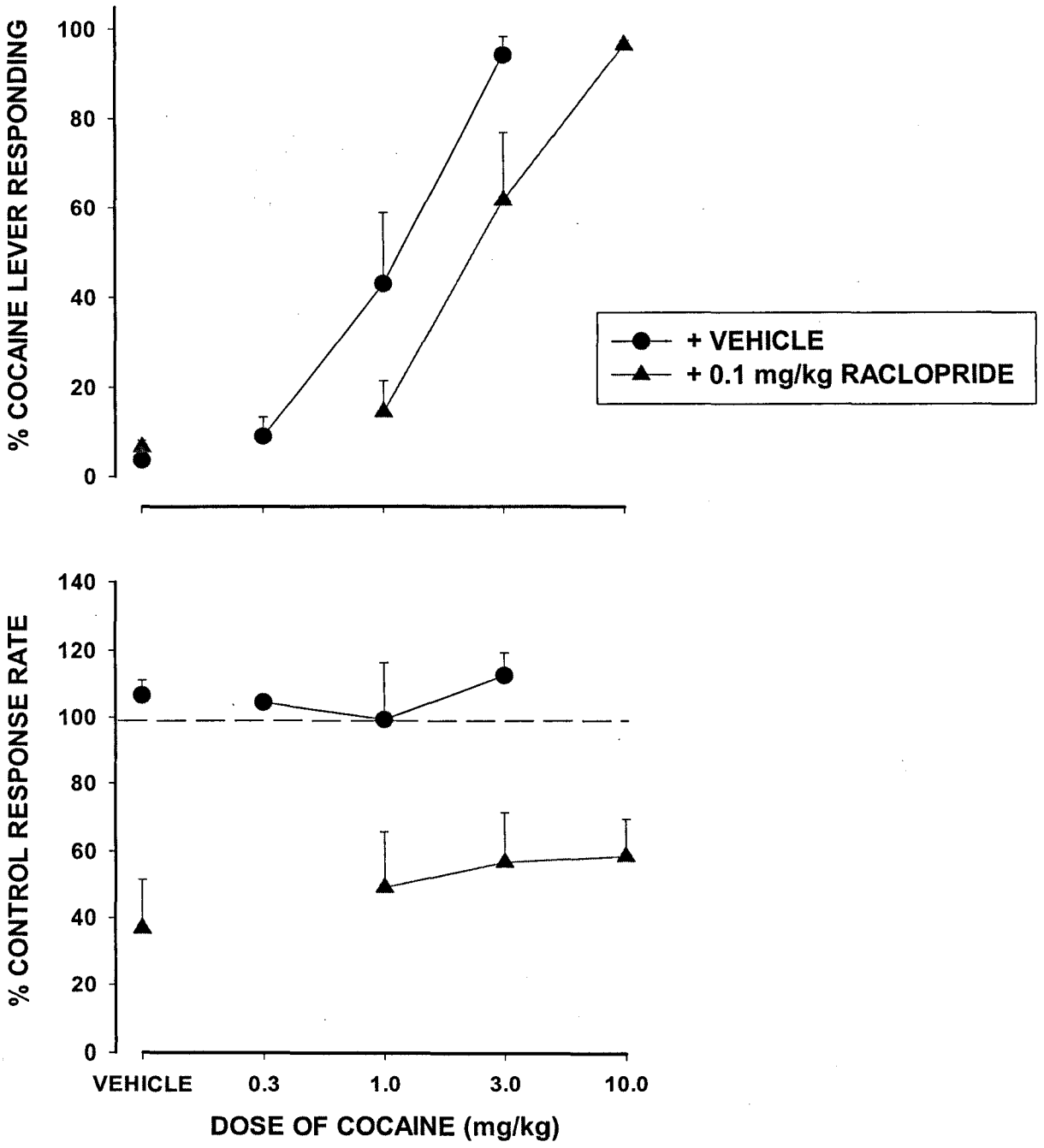

Figure 3.13 Upper panel: Effects of the DA D2-like receptor antagonist raclopride alone and in combination with 1, 3 and 10 mg/kg of cocaine in rats trained at 3 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents the mean of at least three out of seven rats tested at each dose (upper panel)

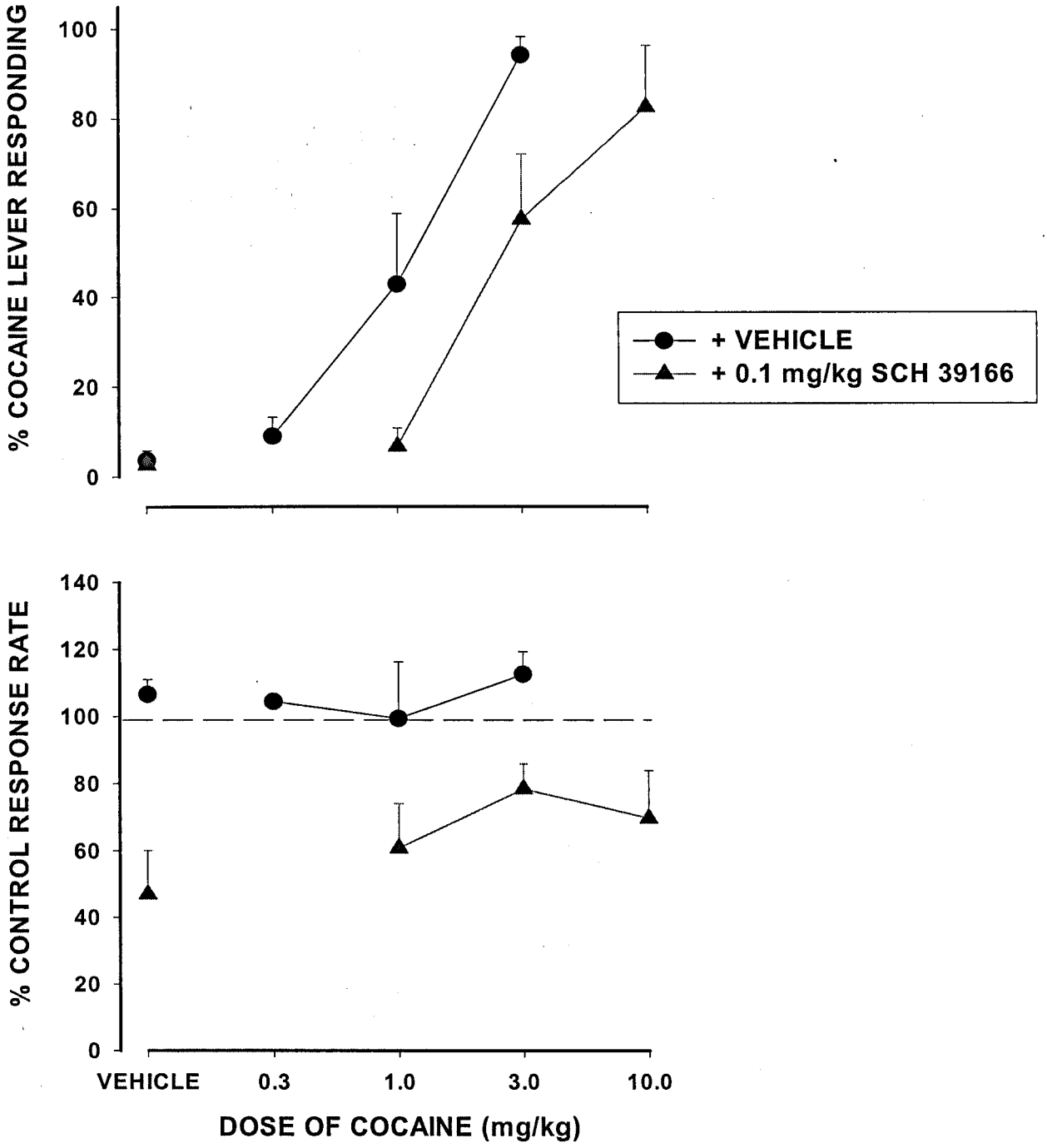

Figure 3.14 Upper panel: Effects of the DA D1-like receptor antagonist SCH 39166 alone and in combination with 1, 3 and 10 mg/kg of cocaine in rats trained at 3 mg/kg. The antagonist was administered 30 min before cocaine (or vehicle). Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents the mean of at least three out of seven rats tested at each dose (upper panel)

TABLE 3.3

DRUGS	ED_{50} (95 % CL)	POTENCY RELATIVE TO COCAINE
TRAINING DOSE (10 mg/kg) 2ND GROUP		
DOPAMINE D2-LIKE RECEPTOR ANTAGONISTS		
COCAINE ALONE (N=6)	2.75 (1.95 - 3.89)	1.00
+ 3 mg/kg L-741,626	4.49 (3.66 - 5.49)	0.62 (0.42 - 0.88)
+ 6 mg/kg L-741,626	8.55 (5.05 - 14.40)	0.32 (0.17 - 0.58)
COCAINE ALONE (N=8)	2.49 (1.86 - 3.29)	1.00
+ 0.3 mg/kg haloperidol	8.27 (6.43 - 10.64)	0.30 (0.20 - 0.46)
COCAINE ALONE (N=8)	2.49 (1.86 - 3.29)	1.00
+ 0.3 mg/kg raclopride	5.17 (3.18 - 8.38)	0.46 (0.29 - 0.72)
TRAINING DOSE (3 mg/kg)		
DOPAMINE D2-LIKE RECEPTOR ANTAGONISTS		
COCAINE ALONE (N=6)	0.95 (0.66 - 1.38)	1.00
+ 1 mg/kg L-741,626	2.08 (1.97-2.18)[a]	0.47 (0.34-0.65)[a]
+ 5 mg/kg L-741,626	b	0.34 (0.20 - 0.68)
COCAINE ALONE (N=8)	1.00 (0.71 - 1.40)	1.00
+ 0.1 mg/kg haloperidol	1.64 (0.84 - 3.19)	0.69 (0.34 - 1.26)
+ 0.3 mg/kg haloperidol	3.91 (2.40 - 6.37)	0.29 (0.16 - 0.50)
COCAINE ALONE (N=7)	1.02 (0.68 - 1.52)	1.00
+ 0.1 mg/kg raclopride	2.54 (1.83 - 3.54)	0.40 (0.24 - 0.68)
DOPAMINE D1-LIKE RECEPTOR ANTAGONIST		
COCAINE ALONE (N=7)	1.02 (0.68 - 1.52)	1.00
+ 0.1 mg/kg SCH 39166	3.22 (2.25 - 4.62)	0.32 (0.18 - 0.54)

a = Significant deviation from linearity

b = insufficient data to calculate ED_{50} value

ED_{50} values (mg/kg) and relative potencies for the DS effects of 10 mg/kg and 3 mg/kg cocaine in combination with DA D2-like (L-741,626, haloperidol and raclopride) and DA D1-like (SCH 39166) receptor antagonists.

TABLE 3.4

	Training Dose (mg/kg)	
	3.0	**10.0**
D2-like antagonists		
L-741,626	**5.61**	**4.97**
Haloperidol	**6.51**	**6.50**
Raclopride	**6.87**	**6.25**
D1-like antagonist		
SCH 39166	**6.88**	**7.16**

Schild analysis results: mean pA2 values for the effects of the D2-like antagonists and the D1-like antagonist on the DS effects of two different training doses of cocaine.

3.3.3 Substitution tests with a selective NE/5-HT re-uptake inhibitor, venlafaxine, on high (10 mg/kg) and low (3 mg/kg) cocaine trained rats.

The selective NE/5-HT re-uptake inhibitor, venlafaxine, produced partial substitution for cocaine's DS effects in both training conditions, with ED_{50} values of 37.24 and 8.11 mg/kg for the high and low dose groups, respectively (Figures 3.15-3.16). However, the slopes of the dose-effect curves for the two training doses deviated significantly from linearity, and the lack of parallelism between the cocaine curve suggests that venlafaxine maybe producing its DS effects through mechanisms different from those of cocaine. When tested in the high training dose condition, venlafaxine produced a maximum of 55 % substitution, whereas in the low training dose it produced a maximum of 68 % cocaine-appropriate responding. Venlafaxine was tested across a range of doses, from those that were inactive to those that decreased response rates to less than 40 and 30 % of control rates for the high and low training dose conditions, respectively.

The slope of the dose-effect curve also deviated significantly from parallelism with the cocaine dose-effect curve in both training conditions, preventing a reliable estimate of relative potency. Comparing the two curves for each training condition, it is evident from the ED_{50} values obtained that there is an apparent potency shift with training dose. The potency to substitute for cocaine increases as the training dose decreases, approximately 4-fold.

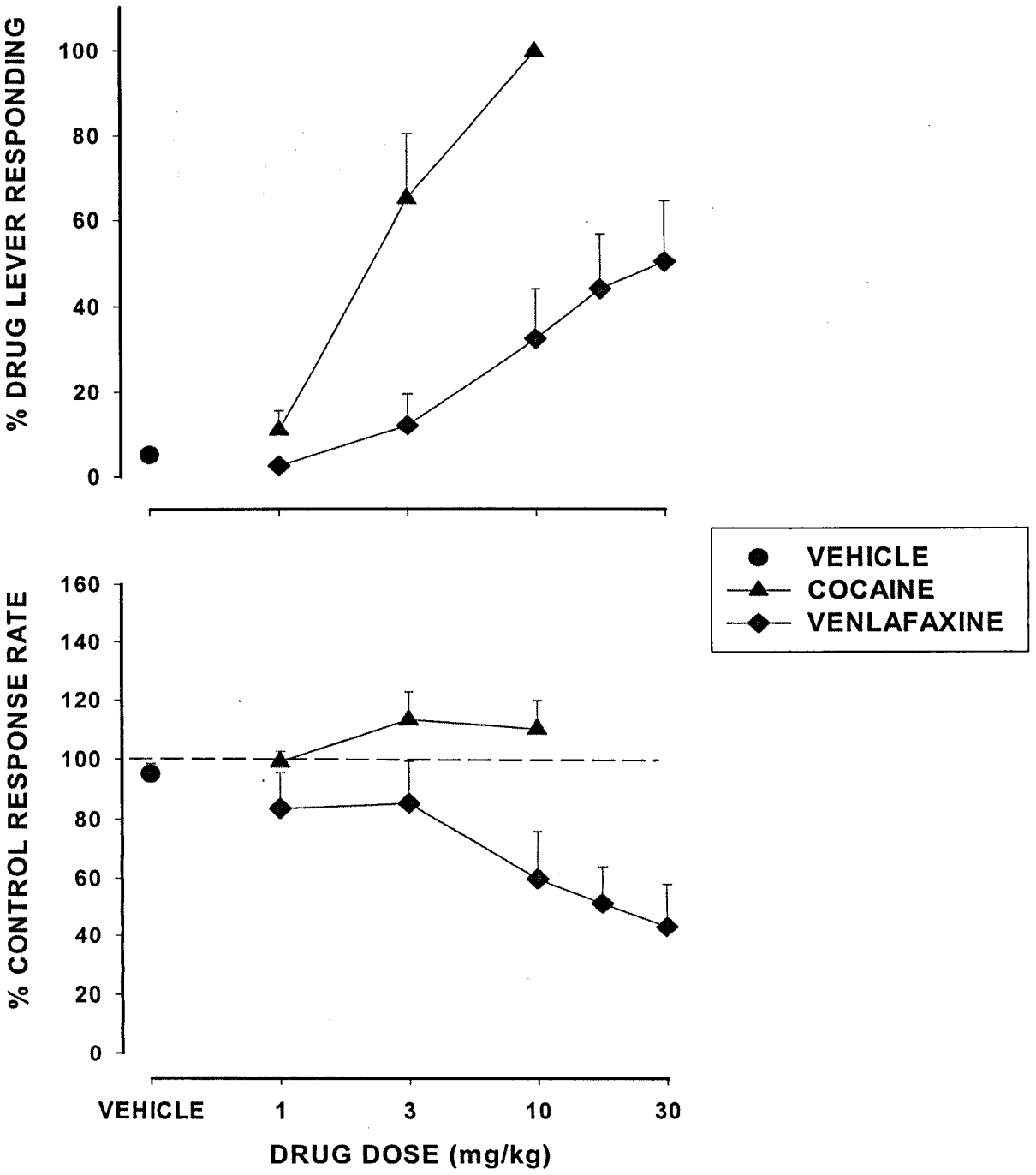

Figure 3.15 Upper panel: Effects of cocaine and venlafaxine (administered 30 min pre-test) on the percentages of responding on the cocaine-correlated lever for rats trained at 10 mg/kg. Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents mean performance of at least three out of eight rats tested at each dose (upper panel)

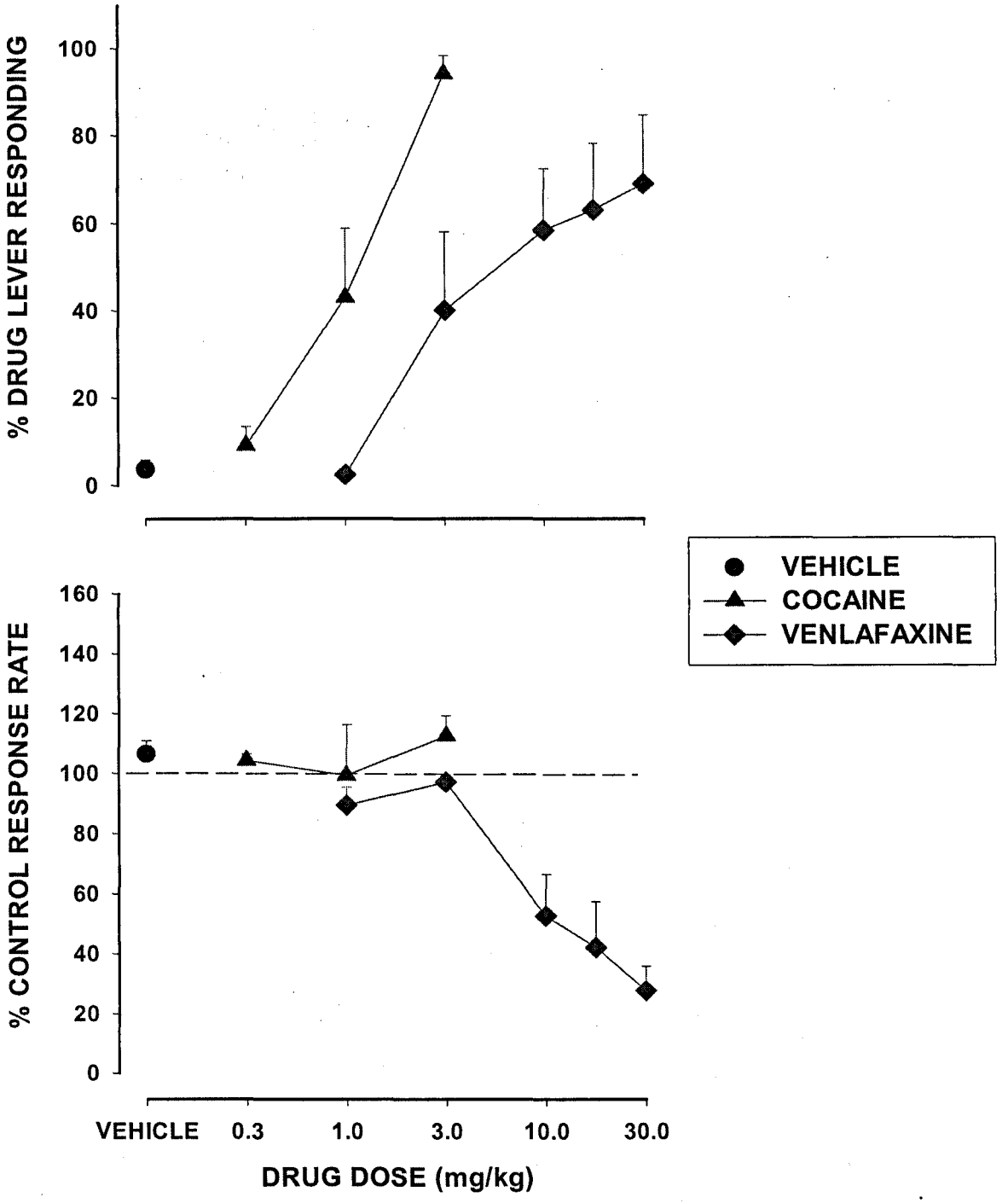

Figure 3.16 Upper panel: Effects of cocaine and venlafaxine (administered 30 min pre-test) on the percentages of responding on the cocaine-correlated lever for rats trained at 3 mg/kg. Lower panel: Rates of responding expressed with respect to vehicle response rates. Each point represents mean performance of at least three out of seven rats tested at each dose (upper panel)

3.4 Discussion

Reliable discriminative control of performance by cocaine was maintained at a high level throughout the study at two different training doses. D-amphetamine, another psychomotor stimulant, substituted fully for cocaine in rats trained at the high dose. However, neither a benzodiazepine, CDP, nor a barbiturate, pentobarbitone, elicited full cocaine-appropriate responding, although partial substitution was obtained at the highest dose of CDP tested. Each of these drugs was tested across a range of doses, from those that had no effect on response rates to those that decreased response rates to less than 20 % of control.

D-amphetamine is a prototypic stimulant that has a well-established potential for abuse. It produces a range of neurochemical effects including the release of DA and NE from pre-synaptic terminals and blockade of transmitter re-uptake from the synapse (Harris and Baldessarini, 1973; Azzaro et al., 1974; Taylor and Ho, 1978). It is well known that cocaine and phenylethylamines such as d-amphetamine and methamphetamine can produce similar behavioural effects (e.g. hyperlocomotion, stereotyped behaviours and reinforcing effects). Cocaine and amphetamine or methamphetamine have been shown to cross-generalize or exhibit cross-tolerance in drug discrimination procedures (Colpaert et al., 1978; Wood and Emmett-Oglesby, 1986; Woolverton and Cervo, 1986; Suzuki et al., 1996). The present results are consistent with others showing that the DS effects of d-amphetamine are similar to those of cocaine.

Pentobarbitone is a sedative from the barbiturate class and has no common mechanism of action with cocaine: pentobarbitone did not substitute for cocaine, supporting the pharmacological specificity of the cocaine discrimination. The failure of pentobarbitone to substitute for cocaine is in accordance with results indicating that GABAergic mechanisms are minimally involved in the DS effects of cocaine (Kleven et al., 1990; Cunningham and Callahan, 1991, Baker et al., 1991; Terry et al., 1994). It was therefore surprising to obtain partial substitution (albeit weak: 35 % maximum substitution) of CDP for cocaine in the present study, since both pentobarbitone and CDP share some similarities in terms of mechanisms of action. CDP is a benzodiazepine, widely used as a sedative or anxiolytic. Benzodiazepines are believed to exert their actions (anxiolytic, sedative, anticonvulsant, muscle relaxant) by

enhancing the inhibitory activity of gamma aminobutyric acid (GABA) at $GABA_a$ inhibitory synapses (Choi et al., 1977; MacDonald and Barker, 1978). One possible explanation for its variety of effects is that there are at least three different benzodiazepine binding sites: BZ1, BZ2 and BZ3 (occasionally known as ω1, ω2, ω3). The attachment of a benzodiazepine to its binding site causes an allosteric change in the $GABA_a$ receptor such that GABA binding is enhanced (Choi et al., 1977; MacDonald and Barker, 1978). However, studies are emerging to suggest a modulatory role for GABA on mesolimbic dopaminergic pathways (Kita and Kitai, 1988; Bernath and Zigmund, 1989; Pirot et al., 1992; Seutin et al., 1994). GABA neurons in both the nucleus accumbens (NAC) and ventral pallidum project to the A10 region of the ventral tegmental area (VTA), providing an inhibitory feedback loop (Henry et al., 1989).

No other studies so far have examined the role of benzodiazepine binding sites in the DS effects of cocaine. However, in the only study to-date looking at the effects of CDP on the reinforcing effects of cocaine, Goeders et al. (1989) showed that it could modify cocaine self-administration in rodents. The results suggested that CDP decreased the reinforcing efficacy of cocaine through indirect GABAergic mechanisms by binding to benzodiazepine sites located near to the GABA site which enhanced its overall effect. This work has been supported by recent findings that baclofen (a $GABA_b$ agonist) could selectively attenuate the reinforcing effects of cocaine in rats (Roberts et al., 1996; Shoaib et al., 1998). Therefore, the partial substitution for the DS effects of cocaine reported within may be produced by indirect GABA activation and release of DA from the mesolimbic dopaminergic pathways. Self-administration data strongly suggest that $GABA_b$ agonists should be considered as candidate pharmacotherapeutic agents for cocaine addiction. Guderman et al. (1996) have investigated the effects of baclofen (20-60 mg/day) in an open trial. Very preliminary data suggest that baclofen may suppress cocaine craving during the early phase of cocaine withdrawal. There is, therefore, a need for more experiments with direct $GABA_{a/b}$ agonists on the DS effects of cocaine in order to understand better the possible involvement of GABA. The finding that pentobarbitone (which also modulates GABA transmission) does not substitute for the DS effects of cocaine, suggests that the barbiturate receptor site is not critical for GABA-DA modulation.

The present study examined the role of novel DA D2-like receptor subtype antagonists (with some specificities for the D3 and D4 receptor subtypes) on the DS effects of cocaine. The results of these experiments indicate that U-99194A does not produce stimulus effects similar to those of cocaine. The present findings also indicate that U-99194A (up to 10 mg/kg) does not block cocaine's DS effects. These results support those of Baker et al. (1997), who showed that U-99194A did not significantly block cocaine's DS effects in rats trained at 10 mg/kg, nor did they see any potentiation of cocaine's DS effects by the D3 antagonist. The doses of the antagonist in Baker's study corresponded with those in the present one (1-10 mg/kg). Support that behaviourally-active doses were used is provided by the drug's effect on response rate, i.e. U-99194A at the highest dose reduced response rates to < 60% control. Support also comes from other behavioural experiments, in which it has been shown that doses of U-99194A needed to affect locomotor activity (Smith et al., 1999a) and progressive ratio responding (Smith et al., 1999b) are in the range of 2-10 mg/kg. Two other D3-preferring antagonists, (+)-UH 232 and (+)-AJ76, also fail to produce DS effects like those of cocaine, nor do they reduce cocaine discrimination to the extent that has been observed with DA D1-like or other D2-like DA antagonists (Clark et al., 1995; also see Table 2.3, section 2.4).

In contrast, the DA D3-preferring agonists, 7-OH-DPAT and (+)-PD 128907, were recently shown to produce stimulus generalisation in rats trained to discriminate cocaine from saline, although at doses that markedly decreased response rate (Acri et al., 1995). However, the extent to which the DS effects of these agents were produced by D3 receptor-mediated actions was examined in two separate studies by testing these putative D3 agonists in combination with the more selective DA D3 antagonist U-99194A. Depoortere et al. (1998) recently showed that U-99194A failed to antagonise the DS effects of the DA D3/D2 receptor agonist 7-OH-DPAT. It was found instead to markedly shift the 7-OH-DPAT generalisation curve to the left, suggesting that it potentiated the DS effects of 7-OH-DPAT. Baker (1998) showed only partial stimulus generalisation by 7-OH-DPAT in rats trained to discriminate 5 mg/kg cocaine from saline, but complete stimulus generalisation in rats trained to discriminate d-amphetamine from saline. U-99194A, on the other hand, produced partial substitution for both training drugs but failed to block the stimulus

generalisation produced by 7-OH-DPAT in rats trained to discriminate d-amphetamine. These results suggest that the DS effects of psychostimulants and their similarities to 7-OH-DPAT are not critically dependent on D3 receptor activation. This is supported by Boulay et al. (1998) who showed that the in vivo effects of 7-OH-DPAT, cocaine and U-99194A were still present in DA D3 knock-out mice suggesting that these compounds (7-OH-DPAT and U-99194A) can produce behavioural effects which are not mediated by activity at DA D3 receptors.

The DA D4 receptor antagonist, L-745,870, failed to alter the DS effects of cocaine. Confirmation that the dose-range of the antagonist extended to behaviourally-active doses was provided by the drug's dose-dependent effect on response rate. The lack of any interaction with cocaine dose on response rates also suggests independence of mechanism in the regulation of this aspect of behaviour. The effect on response rate is one of few behavioural effects identified for drugs that selectively block DA D4 receptors. In terms of cocaine's DS effects, Spealman (1996) found no relationship between the substitution potencies of various DA receptor agonists and their intrinsic efficacies or binding affinities at DA D4 receptors: instead, drug action at DA D3 receptors seemed most clearly relevant. In that study, it was shown that the cocaine-like DS effects of the most selective of the D3-agonists, PD128907 were attenuated by D2-like receptor antagonists with an order of potency: nemonapride > eticlopride > YM 436611 which corresponded more closely to their reported order of affinity at cloned human D3 than either D2 or D4 receptors. Although such findings point to a role for D3 receptors in the DS effects of cocaine, the evidence is so far inferential since, as mentioned, the present study and Baker et al. (1997) have shown that the DS effects of cocaine cannot be blocked by a selective D3 receptor antagonist, and other results suggest that substitution for cocaine by putative D3 agonists may not be mediated by the D3 receptor.

The study is the first to investigate DA D4 receptor involvement in the DS effects of cocaine. No evidence for involvement was found. DA D4 receptor distribution among brain regions still requires clarification: there is agreement that such receptors are expressed in midbrain nuclei, amygdala and frontal cortex (e.g. Van Tol et al., 1991), but there are conflicting reports as to whether they are expressed in nucleus accumbens and basal ganglia (e.g. Defagot and Antonelli, 1997). The brain

substrates of cocaine's DS effects have not been widely studied, but intra-accumbens infusions of cocaine and DA receptor antagonists support a critical role for this region (Callahan et al., 1997). Limited expression of DA D4 receptors in nucleus accumbens (if confirmed) might therefore explain the ineffectiveness of L-745,870 here. However, recent studies have shown that the DS effects of cocaine are also dependent upon at least two brain regions that have consistently shown DA D4 receptor expression: the amygdala and frontal cortex (Callahan et al., 1995; 1997). DA D1-like receptor antagonists injected into these regions block cocaine's DS effects. The present findings therefore suggest that DA D4 receptors in the amygdala and frontal cortex are probably not important to cocaine's DS effects. So far, no other compounds specific to this receptor subtype have been tested on the DS effects of cocaine. Experiments using other selective DA D4 receptor antagonists and agonists (when available) should be conducted to confirm this outcome. However, L-745,870 has been used as a tool to elucidate the DS properties of the atypical neuroleptic clozapine, a drug with some affinity for the DA D4 receptor (Goudie et al., 1998). In rats trained to discriminate clozapine (5 mg/kg i.p.) from saline, L-745,870 only produced 14 % generalisation, suggesting that DA D4 receptors are not involved in clozapine's DS effects. Hence clozapine should not be used as a putative D4 antagonist.

Using the selective DA D1-like receptor antagonist SCH 39166 for comparison with the D2-like compounds confirmed previous studies, demonstrating the importance of DA D1-like receptors to the DS effects of cocaine (e.g. Callahan et al., 1997). Previous studies with rats have only tested antagonists against the training dose of cocaine (e.g. Barrett and Appel, 1989; Spealman et al., 1991; Witkin et al., 1991; Callahan et al., 1991; Baker et al., 1993; Callahan et al., 1997). Our results, testing the full cocaine dose-response curve, suggests that DA D1-like antagonists are sufficient to produce surmountable antagonism of cocaine's DS effects: tests using monkeys have yielded similar results (e.g. Kleven et al., 1990; Spealman et al., 1991).

DA D2-like antagonists that are less selective than U-99194A and L-745,870 have been shown to attenuate the DS effects of indirect DA agonists in both rats (Arnt, 1988; Callahan et al., 1991) and monkeys (Kleven et al., 1990; Melia and Spealman, 1991; Spealman et al., 1991). However, as stated earlier (section 2.4),

several studies have reported that DA D2-like antagonists do not consistently block the DS properties of cocaine or amphetamine (e.g. Barrett and Appel, 1989; Kamien and Woolverton, 1989; Witkin et al., 1991). Terry et al. (1994) also showed that training dose may be an important determinant of the extent to which DA D2-like receptors are involved in the DS effects of cocaine.

In the present study, haloperidol, raclopride and L-741,626 (three centrally acting D2-like receptor antagonists) attenuated the DS effects of cocaine in high dose training conditions with an order of potency: haloperidol > = raclopride > L-741,626. As stated (section 2.4), several studies have reported that haloperidol does not consistently block the DS properties of cocaine (Barrett and Appel, 1989; Kamien and Woolverton, 1989; Witkin et al., 1991) even at doses much higher than used in the present study (0.3-1.6 mg/kg). As remarked earlier (section 2.4), in some cases, pharmacodynamic and/or pharmacokinetic variables may account for incomplete blockade of the stimulus effects of cocaine by haloperidol. For example, Callahan and Cunningham (1993) showed that by increasing the interval from 30 min to 120 min between injection of haloperidol (0.5 mg/kg) and testing for recognition of the cocaine cue, cocaine antagonism increased from 20 to 85 %. This result contrasts with the present one, as not only did we see a clear attenuation with haloperidol at 0.3 mg/kg, but we also used a pre-treatment time of 30 min. In contrast, the only study using raclopride in drug discrimination was by Johanson and Barrett (1993), where it was shown that raclopride (1-1.7 mg/kg) attenuated the DS effects of cocaine in pigeons. The result is similar to the present study, albeit with different doses and species. The most obvious difference between studies using haloperidol that failed to produce any antagonism and the present study is the procedure for testing antagonism. Unlike previous studies, which tested antagonists only against the training dose of cocaine, the present study tested the antagonists against the full cocaine dose-response curve, which offers the advantage of minimal disruption of response rates when tested against cocaine (also see section 1.6.3).

Looking at the effects of the D2-like antagonists in the low dose training group, the profiles observed were very similar, in that haloperidol and raclopride were more potent in attenuating cocaine's DS effects than L-741,626. The present study is the first to investigate DA antagonist compounds at the 3 mg/kg training dose of

cocaine. Unlike Terry et al. (1994), who used D1-like and D2-like agonists, a different picture is observed using antagonist compounds in terms of attributing receptor subtype involvement in cocaine's DS effects. Not only does the present study support D1-like receptor subtype involvement in low dose training conditions (as shown by the selective blockade by the D1-like antagonist SCH 39166), but it also supports D2-like receptor subtype involvement in the low dose DS effects.

In characterising further the receptor subtypes at which these antagonists are acting to block the DS properties of cocaine, 'Schild analysis' was employed to calculate pA2 values for the individual DA antagonist compounds. This information tells us, by comparing the pA2 values obtained for each compound, whether they are acting on the same receptor subtypes. The results in table 3.4 make it difficult to conclude that the DA receptor antagonists employed in the present study are acting at different receptors. However, it is interesting to note that although L-741,626, haloperidol and raclopride are each effective in attenuating cocaine's DS effects in high dose training conditions, the actions of L-741,626 may be mediated via different receptor subtypes (i.e. pA2 values obtained are 4.97, 6.50 and 6.87, respectively). This result fits with the drug's binding affinities, since haloperidol and raclopride not only display affinity for different receptors within the D2-like receptor subfamily, but they also bind to other receptors (as mentioned previously), unlike L-741,626 which is reported to act exclusively at the DA D2 receptor subtype.

Since our antagonist studies argue against a role for DA D4 receptors, and whilst clarification using more selective D2 and D3 receptor antagonists is still needed, these data suggest that the DA D2 receptor is the critical subtype within the D2-like subfamily that is involved in the DS effects of cocaine. Also, the similar effectiveness of haloperidol (i.e. D2=D3=D4) and raclopride (i.e. D2=D3>>D4) at blocking the cocaine cue also supports the absence of a role for DA D4 receptors in cocaine's DS effects. Once again, pA2 values obtained in the low dose training conditions paralleled those of the high dose training group, suggesting that similar mechanisms of action were engaged in the two training groups.

Finally, in an additional experiment, the selective NE/5-HT re-uptake inhibitor venlafaxine partially substituted for the DS effects of cocaine, suggesting that neurotransmitters other than DA may be involved in cocaine's DS effects. Although

significant deviations from parallelism were observed in both training conditions (suggesting that venlafaxine may be producing its effects via a different mechanism of action compared to cocaine), more cocaine-appropriate responding was found in the low dose training condition. Venlafaxine is a potent inhibitor of 5-HT and NE re-uptake, but it may also inhibit the re-uptake of DA in vitro at concentrations only 10-fold higher (Holliday and Benfield, 1995). However, as doses were used in a range where the dopaminergic effects of venlafaxine are not apparent (≤ 30 mg/kg; Reneric and Lucki, 1998) one can probably rule out any dopaminergic involvement in the present results. This is supported by other behavioural assays where venlafaxine has been used as a selective NE/5-HT inhibitor at doses of 10 and 30 mg/kg p.o. (Jackson et al., 1997). It would be interesting to see whether a sub-maximal dose of venlafaxine can potentiate the DS effects of cocaine. If so, venlafaxine may be worth investigating further as a therapeutic agent for cocaine abuse.

Because cocaine can be considered a strong inhibitor of 5-HT re-uptake, it is conceivable that this monoamine could be involved in its DS effects and may contribute to the substitution of venlafaxine for cocaine. However, 5-HT receptor antagonists do not block the DS effects of cocaine (Meert and Janssen, 1992; Peltier et al., 1994), and moreover the selective 5-HT re-uptake inhibitors have been shown not to substitute for cocaine. For example, citalopram (Baker et al., 1993) and fluoxetine (Kleven et al., 1990; Cunningham and Callahan, 1991; Baker et al., 1993) do not mimic the stimulus effects of cocaine. However, pre-treatment with either fluoxetine or sertraline can shift the dose-effect curve for cocaine to the left; for example, both 1.25 and 4 mg/kg fluoxetine enhances the stimulus properties of cocaine in rats (Cunningham and Callahan, 1991; Kleven and Koek, 1998) and in squirrel monkeys (Schama et al., 1997). In this context, the in vivo 5-HT re-uptake blocking properties of cocaine contribute little to its DS effects. Even so, it is clear that combined administration of cocaine and 5-HT re-uptake blockers influences its behavioural effects (Walsh and Cunningham, 1997) which suggests that 5-HT could play a modulatory role.

Several recent pharmacological characterisations of the DS effects of low doses of cocaine indicate that NE involvement may be more important than previously believed. That is, NE re-uptake blockers (e.g. desipramine, nisoxetine or

talsupram) engender more complete substitution for the DS effects of a low training dose in either rats (Terry et al., 1994) or monkeys (Spealman, 1995), in contrast to findings obtained in animals trained with higher doses (Broadbent et al., 1991; Baker et al., 1993; Spealman, 1995). It could be that species and/or training conditions are important determinants of the extent that NE is involved in the DS effects of cocaine. Taken together, the study with venlafaxine suggests that although it may not produce full substitution for cocaine in the high dose training group, there seems to be a potency shift as the training dose is decreased. The apparent blockade of NE and 5-HT re-uptake by venlafaxine does not seem to produce a greater effect compared to when these selective NE and 5-HT re-uptake inhibitors are used alone. The possibility that venlafaxine may potentiate the DS effects of low doses of cocaine still needs to be determined in order to characterise fully the involvement of NE/5-HT in the DS effects of cocaine.

In summary, it can be stated conclusively that the DA D3 and D4 receptor subtypes do not contribute to the DS effects of cocaine. Instead, the present study using selective antagonists within the D1-like and D2-like receptor subtype families has suggested a role for the D1-like and the D2 receptor subtype specifically. In addition, it also suggests that D1-like and D2 receptor subtype antagonists are not differentially effective between the two training doses. Thus for DA D2-like receptor antagonists with different affinities for receptors within the D2-like subfamily, blockade of cocaine's DS effects cannot be attributed to the D3 or D4 receptor subtypes: the D2 receptor is likely to be critical.

CHAPTER 4:

'THE ROLE OF DOPAMINE ON FEEDING AND LOCOMOTOR ACTIVITY IN THE RAT'

CHAPTER 4:

THE ROLE OF DOPAMINE ON FEEDING AND LOCOMOTOR ACTIVITY IN THE RAT

4.1 Introduction

In the previous chapter, it was concluded that DA D3 and D4 receptors play a negligible role in the DS effects of cocaine. It would now be useful to assess the generality of these results by examining other behavioural effects of cocaine. For example, cocaine has been shown to suppress appetite (Bedford et al., 1980; Blavet and De Feudis, 1982; Foltin et al., 1983) and to increase activity (e.g. Reith and Fischette, 1991). At still higher doses, repetitive motor activity (stereotyped behaviour) often occurs (e.g. Ujike et al., 1990). Any or all of these behaviours could be used to investigate DA receptor subtype involvement in cocaine's effects (e.g. Scheel-Kruger et al., 1977; Costall and Naylor, 1979; Beninger, 1983; Rapoza and Woolverton, 1991). Although DA clearly plays a major role in several of the behavioural effects of cocaine, the involvement of the recently cloned D3 and D4 receptors in such effects of cocaine has not been extensively examined. At the same time, the in vivo effects of DA D3 and D4 receptor antagonists themselves have not yet been determined. Since DA has been implicated in many different processes, such as ingestive and motor behaviours (section 1.5), the need to investigate the effects of these antagonists alone is an essential prelude to combination studies with cocaine. In section 1.5, the effects of selective DA receptor agonists and antagonists on locomotor activity were described; these are also summarised in Table 4.1. The following section will now briefly describe the effects that agonists and antagonists of these receptor subfamilies have on ingestive behaviour.

4.2 Dopamine and Food Reward

There is considerable evidence for an association between feeding behaviours and the release of DA in the nucleus accumbens (Hernandez and Hoebel, 1988a; McCullough and Salamone, 1992; Yoshida et al., 1992). The nucleus accumbens is located within the ventromedial striatum and is a critical neural substrate for reinforcement processes and appetitive behaviour. The anatomical organisation of the nucleus accumbens is well-suited for this role. Information in the form of affective

and motivational states from the limbic structures such as the amygdala, hippocampus, prefrontal cortex and brainstem autonomic centres (Kelley et al., 1982) converges on the nucleus accumbens. From here, it has extensive connections to motor output systems (Groenewegen et al., 1980). Hence the nucleus accumbens has been viewed as an interface between the corticolimbic and motor systems where processing of affective information is translated into motor actions (Mogenson et al., 1980). The nucleus accumbens is no longer considered to be a homologous structure but to comprise of three major sub-territories, termed the core, shell and rostral pole. The core subregion connects extensively to classic basal ganglia output structures such as the ventral pallidum, subthalamic nucleus and substantia nigra. The shell subregion, in contrast, projects most heavily to subcortical limbic regions such as the lateral hypothalamus and ventral tegmental area (Deutch and Cameron, 1992). A functional link may therefore exist between these brain regions, whereby the shell subregion may exert an important modulating influence on the lateral hypothalamic neurones which regulates food intake. It may also be that cocaine has a direct effect on food intake per se by signalling satiety signals within the hypothalamus. A recent study by Kristensen et al. (1998) has shown that a brain-located peptide named CART (cocaine and amphetamine regulated transcript) is a satiety factor closely associated with the actions of two important regulators of food intake, leptin and neuropeptide Y. When injected intracerebroventricularly into rats, recombinant CART peptide inhibits both normal and starvation-induced feeding, and completely blocks the feeding response caused by neuropeptide Y.

As stated earlier, there are also a number of studies suggesting a specific link between reward and the mesolimbic dopamine system. For example, accumbens DA release has been shown to be potentiated by many non alimentary rewarding stimuli such as psychostimulant drug administration (Kiyotkin, 1993), sexual behaviour (Damsma et al., 1992), and electrical stimulation of the lateral hypothalamus (Hernandez and Hoebel, 1988b). Compounds that facilitate DA neurotransmission, such as cocaine, cause an overall increase in DA levels in the nucleus accumbens and have been shown to reduce food ingestion. Compounds which lower DA tone within the system such as DA receptor antagonists (Hodge et al., 1994; Hsiao and Smith, 1995) also reduce ingestion of palatable foods. Hence, there is a relationship between

mesolimbic DA system activity and reward, including food reward (Martel and Fantino, 1996); basal DA tone within the system appears to contribute to the regulation of food intake.

4.3 Role of Dopamine Receptor Subtypes in Ingestive Behaviour

4.3.1 'D1-Like' and 'D2-Like' Agonists

To date, studies of the effects of dopaminergic drugs on ingestive behaviour have utilised compounds that are selective for either the DA D1-like or D2-like receptor sub-families (cf. Terry, 1996). While both D1-like and D2-like receptor agonists reduce the size of meals, it is quite clear that they achieve this effect by different means. Gilbert and Cooper (1985) first reported that a selective D1-like receptor agonist, the benzazepine SK&F 38393, produced a dose-dependent suppression of palatable food consumption in non-deprived rats. A microstructural analysis of the anorectic effect of SK&F 38393 revealed that it reduced the duration and rate of eating (Cooper et al., 1990). However, as the selective D1-like receptor antagonist SCH 23390 was unable to antagonise the anorectic effect of SK&F 38393 (Terry and Katz, 1992), questions were raised as to whether the compound's effect is exclusively due to action at D1/D5 sites. The introduction of the potent and selective isochroman D1-like receptor agonist A-68930 (DeNinno et al., 1990) provided further opportunity to study the role of this receptor subtype in feeding behaviour. Al-Naser and Cooper (1994), using microstructural analysis, showed it to significantly reduce food consumption. In particular, it had a modest effect on eating rate but markedly reduced the frequency of feeding bouts. Moreover, it significantly increased grooming.

Unlike D1-like agonists, which only inhibit food intake, D2-like agonists have been shown to produce biphasic effects. For example, selective D2-like agonists such as N-0437 and PHNO increase eating at low doses (Martin-Iversen and Dourish, 1988; Clifton et al., 1989; Rusk and Cooper, 1989b), but have been shown to inhibit eating at high doses (Clifton et al., 1989). Their main anorectic effect, within a meal, is to reduce the rate of eating (Rusk and Cooper, 1989b). In addition to its effect on feeding, N-0437 was also shown to suppress grooming, but stimulated oral responses such as licking and gnawing (Rusk and Cooper, 1989b). Table 4.1 summarises the

main findings. Most of the relevant behavioural pharmacology so far has involved DA receptor subtype stimulation, i.e. the use of selective agonists. However, antagonists of each receptor subtype also produce clear behavioural effects.

4.3.2 'D1-Like' and 'D2-Like' Antagonists

The D1-like antagonist SCH 23390 has been shown to reduce food intake both in food-deprived rats (Gilbert and Cooper, 1985; Zarrindast et al., 1991; Terry and Katz, 1992) and in free-feeding animals (Clifton et al., 1991; Naruse et al., 1991). There have also been reports that SCH 23390 reduces operant responding for food reinforcement (e.g. Rusk and Cooper, 1994). In contrast, D2-like antagonists can produce biphasic effects on food intake, with low doses enhancing food intake and high doses suppressing it, e.g. spiperone (Cooper and Sweeney, 1980) sulpiride (Parada et al., 1988) haloperidol (Hobbs et al., 1994) and raclopride (Terry, 1996). Smith and Hsiao (1995) also showed that raclopride can reduce sucrose preference in rats, indicating that central DA D2-like mechanisms may be involved in the mediation of food reward.

D1-like and D2-like antagonists have not been studied thoroughly in terms of the microstructural parameters that contribute to their anorectic effects. In the only study to date, Terry (1996) showed that the D1-like antagonist SCH 39166 did not affect total food intake, but significantly reduced feeding bout frequency. This reduction was compensated by a dose-dependent (but non-significant) increase in the mean duration of each bout. However, changes in other aspects of motor behaviour such as locomotion, rearing and grooming make the results difficult to interpret with regards to specificity of the behavioural effects on feeding.

D2-like antagonists present further problems due to their biphasic dose-effect on feeding behaviour. Studying the effect of raclopride on palatable food consumption by non-deprived rats, Terry (1996) showed that the low dose hyperphagic effect resulted mainly from increases in the number of feeding bouts and mean bout duration, whereas the high dose hypophagia was a product of increased latency to eat and reduced number of feeding bouts. However, as the hypophagic effect occurred at and above doses of raclopride that significantly disrupted other aspects of motor behaviour, it is difficult to assume behavioural specificity of the D2-like antagonist anorectic effect. Studies with the D2-like antagonist YM-09151-2, have shown little

TABLE 4.1

Summary of effects of selective dopamine receptor subtype agonists and antagonists on feeding and locomotor activity in the rat (except where mentioned).

ACTION	DRUG	FEEDING BEHAVIOUR	LOCOMOTOR ACTIVTY
D1-like AGONISTS:			
	SKF 38393	↓ Gilbert & Cooper (1985) Martin-Iversen & Dourish (1988) Rusk & Cooper (1989a)	↑ Szechtman et al (1991) ↑ &↓ Tirelli & Terry (1993) [Mice]
	SKF 75760	↓ Rusk & Cooper (1989a)	
	SKF 82958 & SKF 77434	↓ Terry & Katz (1992)	
	A-68930	↓ Cooper & Al-Naser (1994)	
D2-like AGONISTS:			
	Apomorphine	↓ Barzaghi et al; (1973)	↑ &↓ Stahle & Ungerstedt (1986)
	Bromocriptine	↓ Hawkins et al; (1986)	
	N-0437	↑ (low doses) ↓ (high doses) Clifton, Rusk & Cooper (1989) Rusk & Cooper (1989b)	↓ (low doses) Rusk & Cooper (1989b)
	PHNO	↑(low doses) ↓ (high doses) Martin-Iversen & Dourish (1988)	
D1-like ANTAGONISTS:			
	SCH 23390	↓ Koechling et al; (1988) Naruse et al; (1991) Clifton Rusk & Cooper (1991)	↓ Bruhwyler et al; (1990) Cabib et al; (1991) [mice] Meyer at al; (1993)
	SCH 39166	↓ Terry & Katz (1994) Panocka et al; (1995)	
D2-like ANTAGONISTS:			
	Haloperidol	↓ Naruse et al; (1991)	↓ Bakshi & Kelley (1991)
	YM-09151-2 Remoxipride & Raclopride	↓ feeding rate ↑ meal size ↑ food intake Clifton, Rusk & Cooper (1991)	
	Raclopride	↓ sucrose preference Smith et al; (1995)	↓ Horita et al; (1995) Wise & Carlezon (1994)
	Sulpiride	↑ Parada et al; (1988)	↓ Cabib et al; (1991) [mice]

↑ / ↓ = Increase or Decrease behaviour.

effect on total food intake, but cause substantial dose-related decreases in feeding rate and an enhancement of both meal size and duration (Clifton et al., 1991). In the same study, a second DA D2-like antagonist, remoxipride, produced similar effects on feeding patterns, and in addition raised food intake during the 2 hr after drug administration. Another D2-like antagonist, raclopride, produced a short-term rise in food intake and a slowing of feeding rate, with a non-significant increase in meal size at intermediate doses.

In summary, D1-like agonists consistently produce anorexia by specifically affecting meal duration through an effect on meal frequency, but more drugs need to be studied. Similarly, D1-like antagonists can reduce food intake, perhaps also through an effect on meal frequency, but disruptions of feeding occur in conjunction with decrements in motor behaviour. D2-like agonists and antagonists present greater interpretative difficulties: both classes of drugs produce biphasic effects on intake, with low doses enhancing food intake and high doses suppressing it. Both classes tend to reduce eating rate, and the enhanced feeding reported following low doses of a D2-like antagonist most often reflects an increase in average meal duration.

4.4 Animal Models to Measure Feeding and Locomotor Activity

4.4.1 Feeding Behaviour

A plethora of drugs inhibit food intake when administered to experimental animals. One of the most contentious issues in the psychopharmacology of appetite concerns the identification of mechanisms underlying an observed reduction of eating. Often in such experiments the only measure taken is the amount of food consumed over brief time periods following acute drug treatment. This information is important, but it is insufficient to allow any conclusions to be drawn about the specificity of action of the drug on feeding behaviour.

A. Food Deprivation, Food Intake and Drug Effects

The simplest experimental paradigm that has been used to examine drug effects on food intake involves administration of the drug to food-deprived rats and the subsequent determination of food intake over a brief test period. This procedure is simple and convenient for the experimenter, but can be criticised on several grounds.

1. A period of food deprivation followed by a brief period of food access does not involve a normal behavioural repertoire.
2. Deprivation may result in physiological changes, both in the periphery and the brain, which may impair the efficacy of the drugs used to alter feeding, e.g. periods of food deprivation have been shown to influence brain neurotransmitter synthesis and metabolism (Fletcher, 1989).
3. Finally, and perhaps most important, the observation that a drug alters total food intake does not provide any insight into the specific effects of the drug on behaviour.

Feeding behaviour is different from food intake in the sense that it can be defined accordingly to 'contextual', and 'temporal' dimensions. In the rat, feeding is an episodic activity in which periods of eating (meals) are separated from each other by periods of other behaviours. A meal has a clearly determined beginning and end, and can be defined operationally in terms of its initiation, duration and termination. Blundell (1979) suggests that the terms hunger, appetite, satiation and satiety can be related to these separate facets of the meal. Hunger is defined as the process that indicates that eating is imminent, and is inferred from the initiation of a meal. Appetite is the process that maintains feeding and guides the selection of foods based upon the hedonic value of those foods. Satiation refers to the termination of feeding resulting from the act of food ingestion itself, whereas satiety is the state of inhibition over further eating. In order to understand the nature of the effects of drugs on feeding behaviour, it is necessary to use experimental procedures that supply more information than just the quantity of food consumed in a brief period of time.

B. The Microstructure of Feeding

Blundell and Latham (for review see Blundell, 1983) have pioneered the use of a technique to examine the continuous changes in the 'microstructure' of feeding behaviour. The technique involves recording instances and durations of feeding bouts and the intervals between feeding bouts. The typical experimental procedure involves adapting the animal to a food-deprivation schedule. Once food intake has stabilised, the animal is injected with drug and placed in the test cage with access to food. The animal is then observed, usually for 30 min or 1h, and the occurrence and duration of

various behaviours (e.g. Table 4.2) are recorded either manually or with a keyboard connected to a microcomputer. In addition to recording feeding bouts, it is possible to record instances of general activity, grooming, resting and drinking. At the end of the test session, the amount of food consumed is determined. Once data collection has been completed, the data can be broken down into various behavioural categories and feeding parameters. Essentially, each behaviour is described in terms of its duration and frequency across the test period. Several other parameters can be derived to describe feeding, and these are listed in Table 4.3.

Microstructural analysis of feeding behaviour permits identification of differing mechanisms of drug action not apparent from pharmacological studies of food intake alone. This procedure, therefore, provides a methodology for the preliminary identification of drugs that appear to reduce food intake via processes linked to natural mechanisms of satiety.

4.4.2 Locomotor Activity

In investigating a particular neural system or a certain class of drugs, most information can be gained by studying a range of behaviours. Not only can one measure a drug's effects on motivational behaviours such as feeding, as mentioned above, analysis of spontaneously emitted behaviour such as locomotor activity can also produce useful information. The term 'locomotion' means movement from one location to another. In rodents, an important component of spatial exploration is locomotion, and it is one of the most prominent in the rat's repertoire of spontaneous behaviours. The most commonly-used procedure for measuring locomotor activity is the photocell cage (Figure 4.1). The prototype consists of a box or cage, usually rectangular and large enough for the rat to walk around, with two photocells placed on the long axis of the cage, each consisting of an emitter and receiver interfaced to a microcomputer. Each photocell beam, when broken, registers a count which is recorded by the computer. The scores are usually collected in specified bins. This method is adopted here. There are many factors that influence the general activity level of animals; these factors must be taken into account when testing for drug effects (Cambell, 1960; Prescott, 1970; File and Day, 1972; Evans, 1974; Robbins, 1977; File, 1978; File, 1985) .

Table 4.2. Behavioural categories used for observational analysis. (From: Halford, Wanninayoke and Blundell, 1998).

Eating	Biting, gnawing or swallowing food from wet mash directly or from front paws
Grooming	Licking of the body, feet and/or genitals. Scratching of coat or head with hind leg. Stroking whiskers with paws, biting of the tail
Locomotion	Walking around the cage or circling. Movements involving all four limbs
Rearing	Front paws raised from the tank floor and either placed on the side of the tank or placed in front of the body
Resting	Relaxed position with head curled to body or resting on the bottom of the cage, stretched out either on side or belly

Table 4.3. Parameters derived from the microstructural analysis of feeding behaviour.

Total food intake (g)
Total time spent eating (min)
Latency to onset of first feeding bout (min)
Number of feeding bouts
Bout size (g)
Bout duration (min)
Rate of eating (g/min)

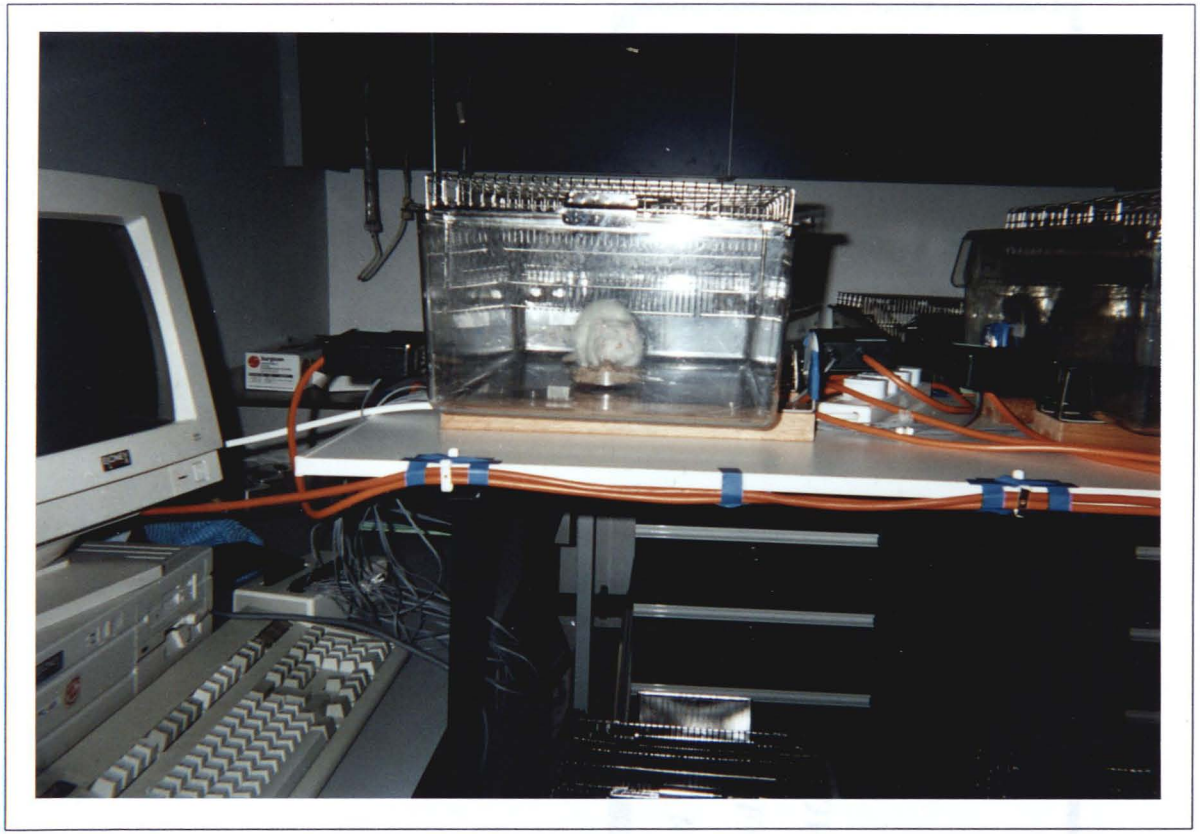

Figure 4.1. **Prototype model for measuring locomotor activity in a photocell cage. The photocells are placed along the axis of the cage where 'beam' breakages register a count which is received by a microcomputer.**

CHAPTER 5:

'A COMPARISON OF THE EFFECTS OF NOVEL 'D2-LIKE' RECEPTOR SUBTYPE ANTAGONISTS ON FEEDING AND LOCOMOTOR ACTIVITY IN THE RAT'

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A COMPARISON OF THE EFFECTS OF NOVEL 'D2-LIKE' RECEPTOR SUBTYPE ANTAGONISTS ON FEEDING AND LOCOMOTOR ACTIVITY IN THE RAT

5.1 Aims

The main purpose of the experiments described in the present chapter is to investigate the effects of two novel D2-like receptor subtype antagonists on feeding and locomotor activity in the rat. The contributions of DA D3 and D4 receptors to these effects have not been determined due to the limited availability of compounds acting specifically at these receptors. The development of two drugs selective for these receptors, U-99194A and L-745,870, respectively, now allows for such characterisation to be made. These compounds were subsequently compared with the prototypical D2-like antagonist sulpiride (Kebabian and Calne, 1979; Seeman and Van Tol, 1994) and the recently developed antagonist amisulpride, a structural analog to sulpiride. No previous studies have looked at the roles of these novel D2-like receptors in feeding behaviour. The receptor binding profiles of U-99194A and L-745,870 have been described in section 3.1; however, it is necessary to describe briefly the binding affinities of the comparison compounds of sulpiride and amisulpride.

Sulpiride has been hypothesised to have a higher affinity for presynaptic than for postsynaptic D2 receptors at low doses (Robertson and MacDonald, 1985), and to show a similar affinity for D2 and D3 postsynaptic receptors at high doses (Sokoloff, 1990). Sulpiride has also been widely used as a prototypical D2-like antagonist in many behavioural studies (e.g. Arnt, 1985; Parada et al., 1988; Cabib et al., 1991; Bakshi and Kelley, 1991; Inoue et al., 1995; Neisewander et al., 1995; Baker et al., 1996). Amisulpride is a novel antipsychotic agent that shows clinical efficacy against both the positive and negative symptoms of schizophrenia. It is a benzamide derivative with a unique neurochemical and psychopharmacological profile (Perrault et al., 1997). The compound has selective affinity for human D3 and D2 receptor subtypes in vitro, and ex-

vivo binding studies have shown it to be twice as selective for D3 as for D2 receptors (Scatton et al., 1997). At low doses it preferentially blocks presynaptic DA autoreceptors (therefore increasing DA release) while postsynaptic DA receptor antagonism is evident at higher doses (Scatton et al., 1997; Schoemaker et al., 1997). Moreover, it has been claimed that amisulpride interacts preferentially with limbic DA D2-like receptors rather than with striatal D2-like receptors (Schoemaker et al., 1997) a characteristic that may explain its low incidence of extrapyramidal side effects (Perrault et al., 1997).

Ligand binding studies in vitro using rat brain have shown that both sulpiride and amisulpride have a high affinity for DA receptors but no significant affinity for 5-HT receptors, histamine H1, muscarinic and α-adrenergic receptors, or a variety of other receptors and drug recognition sites (Scatton et al., 1994). Amisulpride selectively recognises the DA D2 and D3 subtypes with similar affinity (Ki values of 2.8 and 3.2 nM respectively) but has no affinity (Ki value of > 1000 nM) for D1, D4 and D5 receptor subtypes (Scatton et al., 1997). Ki values for sulpiride at D2 and D3 receptor subtypes have been reported as 15 and 13 nM, respectively (Seeman and Van Tol, 1994). In effect, we have four different compound which all belong to the D2-like subfamily but show differing specificities for the various DA receptor subtypes, i.e. amisulpride, with equal affinity for D2/D3 sites at both pre and post-synaptic receptors; sulpiride, also with equal affinity for D2/D3 sites, but which binds specifically to D2 sites pre-synaptically; U-99194A, selective for D3 receptors; and L-745,870 selective for D4 receptors.

All drugs were compared using a similar procedure in which locomotor activity was measured at the same time as feeding to see if drug effects on feeding occurred at doses that produced no effects on simple measures of motor behaviour. Also, locomotor activity tends to be among the first behaviours affected by drug action when measuring multiple behaviours. These experiments were intended to characterise the overall effects of these compounds on feeding and locomotion as a prelude to experiments using a microstructural analysis of behaviour.

5.2 Materials and methods

5.2.1 Subjects:

Male Sprague-Dawley rats, eight per experiment (Charles River, Margate, Kent) and weighing 320-360g, were housed in pairs with free access to water under a 12 hr light/dark cycle (lights on 07:00). They were maintained on a restricted diet of 15g standard food pellets, adjusted on test days so that regardless of any effect of the test drug, all rats received the same food allowance per day. All testing was between 12:00 and 14:00 hours.

5.2.2 Apparatus:

The test food was presented in a shallow plastic petri-dish, 9 cm in diameter, with a rim 1.5cm high and weighing approximately 10 g; it was secured with 'velcro-tape' to the floor of the experimental cages (Ebenezer, 1990). Testing was conducted in experimental cages measuring 53 × 32 × 18 cm high. Water was available ad lib in the test boxes from a spout connected to an externally mounted bottle. The highly palatable diet (Gilbert and Cooper, 1985) consisted of 50 ml sweetened condensed milk (Nestle) mixed with 200 ml tap water and 150 ml powdered rodent lab chow (No1 ground rat maintenance diet, Special Diet Services Ltd; Essex, UK). Locomotor activity was measured in the same test boxes, each bisected by two photo-cell beams (Retroreflective Scan System, model RS 348-38, RS Components, Corby, Northants, UK). The photobeams were fixed horizontally at a height of 7 cm and spaced 25 cm apart from each other and 3.5 cm from each end of the box. One locomotor activity count was registered each time a rat crossed between the two beams. Multiple interruptions of the same beam were recorded but not analysed here.

5.2.3 Procedure:

Rats were removed from the colony room to a separate room in their home cages and given familiarisation sessions in the experimental cages prior to drug studies. They were adapted to eating the sweetened wet mash for 120 min in the absence of external disturbance until baseline levels of food intake were consistent (4 sessions with at least 48

hrs between testing). On experimental sessions, rats were injected i.p. with either physiological saline (control) or the test drug and placed back in their home cages. The test food was presented to the rats 30 min after injection of the antagonists, with the exception of sulpiride, for which there was a 60 min interval. The dishes and their contents were weighed before being presented to the rats and at 30, 60, 90 and 120 min afterwards. The subtraction of post-from pre-feeding values was the measure of food intake. Water consumption was also measured at the same time points. Locomotor crossings were recorded automatically by PC throughout the 2 hr test session, and then collated into 30 min intervals for analysis using a purpose-written data extraction programme. A repeated measures design was used, with each rat receiving all treatments in a random order and with a period of at least 48 hrs between successive tests.

5.2.4 Drugs:

Amisulpride (Synthelabo Recherche, Bagneaux, France) was dissolved in distilled water with a few drops of 1M HCl solution and NaOH to give a pH of 7.5. (-) Sulpiride (Tocris Cookson, Bristol, England , UK) was dissolved in distilled water and a few drops of acetic acid to give a pH of 6.5. L-745,870 (3-([4-chlorophenyl) piperazin-1-yl] methyl)-1H-pyrrolo [2,3-b] pyridine; Tocris Cookson, Bristol, England, UK) and U-99194A Maleate (5,6,-Dimethoxy-2-(di-n-propylamino)indan maleate; RBI Chemicals, Natick MA, USA) were dissolved in distilled water. All control conditions used appropriate vehicles and all drugs were injected i.p. at 1 ml/kg.

5.2.5 Data analysis:

Results were analysed using a two-way analysis of variance for repeated measures (ANOVA) followed by *post-hoc* t-tests (Student-Newman Keuls and Dunnett's multiple comparisons tests). Time samples were analysed not only for the entire 2 hr period (in 30 min bins) but also for the first 30 min of the test session, since 20-30 min has been a common test duration in similar studies using other drugs, and a 30 min period is also to be used for microstructural studies later. ED_{50} values and their 95% confidence limits were calculated using standard ANOVA and linear regression techniques on data from the

linear portion of the dose-response curves. A significant difference in ED_{50} values was obtained when comparisons presented non-overlapping 95% confidence limits. Relative potency estimates were calculated to give the dose of standard drug (mg/kg) equal to 1 mg/kg of comparison drug; a significant difference was assumed if the 95% confidence limits did not include 1.0.

5.3 Results

5.3.1 Experiment 1: Effects of U-99194A and L-745,870.

The effects of U-99194A on food intake and locomotor activity are shown in Figure 5.1. Administration of U-99194A did not affect food intake over 2 hrs [Main effect of dose: $F(3,21) = 1.89$, $P = 0.162$ NS]. However, there was a significant time effect [$F(3,21) = 311.68$, $P<0.01$] and a dose×time interaction [$F(9,63) = 3.34$, $P<0.01$]. *Post-hoc* tests did not reveal any significant differences between individual doses of U-99194A and vehicle control over the 2 hr test session. However, when the data for the first half hour of the test session were analysed, an effect of drug on food intake was seen [$F(3,21) = 5.25$, $P<0.01$]. *Post-hoc* tests, on the other hand, did not reveal any significant differences between individual doses of U-99194A and vehicle control.

U-99194A did not significantly affect locomotor activity over the full experiment [$F(3,21) = 2.79$, $P>0.05$]. There was a significant time effect [$F(3,21) = 26.98$, $P<0.01$] but no dose×time interaction [$F(9,63) = 1.14$, $P = 0.352$ NS]. None of the doses of U-99194A affected total locomotor activity over the full 2 hr test period. Data analysed over the first half hour, however, indicated a main effect on locomotor activity [$F(3,21) = 3.19$, $P<0.05$], with *post-hoc* tests indicating that the 10.0 mg/kg dose caused a significant reduction in locomotor activity over the first 30 min ($P<0.05$).

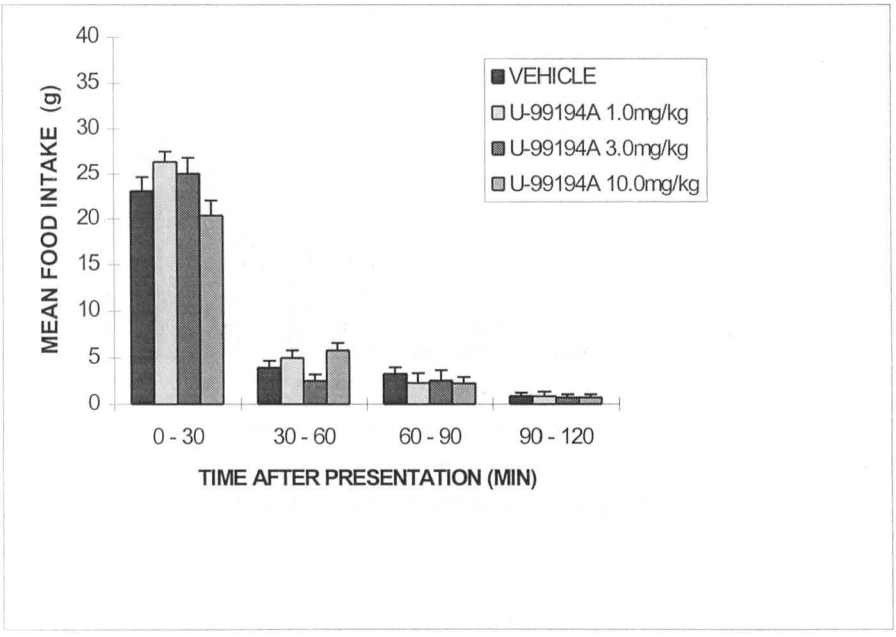
The effects of L-745,870 on food intake and locomotor activity are shown in Figure 5.2. Administration of L-745,870 reduced food intake; main effect of dose: $F(3,21) = 3.50$, $P<0.05$. There was also a significant time effect [$F(3,21) = 102.08$, $P<0.01$] and a dose×time interaction [$F(9,63) = 4.08$, $P<0.01$]. *Post-hoc* tests revealed that

the 10.0 mg/kg dose of L-745,870 produced a significant decrease in feeding ($P<0.01$) compared with vehicle controls over the full test session. When the data within the first 30 min of the test session were analysed, an effect of drug on food intake was also seen [$F(3,21) = 13.20$, $P<0.01$]. *Post-hoc* tests again showed that the 10.0 mg/kg dose of L-745,870 produced a significant decrease in feeding ($P<0.01$) compared with vehicle controls.

Administration of L-745,870 also resulted in a dose-related decrease in locomotor activity [$F(3,21) = 17.36$, $P<0.01$]; there was also a significant time effect [$F(3,21) = 18.29$, $P<0.01$] and a dose×time interaction [$F(9,63) = 3.73$, $P<0.01$]. *Post-hoc* tests revealed that the 3.0 and 10.0 mg/kg doses of L-745,870 produced significant reductions in locomotor activity ($P<0.01$) compared with vehicle controls over the full test session. Data analysed within the first 30 min also revealed an effect of L-745,870 [$F(3,21) = 34.78$, $P<0.01$], and *post-hoc* tests showed that the 3.0 and 10.0 mg/kg doses produced significant reductions in locomotor activity over 30 min ($P<0.01$) compared with vehicle controls, mirroring the effects over the full test period.

ED_{50} values could not be calculated for the effects of U-99194A due to the drug's inability to reduce behaviours below 50% of vehicle control levels. Higher doses of the drug were not used because they are unlikely to be D3 selective. With regards to L-745,870, only locomotor activity was reduced enough for its ED_{50} value to be calculated (Table 5.1). This dose, 3.43 mg/kg is above the range considered selective for the D4 receptor (doses of < 1 mg/kg being selective for the site, Patel et al., 1996a; 1997).

No effect of these compounds on water intake was found (results not shown).



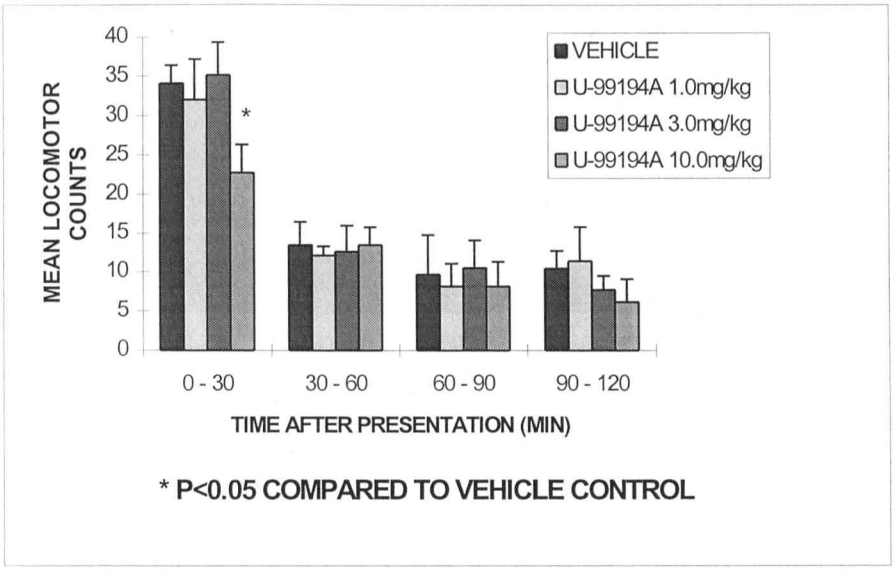
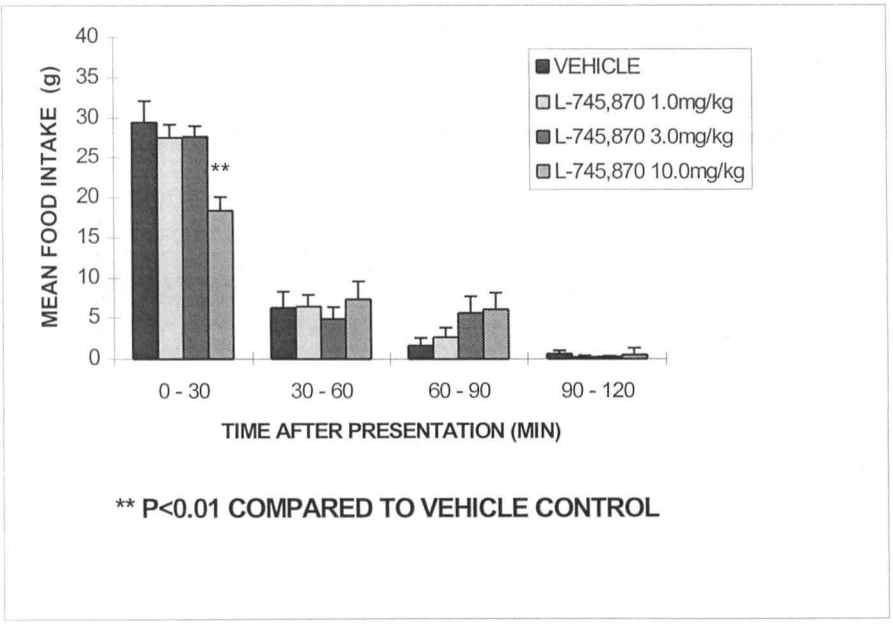


Figure 5.1. Effects of the D3 receptor antagonist U-99194A on feeding (top panel) and locomotor activity (bottom panel) in food-deprived rats. N = 8 at all points. Data were recorded from entry to the experimental cages (time 0 min) onwards.



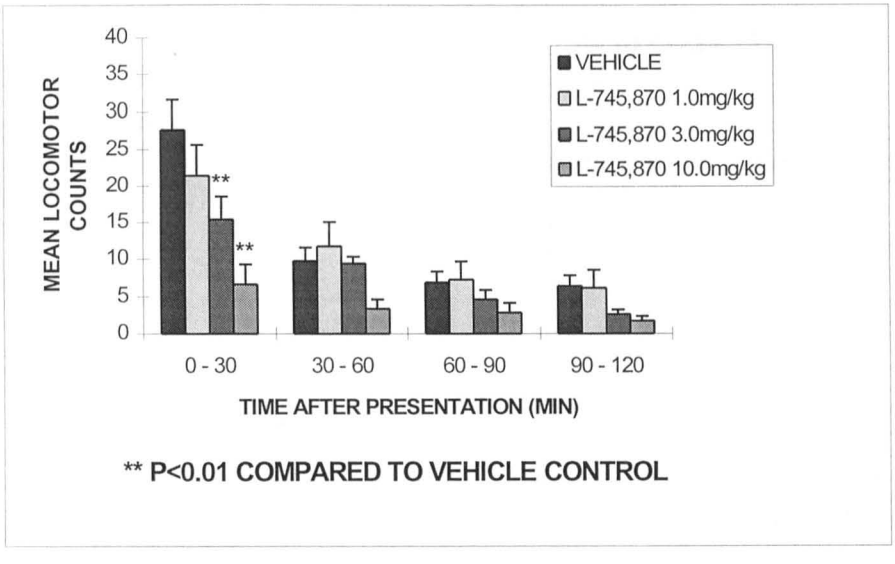


Figure 5.2. Effects of the D4 receptor antagonist L-745,870 on feeding (top panel) and locomotor activity (bottom panel) in food-deprived rats. N = 8 at all points. Data were recorded from entry to the experimental cages (time 0 min) onwards.

TABLE 5.1.

ED_{50} values (mg/kg) for the effects of four dopamine receptor subtype antagonists on two behaviours (feeding and locomotor activity) during the first 30 min of the test session.

	FEEDING	LOCOMOTOR ACTIVITY
DRUG	**ED_{50} (95% CL)**	**ED_{50} (95% CL)**
U-99194A	-	-
L-745,870	-	3.43 (2.40-4.90)
Amisulpride	-	13.49 (7.47-24.36)
Sulpiride	-	68.64 (10.05-468.97)
R.P.	-	**2.94 (0.51-29.77)**

R.P. = Relative potency.
(-) = No value calculated due to food intake or locomotor activity not being altered by more than 50% of control levels.

5.3.2 Experiment 2: Effects of sulpiride and amisulpride.

The effects of sulpiride on food intake and locomotor activity are shown in Figure 5.3. Administration of sulpiride did not result in a main effect of drug on food intake [$F(3,21) = 1.33$, $P = 0.292$ NS], although there was a significant time effect [$F(3,21) = 151.38$, $P<0.01$] and a dose×time interaction [$F(9,63) = 2.58$, $P<0.05$]. There was no significant effect of any dose on total food consumed over the 2 hr test period. However, analysing the data over the first 30 min alone revealed an effect of drug on food intake [$F(3,21) = 3.77$, $P<0.05$]. *Post-hoc* tests revealed that the 5.0 mg/kg and 50.0 mg/kg doses of sulpiride produced significant increases in feeding ($P<0.05$) compared with vehicle control conditions.

In contrast, administration of sulpiride resulted in a decrease in locomotor activity over the 2 hr test period [$F(3,21) = 14.35$, $P<0.01$] with a significant time effect [$F(3,21) = 34.46$, $P<0.01$] and a dose×time interaction [$F(6,63) = 2.18$, $P<0.05$]. Total locomotor activity was suppressed at 50.0 mg/kg ($P<0.01$) compared with vehicle controls. Analysing the data over the first 30 min alone also revealed a main effect of drug on locomotor activity [$F(3,21) = 18.13$, $P<0.01$]. *Post-hoc* tests showed that 50.0 mg/kg produced a significant reduction in locomotor activity ($P<0.01$).

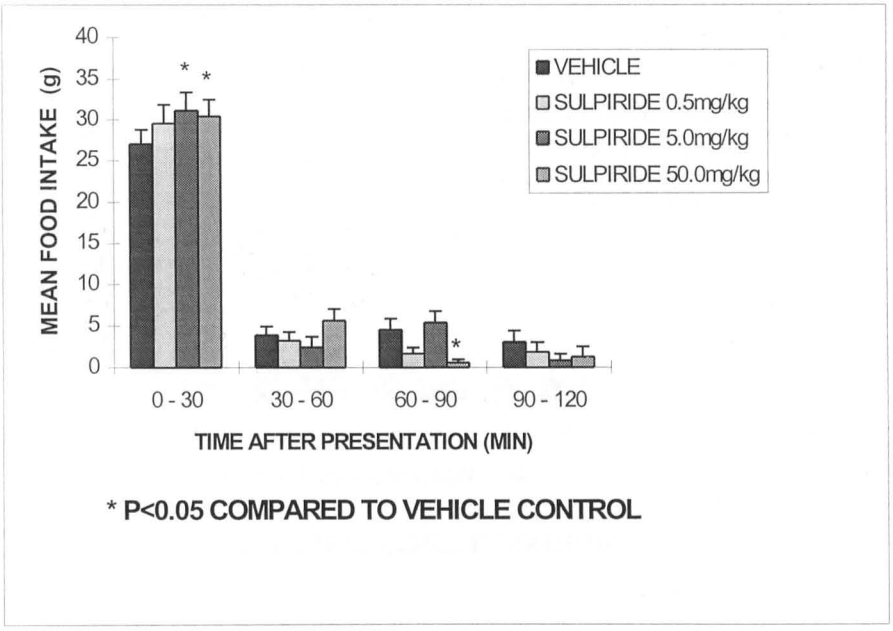
The effects of amisulpride on food intake and locomotor activity are shown in Figure 5.4. Administration of amisulpride did not produce a main effect on food intake over the full 2 hr test session [$F(3,21) = 1.93$, $P = 0.156$ NS]. There was a significant time effect [$F(3,21) = 250.10$, $P<0.01$] and a dose×time interaction [$F(6,63) = 2.22$, $P<0.05$]. Similar to the results obtained with sulpiride, amisulpride failed to significantly affect total food intake over the 2 hr test period at any of the doses used. However, analysing the data from the first 30 min revealed an effect on food intake [$F(3,21) = 3.71$, $P<0.05$]: *Post-hoc* tests showed this to be due to a significant increase in feeding produced by the 5.0 mg/kg dose of amisulpride ($P<0.05$ compared with vehicle control).

In contrast, administration of amisulpride resulted in a decrease in locomotor

activity [Main effect: $F(3,21) = 10.24$, $P<0.01$], a significant time effect [$F(3,21) = 44.27$, $P<0.01$] and a dose×time interaction [$F(9,63) = 5.11$, $P<0.01$]. Total locomotor activity was significantly suppressed at 50.0 mg/kg ($P<0.01$) compared with vehicle controls. Analysing the data from the first 30 min also revealed an effect on locomotor activity [Main effect: $F(3,21) = 23.63$, $P<0.01$]. *Post-hoc* tests showed that the 50.0 mg/kg dose also produced a significant reduction in locomotor activity compared with vehicle controls ($P<0.01$) over the first 30 min of the test session.

A further experiment was carried out using 100 mg/kg of amisulpride (results not shown), a dose believed to block postsynaptic receptors (Scatton et al., 1997). The results showed no significant decrease in food intake either in the first 30 minutes (Vehicle: 29.6 g mean food intake; amisulpride: 26.7 g), or over the whole session. However, there was a significant decrease in locomotor activity ($P<0.01$) in comparison with vehicle control.

ED_{50} values for the two drugs (Table 5.1) showed that amisulpride is more potent (~ 3 times) than sulpiride in suppressing locomotor activity. No value could be calculated for feeding due to the drugs' failure to change food intake by more than 50%.



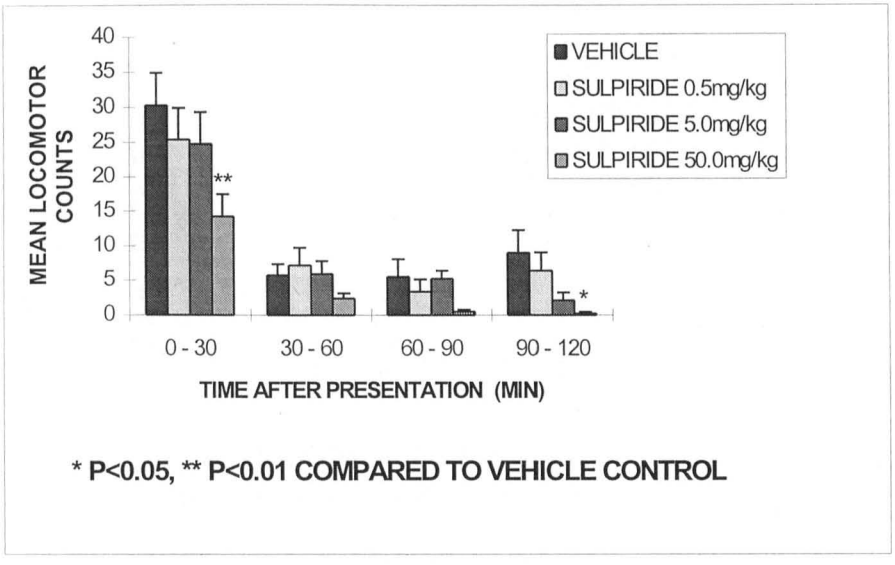
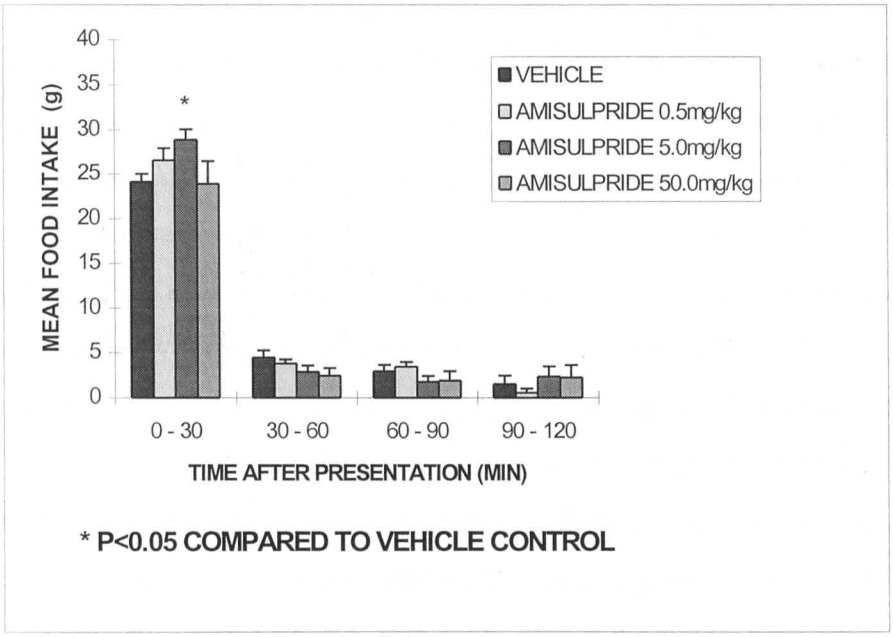


Figure 5.3. Effects of the D2-like receptor antagonist Sulpiride on feeding (top panel) and locomotor activity (bottom panel) in food-deprived rats. N = 8 at all points. Data were recorded from entry to the experimental cages (time 0 min) onwards.



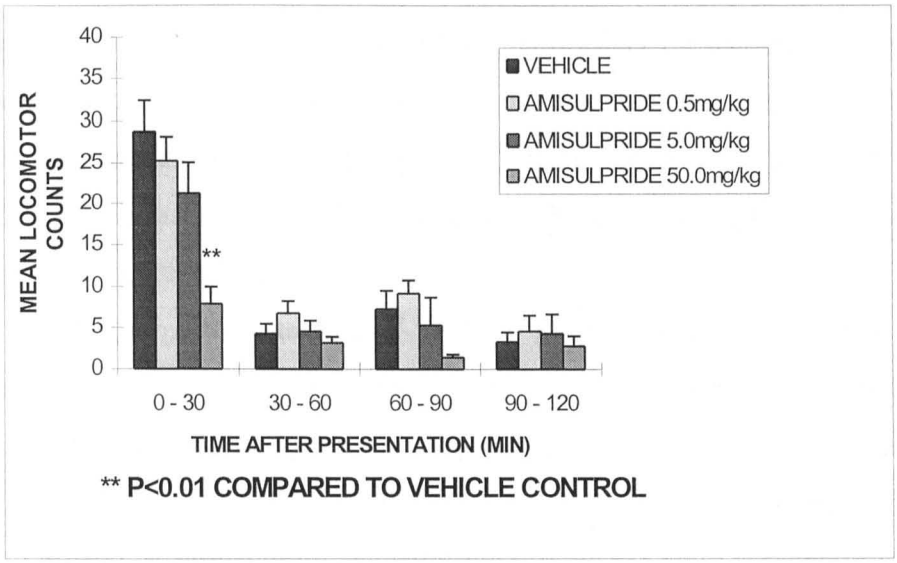


Figure 5.4. Effects of the D2-like receptor antagonist Amisulpride on feeding (top panel) and locomotor activity (bottom panel) in food-deprived rats. N = 8 at all points. Data were recorded from entry to the experimental cages (time 0 min) onwards.

5.4. Discussion

The results show that sulpiride has similar effects to amisulpride on feeding behaviour, producing an increase in food intake at low doses but no apparent effect at the higher doses tested. Also, the locomotor activity data showed that both drugs caused a dose-dependent decrease in activity. Other D2-like antagonist drugs have been shown previously to produce a similar biphasic effect on eating, i.e. an increase in food intake at low doses followed by a decrease at higher doses. Hence, the effects of the standard D2-like compounds used in the present study are typical of what has been found before. For example, spiperone (Cooper and Sweeney, 1980), pimozide (Lawson et al., 1984), YM 09151-2 and remoxipride (Clifton et al., 1991), haloperidol (Hobbs et al., 1994) and raclopride (Terry,1996) have all been shown to increase feeding when administered at low doses. Similarly, many of the above D2-like compounds have been shown to decrease locomotor activity (e.g. Bakshi and Kelley, 1991; Wise and Carlezon, 1991; Horita et al., 1995).

Here, we report for the first time the effects of U-99194A and L-745,870, drugs which display relatively high affinity and selectivity for D3 and D4 receptors respectively. U-99194A produced a significant main effect on feeding over the first 30 min, but did not produce any significant changes in food intake over the full 2 hr test session. There was also a small but significant reduction in locomotor activity at the highest dose. In contrast, L-745,870 inhibited food intake at the highest dose, and caused a dose-dependent decrease in locomotor activity.

It is interesting to note that U-99194A showed a similar feeding profile to sulpiride and amisulpride. All three drugs (but not L-745,870) produced an increase in food intake at low doses and a decrease at higher doses. These results may suggest that the D3 receptor subtype within the D2-like receptor subfamily may be responsible for this behavioural profile, as amisulpride and sulpiride bind to D3 and D2 receptor subtypes. A microstructural study of the effects of U-99194A is needed in order to identify the features of behaviour responsible for the drug's effect on feeding (Chapter 6), and to

determine whether it operates in a similar manner to other D2-like antagonists.

In contrast, a number of studies have investigated the role of the D3 receptor in locomotor activity. Other drugs sometimes considered to be D3 receptor antagonists, such as (+)-AJ76 and (+)-UH232, which display 3-5 times higher affinity for D3 than D2 receptors, have been shown to produce dose-dependent increases in locomotor activity accompanied by increases in DA release (Svensson et al., 1986), suggesting the involvement of presynaptic receptors. Recently, the finding that U-99194A (30-fold D3 over D2 selectivity) increases locomotor activity without any concomitant effects on DA release has led to the hypothesis that the D3 receptor is postsynaptic, and that its blockade leads to stimulation of locomotor activity (Waters et al., 1993; Waters et al., 1994). Kling-Petersen et al. (1995) also showed that U-99194A produced an increase in activity when injected into the NAC or the lateral ventricle, but not in the ventral tegmental area. The findings by these authors conflict with the present results, probably due to methodological differences between the studies. In one study (Waters et al., 1993), U-99194A increased locomotor activity in rats at doses of 25 μmol/kg (s.c.) and above; this minimum dose corresponds to approximately 10 mg/kg in the present study, a dose that decreases locomotor activity. In this study, locomotor activity and food intake were measured simultaneously, whereas Waters et al. (1993) measured locomotor activity in rats which were habituated to their environment for 60 minutes before administration of the drug. Gendreau et al. (1997), while studying the effects of D3 receptor involvement on emotional reactivity in mice, found that after habituation to the activity chamber prior to drug administration, U-99194A increased locomotion and rearing at lower doses (5, 10 mg/kg) but reduced these behaviours at higher doses (20, 30 mg/kg) . However, in mice exposed to the activity chamber for the first time, the same drug produced only weak motor activation at lower doses and an early decrease in motor behaviour at higher doses that was followed by an increase in locomotion later in the test session. The latter study and the present one are the only ones to date to suggest that the selective D3 antagonist U-99194A can reduce motor activity, albeit using different doses and species. Clearly there are discrepancies in the results for this compound as to whether or not it stimulates

locomotor activity. A microstructural study of this compound could again help identify the nature of the drug's effect on locomotor activity.

We also examined for the first time the effects of the selective D4 receptor antagonist L-745,870 on feeding and locomotor activity. Given the drug's negligible effect in behavioural tests of antipsychotic activity (Bristow et al., 1996) and on cocaine's discriminative stimulus effects (reported in Chapter 3), and the limited effects of other selective D4 antagonist compounds such as U-101387 (Merchant et al., 1996), it was surprising to see this drug affecting feeding in the present study. However, caution is needed since the decrease in food intake coincided with a decrease in locomotor activity. More importantly, the effects on feeding occurred at a dose unlikely to be D4-specific, and at which other D2 receptor subtypes are occupied (Bristow, personal communication). Similarly, the ED_{50} value obtained for locomotor activity (3.43 mg/kg) is at the upper end of the selectivity range, i.e. doses of below 1 mg/kg are presumed selective for the D4 receptor subtype (Bristow, personal communication). Again, this result reinforces the finding that the decreases in feeding and locomotor activity which occurred with compounds belonging to the D2-like subfamily may be attributed to binding at the D2/D3 receptor subtypes and not the D4 receptor subtype.

It has been suggested that activation of central D2-like receptors is necessary for the normal positive reinforcing effects of sucrose on sham feeding, since selective D2-like receptor antagonists (including sulpiride) suppress sucrose sham-feeding in the rat (Schneider et al., 1986). This effect appears to be centrally mediated because domperidone, a DA antagonist restricted to peripheral receptors, has no effect on either sham or real feeding (Duong and Weingarten, 1993). However, sulpiride and amisulpride have been shown to potentiate food-induced conditioned place preference, perhaps indicating enhanced incentive properties of the primary reinforcer (Guyon et al., 1993). The authors hypothesised that neuroleptics such as amisulpride and sulpiride can increase the ability of rats to experience pleasure through potentiation of the release of DA triggered by food presentation. This could be due to the higher affinity of these compounds for D2 (or D3) autoreceptors which were blocked at concentrations below

those that block postsynaptic receptors.

Amisulpride blocks the terminal DA autoreceptor and behavioural experiments are consistent with this finding. For example, there is evidence that apomorphine-induced yawning and hypolocomotion maybe presynaptically mediated (e.g. Di Chiara et al., 1976; Yamada and Furukawa, 1980; Dourish and Hutson, 1985), and amisulpride preferentially blocks these effects at low doses (Perrault et al., 1997). The wide separation between doses of amisulpride that antagonise the different apomorphine-induced effects may be related to a preferential blockade of pre-synaptic D2/D3 receptors involved in apomorphine-induced hypomotility and yawning (Schoemaker et al., 1997). This hypothesis is supported by neurochemical studies in vivo showing that amisulpride increases the release of DA in the olfactory tubercle evoked by electrical stimulation of the ascending dopaminergic pathways in the rat (an index of nerve terminal D2/D3 autoreceptor blockade) at doses very close to those that antagonise hypomotility induced by apomorphine, i.e. ~ 10 mg/kg (Schoemaker et al., 1997). An interaction of amisulpride at low doses with presynaptic DA autoreceptors was also supported by an increase in extracellular DA levels in the striatum and nucleus accumbens of the rat (Schoemaker et al., 1997). Much higher doses were needed (i.e. 40-80 mg/kg) to decrease striatal ACh levels (an index of postsyanptic D2 receptor blockade), as well as to reverse apomorphine-induced hypermotility or stereotypies (Schoemaker et al., 1997).

This may help explain the behavioural effects of this drug on feeding behaviour: at low doses there is an increase in food intake as a result of D2/D3 pre-synaptic blockade. But this doesn't explain why its structural analog sulpiride at relatively high doses (50 mg/kg, presumably a preferential postsynaptic dose) caused an increase in food intake and did not suppress feeding as would be expected from this hypothesis. Clearly, there is a distinction in behavioural effects between the low and high doses of these D2-like antagonists on feeding behaviour. However, there are claims that autoreceptors may not be involved in these sorts of effect (for review, see Stahle, 1992). Stahle (1992) argues against the hypothesis that stimulation of DA autoreceptors is the mechanism by which DA agonists induce yawning and suppression of exploration. The relation between

reduced extracellular DA levels (assessed by microdialysis) and the behavioural effects of DA agonists, a DA synthesis inhibitor and a granule storage blocker have been shown to be highly inconsistent (Stahle, 1992). In addition, amphetamine co-treatment can increase DA levels while yawning and suppression of exploration are still induced. Stahle (1992) suggested that autoreceptors are not the mediators of these behavioural effects, and proposed instead that postsynaptic receptors mediate DA agonist-induced yawning and suppression of exploration. Hence, we cannot be certain that the stimulation of feeding obtained here is necessarily due to autoreceptor-mediated action.

The exact anatomical sites for feeding behaviour cannot be determined from this study; however, Parada at al. (1988) reported that sulpiride injected into the lateral hypothalamus caused an increase in feeding at low doses. While dose-dependent enhancement of feeding duration was also obtained following injection of haloperidol into the nucleus accumbens, injection of sulpiride in the ventrolateral striatum reduced feeding duration (Bakshi and Kelley, 1991; Inoue et al., 1995).

With regards to locomotor activity, no biphasic effects were obtained with either sulpiride or amisulpride: both drugs caused dose-dependent decreases in activity. This conflicts with the findings by Perrault et al. (1997) who reported only a slight decrease in locomotor activity at 100 mg/kg amisulpride, with an ED_{50} > 100 mg/kg compared with 13.49 mg/kg obtained in the present study. Their explanation for the drug's low potential for decreasing locomotor activity highlighted three biochemical properties which characterise this drug: (A) high specificity for D2 and, perhaps more importantly, D3 limbic receptors. By using [^{3}H] raclopride, a high affinity radioligand for the DA D2 and D3 receptors, Schoemaker et al. (1997) showed that amisulpride (and its analog sulpiride) displayed a preferential affinity for limbic D2/D3 receptors; (B) selectivity for limbic areas: for example, few D3 receptors are found in the striatum. The drug's ED_{50} for inhibiting in vivo [^{3}H] raclopride-specific binding in the rat striatum was 43.6 mg/kg, compared with 17.3 mg/kg in the limbic system; and (C) preferential blockade of pre-synaptic D2/D3 receptors (as mentioned earlier); by increasing DA release, it may relieve blockade of striatal postsynaptic D2 receptors. As amisulpride and sulpiride decreased

locomotor activity in the present study at doses believed to be acting post-synaptically (i.e. 40-80 mg/kg, and at doses which are believed to occupy 70-80 % striatal D2 receptors) this decrease in behaviour can only be attributed to blockade of striatal D2 receptors.

Amisulpride was more potent than sulpiride in suppressing locomotor activity, and more potent than in the study by Perrault et al. (1997). However, it is difficult to compare the present results with those of Perrault, since in their study food intake was not measured simultaneously with locomotor activity. Also, their rats were not habituated as they were in the present study, and so they produced high baseline levels of activity which makes comparisons difficult to conclude. However, it is worth noting the difference in potency between amisulpride and sulpiride. This may be due to amisulpride having a greater affinity for the D2/D3 receptor subtypes (5-fold more selective compared to sulpiride): its behavioural potency mirrors this effect, amisulpride being nearly three times more potent than sulpiride at suppressing locomotor activity.

In conclusion, the experiments suggest that the D3 receptor antagonist U-99194A produces similar behavioural effects to other D2-like receptor antagonists. However, the D4 receptor antagonist L-745,870, at doses specific for the D4 receptor subtype, does not produce a similar profile; only at doses that occupy D2 receptors were significant effects on feeding and locomotor activity observed.

CHAPTER 6:

'DOPAMINE D3 AND D4 RECEPTOR ANTAGONISTS: MICROSTRUCTURAL ANALYSES OF THEIR EFFECTS ON FEEDING AND ASSOCIATED BEHAVIOURS IN THE RAT'

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6.1 Introduction And Aims Of Experiments

Studies focusing on DA and ingestive behaviour have largely been concerned with the effects of DA D2-like receptor antagonists. Due to the lack of selective drugs, the possible involvement of D3 and D4 receptor subtypes has been overlooked. However, the data from the previous chapter indicated that U-99194A produced a significant effect on food intake in the first 30 min of the test session. The same drug also caused a slight reduction in locomotor activity at the highest dose tested (10.0 mg/kg). The selective D4 antagonist, L-745,870, in comparison, also produced an effect on food intake, evident at 10.0 mg/kg, and a dose-related decrease in locomotor activity.

Although the measurement of food consumption by quantity may shed light upon certain features of energy balance, the moment-to-moment, psychological processes that control feeding behaviour cannot be obtained by such measures. Consequently, feeding can be described in greatest detail by taking account of its microstructure (Blundell, 1983). The procedure involves exhaustively observing the behaviour of rats under drug during a fixed time-period and recording various categories of behaviour (Table 4.2 and 4.3). The purpose of such fine analysis is to reveal potentially subtle differences between the effects of drugs that may not be revealed by a simple measure of the amount of food consumed. The microstructural approach therefore generates results which can be used not only to discriminate between drug actions, but also to suggest their underlying behavioural mechanisms of action. In this respect, the involvement of D3 and D4 receptors in feeding and locomotor activity requires further investigation.

The aim of the next experiments is to assess the effects of U-99194A and L-745,870 using a microstructural analysis of behaviour. Comparisons will be drawn with the effects of other DA D2-like antagonists on food intake. In addition to feeding, a number of other behavioural responses were observed. This allows more detailed

comparisons to be made with other DA D2-like antagonists, and more generally provides a fuller characterisation of the behavioural profiles associated with the actions of the drugs. The information obtained will not only be informative per se, but will also provide data concerning doses that are necessary prior to drug combination studies with cocaine (chapter 8).

6.2 Materials and methods

6.2.1 Subjects:

Sixteen male Sprague-Dawley rats, eight per experiment, (Charles River, Margate, Kent), weighing 320-360g were housed in pairs with free access to water under a 12 hr light/dark cycle, lights on 07:00. They were all maintained on a restricted diet of 15g standard food pellets per day, adjusted on test days so that regardless of the effect of drug all rats received the same food allowance per day. All testing was between 12:00 and 14:00 hours.

6.2.2 Drugs:

U-99194A Maleate (5,6,-Dimethoxy-2-(di-n-propylamino)indan maleate) and L-745,870 (3-([4-chlorophenyl) piperazin-1-yl] methyl) -1H-pyrrolo [2,3-b] pyridine) were obtained from RBI Chemicals (Natick MA, USA) and Tocris Cookson (Bristol, UK) respectively. They were both dissolved in distilled water, administered intraperitoneally at doses of 1, 3 and 10 mg/kg and injected 30 min prior to test.

6.2.3 Procedure:

The pre-treatment adaptation period, food preparation and apparatus were the same as described in section 5.2.3. Thirty minutes following drug administration, the animal was transferred to the observation box containing a plastic petri dish with a freshly-prepared sample of the sweetened mash. Throughout the 30 min observation period, an observer kept a continuous record of the animal's behaviour. Each episode of behaviour was recorded by entering the start and end into a microcomputer (Elonex PC) where a purpose-built software programme recorded each observed behaviour. The software logged the duration and sequence of each key-press. Five keys were assigned to five non-

overlapping response categories, which consisted of: (i) feeding, i.e. biting, chewing and ingesting the sweetened mash; (ii) locomotor activity, horizontal activity, horizontal movement about the test box employing all four limbs; (iii) rearing, front limbs raised or resting on the side of the observation box; (iv) grooming, i.e. licking, scratching or biting at any part of the body surface; (v) stationary, resting or immobile, i.e. not engaged in any of the preceding activities. The software provided an analysis of the total duration (min) for each response category, the frequency of individual bouts for each category, and the mean duration of individual bouts for 5-min interval bins over a total duration of 30 minutes. Food intake was calculated by successive weighings (to the nearest 0.1 g, any spillage was collected). The local rate of eating (g/min) was calculated for each test by dividing the amount of food consumed by the total duration of feeding. The latency to initiate feeding was timed from the start of the session until the moment the animal made contact with the sweetened mash. The observers were pre-trained to recognise and record all behavioural categories as shown in Table 4.2.

6.2.4 Design and statistical analysis:

The data were analysed by one-way analysis of variance (ANOVA) for repeated measures where the drug doses were administered to the rats in a 'Latin Square' design. Comparisons between the results for individual drug-treatment conditions and the vehicle control condition were made using Dunnett's t-test.

6.3 Results

6.3.1 The D3 antagonist

(a) Food Intake and Feeding Microstructure.

U-99194A (1-10 mg/kg) produced a significant effect on food intake over the 30-min observation period [$F(3,21) = 4.83$, $P<0.05$], although post-hoc tests revealed that only the highest dose differed significantly from vehicle (a modest 20 % reduction with respect to control levels; Fig. 6.1a). There was no significant effect of the drug on the latency to initiate feeding (Fig. 6.1b); a trend towards an increase is shown, but the effect failed to reach statistical significance ($P>0.05$). Likewise, analysing the other feeding parameters, U-99194A failed to affect total time spent feeding (Fig. 6.1d), mean bout duration (Fig. 6.1e) or bout frequency (Fig. 6.1f and Table 6.1). However, even though there were no significant effects on mean bout duration or bout frequency, a noticeable trend was apparent for both measures. While mean bout duration showed a biphasic response, i.e. an increase to 3 mg/kg followed by a decrease at 10 mg/kg, bout frequency showed the opposite trend. The only microstructural parameter significantly affected was local rate of eating [$F(3,21) = 3.90$, $P<0.05$; Fig. 6.1c], reduced by 17 % with respect to control levels at 10 mg/kg ($P<0.05$).

(b) Locomotor Activity.

U-99194A produced no significant effects on any of the parameters of locomotor activity during the observation period (Table 6.1). However, duration of locomotor activity and bout frequencies did show biphasic profiles, tending to decrease and then increase to baseline levels.

(c) Rearing.

U-99194A significantly reduced the duration of rearing activity [$F(3,21) = 3.79$, $P<0.05$], largely due a significant reduction in the frequency of individual bouts [$F(3,21) = 5.06$, $P<0.01$; Table 6.1]. Duration of individual rearing bouts showed little change with dose. Total rearing duration decreased by 60 % at 10 mg/kg ($P<0.01$), and at the same dose, mean bout frequencies also decreased by 60 % ($P<0.01$).

(d) Grooming.

U-99194A had no significant effect on any of the parameters of grooming behaviour (Table 6.1).

(e) Immobility.

U-99194A had no significant effect on any parameter of immobility behaviour during the observation period (Table 6.1).

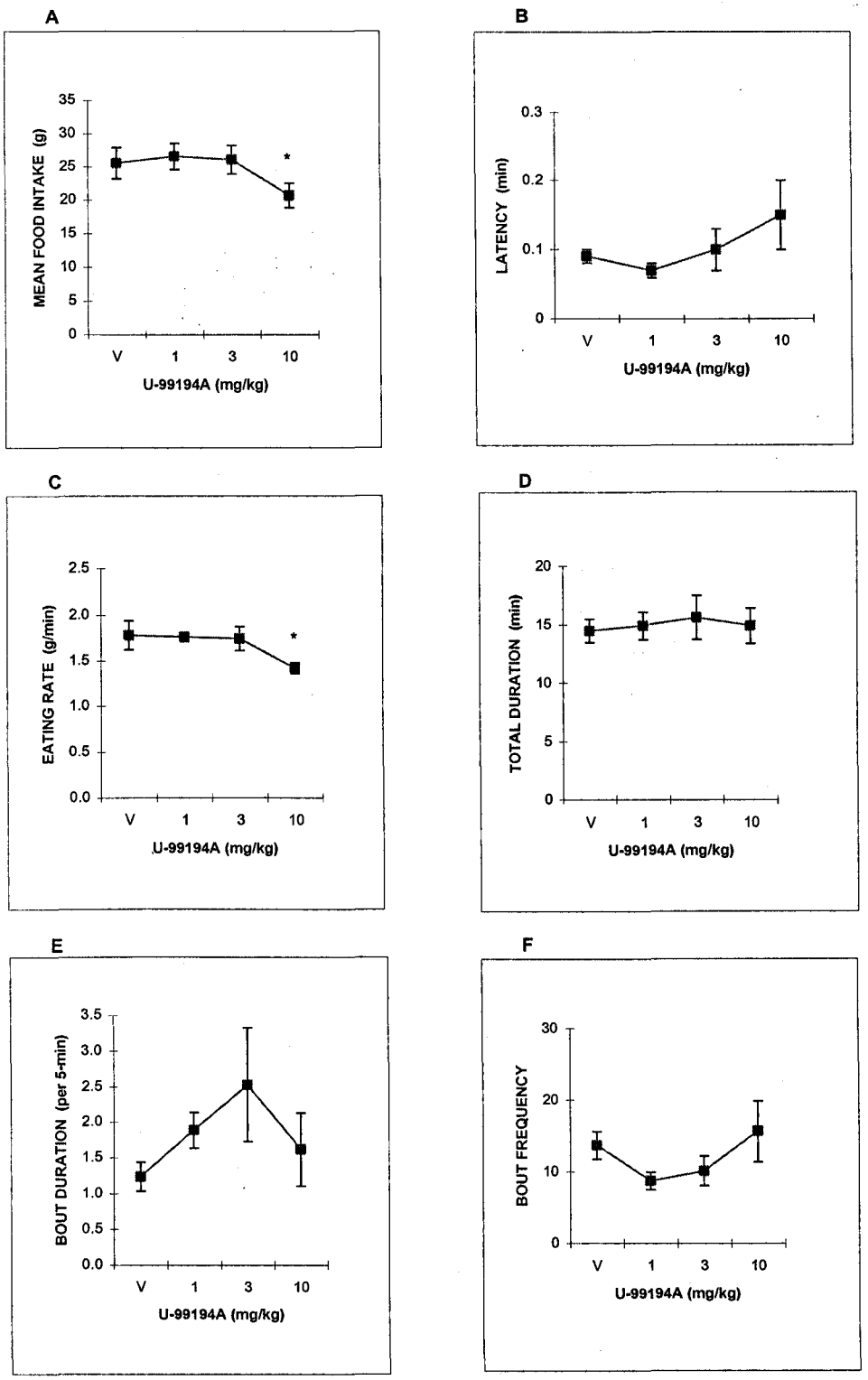

Figure 6.1. Effects of the dopamine D3 receptor antagonist U-99194A on feeding parameters : **A**: Total food intake **B**: Latency to start eating **C**: Eating rate **D**: Total duration **E**: Mean bout duration **F**: Bout frequency. N= 8 at all points; * $P<0.05$, ** $P<0.01$ compared to vehicle control, V (comparisons by Dunnett's t-tests).

	U-99194A (mg/kg)				F-VALUE	P-VALUE
BEHAVIOURAL RESPONSE/CATEGORIES	0	1	3	10		
LOCOMOTOR ACTIVITY						
TOTAL DURATION (min)	2.10 +/- 0.26	1.49 +/- 0.08	1.66 +/- 0.30	2.29 +/- 0.61	1.001	NS
BOUT DURATION (per 5-min)	0.07 +/- 0.01	0.08 +/- 0.01	0.07 +/- 0.01	0.09 +/- 0.01	1.388	NS
BOUT FREQUENCY	30.75 +/- 4.22	19.75 +/- 2.22	23.50 +/- 4.68	25.50 +/- 5.70	1.404	NS
REARING						
TOTAL DURATION (min)	2.54 +/- 0.46	1.62 +/- 0.28	1.73 +/- 0.42	1.03 +/- 0.31 **	3.789	P<0.05
BOUT DURATION (per 5-min)	0.12 +/- 0.01	0.12 +/- 0.01	0.12 +/- 0.02	0.12 +/- 0.01	0.089	NS
BOUT FREQUENCY	21.25 +/- 2.99	13.00 +/- 1.73	16.38 +/- 4.12	8.63 +/- 2.48 **	5.057	P<0.01
GROOMING						
TOTAL DURATION (min)	0.99 +/- 0.25	1.58 +/- 0.53	1.06 +/- 0.26	1.51 +/- 0.54	0.64	NS
BOUT DURATION (per 5-min)	0.21 +/- 0.04	0.28 +/- 0.07	0.26 +/- 0.05	0.23 +/- 0.05	0.595	NS
BOUT FREQUENCY	4.88 +/- 0.77	5.25 +/- 0.90	4.13 +/- 0.72	6.25 +/- 1.61	0.652	NS
RESTING/IMMOBILITY						
TOTAL DURATION (min)	9.68 +/- 0.77	9.82 +/- 1.08	9.48 +/- 1.46	9.57 +/- 1.35	0.043	NS
BOUT DURATION (per 5-min)	1.20 +/- 0.23	1.67 +/- 0.41	1.99 +/- 0.44	0.99 +/- 0.13	2.529	NS
BOUT FREQUENCY	9.25 +/- 1.10	7.88 +/- 1.23	5.75 +/- 0.84	10.50 +/- 1.43	2.794	NS

Results are shown in terms of Mean +/- S.E.M. (N=8). F-ratios and levels of significance are shown for one-way ANOVA.

Levels of significance for individual dose comparisons against control conditions : * P <0.05; ** P <0.01 (Dunnett's t-test).

Table 6.1. Microstructural analysis of non-feeding behaviours following administration of the D3 antagonist U-99194A. Behaviours significantly affected are highlighted in grey.

6.3.2. The D4 antagonist

(a) Food Intake and Feeding Microstructure.

L-745,870 (1-10 mg/kg i.p.) had no effect on total food intake during the 30-min observation period [$F(3,21) = 2.59$, NS; Fig. 6.2a]. It also had no effect on the latency to initiate feeding or the total time spent feeding (Table 6.2; Fig. 6.2b and 6.2d). There was a significant reduction in the local rate of eating [$F(3,21) = 4.57$, $P<0.05$; Fig. 6.2c], with a reduction by 30 % at 10 mg/kg. In addition, the frequency of feeding bouts was significantly reduced [$F(3,21) = 12.02$, $P<0.001$; Fig. 6.2f]. Surprisingly, mean bout duration showed an opposite trend [$F(3,21) = 4.49$, $P<0.05$; Fig. 6.2e]; at 10 mg/kg, L-745,870 significantly increased the mean bout duration. Overall, the reductions in eating rate and bout frequency were cancelled out by an increase in mean bout duration, resulting in a negligible net effect on food intake.

(b) Locomotor activity.

At doses of 3 and 10 mg/kg, L-745,870 significantly reduced the duration of locomotor activity, as a result of a reduction in the frequency of individual bouts (Table 6.2), with duration of individual bouts of locomotion showing little change. Mean bout frequency decreased by 37 % at 3 mg/kg and 63% at the highest dose of 10 mg/kg. At corresponding doses, total duration of locomotor activity decreased by 47 % and 70 % respectively.

(c) Rearing.

L-745,870 had no significant effect on the total duration of rearing or mean bout duration, although there was a significant reduction in the frequency of rearing (Table 6.2). This occurred at the highest dose of 10 mg/kg, and corresponded to a reduction of approximately 58 %.

(d) Grooming.

There was a significant decrease in total duration of grooming, although the frequency of grooming bouts was very similar between doses; there was a significant decrease in mean bout duration with the highest dose having a prominent effect (Table 6.2).

(e) Immobility.

L-745,870 (1-10 mg/kg) had no significant effect on any parameters of immobility during the observation period (Table 6.2).

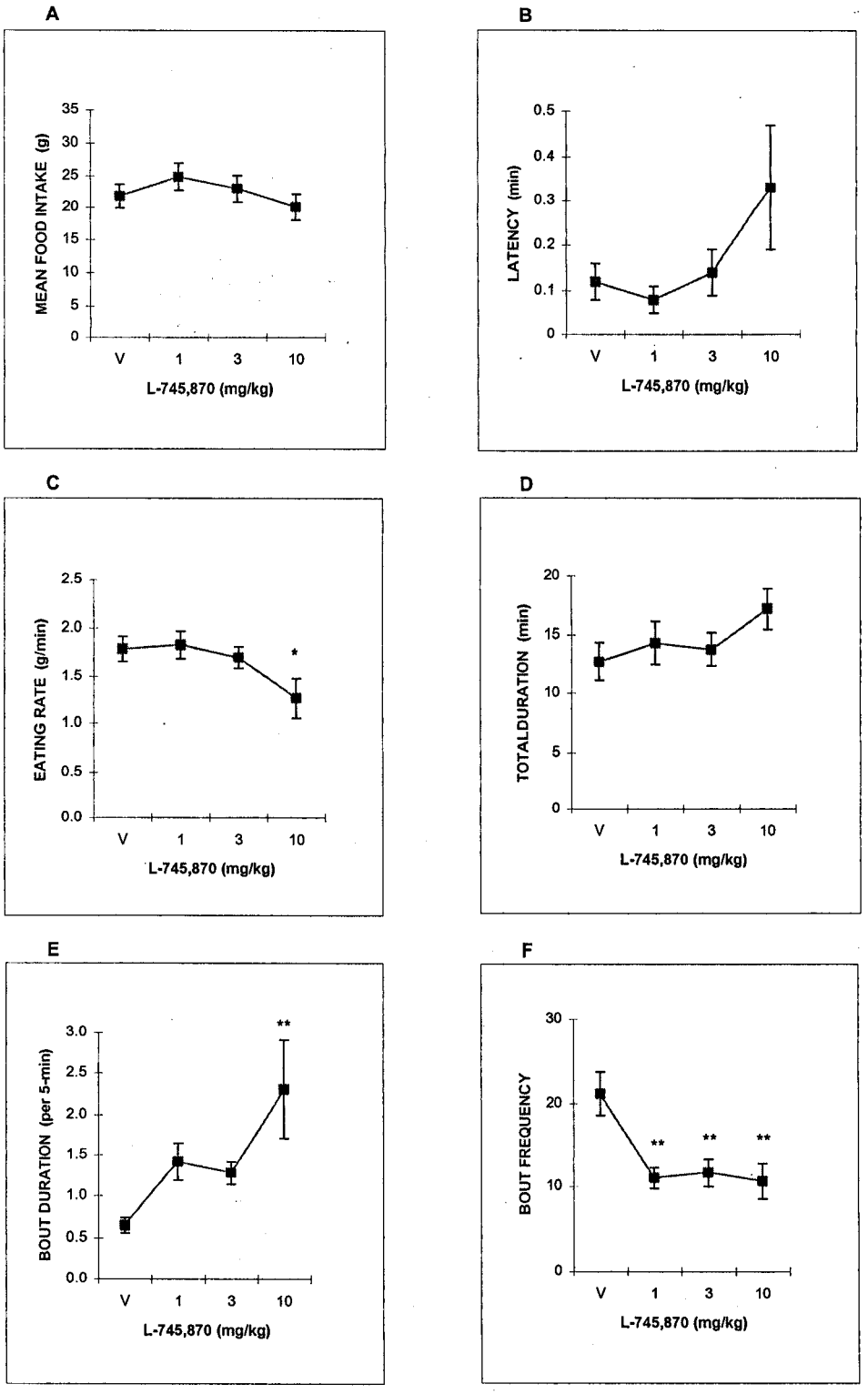

Figure 6.2. Effects of the dopamine D4 receptor antagonist L-745,870 on feeding parameters : **A**: Total food intake **B**: Latency to start eating **C**: Eating rate **D**: Total duration **E**: Mean bout duration **F**: Bout frequency. N= 8 at all points; * $P<0.05$, ** $P<0.01$, compared to vehicle control, V (comparisons by Dunnett's t-tests).

BEHAVIOURAL RESPONSE/CATEGORIES	L-745,870 (mg/kg) 0	1	3	10	F-VALUE	P-VALUE
LOCOMOTOR ACTIVITY						
TOTAL DURATION (min)	3.46 +/- 0.38	2.79 +/- 0.42	1.85 +/- 0.32 **	1.03 +/- 0.16 **	11.529	P<0.0001
BOUT DURATION (per 5-min)	0.09 +/- 0.02	0.10 +/- 0.01	0.07 +/- 0.01	0.08 +/- 0.01	1.61	NS
BOUT FREQUENCY	42.25 +/- 5.48	30.38 +/- 5.34	26.63 +/- 4.48 *	15.78 +/- 3.43 **	8.109	P<0.001
REARING						
TOTAL DURATION (min)	4.29 +/- 0.97	3.46 +/- 0.98	3.64 +/- 0.95	1.77 +/- 0.47	2.814	NS
BOUT DURATION (per 5-min)	0.15 +/- 0.01	0.14 +/- 0.02	0.15 +/- 0.03	0.14 +/- 0.03	0.011	NS
BOUT FREQUENCY	29.0 +/- 4.68	23.50 +/- 6.41	20.63 +/- 4.78	12.13 +/- 3.39 **	4.627	P<0.05
GROOMING						
TOTAL DURATION (min)	1.15 +/- 0.29	0.67 +/- 0.14	0.52 +/- 0.12 *	0.56 +/- 0.18 *	3.728	P<0.05
BOUT DURATION (per 5-min)	0.26 +/- 0.04	0.22 +/- 0.05	0.18 +/- 0.02	0.13 +/- 0.03 *	3.308	P<0.05
BOUT FREQUENCY	4.63 +/- 0.99	4.75 +/- 1.60	3.13 +/- 0.77	3.63 +/- 0.92	0.912	NS
RESTING/IMMOBILITY						
TOTAL DURATION (min)	7.12 +/- 1.64	7.31 +/- 1.49	9.71 +/- 1.64	8.66 +/- 1.32	1.233	NS
BOUT DURATION (per 5-min)	1.15 +/- 0.35	1.24 +/- 0.23	2.45 +/- 0.98	1.22 +/- 0.36	1.449	NS
BOUT FREQUENCY	8.13 +/- 1.95	7.75 +/- 1.92	8.38 +/- 2.28	9.75 +/- 1.71	0.46	NS

Results are shown in terms of Mean +/- S.E.M. (N=8). F-ratios and levels of significance are shown for one-way ANOVA.

Levels of significance for individual dose comparisons against control conditions : * P <0.05; ** P <0.01 (Dunnett's t-test).

Table 6.2. Microstructural analysis of non-feeding behaviour following administration of the D4 antagonist L-745,870. Behaviours significantly affected are highlighted in grey.

6.4. Discussion

Microstructural analysis helps us to identify the behavioural changes which underlie the effects of drugs on feeding behaviour. The present results provide a detailed behavioural analysis of the effects of selective DA D3 and D4 receptor antagonists in food-deprived rats, and can be compared with results for compounds that are less selective within the DA D1-like and D2-like receptor sub-families (Table 6.3).

The results indicate that a significant anorectic effect was detected at 10 mg/kg U-99194A. The reduction in feeding could not be attributed to a reliable increase in the latency to initiate feeding, or to effects on bout duration or frequency, but seems primarily due to a significant reduction in the rate of feeding. With regards to the DA D4 antagonist, there was no evidence that L-745,870 (1-10 mg/kg) significantly affected total food intake. The overall duration of feeding and the latency to initiate feeding were unaffected by the D4 antagonist. Nevertheless, at 1, 3 and 10 mg/kg, L-745,870 significantly reduced feeding bout frequency, with the highest dose also decreasing eating rate. Paradoxically, mean bout duration was shown to increase, therefore tending to cancel out the effect of frequency and resulting in a negligible net effect on total food intake. This finding goes against our previous results where we showed that the 10 mg/kg dose of L-745,870 produced a significant decrease in feeding ($P<0.01$) that continued over the full 2 hr test period. Nevertheless, the present results do suggest a trend towards a decrease, so the highest dose may be 'borderline' in terms of reliably reducing food intake.

Comparisons with the effects of less-selective D2-like receptor antagonists suggest that D3 or D4 receptor blockade is not an important contributor to the effects of these drugs (Table 6.3). Thus traditional D2-like antagonists have only one feature in common with the present results for the D3 antagonist: a decrease in the local rate of eating (Clifton et al., 1991). Terry (1996), looking at palatable food consumption by non-deprived rats, demonstrated a biphasic dose-effect of raclopride (D2/D3 antagonist) on feeding, with increased intake at low doses; the only parameters significantly affected

	"D1-Like" Receptor Subfamily		*"D2-Like" Receptor Subfamily*		*Present results*	
	Agonist	Antagonist	Agonist	Antagonist	D3	D4
Behavioural Responses:					(antagonists)	
Food Intake	↓	0	↓	↑(LD) ↓(HD)	↓	0
Latency	0	0	0	↑(HD)	0	0
Eating Rate	↓	0	↓	↓	↓	↓
Total Duration	↓	0	0	0	0	0
Mean Bout Duration	↑	↑	0	↑(LD)	0	↑
Bout Frequency	↓	↓	0	↑(LD) ↓(HD)	0	↓

KEY: ↑ = Increase ↓ = Decrease 0 = No Effect HD = High Dose LD = Low Dose

Table 6.3. Summary of effects of dopaminergic drugs on ingestive behaviour (from Terry, 1996; and present results)

were feeding duration and the latency to start eating. The low-dose hyperphagia reflected modest increases in the number of feeding bouts and mean bout duration, whereas the high dose hypophagia was a product of increased latency to eat and reduced number of feeding bouts. This behavioural profile is very different from that of U-99194A, which suggests that even though raclopride binds to the D3 receptor subtype, its effect on feeding cannot be attributed exclusively to action at the D3 receptor subtype within this subfamily. Analysing U-99194A's effects on feeding microstructurally therefore suggests that the drug affects feeding via different processes in comparison with less selective D2-like antagonists, even though all such drugs have a similar feeding profile.

In fact, U-99194A produces effects most similar to those of the D2-like agonists (Table 6.3; also Terry, 1996). In a very recent study that makes an ethologically-based comparison of the behavioural effects of GR 103691, nafadotride and U-99194A (all putative selective DA D3 receptor antagonists) it was shown that U-99194A caused a dose-dependent stimulation of episodes of non-stereotyped sniffing, locomotion, chewing and eating of faecal pellets, with some stimulation of rearing and reduced levels of grooming (Clifford and Waddington, 1998). The topography of response to U-99194A overlapped somewhat with the profiles of D2-like agonists (as is the case for the feeding profiles reported in the present study) and D1-like agonists. In addition, the behavioural effects of U-99194A were antagonised by pre-treatment with the prototypical D2-like antagonist, haloperidol. On this basis, the ethogram of U-99194A is more similar to, though not identical with, that of the D2-like agonists. As yet, however, there is little direct evidence to suggest either agonist or partial agonist activity for U-99194A at any of the D2-like receptors, although the possibility cannot be excluded.

The selective D4 antagonist, L-745,870, on the other hand, produced effects most similar to those of D1-like antagonists. However, certain features of the behavioural effects did display some similarities with ligands for other DA receptor subtypes. Whereas a decrease in the rate of feeding mirrored D2-like agonist effects (Rusk and Cooper, 1989) the closest overall similarity of effects was with a DA D1-like antagonist. Terry and colleagues demonstrated that SCH 39166 (a selective D1-like antagonist) caused a decrease in the number of meals consumed, which was compensated by a dose-

dependent increase in mean duration of eating bouts (Terry, 1996). There has been considerable interest in the possibility that D1-like and D2-like receptors interact to modulate each other's effects. The similarity of behavioural effects induced by these selective DA receptor subtype antagonists suggests a possible D1-D4 interaction in the control of food intake. This may be supported by the fact that D4 receptors are expressed in midbrain nuclei, amygdala and frontal cortex (Hadley, 1996), while there is still debate as to whether they are expressed in nucleus accumbens and basal ganglia (Defagot and Antonelli, 1997); thus the two receptors may show co-localisation. Further experiments to see whether the selective D4 antagonist, L-745,870, can attenuate the hypophagic effects of D1-like agonists may provide evidence for such an interaction.

Alternatively, a compensatory mechanism may be initiated by the drug, such that a decrease in the number of meals is compensated by increases in mean duration as a result of a non-specific response to disruption of feeding. However, as yet, there are no reports of other classes of anorectic compound showing a similar profile.

U-99194A had no effect on any parameter of locomotor activity at doses up to 10 mg/kg. Once again, the results conflict with previous work (see Chapter 5), in which U-99194A was shown to increase locomotor activity without any concomitant effects on DA release. This has led to the claim that the D3 receptor subtype is functionally relevant at the post-synaptic level, and that its blockade leads to activation of locomotor activity (Waters et al., 1993, 1994). The results also contrast with the previous experiment, in which a decrease in photocell beam interruptions was recorded at 10 mg/kg. Again this could be due to methodological differences, since the previous study measured photocell beam disruptions; the present experiment characterised the behaviour by continuous observation in terms of total duration, mean bout duration and bout frequencies. However, further analysis of data from the previous study, counting individual beams breaks rather than consecutive beam disruptions, showed that a decrease did not occur for this measure (72 beam crossings for vehicle control, versus 69, 76 and 64 for the 1, 3 and 10mg/kg doses, respectively). This is consistent with the lack of effect across doses obtained here, and suggests that at the highest dose the animals were moving predominantly locally, at one end of the experimental cage. Clearly there are

discrepancies for this compound as to whether or not (and how) it affects locomotor activity; as mentioned earlier (section 5.4), the effect may depend not only on methods of analysis but also the species used and the test procedure.

Other anomalous results were obtained for rearing and grooming behaviour. U-99194A clearly caused a dose-dependent decrease in total rearing duration and bout frequency; however, Accili et al. (1996) reported an increase in locomotor activity and rearing in an exploratory test using D3 'knock-out' mice. This suggests that blockade of the D3 receptor subtype by antagonist compounds such as U-99194A produces a different behavioural profile from that which occurs when the receptor is 'knocked out' genetically. A direct comparison between the studies is difficult to make. It could be that the null-mutant organism might not only lack the product of a single gene for the D3 receptor subtype, but might also express a number of physiological and behavioural processes that have been altered during development to compensate for the effect of the null mutation. Therefore, one might expect an array of complex phenotypical changes not directly related to the function of the gene of interest. However, studies investigating the effects of D3 antagonists in D3 'knockout' animals would be useful to confirm whether these compounds are genuinely specific for these receptors.

L-745,870 caused a substantial reduction in the overall time devoted to locomotor activity and in locomotor bout frequency. This cannot be attributed to more time spent feeding or resting, as the animals spent similar time intervals for each behavioural parameter regardless of dose used, and there were no significant differences between them (Table 6.2). The results may therefore suggest a specific behavioural effect: reducing locomotor activity via DA D4 receptor blockade, since it occurred at the low dose of 3 mg/kg. However, this is still a high dose in terms of selectivity for the D4 receptor subtype, and the involvement of sigma sites and D2 receptors cannot be ruled out (see Table 4.1). The D4 antagonist decreased grooming bout duration and total time spent grooming; this occurred at the highest dose and may therefore have involved blockade of D2 receptors. This is in agreement with work by Terry (1996), in which the DA D2/D3 antagonist raclopride reduced all parameters of grooming behaviour. Not only is this

evident with D2-like antagonists, it also occurs with D2-like agonists such as N-0437 (Rusk and Cooper, 1989).

This is the only study so far that has looked (microstructurally) at the behavioural effects of a D4 antagonist. However, one study using a different selective DA D4 antagonist, NRA0045, reported that it decreased spontaneous locomotor activity at doses up to 10 mg/kg i.p. (but not below 50% of control levels). In addition, locomotor hyperactivity induced by methamphetamine was dose-dependently antagonised by NRA0045 (ED_{50} = 0.4 mg/kg), whereas methamphetamine-induced stereotyped behaviour (at a higher dose) was not affected by NRA0045 (Okuyama et al., 1997a,b). As this drug also blocks 5-HT2a receptors, and the authors fail to specify the drug's selectivity for D4 receptors at this dose, it is difficult to conclude that the effects are mediated solely by binding to the D4 receptor subtype. The present results suggest that this is unlikely.

Since the identification of the DA D3 and D4 receptor subtypes, some progress has been made in understanding the functional roles of these novel receptors (e.g. for a review concerning D3 receptors: Shafer and Levant, 1998). In summary, the present study examined the effects of the selective D3 antagonist U-99194A and the selective D4 antagonist L-745,870 on feeding and other behaviours. At doses that occupy post-synaptic D3 receptors, feeding is marginally affected by U-99194A; effects on intake and rate of eating only occurred at the highest dose. The drug produced negligible effects on locomotor activity and grooming, but rearing was significantly reduced at the highest dose. Comparisons with the effects of less selective D2-like receptor antagonists suggest that D3 receptor blockade is not an important contributor to the effects of D2-like antagonists when analysed microstructurally. D4 receptor blockade produced a different profile of effects. There was no reduction in food intake at D4 receptor-selective doses; however, microstructural analysis showed that a significant decrease in the number of meals consumed was compensated by an increase in mean duration of feeding bouts. The local rate of eating declined only at the highest dose. Unlike the D3 antagonist, L-745,870 also caused a substantial reduction in locomotor activity at doses that did not affect rearing. Comparisons with the effects of less-selective D2-like receptor antagonists

suggest that D4 receptor blockade is not an important contributor to those effects. In fact, L-745,870 produced effects more similar to those of D1-like antagonists.

The results reveal clear dissociations between the effects of D3 and D4 receptor blockade on specific behaviours, but suggest only subtle effects on feeding. Neither D3 nor D4 blockade alone mimics the effects of previously-tested D2-like receptor antagonists.

CHAPTER 7:

'CHARACTERISATION OF THE EFFECTS OF COCAINE ON FEEDING AND ASSOCIATED BEHAVIOURS IN THE RAT'

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CHARACTERISATION OF THE EFFECTS OF COCAINE ON FEEDING AND ASSOCIATED BEHAVIOURS IN THE RAT

7.1 Introduction And Aims

A decrease in food intake following acute administration of cocaine has been demonstrated using a variety of feeding paradigms (Heffner et al., 1977; Woolverton et al., 1978; Balapole et al., 1979; Bedford et al., 1980; Blavet and De Feudis, 1982). Specifically, Balapole et al. (1979) showed that the effect of cocaine (10-25 mg/kg i.p.) on food intake was relatively transient, occurring only in the first hour post-injection. Others have shown dose-related (3.45-27.6 mg/kg i.p.) reductions in food consumption produced by cocaine in food-deprived rats (Heffner et al., 1977; Bedford et al., 1980), and Foltin et al. (1983) found that cocaine (4-32 mg/kg i.p.) could also produce dose-dependent reductions in the consumption of sweetened milk by non-deprived rats.

Cooper and Van der Hoek (1993) were the first to investigate the microstructutral changes in feeding that occur following cocaine administration (5.6-30 mg/kg). They found that cocaine increases the latency to initiate feeding, but did not affect the local rate of ingestion when feeding occurred. The authors concluded that cocaine may act during the appetitive phase that precedes consumption to reduce food incentive, but that food ingestion that occurs during the consummatory phase is not necessarily affected. They also reported increases in the frequency of locomotion.

Rapoza and Woolverton (1991) reported that cocaine 16 mg/kg suppressed milk intake to a greater extent than water intake, suggesting that cocaine has a more specific inhibitory effect on food intake. Wolgin and Hertz (1995) addressed this issue by investigating the effects of acute and chronic cocaine on milk intake in bottle-fed (dependent on appetitive behaviour) and cannula-fed (consummatory behaviour) rats; they showed decreased intakes in cannula-fed rats at the higher dose, suggesting that cocaine can also affect consummatory responses.

Although a microstructural approach to studying the effects of cocaine has been presented, to date no reports have provided behavioural data relevant to identify the

receptor subtypes by which cocaine affects feeding and associated behaviours in the rat; this is an issue that warrants further research. In addition to its anorectic effect, one of the defining behavioural characteristics of cocaine as a psychomotor stimulant is its ability to elicit increases in motor activity. These motor-increasing effects in mammals are similar to those produced by other psychostimulants, particularly amphetamine. At low doses, these drugs produce an alerting response, consisting of increases in exploration, locomotion, grooming and rearing (Scheel-Kruger et al., 1977; Snoddy and Tessel, 1985). As the dose is increased, locomotor activity decreases and the behavioural patterns become stereotyped, often involving continuous repetition of one or a few items of behaviour. In rats, the repetitive behaviours induced by amphetamine and cocaine include head bobbing, gnawing, sniffing and licking (Scheel-Kruger et al., 1977). The neurobiology of the motor-activating effects of cocaine has been the subject of numerous investigations for over two decades. The majority of evidence indicates that the neurochemical effects of cocaine underlying its ability to increase motor activity involve dopaminergic systems (Costall and Naylor, 1979; Scheel-Kruger et al., 1977; Beninger, 1983). There is now substantial evidence linking cocaine-induced locomotor activation to blockade of DA reuptake (Scheel-Kruger, 1971; Scheel-Kruger et al., 1977; Snoddy and Tessel, 1985). The most recent and compelling evidence for this hypothesis comes from studies of DA transporter 'knockout' mice. In contrast to wild-type mice, the mutant mice showed no change in locomotor activity when administered a high dose of cocaine (Giros et al., 1996). This is presumably because, in the absence of the DA transporter, cocaine had no effect on extracellular DA concentration. The locomotor stimulating effects of cocaine have been ascribed largely to enhancement of dopaminergic transmission in the nucleus accumbens. This was first suggested by Kelly and Iversen (1976), who demonstrated reductions in cocaine-induced locomotor behaviour following 6-OHDA lesions of the mesolimbic DA pathway. Further supporting this hypothesis are more recent studies showing that locomotor activity can be elicited by direct microinjection of cocaine into the nucleus accumbens (Delfs, Schreiber and Kelley, 1990), and that the locomotor effects of systemically-administered cocaine can be significantly attenuated by

injection of sulpiride (the D2-like antagonist) into the accumbens (Neisewnader, O'Dell and Redmond, 1995).

However, as for the effects of cocaine on feeding, no studies have been carried out looking at the involvement of novel D2-like receptor subtypes on such behaviours. Since cocaine has been reported to affect feeding behaviour in a variety of feeding paradigms and to elicit increases in motor activity, the initial experiments of this chapter were carried out to investigate whether these results could be replicated in our laboratory using a paradigm which has yielded consistent effects in previous studies. An experiment was conducted to characterise the dose(s) of cocaine which reliably produce anorexigenic effects in the rat. Locomotor activity was measured at the same time as feeding to see if effects on feeding occurred at doses which also elicit increases in activity. An additional experiment was carried out looking at the effects of cocaine on locomotor activity without the presence of food. This was to examine whether concomitant feeding alters cocaine's dose-response profile for locomotion. Finally, a microstructural study of cocaine's effects on feeding and a range of typical behaviours was conducted. The data from this final experiment will be important for the later studies into the receptor mechanisms by which cocaine affects feeding and associated responses; specifically, it will be used to justify dose selection for experiments to test the involvement of DA D2-like receptors in the cocaine-induced behaviours.

7.2 Materials and methods

7.2.1 Subjects:

Twenty-four male Sprague-Dawley rats (eight per study; Charles River, Margate, Kent) weighing 320-360g were housed in pairs with free access to water under a 12 hr light/dark cycle, lights on 07:00. They were all maintained on a restricted diet of 15 g of standard food pellets, adjusted on test days so that, regardless of the effect of drug, all rats received the same food allowance per day. All testing was between 12:00 and 14:00 hours.

7.2.2 Apparatus and procedure:

The apparatus and procedure for experiment 1 were as described in sections 5.2.2 and 5.2.3. The apparatus and procedure for microstructural analysis in experiment 2 was as described in section 6.2.3.

7.2.3 Drugs:

(-)-Cocaine HCl (Sigma Chemicals, Poole Dorset) was dissolved in 0.9% saline solution and administered intraperitoneally at doses of 3, 10 and 30 mg/kg. Cocaine was injected at a volume of 1 ml/kg, 10-min prior to the rat being placed inside the experimental cages.

7.2.4 Data analysis:

The data were analysed by two-way analysis of variance (ANOVA) for repeated measures where the drug doses were administered to the rats in a 'Latin Square' design: the two factors were drug and time in the first experiment. The microstructural analysis data for the second experiment were analysed using one-way ANOVA. Subsequent comparisons were by Dunnett's post-hoc t-tests for simple main effects and main effects of dose. All comparisons between the results for individual drug treatments and the saline control condition for both experiments were also made using Dunnett's post-hoc t-tests. The Greenhouse-Geisser correction for violations of compound symmetry and sphericity (Kirk, 1982) did not alter the final significant F-values, so for clarity the original F-values and degrees of freedom are given.

7.3 Results

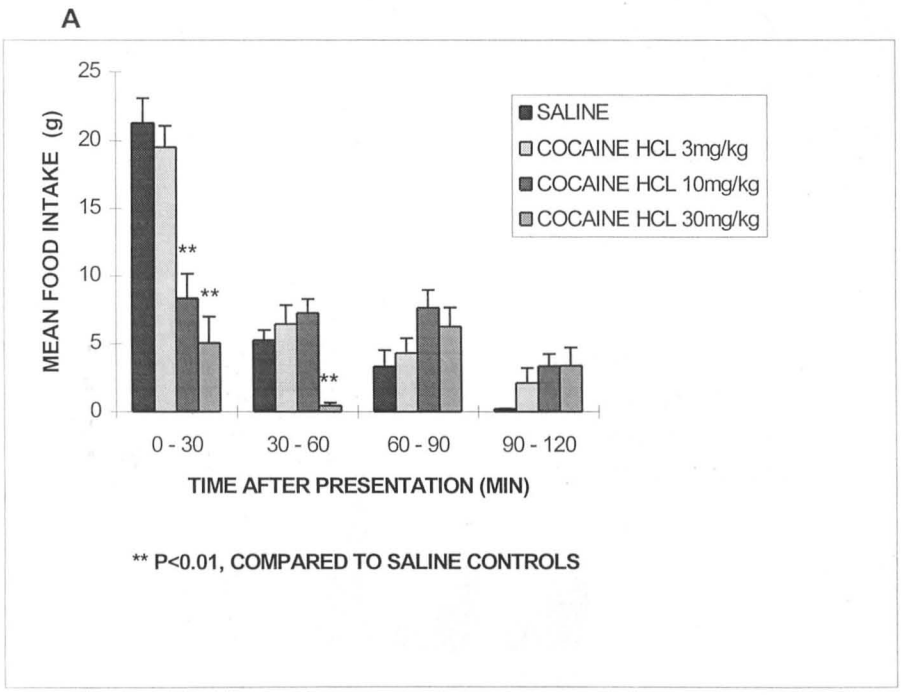
7.3.1 EXPERIMENT 1: Effect of cocaine on food intake and locomotor activity.

The effects of cocaine on food intake and locomotor activity are shown in Figs. 7.1 and 7.2. Administration of cocaine resulted in a dose-related decrease in food intake [$F(3,21) = 15.13$, $P<0.01$]. There was a significant time effect [$F(3,21) = 40.49$, $P<0.01$] and a dose×time interaction [$F(9,63) = 12.73$, $P<0.01$]. *Post-hoc* tests revealed that the 10 mg/kg and 30 mg/kg doses of cocaine produced significant reductions in feeding during

the first 30 min after administration ($P<0.01$), and the 30 mg/kg dose continued to have an effect throughout the first 60 min of the test session (Fig. 7.1A). Administration of cocaine also resulted in a dose-related increase in locomotor activity [$F(3,21) = 9.84$, $P<0.01$) with a significant time effect [$F(3,21) = 60.15$, $P<0.01$] and a dose×time interaction [$F(9,63) = 5.19$, $P<0.01$].

To investigate the extent to which behavioural competition from feeding may have influenced the locomotor effects of cocaine, an additional experiment was carried out where locomotor activity was measured without the presence of food (Fig. 7.2). The effect of dose was greater when locomotor activity was measured on its own. When locomotor activity was measured without feeding, 30 mg/kg produced more consecutive beam interruptions (~287) compared to when feeding and locomotor activity were measured simultaneously (~158) in the first 30 min of the test session. The dose effect was significant [$F(3,21) = 13.44$, $P<0.01$], as were the time effect and the dose×time interaction [$F(3,21) = 17.22$, $P<0.01$ and $F(9,63) = 3.35$, $P<0.01$, respectively]. *Post-hoc* tests revealed that only the 30 mg/kg dose produced significant increases in locomotor activity ($P<0.01$) in both experiments (with and without the presence of food) and this was the case for the first 90 and 45 min of the respective experiments (Fig. 7.1B and Fig. 7.2, respectively). Planned comparisons revealed that total food intake over the full test period was only significantly suppressed at the highest dose of cocaine ($P<0.01$). Locomotor activity was also increased significantly only at the highest dose in both experiments.

In summary, cocaine produced two distinct effects: hypophagia and hyperlocomotion. Locomotor activity was most sensitive to the highest dose of cocaine, with activity being increased throughout the 90 min test session. Feeding was affected in the first 30 min of the session at both 10 and 30 mg/kg. By comparing the two experiments, it can be concluded that cocaine causes hypophagia and hypermotility in rats, and concomitant feeding does not qualitatively alter cocaine's dose-response profile for locomotion.



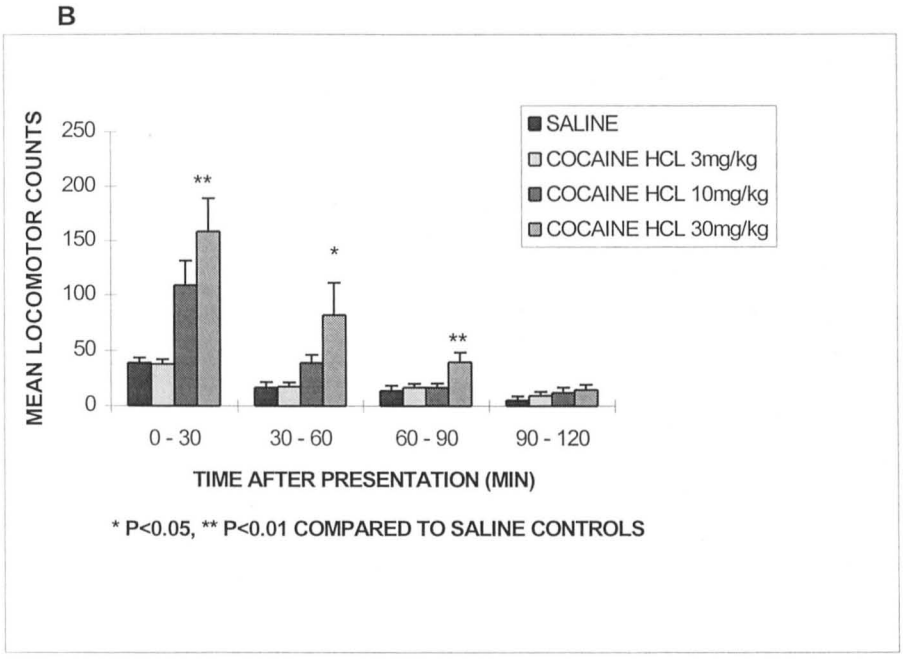


Figure 7.1. (A) The effect of cocaine on food intake in food-deprived rats (top panel). N=8 at all points. (B) The effect of cocaine HCl on locomotor activity in food deprived rats (bottom panel). N=8 at all points. Data were recorded from entry to the experimental cages (time 0 min) onwards.

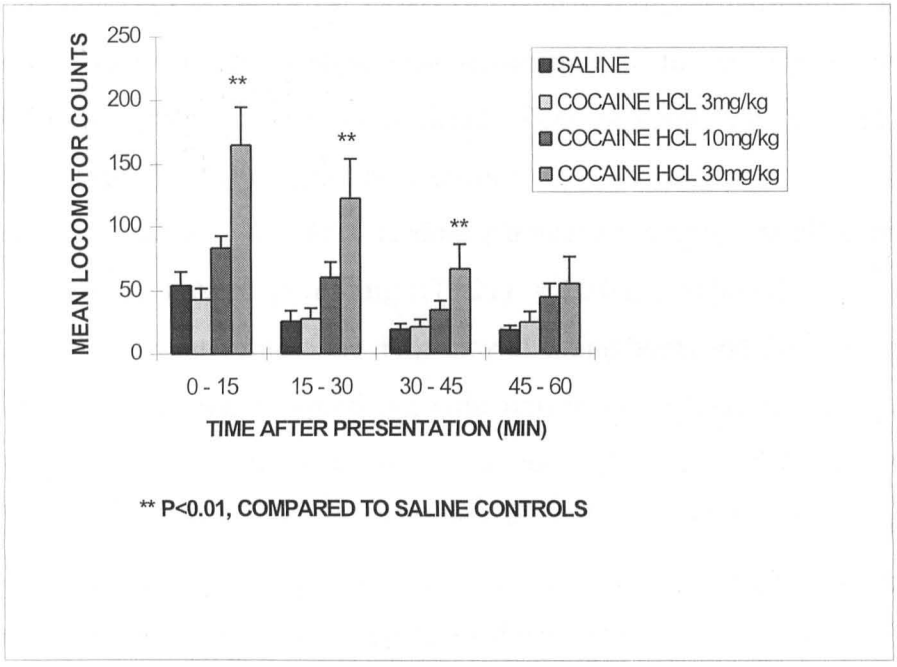


Figure 7.2. The effect of cocaine on locomotor activity without the presence of food in food-deprived rats. N=8 at all points. Data were recorded from entry to the experimental cages (time 0 min) onwards.

7.3.2 EXPERIMENT 2: A microstructural analysis of the effects of cocaine.

(a) Food Intake and Eating Microstructure.

During the adaptation period in which the animals were trained to eat the sweetened wet mash diet, the food-deprived rats consistently consumed over 20 g within the 30 min test period. Cocaine (3-30 mg/kg i.p.) produced a dose-dependent reduction in food consumption [$F(3,21) = 17.14$, $P<0.0001$] with significant reductions at all doses (Fig. 7.3a(i)). There was also a significant lengthening of the latency to start feeding [$F(3,21) = 21.06$, $P<0.0001$] and a significant effect on eating rate [$F(3,21) = 19.05$, $P<0.0001$]; see Figs. 7.3a(ii) and (iii), respectively.

Likewise, analysing the other feeding parameters, cocaine resulted overall in a reduction in the time devoted to feeding [$F(3,21) = 10.04$, $P<0.0005$]. Total duration of feeding can be divided further into the frequency of eating bouts and the mean duration of individual bouts. There was a significant reduction in mean bout duration [$F(3,21) = 3.16$, $P<0.05$], and in the frequency of eating bouts [$F(3,21) = 5.79$, $P<0.005$; Figs. 7.3a(iv), (v) and (vi)]. Hence cocaine's ability to prolong the latency to start feeding, together with the reduction in mean bout duration and the frequency of eating episodes resulted overall in a reduction in the time devoted to eating.

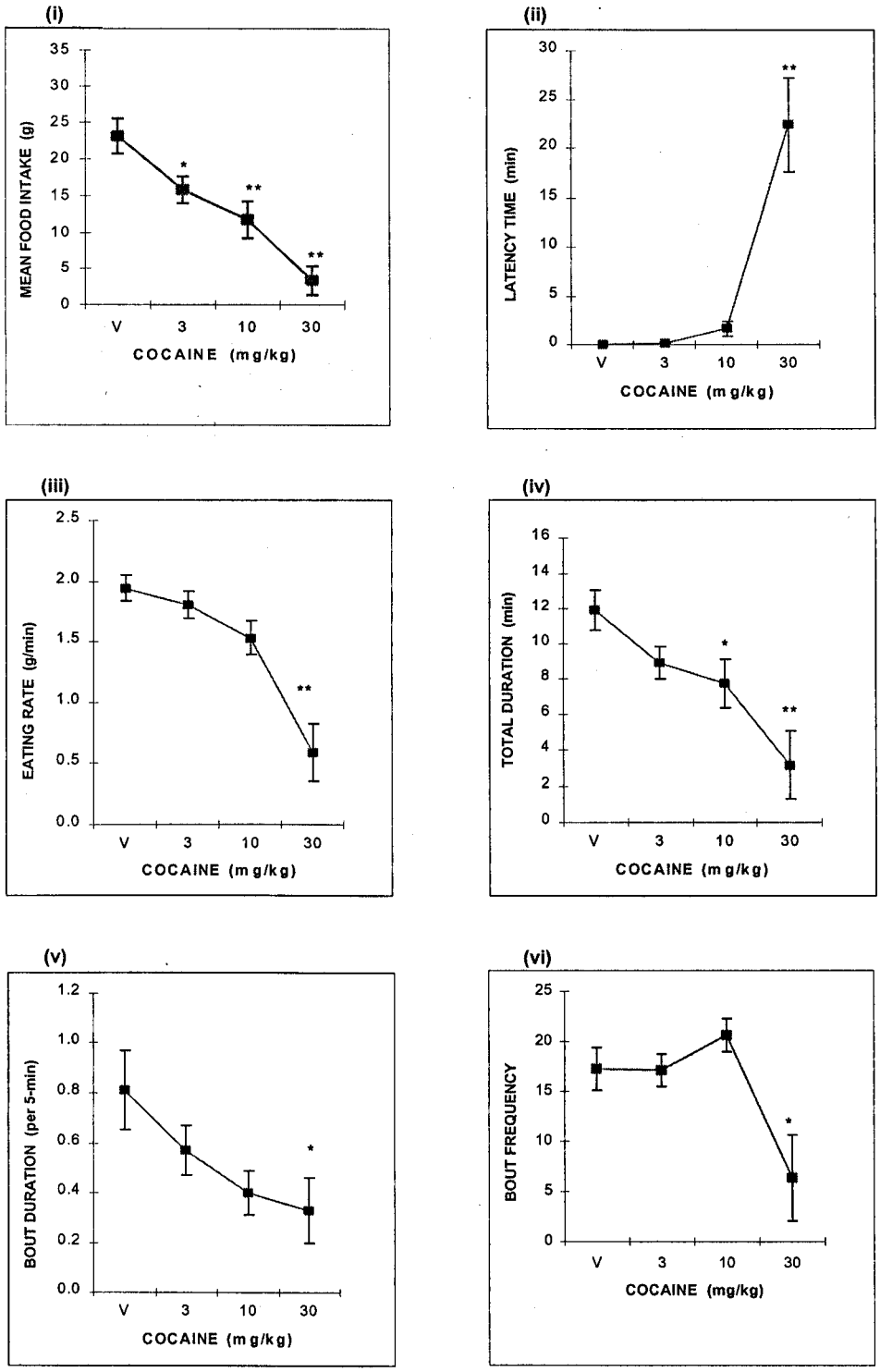


Figure 7.3(a) Effects of cocaine on feeding parameters: (i) total food intake (ii) latency to start eating (iii) eating rate (iv) total duration (v) mean bout duration (vi) bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to vehicle control, V (comparisons by Dunnett's t-tests)

(b) Locomotor Activity.

Cocaine significantly increased the total duration of locomotor activity [$F(3,21) = 14.92$, $P<0.0001$], with significant effects being observed at 10 and 30 mg/kg (Fig. 7.3b(i)). Cocaine also produced a significant increase in mean bout duration [$F(3,21) = 4.39$, $P<0.05$] for which the highest dose alone showed a significant effect by post-hoc tests (Fig. 7.3b(ii)). There was also a significant effect on the frequency of locomotor bouts [$F(3,21) = 12.94$, $P<0.0001$] with 10 mg/kg producing a significant effect in comparison with saline (Fig. 7.3b(iii)).

(c) Rearing.

There was no effect of cocaine on total duration or mean bout duration of rearing behaviour (Fig. 7.3c(i) and (ii)), but cocaine significantly altered rearing bout frequency [$F(3,21) = 8.06$, $P<0.001$]; this effect was primarily attributable to the 10 mg/kg dose (Fig. 7.3c(iii)) which was compensated in terms of total duration by the reduced mean bout duration.

(d) Grooming and resting/immobility.

There was a significant reduction in total duration of grooming [$F(3,21) = 6.98$, $P<0.005$]. This change was largely due to dose dependent reductions in the frequency of grooming episodes [$F(3,21) = 10.26$, $P<0.0005$] and in individual bout duration [$F(3,21) = 8.50$, $P<0.001$]. Significant effects were seen at the highest dose only (Fig. 7.3d(i),(ii),(iii)). The two highest doses of cocaine also resulted in a decrease in time spent immobile (Fig. 7.3e(i),(ii),(iii)) presumably due to the increases in time devoted to locomotor activity and rearing.

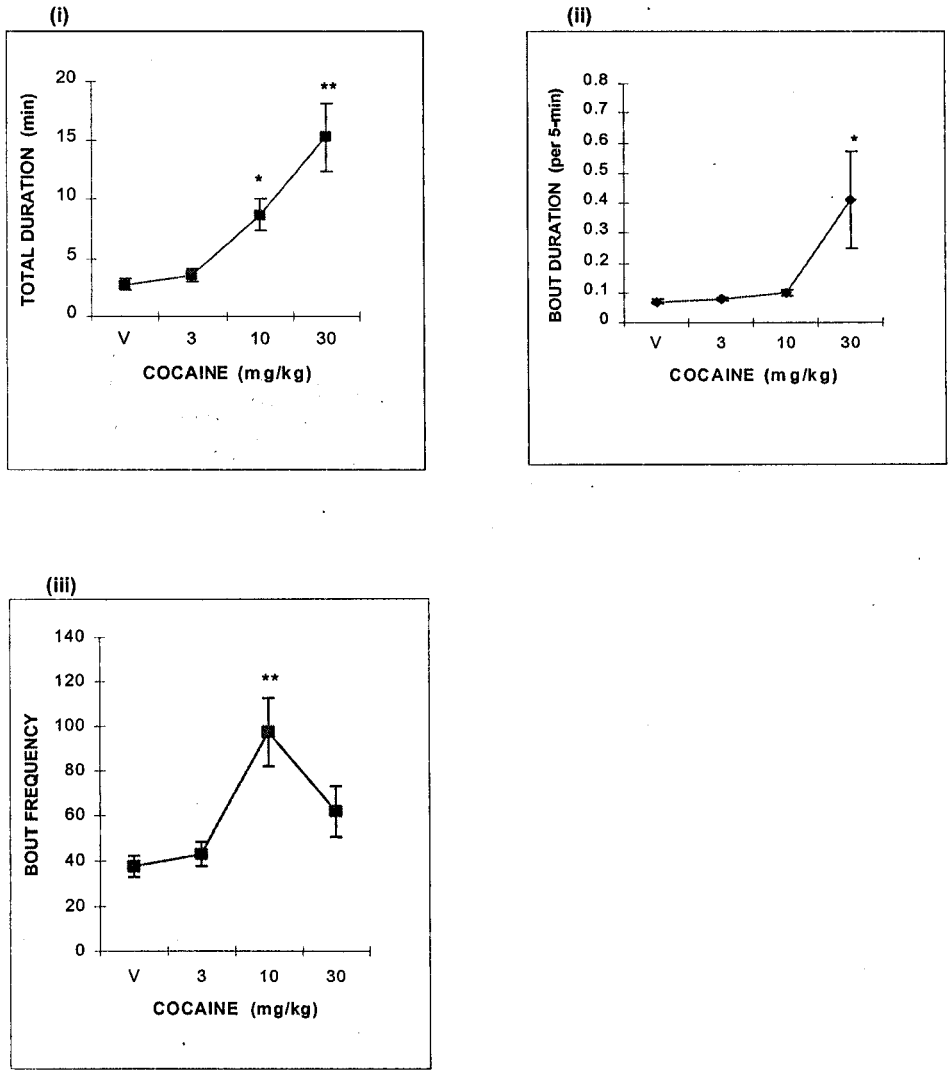


Figure 7.3(b) Effects of cocaine on locomotor activity parameters: (i) total duration (ii) mean bout duration (iii) bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to vehicle control, V (comparisons by Dunnett's t-tests)

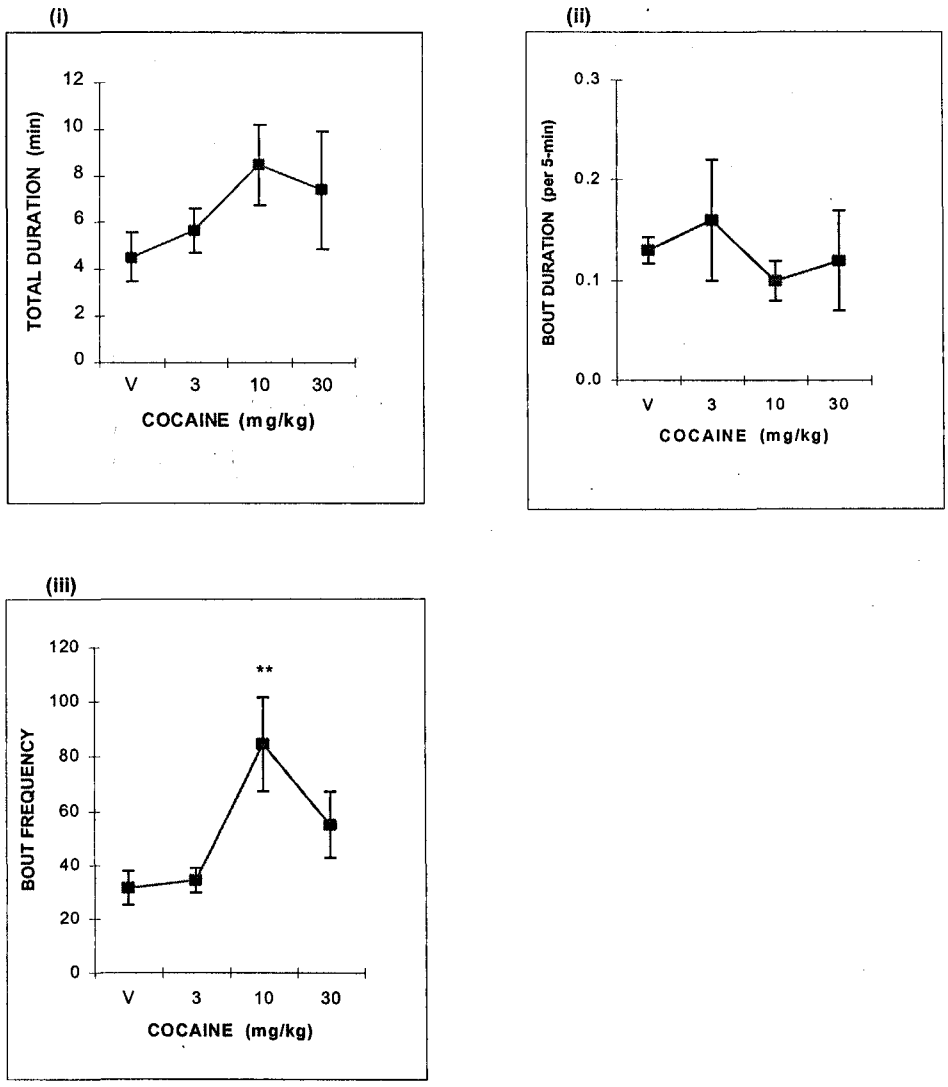


Figure 7.3(c) Effects of cocaine on rearing activity parameters: (i) total duration (ii) mean bout duration (iii) bout frequency. N=8 at all points; ** $P<0.01$ compared to vehicle control, V (comparisons by Dunnett's t-tests)

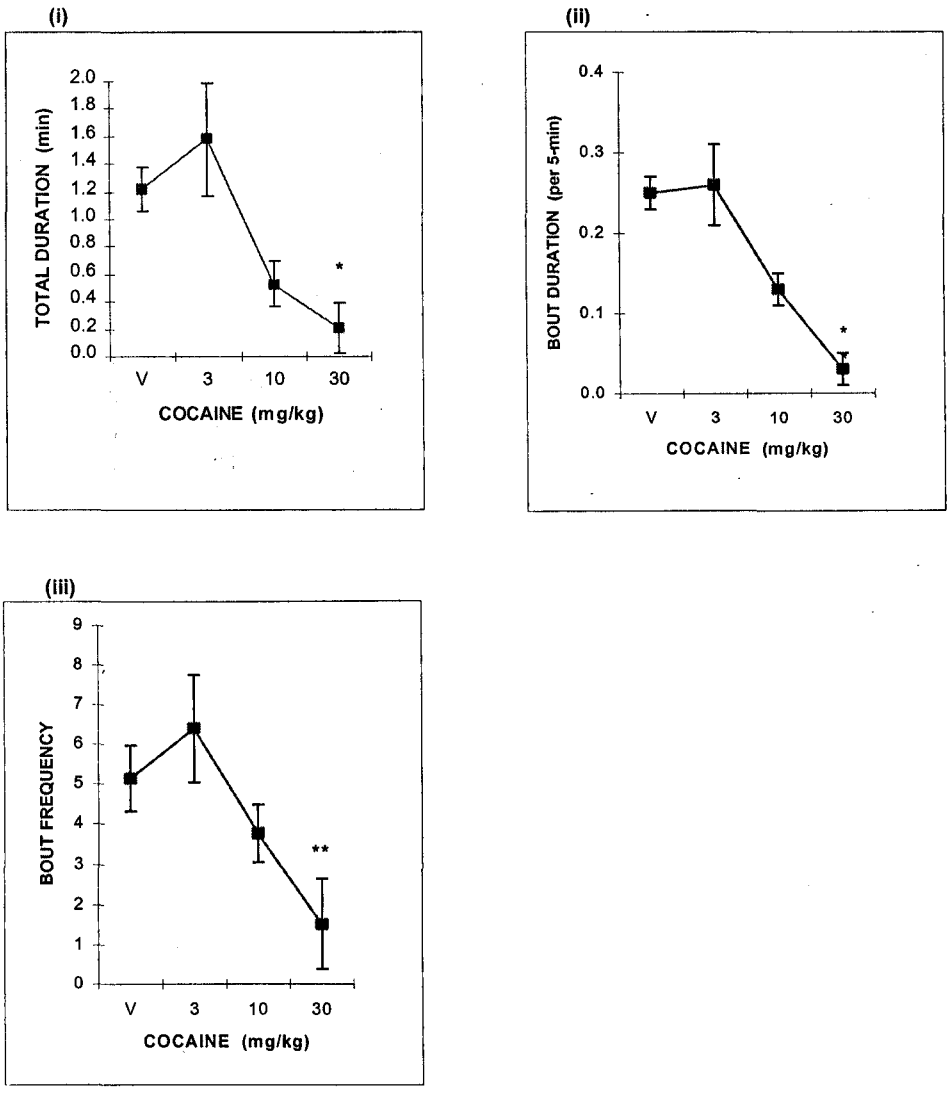


Figure 7.3(d) Effects of cocaine on grooming activity parameters: (i) total duration (ii) mean bout duration (iii) bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to vehicle control, V (comparisons by Dunnett's t-tests)

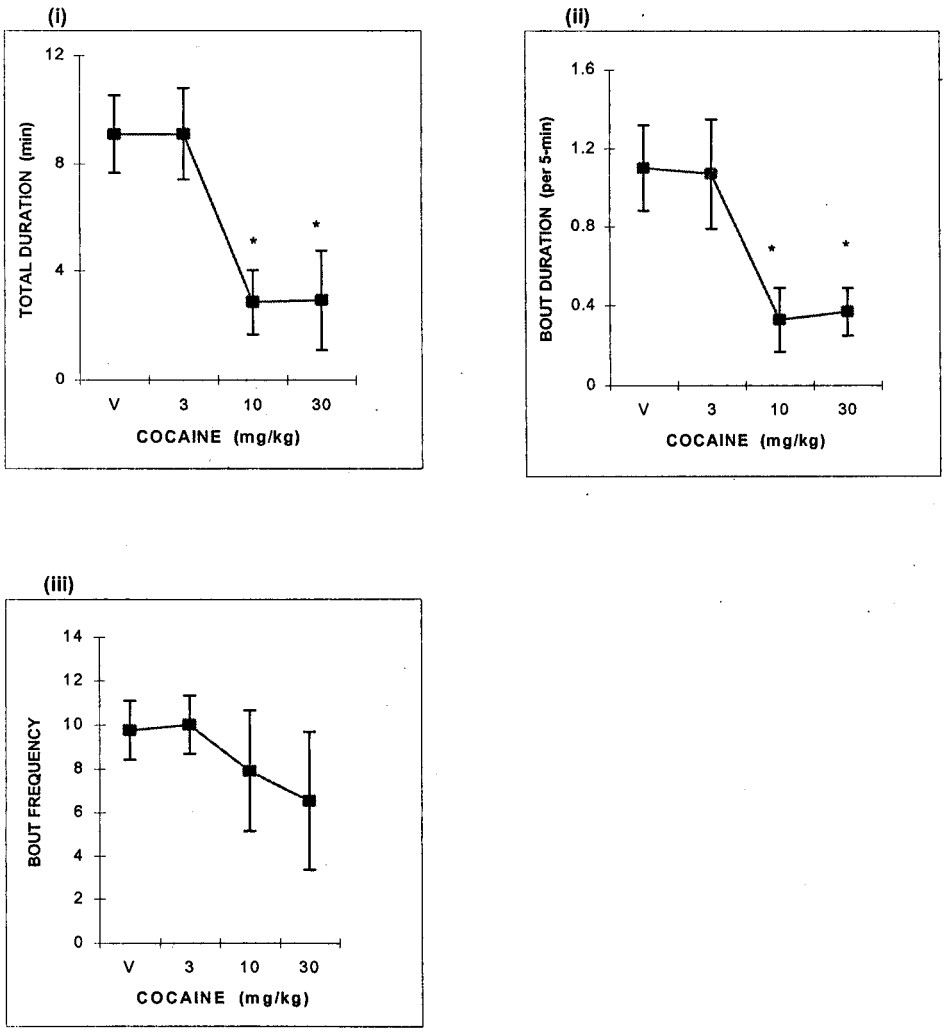


Figure 7.3(e) Effects of cocaine on immobility activity parameters: (i) total duration (ii) mean bout duration (iii) bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to vehicle control, V (comparisons by Dunnett's t-tests)

7.4 Discussion

Cocaine (3-30 mg/kg) produced a dose-dependent reduction in palatable food consumption in food-deprived rats. These results are consistent with earlier findings (See Section 7.1). The minimum dose to produce a significant reduction was 3 mg/kg. Locomotor activity was measured at the same time as feeding to see if the effects on feeding occurred at doses which could elicit increases in activity. The findings from this experiment also confirm previous work that cocaine causes an increase in activity. An additional experiment was also carried out looking at the effects of cocaine on locomotor activity without the presence of food reward. Comparing the two experimental procedures, locomotor activity counts were much higher in the latter (158 vs. 280 in the first 30-min). It has been suggested that food-deprivation can increase the reinforcing efficacy of drugs of abuse, including cocaine (Carroll et al., 1981). Bell et al. (1997) also showed that relative to food-satiated rats, food-deprived rats can exhibit greater conditioned preference for a cocaine-paired environment during tests of conditioned place preference and greater cocaine-induced locomotor activity. The food-deprivation variable may partly explain the increase in locomotor activity observed in experiment 2 where food was not available to the animal. However, the main reason for the difference must be attributed to the presence of a competing behaviour. Nevertheless, two distinct behaviours were observed in experiment one: cocaine causes hypophagia and hypermotility in rats and concomitant feeding does not alter cocaine's dose-response profile for locomotion. A microstructural study of cocaine was then conducted to identify the features of behaviour responsible for cocaine's effect on feeding and locomotor activity following cocaine administration.

The findings from this experiment showed that the reduction in food consumption at the highest dose tested was due to a contribution of factors: a prolonged latency to induce feeding together with a reduction in the rate of eating. Furthermore, a significant reduction in the time devoted to feeding was also observed. The significant reductions in the frequency of feeding bouts and mean bout duration were major contributors to the reduction in feeding duration at 30 mg/kg. Ultimately, the anorectic effect of cocaine at the highest dose depended on the latency and the rate of eating, this bringing about the

reduction in the number of eating bouts and mean bout duration. The reductions at 3 and 10 mg/kg, on the other hand, were caused by a dose-related change in the rate of eating and a reduction in the time devoted to feeding. The frequency of feeding bouts was not a contributor to the reduction in feeding duration at these doses, but rather due to a dose-dependent reduction in mean bout duration.

The locomotor stimulant effects of cocaine were also measured; locomotor activity duration was increased at 10 and 30 mg/kg. At 30 mg/kg cocaine, the mean bout duration was markedly increased leading to large increases in total duration of locomotion. At 10 mg/kg cocaine, the frequency of bouts of locomotion was responsible for an increase in total duration. In contrast, there were no significant effects of cocaine on total duration or mean bout duration of rearing behaviour, but cocaine significantly affected rearing bout frequency, an effect that was primarily attributable to the 10 mg/kg dose. Cocaine also reduced grooming throughout the 30-min test period, and completely suppressed it at 30 mg/kg. This was due to cocaine shortening both mean bout duration and bout frequency.

The results of the present study differ only slightly from those of Cooper and van der Hoek (1993). In their study, it was shown that cocaine suppressed feeding in a dose-dependent manner (significantly at 10 mg/kg and greater), and this was due in the main to a reduction in the frequency of feeding bouts. In contrast, the mean bout duration of eating bouts was unaffected, except at the highest dose, 30 mg/kg. In addition, the rate of eating was not significantly affected by cocaine at any dose. In effect, cocaine delayed the initiation of feeding, thus bringing about the reduction in the number of eating bouts. The effect of cocaine on locomotor activity and rearing was very much the same as in the present study. Another feature of their study was the marked suppression of grooming at the lowest dose of 5.6 mg/kg, whereas this was only observed at the highest dose in the present study. Comparing each other's data shows that results obtained by Cooper and van der Hoek (1993) are very similar to the present study, even though different procedures were used: Cooper and van der Hoek (1993) used non-deprived rats, whereas the present study used food-deprived rats of a different strain (i.e. Sprague-Dawley vs. Male Hooded rats).

Comparisons with other DA re-uptake inhibitors and selective DA D1-like and D2-like agonists reveal similarities and differences between them. Microstructural analyses of the rat's anorectic response to amphetamine have shown it to be primarily due to a delay in initiation of feeding (Blundell and Latham, 1980; Cooper and Sweeney, 1980; Willner and Towell, 1982). The more selective DA uptake inhibitor, GBR 12909, also reduced food intake through a reduction in feeding duration and produced a non-significant trend towards an increased eating rate (Van der Hoek and Cooper, 1994). The latency parameter, however, was not reported. Also, other aspects of behaviour were significantly changed; in particular, there was a marked increase in stereotyped sniffing. Thus, it is possible that behavioural competition could account for the anorectic effects of GBR 12909. The microstructural data for cocaine also differ from those reported for the selective DA D2-like receptor agonist N-0437 (Rusk and Cooper, 1989) and the selective DA D1-like receptor agonist SK&F 38393 (Cooper et al., 1990). N-0437 did not reduce the duration of feeding, but caused a decrease in food intake by selectively reducing the rate of eating. SK&F 38393, on the other hand, reduced the frequency of feeding bouts together with the local rate of eating, effects that were accompanied by a compensatory increase in the mean bout duration. Therefore, cocaine's effects cannot be matched with the effects of either selective DA D1-like or DA D2-like receptor stimulation. At the time of writing, no selective agonist drugs specific for DA D2, D3 or D4 receptors have been synthesised. However, DA antagonist drugs have been developed that bind to these receptor subtypes; the next experiments were designed to investigate D2-like receptor involvement in cocaine's anorectic and stimulant effects using receptor-subtype selective antagonists.

CHAPTER 8:

'MICROSTRUCTURAL ANALYSIS OF 'D2-LIKE' RECEPTOR SUBTYPE ANTAGONISTS IN COMBINATION WITH COCAINE'

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MICROSTRUCTURAL ANALYSIS OF 'D2-LIKE' RECEPTOR SUBTYPE ANTAGONISTS IN COMBINATION WITH COCAINE

8.1 Introduction And Aims

Although a microstructural analysis of cocaine's effects has been presented, to date no studies have examined DA receptor subtype involvement in cocaine's effects on feeding and associated behaviours in the rat. As cocaine is an indirect DA agonist, its behavioural effects may involve activation of one or a subset of the DA receptor subtypes. Previous experiments in this thesis looking at the DS effects of cocaine have shown that DA D2-like receptors are differently involved (Chapter 3); it is now possible to assess the generality of these results by examining other behavioural effects of cocaine.

In the previous chapter, a microstructural approach to the study of feeding was used to characterise the dose(s) at which cocaine affects feeding and related behavioural responses. It was concluded from these data that cocaine produces a dose-dependent reduction in palatable food consumption, together with an increase in locomotor and rearing activity, accompanied by a marked suppression of grooming. The minimum dose to produce these effects was 10 mg/kg, and so this dose was chosen here. This was also the main training dose used in the earlier drug discrimination experiments (as presented in Chapter 3).

The necessity of D2-like stimulation for the expression of cocaine's effects on feeding and related behaviours was examined by administering the D2/D3 selective antagonist raclopride (Kohler et al., 1985; Anderson, 1988) in combination with cocaine, and comparing its effects for the first time with the effects of the selective D3 and D4 antagonists, U-99194A and L-745,870, respectively. However, a common feature of D2-like antagonists is a biphasic action on ingestive behaviour (see section 4.3.2). This presents special difficulties in the design and interpretation of combination studies with D2-like antagonists. Doses used for the combination experiments were chosen from studies that specifically characterised the effects of these drugs on the microstructure of feeding and associated behaviours. For example, using an identical procedure, Terry (1996) demonstrated a biphasic dose-effect of

raclopride on feeding, and the only dose which consistently did not significantly alter feeding or other aspects of motor behaviour was 0.1 mg/kg; i.e. this was the highest dose which produced negligible effects on its own. Similarly, for the D3 and D4 antagonist compounds U-99194A and L-745,870 (characterised in Chapter 6) doses of 3 and 1 mg/kg, respectively, were chosen for the combination experiments, since these were at the threshold of being behaviourally active on their own, and more importantly are selective for their respective receptor subtypes.

For comprehensive analysis of the interactive effects, the 10 mg/kg dose of cocaine was compared with the combination of the submaximal dose of the D2-like antagonists with cocaine, also with each of the D2-like antagonists when tested alone, and with vehicle. There were six time intervals. Therefore, the data were analysed using a 2 (cocaine vs. saline) × 2 (antagonist vs. vehicle) × 6 (time intervals) ANOVA.

8.2 Materials and methods

8.2.1 Subjects:

Twenty-four male Sprague-Dawley rats (eight per study; Charles River, Margate, Kent) weighing 320-360 g were housed in pairs with free access to water under a 12 hr light/dark cycle, lights on 07:00. They were all maintained on a restricted diet of 15 g of standard food pellets, adjusted on test days so that, regardless of the effect of drug, all rats received the same food allowance per day. All testing was between 12:00 and 14:00 hours.

8.2.2 Apparatus and procedure:

The pre-treatment adaptation procedure and food preparation were as described in section 5.2.3. The following experiments were carried out using a similar procedure to that described in section 6.2.3 for microstructural analysis, except that the software used to log the duration and sequence of each key press was now the "Observer" (Version 3.0, Noldus, Netherlands). This offered the advantage for the data to be recorded in 5-min interval bins for a total of 30 minutes, which was not possible in the previous studies. Locomotor activity was also measured the same way as described in section 5.2.3, so allowing comparison with the visual observations; again the data were analysed in 5- min intervals using a purpose-written data extraction programme.

8.2.3 Drugs:

(-)-Cocaine HCl, (Sigma Chemicals, Poole Dorset) and S (-)-Raclopride L-tartrate (RBI chemicals, St. Albans, Herts, UK) were dissolved in 0.9% saline solution and administered intraperitoneally at doses of 10 (cocaine) and 0.1 mg/kg (raclopride) respectively. U-99194A Maleate (5,6,-Dimethoxy-2-(di-n-propylamino) indan maleate; RBI Chemicals, Natick MA, USA) and L-745,870 (3-([4-chlorophenyl) piperazin-1-yl] methyl)-1H-pyrrolo [2,3-b] pyridine; Tocris Cookson, Bristol, England, UK) were dissolved in distilled water and administered intraperitoneally at doses of 3 and 1 mg/kg respectively. All drugs were injected at a volume of 1 ml/kg and all the DA D2-like receptor subtype antagonists were injected 30 min prior to the cocaine injection.

8.2.4 Data analysis:

The data were analysed by a 2 × 2 × 6 model ANOVA for repeated measures. The ANOVAs incorporated cocaine dose and antagonist dose as between-subject factors (two levels each) and time interval (30 min duration; six levels: 5-min intervals) as a within-group factor. All drug doses were administered to the rats in a 'Latin Square' design and comparisons between the results for individual drug-treatments and vehicle controls were made using Dunnett's post-hoc t-tests. Post-hoc analyses were only carried out if there were significant treatment interactions.

8.3 RESULTS

8.3.1 EXPERIMENT 1: Combination study with the D2/D3 antagonist. (a) Food Intake and Eating Microstructure.

Effect of Cocaine:

During the pre-test adaptation period, the food deprived rats consumed on average 20 g within the 30 min test session. Table 8.1 revealed that cocaine produced a pronounced reduction in food consumption [$F(1,7) = 81.93$, $P<0.01$] (Fig. 8.1a(i)). These reductions were not due to any significant change in the local rate of eating, as indicated in Fig. 8.1a(iii), but cocaine's effect on the latency to start feeding was very close to being significant [$F(1,7) = 4.93$, $P = 0.065$] (Fig. 8.1a(ii)). As Table 8.1 indicates, the explanation for the reduction in food intake is that cocaine produced a reduction in the total duration of feeding [$F(1,7) = 66.39$, $P<0.01$] (Fig. 8.1 a(iv)A&B). The total duration can be further subdivided into the frequency of eating bouts and their mean bout duration. Cocaine significantly suppressed mean bout duration [$F(1,7) = 32.83$, $P<0.01$] (Fig. 8.1a(v)A&B), but had no effect on the frequency of eating bouts (Fig. 8.1a(vi)A&B). Hence, cocaine acted to increase the latency to start feeding and to reduce mean bout duration, and this in turn led to a reduction in the time devoted to feeding.

Effect of Antagonist:

The analysis indicates that the administration of raclopride did not have an effect on any feeding parameter (Table 8.1).

Cocaine and Antagonist Interaction:

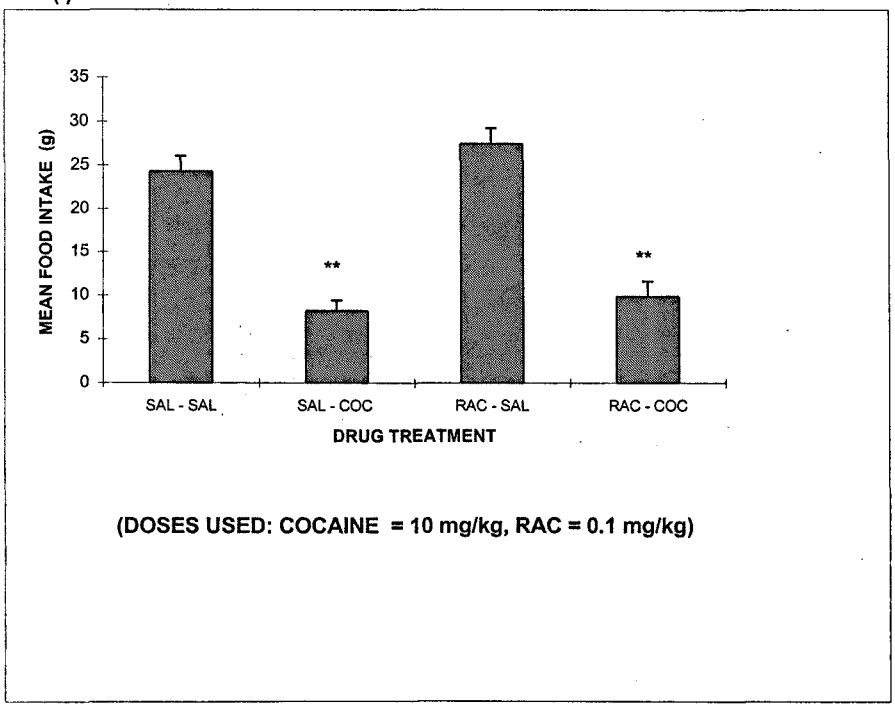
Analysing the results using $2 \times 2 \times 6$ ANOVA with factors antagonist, cocaine and time (Table 8.1) revealed no significant interactions between raclopride and cocaine for total duration nor mean bout duration. Instead, the analysis showed a significant interaction between raclopride and cocaine and time for feeding bout frequency only [$F(5,35) = 5.64$, $P<0.01$]. *Post-hoc* t-tests revealed that pre-treatment with raclopride at 0.1 mg/kg attenuated the cocaine-induced decrease in feeding bout frequency at time intervals 5-10 and 10-15 min (Fig. 8.1a(vi)A). Collapsing across the 30 min period for this behavioural parameter also revealed an interaction between raclopride and cocaine [$F(1,7) = 7.29$, $P<0.01$]. However, *post-hoc* t-tests revealed no

significant difference between raclopride plus cocaine and cocaine alone when total bout frequency was collapsed over the 30 min session (Fig. 8.1a(vi)B).

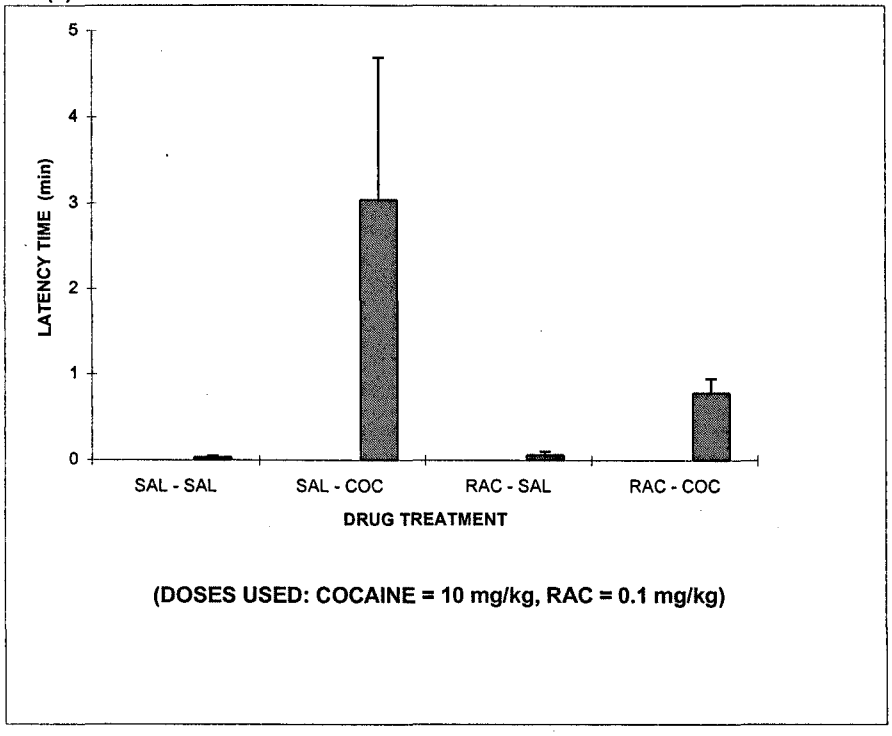
In effect, raclopride at 0.1 mg./kg only affected cocaine moderately on one parameter of feeding microstructure, that of feeding bout frequency. However, as other parameters were not affected by pre-treatment with raclopride, the effect on feeding bout frequency was not sufficient to attenuate the overall effect of cocaine on food intake.

Figure 8.1a.

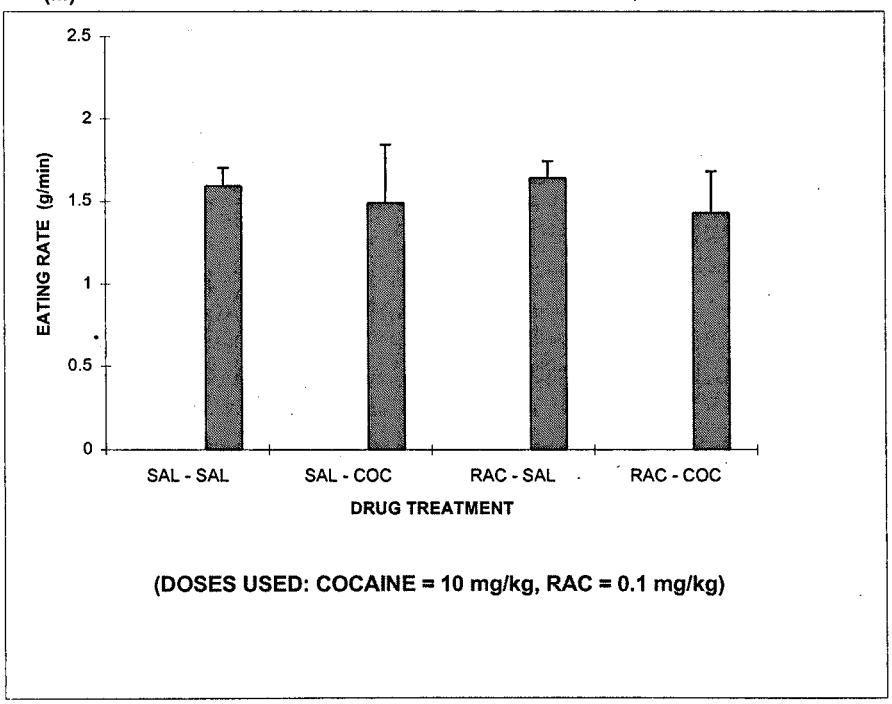
(i)



(ii)

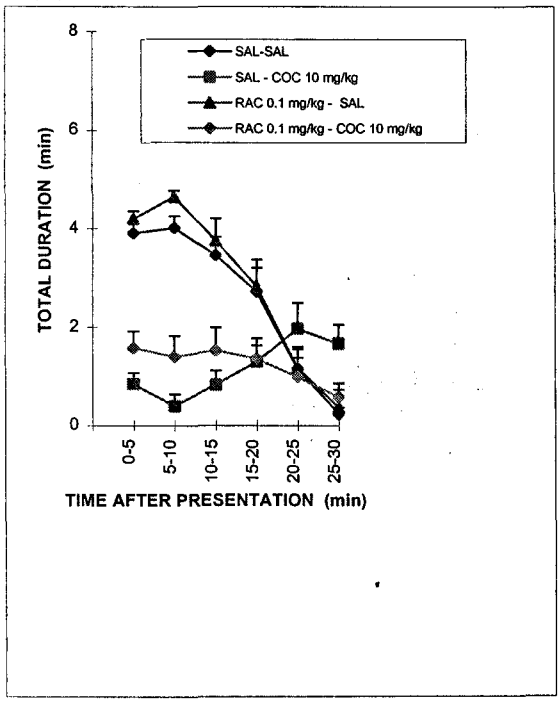


(iii)

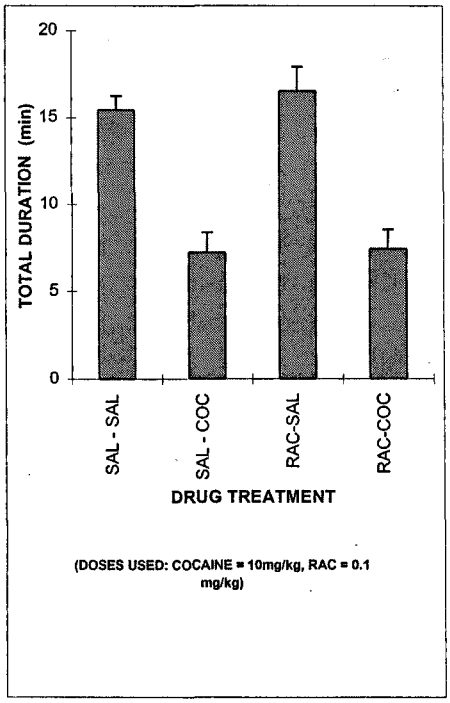


(DOSES USED: COCAINE = 10 mg/kg, RAC = 0.1 mg/kg)

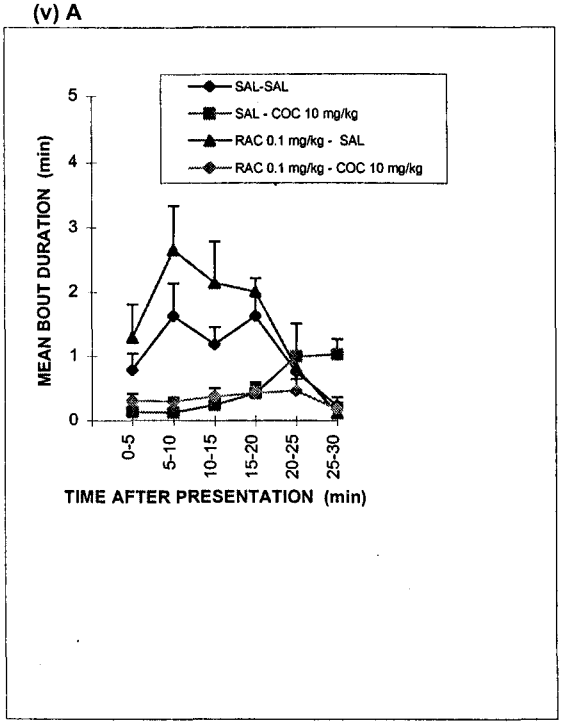
(iv) A


SAL-SAL
SAL - COC 10 mg/kg
RAC 0.1 mg/kg - SAL
RAC 0.1 mg/kg - COC 10 mg/kg
TOTAL DURATION (min)
0
2
4
6
8
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

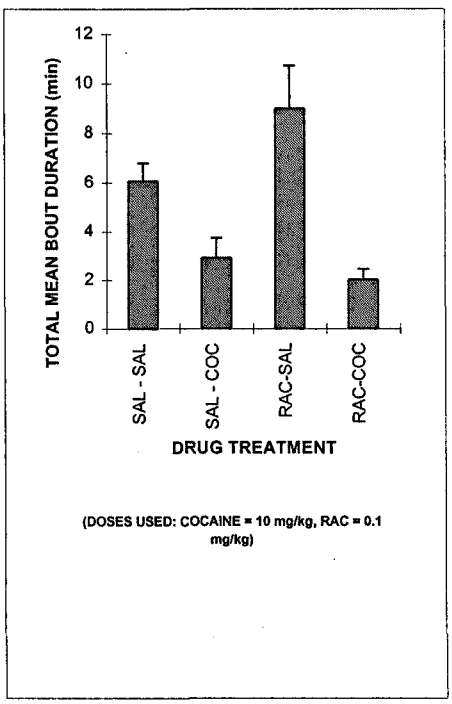
B


TOTAL DURATION (min)
0
5
10
15
20
SAL - SAL
SAL - COC
RAC-SAL
RAC-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10mg/kg, RAC = 0.1 mg/kg)

(v) A


SAL-SAL
SAL - COC 10 mg/kg
RAC 0.1 mg/kg - SAL
RAC 0.1 mg/kg - COC 10 mg/kg
MEAN BOUT DURATION (min)
0
1
2
3
4
5
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

B


TOTAL MEAN BOUT DURATION (min)
0
2
4
6
8
10
12
SAL - SAL
SAL - COC
RAC-SAL
RAC-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, RAC = 0.1 mg/kg)

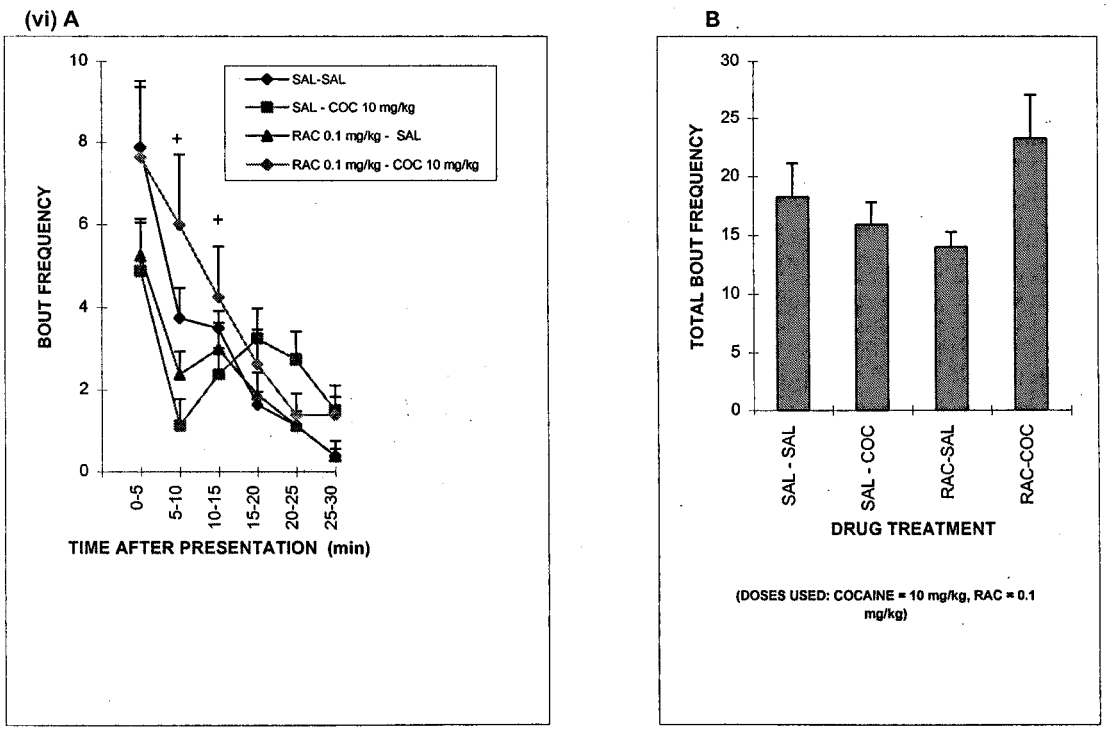


Figure 8.1(a) Effects of pre-treatment with a D2/D3 antagonist on cocaine-induced feeding parameters: (i) total food intake (ii) latency to start eating (iii) eating rate (iv)A&B total duration (v)A&B mean bout duration (vi)A&B bout frequency. N=8 at all points; ** $P<0.01$ compared to saline-controls; + $P<0.05$ compared to saline-cocaine (comparisons by Dunnett's t-tests)

TABLE 8.1. 2 (cocaine vs. saline) × 2 (antagonist vs. saline) × 6 (time intervals) ANOVA on behavioural scores from microstructural analysis of raclopride in combination with cocaine

RESPONSE CATEGORY	Antag. $F_{(1,7)}$	Coc. $F_{(1,7)}$	Antag. * Coc. $F_{(1,7)}$	Time $F_{(5,35)}$	Antag. * Time $F_{(5,35)}$	Coc. * Time $F_{(5,35)}$	Antag. * Coc. * Time $F_{(5,35)}$
FEEDING							
FOOD INTAKE	1.56 NS	81.93 P<0.01	0.21 NS				
LATENCY	1.82 NS	4.93*NS	2.03 NS				
RATE	0.93 NS	1.15 NS	1.05 NS				
TOTAL DURATION	1.28 NS	66.39 P<0.01	0.39 NS	16.05 P<0.01	2.60 P<0.05	24.96 P<0.01	1.94 NS
BOUT DURATION	0.71 NS	32.83 P<0.01	2.33 NS	4.45 P<0.01	1.77 NS	9.37 P<0.01	0.17 NS
BOUT FREQUENCY	0.37 NS	1.55 NS	7.29 P<0.01	27.18 P<0.01	1.09 NS	0.39 NS	5.64 P<0.01
LOCOMOTOR ACTIVITY							
LOCOMOTOR COUNTS	4.71* NS	44.03 P<0.01	1.36 NS	58.29 P<0.01	1.07 NS	14.81 P<0.01	0.35 NS
TOTAL DURATION	7.84 P<0.05	51.90 P<0.01	2.93 NS	27.00 P<0.01	1.03 NS	19.25 P<0.01	0.98 NS
BOUT DURATION	2.74 NS	0.09 NS	0.03 NS	1.45 NS	0.76 NS	1.46 NS	1.49 NS
BOUT FREQUENCY	5.46 P<0.05	63.34 P<0.01	3.72 NS	37.09 P<0.01	1.11 NS	17.58 P<0.01	0.60 NS
REARING							
TOTAL DURATION	3.85 NS	23.21 P<0.01	2.47 NS	3.93 P<0.01	0.13 NS	4.44 P<0.01	0.13 NS
BOUT DURATION	6.43 P<0.05	0.31 NS	0.54 NS	2.56 P<0.05	0.50 NS	2.39 NS	1.03 NS
BOUT FREQUENCY	5.17 P<0.05	43.81 P<0.01	5.69 P<0.05	20.31 P<0.01	1.84 NS	12.53 P<0.01	0.84 NS
GROOMING							
TOTAL DURATION	2.40 NS	0.73 NS	4.01 NS	2.71 P<0.05	0.65 NS	0.37 NS	0.93 NS
BOUT DURATION	1.35 NS	0.01 NS	6.86 P<0.05	2.63 P<0.05	0.44 NS	0.46 NS	0.97 NS
BOUT FREQUENCY	2.88 NS	3.20 NS	15.91 P<0.01	4.04 P<0.01	0.47 NS	1.70 NS	1.47 NS

(* P = 6.50)

(b) Locomotor Activity.

Effect of Cocaine:

Analysing the results using 2 × 2 × 6 ANOVA (Table 8.1) revealed that cocaine significantly increased mean locomotor counts throughout the first 25 min of the test session [$F(1,7) = 44.03$, $P<0.01$] (Fig. 8.1b (i)A&B). The duration of individual bouts showed little change, but there were large increases in the frequency of bouts [$F(1,7) = 63.34$, $P<0.01$] which was the sole contributor to cocaine significantly increasing the total duration of locomotor activity [$F(1,7) = 51.90$, $P<0.01$]. The time-course for cocaine's effects indicates that total duration and bout frequency of locomotor activity were enhanced throughout the observation period (Fig. 8.1b(ii)A&B and Fig. 8.1b(iv)A&B respectively.

Effect of Antagonist:

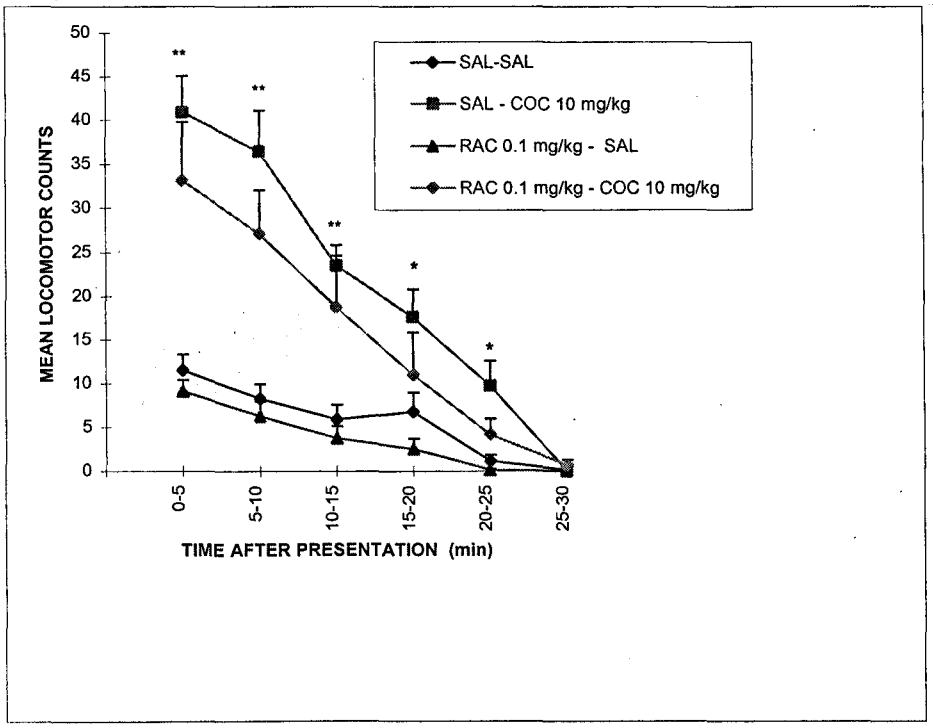
The analysis also indicates that administration of raclopride at 0.1 mg/kg is producing a significant effect on total duration and bout frequency of locomotor activity [respectively; $F(1,7) = 7.84$, $P<0.05$; $F(1,7) = 5.46$, $P<0.05$]. However, this was not sufficient to produce an overall effect of raclopride on locomotor counts, but the value was close to being significant [$F(1,7) = 4.71$, $P = 0.065$]. The respective graphs show that raclopride causes a decrease in locomotor activity compared with vehicle treatment, but *post-hoc* t-tests did not reveal any significant effects at any specific time intervals, nor when the data was collapsed across the 30 min period (Fig. 8.1b(ii)A&B and Fig. 8.1b(iv)A&B, respectively).

Cocaine and Antagonist Interaction:

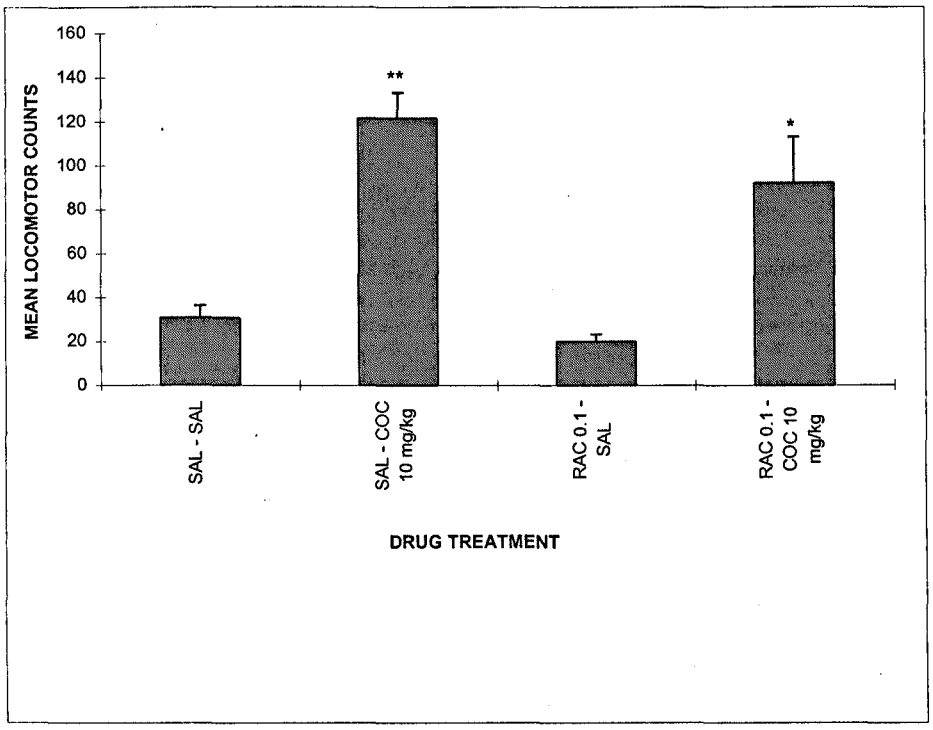
Table 8.1 revealed no significant interactions between raclopride and cocaine, nor between cocaine, antagonist and time on any behavioural parameter for locomotor activity.

Figure 8.1b.

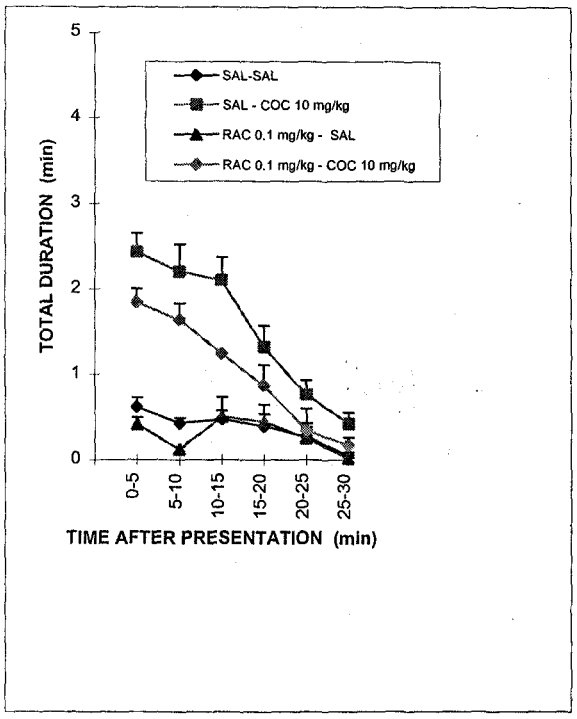
(i) A



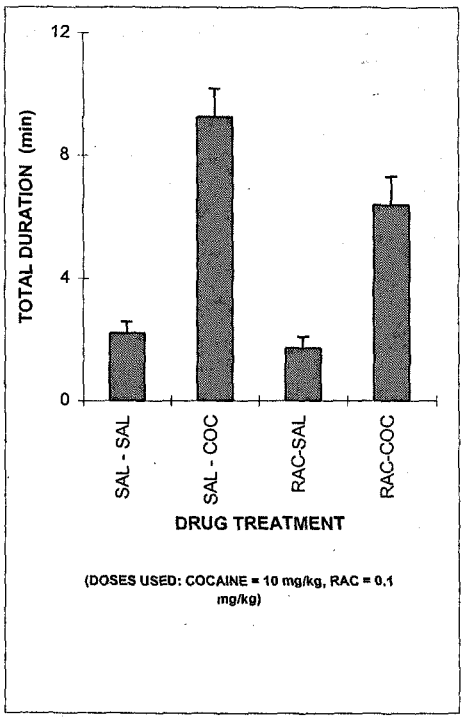
B



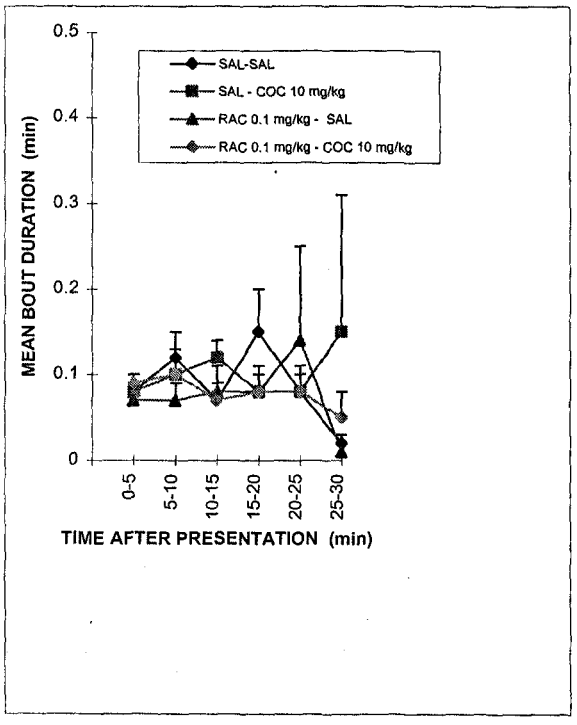
(ii) A


SAL-SAL
SAL - COC 10 mg/kg
RAC 0.1 mg/kg - SAL
RAC 0.1 mg/kg - COC 10 mg/kg
TOTAL DURATION (min)
0
1
2
3
4
5
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

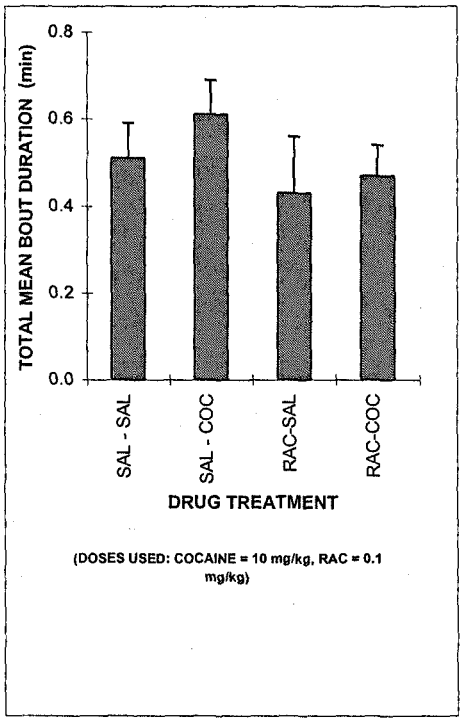
B


TOTAL DURATION (min)
0
4
8
12
SAL - SAL
SAL - COC
RAC-SAL
RAC-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, RAC = 0.1 mg/kg)

(iii) A


SAL-SAL
SAL - COC 10 mg/kg
RAC 0.1 mg/kg - SAL
RAC 0.1 mg/kg - COC 10 mg/kg
MEAN BOUT DURATION (min)
0
0.1
0.2
0.3
0.4
0.5
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

B


TOTAL MEAN BOUT DURATION (min)
0.0
0.2
0.4
0.6
0.8
SAL - SAL
SAL - COC
RAC-SAL
RAC-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, RAC = 0.1 mg/kg)

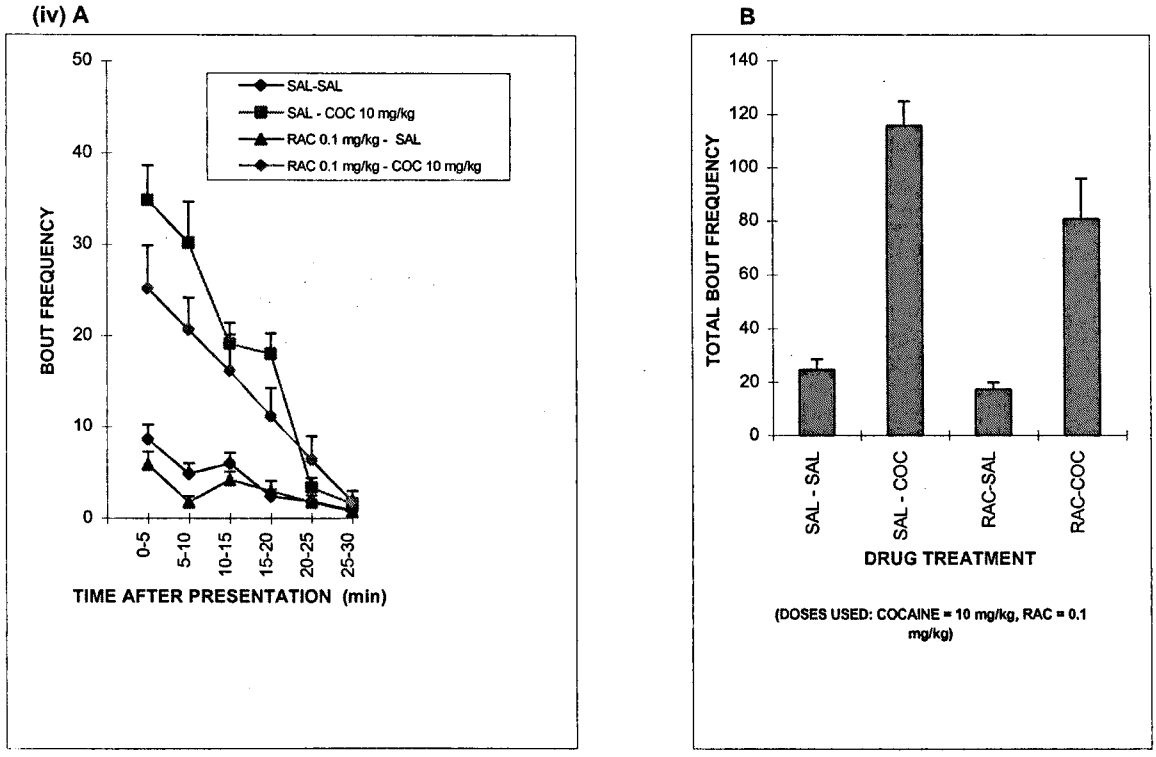


Figure 8.1(b) Effects of pre-treatment with a D2/D3 antagonist on cocaine-induced locomotor activity parameters: (i)A&B total locomotor counts (ii)A&B total duration (iii) A&B mean bout duration (iv) A&B bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to saline-controls (comparisons by Dunnett's t-tests)

(c) Rearing.

Effect of Cocaine:

Cocaine increased the total duration of rearing [$F(1,7) = 23.21$, $P<0.01$] which corresponded with increases in the frequency of rearing bouts [$F(1,7) = 43.81$, $P<0.01$]. There was no significant effect of mean bout duration. Time course analysis indicates the cocaine's effects on total duration and bout frequency of rearing activity were enhanced throughout the observation period (Fig. 8.1c(i)A&B and Fig. 8.1c(iii)A&B respectively).

Effect of Antagonist:

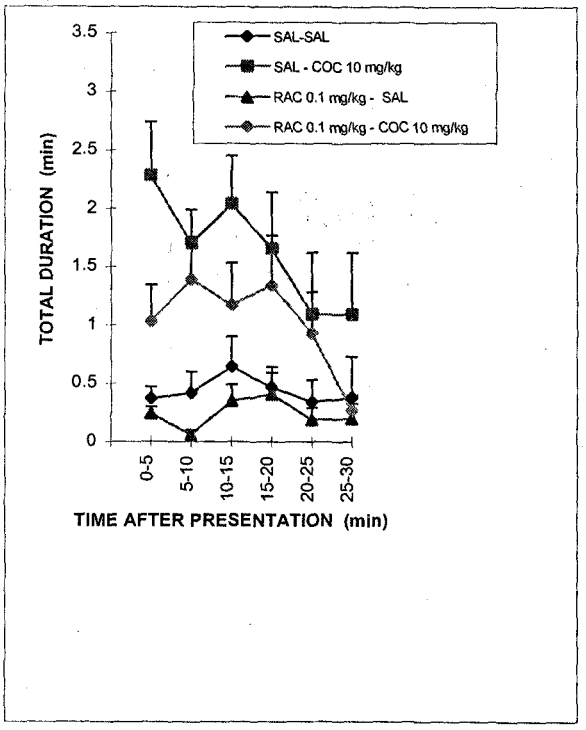
Raclopride produced significant effects on mean bout duration and bout frequency [respectively; $F(1,7) = 6.34$, $P<0.05$; $F(1,7) = 5.17$, $P<0.05$]. However, there was no overall effect of raclopride on total duration of rearing activity (Table 8.1).

Cocaine and Antagonist Interaction:

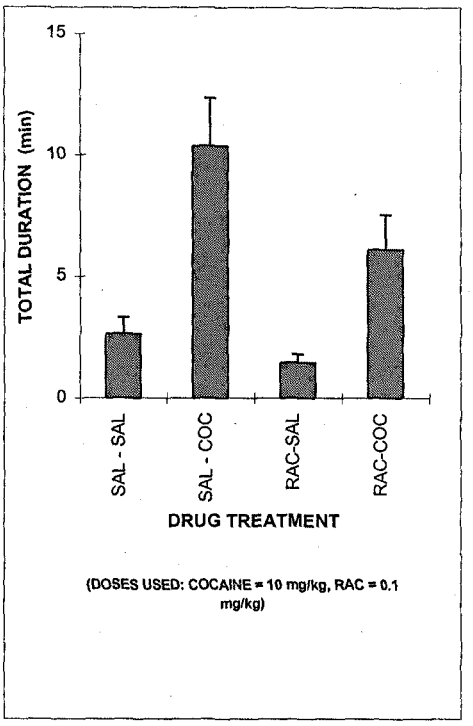
There was no significant interaction between raclopride and cocaine, nor between cocaine, antagonist and time for total duration and mean bout duration for rearing activity (Table 8.1). However, when behavioural counts were collapsed across the 30 min period, an interaction between raclopride and cocaine was seen for rearing bout frequency [$F(1,7) = 5.69$, $P< 0.05$]. *Post-hoc* t-tests revealed that pre-treatment with raclopride at 0.1 mg/kg produced a significant overall attenuation of the effects of cocaine for rearing bout frequency (Fig. 8.1c(iii)B). However, there was no interaction with time for this behavioural parameter [$F(5,35) = 0.84$, NS] as shown in Fig. 8.1c(iii)A.

Figure 8.1c.

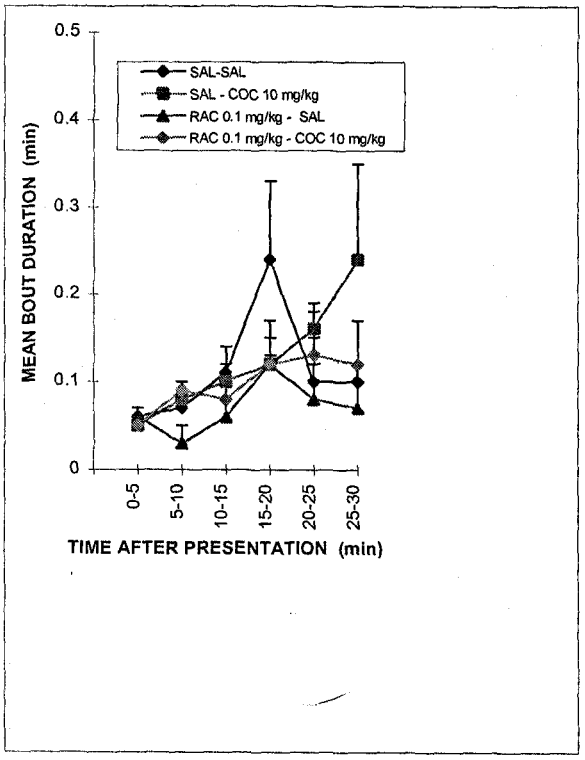
(i) A



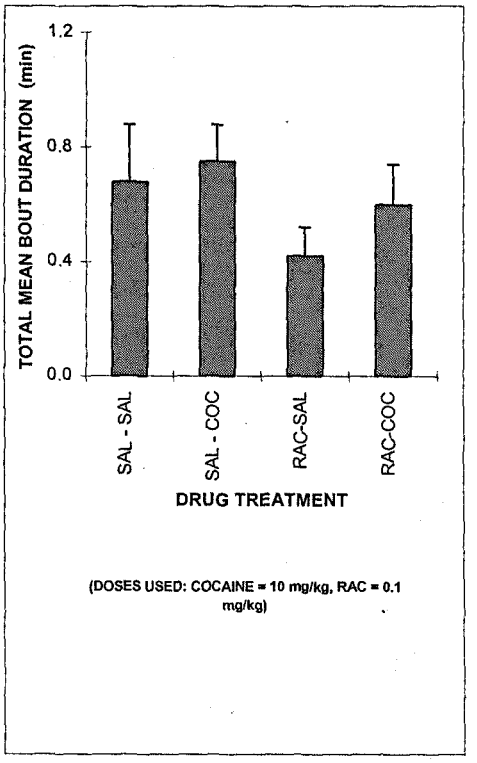
B



(ii) A



B



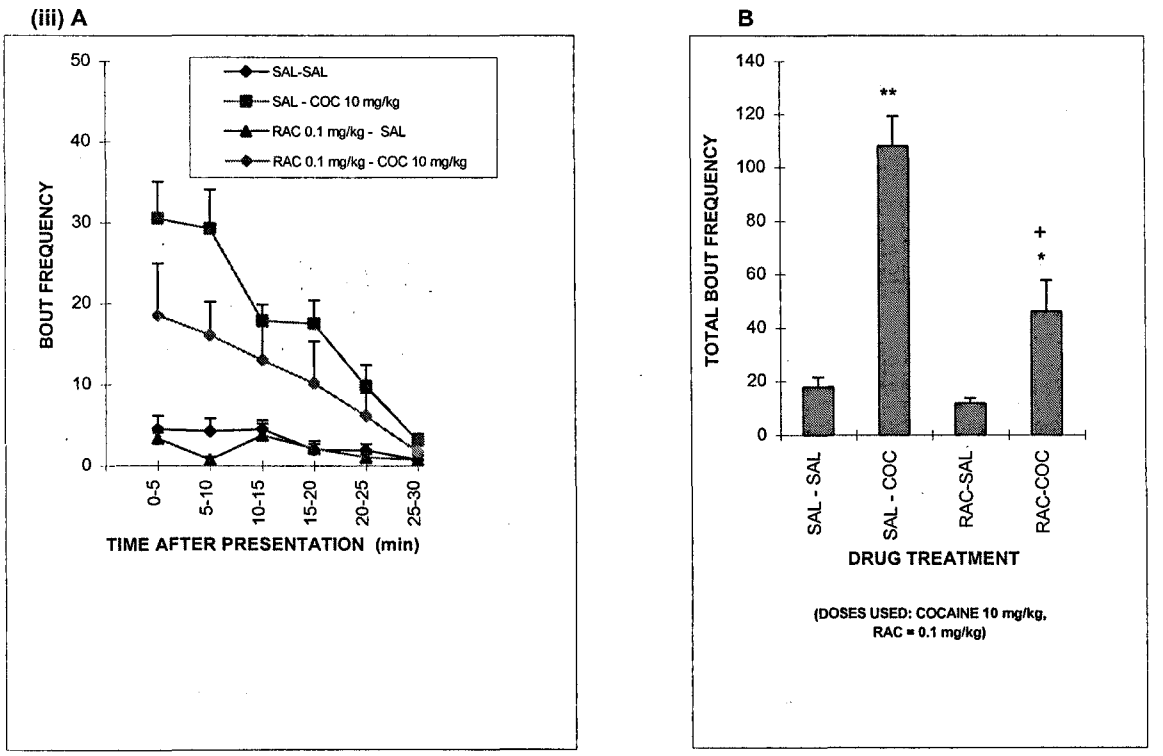


Figure 8.1(c) Effects of pre-treatment with a D2/D3 antagonist on cocaine-induced rearing activity parameters: (i)A&B total duration (ii)A&B mean bout duration (iii)A&B bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to saline-controls; + $P<0.05$ compared to saline-cocaine (comparisons by Dunnett's t-tests)

(d) Grooming.

Effect of Cocaine:

ANOVA revealed that cocaine did not significantly affect any parameter of grooming activity (Table 8.1).

Effect of Antagonist:

ANOVA showed that raclopride did not significantly affect any parameter of grooming activity (Table 8.1).

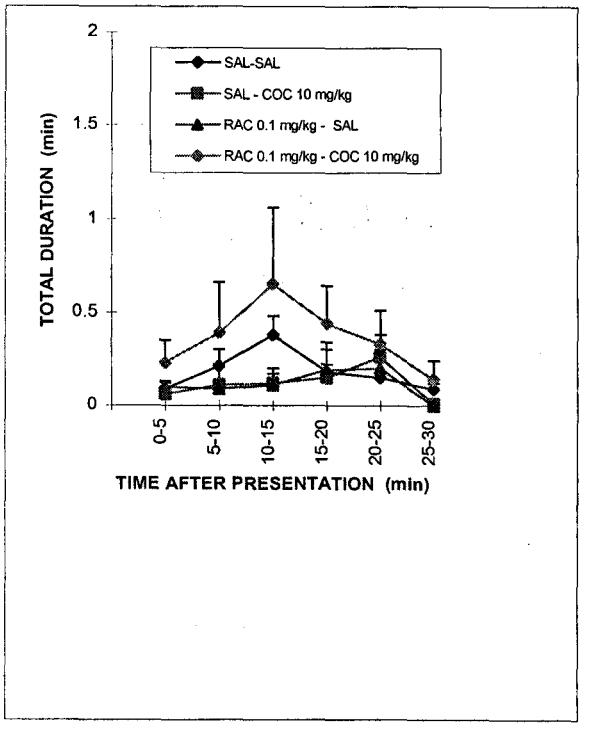
Cocaine and Antagonist Interaction:

A significant interaction was observed for mean bout duration [$F(1,7) = 6.86$, $P<0.05$] and bout frequency [$F(1,7) = 15.91$, $P<0.01$], but not for total duration, over the 30 min period (Fig. 8.1d(ii)B and Fig. 8.1d(iii)B, respectively). *Post-hoc* t-tests revealed that pre-treatment with raclopride at 0.1 mg/kg produced a significant attenuation of the effects of cocaine on grooming mean bout duration ($P<0.05$) and bout frequency ($P<0.01$).

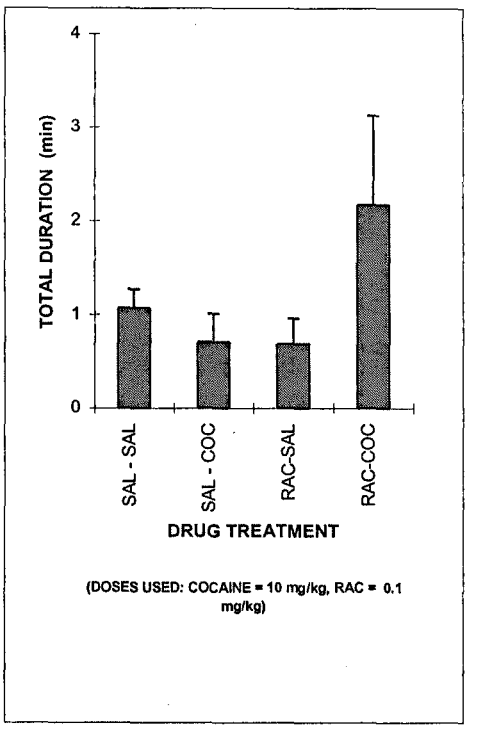
This interaction may explain why a significant effect of cocaine on grooming, which was strongly evident in Chapter 7, was not shown in the present analysis. The results shown in Fig. 8.1d indicate that cocaine produced a decrease in grooming activity, as did raclopride when administered alone. However, pre-treatment with raclopride produced an attenuation of cocaine's effects and hence an interaction between the two drugs. When the mean effect of cocaine treatment is compared to all conditions where cocaine is not administered, the values are very equal resulting in a non-significant main effect of cocaine on grooming activity.

Figure 8.1d.

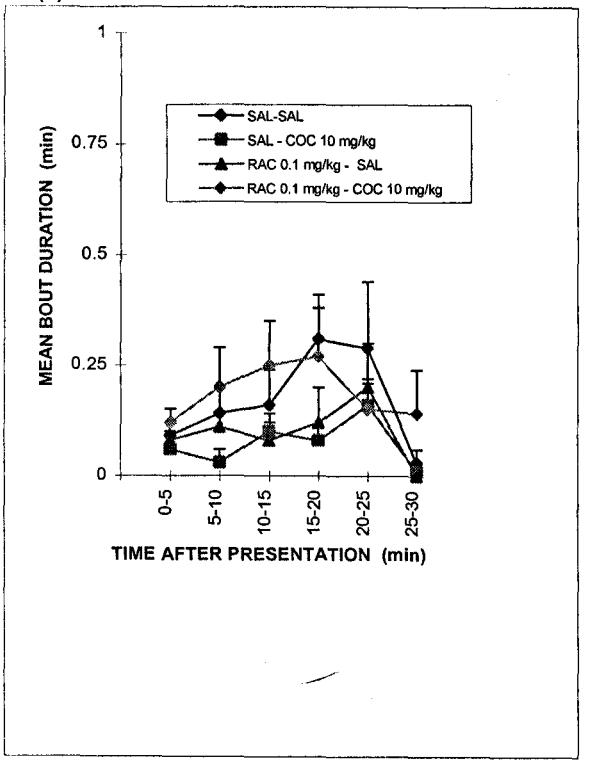
(i) A



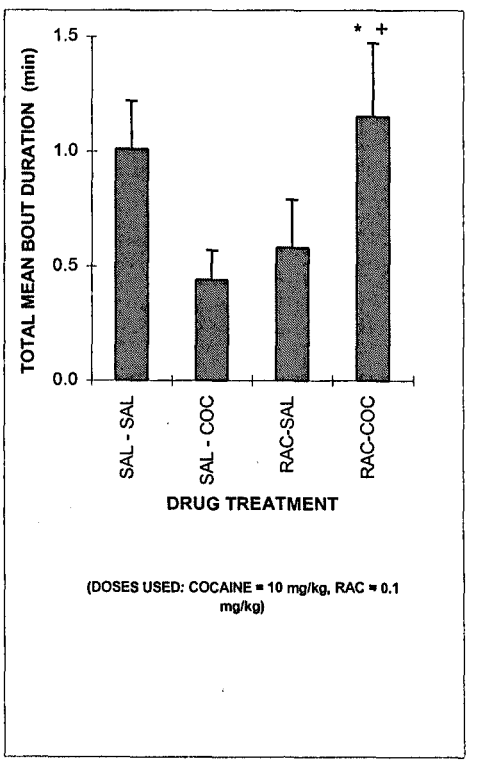
B



(ii) A



B



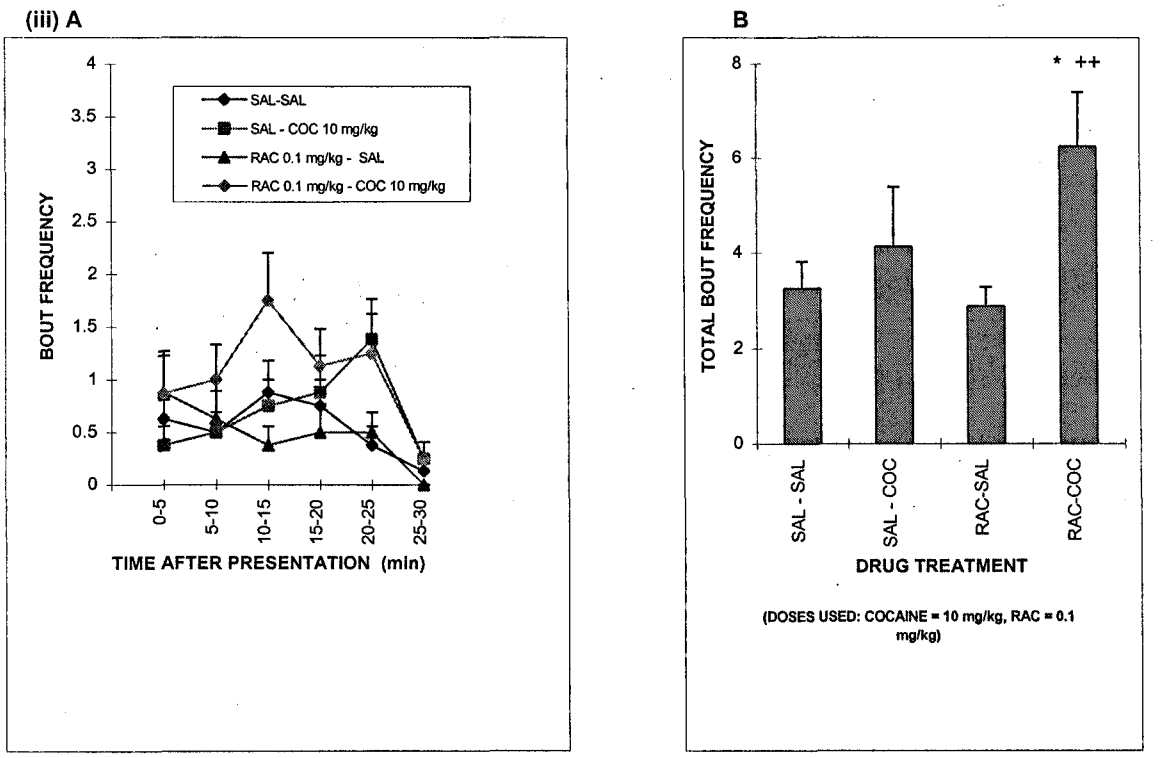


Figure 8.1(d) Effects of pre-treatment with a D2/D3 antagonist on cocaine-induced grooming activity parameters: (i)A&B total duration (ii)A&B mean bout duration (iii)A&B bout frequency. N=8 at all points; * $P<0.05$ compared to saline-controls; + $P<0.05$, ++ $P<0.01$ compared to saline-cocaine (comparisons by Dunnett's t-tests)

8.3.2 EXPERIMENT 2: Combination study with the selective D3 antagonist, U-99194A. (a) Food Intake and Eating Microstructure.

Effect of Cocaine:

Analysing the results by a 2 × 2 ×6 ANOVA with factors antagonist, cocaine and time (Table 8.2) revealed that cocaine produced a reduction in food consumption [$F(1,7) = 41.14$, $P<0.01$; Fig. 8.2a(i)]. Similar to the results from Experiment 1, the reduction was due to a combined effect on the latency to induce feeding [$F(1,7) = 7.60$, $P<0.05$; Fig. 8.2a(ii)] and a reduction in the total duration of feeding [$F(1,7) = 43.60$, $P<0.01$; Fig. 8.2a(iv)A&B]. The primary cause of the effect on total duration of feeding was the significant effect on mean bout duration [$F(1,7) = 11.30$, $P<0.05$; Fig. 8.2a(v)A&B], as there was no significant effect of cocaine on the frequency of eating bouts (Table 8.2).

Effect of Antagonist:

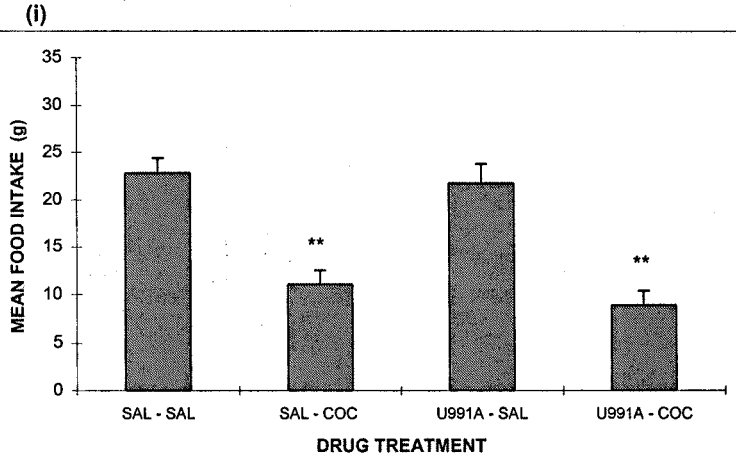
The analysis indicates that administration of the selective D3 antagonist U-99194A at 3 mg/kg did not affect any of the behavioural parameters for feeding, except for mean bout duration which decreased [$F(1,7) = 6.62$, $P<0.05$; Fig. 8.2a(v)A; Table 8.2]. These results are consistent with the results described in section 6.3.1, except for the effect on mean bout duration.

Cocaine and Antagonist Interaction:

Analysing the results by a 2 × 2 × 6 ANOVA with factors antagonist, cocaine and time (Table 8.2) revealed no significant interactions between U-99194A and cocaine for any behavioural parameter of feeding over the whole 30 min period. Instead, the analysis showed a significant interaction between U-99194A, cocaine and time for total duration [$F(5,35) = 4.53$, $P<0.01$] and mean bout duration [$F(5,35) = 3.40$, $P<0.05$], but not for bout frequency. However, *post-hoc* t-tests failed to show any significant differences between pre-treatment with U-99194A plus cocaine and cocaine plus saline at any time interval for either parameter. Perhaps some potentiation of the decreases in scores occurred when U-99194A was combined with cocaine at certain time points, both for total duration (Fig. 8.2a(iv)A) and mean bout duration parameters (Fig. 8.2a(v)A). This might contribute to the interaction observed for these parameters for U-99194A, cocaine and time. However, from the figures it is clear that the effects are complex.

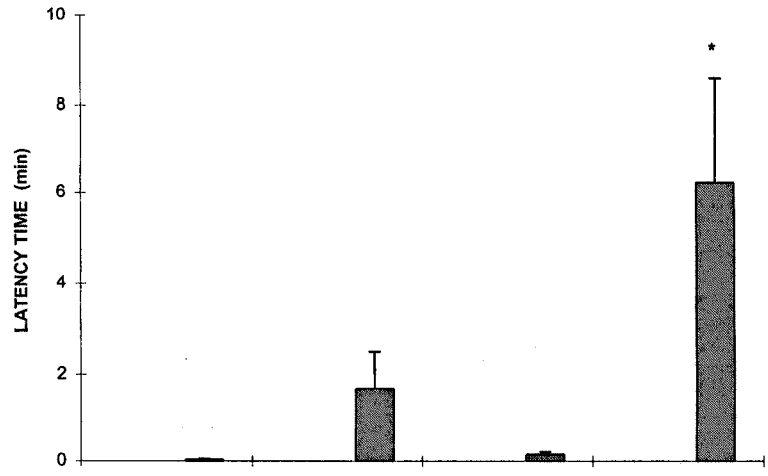
Figure 8.2a.

(i)



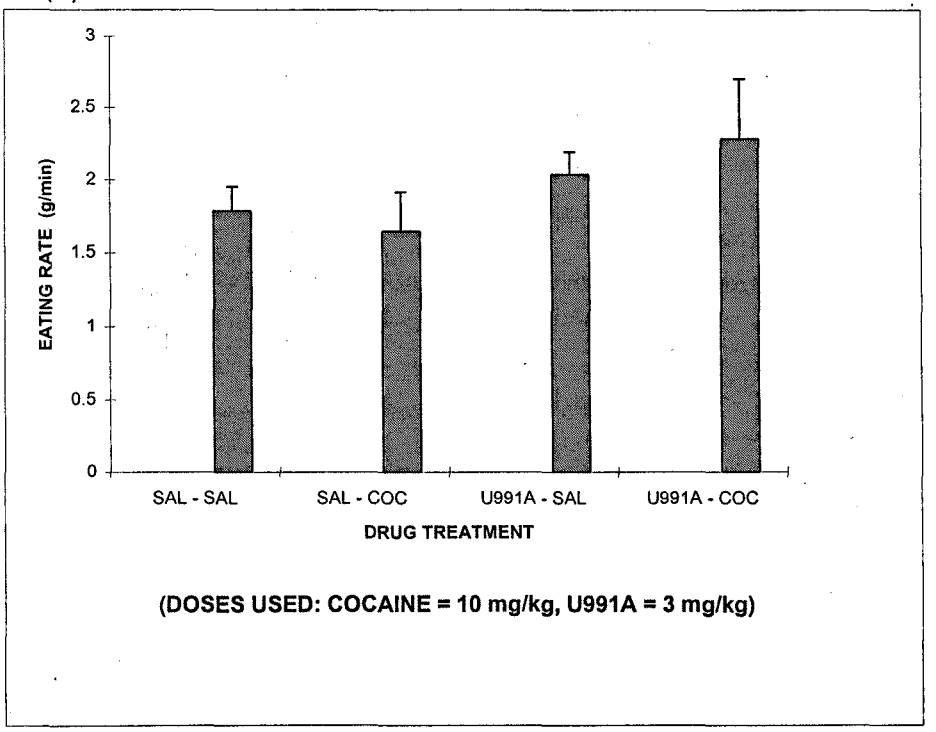
(DOSES USED: COCAINE = 10 mg/kg, U991A = 3 mg/kg)

(ii)



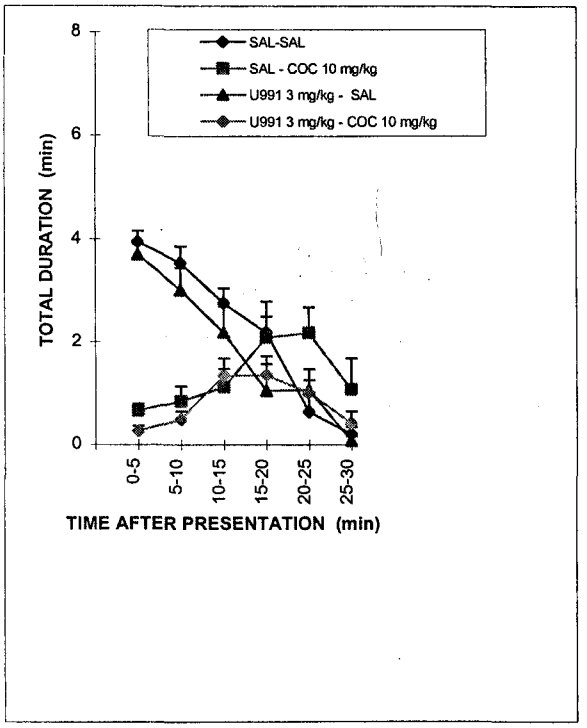
(DOSES USED: COCAINE = 10 mg/kg, U991A = 3 mg/kg)

(iii)

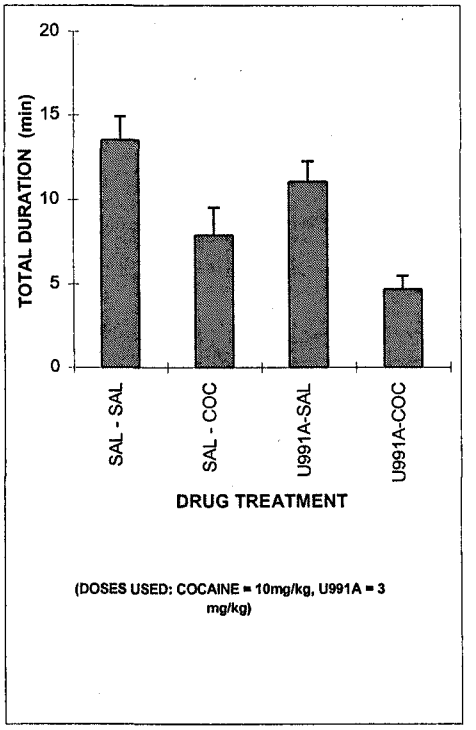


(DOSES USED: COCAINE = 10 mg/kg, U991A = 3 mg/kg)

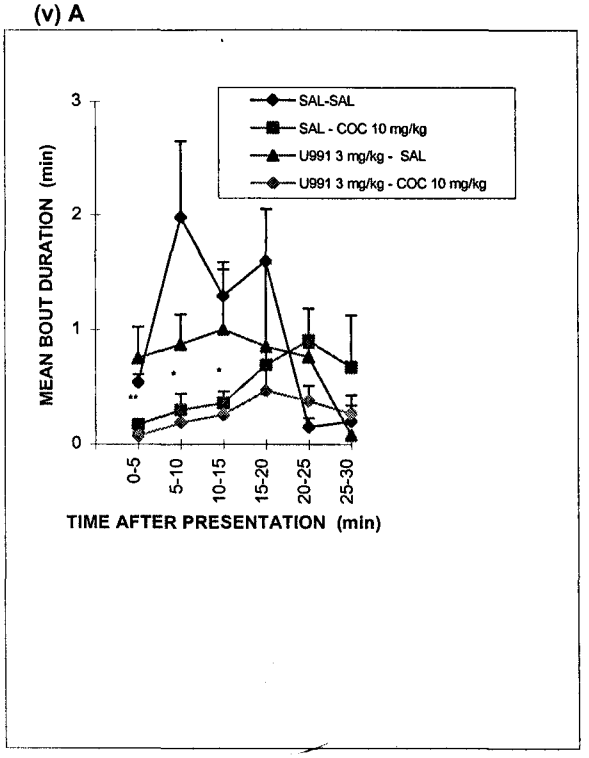
(iv) A


SAL-SAL
SAL - COC 10 mg/kg
U991 3 mg/kg - SAL
U991 3 mg/kg - COC 10 mg/kg
TOTAL DURATION (min)
0
2
4
6
8
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

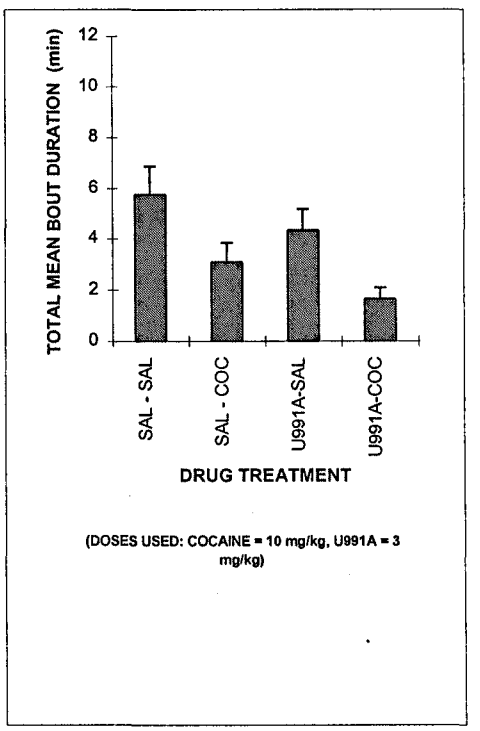
B


TOTAL DURATION (min)
0
5
10
15
20
SAL - SAL
SAL - COC
U991A-SAL
U991A-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10mg/kg, U991A = 3 mg/kg)

(v) A


SAL-SAL
SAL - COC 10 mg/kg
U991 3 mg/kg - SAL
U991 3 mg/kg - COC 10 mg/kg
MEAN BOUT DURATION (min)
0
1
2
3
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

B


TOTAL MEAN BOUT DURATION (min)
0
2
4
6
8
10
12
SAL - SAL
SAL - COC
U991A-SAL
U991A-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, U991A = 3 mg/kg)

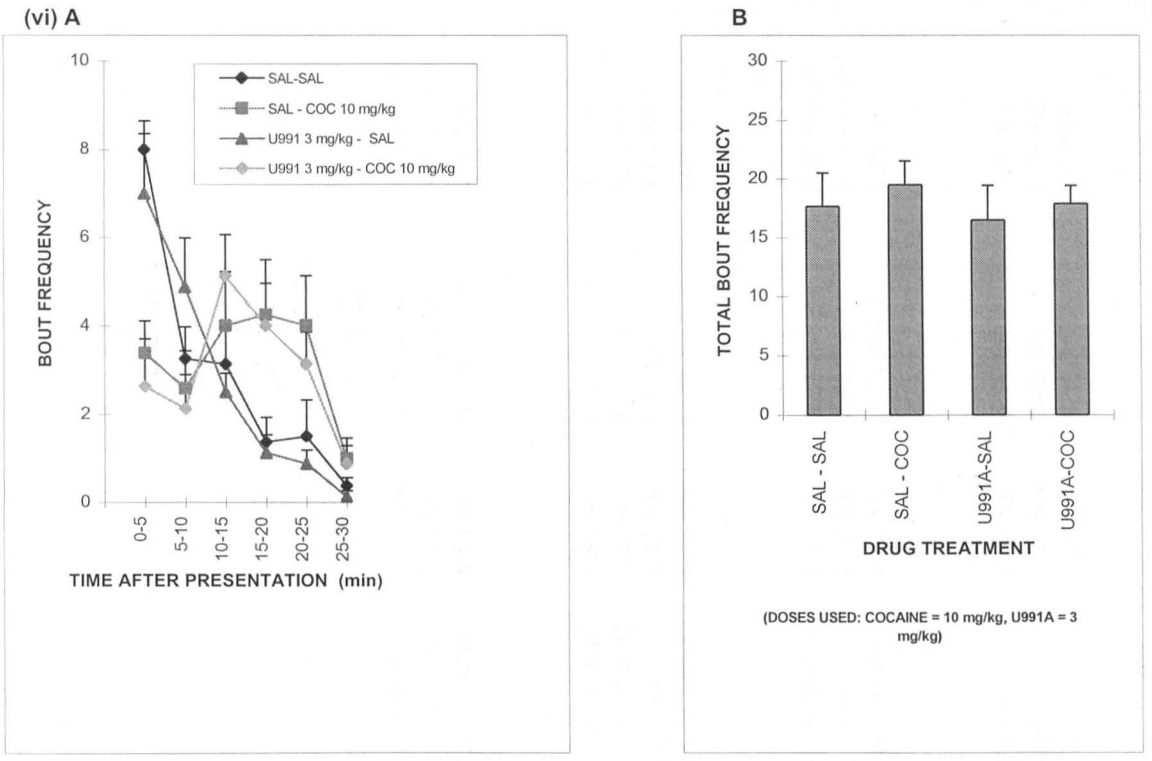


Figure 8.2(a) Effects of pre-treatment with a selective D3 antagonist on cocaine-induced feeding parameters: (i) total food intake (ii) latency to start eating (iii) eating rate (iv)A&B total duration (v)A&B mean bout duration (vi)A&B bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to saline-controls (comparisons by Dunnett's t-tests)

TABLE 8.2. 2 (cocaine vs. saline) × 2 (antagonist vs. saline) × 6 (time intervals) ANOVA on behavioural scores from microstructural analysis of U-99194A in combination with cocaine

RESPONSE CATEGORY	Antag. $F_{(1,7)}$	Coc. $F_{(1,7)}$	Antag. * Coc. $F_{(1,7)}$	Time $F_{(5,35)}$	Antag. * Time $F_{(5,35)}$	Coc. * Time $F_{(5,35)}$	Antag. * Coc. * Time $F_{(5,35)}$
FEEDING							
FOOD INTAKE	1.01 NS	41.14 P<0.01	0.03 NS				
LATENCY	4.19 NS	7.60 P<0.05	3.75 NS				
RATE	2.51 NS	0.07 NS	1.16 NS				
TOTAL DURATION	4.59 NS	43.60 P<0.01	0.10 NS	13.26 P<0.01	0.76 NS	23.23 P<0.01	4.53 P<0.01
BOUT DURATION	6.62 P<0.05	11.30 P<0.05	0.0 NS	3.38 P<0.05	0.89 NS	4.63 P<0.01	3.40 P<0.05
BOUT FREQUENCY	0.29 NS	1.19 NS	0.02 NS	12.27 P<0.01	0.50 NS	10.93 P<0.01	1.07 NS
LOCOMOTOR ACTIVITY							
LOCOMOTOR COUNTS	4.10 NS	21.59 P<0.01	3.76 NS	63.52 P<0.01	1.58 NS	14.23 P<0.01	0.88 NS
TOTAL DURATION	5.44 P<0.05	42.60 P<0.01	1.26 NS	37.25 P<0.01	1.25 NS	9.07 P<0.01	0.42 NS
BOUT DURATION	0.84 NS	9.33 P<0.05	0.22 NS	1.61 NS	0.22 NS	1.99 NS	1.18 NS
BOUT FREQUENCY	11.08 P<0.05	23.31 P<0.01	2.30 NS	46.20 NS	0.25 NS	12.86 P<0.01	1.48 NS
REARING							
TOTAL DURATION	0.16 NS	16.73 P<0.01	0.67 NS	14.74 P<0.01	0.47 NS	5.23 P<0.01	3.10 P<0.05
BOUT DURATION	2.40 NS	0.93 NS	1.57 NS	2.34 NS	0.56 NS	0.54 NS	2.62 P<0.05
BOUT FREQUENCY	6.09 P<0.05	19.05 P<0.01	1.93 NS	56.78 P<0.01	0.20 NS	15.24 P<0.01	1.29 NS
GROOMING							
TOTAL DURATION	0.45 NS	25.25 P<0.01	0.46 NS	1.33 NS	1.70 NS	0.86 NS	0.85 NS
BOUT DURATION	0.05 NS	45.09 P<0.01	0.05 NS	1.17 NS	2.47 NS	0.46 NS	1.15 NS
BOUT FREQUENCY	0.03 NS	15.75 P<0.01	0.03 NS	1.05 NS	2.04 NS	2.80 P<0.01	0.63 NS

(b) Locomotor Activity.

Effect of Cocaine:

Table 8.2 show that, as in experiment 1, cocaine significantly increased mean locomotor counts [$F(1,7) = 21.59$, $P<0.01$; Fig. 8.2b(i) A&B]. This was due to an increase in total duration of locomotor activity [$F(1,7) = 42.60$, $P<0.01$], which was accompanied by an increase in mean bout duration [$F(1,7) = 9.33$, $P<0.05$] and an increase in the frequency of bouts [$F(1,7) = 23.31$, $P<0.01$]. The time-course for cocaine's effects indicates that total duration and bout frequency of locomotor activity were enhanced throughout the observation period (Fig. 8.2b(ii)A&B and Fig. 8.2b(iv)A&B, respectively].

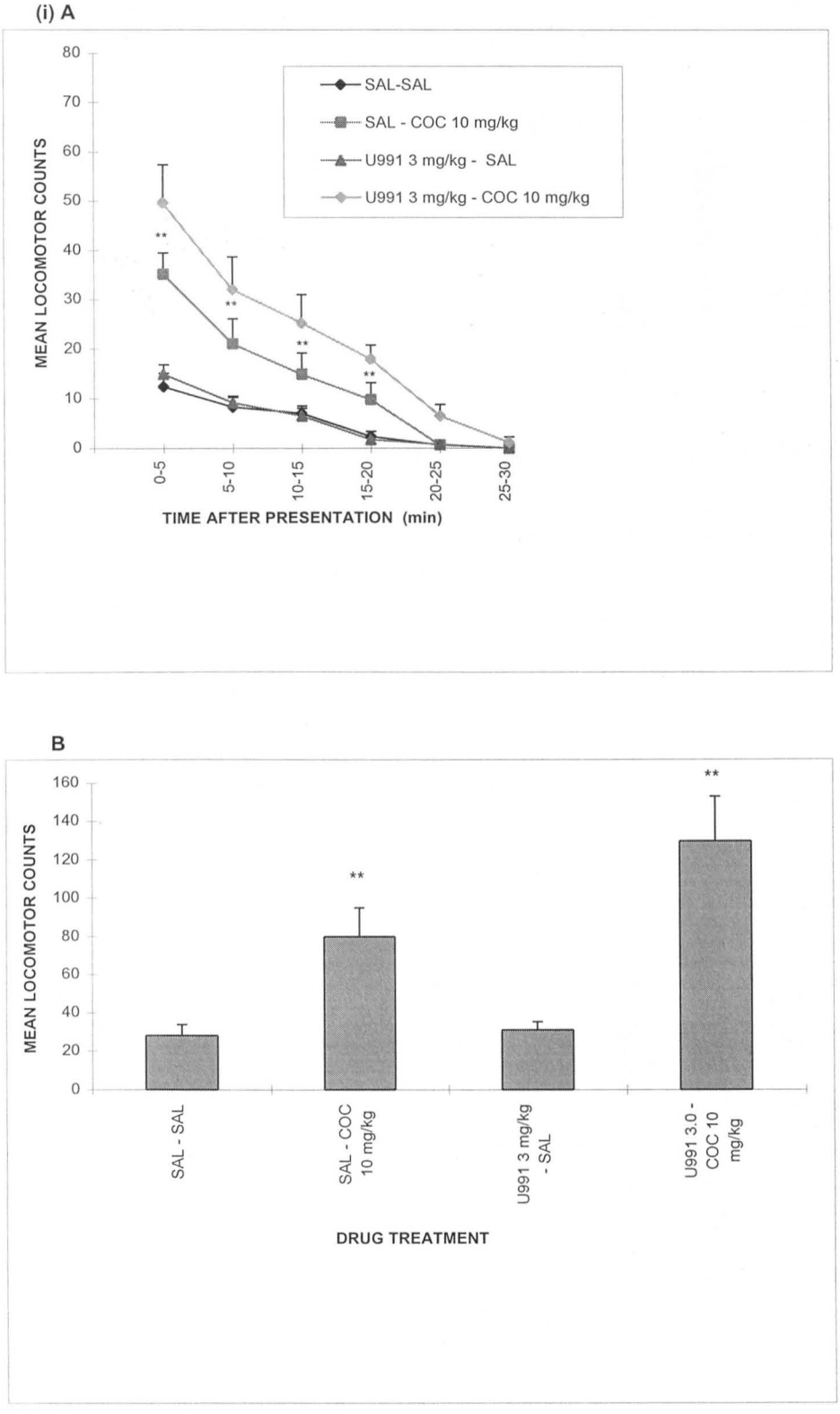
Effect of Antagonist:

Administration of U-99194A produced a significant increase in total duration and bout frequency of locomotor activity [respectively: $F(1,7) = 5.44$, $P<0.05$; $F(1,7) = 11.08$, $P<0.05$] that were evident in the first 10 min of the test session (Fig. 8.2b(ii&iv)A). However, this was not sufficient to produce an overall effect of U-99194A on locomotor counts [$F(1,7) = 4.10$, NS]. This conflicts with the results described in section 6.3.1, where no significant effects on locomotor activity were seen with any dose. The discrepancy in results may be explained due to the different procedures used for the respective studies. Whereas in the present study one characterised one dose in 5 min bins; the results in chapter 6 analysed a number of doses of the drug and the data were collapsed in a 30 min session. The subtle effects of the drug observed in the 5 min bins undoubtedly would be lost in the latter procedure.

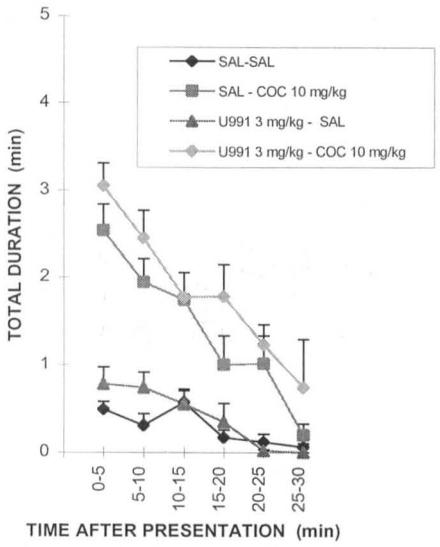
Cocaine and Antagonist Interaction:

As shown in Table 8.2, there were no significant interactions between U-99194A and cocaine, nor between cocaine, antagonist and time on any behavioural parameter for locomotor activity. The results shown in Fig. 8.2b(i)A&B suggests that the U-99194A pre-treatment did not attenuate the hypermotility effects of cocaine, instead U-99194A appeared to potentiate locomotor activity when combined with cocaine, an effect not produced with raclopride.

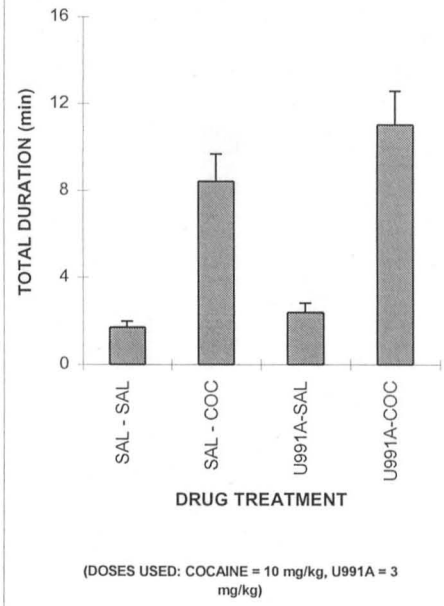
Figure 8.2b.


(i) A
MEAN LOCOMOTOR COUNTS
80
70
60
50
40
30
20
10
0
SAL-SAL
SAL - COC 10 mg/kg
U991 3 mg/kg - SAL
U991 3 mg/kg - COC 10 mg/kg
**
**
**
**
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)
B
MEAN LOCOMOTOR COUNTS
160
140
120
100
80
60
40
20
0
**
**
SAL - SAL
SAL - COC 10 mg/kg
U991 3 mg/kg - SAL
U991 3.0 - COC 10 mg/kg
DRUG TREATMENT

(ii) A

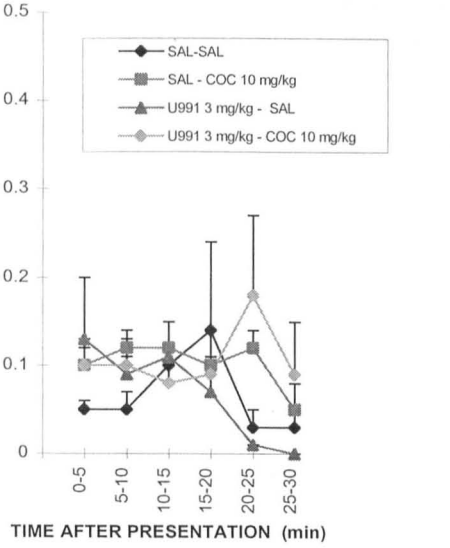


B

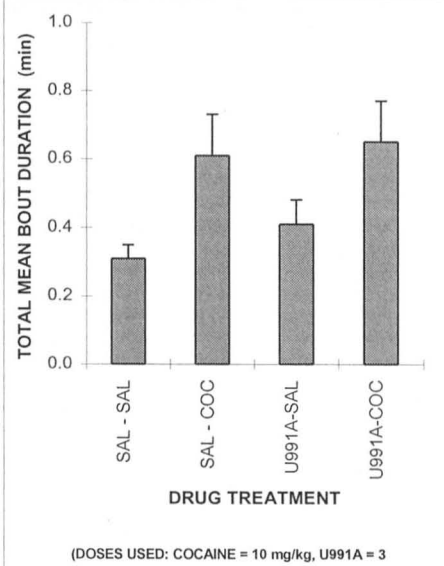


(DOSES USED: COCAINE = 10 mg/kg, U991A = 3 mg/kg)

(iii) A



B



(DOSES USED: COCAINE = 10 mg/kg, U991A = 3 mg/kg)

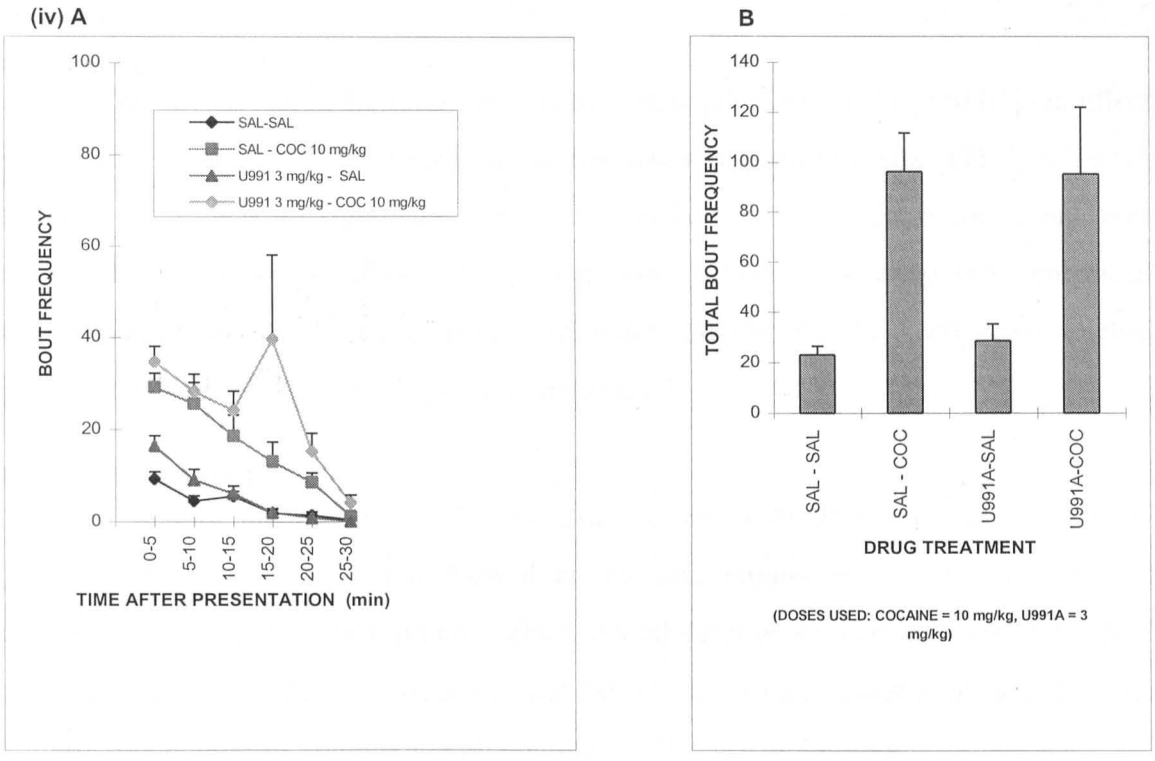


Figure 8.2(b) Effects of pre-treatment with a selective D3 antagonist on cocaine-induced locomotor activity parameters: (i)A&B total locomotor counts (ii)A&B total duration (iii)A&B mean bout duration (iv)A&B bout frequency. N=8 at all points; ** $P<0.01$ compared to saline-controls (comparisons by Dunnett's t-tests)

(c) Rearing.

Effect of Cocaine:

Cocaine increased the total duration of rearing [$F(1,7) = 16.73$, $P<0.01$], an effect which corresponded with increases in the frequency of rearing bouts [$F(1,7) = 19.05$, $P<0.01$]. There was no significant effect of mean bout duration. Time course analyses indicated that cocaine's effects on total duration and bout frequency were enhanced throughout the observation period (Fig. 8.2c(i)A&B and Fig. 8.2c(iii)A&B, respectively). This is consistent with the results from Experiment 1.

Effect of Antagonist:

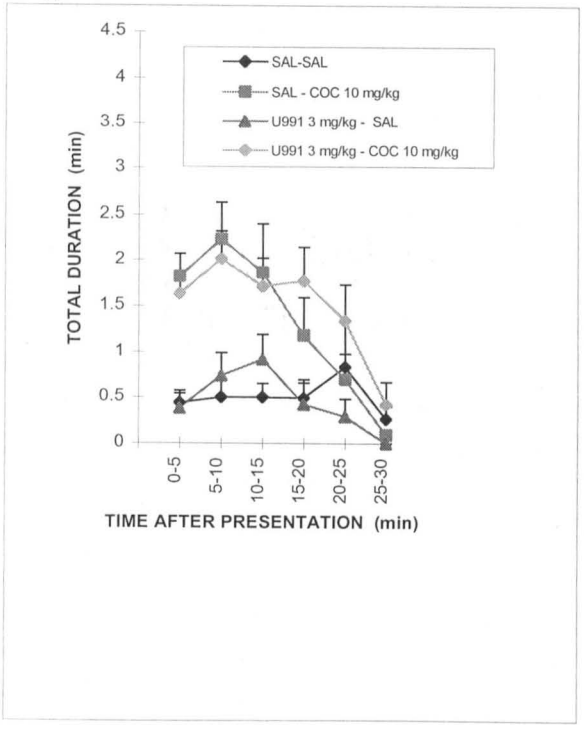
The only parameter significantly affected by U-99194A was bout frequency [$F(1,7) = 6.09$, $P<0.05$] which showed an increase largely due to the cocaine and antagonist condition; but this did not influence total duration of rearing (Table 8.2). Bout frequency was significantly reduced in the earlier experiment (chapter 6), albeit at the slightly higher dose of 10 mg/kg, with not much difference at 3 mg/kg.

Cocaine and Antagonist Interaction:

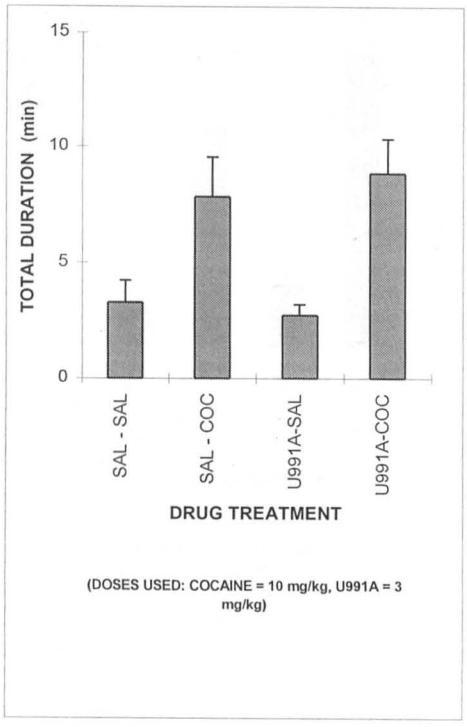
There was a significant interaction between U-99194A, cocaine and time for total duration and mean bout duration [respectively: $F(5,35) = 3.10$, $P<0.05$; $F(5,35) = 2.62$, $P<0.05$]. *Post-hoc* t-tests, on the other hand, did not reveal any significant differences between the combination of U-99194A plus cocaine and cocaine treatment only, for either parameter, at any specific time interval (Fig. 8.2c(i)A and Fig. 8.2c(ii)A). Collapsing data across the 30 min period did not reveal any significant interactions between U-99194A and cocaine on any parameter of rearing activity.

Figure 8.2c.

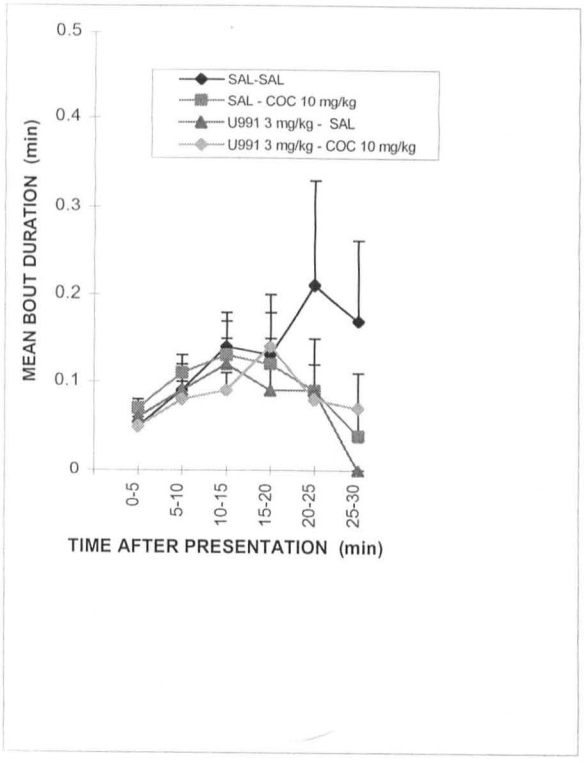
(i) A



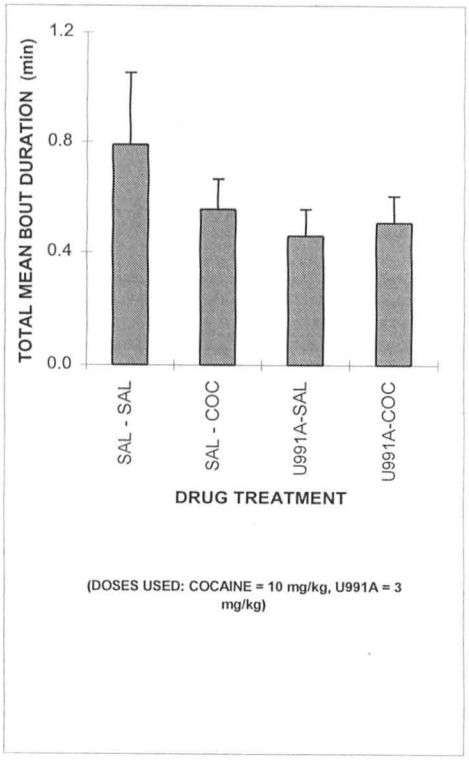
B



(ii) A



B



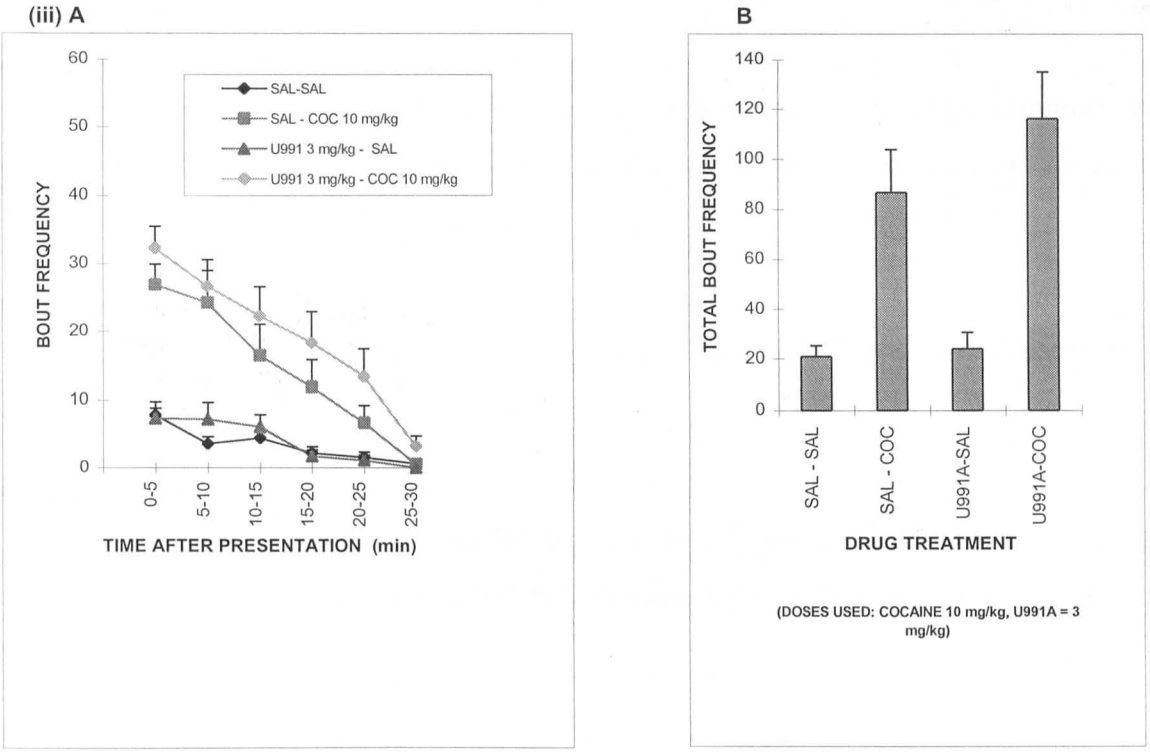


Figure 8.2(c) Effects of pre-treatment with a selective D3 antagonist on cocaine-induced rearing activity parameters: (i)A&B total duration (ii)A&B mean bout duration (iii)A&B bout frequency. N=8 at all points

(d) Grooming.

Effect of Cocaine:

Table 8.2 shows that cocaine produced a decrease in the total duration of grooming [$F(1,7) = 25.25$, $P<0.01$] which corresponded with decreases in mean bout duration [$F(1,7) = 45.09$, $P<0.01$] and bout frequency [$F(1,7) = 15.75$, $P<0.01$].

Effect of Antagonist:

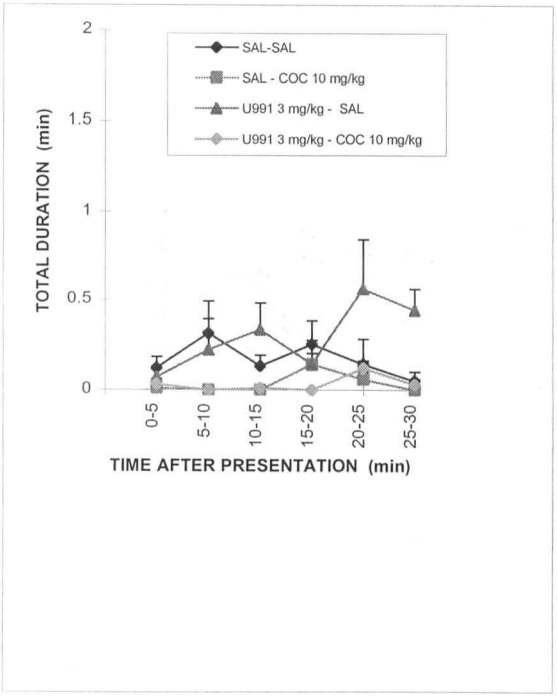
U-99194A did not produce any significant effects on any behavioural parameter of grooming activity.

Cocaine and Antagonist Interaction:

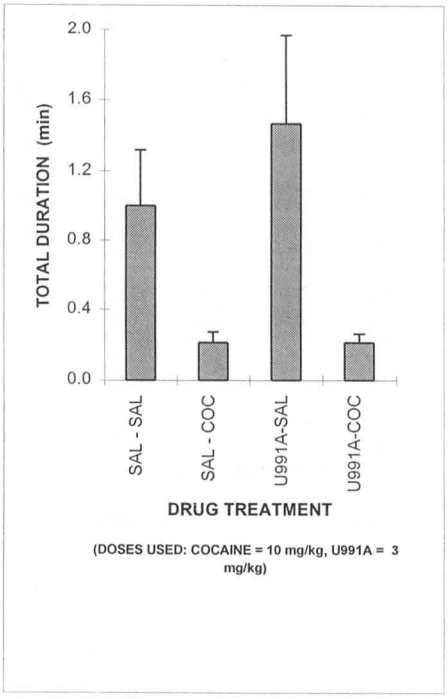
The analysis revealed no significant interactions between U-99194A and cocaine for grooming activity, or between U-99194A, cocaine and time (Fig. 8.2d(i-iii) and Table 8.2).

Figure 8.2d.

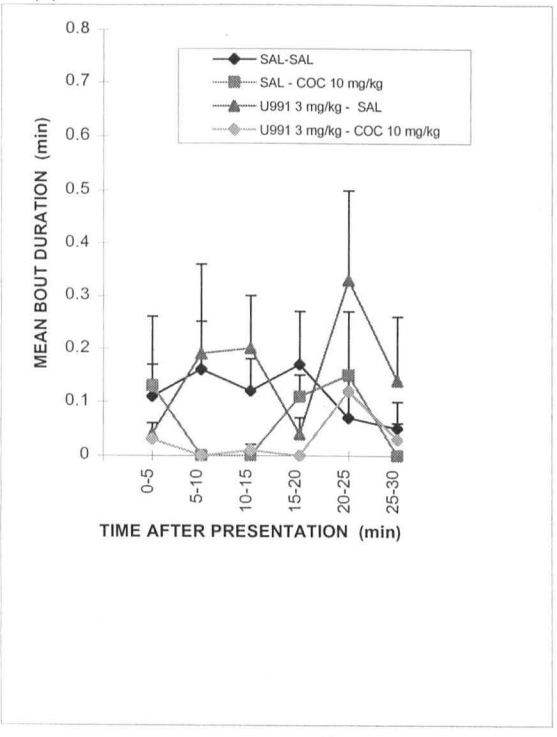
(i) A



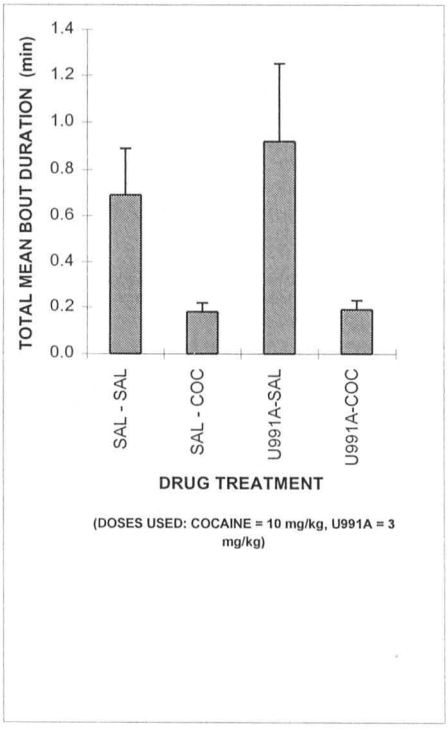
B



(ii) A



B



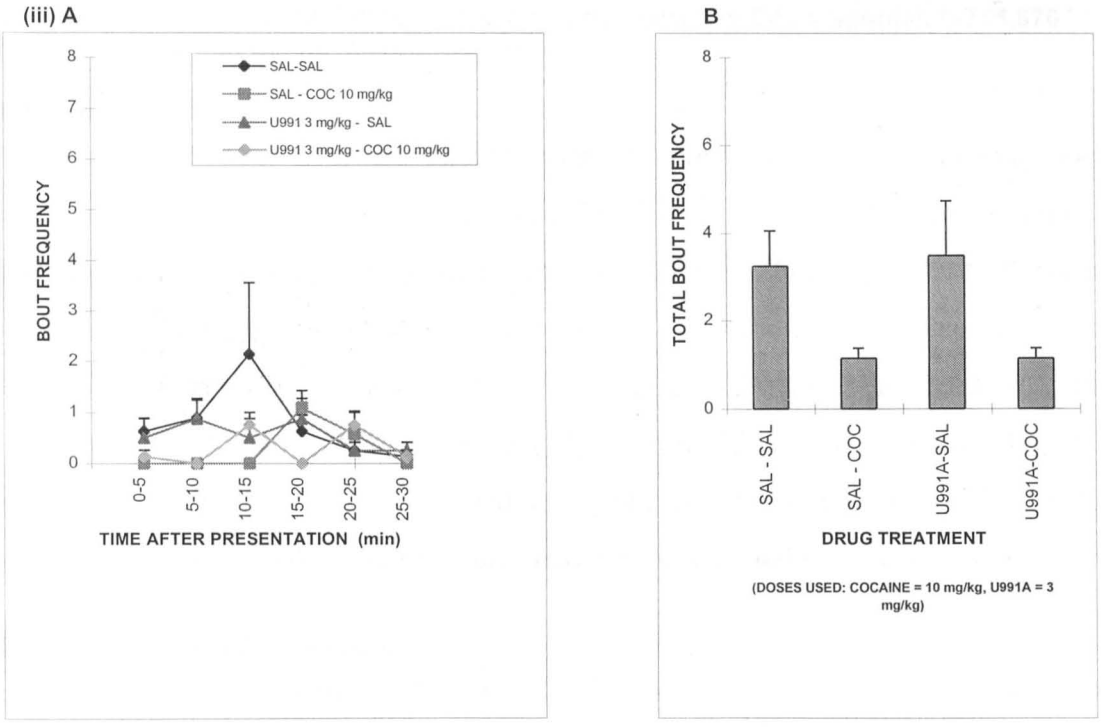


Figure 8.2(d) Effects of pre-treatment with a selective D3 antagonist on cocaine-induced grooming activity parameters: (i)A&B total duration (ii)A&B mean bout duration (iii) A&B bout frequency. N=8 at all points

8.3.3 EXPERIMENT 3: Combination study with the selective D4 antagonist, L-745,870. (a) Food Intake and Eating Microstructure.

Effect of Cocaine:

In agreement with the results from the previous two experiments, cocaine produced a reduction in food consumption [$F(1,7) = 11.79$, $P<0.05$]. This was due to a combined effect of an increased latency to induce feeding [$F(1,7) = 6.02$, $P<0.05$] and a reduction in the total duration of feeding [$(F1,7) = 9.30$, $P<0.05$]. The only notable exception is that for this group, there was no effect of mean bout duration, rather a significant effect on the rate of eating was observed [$(F1,7) = 9.83$, $P<0.05$]; (Fig. 8.3a(i-vi)). However, *post-hoc* t-tests revealed no significant effects between cocaine treatment and vehicle controls when specific comparisons between conditions were made.

Effect of Antagonist Treatment:

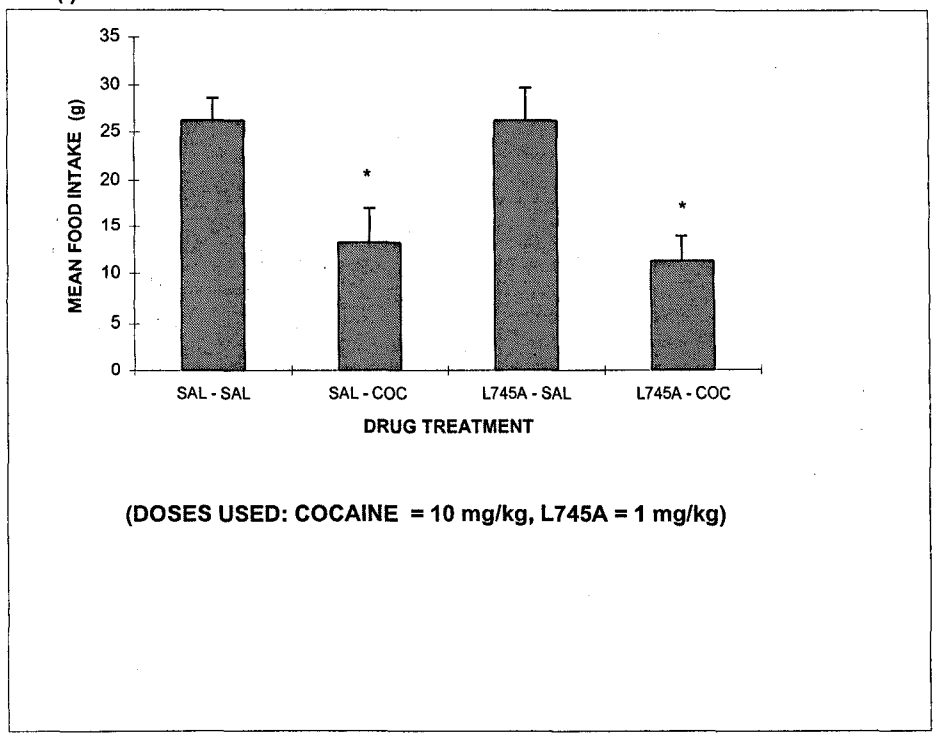
The analysis indicated that L-745,870 did not affect any of the behavioural parameters for feeding (Table 8.3).

Cocaine and Antagonist Interaction:

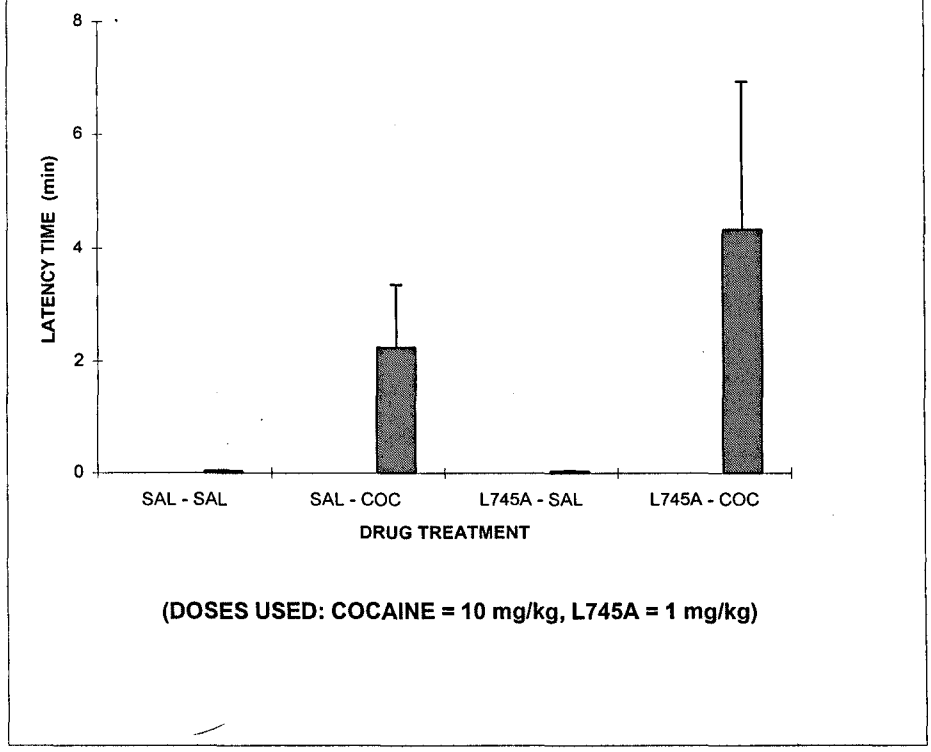
Analysing the results using $2 \times 2 \times 6$ ANOVA with factors antagonist, cocaine and time (Table 8.3) revealed no significant interactions between L-745,870 and cocaine or between L-745,870, cocaine and time for any behavioural parameter of feeding (Fig. 8.3a(iv-vi)A&B).

Figure 8.3a.

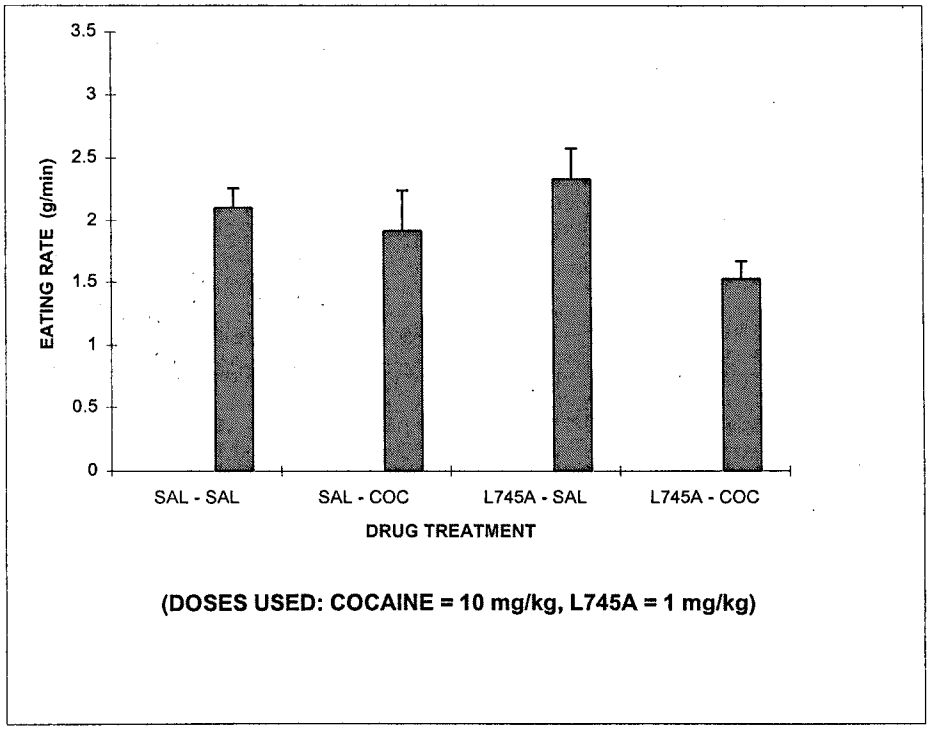
(i)



(ii)

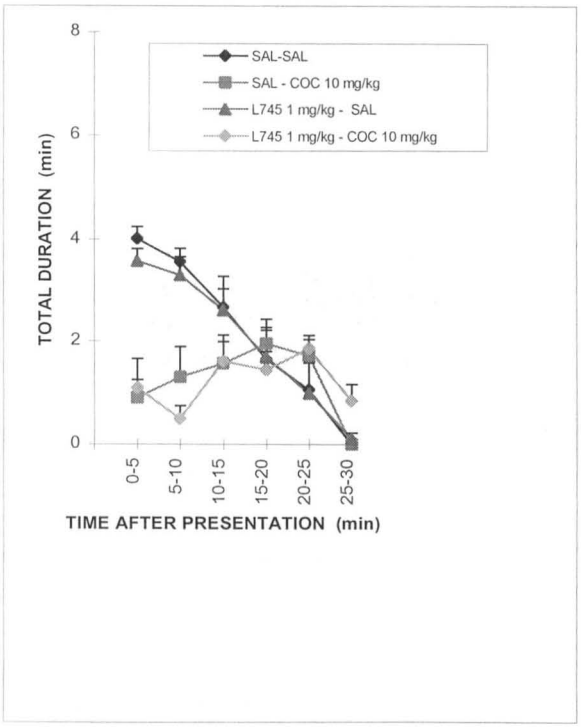


(iii)

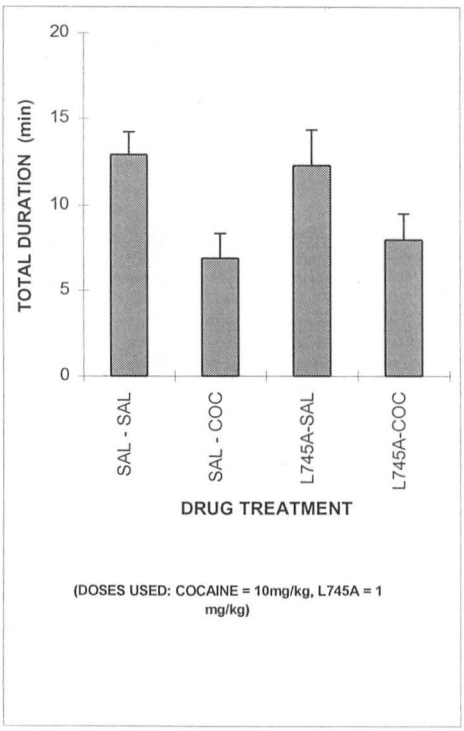


(DOSES USED: COCAINE = 10 mg/kg, L745A = 1 mg/kg)

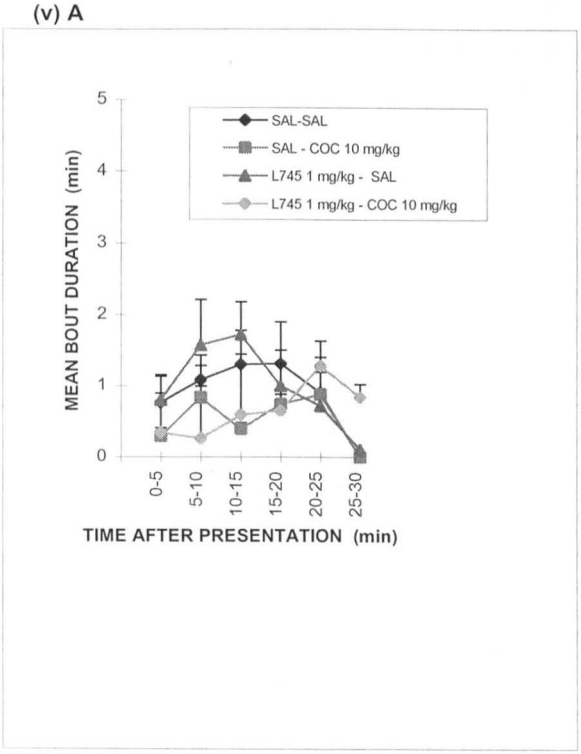
(iv) A


SAL-SAL
SAL - COC 10 mg/kg
L745 1 mg/kg - SAL
L745 1 mg/kg - COC 10 mg/kg
TOTAL DURATION (min)
0
2
4
6
8
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

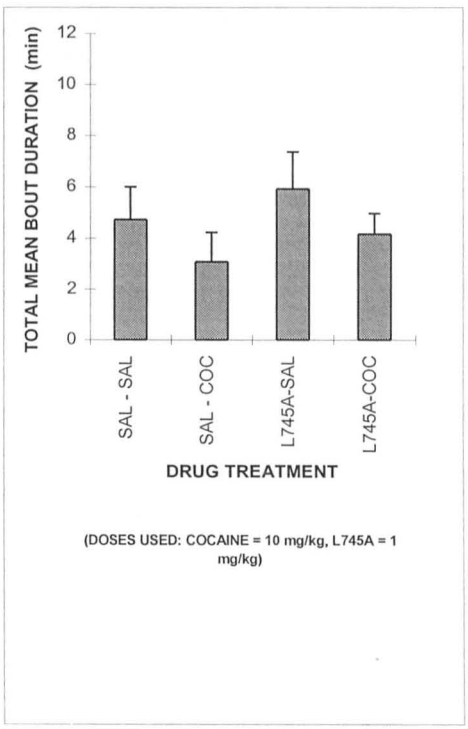
B


TOTAL DURATION (min)
0
5
10
15
20
SAL - SAL
SAL - COC
L745A-SAL
L745A-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10mg/kg, L745A = 1 mg/kg)

(v) A


SAL-SAL
SAL - COC 10 mg/kg
L745 1 mg/kg - SAL
L745 1 mg/kg - COC 10 mg/kg
MEAN BOUT DURATION (min)
0
1
2
3
4
5
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

B


TOTAL MEAN BOUT DURATION (min)
0
2
4
6
8
10
12
SAL - SAL
SAL - COC
L745A-SAL
L745A-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, L745A = 1 mg/kg)

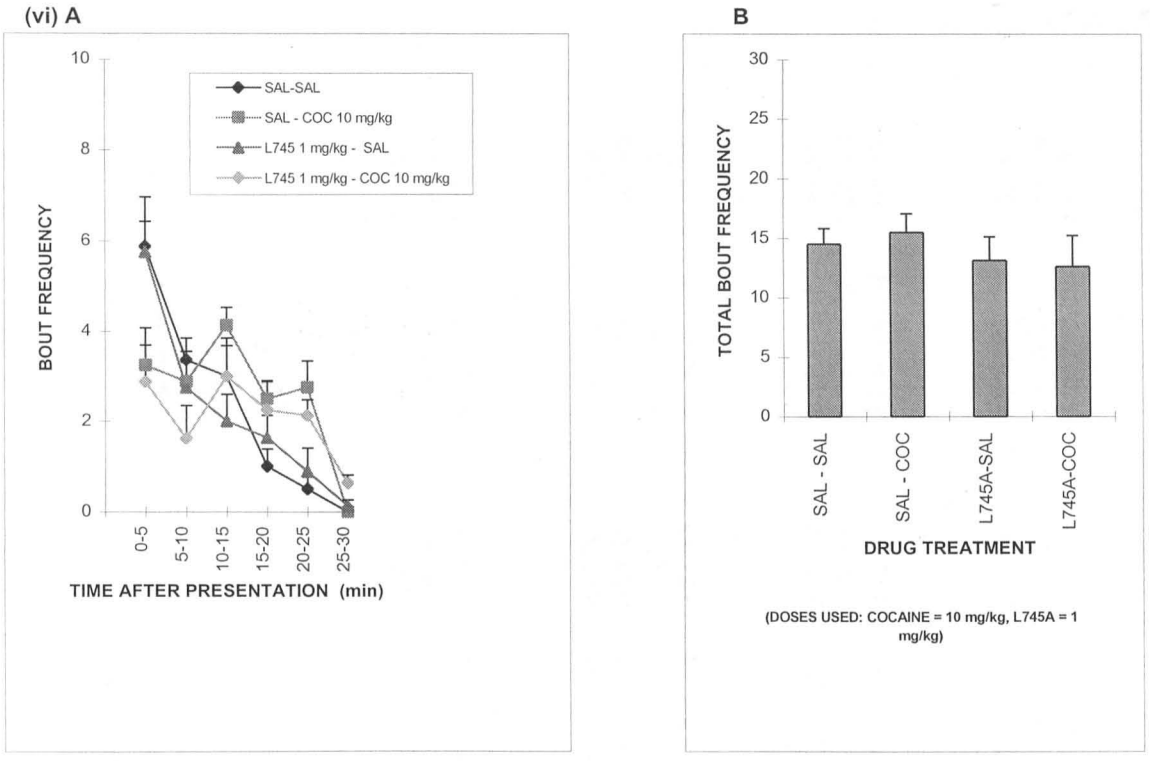


Figure 8.3(a) Effects of pre-treatment with a selective D4 antagonist on cocaine-induced feeding parameters: (i) total food intake (ii) latency to start eating (iii) eating rate (iv)A&B total duration (v)A&B mean bout duration (vi)A&B bout frequency. N=8 at all points; * $P<0.05$ compared to saline-controls (comparisons by Dunnett's t-tests)

TABLE 8.3. 2 (cocaine vs. saline) × 2 (antagonist vs. saline) × 6 (time intervals) ANOVA on behavioural scores from microstructural analysis of L-745,870 in combination with cocaine

RESPONSE CATEGORY	Antag. $F_{(1,7)}$	Coc. $F_{(1,7)}$	Antag. * Coc. $F_{(1,7)}$	Time $F_{(5,35)}$	Antag. * Time $F_{(5,35)}$	Coc. * Time $F_{(5,35)}$	Antag. * Coc. * Time $F_{(5,35)}$
FEEDING							
FOOD INTAKE	0.02 NS	11.79 P<0.05	0.89 NS				
LATENCY	0.49 NS	6.02 P<0.05	0.49 NS				
RATE	0.14 NS	9.83 P<0.05	2.05 NS				
TOTAL DURATION	0.08 NS	9.30 P<0.05	0.03 NS	16.42 P<0.01	1.20 NS	15.51 P<0.01	0.90 NS
BOUT DURATION	0.71 NS	3.67 NS	0.02 NS	3.07 P<0.05	1.03 NS	3.31 P<0.05	1.02 NS
BOUT FREQUENCY	1.75 NS	0.01 NS	0.51 NS	27.01 P<0.01	1.09 NS	9.61 P<0.01	0.31 NS
LOCOMOTOR ACTIVITY							
LOCOMOTOR COUNTS	0.38 NS	16.01 P<0.01	0.06 NS	29.37 P<0.01	0.93 NS	9.60 P<0.01	0.30 NS
TOTAL DURATION	0.04 NS	45.89 P<0.01	0.19 NS	48.09 P<0.01	0.20 NS	8.89 P<0.01	1.52 NS
BOUT DURATION	0.04 NS	6.23 P<0.05	0.49 NS	0.70 NS	0.74 NS	1.23 NS	1.21 NS
BOUT FREQUENCY	0.14 NS	20.48 P<0.01	0.18 NS	27.88 P<0.01	0.21 NS	14.56 P<0.01	0.65 NS
REARING							
TOTAL DURATION	0.09 NS	20.50 P<0.01	1.00 NS	9.70 P<0.01	0.69 NS	2.57 P<0.05	1.08 NS
BOUT DURATION	0.76 NS	0.99 NS	1.07 NS	3.27 P<0.05	0.20 NS	2.76 P<0.05	1.35 NS
BOUT FREQUENCY	0.04 NS	22.54 P<0.01	0.26 NS	22.57 P<0.01	0.15 NS	13.44 P<0.01	0.65 NS
GROOMING							
TOTAL DURATION	1.36 NS	11.96 P<0.05	1.32 NS	1.92 NS	2.36 NS	1.12 NS	2.77 NS
BOUT DURATION	0.10 NS	4.57 NS	0.07 NS	2.44 NS	1.89 NS	1.94 NS	1.96 NS
BOUT FREQUENCY	1.89 NS	6.94 P<0.05	0.14 NS	1.47 NS	1.12 NS	3.29 P<0.05	1.41 NS

(b) Locomotor Activity.

Effect of Cocaine:

Once again, as in the previous two experiments, cocaine significantly increased mean locomotor counts [$F(1,7) = 16.01$, $P<0.01$] (Fig. 8.3b(i)A&B). This was again due to an increase in total duration of locomotor activity [$F(1,7) = 45.89$, $P<0.01$], which was accompanied by an increase in mean bout duration [$F(1,7) = 6.23$, $P<0.05$] and an increase in the frequency of bouts [$F(1,7) = 20.48$, $P<0.05$; Fig. 8.3b(ii-iv)A&B]. The time course for cocaine's effects also mirrored previous experiments in that total duration and bout frequency of locomotor activity were enhanced throughout the observation period.

Effect of Antagonist:

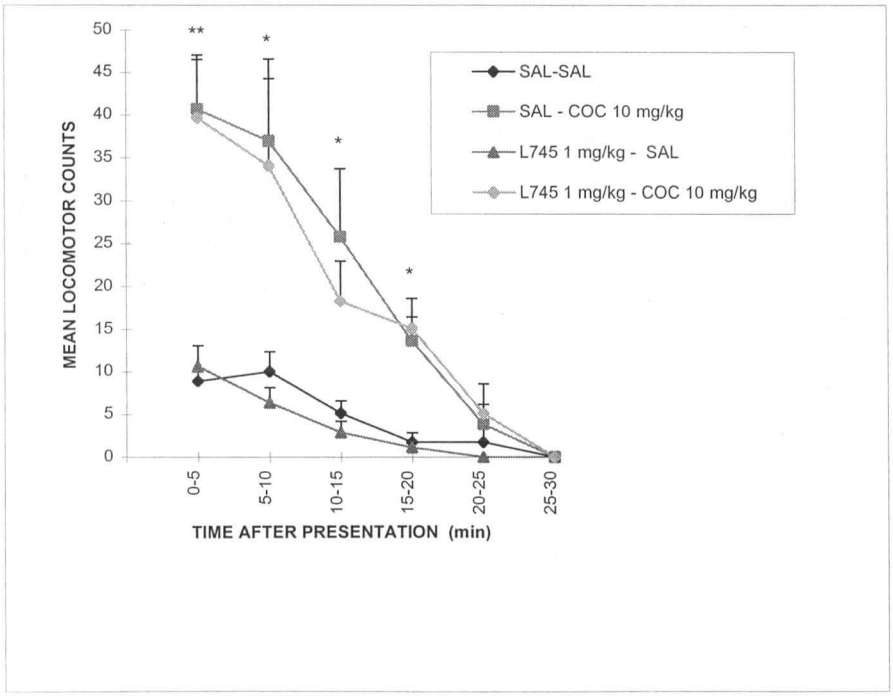
L-745,870 did not affect any of the parameters of locomotor activity (Table 8.3).

Cocaine and Antagonist Interaction:

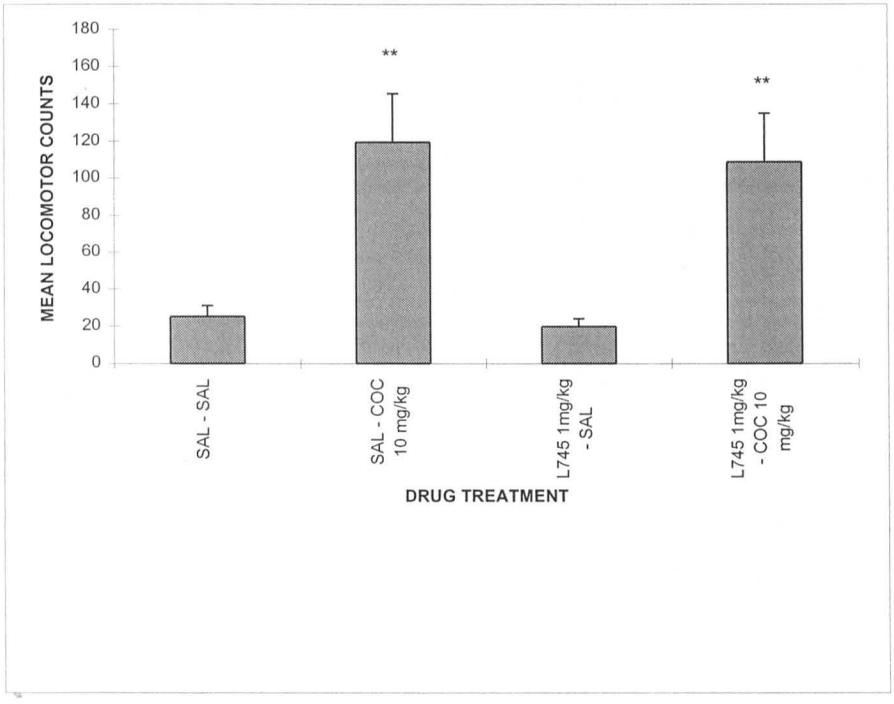
The $2 \times 2 \times 6$ ANOVA also revealed no significant interactions between L-745,870, cocaine and time, or between L-745,870 and cocaine, for any parameter of locomotor activity (Fig. 8.3b(ii-iv)A&B).

Figure 8.3b.

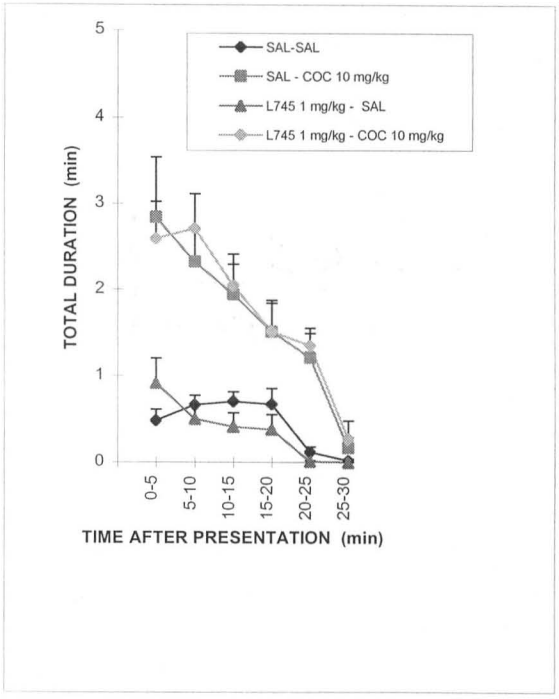
(i) A



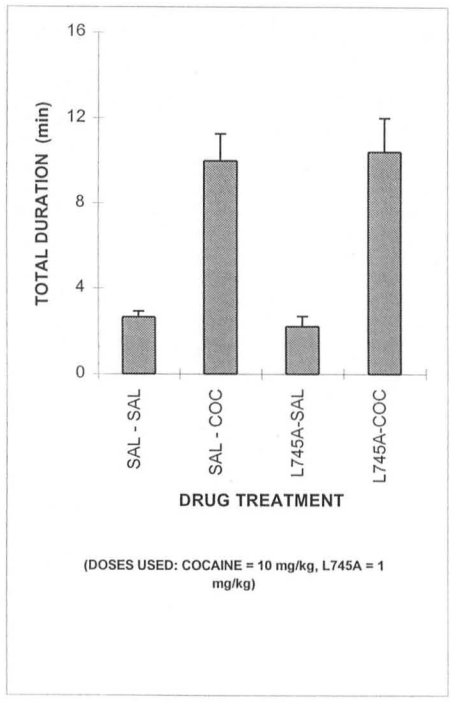
B



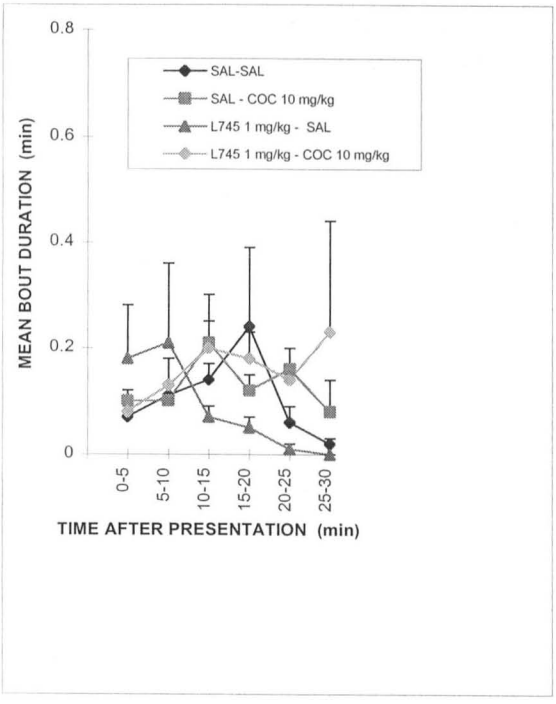
(ii) A


SAL-SAL
SAL - COC 10 mg/kg
L745 1 mg/kg - SAL
L745 1 mg/kg - COC 10 mg/kg
TOTAL DURATION (min)
0
1
2
3
4
5
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

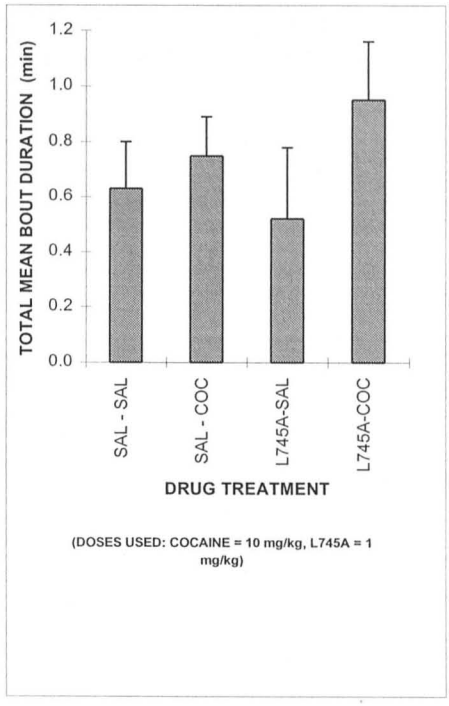
B


TOTAL DURATION (min)
0
4
8
12
16
SAL - SAL
SAL - COC
L745A-SAL
L745A-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, L745A = 1 mg/kg)

(iii) A


SAL-SAL
SAL - COC 10 mg/kg
L745 1 mg/kg - SAL
L745 1 mg/kg - COC 10 mg/kg
MEAN BOUT DURATION (min)
0
0.2
0.4
0.6
0.8
0-5
5-10
10-15
15-20
20-25
25-30
TIME AFTER PRESENTATION (min)

B


TOTAL MEAN BOUT DURATION (min)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
SAL - SAL
SAL - COC
L745A-SAL
L745A-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, L745A = 1 mg/kg)

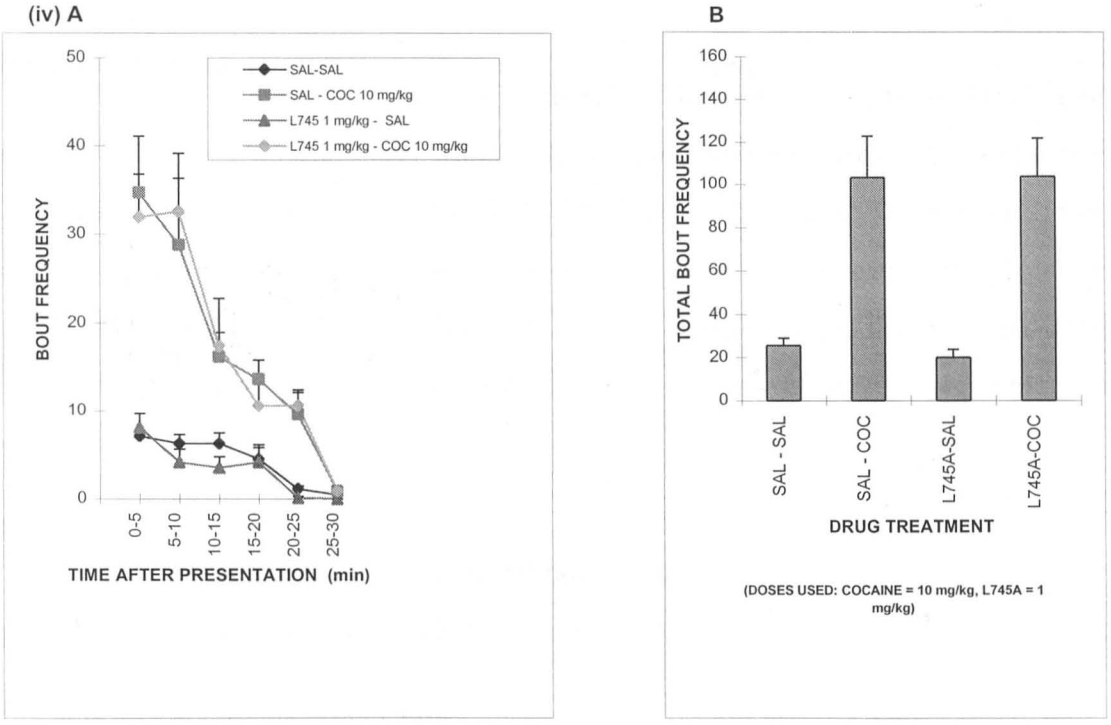


Figure 8.3(b) Effects of pre-treatment with a selective D4 antagonist on cocaine-induced locomotor activity parameters: (i)A&B total locomotor counts (ii)A&B total duration (iii)A&B mean bout duration (iv)A&B bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to saline-controls (comparisons by Dunnett's t-tests)

(c) Rearing.

Effect of Cocaine:

Table 8.3 shows that cocaine produced an increase in the total duration of rearing [$F(1,7) = 20.50$, $P<0.01$] which corresponded with increases in the frequency of rearing bouts [$F(1,7) = 22.54$, $P<0.01$]. Time course analysis indicates that cocaine's effects on total duration and bout frequency of rearing activity were enhanced throughout the observation period (Fig. 8.3c(i)A&B and Fig. 8.3c(iii)A&B, respectively). This is also consistent with the results from the previous two experiments.

Effect of Antagonist:

The D4 antagonist L-745,870 did not produce any significant changes in any parameter of rearing activity (Table 8.3).

Cocaine and Antagonist Interaction:

There were no significant interactions between L-745,870, and cocaine and time, or between L-745,870 and cocaine, for any parameter of rearing activity (Fig. 8.3c(i-iii) and Table 8.3).

(d) Grooming.

Effect of Cocaine:

As in experiment 2, cocaine produced a decrease in the total duration of grooming [$F(1,7) = 11.96$, $P<0.05$] which corresponded with a decrease in bout frequency [$F(1,7) = 6.94$, $P<0.05$; Table 8.3].

Effect of Antagonist:

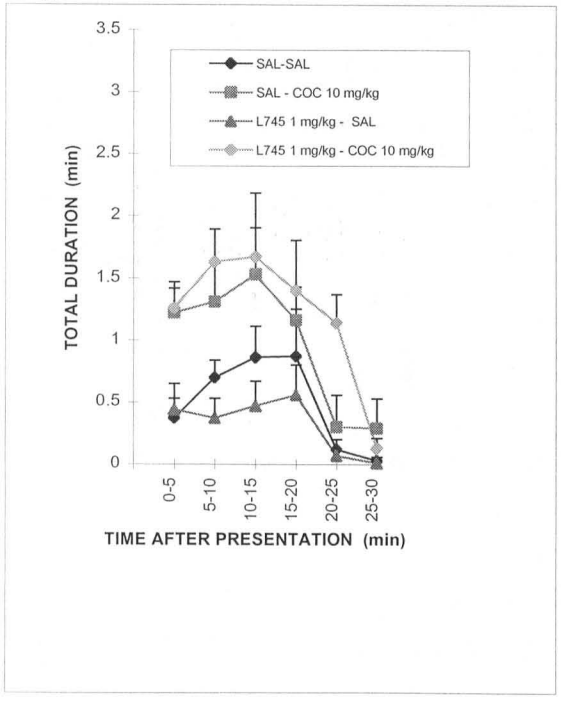
L-745,870 did not produce any significant effects on any behavioural parameter of grooming (Table 8.3).

Cocaine and Antagonist Interaction:

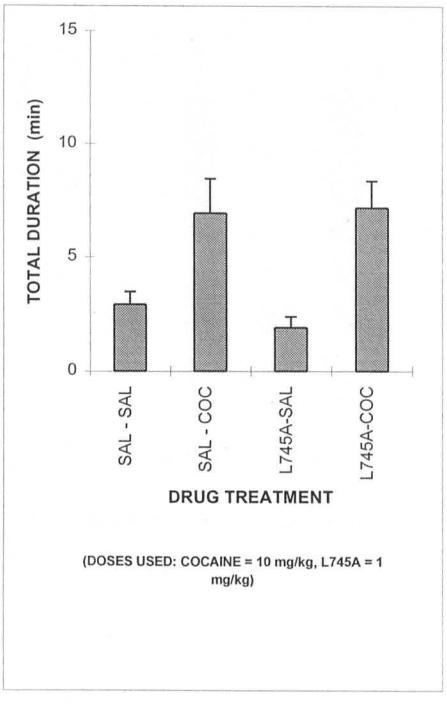
There were no significant interactions between the two drugs, or between the two drugs and time (Fig. 8.3d(i-iii) and Table 8.3).

Figure 8.3c.

(i) A



B



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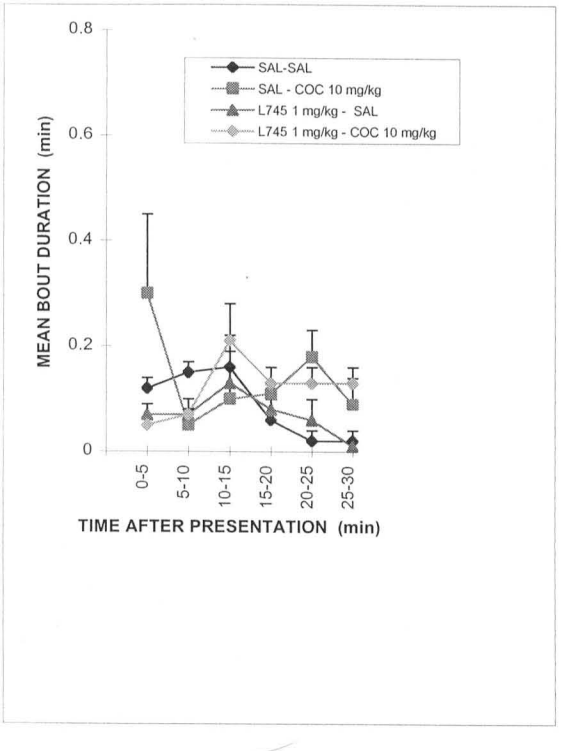

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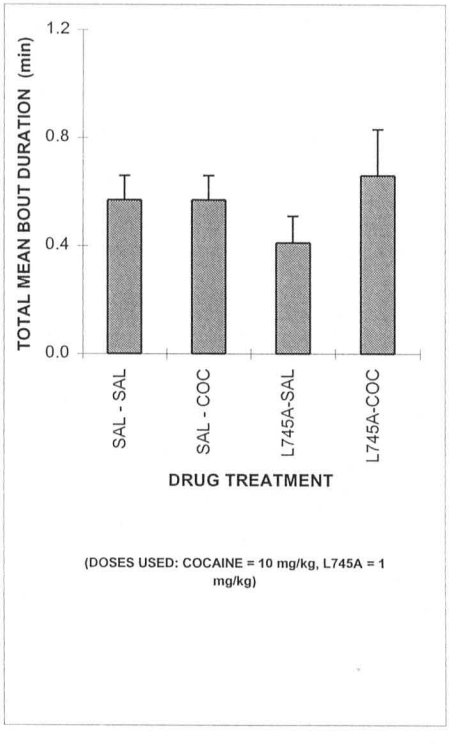


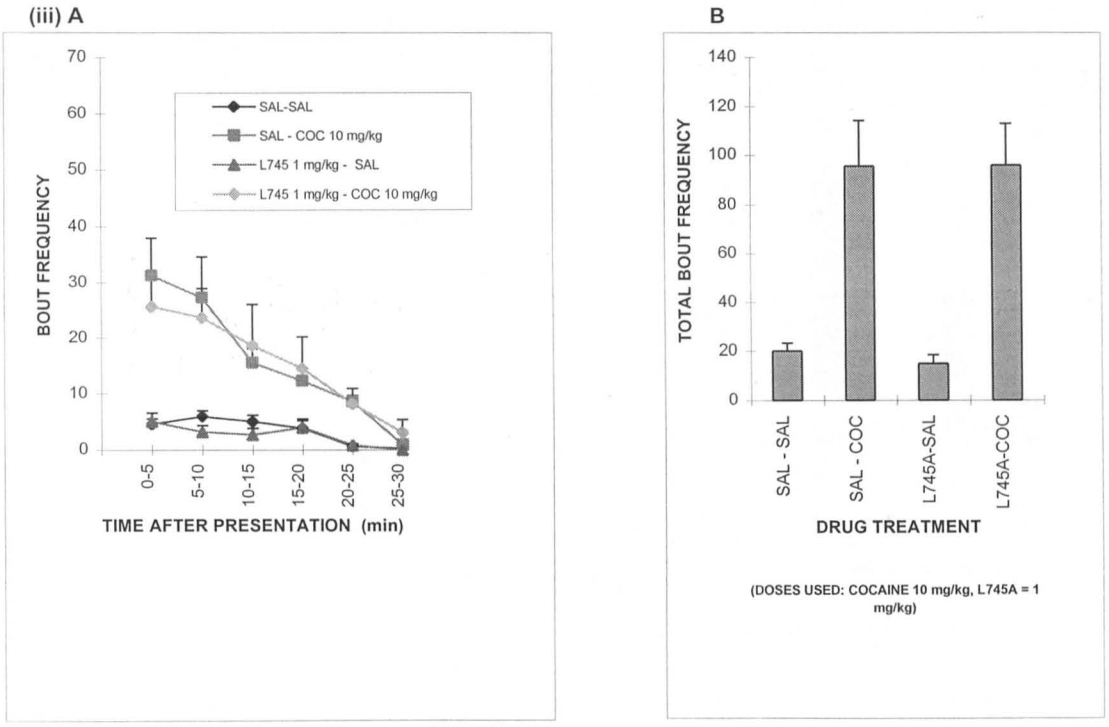
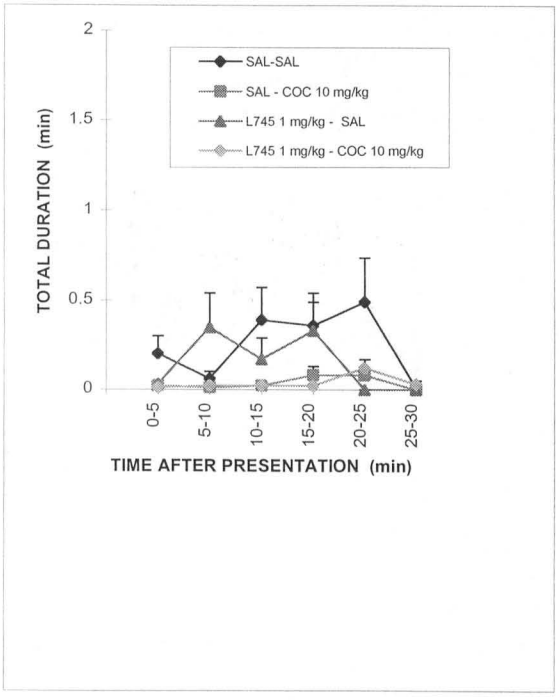


Figure 8.3(c) Effects of pre-treatment with a selective D4 antagonist on cocaine-induced rearing activity parameters: (i)A&B total duration (ii)A&B mean bout duration (iii)A&B bout frequency. N=8 at all points

Figure 8.3d.

(i) A



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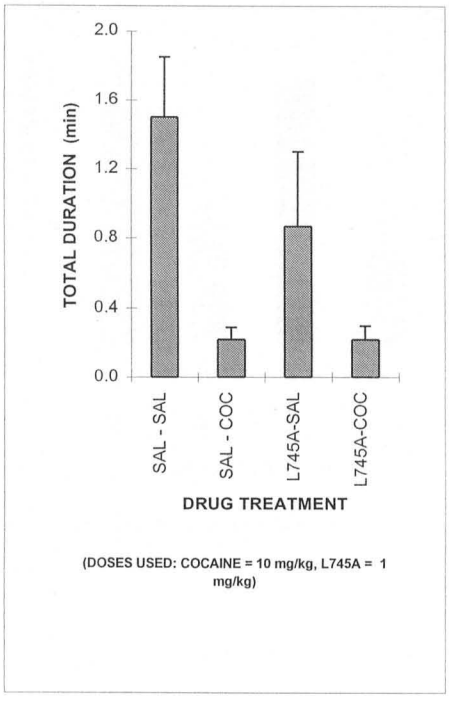
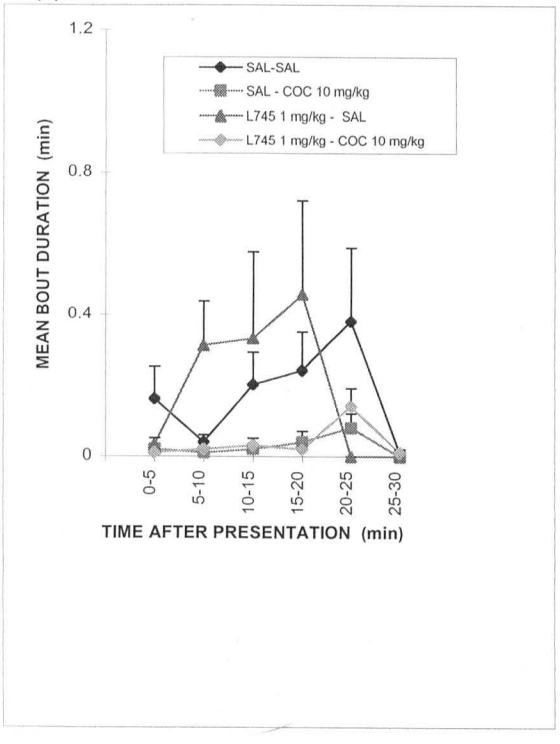


(ii) A



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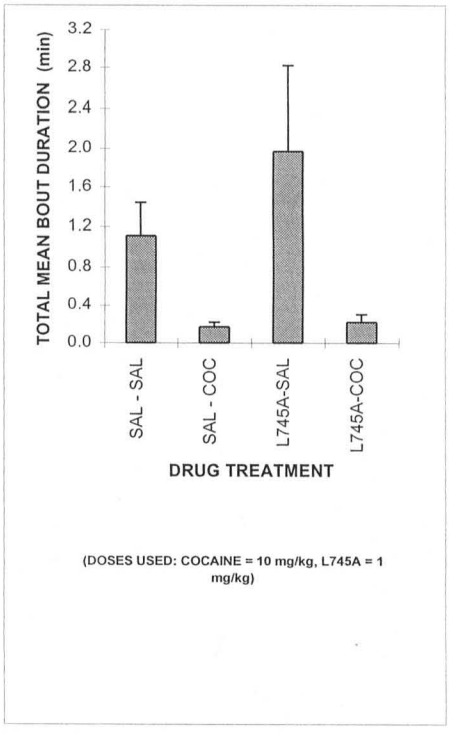

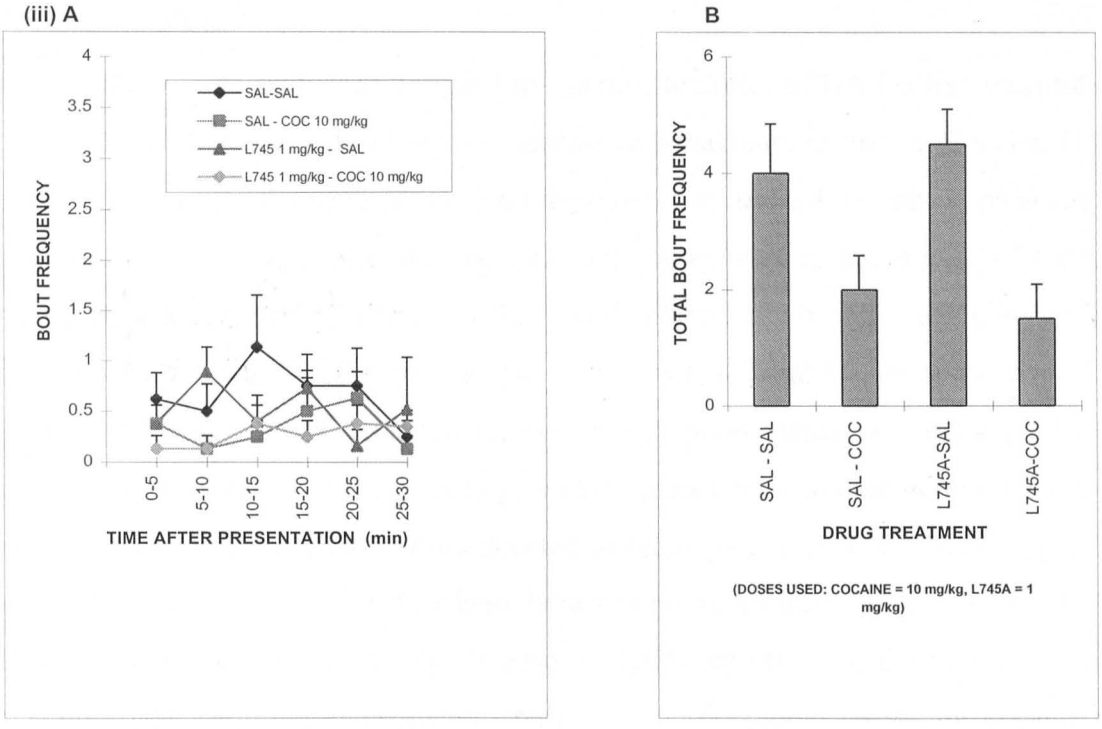

Figure 8.3(d) Effects of pre-treatment with a selective D4 antagonist on cocaine-induced grooming activity parameters: (i)A&B total duration (ii)A&B mean bout duration (iii)A&B bout frequency. N=8 at all points

8.4 Discussion

The present study was designed to examine the roles of DA D2-like receptors in cocaine's effects on food intake and associated behaviours in the rat. Cocaine (10 mg/kg, i.p.) was administered to food-deprived rats trained to eat a palatable, sweetened mash. Food intake and associated behaviours were recorded over a 30 min period. Cocaine produced distinct behavioural effects which were generally very consistent throughout the experiments (see Tables 8.1, 8.2 and 8.3 for comparisons). There was a pronounced reduction in food consumption, which was caused by an increase in the latency to induce feeding, with decreases in mean bout duration, which overall resulted in a decreased time devoted to feeding. The only notable exception was in Experiment 3 (Table 8.3), where there was no indication of a significant effect on mean bout duration by cocaine. Instead, a significant effect on the rate of eating was observed. Despite this anomalous result, the observational results for cocaine's feeding profile were consistent throughout of three independent experiments. This was also the case for the experiment described in section 7.3.2 although latency was significantly affected at 30 mg/kg rather than 10 mg/kg. Time course data indicated that cocaine's duration of action was 20 min. Measures of total duration and mean bout duration were suppressed initially, and then rose to baseline levels towards the end of the test. Vehicle treatment, on the other-hand, produced the highest level of feeding at the start of the test, with the level of feeding subsequently diminishing as the animals became satiated.

Cocaine also significantly increased mean locomotor counts throughout the first 25 min of the test session. The duration of individual bouts showed little change in the first experiment (Table 8.1), however significant effects of this parameter were found in the latter two experiments (Table 8.2 and 8.3). Consistently throughout, there were large increases in the frequencies of bouts, which was the sole contributor to cocaine significantly increasing the total duration of locomotor activity at 10 mg/kg. The time course for cocaine's effects indicates that total duration and bout frequency of locomotor activity were enhanced throughout the observation period. Once again, the effects of cocaine on locomotor activity mirrored the earlier results (see section 7.3.2). A similar profile also occurred for rearing activity, which corresponded with increases in the frequency of rearing bouts. There was no significant effect of mean

bout duration (also see section 7.3.2). Time course analysis indicated that cocaine's effects on total duration and bout frequency of rearing activity were enhanced throughout the observation period.

Grooming behaviour was also affected by cocaine, with significant suppression of nearly all parameters recorded, the only exception being in the first experiment (Table 8.1). This may well be due to the effect being masked by the strong interaction with raclopride. Although no significant outcomes were reported in section 7.3.2 for the effects of 10 mg/kg cocaine on grooming, a similar profile was obtained.

The effects of a series of D2-like receptor antagonists with differing selectivities for D2, D3 and D4 receptors, given as pre-treatments to cocaine, were then compared. The D2/D3 antagonist raclopride (0.1 mg/kg) produced only a marginal attenuation of cocaine's effects on feeding. In effect, raclopride only altered one parameter of cocaine's effect on feeding microstructure, that of feeding bout frequency. There was a significant interaction between raclopride, cocaine and time for this parameter, with *post-hoc* t-tests revealing attenuation by raclopride at time intervals 5-10 and 10-15 min. Collapsing across the 30 min period, there was also an interaction between raclopride and cocaine for this parameter. Because other parameters of feeding, such as total duration and mean bout duration, were not affected by pre-treatment with raclopride, the effect on feeding bout frequency was not sufficient to attenuate overall the effect of cocaine on food intake.

Recently, Rapoza and Woolverton (1991) examined the role of DA receptors in the effect of cocaine on sweetened milk intake. Cocaine produced dose-dependent decreases in milk consumption, and raclopride attenuated the effects of at least one of cocaine's effective doses. Stimulation of D2/D3 receptors, therefore, may be involved in cocaine's suppressant effects on milk ingestion. However, the present results suggests that D2-like receptors play a minor role in cocaine's suppressant effects on food intake. The differences in results between their study and the present one might relate to the differences in the diets used in the two studies (i.e. liquid diet of sweetened condensed milk versus a mash diet). However, it might also be a result of the more conservative method of selecting behaviourally inactive doses used here: in this study a dose was used that had minimal effects on its own (Terry, 1996); Rapoza and Woolverton reported attenuation by doses of the antagonist which themselves

induced hypophagia (i.e. 0.3 and 1.0 mg/kg). Combination studies using selective antagonists with cocaine become difficult to interpret when the antagonist itself has strong, significant effects when administered alone. This is particularly relevant when choosing compounds from the D2-like subfamily, due to their common feature of inducing biphasic effects on food intake (see section 4.3). The need to characterise raclopride administered alone is therefore essential when carrying out full interaction studies with cocaine. This is particularly important in relation to antagonism of cocaine's anorectic effect. The only dose of raclopride which consistently produced minimal disruption of the parameters of feeding and other aspects of motor behaviour was 0.1 mg/kg (Terry, 1996), and so it was chosen as the optimum dose for the combination study here. Table 8.1 reinforces this dose selection as the results clearly show that raclopride did not affect any feeding parameters when this dose was administered alone although it is apparent that the dose has marginal behavioural activity from its effects on locomotion and rearing. Other D2-like antagonist drugs, such as sulpiride and haloperidol, were not used as no data for such drugs have been published in terms of their microstructural profiles of effects on feeding and associated behaviours; therefore, choosing a reliable dose for the combination study would have presented difficulties. Additionally, one advantage of choosing raclopride over sulpiride concerns pharmacokinetics rather than pharmacodynamic effects (sulpiride does not cross the blood brain barrier as easily as raclopride, and it requires a 60 min pre-treatment time, therefore presenting methodological problems in observing the individual animal's behaviour within a suitable time period).

In agreement with the feeding data, raclopride produced only a marginal attenuation of cocaine's effects on locomotor and rearing activity, with the only interactions observed being for rearing bout frequency when behavioural counts were collapsed across the 30 min period. The results imply that the blockade of D2/D3 receptors should not be effective in altering cocaine-induced locomotor and rearing activity. There are reports in both mice and rats that show other D2-like antagonists (e.g. sulpiride and haloperidol, which have similar affinities for D2 and D3 receptors, when compared with raclopride) to be less effective at reversing cocaine-induced locomotor behaviours at non-sedative doses (Cabib et al., 1991; Ushijima et al., 1995). But there is some inconsistency in the literature, since some other studies have

demonstrated effective antagonism by D2-like antagonists. In particular, localisation studies by Tella (1994) and Neisewander et al. (1995) have produced conflicting results compared with systemic administration studies. Infusion of cocaine directly into the nucleus accumbens can elicit locomotor activity that can be reversed by systemic administration of the non-selective DA antagonist cis-flupenthixol (Delfs et al., 1990). Sulpiride not only reverses cocaine-induced stereotypies when infused into the caudate putamen (Arnt, 1985), it also reverses cocaine-induced locomotion when infused into the nucleus accumbens (Neisewander et al., 1995; Baker et al., 1996). The results of these studies suggests that blockade of D2-like receptors by sulpiride, in the nucleus accumbens, is sufficient to reverse cocaine-induced locomotion. The discrepancy as to whether D2-like antagonists can attenuate cocaine-induced locomotor activity seems to depend on whether these compounds are administered systemically or locally into specific regions of the brain.

Consistent with the present data suggesting that D2/D3 receptors may be marginally involved in cocaine-induced motor behaviours, Tirelli at al. (1997) have demonstrated negligible interactions between cocaine and D2-like agonists on locomotor activity, suggesting a lack of involvement of D2-like receptors in such behaviour. In their study, cocaine did not reliably alter the hypoactivity produced by any of three D2-like agonists: 7-OH-DPAT, quinpirole and RU 24213. These compounds are all D2/D3 agonists (Sokoloff et al., 1990; Levesque et al., 1992; Levant et al., 1995) and target similar receptors to raclopride. By demonstrating negligible interactions between cocaine and D2-like agonists in mice, the study is consistent with the present one failing to demonstrate any necessary involvement of D2/D3 receptors in one of the behavioural effects of cocaine, using different species and investigating antagonist drugs instead of agonists.

One of the most striking effects of raclopride pre-treatment with cocaine was its marked attenuation of cocaine-induced decreases in grooming behaviour. A significant interaction was observed for mean bout duration and bout frequency, but not for total duration. The data implies that grooming behaviour may provide a particularly sensitive index of cocaine's action at D2/D3 receptors. Since cocaine significantly suppresses grooming and raclopride was able to attenuate this profile, it is possible that stimulation of D2/D3 receptors exclusively is sufficient to account for

cocaine's effects on grooming. Consistent with the idea that this may be a D2-like mediated effect, D2-like receptor agonists such as N-0437 have been shown to suppress grooming significantly (Rusk and Cooper, 1989); indeed, residual D1-like stimulation by cocaine-potentiated dopamine after D2/D3 blockade may be expected to elevate grooming.

Behavioural and pharmacological studies of the DA D3 receptor have prompted several hypotheses regarding its function. The D3 antagonist, U-99194A at 3 mg/kg, produced negligible attenuation of cocaine's effects on food intake. If anything, the results suggested a potentiation of the anorectic effect when U-99194A was given with cocaine. This profile was evident at certain time intervals (particularly late in the session) for total duration and mean bout duration, which led to significant interactions between U-99194A, cocaine and time for these parameters, but not for bout frequency. In comparison with raclopride, which also has affinity for D3 receptors, U-99194A plus cocaine produced a different profile on food intake. This potentiation by U-99194A of cocaine's effects on food intake suggests that concomitant D2 blockade by raclopride eliminates this trend.

Recently, reports have emerged that a post-synaptic sub-population of DA D2-like receptors (possibly the D3 molecular isoform of the receptor) can mediate decreases in spontaneous locomotor activity (see section 1.3.2). This is based on the evidence that purported antagonists with selectivity for the D3 receptor subtype, such as U-99194A, sometimes increase locomotor activity without affecting DA release (Waters et al., 1993; Svensson et al., 1994). Therefore, it was interesting to note the behavioural effects of pre-treatment with a sub-maximal dose of U-99194A with cocaine. There were no significant interactions between U-99194A and cocaine on any parameter for locomotor activity, although U-99194A appeared to potentiate (to some extent) locomotor activity when combined with cocaine, an effect not produced by raclopride. In contrast, there was a significant interaction between U-99194A, cocaine and time for total duration and mean bout duration for rearing activity, for which a potentiating profile was observed. This is the first report of a potentiating effect of a DA D3 receptor antagonist with cocaine, albeit rather modest.

There is evidence in the literature, however, showing that DA putative D3-preferring agonists interact with effects mediated by other DA receptors. For

example, Thorn et al. (1997) demonstrated that low doses of the DA D3/D2 receptor agonist, quinelorane, could antagonise amphetamine-stimulated-hyperactivity. In addition, quinelorane had no significant effect on amphetamine-enhanced DA release suggesting that the behavioural response to quinelorane is not evoked by autoreceptor stimulation. In another study, the putative DA D3-receptor agonists 7-OH-DPAT and PD 128907 significantly suppressed SK&F 81297 (D1-like agonist) induced hyperactivity at high doses, suggesting that these D3 agonists may influence specifically D1-receptor mediated behaviours via post-synaptic D3 receptors (Mori et al., 1997). Thus, the D3 receptor may have some modulatory control over the behavioural effects of cocaine, perhaps rearing activity. In particular, by removing this tonic control by administration of an antagonist selective for this receptor subtype, greater stimulation of either D1-like or D2-like receptor subtypes may occur, and hence the effects of cocaine may be potentiated. The results also revealed no significant interactions between U-99194A and cocaine for grooming activity, suggesting that the decrease in grooming induced by cocaine is not mediated by the D3 receptor subtype. Instead, the attenuation produced by raclopride reinforces the conclusion that this is a D2 receptor-mediated effect.

Finally, the D4 receptor antagonist L-745,870 failed to antagonise any of the effects of cocaine on behaviour. This is consistent with previous work on this compound where it has been shown to have no effects in tests of antipsychotic activity (Bristow et al., 1996) nor on cocaine's DS effects (Chapter 3). For L-745,870 to act exclusively as a DA D4 receptor antagonist in vivo is dependent on the dose administered. At doses up to 1 mg/kg p.o. in rats, any effects observed with this compound are likely to be mediated via D4 receptor antagonism, since at this dose L-745,870 will occupy > 90% of D4 receptors in the CNS (Patel et al., 1996b). Therefore, the results suggest that the D4 receptor subtype does not contribute to cocaine-induced behaviours, and this lack of effect cannot be attributed to the dose of L-745,870 being non-selective or having no significant receptor occupancy.

In conclusion, the results of the present study suggests that indirect stimulation of receptors of the D2-like subfamily may not be important to cocaine's effects on food intake and locomotor activity. The D2/D3 antagonist raclopride produced only marginal attenuation of cocaine's effects on food intake, locomotor and rearing

activity, but it clearly attenuated suppression of grooming by cocaine, strongly suggesting that this is a D2-mediated effect. The D3 antagonist U-99194A, produced a modest potentiating effect on certain parameters of cocaine-induced hypophagia and rearing. This result implies that stimulation of D3 receptors by cocaine may inhibit activity, and removing this effect by blocking the D3 receptor may subtly potentiate some cocaine-induced behaviours. Finally, the selective D4 antagonist L-745,870 failed to alter any of the behavioural effects induced by cocaine, and in agreement with studies of cocaine's DS effects, suggests a total lack of involvement of this receptor subtype in cocaine's effects on feeding, locomotor activity, rearing or grooming.

CHAPTER 9:

'MICROSTRUCTURAL ANALYSIS OF A 'D1-LIKE' RECEPTOR SUBTYPE ANTAGONIST IN COMBINATION WITH COCAINE'

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MICROSTRUCTURAL ANALYSIS OF A 'D1-LIKE' RECEPTOR SUBTYPE ANTAGONIST IN COMBINATION WITH COCAINE

9.1 Introduction And Aims

In the previous chapter, it was demonstrated that DA D2-like receptors do not seem to be necessarily involved in certain effects of cocaine on feeding, locomotor and rearing in the rat, since blockade of these receptors is not always sufficient to reverse cocaine-induced effects. The effect most strongly attenuated by the D2/D3 antagonist raclopride was cocaine's suppression of grooming. The limited abilities of the D2-like antagonists to attenuate the behaviours induced by cocaine prompts an assessment of the specificity of these results by investigating the involvement of DA D1-like receptors using similar methods. The purpose of the present study was therefore to examine whether DA D1-like receptors play a key part in cocaine's effects on such behaviours. This was examined by administering the D1-like selective antagonist SCH 39166 (Chipkin et al., 1988). SCH 39166 was chosen over the other prototypical D1-like receptor antagonist SCH 23390 (Iorio et al., 1983) because SCH 39166 possesses a higher selectivity for D1 vs. 5-HT_2 and 5-HT_{1c} receptors. The ratio of Ki's for 5-HT_2 and D1 receptors is about 90 for SCH 39166 and about 30 for SCH 23390. Moreover, the ratio of Ki's for 5-HT_{1c} and D1 is about 700 for SCH 39166 and about 75 for SCH 23390 (Chipkin et al., 1988; McQuade et al., 1991).

As described in section 4.3.2, D1-like antagonists have been shown to reduce food intake both in food-deprived rats (Gilbert and Cooper, 1985; Zarrindast et al., 1991; Terry and Katz, 1994) and in free-feeding animals (Clifton et al., 1991; Naruse et al., 1991). Biphasic effects seen with the D2-like antagonists on ingestive behaviour do not arise with drugs acting at D1-like receptors. Therefore, for food intake, the D1-like antagonist effect on feeding is less problematic when designing combination studies with cocaine, since both drugs act in the same direction, i.e. decrease intake. For example, Terry and Katz (1994), investigating the effects of the D1-like receptor antagonists SCH 23390 and SCH 39166 on feeding behaviour, showed that doses of 0.3 mg/kg and above produced inhibition of food intake. Consistent with this notion, Terry (1996) characterised further the anorectic effects of SCH 39166 using a

microstructural study and showed that 0.3 mg/kg significantly reduced some aspects of feeding (notably, a significant reduction in the number of meals consumed). In addition, this effect coincided with significant reductions in other kinds of motor behaviour: locomotion, rearing and grooming. Therefore, based on these prior studies, it was decided that 0.03 and 0.1 mg/kg doses of SCH 39166 would be used for combination with cocaine (in a balanced 'Latin Square' design for repeated measures) as microstructural analyses have shown these to be below the threshold of behaviourally activity when administered alone.

9.2 Materials and methods

9.2.1 Subjects:

Eight male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 320-360 g were housed in pairs with free access to water under a 12 hr light/dark cycle, lights on 07:00. They were all maintained on a restricted diet of 15 g of standard food pellets, adjusted on test days so that, regardless of the effect of drug, all rats received the same food allowance per day. All testing was between 12:00 and 14:00 hours.

9.2.2 Apparatus and procedure:

The experiment used a procedure similar to that described in section 8.2.2.

9.2.3 Drugs:

(-)-Cocaine HCl, (Sigma Chemicals, Poole Dorset) and SCH 39166 HCl ((-)-trans -6,7,7a,8,9,13b-hexahydro-3-chloro-2 hydroxy-N-methyl -5H-benzo [d] naptho-{2,1-b}azepine) (Schering Corporation, Bloomfield NJ, USA) were dissolved in 0.9% saline solution and administered intraperitoneally at doses of 10 mg/kg cocaine and 0.03, 0.1 mg/kg SCH 39166. All drugs were injected at a volume of 1 ml/kg and the DA D1-like receptor subtype antagonist was injected 30 min prior to the cocaine injection.

9.2.4 Data analysis:

The data were analysed by two-way ANOVA for repeated measures, where drug treatment (4 levels) and time (6 levels) were incorporated as within-subject factors. A one-way ANOVA was calculated when behavioural data were collapsed across the 30-min period. All drug doses were administered to the rats in a 'Latin Square' design

and comparisons between the results for individual drug treatments against vehicle controls were made using 'Dunnett's post-hoc t-tests.

9.3 Results

(a) Food Intake and Eating Microstructure.

During the pre-test adaptation period, the food-deprived rats consumed on average 20 g wet mash within the 30 min test session. One-way ANOVA revealed an overall effect of treatment on food consumption [$F(3,21) = 12.55$, $P<0.01$]; (Fig. 9.1a(i)). Pre-treatment with SCH 39166 at 0.1 mg/kg produced a significant attenuation of cocaine-induced hypophagia ($P<0.05$). In fact, mean food intake after pre-treatment with SCH 39166 was 18.7 g, a value close to saline levels (20.9 g). At 0.03 mg/kg, SCH 39166 failed to affect cocaine-induced hypophagia. Although non-significant results were obtained for latency to induce feeding [$F(3,21) = 1.55$, NS; Fig. 9.1a(ii)], the results suggests an increase in the latency to start feeding after cocaine, and a dose-related attenuation by the D1-like antagonist. There was a significant effect on eating rate [$F(3,21) = 3.72$, $P<0.05$; Fig. 9.1a(iii)]; *post-hoc* t-tests revealed that there were no significant differences between vehicle controls and cocaine treatment, or cocaine plus the D1-like antagonist, on the rate of eating (Fig. 9.1a(iii)).

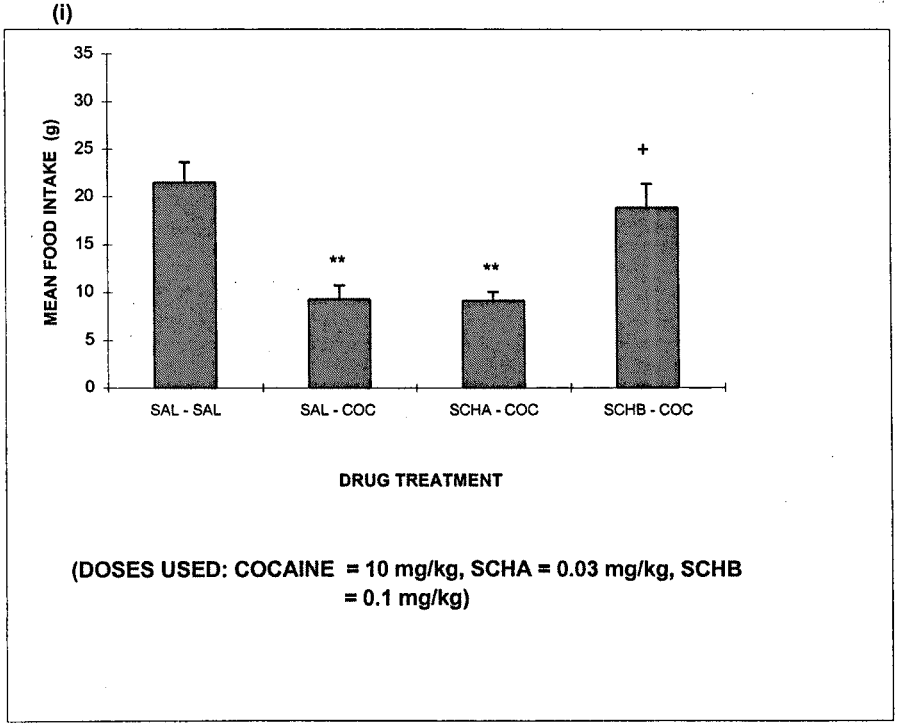
A two-way ANOVA for total duration of feeding revealed a significant effect of treatment [$F(3,21) = 18.86$, $P<0.01$]. However, there was no significant effect of time [$F(5,35) = 1.78$, NS] but a significant drug×time interaction [$F(15,105) = 6.28$, $P<0.01$]. Fig. 9.1a(iv) illustrates the effect of treatment on total feeding duration. Saline treated rats spent most time feeding early in the test session, with decreasing levels through to the end of the session. In contrast, *Post-hoc* t-tests revealed that cocaine treated rats spent little time feeding in the early phase of the session (probably related to the latency effect), with some indication of recovery in the latter stages. Pre-treatment with SCH 39166 at 0.1 mg/kg significantly attenuated cocaine's effects throughout the test session, with baseline levels being restored during time-bins 10-15 min. As the session entered the latter stages, the lowest dose of SCH 39166 also attenuated the effects of cocaine at times 15-25 min ($P<0.01$ compared to Sal-Coc data).

Feeding mean bout duration and bout frequency also followed a similar profile. There were significant effects of treatment for mean bout duration and bout frequency [respectively; $F(3,21) = 16.12$, $P<0.01$ and $F(3,21) = 3.65$, $P<0.05$]. A significant time effect was also shown for the two parameters [respectively; $F(5,21) = 3.06$, $P<0.05$ and $F(5,21) = 14.72$, $P<0.01$], and significant drug×time interactions [respectively; $F(15,105) = 2.39$, $P<0.01$ and $F(15,105) = 3.59$, $P<0.01$)] (Fig. 9.1a(v)&(vi)). For these parameters, an unusual profile was observed after pre-treatment with the D1-like antagonist. For mean bout duration, SCH 391666 at 0.1 mg/kg produced unusually high scores throughout the observation period, which was not replicated for the bout frequency parameter. On the other hand, the low dose of SCH 39166 given with cocaine produced high scores for bout frequencies, again throughout the test session. However, as the mean bout duration was not affected by this low dose, it's effect on bout frequency was not sufficient to attenuate cocaine's effect on intake overall. The mechanism by which the highest dose attenuated cocaine-induced hypophagia was the increase in total duration of feeding, caused mainly by an effect on mean bout duration and a small effect on bout frequency.

These trends were reflected in the data when behavioural counts were collapsed across the 30 min period: *Post-hoc* t-tests revealed that pre-treatment with the highest dose of SCH 39166 attenuated cocaine's effects on total duration and mean bout duration ($P<0.01$), but had no effect on bout frequency (Fig. 9.1(iv-vi)B).

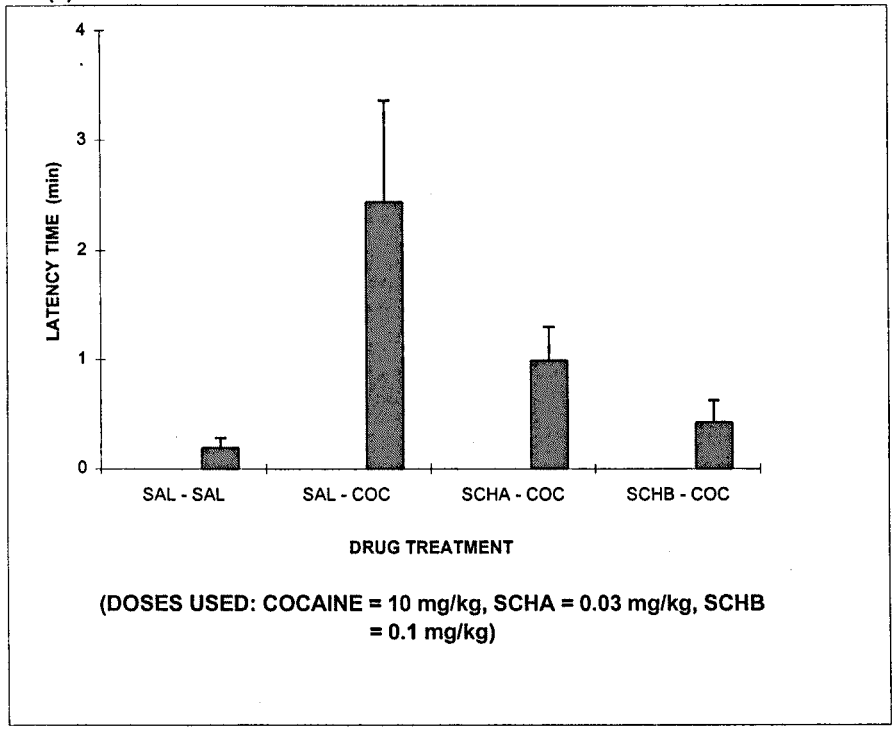
Figure 9.1a.

(i)



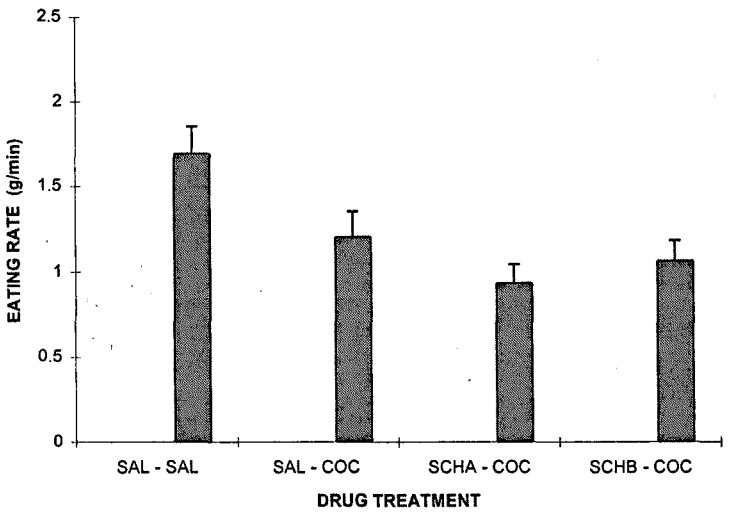
(DOSES USED: COCAINE = 10 mg/kg, SCHA = 0.03 mg/kg, SCHB = 0.1 mg/kg)

(ii)



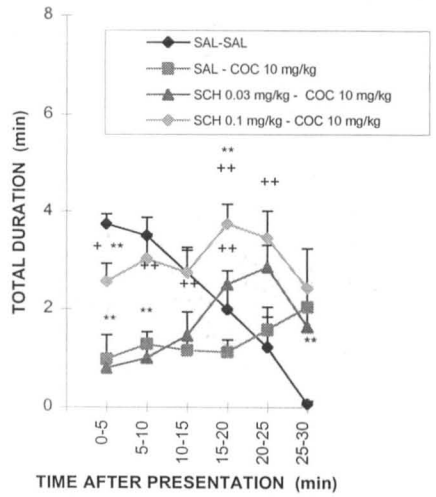
(DOSES USED: COCAINE = 10 mg/kg, SCHA = 0.03 mg/kg, SCHB = 0.1 mg/kg)

(iii)

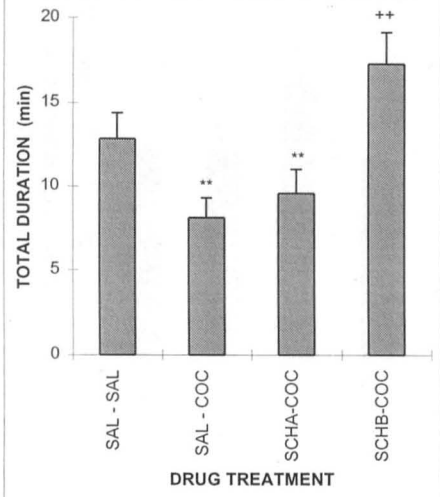


(DOSES USED: COCAINE = 10 mg/kg, SCHA = 0.03 mg/kg, SCHB = 0.1 mg/kg)

(iv) A

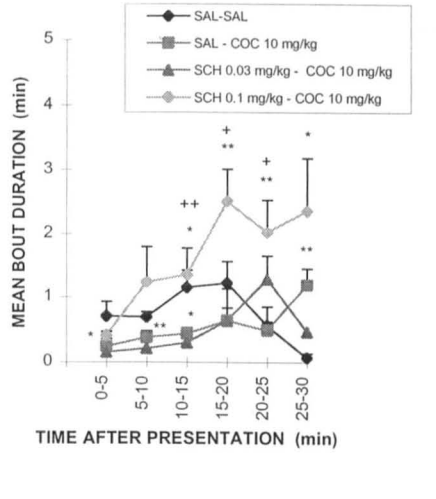


B

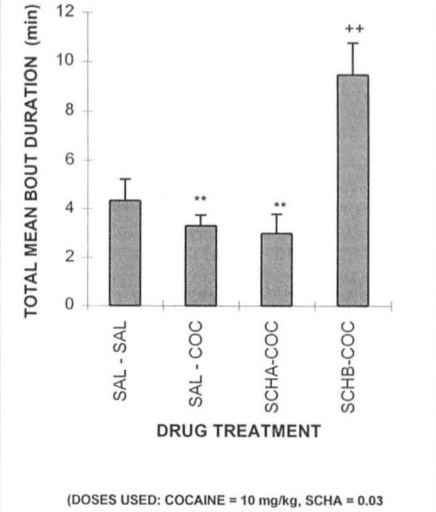


(DOSES USED: COCAINE = 10mg/kg, SCHA = 0.03 mg/kg, SCHB = 0.1 mg/kg)

(v) A



B



(DOSES USED: COCAINE = 10 mg/kg, SCHA = 0.03 mg/kg, SCHB = 0.1 mg/kg)

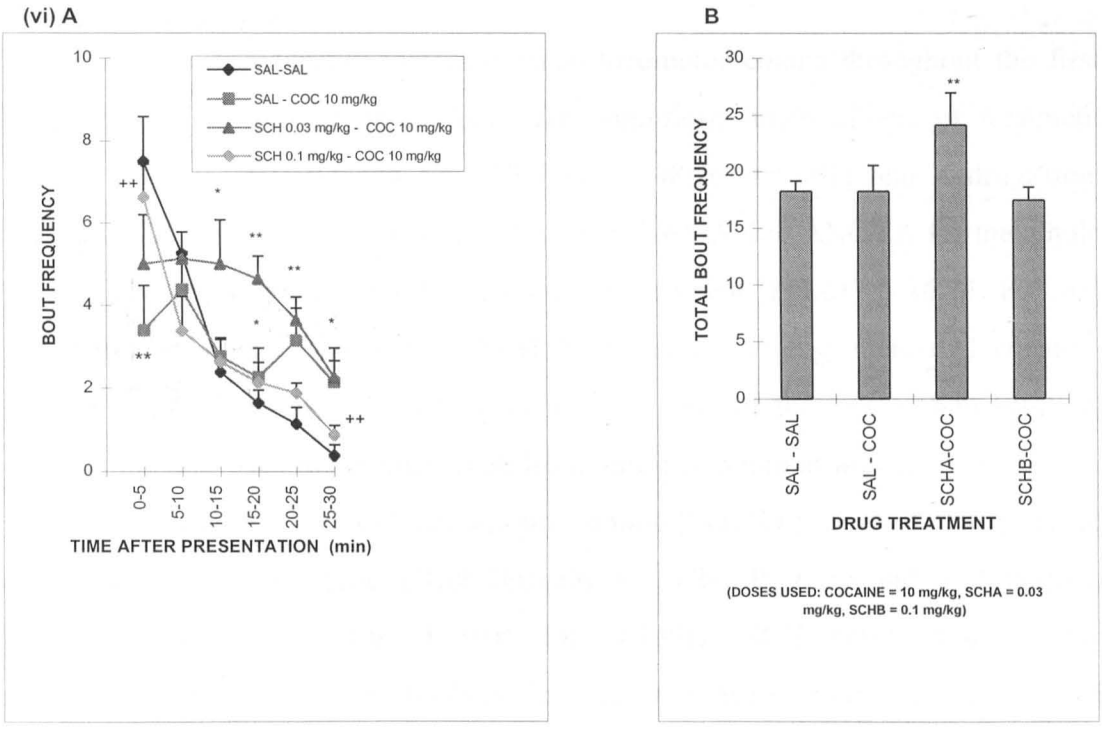


Figure 9.1(a) Effects of pre-treatment with a selective D1-like antagonist on cocaine-induced feeding parameters: (i) total food intake (ii) latency to start eating (iii) eating rate (iv)A&B total duration (v)A&B mean bout duration (vi)A&B bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to saline-controls; + $P<0.05$, ++ $P<0.01$ compared to saline-cocaine (comparisons by Dunnett's t-tests)

(b) Locomotor Activity.

Cocaine significantly increased mean locomotor counts throughout the first 20-25 min of the test session. There were significant main effects of treatment [$F(3,21) = 17.0$, $P<0.01$] and time [$F(5,35) = 48.76$, $P<0.01$] and a drug×time interaction [$F(15,105) = 4.57$, $P<0.01$] (Fig. 9.1b(i)A). A 4×6 ANOVA for the whole test session also showed a significant effect of treatment [$F(3,21) = 16.74$, $P<0.01$] (Fig. 9.1b(i)B). Pre-treatment with SCH 39166 at 0.1 mg/kg attenuated cocaine-induced locomotor activity at time intervals 5-20 min of the test session bringing locomotor counts back to baseline levels from time 10-15 min onwards.

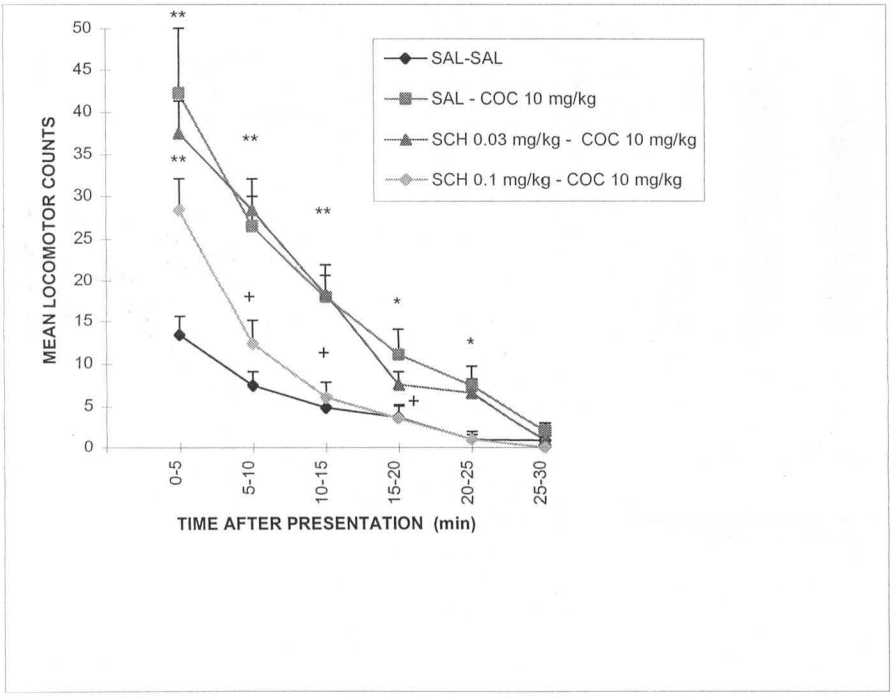
There was an effect of treatment on duration [$F(3,21) = 18.34$, $P<0.01$]. There was also a significant time effect [$F(5,35) = 35.23$, $P<0.01$] and a drug×time interaction [$F(15,105) = 4.62$, $P<0.01$; Fig. 9.1b(ii)]. SCH 39166 at 0.1 mg/kg attenuated total time spent on this behaviour, reducing it to saline levels from 10-15 min onwards. For mean bout duration, there was a significant main effect of treatment [$F(3,21) = 3.23$, $P<0.05$], a significant time effect [$F(5,35) = 3.74$, $P<0.01$] but no drug×time interaction [$F(15,105) = 0.83$, NS] (Fig. 9.1b(iii)).

A similar profile was shown for locomotor bout frequency (Fig. 9.1b(iv)) as was shown for total duration of locomotor activity. There was a significant main effect of treatment [$F(3,21) = 17.34$, $P<0.01$] a significant time effect [$F(5,35) = 59.35$, $P<0.01$] and a significant drug×time interaction [$F(15,105) = 4.13$, $P<0.01$]. Once again, SCH 39166 at 0.1 mg/kg produced significant attenuation of cocaine induced increases in bout frequencies. Attenuation was greatest for this parameter, in that it persisted from the start of the test session through to the end.

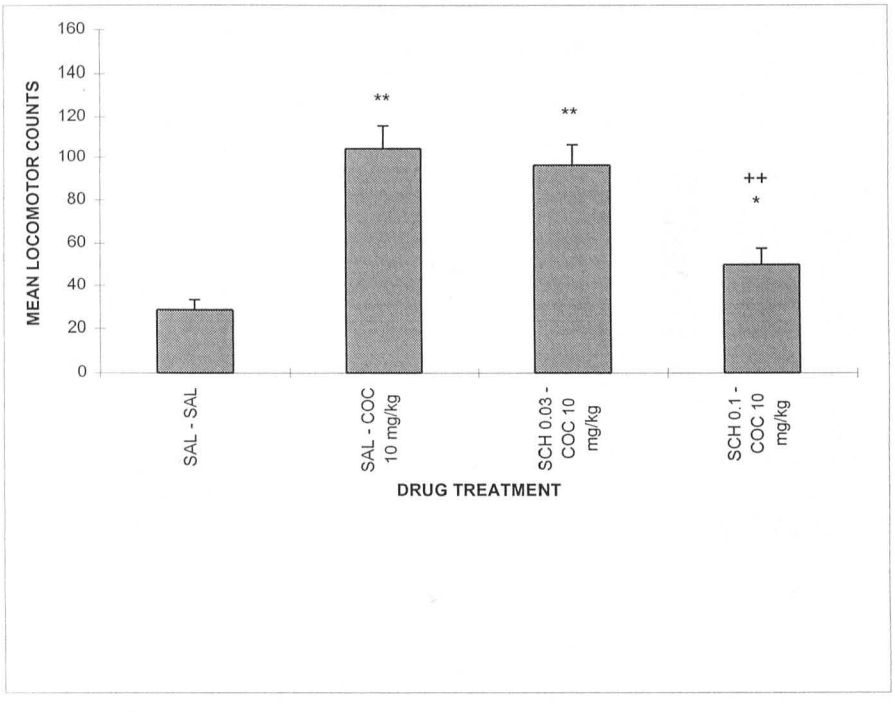
Hence, locomotor bout frequency was the parameter responsible for cocaine's effects on locomotor activity counts and total duration for this behaviour. Upon administration of the D1-like antagonist, there was a clear antagonism of this parameter, which as a result led to a decrease in total duration (and consequently locomotor counts) throughout the observation period. This was also the case when behavioural counts were collapsed across the 30 min period (Fig. 9.1b(ii&iv)B).

Figure 9.1b.

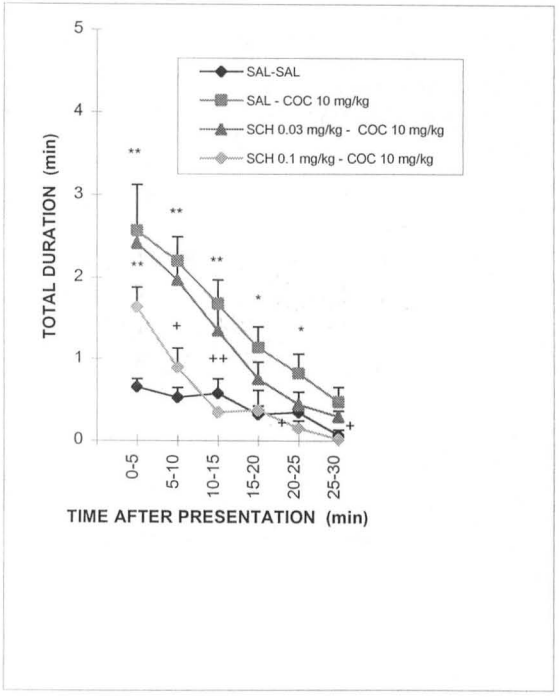
(i) A



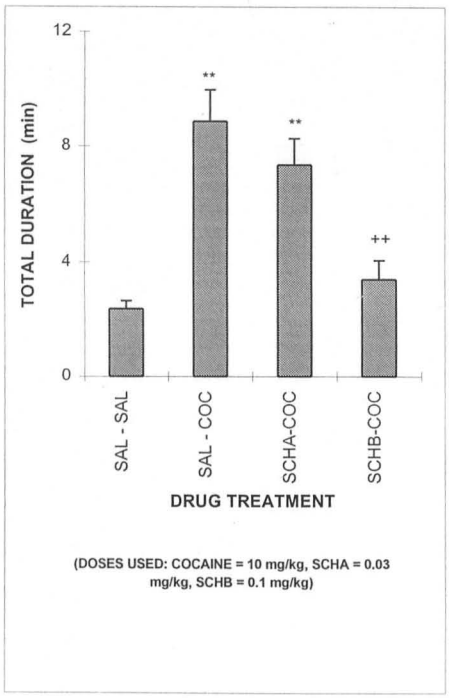
B



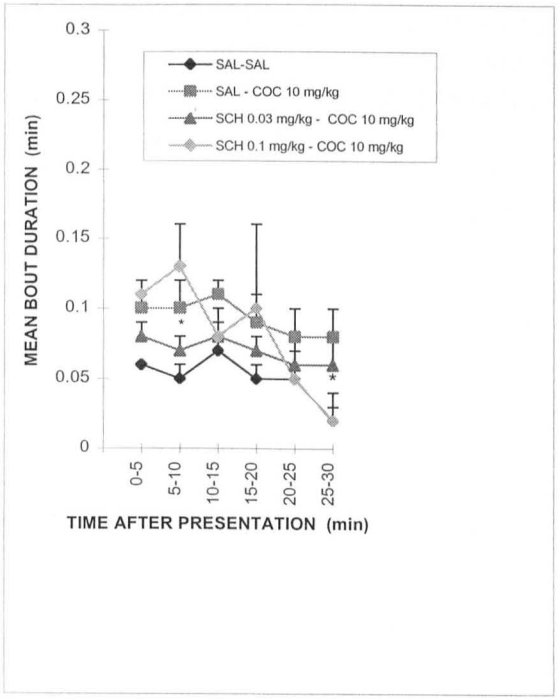
(ii) A


SAL-SAL
SAL - COC 10 mg/kg
SCH 0.03 mg/kg - COC 10 mg/kg
SCH 0.1 mg/kg - COC 10 mg/kg
TOTAL DURATION (min)
TIME AFTER PRESENTATION (min)
0-5
5-10
10-15
15-20
20-25
25-30

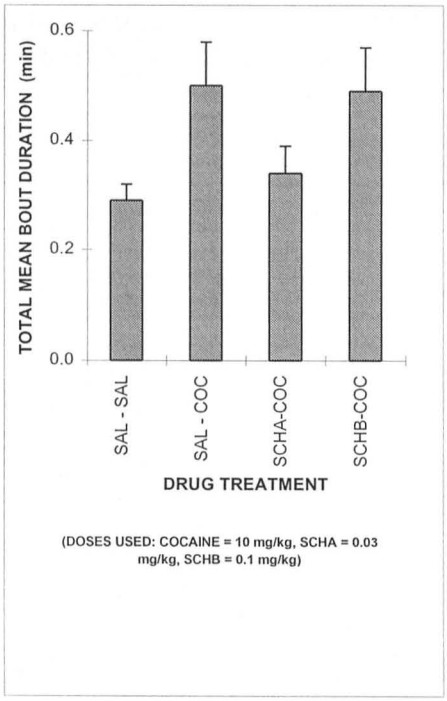
B


TOTAL DURATION (min)
SAL - SAL
SAL - COC
SCHA-COC
SCHB-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, SCHA = 0.03 mg/kg, SCHB = 0.1 mg/kg)

(iii) A


SAL-SAL
SAL - COC 10 mg/kg
SCH 0.03 mg/kg - COC 10 mg/kg
SCH 0.1 mg/kg - COC 10 mg/kg
MEAN BOUT DURATION (min)
TIME AFTER PRESENTATION (min)
0-5
5-10
10-15
15-20
20-25
25-30

B


TOTAL MEAN BOUT DURATION (min)
SAL - SAL
SAL - COC
SCHA-COC
SCHB-COC
DRUG TREATMENT
(DOSES USED: COCAINE = 10 mg/kg, SCHA = 0.03 mg/kg, SCHB = 0.1 mg/kg)

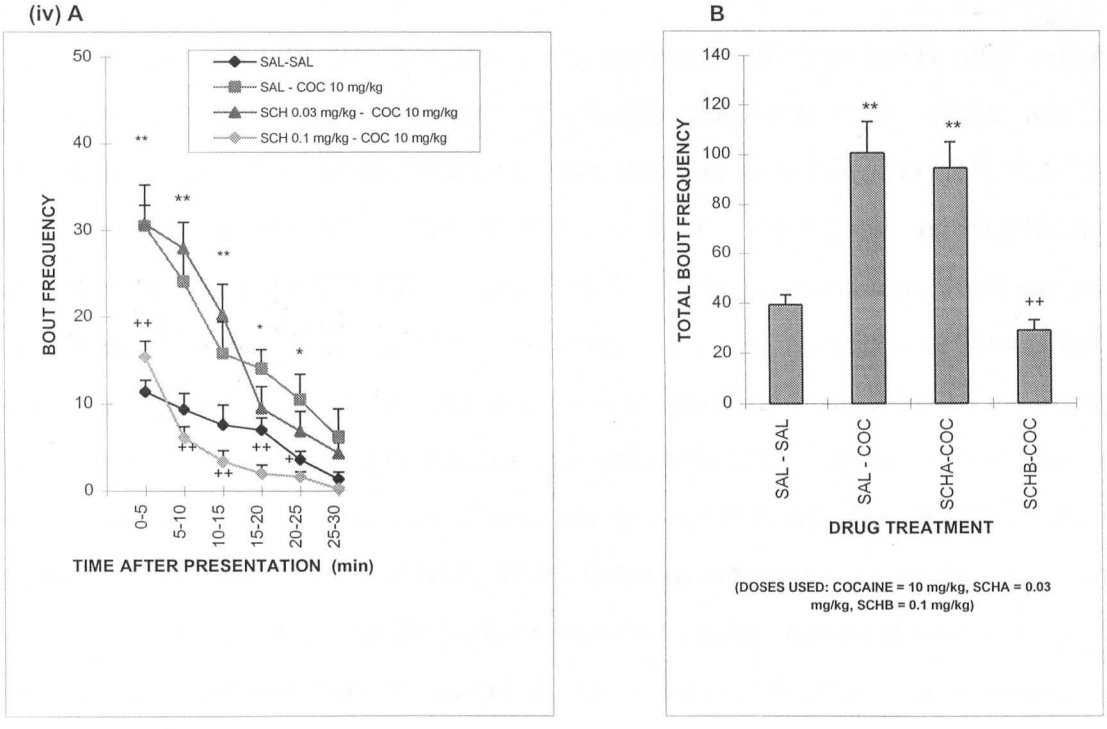


Figure 9.1(b) Effects of pre-treatment with a selective D1-like antagonist on cocaine-induced locomotor activity parameters: (i)A&B total locomotor counts (ii)A&B total duration (iii)A&B mean bout duration (iv)A&B bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to saline-controls; + $P<0.05$, ++ $P<0.01$ compared to saline-cocaine (comparisons by Dunnett's t-tests)

(c) Rearing.

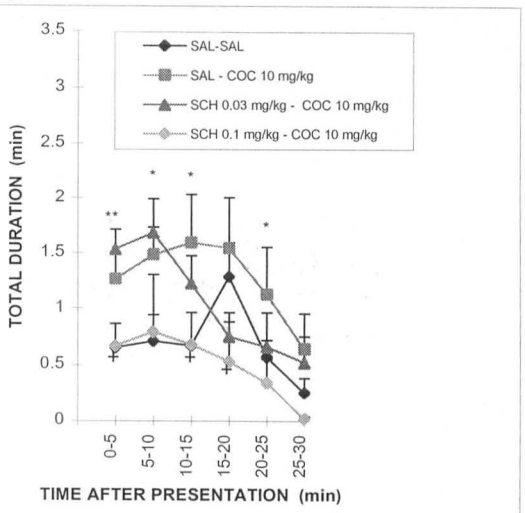
Rearing activity mirrored locomotor activity, with low levels after saline treatment and an increase produced by cocaine (Fig. 9.1c(i-iii)). There was a significant main effect of treatment for total duration of rearing [$F(3,21) = 5.37$, $P<0.01$] a significant time effect [$F(5,35) = 5.29$, $P<0.01$] but no significant dose×time interaction [$F(15,105) = 1.20$, NS]. For mean bout duration, there was no significant main effect of treatment, nor a time effect or a drug×time interaction. However, for rearing bout frequency there was a significant main effect of treatment [$F(3,21) = 13.60$, $P<0.01$], a significant time effect [$F(5,35) = 34.68$, $P<0.01$] and a significant drug×time interaction [$F(15,105) = 4.0$, $P<0.01$] (Fig. 9.1c(iii)). Pre-treatment with the low dose of SCH 39166 failed to antagonise cocaine's effects on any parameters of rearing, but the higher dose of 0.1 mg/kg attenuated total time spent rearing and mean bout frequency during the early stages of the test session. Therefore the antagonism produced by SCH 39166 of cocaine's effects on total time spent rearing was via a specific action on bout frequency, not via mean bout duration

(d) Grooming and resting/immobility.

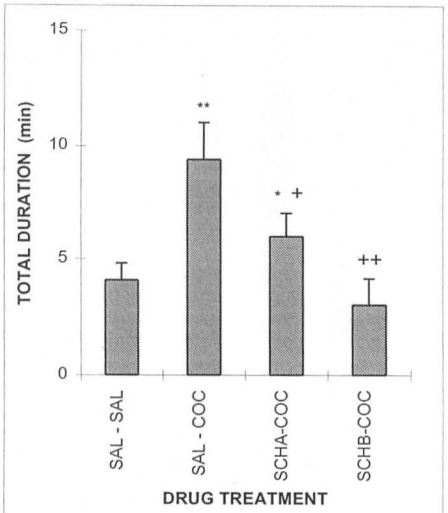
None of the behavioural parameters for grooming were affected by any drug treatment when compared with saline treatment (data not shown). There was also no significant main effect of time, and no drug×time interaction for any parameter. However, cocaine on its own did produce a decrease in the total duration of grooming ($P<0.05$) which corresponded with decreases in mean bout duration and bout frequency ($P<0.05$ for both) when compared to saline treatment across the 30 min period.

Figure 9.1c.

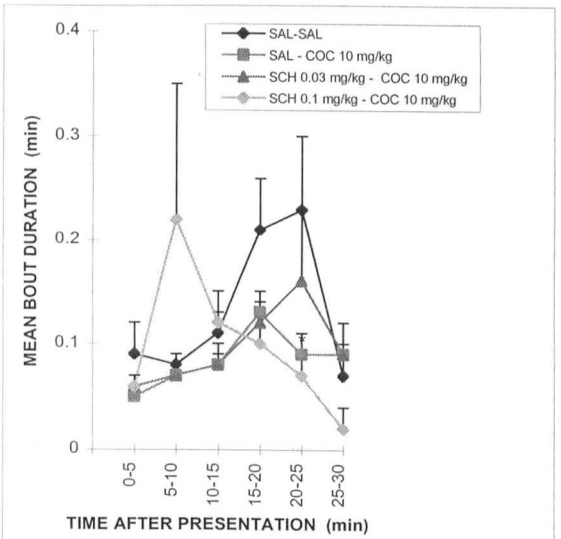
(i) A



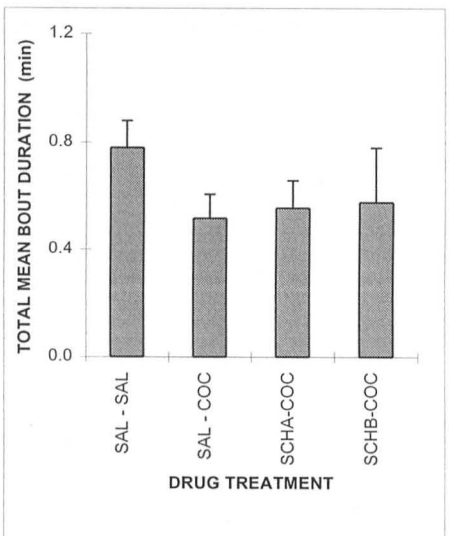
B



(ii) A



B



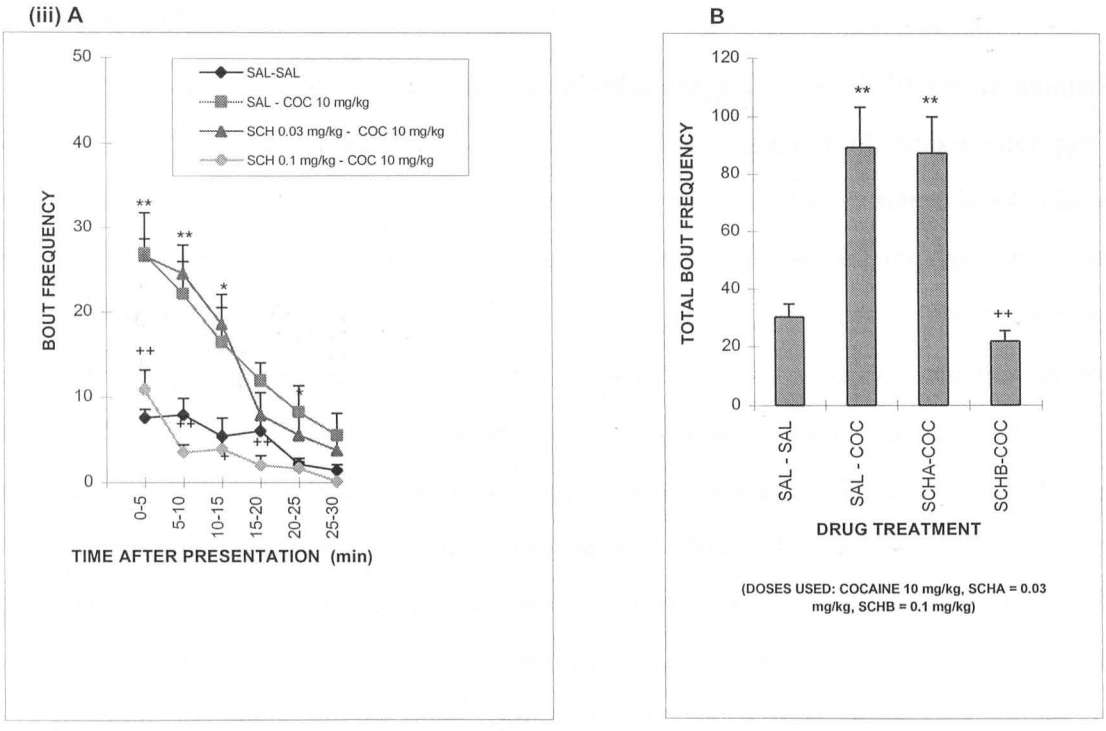


Figure 9.1(c) Effects of pre-treatment with a selective D1 antagonist on cocaine-induced rearing parameters: (i)A&B total duration (ii)A&B mean bout duration (iii)A&B bout frequency. N=8 at all points; * $P<0.05$, ** $P<0.01$ compared to saline-controls; + $P<0.05$, ++ $P<0.01$compared to saline-cocaine (comparisons by Dunnett's t-tests)

9.4 Discussion

The primary finding was that the D1-like antagonist SCH 39166 attenuated cocaine's suppressant effects on food intake. In fact, mean food intake after pre-treatment with SCH 39166 (0.1 mg/kg) was 18.7 g, a value close to saline levels (20.9 g). At 0.03 mg/kg, SCH 39166 failed to affect cocaine-induced hypophagia. The mechanism by which the highest dose attenuated cocaine-induced hypophagia was attributed to an increase in the total duration of feeding. This was caused mainly by the effect on mean bout duration, a large effect on bout frequency at the start of the test session, and the probable contribution of a non-significant reduction in the latency to start feeding. However, an unusual profile was observed after pre-treatment with the D1-like antagonist on these parameters. SCH 39166 at 0.1 mg/kg produced unusually high scores for mean bout duration throughout the observation period, but not for bout frequency. On the other hand, the low dose of SCH 39166 (which had no effect on food intake per se) produced high scores for bout frequency when combined with cocaine. As mean bout duration was not affected by this dose, the effect on bout frequency was not sufficient to attenuate cocaine's effects on feeding overall, thereby explaining the ineffectiveness of this dose.

Comparing the effects of the D1-like antagonist with those of the D2-like antagonists (see Table 9.1) suggest that D1-like receptors play a more important role in cocaine's suppressant effects on food intake. In the only study to date looking at DA receptor involvement of cocaine's effect on feeding, Rapoza and Woolverton (1991) examined the effects of a selective DA D1-like receptor antagonist, SCH 23390, and a D2/D3 receptor antagonist, raclopride, on milk consumption. Both antagonists attenuated the effects of at least one of cocaine's effective doses. However, as mean food intake was measured only in their study, it is not clear how these drugs attenuated cocaine's effects. Also, the use of behaviourally active doses (i.e. 0.25 & 0.5 mg/kg SCH 23390, and 0.3 & 1.0 mg/kg raclopride, doses which themselves induced hypophagia) makes the comparison between studies difficult to interpret. Nevertheless, the result showing that our more conservative behaviourally inactive dose of SCH 39166 (0.1 mg/kg) attenuated cocaine's suppressant effects on food intake, unequivocally demonstrates that the D1-like receptor subtype is an important contributor to the mechanisms by which cocaine produces its action.

Table 9.1. A comparison of the effects of pre-treatment with selective D1-like and D2-like antagonists on the microstructural effects of cocaine.

BEHAVIOURAL RESPONSE CATEGORY	COCAINE'S EFFECTS	D1-like Antag.	D2/D3 Antag.	D3 Antag.	D4 Antag.
FEEDING					
FOOD INTAKE	↓	+++	0	0	0
LATENCY	↓	0	0	0	0
RATE	↓	0	0	0	0
TOTAL DURATION	↓	+++	+	-	0
MEAN BOUT DURATION	↓	+	+	-	0
BOUT FREQUENCY	↓	+++	+	0	0
LOCOMOTOR ACTIVITY					
TOTAL DURATION	↑	++	+	-	0
MEAN BOUT DURATION	0	0	0	0	0
BOUT FREQUENCY	↑	+++	+	-	0
REARING					
TOTAL DURATION	↑	++	+	-	0
MEAN BOUT DURATION	0	0	0	0	0
BOUT FREQUENCY	↑	+++	+	-	0
GROOMING					
TOTAL DURATION	↓(HD)	0	++	0	0
MEAN BOUT DURATION	↓(HD)	0	+++	0	0
BOUT FREQUENCY	↓(HD)	0	+++	0	0

KEY: (↓) = decrease (↑) = increase (+) = antagonises (-) = potentiates (0) = no effect (+++) = strong (+) = weak (HD) = high dose

In agreement with the feeding data, DA D1-like receptor blockade also had a greater effect than DA D2-like receptor blockade in attenuating cocaine induced hyperactivity and rearing behaviour (also see Table 9.1). This is consistent with reports in both mice and rats that D1-like antagonists are more effective than D2-like antagonists in altering cocaine-induced locomotor behaviours at non-sedative doses (e.g. Cabib et al., 1991; Ushijima et al., 1995; Le et al., 1997; O'Neil and Shaw, 1999). In all these studies it was shown that the D1-like antagonists (notably SCH 23390) could reduce hyperlocomotion induced by cocaine at doses that did not consistently alter spontaneous activity. Whereas, the D2-like antagonists either had no effect at all (spiperone: Le et al., 1997) or could only reduce the hyperlocomotor effects of cocaine at doses in excess of the minimum dose required to attenuate spontaneous locomotor activity significantly (e.g. Cabib et al., 1991; Ushijima et al., 1995, O'Neil and Shaw, 1999). These results along with the present study therefore indicate that D1-like but not D2-like receptors play a significant role in cocaine-induced hyperactivity. It has also been reported that D1-like receptors are important to a range of other cocaine-induced behaviours. For example, McCreary and Marsden (1993) showed that DA D1-like receptors may be more important in the expression of sensitisation to the conditioned behavioural effects of cocaine, as behavioural sensitisation was antagonised by the selective DA D1-like antagonist compound, SCH 23390, but not by the DA D2-like antagonist, haloperidol. This was supported by DeVries et al. (1998) who showed that infusion of a D1-like receptor agonist into the nucleus accumbens enhances cocaine-induced behavioural sensitisation. However, there is some inconsistency in the literature, as Mattingly et al. (1996) showed that co-administration of either haloperidol or SCH 23390 can block the acute locomotor-activating effects of cocaine, but only haloperidol prevented the development of behavioural sensitisation. Localisation studies have also produced mixed results: Neisewander et al. (1998) and Baker et al. (1998) looked at the role of DA D1-like receptors in cocaine-induced behaviours and found, in agreement with previous work, that systemic administration of SCH 23390 reversed cocaine-induced locomotion, sniffing and cocaine-conditioned place preference, suggesting that stimulation of DA D1-like receptors is necessary for these behavioural changes. However, intra-accumbens administration of SCH 23390 did not alter cocaine-induced locomotor

activity, although it did reverse cocaine-conditioned place preference despite occupying 40-60 % of D1-like receptors in the anterior nucleus accumbens core and shell. Hence D1-like receptors located in structures other than the nucleus accumbens may be important for cocaine's stimulant properties, which would explain the ineffectiveness of intra-accumbens administration of SCH 23390 by Neisewander and Baker. A problem that still needs to be resolved concerns the subtypes of D1-like receptors necessary for cocaine-induced locomotion. Using selective antisense oligonucleotides, it has been shown that the DA D1 receptor subtype may play a facilitatory role in locomotor activity, whereas the DA D5 receptor subtype within the D1-like subfamily may exert an inhibitory effect (Dziewczapolski et al., 1998). However, as there are no drugs selective for this receptor subtype to date, the roles of the DA D1 and D5 receptor subtypes in cocaine-induced behaviours remains poorly characterised.

The only effect of cocaine that SCH 39166 failed to attenuate was inhibition of grooming. SCH 39166 at a dose of 0.1 mg/kg had no effect on any of the behavioural parameters for grooming, in contrast with the D2/D3 antagonist raclopride, which had strongly attenuated cocaine-induced decreases in grooming. The results here therefore reinforce the conclusion from the previous chapter and suggest that inhibition of grooming must be exclusively a D2 mediated effect. It is interesting to note that both the selective D2-like receptor agonist N-0437 (Rusk and Cooper, 1989b) and cocaine (Cooper et al., 1993, and section 7.3.2) significantly reduced grooming in similar tests of palatable food consumption. Yet the effects were achieved by different means. N-0437 reduced the frequency of grooming bouts and had no effect on the mean duration of grooming; in contrast Cooper at al. (1993) reported that cocaine reduces the bout duration of grooming while having little effect on the bout frequency. The results described in section 7.3.2 and in the previous chapter also differ with the above two studies suggesting that cocaine decreases grooming by disrupting all parameters. Early reports on D1-like agonists, on the other hand, demonstrate a different picture altogether. The D1-like agonist SKF 38393 induces non-stereotyped grooming (Molloy and Waddington, 1984); the induction of grooming by SKF 38393 has been widely confirmed, and has been replicated using other D1-like agonists such as A-68930 (Waddington and Daly, 1993; Naser and Cooper, 1994).

Hence, although D1-like agonists (SKF 38393 and A-68930), N-0437 and cocaine all reduce feeding behaviour, there is a disjunction regarding grooming. D1-like agonists induce grooming, whereas N-0437 and cocaine suppresses it. Therefore the ability of raclopride to reverse cocaine-induced suppression of grooming (section 8.3.1), without affecting locomotion or rearing very much, suggests that inhibition of grooming by cocaine is not due to indirect increases in other behaviours (i.e. to behavioural competition). This generalisation can be extended by looking at other results in the present study using the D1-like antagonist. Normalising locomotor activity, with SCH 39166 in combination with cocaine, does not allow recovery of grooming. Therefore, these behaviours induced by cocaine must be independent and mediated via specific receptors, an outcome that has not been demonstrated before.

In conclusion, at a behaviourally inactive dose the selective D1-like antagonist, SCH 39166, attenuated cocaine-induced hypophagia, hyperactivity and rearing, but had no effect on suppression of grooming. These results suggest that indirect stimulation of receptors of the D1-like subfamily may be more important than stimulation of receptors of the D2-like subfamily to the locomotor, rearing and anorectic effects of cocaine.

CHAPTER 10:

'SUMMARY AND FINAL CONCLUSIONS'

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SUMMARY AND FINAL CONCLUSIONS

The recent escalation of cocaine abuse has increased awareness of the need to understand the behavioural effects of cocaine and the determinants of those effects. It has become widely accepted that CNS DA plays a primary role in the behavioural effects of cocaine. However, little is known regarding the DA receptor subtypes that are involved in different cocaine-induced behaviours. In animal studies, cocaine can elicit hypophagia, an increase in locomotor activity, stereotyped behaviour and it can also function as a discriminative stimulus. The aim of this thesis was to examine DA receptor subtype involvement in these behavioural effects, in particular the roles of D2-like receptors. The major findings and implications of the thesis are described below.

10.1 Dopamine receptor subtypes and cocaine-induced behaviours

10.1.1 Involvement in the discriminative stimulus effects of cocaine:

The DS effects of cocaine are primarily mediated by indirect stimulation of DA receptors, but the relative contributions of receptor subtypes within D1-like and D2-like receptor subfamilies have not been fully determined as yet. The first study tested the effects of antagonists selective for receptors within the D2-like subfamily (D3 or D4) on the DS effects of cocaine, and compared them with the effects of a D1-like receptor antagonist. A second study, using two different training doses (10 mg/kg vs. 3.0 mg/kg), tested the effects of three centrally acting D2-like antagonists on cocaine's DS effects: L-741,626; haloperidol; and raclopride. L-741,626 is a highly selective antagonist of the D2 receptor within the D2-like receptor subfamily; the other two drugs have different binding affinities for the various D2-like receptors. The effects of the D2-like antagonists were then compared with the effects of the D1-like receptor antagonist, SCH 39166, on cocaine's full dose response curve.

Neither the D4 receptor antagonist L-745,870 (1–10 mg/kg), nor the D3 receptor antagonist U-99194A (1–10 mg/kg), substituted for cocaine, and in combination with cocaine neither drug shifted the dose-response function for cocaine at any dose, although

both reduced rates of responding. If anything, there was perhaps some indication that the highest dose of U-99194A potentiated the effect of a low dose of cocaine. On the other hand, and in striking contrast, pre-treatment with the D1-like antagonist SCH 39166 (0.1, 0.3 mg/kg) produced significant, dose-related rightward shifts in the cocaine generalisation curve, indicating effective, surmountable antagonism. The results suggest negligible involvement of D4 receptors in particular, and support an important role for D1-like receptors in cocaine's DS effects.

Most studies assessing the DS effects of cocaine in rats have used a training dose of 10 mg/kg. Typically, such studies have suggested that DA D1-like and D2-like receptors both contribute to the DS effects of cocaine. However, the relative contributions of DA receptor subtypes to the effects of a lower training dose of cocaine have not been so well characterised. Comparing between the two different training doses of cocaine, the D1-like and D2-like receptor antagonists were not differentially effective between the two training groups. Regardless of cocaine training dose, all four antagonists produced significant, parallel, rightward shifts in the dose response function for cocaine's DS effects, indicating competitive antagonism. L-741,626 was less potent than haloperidol or raclopride, and pA_2 analysis suggested that antagonism of cocaine's effects by L-741,626 was by a different mechanism from that of haloperidol and raclopride.

DA D1-like and D2-like receptors within the mesocorticolimbic system have been shown to play a significant role in mediating the DS effects of cocaine, with particular importance attributed to the roles of the nucleus accumbens and the amygdala, subsites where DA D3 and D4 receptors are expressed, respectively. The present findings showing that L-745,870 does not alter the DS effects of cocaine therefore suggests that DA D4 receptors in amygdala and frontal cortex are probably not important to cocaine's DS effects. Further experiments using selective D4 antagonists, or substitution tests using selective D4 agonists, when they become available, are needed to determine whether the lack of effects is specific to this compound. The finding of negligible involvement of DA D3 receptors in cocaine's DS effects contrasts with the current consensus; it challenges the orthodox view of the importance of D3 receptors to cocaine's DS effects. Other

selective D3 receptor antagonists should therefore also be examined as they become available for study.

In summary, the experiments suggest that DA D1-like receptors, but not D3 or D4 receptors from the D2-like subfamily, are critically involved in the DS effects of cocaine at high dose training conditions. The experiments also suggest that D2 receptors within the D2-like receptor subfamily are the critical sites mediating the DS effects of cocaine. In addition, neither D2 nor D1-like receptor subtypes have preferential involvement at either high or low training doses of cocaine. This is an important finding, as Terry et al. (1994) suggested that training dose may be an important determinant of the extent to which DA receptor subtypes are involved in the DS effects of cocaine. The present results may suggest that some of the D2-like agonists used by Terry et al. (1994) may have more complex DS effects (quinpirole showed near-full substitution) and that the use of selective D2-like receptor antagonists may generate more reliable results. Alternatively, the results may reflect an interaction between D1-like and D2-like receptors, whereby blockade of D2 receptors reduces D1-like receptor involvement. Given that quinpirole substitutes for cocaine at low training doses, the former explanation may be more likely.

Before a series of behavioural studies could be developed to investigate DA receptor involvement in cocaine-induced hypophagia and other concurrent behaviours, it was necessary to examine the effects of selective DA receptor subtype antagonists alone on food intake associated behaviours.

10.1.2 DA D2-like receptor subtypes and ingestive behaviour:

The contributions of DA D3 and D4 receptors to the effects of non-selective dopaminergic drugs on feeding have not been determined due to the limited availability of compounds acting specifically at these receptors. The development of drugs selective for DA D3 and D4 receptors allowed for characterisation of the effects of drugs that target specific receptors within the D2-like receptor subfamily. These novel D2-like receptor subtype antagonists were compared with the prototypical D2-like antagonist sulpiride, and to a structural analog, amisulpride. At doses that occupy postsynaptic D3 receptors, feeding was only marginally affected by U-99194A (1–10 mg/kg); effects on

intake and rate of eating only occurred at the highest dose. The drug produced negligible effects on locomotor activity and grooming, but rearing was significantly reduced at the highest dose. Comparisons with the effects of less-selective D2-like receptor antagonists suggested that D3 receptor blockade is not an important contributor to those effects. In fact, U-99194A produces effects most similar to those of D2-like agonists. D4 receptor blockade by L-745,870 produced a different profile of effects. There was no reduction in food intake at D4 receptor-selective doses (1–3 mg/kg); however, microstructural analysis showed that a significant decrease in the number of meals consumed was compensated by an increase in mean duration of feeding bouts. The local rate of eating declined only at the highest dose. Unlike the D3 antagonist, L-745,870 also caused a substantial reduction in locomotor activity at doses that did not affect rearing. Comparisons with the effects of less-selective D2-like receptor antagonists suggest that D4 receptor blockade is not an important contributor of those effects. In fact, L-745,870 produces effects most similar to those of D1-like antagonists.

Sulpiride, a prototypical D2-like antagonist, produced a dose-related increase in feeding 30 min after administration (significant at 5.0 and 50.0 mg/kg) and a decrease in locomotor activity at the highest dose of 50.0 mg/kg. ED_{50} values showed that amisulpride was roughly three times more potent than sulpiride in suppressing locomotor activity, a value that is similar to the difference in their binding potencies. Amisulpride produced similar results to sulpiride: an increase in feeding 30 min after administration, significant at a dose of 5.0 mg/kg, but higher doses (reported to antagonise postsynaptic receptors) had no significant effect on food intake, although some decrease was evident. In contrast, amisulpride caused a decrease in locomotor activity 30 min after administration, evident only at 50.0 mg/kg. The results therefore suggests that as amisulpride and sulpiride shared a similar behavioural profile, the additional D3 binding affinity reported for amisulpride may not have much bearing on its effects on feeding and locomotor behaviour. The results also reveal clear dissociations between the effects of D3 and D4 receptor blockade on specific behaviours, but suggest only subtle effects on feeding. Neither D3 nor D4 blockade alone mimics the effects of previously-tested D2-like receptor antagonists.

10.1.3 Dopamine receptor Involvement in cocaine-induced hypophagia and concurrent behaviours:

Cocaine administered to food-deprived rats trained to eat a palatable sweetened mash produced distinct behavioural effects: hypophagia, locomotor hyperactivity, increased rearing and decreased grooming. Cocaine suppressed food intake by delaying the onset of feeding and suppressing the rate of eating, but this latter parameter was only significant at the highest dose, 30 mg/kg. In effect, cocaine's ability to prolong the latency to start feeding, together with a reduction in the frequency of eating episodes, resulted in an overall reduction in the time devoted to feeding. Cocaine also produced a dose-dependent increase in time devoted to locomotor activity and rearing, corresponding with increases in bout frequencies for these behaviours. The opposite was the case for grooming behaviour: cocaine significantly reduced total duration, largely due to a dose-dependent reduction in the frequency of grooming episodes and mean bout duration.

The effects of a series of D2-like antagonists given as pre-treatments to cocaine were then compared. Drugs with differing selectivities for D2, D3 and D4 receptors (raclopride, U-99194A and L-745,870) produced different outcomes. The D3 (U-99194A) and the D4 (L-745,870) antagonists failed to alter any of the behavioural effects induced by cocaine. In contrast, the D2/D3 antagonist raclopride produced only marginal attenuation of cocaine's effects on food intake, locomotion and rearing activity. However, it clearly attenuated suppression of grooming by cocaine, strongly suggesting that this is a D2-mediated effect. This therefore implies that grooming behaviour may provide a particularly sensitive index of D2/D3 receptor involvement of cocaine's suppressant effects. A comparison between U-99194A and raclopride (which also has selectivity for DA D3 receptors) suggests that raclopride's marginal attenuation of cocaine's suppression of eating, locomotor and rearing hyperactivity must be mediated by DA D2 receptors.

The selective D1-like antagonist SCH 39166 at 0.1 mg/kg, on the other hand, reversed cocaine-induced hypophagia by increasing the time devoted to feeding and bout frequencies. It also attenuated cocaine-induced hyperactivity and rearing behaviour by decreasing the same parameters, but had no effect in attenuating cocaine's suppressant

effects on grooming. These results suggest that indirect stimulation of receptors of the D1-like subfamily may be more important than stimulation of receptors of the D2-like subfamily in locomotor activity, rearing and suppression of eating. The results also show how different behavioural effects of cocaine can be regarded as independent, and under independent receptor control. The ability of raclopride to recover grooming without affecting locomotor or rearing activity very much suggests that inhibition of grooming by cocaine is not due to indirect increases in other behaviours. This is supported by the results from the combination study with SCH 39166 and cocaine, where the D1-like antagonist normalised all behaviours induced by cocaine apart from grooming. Therefore one can say that these behaviours induced by cocaine must be working independently and through specific receptor control. These are the first results to show such outcome.

10.1.4 Commonalities between drug discrimination and feeding studies:

The thesis has focused on the neurobiology of the acute behavioural effects of cocaine. The information gained has not only contributed to our understanding of the neuropharmacology of abused drugs such as cocaine, but also to our understanding of the effects of such compounds on natural rewards such as provided by feeding. The activation of ML DA systems appears to be linked to the process of reward (see section 1.3.2). For example, dopaminergic activity is increased by presentation of a broad range of natural rewards (e.g. food, water, sex) and by associated conditioned stimuli (Phillips et al., 1991; Damsma et al., 1991; Apicella et al., 1991; Schultz et al., 1992). DA is increased in both the nucleus accumbens and neostriatum by a continuous stimulus for food prior to eating and throughout a meal, decreasing to baseline 10-30 min after the meal (Phillips et al., 1991). Therefore, one can ask the question is there an association between reinforcement produced by "natural" reinforcers (such as food) and the reinforcing effects of drugs of abuse? A common assumption is that food cravings experienced with food consumption are due to intense likings for specific food (Berridge, 1996); in the same way that administration of cocaine has been reported to be followed by an increase in cocaine craving (Berridge, 1996); an effect that may increase the likelihood of additional cocaine consumption. If hyperactivation of the DA-related

system of 'liking' were induced selectively, the result would be a focused, intense craving for a specific target. However, an important difference between drug cravings and food cravings is that psychostimulant drugs produce sensitisation of DA-related systems, a process that might be expected to greatly amplify craving (Berridge, 1996). This gives drug craving in addiction a degree of intensity that is unlikely to be matched by most food cravings, although the process may otherwise be quite similar and therefore would provide two behavioural models in which the DA receptor subtype involvement could be studied.

There are several reasons for believing that the DS effects of a drug are important processes in addiction. The first relates to the proposed relationship between discriminative and subjective effects. Both theory and empirical data support the view that drug discrimination procedures in animals are the closest available experimental model for such effects (Stolerman, 1992). Secondly, the discriminative effects of drugs may directly promote drug-seeking behaviour because such behaviour has been associated previously with the perceived effects of drugs. Thus, the ability to perceive and identify the characteristic subjective effects of drugs may encourage drug-seeking behaviour by indicating the effects and potencies of substances that are sampled, and by directing the organism towards one substance rather than another. Therefore, analyses of the DS effects of cocaine can indicate central actions involved in its subjective effects. Since the latter effects of drugs contribute to their abuse, investigation of the DS effects of cocaine should help establish the central mechanisms involved in cocaine abuse. It is very much the case that repeated drug administration best mirrors normal, social patterns of cocaine use; however it is still unclear as to whether either tolerance or sensitisation develops to the reinforcing effects of cocaine. What has been shown is that tolerance or sensitisation to the DS effects of cocaine does not develop in rats (Wood and Emmett-Oglesby, 1986; also see section 3.3). Hence, in practice, the drug discrimination model can be considered a kind of acute administration protocol, and it is therefore justifiable to compare the results from the model with those from acute behavioural tests, such as was done here using feeding and locomotor activity.

10.2 Future Studies

In order to effectively study the roles of DA receptor subtypes in the behavioural effects of cocaine, appropriately selective pharmacological tools will be necessary. As yet, a sufficient number of agonists and antagonists selective for DA D2, D3 and D4 receptors have not been investigated in a wide enough range of behavioural tests for definitive conclusions to be made concerning the functions of these receptor subtypes in cocaine-induced behaviours. Hence, to unequivocally prove that the DA D3 and D4 receptors from the D2-like subfamily are not involved in the DS effects of cocaine, experiments using other selective receptor antagonists and agonists (when available) should be conducted. It would also be of interest to see whether the ineffectiveness of these receptor subtype antagonists on cocaine's DS effects can also be generalised to other models relevant to cocaine abuse, such as self-administration and conditioned place preference.

Also, as an adjunct to the drug discrimination studies, an interesting result observed in these experiments was the limited involvement of D2-like receptors in cocaine-induced hypophagia and locomotor hyperactivity, in comparison with D1-like receptors. The results contrast with many local application studies using D2-like antagonist compounds, in which the involvement of these receptors was clearly indicated. Therefore, applying these antagonists locally to specific brain regions in studies of cocaine's effects on feeding and locomotor activity (perhaps using a similar model to that of chapter 8) may shed more light on the discrepancy. In particular, studies using the very recently-developed D2-selective antagonist L-741,626 may help to address these issues, as well to substantiate the novel finding that cocaine-induced decreases in grooming is a D2-mediated effect. It would also be of great interest to expand these experiments to see whether other stereotyped behaviours induced by cocaine, such as gnawing, scratching and head-bobbing, are also mediated by the DA D2-receptor subtype.

More generally, recent progress in molecular biology has offered an alternative to the use of pharmacological antagonism in the elucidation of the receptor effects of cocaine. It is now possible, through selective targeting of the genome, to obtain mice that lack a functional type of receptor ('knockout' mice). With gene-targeting, one can knock

out a gene in vivo and create a mutant organism that completely lacks the gene product. The promise of gene-targeting is to reveal the in vivo function of the genes of interest (e.g. for the DA receptor subtypes) and eventually to conduct experiments similar to those described in this thesis to further test whether particular receptor subtypes are necessarily involved in the behavioural effects of cocaine. Clearly, a null-mutant organism might not only lack the product of a single gene but might also possess a number of physiological or behavioural traits that have been altered to compensate for the effect of the null mutation. Therefore, comparisons would still be needed with the effects of selective agonists and antagonists for each receptor subtype in order to understand fully the involvement of such receptors in the behavioural effects of cocaine.

REFERENCES:

REFERENCES

Accili, D., C.S. Fishburn, J. Drago, H. Steiner, J.E. Lachowicz, B.H. Park, E.B. Gauda, E.J. Lee, M.H. Cool, D.R. Sibley, C.R. Gerfen, H. Westpahl and S. Puchs (1996) A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. Proc Natl Acad Sci USA **93:** 1945-1949

Acri, J.B., S.R. Carter, K. Alling, B. Geter-Douglass, D. Dijksta, H. Wikstrom, J.L. Katz and J.M. Witkin (1995) Assessment of cocaine-like discriminative stimulus effects of dopamine D3 receptor ligands. European Journal of Pharmacology **281:** R7-R9

Ahlenius, S. and P.Salmi (1994) Behavioural and biochemical effects of the dopamine D3 receptor-selective ligand, 7-OH-DPAT, in the normal and the reserpine-treated rat. European Journal of Pharmacology **260:** 177-181

Ahlenius, S. and K. Larsson (1995) Effects of the dopamine D3 receptor ligand 7-OH-DPAT on male rat ejaculatory behaviour. Pharmacology Biochemistry and Behaviour **51:** 545-547

Albin, R.L., A.B. Young and J.B. Penney (1989) The functional anatomy of basal ganglia disorders. Trends in Neuroscience **12:** 366-375

Alexander, G.E. and M.D. Crutcher (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends in Neuroscience **13:** 266-271

Al-Naser, H.A. and S.J. Cooper (1994) A-68930, a novel, potent dopamine D1 receptor agonist: a microstructural analysis of its effects on feeding and other behaviour in the rat. Behavioural Pharmacology **5:** 210-218

Altman, J., B. J. Everitt, S. Glautier, A. Markou, D. Nutt, R. Oretti, G.D. Philips and T.W. Robbins (1996) The biological, social and clinical bases of drug addiction: commentary and debate. Psychopharmacology **125:** 285-345

Andersen, P.H., J.A. Gingrich, M.D. Bates, A. Dearry, P. Falardeau, S.E. Senogles and M.G. Caron (1990) Dopamine receptor subtypes: Beyond the D1/D2 classification. Trends in Pharmacological Sciences **11:** 231-23

Andersen, P.H. and J.A. Jansen (1990) Dopamine receptor agonists: Selectivity and dopamine D1 receptor efficacy. European Journal of Pharmacology **188:** 335-347

Anderson, P.H. (1988) Comparison of the pharmacological characteristics of [3H] raclopride and [3H] SCH 23390 binding to dopamine receptors in vivo in mouse brain. European Journal of Pharmacology **146:** 113-120

Apicella, R., T. Ljungberg, E. Scarnati and W. Schultz (1991) Responses to reward in monkey dorsal and ventral striatum. Experimental Brain Results **85:** 491-500

Aretha, C.W., A. Sinha and M.P. Galloway (1995) Dopamine D3 - preferring ligands act at synthesis modulating autoreceptors. Journal of Pharmacology and Experimental Therapeutics **274:** 609-613

Aretha, C.W. and M.P. Galloway (1996) Dopamine autoreceptor reserve in vitro: possible role of dopamine D3 receptors. European Journal of Pharmacology **305:** 119-122

Arnt, J. (1985) Antistereotypic effects of D1 and D2 antagonists after intrastriatal injections in rats. Pharmacological and regional specificity. Naunyns-Schmiedeberg Arch **330:** 97-104

Arnt, J. (1988) The discriminative stimulus properties of the D1 agonist SKF 38393 and the D2 agonist (-)-NPA are mediated by separate mechanisms. Life Sciences **42:** 565-574

Azzaro, A.J., R.J. Ziance and C.O. Routledge (1974) The importance of neuronal uptake of amines for amphetamine-induced release of 3H-norepinephrine from isolated brain tissue. Journal of Pharmacology and Experimental Therapeutics **189:** 110-118

Baker, D.A., T.V. Khroyan, L.E. O'Dell, R.A. Fuchs and J.L. Neisewander (1996) Differential effects of intra-accumbens sulpiride on cocaine-induced locomotion and conditioned place preference. Journal of Pharmacology and Experimental Therapeutics **279:** 392-402

Baker, D.A., R.A. Fuchs, S.E. Specio, T.V. Khroyan and J.L. Neisewander (1998) Effects of intra-accumbens administration of SCH 23390 on cocaine-induced locomotion and conditioned place preference. Synapse **30:** 181-193

Baker, L.E., E.E. Riddle, R.B. Saunders and J.B. Appel (1993) The role of monoamine uptake in the discriminative stimulus effects of cocaine and related compounds. Behavioural Pharmacology **4:** 69-79

Baker, L.E., M.E. Miller and K.A. Svensson (1997) Assessment of the discriminative stimulus effects of the D3 dopamine antagonist PNU-99194A in rats: Comparison with psychomotor stimulants. Behavioural Pharmacology **8:** 243-252

Baker, L.E. (1998) (+)-7-OH-DPAT produces stimulus generalisation in rats trained to discriminate d-amphetamine: Lack of D3 receptor involvement. Behavioural Pharmacology **9:** S104

Bakshi, V.P. and A.E. Kelley (1991) Dopaminergic regulation of feeding behaviour: I. Differential effects of haloperidol microinfusion into three striatal subregions. Psychobiology **19:** 223-232

Balapole, D.C., C.D. Hansult and D. Dorph (1979) The effect of cocaine on food intake in rats. Psychopharmacology **64:** 121-122

Bardo, M.T., J.L. Neisewander and T.S. Miller (1986) Repeated testing attenuates conditioned place preference with cocaine. Psychopharmacology **89:** 239-243

Barrett, R.L. and J.B. Appel (1989) Effects of stimulation and blockade of dopamine receptor subtypes on the discriminative stimulus properties of cocaine. Psychopharmacology **99:** 13-16

Barzaghi, R., A. Groppetti, P. Mantegazza and E. Muller (1973) Reduction of food intake by apomorphine: a pimozide-sensitive effect. Journal of Pharmacy and Pharmacology **25:** 909-911

Beckstead, R.M., V.B. Domesick and W.J.H. Nauta (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res **175:** 191-217

Bedford, J.A., D.K. Lovell, C.E. Turner, M.A. Elsohly and M.C. Wilson (1980) The anorectic and actometric effects of cocaine and two coca extracts. Pharmacology Biochemistry and Behaviour **13:** 403-408

Beninger, R.J. (1983) The role of dopamine in locomotor activity and learning. Brain Res Rev **6:** 1733

Bergman, J., B.K. Madras, S.E. Johnson and R.D. Spealman (1989) Effects of cocaine and related drugs in nonhuman primates. II. Self administration by squirrel monkeys, Journal of Pharmacology and Experimental Therapeutics **251:** 150-155

Bergman, J., J.B. Kamien and R.D. Spealman (1990) Antagonists of cocaine self-administration by selective dopamine D1 and D2 antagonists. Behavioural Pharmacology **1:** 355-362

Bernath, S. and M.J. Sigmund (1989) Dopamine may influence striatal GABA release via three separate mechanisms. Brain Res **476:** 373-376

Berridge, K.C. (1996) Food Reward: Brain substrates of wanting and liking. Neurosciences and Biobehavioural Reviews **20:** 1-25

Billard, S., V. Ruperto, G. Crosby, L.C. Iorio and A. Barnett (1984) Characterisation of the binding of ^{3}H-SCH 23390, a selective D1 receptor antagonist ligand, in rat striatum. Life Science **35:** 1885-1893

Bischoff, S., M. Heinrich, J.M. Sonntag and J. Krauss (1986) The D1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT_2) receptors. European Journal of Pharmacology **129:** 367-370

Blavet, N. and F.V. DeFeudis (1982) Inhibition of food intake in the rat. Neurochem Res **7:** 339-348

Blundell, J.E. (1979) Hunger, Appetite and Satiety - Constructs in search of identities, in lifestyles in nutrition; M.R. Turner (ED) Applied Science, London

Blundell, J.E. and C.J. Latham (1980) Characterisation of adjustments to the structure of feeding behaviour following pharmacological treatments: effects of amphetamine and fenfluramine and the antagonism by pimozide and methergoline. Pharmacology Biochemistry and Behaviour **12:** 717-722

Blundell, J.E. (1983) Structure, Process and Mechanism: Case studies in the Psychopharmacology of feeding, In: L.L. Iversen, S.D. Iversen and S.H. Snyder (EDS) Handbook of Psychopharmacology, Plenum Press, London

Blundell, J.E. (1988) Chp. 3: Structure, Process and Mechanism: Case Studies In The Psychopharmacology of Feeding. In: Handbook of Psychopharmacology. Vol.16. (Eds: Iversen, LL; Iversen, SD; Snyder, SH) Plenum Press, New York and London

Bonhaus, D.W., K.K. Weinhardt, M. Taylor, A. DeSouza, P.M. McNeeley, K. Szczepanski, D.J. Fontana, J. Trinh, C.L. Rocha, M.W. Dawson, L.A. Flippin and R.M. Eglen (1997) RS-10221: a novel high affinity and selective, 5-HT_{2c} receptor antagonist. Neuropharmacology **36:** 621-629

Bordi, F. and E. Meller (1989) Enhanced behavioural stereotypies elicited by intrastriatal injection of D1 and D2 dopamine agonists in intact rats. Brain Res **504:** 276-283

Boulay, D., R. Depoortere, G.H. Perrault and D.J. Sanger (1998) No evidence for differences between dopamine D3 receptor knock-out and wild-type mice. Psychopharmacology **12:** Suppl A. A7

Bouras, C., P. Schultz, J. Constantinidis and R. Tissot (1986) Differential effects of acute and chronic administration of haloperidol on substance P and enkephalins in diverse rat brain areas. Biological Psychiatry **16:** 169-174

Bouthenet, M.L., E. Souill, M.P. Martres, P. Sokoloff, B. Giros and J.C. Schwartz (1991) Localisation of dopamine D3 receptor mRNA in the rat brain using in situ hybridisation histochemistry: comparison with dopamine D2 receptor mRNA. Brain Res **564:** 203-219

Bowery, B.J., Z. Razzaque, F. Emms, S. Patel, S. Freedman, L. Bristow, J. Kulagowski and G.R. Seabrook (1996) Antagonism of the effects of (+)-PD 128907 on midbrain dopamine neurones in rat brain slices by a selective D2 receptor antagonist L-741,626. British Journal of Pharmacology **119:** 1491-1497

Boyson, S.J., P. McGanigle and P.B. Molinoff (1986) Quantitative autoradiographic localisation of the D1 and D2 subtypes of dopamine receptors in rats brain. Journal of Neuroscience **6:** 3177-3188

Bozarth, M.A. and R.A Wise (1985) Toxicity associated with long term intravenous heroin and cocaine self-administration in the rat. JAMA **254:** 81-83

Bozarth, M.A. (1990) Drug addiction as a psychological process, In: Addiction Controversies, D.M. Warburton (ED) Harwood Academic Publishers

Brady, K.T., R.B. Lydiand, R. Malcolm and J.C. Bellenger (1991) Cocaine-induced Psychosis. Journal of Clinical Psychiatry **52:** 509-512

Brauer, L.H., A.J. Goudie and H.C. De Wit (1997) Dopamine ligands and the stimulus effects of amphetamine. Animal models versus human laboratory data. Psychopharmacology **130:** 2-13

Brewster, W.K., D.E. Nichols, R.H. Rigg, D.M. Mettoli, T.W. Lovenberg, H.H. Lewis and R.B. Malcolm (1990) trans-10,11-dihydroxy-5,6,6a7,8,12b hexahydrobenzo [a] phenanthioline: A highly potent selective dopamine D1 full agonist. J Med Chem **33:** 1756-1764

Bristow, L.J., K.L. Saywell, G.P. Cook, J.J. Kulagowski, P.D. Leeson, C.I. Ragan and M.D. Tricklebank (1996) Lack of effects of the dopamine D4 receptor antagonist, L-745,870 on amphetamine-induced behaviours in rodents. British Journal of Pharmacology **119:** 210P

Bristow, L.J., G.P. Cook, S. Patel, N. Curtis, I. Mower and J.J. Kulagowski (1998) Discriminative stimulus properties of the putative dopamine D3 receptor agonist, (+)-PD 128907: role of pre-synaptic dopamine D2 autoreceptors. Neuropharmacology **37:** 793-80

Broadbent, J., E.K. Riddle and J.B. Appel (1991) Involvement of dopamine uptake in the discriminative stimulus effects of cocaine. Behavioural Pharmacology **2:** 187-197

Bruhwyler, J., E. Chleide, J.F. Liegeois, J. Delarge and M. Mercier (1991) Effects of specific dopamine agonists and antagonists in the open-field test. Pharmacology Biochemistry and Behaviour **39:** 367-371

Bunzow, J.R., H.H.M. Van Tol, D.K. Grandy, P. Albert, J. Solon, M. Christie, C.A. Machida, K.A. Neve and O. Civelli (1988) Cloning and expression of a rat D2 dopamine receptor cDNA. Nature **336:** 783-787

Cabib, S., C. Castellano, V. Cestari, V. Filibeck and S. Puglisi-Allegra (1991) D1 and D2 receptor antagonists differently affect cocaine-induced locomotor hyperactivity in the mouse. Psychopharmacology **105:** 335-339

Cador, M., T.W. Robbins and B.J. Everitt (1989) Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. Neuroscience **30:** 77-86

Caine, S.B. and G.F. Koob (1993) Modulation of cocaine self-administration in the rat through D3 dopamine receptors. Science **260:** 1814-1816

Caine, S.B. and G.F. Koob (1994) Effects of dopamine D1 and D2 antagonists on cocaine self-administration under different schedules of reinforcement in the rat. Journal of Pharmacology and Experimental Therapeutics **270:** 209-217

Caine, S.B. and G.F. Koob (1995) Pre-treatment with the dopamine agonist 7-OH-DPAT shifts the cocaine self-administration dose-effect function to the left under different schedules in the rat. Behavioural Pharmacology **6:** 331-347

Caine, S.B., S. Stevens Negus, N.K. Mello, L. Bristow, J. Kulagowski and S. Patel (1997) Effects of D2, D3 or D4 receptor blockade on self-administration of cocaine

and quinelorane, Abstract, Presented at the College on the Problems of Drug Dependence

Calcagretti, D.J. and M.D. Schechter (1992) Psychostimulant-induced activity is attenuated by two putative dopamine release inhibitors. Pharmacology Biochemistry and Behaviour **43:** 1023

Callahan, P.M., J.B. Appel and K.A. Cunningham (1991) Dopamine D1 and D2 mediation of the discriminative stimulus properties of d-amphetamine and cocaine. Psychopharmacology **103:** 50-55

Callahan, P.M. and K.A. Cunningham (1993) Discriminative stimulus properties of cocaine in relation to dopamine D2 receptor function in rats. Journal of Pharmacology and Experimental Therapeutics **266:** 585-592

Callahan, P.M., R. De La Garza II and K.A. Cunningham (1994) Discriminative stimulus properties of cocaine: modulation by dopamine D1 receptors in the nucleus accumbens. Psychopharmacology **115:** 110-114

Callahan, P.M., S.K. Bryan and K.A. Cunningham (1995) Discriminative stimulus effects of cocaine: antagonism by dopamine D1 receptor blockade in the amygdala. Pharmacology Biochemistry and Behaviour **51:** 759-766

Callahan, P.M., R. De La Garza and K.A. Cunningham (1997) Mediation of the discriminative stimulus properties of cocaine by mesocorticolimbic dopamine systems. Pharmacology Biochemistry and Behaviour **57:** 601-607

Campbell, B.A. (1960) Effects of water deprivation on random activity. J Comp Physiol Psychol **53:** 240-241

Cao, B.J. and R.J. Rodgers (1997) Dopamine D4 receptor and anxiety: Behavioural profiles of clozapine, L-745,870 and L-741,742 in the mouse plus-maze. European Journal of Pharmacology **335:** 117-125

Carboni, E., A. Imperato, L. Perrezzani and G. Di Chiara (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentration preferentially in the nucleus accumbens of freely moving rats. Neuroscience **28:** 653-661

Carr, G.P., H.C. Fibiger and A.C. Philips (1989) Conditioned place preference as a means of drug reward. In: Liebman, J.M. and Cooper, S.J. NewYork. Oxford University Press. 264-319

Carroll, M.E., S.T. Lac and S.T. Nygaard (1989) A concurrently available non-drug reinforcer prevents the acquisition or decreases the maintenance of cocaine-reinforced behaviour. Psychopharmacology **97:** 23-29

Chandler, C.J., W. Wohab, B.S. Starr, and M.S. Starr (1990) Motor depression: a new role for D1 receptors? Neuroscience **38:** 437-445

Charuchinda, C., P. Supavilai, M. Karoboth and J.M. Palacios (1987) Dopamine D2 receptors in the rat brain: Autoradiographic visualisation using a high affinity selective agonist ligand. Journal of Neuroscience **7:** 1352-1360

Chipkin, R.E., L.C. Iorio, V.L. Coffin, R.D. McQuade, J.G. Berger and A. Barnett (1988) Pharmacological profile of SCH 39166: A dopamine D1 selective benzonaphthazine with potential antipsychotic activity. Journal of Pharmacology and Experimental Therapeutics **247:** 1093-1102

Choi, D.W., D.H. Farb and G.D. Fischbach (1977) Chlordiazepoxide selectively augments GABA activity in spinal cord cultures. Nature **269:** 347-349

Clark, D. (1990) Discriminative properties of drugs of abuse, Chp. 14 Pg. 185-200. In: David M. Warburton (ED), Addiction Controversies, Howard Academic Publishers

Clark, D., M. Exner, L.J. Furmidge, K. Svensson and C. Sonesson (1995) Effects of the dopamine autoreceptor antagonist (-)-DS121 on the discriminative stimulus properties of d-amphetamine and cocaine. European Journal of Pharmacology **275:** 67-74

Clifford, J.J. and J.L. Waddington (1998) Heterogeneity of behavioural profile between three new putative selective D3 dopamine receptor antagonists using an ethologically based approach. Psychopharmacology **136:** 284-290

Clifton, P.G., I.N. Rusk and S.J. Cooper (1989) Stimulation and inhibition of food intake by the selective dopamine D2 agonist, N-0437: a meal pattern analysis. Pharmacology Biochemistry and Behaviour **33:** 21-26

Clifton, P.G., I.N. Rusk and S.J. Cooper (1991) Effects of dopamine D1 and dopamine D2 antagonists on the free feeding and drinking patterns of rats. Behavioural Neuroscience **105:** 272-281

Cohen, A.T., R.D. Todd, S. Harmaon and K.L. O'Malley (1992) Photoreceptors of mouse retina possess D4 receptors coupled to adenylyl cyclase. Proc Natl Acad Sci. USA **89:** 12093-12097

Colpaert, F.C., C.J.E. Niemegeers and P.A.J. Janssen (1976) Cocaine cue in rats as is relates to subjective drug effects: A preliminary report. European Journal of Pharmacology **40:** 195-199

Colpaert, F.C., C.J.E. Niemegeers and P.A.J. Janssen (1978) Discriminative stimulus properties of cocaine and d-amphetamine, and antagonism by haloperidol. A comparative study. Neuropharmacology **17:** 120-125

Cooper, S.J. and K.F. Sweeney (1980), Effects of spiperone alone and in combination with anorectic agents on feeding parameters in the rat. Neuropharmacology **19:** 997-1003

Cooper, S.J., I.N. Rusk and D.J. Barber (1989) Sucrose sham-feeding in the rat after administration of the selective dopamine D2 receptor agonist N-0437, d-amphetamine or cocaine. Pharmacology Biochemistry and Behaviour **32:** 447-452

Cooper, S.J., J. Francis and I.N. Rusk (1990) The anorectic effect of SK&F 38393, a selective dopamine D1 receptor agonist: a microstructural analysis of feeding and related behaviour. Psychopharmacology **100:** 182-187

Cooper, S.J. and G.A. Van der Hoek (1993) Cocaine: a microstructural analysis of its effects on feeding and associated behaviour in the rat. Brain Research **608:** 45-51

Cooper, S.J. and H.A. AL-Naser (1994) A-68930, a novel potent dopamine D1 receptor agonist: a microstructural analysis of its effects on feeding and other behaviours in the rat. Behavioural Pharmacology **5:** 210-218

Corbin, A.E., T.A. Pugsley, H.C. Akunne, S.Z. Whetzel, K.T. Zaski, L.M. Georgic, C.B. Nelson, J.L. Wright, L.D. Wise and T.G. Heffner (1998) Pharmacological characterisation of PD 152255, a novel dimeric benzimidazole dopamine D3 antagonist. Pharmacology Biochemistry and Behaviour **59:** 487-493

Costall, B. and R.J. Naylor (1979) Behavioural aspects of dopamine agonists and antagonists. In the neurobiology of dopamine, (ED) A.S. Horn and J. Korf and B.H.C. Westerink. pp 555-576, Academic Press, London

Cunningham, K.A. and P.M. Callahan (1991) Monomaine re-uptake inhibitors enhance the discriminative state induced by cocaine in the rat. Psychopharmacology **104:** 177-180

Daly, S.A. and J.L. Waddington (1993) Behavioural effects of the putative D3 dopamine receptor agonist 7-OH-DPAT in relation to other 'D2-like' agonists. Neuropharmacology **32:** 509-510

Damsma, G., J.G. Pfaus, D. Wenkstern, A.G. Philips and H.C. Fibiger (1992) Sexual behaviour increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion. Behavioural Neuroscience **106:** 181-191

Damsma, G., T. Bottema, B.H. Westerink, P.G. Tepper, D. Dijkstra, T.A. Pugsley, R.G. MacKenzie, T.G. Heffner and H. Wikstrom (1993) Pharmacological aspects of R-(+)-7-OH-DPAT, a putative dopamine D3 receptor ligand. European Journal of Pharmacology **249:** R9-10

Dearry, A., J.A. Gingrich, R.T. Falardeau, M.D. Bates Jr and M.G. Caron (1990) Molecular cloning and expression of the gene for a human D1 dopamine receptor. Nature **347:** 72-76

Defagot, M.C. and M.C. Antonelli (1997) Autoradiographic localisation of the putative D4 dopamine receptor in rat brain. Neurochem Res **22:** 401

De la Garza, R. and C.E. Johanson (1983) The discriminative stimulus properties of cocaine in the rhesus monkey. Pharmacology Biochemistry and Behaviour **19:** 145-148

De la Garza, R. and C.E. Johanson (1985) Discriminative stimulus properties of cocaine in pigeons. Psychopharmacology **85:** 23-30

Delfs, J.M., L. Schreiber and A.E. Kelley (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. Journal of Neuroscience **10:** 303-310

DeNinno, M.P., R. Schoenlaber, K.E. Asin, R. MacKenzie and J.W. Kebabian (1990) (1R,3S)-1-(aminomethyl0-3,4-dihydro-5,6-di-hydroxy-3-phenyl-1H-2-benzopyran: a potent and selective D1 agonist. Journal of Medicinal Chemistry **33:** 2948-2950

DeNinno, M.P., R. Schoenlaber, R. MacKenzie, D.R. Britta, K.E. Ansin, C. Briggs, J.M. Trogman, M. Ackerman, L. Artman, L. Bednay, R. Bhatt, P. Curzon, E. Gomez, C.H. Kay, J. Stittsworth and J.W. Kebabian (1991) A-68930: a potent agonist selective for the dopamine D1 receptor. European Journal of Pharmacology **199:** 209-219

Depoortere, R., G.H. Perrault and D.J. Sanger (1998) The putative dopamine D3 receptor antagonist PNU-99194A potentiates the discriminative stimulus induced by the dopamine D3/D2 receptor agonist 7-OH-DPAT. Behavioural Pharmacology **9:** Suppl 1. S31

Deutsch, A.Y. and D.S. Cameron (1992) Pharmacological characterisation of dopamine system in the nucleus accumbens core and shell. Neuroscience **46:** 49-56

DeVries, T.J., A.R. Cools, and T.S. Shippenberg (1998) Infusion of a D1 receptor agonist into the nucleus accumbens enhances cocaine-induced behavioural sensitisation. NeuroReport **9:** 1763-1768

Di Chiara, G., M.L. Porceddu, L. Vargiu (1976) Evidence for dopamine receptors mediating sedation in the mouse brain. Nature **264:** 564-567

Di Chiara, G. (1995) The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drugs Alcohol Depend **38:** 95-137

Dickinson, A. and B. Balleine (1994) Motivational control of goal-directed action. Anim Learning Behav **22:** 1-18

Dol Toso, R., B. Sommer, M. Ewert, A. Herb, D.B. Pritchett, A. Bach, B.D. Shivers and P.H. Seeburg (1989) The dopamine D2 receptor: Two molecular forms generated by alternative splicing. EMBOJ. **8:** 4025-4034

Dourish, C.T. and P.H. Hutson (1985) Bilateral lesions of the striatum induced with 6-hydroxydopamine abolish apomorphine-induced yawning in rats. Neuropharmacology **24:** 1051-1055

Duong, A. and H.P. Weingarten (1993) Dopamine antagonists on central, but not peripheral, receptors to inhibit sham and real feeding. Physiology and Behaviour **54:** 449-454

Dyr, W., W.J. McBride, L. Lumeng, T.K. Li and J.M. Murphy (1993) Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high alcohol drinking (HAD) line of rats. Alcohol **10:** 207-212

Dziewczapolski, G., L.B. Menolled, M.C. Garcia, M.A. Mora, O.S. Gershanik and M. Rubinstein (1998) Opposite roles of D1 and D5 dopamine receptors in locomotion revealed by selective antisense oligonucleotides. NeuroReport **9:** 1-5

Ebenezer, I.S. (1990) The effect of intracerebroventricular administration of baclofen on food intake in rats. Neuroreport **1:** 73-76

Edwards, G. (1989) Psychiatry and the addiction. Special Issue of International Reviews of Psychiatry **1:** No. 1/7

Eilam, D., K.V.A. Clements and H. Szechtman, (1991) Differential effects of D1 and D2 dopamine agonists on stereotyped locomotion in rats. Behav Brain Res **45:** 117-124

Eilam, D., H. Talangbayan, G. Canaran and H. Szechtman (1992) Dopaminergic control of mouthing, snout contact, and grooming: opposing roles of D1 and D2 receptors. Psychopharmacology **106:** 447-454

Emmett-Oglesby, M.W., M. Wurst and H. Lol (1983) Discriminative stimulus properties of a small dose of cocaine. Neuropharmacology **22:** 97-101

Evans, H.L. (1971) Influence of light-dark cycle, food presentation and deprivation. Physiol Behav **7:** 455-456

Exner, M. and D. Clark (1992) Agonist and antagonist activity of low efficacy D2 dopamine receptor agonists in rats discriminating d-amphetamine from saline. Behavioural Pharmacology **3:** 609-619

Feldman, R.S., J.S. Meyer and L.F. Quenzer (1997) In: Principles of Neuropsychopharmacology, Chp. 13, Stimulants: Amphetamine and Cocaine, Sinauer Associates, Inc., Publishers

Ferrari, F. and D. Guiliani (1995) Behavioural effects of the dopamine D3 receptor agonist, 7-OH-DPAT in rats. Pharmacol Res **32:** 63-68

File, S.E. and S. Day (1972) Effects of time of day and food-deprivation on exploratory activity in the rat. Anim Behav **20:** 758-762

File, S.E. (1978) The ontogeny of exploration in the rat: Habituation and the effects of handling. Dev Psychobiol **11:** 321-328

File, S.E. (1985) Pharmacological manipulations of response to novelty and their habituation, In: Theories in Psychopharmacology, Vol. 1 (Cooper, SJ; ed) Academic New York

Fischman, M.W. and C.R. Schuster (1982) Cocaine self-administration in humans. Fed Pro. **41:** 241-246

Fischman, M.W. (1984) The behavioural pharmacology of cocaine in humans. In cocaine: Pharmacology, Effects and Treatment of Abuse. (ED) J. Grobowski, pp. 72-91. National Institute of Drug Abuse Research Monograph 50, US. Governing Printing Office, Washington DC

Fischman, M.W., C.R. Schuster, J.I. Javaid, Y. Hotano and J. Davis (1985) Acute tolerance development to the cardiovascular and subjective effects of cocaine. Journal of Pharmacology and Experimental Therapeutics **235:** 677-682

Fletcher, G.H. and M.S. Starr (1988) Intracerebral SCH 23390 and catalepsy in the rat. European Journal of Pharmacology **149:** 175-178

Fletcher, P.J.(1989) Methods for determining the specificity of drug effects on feeding, In: Psychopharmacology, Neuromethods, Vol. 13 (AA Boulton, GA Baker and AJ Green, eds) Humana Press- Clifton-New Jersey

Foltin, R.W., W.L. Woolverton and C.R. Schuster (1983) Effects of psychomotor stimulants, alone and in pairs, on milk drinking in the rat after intraperitoneal and intragastric administration. Journal of Pharmacology and Experimental Therapeutics **226:** 441-448

Freedman, S.B., S. Patel, R. Marwood, F. Emms, G.R. Seabrook, M.R. Knowles and G. McAllister (1994) Expression and pharmacological characterisation of the human D3 dopamine receptor. Journal of Pharmacology and Experimental Therapeutics **268:** 417-426

Furmidge, L., Z.Y. Tang, N. Petry and D. Clark (1991a) Effects of low, autoreceptor selective doses of dopamine agonists on the discriminative cue and locomotor

hyperactivity produced by d-amphetamine. Journal of Neural Transmission [General Section] **86:** 61-70

Furmidge, L., M. Exner and D. Clark (1991b) Role of dopamine D1 and D2 receptors in mediating the d-amphetamine discriminative cue. European Journal of Pharmacology **202:** 191-199

Galloway, M.P. (1988) Neurochemical interactions of cocaine with dopaminergic systems. Trends in Pharmacological Sciences **9:** 451-454

Gawin, F.H. and E.H. Ellinwood (1988) Cocaine and other stimulants: actions, abuse and treatment. N Eng J Med **318:** 1173-1182

Gawin, F.H. (1991) Cocaine addiction: Psychology and Neurophysiology. Science **251:** 1585-1586

Gendreau, P.L., J.M. Petitto, R. Schnauss, K.J. Frantz, C. Van Hartesveldt, JL. Gariepy and M.H. Lewis (1997) Effects of the putative dopamine D3 receptor antagonist PNU 99194A on motor behaviour and emotional reactivity in C57BL/6J mice. European Journal of Pharmacology **337:** 147-155

Gerfen, C.R. (1992) The neostriatal mosaic: multiple levels of compartmental organisation. Trends in Neuroscience **15:** 133-139

Gessa, G.L., M.L. Porceddu, H. Calli, M. Mercui, G. Mercui, M. Serra, E. Ongini and G. Biggio (1985) Sedation and sleep induced by high dose of apomorphine after blocking of D1 receptors by SCH 23390. European Journal of Pharmacology **109:** 269-274

Geter-Douglass, B. and A.L. Riley (1996) Dopamine D1/D2 antagonist combinations as antagonists of the discriminative stimulus effects of cocaine. Pharmacology Biochemistry and Behaviour **54:** 439: 451

Gilbert, D.B. and S.J. Cooper (1985) Analysis of dopamine D1 and D2 receptor involvement in d- and l- amphetamine-induced anorexia in rats. Brain Res Bull **15:** 385-389

Gilbert, D.B. and S.J. Cooper (1995) 7-OH-DPAT injected into the accumbens reduces locomotion and sucrose ingestion: D3 autoreceptor-mediated effects? Pharmacology Biochemistry and Behaviour **52:** 275-280

Giros, B., P. Sokoloff, M.P. Martres, J.F. Rhiou, L.J. Emorine and J.C. Schwartz (1989) Alternative splicing directs the expression of two D2 receptor isoforms. Nature **342:** 923-926

Giros, B., M.P. Martres, P. Sokoloff and J.C. Schwartz (1990) Gene cloning of human dopaminergic D3 receptor and identification of its chromosome. CR Acad Sci III **311:** 501-508

Gleason, S.D. and H.E. Shannon (1998) Meta-chlorophenylpiperazine induced changes in locomotor activity are mediated by 5-HT_1 as well as 5-HT_{2c} receptors in mice. European Journal of Pharmacology **341:** 135-138

Goeders, N.E. and M.J. Kuhar (1987) Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. Alcohol Drug Res **7:** 207-216

Goeders, N.E., M.A. McNulty, S. Mirkis and K.H. McAllister (1989) Chlordiazepoxide alters intravenous cocaine self-administration in rats. Pharmacology Biochemistry and Behaviour **33:** 859-868

Gold, M.S. (1993) In: Cocaine, Chp. 2. The history of cocaine, Plenum Medical Book Company

Goudie, A.J. (1991) Animal models of drug abuse and dependence, Pg. 453-484. In: P. Willner (ED) 'Behavioural Models in Psychopharmacology' Theoretical, industrial and clinical perspectives. Cambridge, C.V.P.

Goudie, A.J. and M.J. Leathley (1993) Drug-discrimination assays, In: Behavioural neuroscience - A practical approach, Vol. 2. A. Sahgal (ED) O.U.P.

Goudie, A.J., J.A. Smith, A. Taylor, M.A.I. Taylor and M.D. Tricklebank (1998) Discriminative stimulus properties of the atypical neuroleptic clozapine in rats: tests with subtype selective receptor ligands. Behavioural Pharmacology **9:** 699-710

Grandy, D.K., Q.Y. Zhou, R.A. Johnson, L. Allen, K. Buck, J.R. Bunzow, J. Salon and O. Civelli (1991) Multiple human D5 dopamine receptor genes: a functional receptor and two pseudogenes. Proc Natn Acad.Sci USA **88:** 9175-9179

Griffiths, R.R., J.V. Brady and J.D. Snell (1978) Progressive ratio performance by drug infusion: comparison of cocaine, diethylpropane, chlorphentaramine and fenfluramine. Psychopharmacology **56:** 5-13

Grinspoon, L. and J.B. Bakalar (1985) Cocaine, a drug and its social evolution (Rev. Ed). Basic Books, New York

Groenewgen, H.J., N.E.H.M. Becker, and A.H.M. Lahman (1980) Subcortical afferents of the nucleus accumbens septi in the cat, studied with retrograde axonal transport of horseradish peroxidase and bisbezimid. Neuroscience **7:** 977-996

Groenewegen, M.J., H.W. Berendse, J.G. Witter and A.H.M. Lohman (1990) The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a small organisation. Prog Brain Res **85:** 95-118

Gudeman, D., S. Shaptaw, D. Majewska, S. Scherf, D. Yeats and W. Ling (1996) Preliminary report of baclofen as a cocaine craving medication. College on Problems of drug dependence Abstracto **1:** 50

Guyon, A., F. Assouly-Besse, G. Bida, A.J. Puech and M.H. Thiebot (1993) Potentiation by low doses of selected neuroleptics of food-induced conditioned place preference in rats. Psychopharmacology **110:** 460-466

Hadley, S.M. (1996) D4 receptors and their antagonists. Med Res Rev **16:** 507

Haertsen, C.A. (1960) Development of scales based on patterns of drug effects using the Addiction Research Centre Inventory (ARCI). Psychological Reports **18:** 163-194

Halford, J.C.G., S.C.D. Wanninayoke and J.E. Blundell (1998) Behavioural satiety sequence (BSS) for the diagnosis of drug action on food intake. Pharmacology Biochemistry and Behaviour **61:** 157-168

Harris, J.E. and R.J. Baldessarini (1973) Uptake of [3H] - catecholamines by homogenates of rat corpus striatum and cerebral cortex: Effects of amphetamine analogues. Neuropharmacology **12:** 669-679

Hawkins, M.F., C.A. Barkemeyer and R.T. Tulley (1986) Synergistic effects of dopamine agonists and centrally administered neurotensin on feeding. Pharmacology Biochemistry and Behaviour **24:** 1195-1201

Heffner, T.G., M.J. Zigmond and E.M. Stricker (1977) Effects of dopaminergic agonists and antagonists on feeding in intact and 6-hydroxydopamine-treated rats. Journal of Pharmacology and Experimental Therapeutics **201:** 386-399

Heimer, Y.L., D.S. Zohm and G.F. Alheid (1995) Basal Ganglia. In: G. Paxinos (ED) The rat nervous system. Academic Press, Sydney

Henry, D.J., A.G. Margaret and F.J. White (1989) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: repeated administration. Journal of Pharmacology and Experimental Therapeutics **251:** 833-839

Hernandez, L. and B.G. Hoebel (1988a) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Science **42:** 1705-1712

Hernandez, L. and B.G. Hoebel (1988b) Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens. Physiol Behav **44:** 599-606

Hidaka, K., S. Tado, M. Matsumoto, J. Ohmori, K. Maeno and T. Yamaguchi (1996) YM-50001: a novel, potent and selective dopamine D4 receptor antagonist. NeuroReport **7:** 2543-2546

Hobbs, D.J., J.E. Koch and Bodner, R.J. (1994) Naltrexone, dopamine receptor agonists and antagonists, and food intake in rats: 1 Food deprivation. Pharmacology Biochemistry and Behaviour **49:** 197-204

Hodge, C.W., H.H. Samson, G.A. Tolliver and M. Haraguchi (1994) Effects of intra-accumbens injections of dopamine agonists and antagonists on sucrose and sucrose-ethanol reinforced responding. Pharmacology Biochemistry and Behaviour **48:** 141-150

Hoffman, D.C. and R.J. Beninger (1985) The D1 dopamine receptor antagonist SCH 23390 reduces locomotor activity and rearing in rats. Pharmacology Biochemistry and Behaviour **22:** 341-342

Hoffman, D.C. and H. Donovan (1995) Catalepsy as a rodent model for detecting antipsychotic compounds with extrapyramidal side effect liability. Psychopharmacology **120:** 128-133

Holliday, S.M. and P. Benfield (1995) Venlafaxine. A review of its pharmacology and therapeutic potential in depression. Drugs **49:** 280-294

Holtzman, S.G. (1990) Discriminative stimulus effects of drugs: Relationship to potential for abuse. Vol.6 Pg.193-200 In: M.W. Adler and A. Cowan (EDS): Modern Methods in Pharmacology, Testing and Evolution of Drugs of Abuse. Wiley-Liss, Inc.

Horita, A., I. Ushijima and H.A. Carino (1995) Involvement of D1 and D2 dopamine systems in the behavioural effects of cocaine in rats. Pharmacology Biochemistry and Behaviour **52:** 737-741

Hoyer, D., D.E. Clarke, J.R. Fozard, P.R. Hartig, G.R. Martin, E.J. Mylecharane, P.R. Saxenon and P.A. Humphrey (1994) VII. International Union of Pharmacology classification of receptors for 5-Hydroxytryptamine (serotonin). Pharmacol Rev **46:** 158-203

Hsiao, S. and G.P. Smith (1995) Raclopride reduces sucrose preference in rats. Pharmacology Biochemistry and Behaviour **50:** 121-125

Hurd, Y.L., J. Kehr and V. Ungerstedt (1988) In vivo microdialysis as a technique to monitor drug transport: correlation of extracellular cocaine levels and dopamine overflow in the rat brain. Journal of Neurochemistry **51:** 1314-1316

Hyttel, J. (1983) SCH 23390: The first selective dopamine D1 antagonist. European Journal of Pharmacology **91:** 153-154

Inoue, K., N. Kirike, Y. Fujisoki, M. Okuno, H. Ito and S. Yamagami (1995) D2 receptors in the ventrolateral striatum are involved in feeding behaviour in rats. Pharmacology Biochemistry and Biochemistry **50:** 153-161

Iorio, L.C., A. Barnett, F.H. Leitz, V.P. Houser and C.A. Korduba (1983) SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. Journal of Pharmacology and Experimental Therapeutics **226:** 462-468

Izenwasser, S. and J.L. Katz (1993) Differential efficacies of Dopamine D1 receptor agonists for stimulating adenylate cyclase in squirrel monkey and rat. European Journal of Pharmacology **246:**1 39-44

Jackson, D.M., S.B. Ross and S.R. Edwards (1989a) Dopamine D2 agonist-induced behavioural depression is reversed by dopamine D1 agonists. Journal of Neural Transmission **75:** 213-220

Jackson, D.M., S.B. Ross and L.G. Larsson (1989b) Dopamine D2 agonist-induced behavioural depression: critical dependence upon postsynaptic dopamine D1 function. Naunyn-Schmiedeberg's Arch Pharmacol **340:** 355-365

Jackson, D.M., L.P. Martin, L.P. Larsson, R.F. Cox, B.L. Waszczak and S.B. Ross (1990) Behavioural, biochemical and electrophysiological studies on the motor depressant and stimulant effects of bromocriptine. Naunyn-Schmiedeberg's Arch Pharmacol **342:** 290-299

Jackson, D.M. and A. Westlind-Danielsson (1994) Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmac Ther **64:** 291-369

Jackson, H.C., A.M. Needham, L.J. Hutchins, S.E. Mazurkiewicz and D.J. Heal (1997) Comparison of the effects of sibutramine and other monoamine re-uptake inhibitors on food intake in the rat. British Journal of Pharmacology **121:** 1758-1762

Jarbe, T.V.C. (1981) Cocaine cue in pigeons: time course studies and generalisation to structurally related compounds (Norcocaine, Win 35,428 and 35,065-20 and (+)-amphetamine. British Journal of Pharmacology **73:** 843-852

Jarbe, T.V.C. (1984) Discriminative stimulus properties of cocaine: effects of apomorphine, haloperidol, procaine and other drugs. Neuropharmacology **23:** 899-907

Jarbe, T.V.C. (1989) Discrimination learning with drug stimuli: Methods and Applications, In: A.A. Boulton, G.B. Baker and A.J. Greenshaw (EDS) Psychopharmacology, Neuromethods, Vol. 13

Johanson, C.E., R.L. Balster and K. Bonese (1976) Self-administration of psychomotor stimulant drugs: The effects of unlimited access. Pharmacology Biochemistry and Behaviour **4:** 45-51

Johanson, C.E. and W.W. Fischman (1989) The pharmacology of cocaine related to its abuse. Pharmacological Reviews **41:** 3-52

Johanson, C.E. and J.E. Barrett (1993) The discriminative stimulus effects of cocaine in pigeons. Journal of Pharmacology and Experimental Therapeutics **267:** 1-8

Jonas, J.M. and M.S. Gold (1986) Cocaine abuse and eating disorder. Lancet **1:** 390-391

Kalivas, P.W. and P. Duffy (1995) D1 receptors modulate glutamate transmission in the ventral tegmental area. Journal of Neuroscience **15:** 5379-5388

Kamien, J.B. and W.L. Woolverton (1989) A pharmacological analysis of the discriminative stimulus properties of d-amphetamine in rhesus monkeys. Journal of Pharmacology and Experimental Therapeutics **258:** 626-632

Kantak, K.M., M.A. Edwards and R.D. Spealman (1995) Effects of N-methyl-D-aspartate antagonists in rats discriminating different doses of cocaine: comparison with direct and indirect dopamine agonists. Journal of Pharmacology and Experimental Therapeutics **274:** 657-65

Katz, J.L. and J.M. Witkin (1992) Effects of quinpirole and SKF 38393 alone and in combination in squirrel monkeys trained to discriminate cocaine. Psychopharmacology **107:** 217-220

Kaur, P. and S. Ahlenius (1997) Potentiation of DOI-induced forward locomotion in rats by (-)-pindolol pretreatment. Journal of Neural Transmission **104:** 605-614

Kebabian, J.W., G.L. Petzold and P. Greengard (1972) Dopamine-sensitive adenylate cyclase in caudate nucleus of rat brain, and its similarity to the 'dopamine transporter'. Proc Natl Acad.Sci USA **69:** 2145-2149

Kebabian, J.W. and D.B. Calne (1979) Multiple receptors for dopamine. Nature **277:** 93-96

Kebabian, J.W. (1988) The D1 receptor, In: Central D1 dopamine receptors, (Eds: Goldstein, M., K. Fuxe and I. Tabachick) Plenum Press, New York and London

Kebabian, J.W., C. Briggs, D.R. Bratta, K. Asin, R.G. Mackenzie, J.F. Malley and R. Schoenleber (1990) A-68930: a potent and specific agonist for the D1 dopamine receptor. American Journal of Hypertension **3:** 405-425

Kehne, J.H., W.W. Sant and C.A. Sorenson (1981) The effects of radio-frequency lesions of the nucleus accumbens on D-amphetamine-induced locomotor and rearing behaviour in rats. Psychopharmacology **75:** 363-367

Kelley, A.E., V.B. Domesick and W.J.H. Nauta (1982) The amygdalostriatal projection in the rat - an anatomical study by anterograde and retrograde tracing methods. Neuroscience **7:** 615-630

Kelley, A.E., C.G. Long and A.M. Gauthier (1988) Induction of oral stereotypy following amphetamine microinjection into a discrete subregion of the striatum. Psychopharmacology **95:** 556-559

Kelley, A.E. (1993) Locomotor activity and exploration. In: Behavioural Neuroscience. A practical approach, Vol. II (ED) A. Sahgal, Oxford University Press

Kelly, P.H. and S.D. Iversen (1976) Selective 6-OH-DA induced destruction of mesolimbic dopamine neurones: Abolition of cocaine-induced locomotor activity in rats. European Journal of Pharmacology **40:** 45-56

Kennet, G.A. and G. Curzon (1988) Evidence that hypophagia induced by mCPP and TFmPP require 5-HT_{1c} and 5-HT_{1b} receptors: hypophagia induced by RU 24967 only requires 5-HT1b receptors. Psychopharmacology **96:** 93-100

Kennet, G.A. and G. Curzon (1991) Potencies of antagonists indicate that 5-HT_{1c} receptors mediate 1-3(chlorophenyl-piperazine-induced hypophagia. British Journal of Pharmacology **103:** 2016-2020

Khroyan, T.V., D.A. Baker and J.L. Neisewander (1995) Dose-dependent effects of the D3-preferring agonist 7-OH-DPAT on motor behaviours and place conditioning. Psychopharmacology **122:** 351-357

Kilpatrick, G.J., B.J. Jones and M.B. Tyers (1987) Identification and distribution of 5-HT3 receptors in rats brain using radioligand binding. Nature **330:** 746-748

Kiritsy-Ray, J.A., J.B. Holter, S.M. Gordon, M.J. Smith and L.C. Terry (1990) Role of the central nervous system in hemodynamic and sympathodrenal responses to cocaine in rats. Journal of Pharmacology and Experimental Therapeutics **255:** 154-160

Kirk, R.A. (1982) Experimental design for the behavioural sciences 2nd Edition Brooks Cole, Monterey CA

Kita, H. and S.T. Kitai (1988) Glutamate decarboxylase immunoreactive neurones in rat neostriatum: their morphological types and populations. Brain Res **447:** 346-357

Kiyatkin, E.A. (1993) Cocaine enhances the changes in extracellular dopamine in nucleus accumbens associated with reinforcing stimuli: A high speed chronoamperometric study in freely moving rats. European Journal of Neuroscience **5:** 284-291

Kiyatkin, E.A. (1995) Functional significance of mesolimbic dopamine. Neuroscience and Biobehavioural Reviews **19:** 573-598

Kleven, M.S., W. Woolverton, C. Schuster and L. Seiden (1988) Behavioural and neurochemical effects of repeated and continuous exposure to cocaine. NIDA Research monograph **81:** 86-93

Kleven, M.S., E.W. Anthony, L.I. Goldberg and W.L. Woolverton (1988) Blockade of the discriminative stimulus effects of cocaine in rhesus monkeys with the D1 dopamine antagonist SCH 23390. Psychopharmacology **95:** 427-429

Kleven, M.S., E.W. Anthony, and W.L. Woolverton (1990) Pharmacological characterisation of the discriminative stimulus effects of cocaine in rhesus monkeys. Journal of Pharmacology and Experimental Therapeutics **254:** 312-317

Kleven, M.S. and W. Koek (1997) Discriminative stimulus properties of cocaine: enhancement by β-adrenergic receptor antagonists. Psychopharmacology **131:** 307-312

Kleven, M.S. and W. Koek (1998) Discriminative stimulus properties of cocaine: enhancement by monoamine re-uptake blockers. Journal of Pharmacology and Experimental Therapeutics **284:** 1015-1025

Kling-Petersen, T., E. Ljung and K. Svensson (1995) Effects on locomotor activity after local application of D3 preferring compounds in discrete areas of the rat brain. Journal of Neural Transmission [General Section] **102:** 209-220

Koechling, V., M.L. Calle and R.A. Wise (1988) Effects of SCH 23390 on motivational aspects of deprivation-induced feeding. Psychobiology **16:** 207-212

Kohler, C., H. Hall, S.O. Ogren and L. Gawell (1985) Specific in vitro and in vivo binding of 3H-raclopride, a potent substituted benzamide drug with high affinity for dopamine D2 receptors in the rat. Biochem Pharmacol **34:** 2251-2259

Koob, G.F. (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends in Pharmacological Sciences **13:** 177-183

Koob, G.F. (1995) Animal models of drug addiction, Psychopharmacology: The fourth generation of progress, F.E. Bloom, D.J. Kupfer (EDS) Raven Press Ltd. New York

Kotler, M., H. Cohen, R. Segman, I. Gritsenko, L. Nemanov, B. Lever, I. Kramer, M. Zer-Zion, I. Kletz and R.P. Ebstein (1997) Excess dopamine D4 receptor (D4DR) exon III seven repeat allele in opioid-dependent subjects. Molecular Psychiatry **2:** 251-254

Krebs-Thomson, K., M.P. Paulus and M.A. Geyer (1998) Effects of hallucinogens on locomotor and investigatory activity and patterns: influence of 5-HT_{2a} and 5-HT_{2c} receptors. Neuropsychopharmacology **18:** 339-351

Kristensen, P., M.E. Judge, L. Thim, V. Ribel, K.N. Christjansen, B.S. Wulff, J.T. Clausen, P.B. Jensen, O.D. Madsen and N. Vrong (1998) Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature **393:** 72-74

Kuhar, M.J. (1992) Molecular pharmacology of cocaine: a dopamine hypothesis and its implications, In: Cocaine: Scientific and Social Dimensions, John Wiley and Sons Publishers

Kulagowski, J.L., H.B. Broughton, N.R. Curtis, I.M. Mawer, M.P. Ridgill, R. Baker, F. Emms, S.B. Freedman, R. Marwood, S. Patel, S. Patel, I. Ragan and P.D. Leeson (1996) 3-[[4-(4-chlorophenyl)-1-yl]-methyl-1H-pyrrolo[2,3-b]pyridine: An antagonist with high affinity and selectivity for the human D4 receptor. Journal of Medicinal Chemistry **39:** 1941-1942

Kulagowski, J.J. and S. Patel (1997) Dopamine D4 receptor antagonists. Current Pharmaceutical Design **3:** 355-366

Lamas, X., S. Stevens-Negus, M.A. Nader and N.K. Mello (1996) Effects of the putative dopamine D3 receptor agonist 7-OH-DPAT in rhesus monkeys trained to discriminate cocaine from saline. Psychopharmacology **124:** 306-314

Lawson, W.B., J. Byrd and D. Reed (1984) Effects of neuroleptics on food intake. Soc Neuroscience Abstract **10:** 302

Le, A.D., D. Tomkins, G. Higgins, B. Quan and E.M. Sellers (1997) Effects of 5-HT3, D1 and D2 receptor antagonists on ethanol- and cocaine-induced locomotion. Pharmacology Biochemistry and Behaviour **57:** 325-332

Levant, B., D.E. Grigoriadis and E.D. De Say (1995) Relative affinities of dopamine drugs at D2 and D3 receptors. European Journal of Pharmacology **278:** 241-243

Levant, B., G.N. Bancroft and C.M. Selkirk (1996) In vivo occupancy of D2 dopamine receptors by 7-OH-DPAT. Synapse **24:** 60-64

Levant, B. and N.R. Vansell (1997) In vivo occupancy of D2 dopamine receptors by nafadotride. Neuropsychopharmacology **17:** 67-71

Levesque, D., J. Diaz, C. Pilon, M.P. Martres, B. Giros, E. Souil, D. Schott, J.L. Margot, J.C. Schwartz and P. Sokoloff (1992) Identification, characterisation and

localisation of the dopamine D3 receptor in rat brain using 7-(3H) hydroxy-N,N-di-n-propyl-2-aminotetralin. Proc Natl Acad Sci USA **89:** 8155-8159

Lynch, D.R. and M.J. Gallagher (1996) Inhibition of N-Methyl-D-Aspartate receptors by haloperidol: development and pharmacological characterisation in nature and recombinant receptors. Journal of Pharmacology and Experimental Therapeutics **279:** 154-161

MacDonald, R.C. and J.L. Barker (1978) Specific antagonism of GABA-mediated post-synaptic inhibition of cultured mammalian neurones: A common model of convulsant action. Neurology **28:** 323-330

Maisto, S.A., M. Galizio and G.J. Connors (1995) In: Drug use and abuse, Chp. 6: Cocaine and amphetamines, Harcourt Brace College Publishers

Maldonado-Irizarry, C.S., C.J. Swanson and A.E. Kelley (1995) Glutamate receptors in the nucleus accumbens shell control feeding behaviour via the lateral hypothalamus. Journal of Neuroscience **15:** 6779-6788

Malmberg, A.H., D.M. Jackson, A. Eriksson and N.A. Mohell (1993) Unique binding characteristics of antipsychotic agents interacting with human dopamine D2A, D2B and D3 receptors. Molecular Pharmacology **43:** 749-754

Marlatt, A., J.S., Boer, D.M. Donovan and D.R. Kivulahn (1988) 'Addictive behaviours: etiology and treatment'. Annul Review of Psychology **39:** 223-252

Martel, P. and M. Fantino (1996) Influence of the amount of food ingested on mesolimbic dopaminergic system activity: A microstructural study. Pharmacology Biochemistry and Behaviour **55:** 297-302

Martin-Iversen, M.T. and C.T. Dourish (1988) Role of dopamine D1 and D2 receptor subtypes in mediating agonist effects on food consumption in rats. Psychopharmacology **96:** 370-374

Matthews, J.C. and A. Collins (1983) Interactions of cocaine and cocaine congeners with sodium channels. Biochem Pharmacol **32:** 455-460

Mattingly, B.A., J.K. Rowlett, T. Ellison and K. Rase (1996) Cocaine-induced behavioural sensitisation: Effects of haloperidol and SCH 23390 treatments. Pharmacology Biochemistry and Behaviour **53:** 481-486

Mazurski, E.J. and R.J. Beninger (1991) Effects of selective drugs for dopaminergic D1 and D2 receptors on conditioned locomotion in rats. Psychopharmacology **105:** 107-112

McCreary, A.C. and C.A. Marsden (1993) Cocaine-induced behaviour: dopamine D1 receptor antagonism by SCH 23390 prevents expression of conditioned sensitisation following repeated administration of cocaine. Neuropharmacology **32:** 387-391

McCullough, L.D. and J.D. Salamone (1992) Involvement of nucleus accumbens dopamine in the motor activity induced by periodic food presentation: A microdialysis and behavioural study. Brain Res **592:** 29-36

McElroy, J., K. Zeller, K. Amy, K. Ward, J. Cawley, A. Mazzola, W. Keim, and K. Rohrbach (1993) In vivo agonist properties of 7-hydroxy-N-N-di-N-popyl-2-aminotetralin, a dopamine D3-selective receptor ligand. Drug Dev Res **36:** 257-259

McNair, D.M., M. Larr and L.F. Droppleman (1971) Manual for the profile of mood states. Educational and Industrial Testing Service, San Diego

McQuade, R.D., R.A. Duffy, C.C. Anderson, G. Crosby, V.L. Coffin, R.E. Chipkin and A. Barnett (1991) SCH 39166: a new D1-selective radioligand: in vitro and in vivo binding analysis. Journal of Neurochemistry **57:** 2001

Meador-Woodruff, J.H., A. Mansour, D.K. Grandy, S.P. Damask, O. Civelli and S.J. Watson Jr. (1992) Distribution of D5 dopamine receptor mRNA in rat brain. Neuroscience Letters **145:** 209-212

Meert, T.F. and P.A.J. Janssen (1992) Ritanserin, a new therapeutic approach for drug abuse. Part 2: Effects on cocaine. Drug Dev Res **25:** 39-53

Meert, T.F. and G.H. Clincke (1994) 7-OH-DPAT and alcohol consumption, withdrawal and discriminative stimulus properties in rats. Alcohol **29:** 489-492

Meert, T.F., P. De Haes, N. Aerts and G. Clincke (1996) Antagonism of the discriminative stimulus properties of cocaine with the combination of a dopamine D1 and D2 antagonist. Acta Neurobiol Exp **56:** 897-905

Melia, K.F. and R.D. Spealman (1991) Pharmacological and characterisation of the discriminative stimulus effects of GBR 12909. Journal of Pharmacology and Experimental Therapeutics **258:** 626-632

Meltzer, H.Y., S. Motsuborg and J.C. Lee (1989) Classification of typical and atypical antipsychotic drugs on the bases of dopamine D1, D2 and serotonin pKi values. Journal of Pharmacology and Experimental Therapeutics **251:** 238-246

Mendelson, J.C. (1960) Role of hunger in T-maze learning for food by rats. Journal of Comparative Physiology and Psychology **62:** 341-349

Merchant, K.M., G.S. Gill, D.W. Harris, R.M. Huff, M.J. Eaton, K. Lockingland, B.S. Lutzke, R.B. Mccall, M.F. Piercey, P.J.K.D. Schrewer, V.H. Sethy, M.W. Smith, K.A. Svensson, A.H. Tang, P.F. Vanvoightlander and R.E. Tenbrink (1996)

Pharmacological characterisation of U-101387, a dopamine D4 receptor selective antagonist. Journal of Pharmacology and Experimental Therapeutics **279:** 1392

Meyer, M.E., C. Van Hartesveldt and T.J. Potter (1993) Locomotor activity following intra-accumbens microinjections of dopamine D1 agonist SK&F 38393 in rats. Synapse **13:** 310-314

Meyer, M.E., G.A. Cottrell, C. Van Hartesveldt and T.J. Potter (1993) Effects of dopamine D1 antagonists SCH 23390 and SK&F 83566 on locomotor activities in rats. Pharmacology Biochemistry and Behaviour **44:** 429-432

Middaugh, L.D., K.K. McGroaty, C.H. Groseclose and B. Adinoff (1998) Cocaine discrimination: relationship to local anaesthetics and monoamine uptake inhibitors in C57BL/6mice. Psychopharmacology **136:** 44-49

Millan, M.J., V. Audinot, J.M. Rivet, A. Gobert, J. Vion, J.F. Prost, M. Spedding and J.L. Peglion (1994) S-14297, a novel selective ligand at cloned human dopamine D3 receptors, blocks 7-OH-DPAT-induced hypothermia in rats. European Journal of Pharmacology **260:** R3-5

Millan, M.J., J-L. Peglion, J. Vion, J-M. Rivet, M. Brocco, A. Gobert, A. Newman-Tancredi, K. Bervoets, C. Dacquet, S. Girardon, V. Jacques, C. Chaput, and V. Audinet (1995) Functional correlates of dopamine D3 receptor activation in the rat in vivo and their modulation by the selective antagonist, (+) S-14297: 1. Activation of postsynaptic D3 receptors mediates hypothermia, whereas blockade of D2 receptors elicits prolactin secretion and catalepsy. Journal of Pharmacology and Experimental Therapeutics **275:** 885-898

Millan, M.J., A. Newman-Tancredi, M. Brocco, A. Gobert, F. Lejeune, V. Audinot, J.M. Rivet, R. Screiber, A. Dekeyne, M. Spedding, J.P. Nicolas and J.L. Peglion (1998) S 18126 ({2-[4-(2,3-dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-ylmethyl]indan-2-yl}), a potent, selective and competitive antagonist at dopamine D4

receptors: An in vitro and in vivo comparison with L-745,870 (3-{[4-(4-chloro-phenyl) piperazin-1yl]methyl]-1H-pyrrolo[2,3-b]pyridine) and raclopride. Journal of Pharmacology and Experimental Therapeutics **287:** 167-186

Mogenson, G.J., D.L. Jones and C.Y. Yim (1980) From motivation to action: functional interface between the limbic system and the motor system. Progress in Neurobiology **14:** 69-97

Mogenson, G.J. and C.R. Yang (1991) The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. Adv Exp Med Biol **295:** 267-290

Molloy, A.G. and J.L. Waddington (1984) Dopaminergic behaviour stereospecifically promoted by the D1 agonist R-SK&F 38393 and selectivity blocked by the D1 antagonist SCH 23390. Psychopharmacology **82:** 409-410

Molloy, A.G. and J.L. Waddington (1985) Sniffing, rearing and locomotor responses to the D1 dopamine agonist R-SK&F 38393 and to apomorphine: differential interactions with the selective D1 and D2 antagonists SCH 23390 and metoclopromide. European Journal of Pharmacology **108:** 305-308

Molloy, A.G., K.M. O'Boyle, M.T. Pugh and J.L. Waddington (1986) Locomotor behaviours in response to new selective D2 and D2 dopamine receptor agonists, and the influence of selective antagonists. Pharmacology Biochemistry and Behaviour **25:** 249-253

Molloy, A.G. and J.L. Waddington (1987) Assessment of grooming and other behavioural responses to the D1 receptor agonist SKF 38393 and its R- and S-enantiomers in intact adult rats. Psychopharmacology **92:** 164-168

Monsma, F.J., L.C. Mohan, L.D. McVitie, C.R. Gerfen and D.R. Sibley (1990) Molecular cloning and expression of a D1 dopamine receptor linked to adenylyl cyclase activation. Proc Natn Acad Sci USA **87:** 6723-6727

Morency, M.A. and R.J. Beninger (1986) Dopaminergic substrates of cocaine-induced place conditioning. Brain Research **399:** 33-41

Mori, T., K. Murase, J. Tanaka and Y. Ichimaru (1997) Biphasic effects of D3 receptor agonists, 7-OH-DPAT and PD 128907 on the D1-receptor agonist-induced hyperactivity in mice. Japanese Journal of Pharmacology **73:** 251-254

Murray, A.M. and J.L Waddington (1989) The induction of grooming and vacuous chewing by a series of selective D1 dopamine receptor agonists: two directions of D1:D2 dopamine receptor interactions. European Journal of Pharmacology **160:** 377-384

Musto, D.F. (1992) Cocaine's history, especially the American experience, In: Cocaine, scientific and social dimensions, Pg. 7-14, Gregory R. Bock and Julie Whelan (Eds). John Wiley and Sons Publishers

Nader, M. and W. Woolverton (1991) Effects of increasing the magnitude of an alternative reinforcer on drug-choice in a discrete-trials procedure. Psychopharmacology **105:** 169-174

Nader, M. and W. Woolverton (1992) Effects of increasing response requirement on choice between cocaine and food in rhesus monkeys. Psychopharmacology **108:** 295-300

Nader, M. and W. Woolverton (1994) Blockade of the discriminative stimulus effects of d-amphetamine in rhesus monkeys with selective 5-HT_{1a} agonists. Behavioural Pharmacology **5:** 591-598

Naruse, T., H. Amano and Y. Koizumi (1991) Possible involvement of dopamine D1 and D2 receptors in diazepam-induced hyperphagia in rats. Fundam Clin Pharmacol **5:** 677-693

Neisewander, J.L., L.E. O'Dell and J.C. Redmond (1995) Localisation of dopamine receptor subtypes occupied by intra-accumbens antagonists that reverse cocaine-induced locomotion. Brain Res **671:** 201-212

Neisewander, J.L., R.A. Fuchs, L.E. O'Dell and T.V. Khroyan (1998) effects of SCH 23390 on dopamine D1 receptor occupancy and locomotion produced by intra-accumbens cocaine infusion. Synapse **30:** 194-204

Nielsen, E.B., K. Randrup and P.H. Andersen (1989) Amphetamine discrimination: effects of dopamine receptor agonists. European Journal of Pharmacology **160:** 253-262

Nishino, H., T. Oro, K. Sasaki, M. Fukuda and K.I. Muramoto (1984) Caudate unit activity during operant feeding behaviour in monkeys and modulation by cooling prefrontal cortex. Behavioural Brain Results **11:** 21-33

Nomikos, G.G., G. Damsma, D. Wenkstern and H.C. Fibiger (1990) In vivo characterisation of locally applied dopamine uptake inhibitors by striatal microdialysis. Synapse **6:** 106-112

Okuyama, S., S. Choki, N. Kawashima, Y. Suzuki, S.I. Ogawa, T. Kumagai, A. Nokazato, M. Nagamie, K. Yamagouchi and K. Tamisawa (1997a) The atypical antipsychotic profile of NRA0045, a novel dopamine D4 and 5-HT2a receptor antagonist in rats. British Journal of Pharmacology **121:** 515-525

Okuyama, S., S. Chaki, R. Yoshikawa, Y. Suzuki, S.I. Ogawa, Y. Imagawa, N. Kawashima, Y. Ikeda, T. Kumagai, A. Nakazato, M. Hagamine and K. Tomisawa (1997b) In vitro and in vivo characterisation of the dopamine D4 receptor, serotonin

5-HT2a receptor and alpha-1 adrenoceptor antagonist (R)-(+)-2-amino-4-(4-fluorophenyl) -5- [1-[4-(4-fluorophenyl)-4-oxobutyl] pyrrolidin-3-yl] thiazole (NRA0045). Journal of Pharmacology and Experimental Therapeutics **282:** 56-63

O'Malley, K.L., S. Harmon, L. Tang and R.D. Todd (1992) The rat dopamine D4 receptor: sequence gene structure and demonstration of expression in the cardiovascular system. New Biol **4:** 137-146

O'Neill, M.F. and G. Shaw (1999) Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonists C-APB in mice. Psychopharmacology **145:** 237-250

Panocka, I., R. Cicocioppo, M. Mosca, C. Polidori and M. Massi (1995) Effects of the dopamine D1 receptor antagonist SCH 39166 on the ingestive behaviour of alcohol-preferring rats. Psychopharmacology **120:** 227-235

Parada, M.A., L. Hernandez and B.G. Hoebel (1988) Sulpiride injections in the lateral hypothalamus induce feeding and drinking in rats. Pharmacology Biochemistry and Behaviour **30:** 917-923

Patel, S., S. Patel, R. Marwood, F. Emms, D. Marston, P.D. Leeson, N.R. Curtis, J. Kulagowski and S.B. Freedman (1996a) Identification and pharmacological characterisation of [^{125}I] L-750,667, a novel radioligand for the dopamine D4 receptor. Journal of Pharmacology and Experimental Therapeutics **50:** 1658-1664

Patel, S., S. Patel, R. Marwood, A.E. Fletcher, J. Kulagowski, C.I. Ragan, P.D. Leeson and S.B. Freedman (1996b) L-745,870 (3-{[4-(4-chloro-phenyl) piperazin-1yl]methyl]-1H-pyrrolo[2,3-b]pyridine: A high affinity and selective dopamine D4 receptor antagonist. British Journal of Pharmacology **119:** 30P

Patel, S., S.B. Freedman, K.L. Chapman, F. Emms, A.E. Fletcher, M. Knowles, R. Marwood, G. McAllister, J. Myers, S. Patel, N. Curtis, J.J. Kulagowski, P.D. Leeson,

M. Ridgill, M. Graham, S. Matheson, D. Rathbone, A.P. Watt, L.J. Bristow, N.M.J. Rupnick, E. Baskin, J.J. Lynch and C.I. Ragan (1997) Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D4 receptor. Journal of Pharmacology and Experimental Therapeutics **283:** 636-647

Peltier, R.L., T.N.W. Emmett-Oglesby, W.H. Thomas and S. Schenk (1994) Failure of ritanserin to block the discriminative stimulus or reinforcing stimulus effects of cocaine. Pharmacology Biochemistry and Behaviour **48:** 473-478

Perrault, H., R. Depoortere, E. Morel, D.J. Sanger and B. Scatton (1997) Psychopharmacological profile of Amisulpride: an antipsychotic drug with presynaptic D2/D3 dopamine receptor activity and limbic selectivity. Journal of Pharmacology and Experimental Therapeutics **280:** 73-82

Petit, H.O. and J.B. Jr. Justice (1991) Effects of dose on cocaine self-administration behaviour and dopamine levels in the nucleus accumbens. Brain Res **539:** 94-102

Phillips, A.G., J.G. Pfaus and C.D. Blanche (1991) Dopamine and motivated behaviour: Insights provided by in vivo analyses. In: Willner PJ; Scheel-Kruger J; Eds. The mesolimbic dopamine system: from motivation to action. Chichester: Wiley; 199-224

Pirot, S., R. Godbout, J.P. Tassin, J. Glowiski and A.M. Thierry (1992) Inhibitory effects of VTA stimulation of activity of prefrontal cortical neurones: evidence for the involvement of both dopamine and Gabaergic components. Neuroscience **49:**857-865

Pisa, M. (1988) Motor functions of the striatum in the rat: critical role of the lateral region in tongue and forelimb reaching. Neuroscience **24:** 453-463

Prescott, R. (1970) Some behavioural effects of variable which influence the 'general' levels of activity of rats. Anim Behav **18:** 791-796

Pugsley, T.A., M.D. Davis, H.C. Akunne, R.G. MacKenzie, Y.H. Shih, G. Damsma, H. Wilkstrom, S.Z. Whetzel, L.M. Georgic, L.W. Cooke, S.B. Demottes, A.E. Corbin, S.A. Glase, L.D. Wise, D. Dijksta and T.G. Heffner (1995) Neurochemical and functional characterisation of the preferentially selective dopamine D3 agonist PD 128907. Journal of Pharmacology and Experimental Therapeutics **275:** 1355-1366

Rang, H.P. and M.M. Dale (1991) Chp 4: The central nervous system, In: Pharmacology, 2nd Edition, Churchill Livingstone, London

Rapoza, D. and W.L. Woolverton (1991) Attenuation of the effects of cocaine on milk consumption in rats by dopamine antagonists. Pharmacology Biochemistry and Behaviour **40:** 133-137

Reith, M.E., B.E. Meisler, H. Sershen and A. Lajthi (1986) Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotypic behaviour. Biochem Pharmacol **35:** 1127

Reith, M.E. and C.T. Fischette (1991) Sertraline and cocaine-induced locomotion in mice. II. Chronic studies. Psychopharmacology **103:** 306-313

Reneric, J.P. and I. Lucki (1998) Antidepressant behavioural effects by dual inhibition of monoamine re-uptake in the rat forced swimming test. Psychopharmacology **136:** 190-197

Ritz, M.C., R.J. Lamb, S.R. Goldberg and M.J. Kuhar (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science **237:** 1219-1223

Ritz, M.C., E.J. Cone and M.J. Kuhar (1990) Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. Life Sciences **46:** 635-45

Robbins, T.W. (1977) A critique of the methods for the measurement of spontaneous motor activity, In: Handbook of Psychopharmacology, Vol.7, (Iversen, LL; Iversen, SD; Snyder, SH; EDS) Plenum, New York

Robbins, T.W., M. Cador, J.R. Taylor and B.J. Everitt (1989) Limbic-striatal interactions in reward-related processes. Neurosci Biobehav Rev **13:** 155-162

Robbins, T.W. and B.J. Everitt (1996) Neurobehavioural mechanisms of reward and motivation. Current Opinion in Neurobiology **6:** 228-236

Roberts, D.C.S. and G.J. Vickers (1984) Atypical neuroleptics increase cocaine self-administration of cocaine. An evaluation of a behavioural screen for antipsychotic activity. Psychopharmacology **82:** 135-139

Roberts, D.C.S., E.A. Lah and G.J. Vickers (1989) Self-administration of cocaine as a progressive schedule in rats: dose response relationship and effects of haloperidol pre-treatment. Psychopharmacology **97:** 535-538

Roberts, D.C.S., M. Andrew and G.J. Vickers (1996) Baclofen attenuates the reinforcing effects of cocaine in rats. Neuropsychopharmacology **15:** 417-423

Robertson, A. and C. MacDonald (1985) Opposite effects of sulpiride and metoclopromide on amphetamine-induced stereotypy. European Journal of Pharmacology **109:** 81-89

Robinson, T.E. and K.C. Berridge (1993) The neural basis of drug craving: an incentive-sensitisation theory of addiction. Brain Results Reviews **18:** 247-291

Rusk, I.N. and S.J. Cooper (1988) Profile of the selective dopamine D2 receptor agonist N-0437: Its effects on palatability- and deprivation-induced feeding, and operant responding for food. Physiology and Behaviour **44:** 545-553

Rusk, I.N. and S.J. Cooper (1989a) The selective dopamine D1 receptor agonist SK&F 38393: its effects on palatability- and deprivation-induced feeding, and operant responding for food. Pharmacology Biochemistry and Behaviour **34:** 17-22

Rusk, I.N. and S.J. Cooper (1989b) Microstructural analysis of the anorectic effect of N-0437, a highly selective dopamine D2 agonist. Brain Research **494:** 350-358

Rusk, I.N. and S.J. Cooper (1994) Parametric studies of selective D1 and D2 antagonists: effects on appetitive and feeding behaviour. Behavioural Pharmacology **5:** 615-622

Sanberg, P.R. (1980) Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature **284:** 472-473

Sanger, D.J. (1991) Screening for abuse and dependence liabilities, Pg. 485-502 In: P. Willner (ED). 'Behavioural models in Psychopharmacology.' Theoretical, industrial and clinical perspectives. Cambridge, C.V.P.

Sasaki, J., T. Tetham and J. Barrett (1995) The discriminative stimulus properties of amphetamine in pigeons. Psychopharmacology **120:** 303-310

Saunders, J.B. (1990) The great legalisation debate. Drug Alcohol Rev **9:** 3-5

Sautel, F., N. Griffon, P. Sokoloff, J.C. Schwartz, C. Launay, P. Simon, J. Costentin, A. Schoenfelder, F. Garrido, A. Man and C.G. Wermuth (1995) Nafadotride, a potent preferential dopamine D3 receptor antagonist, activates locomotion in rodents. Journal of Pharmacology and Experimental Therapeutics **275:** 1239-1246

Scatton, B., G. Perrault and D.J. Sanger (1994) Pharmacological profile of amisulpride, an atypical neuroleptic which preferentially blocks presynaptic D2/D3 receptor antagonists [abstract]. Neuropsychopharmacology **10:** 242S

Scatton, B., Y. Claustre, A. Cudannec, A. Oblin, G. Perrault, D.J. Sanger and H. Schoemaker (1997) Amisulpride: from animal pharmacology to therapeutic action. International Clinical Psychopharmacology **12:** (Suppl 2) S29-S36

Schama, K.F., L.L. Howell and L.D. Byrd (1997) Serotonergic modulation of the discriminative stimulus effects of cocaine in squirrel monkeys. Psychopharmacology **132:** 27-34

Schannon, H.E., K.G. Bemis, S.C. Peters (1991) Potency and efficacy of dopamine agonists in mouse strains differing in dopamine cell and receptor number. Pharmacology Biochemistry and Behaviour **40:** 103-107

Scheel-Kruger, J. (1971) Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolites of the catecholamines in the brain. Journal of Pharmacology **14:** 47-59

Scheel-Kruger, J., C. Braestrup, M. Nielsen, K. Golembiawski and E. Magilnicka (1977) Cocaine: Discrimination on the role of dopamine in the biochemical mechanisms of action, in: cocaine and other stimulants (ED) E.H. Ellinwood and M.M. Kilbey (Plenum Press, New York) p. 373

Schenk, S., T. Hurt, R. Molovechko, A. Robertson, G. Klikowski and Z. Amit (1986) Differential effects of isolation housing on the conditional place preference produced by cocaine and amphetamine. Pharmacology Biochemistry and Behaviour **24:** 1793-1796

Schneider, L.H., J. Gibbs and G.P. Smith (1986) D2 selective receptor antagonists suppress sucrose sham feeding in the rat. Brain Research Bulletin **17:** 605-611

Schneider, L.H., D. Greenberg and G.P. Smith (1988) Comparison of the effects of selective D1 and D2 receptor antagonists on sucrose sham-drinking, In: Kalivas, P.W.

and C.B. Nemeroff (Eds) The Mesolimbic Dopamine System. New York Academy of Sciences, New York, 534-537

Schoemaker, H., Y. Claustre, D. Fage, L. Rouquier, L. Chergui, O. Curet, A. Oblin, F. Gonon, C. Carter, J. Benavides and B, Scatton (1997) Neurochemical characteristics of amisulpride, an atypical dopamine D2/D3 receptor antagonist with both presynaptic and limbic selectivity. Journal of Pharmacology and Experimental Therapeutics **280:** 83-97

Schultz, W., P. Apicella, E. Scarnati and T. Ljungberg (1992) Neuronal activity in monkey ventral striatum related to the expectation of reward. Journal of Neuroscience **12:** 4595-4610

Schwartz, J.C., B. Giros, M.P. Martres and P. Sokoloff (1993) Multiple dopamine receptors as molecular targets for antipsychotics, In: International Academy of Biomedical Drug Research, Vol. 4. New generation of antipsychotic drugs: Novel mechanisms of action. N. Brunello, J. Mendlewicj and J. Racogni (EDS) Pg. 1-14, Karger, Basel

Seeman, P. (1980) Brain dopamine receptors. Pharmacol Rev **32:** 229-313

Seeman, P. and H.H.M. Van Tol (1994) Dopamine receptor pharmacology. Trends In Pharmacological Sciences **15:** 264-269

Seiden, L., K. Sobol and G. Ricuarte (1993) Amphetamine: effects on catecholamine systems and behaviour. Annu Rev Pharmacol Toxicol **32:** 369-377

Seleman, L.D. and P.S. Goldman-Rakic (1985) Longitudinal topography and internalisation of corticostriatal projections in the rhesus monkey. Journal of Neuroscience **5:** 776-794

Self, D.W. and L. Stein (1992) The D1 agonists SKF 82958 and SKF 77474 are self-administered by rats. Brain Research **582:** 349-352

Setler, P.E., H.H. Sarem, C.L. Zirkler and H.C. Saunders (1978) The central effects of a novel dopamine agonist. European Journal of Pharmacology **50:** 419-430

Seutin, V., S.V. Johnson and R.A. North (1994) Effects of dopamine and baclofen on NMDA induced burst firing in rat VTA neurones. Neuroscience **58:** 201-206

Shafer, R. and R. Levant (1998) The D3 dopamine receptor in cellular and organismal function. Psychopharmacology **135:** 1-16

Sharkey, J., K.A. Glen, S. Wolfe and M.J. Kuhar (1988a) Cocaine binding at sigma receptors. European Journal of Pharmacology **149:** 171-174

Sharkey, J., M.C. Ritz, J.A. Schendon, R.C. Hanson and M.J. Kuhar (1988b) Cocaine inhibits muscarinic cholinergic receptors in heart and brain. Journal of Pharmacology and Experimental Therapeutics **246:** 1048-1052

Sherer, M.A., K.M. Kumor and J.H. Jaffe (1989) Effects of intravenous cocaine are partially attenuated by cocaine. Psychiatry Research **27:** 117-125

Shoaib, M., L.S. Swanner, C.E. Beyer, S.R. Goldberg and C.W. Schindler (1998) The $GABA_B$ agonist baclofen modifies cocaine self-administration in rats. Behavioural Pharmacology **9:** 195-206

Sibley, D.R. and F.J. Monsma JR (1992) Molecular biology of dopamine receptors. Trends In Pharmacological Sciences **13:** 61-69

Sibley, D.R., F.J. Monsma Jr and Y. Shen (1993) Molecular neurobiology of D1 and D2 dopamine receptors, In: D1:D2 dopamine receptor interactions, Harcourt Brace and Company Publishers

Slangen, J.L.(1991) Drug discrimination and animal models, In: Animal models in psychopharmacology, advances in pharmacological sciences, Birkhauser Verlag Basel

Smith, A.G., B. Costall, J.C. Neill and M. Shohid (1999a) Effects of the putative dopamine D3 receptor antagonist, U-99194A on locomotor activity behaviour in the common marmoset. British Journal of Pharmacology **126:** 234P

Smith, A.G., B. Costall, J.C. Neill and M. Shohid (1999b) Effects of the putative dopamine D3 receptor antagonist, U-99194A on progressive ratio responding in the common marmoset. British Journal of Pharmacology **126:** 235P

Smith, G.P. and S. Hsaio (1995) Raclopride reduces sucrose preference in rats. Pharmacology Biochemistry and Behaviour **50:** 121-125

Smith, Y. and A. Parent (1988) Neurons of the subthalamic nucleus in primates display glutamate but not GABA immunoreactivity. Brain Results **453:** 353-356

Snoddy, A.M. and R.E. Tessel (1985) Prazosin: effects on psychomotor-stimulant cues and locomotor activity in mice. European Journal of Pharmacology **116:** 221-228

Sokoloff, P., B. Giros, M.P. Martes, M.L. Bouthenet and J.C. Schwartz (1990) Molecular cloning and characterisation of a novel dopamine receptor (D3) as a target for neuroleptics. Nature **347:** 146-151

Solomone, J.D., M.J. Zigmond and E.M. Stricker (1990) Characterisation of the impaired feeding behaviour in rats given haloperidol or dopamine-depleting brain lesions. Neuroscience **39:** 17-24

Sonesson, C., N. Waters, K. Svennson, A. Carlsson, M.W. Smith, M.F. Piercy, E. Mayer and H. Wikstrom (1993) Substituted 3-phenyl-piperidines: a new centrally acting dopamine autoreceptor antagonist. J Med Chem **36:** 3188

Spealman, R.D., J. Bergman, B.K. Madras and K.F. Melia (1991) Discriminative stimulus effects of cocaine in squirrel monkeys: Involvement of dopamine receptor subtypes. Journal of Pharmacology and Experimental Therapeutics **258:** 945-953

Spealman, R.D. (1995a) Noradrenergic involvement in the discriminative stimulus effects of cocaine in squirrel monkeys. Journal of Pharmacology and Experimental Therapeutics **275:** 53-62

Spealman, R.D. (1995b) Discriminative stimulus effects of cocaine in squirrel monkeys: Lack of antagonism by the dopamine D2 partial agonists Terguride, SDZ 208-911 and SDZ 208-912. Pharmacology Biochemistry and Behaviour **51:** 661-665

Spealman, R.D. (1996) Dopamine D3 receptor agonists partially reproduce the discriminative stimulus effects of cocaine in squirrel monkeys. Journal of Pharmacology and Experimental Therapeutics **278:** 1128-1137

Spotts, J.V. and F.C. Shatz (1980) Cocaine use: A representative case approach. Free Press. New York

Spyraki, C., M.C. Fibiger and A.G. Philips (1982) Cocaine-induced place preference conditioning: lack of effects of neuroleptic and 6-OHDA lesions. Brain Research **253:** 195-203

Stahle, L. and V. Ungerstedt (1986) Differential behavioural patterns induced by the dopamine agonist apomorphine analysed by multivariate statistics. Pharmacology Biochemistry and Behaviour **24:** 291-298

Stahle, L. (1992) Do autoreceptors mediate dopamine agonist-induced yawning and suppression of exploration? A critical review. Psychopharmacology **106:** 1-13

Staley, J.K. and D.C. Mash (1996) Adaptive increases in D3 dopamine receptors in brain reward circuits of human cocaine fatalities. Journal of Neuroscience **16:** 6100-6106

Starr, B.S. and M.S. Starr (1986a) Grooming in the mouse is stimulated by the dopamine D1 agonist SKF 38393 and by low doses of the D1 antagonist SCH 23390, but is inhibited by dopamine D2 agonists, D2 antagonists and high doses of SCH 23390. Pharmacology Biochemistry and Behaviour **24:** 837-839

Starr, B.S. and M.S. Starr (1986b) Differential effects of dopamine D1 and D2 agonists and antagonists on velocity of movement, rearing and grooming in the mouse. Neuropharmacology **25:** 455-463

Stolerman, I. (1992) Drugs of abuse: Behavioural principles, methods and terms. Trends in Pharmacological Sciences **13:** 170-176

Stone, T.W. (1995) Chp.7 Sedatives and anxiolytics In: Neuropharmacology: Biochemical and Medicinal Chemistry Series (ED: John Mann) W.H. Freeman and Company Ltd.

Stoof, J. and J.W. Kebabian (1984) Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sciences **35:** 2281-2296

Strange, P. (1996) The energetics of ligand binding in catecholamine receptors. Trends In Pharmacological Sciences **17:** 238

Stratford, T.R. and A.E. Kelley (1997) GABA in the nucleus accumbens shell participates in the central regulation of feeding behaviour. Journal of Neuroscience **17:** 4434-4440

Sunahara, R.K., H.B. Niznik, D.M. Weiner, T.M. Storman, M.R. Brann, J.L. Kennedy, J.E. Gelernter, R. Razmahel, Y.L. Yang, Y. Israel, P. Seeman and B.F.

O'Dowd (1990) Human dopamine D1 receptor encoded by an intronless gene on chromosome 5. Nature **347:** 80-83

Sunahara, R.K., H.C. Guan, B.F. O'Dowd, P. Seeman, L.G. Laurier, S.R. George, J. Torchia, T.H. Van and H.B. Niznik (1991) Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature **350:** 614-619

Suzuki, T., T. Mori, K. Takamori, K. Onodera and M. Misawa (1996) effects of H1 antagonists on discriminative stimulus effects of cocaine and methamphetamine in rats. Behavioural Pharmacology **7:** 111-118

Svensson, K., A.M. Johansson, T. Magnusson and A. Carlsson (1986) (+)-AJ76 and (+)-UH 232: central stimulants acting as preferential dopamine autoreceptor antagonists. Naunyn-Schmiedebergs Arch Pharmacol **334:** 234-245

Svensson, K., A. Carlsson, R.M. Huff, T. Kling-Petersen and N. Waters (1994a) Behavioural and neurochemical data suggest functional differences between dopamine D2 and D3 receptors. European Journal of Pharmacology **263:** 235-243

Svensson, K., A. Carlsson and N. Waters (1994b) Locomotor inhibition by the D3 ligand R-(+)-7-OH-DPAT is independent of changes in dopamine release. Journal of Neural Transmission **95:** 71-74

Szechtman, H., D. Eilman and K.V.A. Clements (1991) Differential effects of D1 and D2 dopamine agonists on stereotyped locomotion in rats. Behavioural Brain Research **45:** 117-124

Tang, A.H., S.R. Franklin, C.S. Himes, M.W. Smith and R.E. Tenbrink (1997) PNU-96415E, a potential antipsychotic agent with clozapine-like pharmacological properties. Journal of Pharmacology and Experimental Therapeutics **281:** 440-447

Taylor, D. and B.T. Ho (1978) Comparison of inhibition of monoamine uptake by cocaine, methylphenidate and amphetamine. Res Commun Chem Pathol Pharmacol **21:** 67-75

Tella, S.R., C.W. Schindler and S.R. Goldberg (1992) Cardiovascular effects of cocaine in conscious rats: relative significance of central sympathetic stimulation and peripheral neuronal monoamine uptake and release mechanisms. Journal of Pharmacology and Experimental Therapeutics **262:** 602-610

Tella, S.R., C.W. Schindler and S.R. Goldberg (1993) Cocaine: cardiovascular effects in relation to inhibition of peripheral neuronal monoamine uptake and central stimulation of the sympathoadrenal system. Journal of Pharmacological and Experimental Therapeutics **267:** 153-162

Tella, S.R. (1994) Differential blockade of chronic versus acute effects of intravenous cocaine by dopamine receptor antagonists. Pharmacology Biochemistry and Behaviour **48:** 151-159

Terry, P. and J.L. Katz (1992) Differential antagonism of the effects of dopamine D1 receptor agonists on feeding behaviour in the rat. Psychopharmacology **109:** 403-409

Terry, P. and J.L. Katz (1994) A comparison of the effects of the D1 receptor antagonists SCH 23390 and SCH 39166 on suppression of feeding behaviour by the D1 agonist SKF 38393. Psychopharmacology **113:** 328-333

Terry, P., J.M. Witkin and J.L. Katz (1994) Pharmacological characterisation of the novel discriminative stimulus effects of a low dose of cocaine. Journal of Pharmacology and Experimental Therapeutics **270:** 1041-1048

Terry, P. (1996) Chp. 11, Dopamine receptor subtypes and ingestive behaviour. In: Drug receptor subtypes and ingestive behaviour (Eds: Cooper, S.J. and P.G. Clifton) Academic Press Harcourt Brace and Company, London

Terry, P. and J.L. Katz (1997) Dopaminergic mediation of the discriminative stimulus effects of bupropion in rats. Psychopharmacology **134:** 201-212

Thorn, L., T.E. Ashmeade, V.J. Storey, C. Routledge and C. Reavill (1997) Evidence to suggest that agonist modulation of hyperlocomotion is via postsynaptic dopamine D2 or D3 receptors. Neuropharmacology **36:** 787-792

Tiberi, M., K.R. Jarvie, C. Silvia, P. Falardeau, J.A. Gingrich, N. Godinot, L. Bertrand, F.T. Yang, R.J. Fremeau and M.G. Caron (1991) Cloning, molecular characterisation and chromosomal assignment of a gene encoding a second D1 dopamine receptor subtype: differential expression pattern in rat brain compared with the D1a receptor. Proc Natn Acad Sci USA **88:** 7491-7495

Tirelli, E. and P. Terry (1993) Biphasic locomotor effects of the dopamine D1 agonist SKF 38393 and their attenuation in non-habituated rats. Psychopharmacology **110:** 69-75

Tirelli, E., J. Reggers and P. Terry (1997) Dopamine 'D2-Like' receptor agonists in combination with cocaine: absence of interactive effects on locomotor activity. Behavioural Pharmacology **8:** 147-159

Toates, F. (1994) Comparing motivational systems - an incentive motivation perspective, In: Appetite, neural and behavioural bases, C.R. Legg and D.A. Booth (EDS) Oxford Science Publications

Ujike, H., K. Akiyama and S. Otsuki (1990) D2 but not D1 dopamine agonists produce augmented behavioural response in rats after subchronic treatment with methamphetamine on cocaine. Psychopharmacology **102:** 459-464

Ushijima, I., M.A. Carino and A. Horita (1995) Involvement of D1 and D2 dopamine systems in the behavioural effects of cocaine in rats. Pharmacology Biochemistry and Behaviour **52:** 737-741

Valler, L. and J. Meldolesi (1989) Mechanisms of signal transduction at the dopamine D2 receptors. Trends In Pharmacological Sciences **10:** 74-77

Van der Hoek, G.A. and S.J. Cooper (1994) The selective dopamine uptake inhibitor GBR 12909: its effects on the microstructure of feeding in rats. Pharmacology Biochemistry and Behaviour **48:** 135-140

Van Groll, B.J. and Appel, J.B. (1992) Stimulus effects of d-amphetamine 1: DA mechanisms. Pharmacology Biochemistry and Behaviour **43:** 967-973

Vanover, K.E. and Woolverton, W.L. (1994) Behavioural effects of the dopamine autoreceptor agonist PD 128483 alone and in combination with cocaine. Journal of Pharmacology and Experimental Therapeutics **270:** 1049-1056

Van Tol, H.H.M. and O. Civelli (1991) Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature **350:** 610-614

Van Tol, H.H.M., C.M. Wu, H.C. Guan, K. Ohara, J. Bunzow, O.R. Civelli, J. Kennedy, H.B. Niznik and V. Jovanovic (1992) Multiple dopamine D4 variants in the human population. Nature **358:** 149-152

Waddington, J.L. (1986) Behavioural correlates of the action of selective D1 dopamine receptor antagonists. Impact of SCH 23390 and SKF 83566,and functionally interactive D1:D2 receptor systems. Biochem Pharmacol **35:** 3661-3667

Waddington, J.L. and S.A. Daly (1993) Regulation of unconditioned motor behaviour by D1:D2 interactions, In: D1:D2 dopamine receptor interactions, J.L. Waddington (ED) San Diego, Academic Press

Wallach, M.B. and S. Gersa (1991) A Neuropsychopharmacology comparison of d-amphetamine, L-dopa and cocaine. Neuropharmacology **10:** 743

Walsh, S.L. and K.A. Cunningham (1997) Serotonergic mechanisms involved in the discriminative stimulus, reinforcing and subjective effects of cocaine. Psychopharmacology **130:** 41-58

Walters, J.R., D.A. Bergstrom, J.H. Carlson, T.N. Chase and A.R. Braun (1987) D1 dopamine receptor activation required for postsynaptic expression of D2 agonist effects. Science **236:** 719-722

Wanibuchi, F. and S. Usuda (1990) Synergistic effects between D1 and D2 dopamine antagonists on catalepsy in rats. Psychopharmacology **102:** 339-342

Warburton, D.M. (1990) Addiction Controversies, Chp. 3. Heroin, Cocaine and now Nicotine, D.M. Warburton (ED) Harwood Academic Press

Waters, N., K. Svensson, S.R. Haadsma-Svensson, M.W. Smith and A. Carlsson (1993) The dopamine D3 receptor: a post synaptic receptor inhibitory on rat locomotor activity. Journal of Neural Transmission **94:** 11-19

Waters, N., L. Lofberg, S.R. Haadsma-Svensson, K. Svensson and A. Carlsson (1994) Differential effects of dopamine D2 and D3 receptor antagonists in regard to dopamine release, in vivo receptor displacement and behaviour. Journal of Neural Transmission [General Section] **98:** 39-55

Weed, M.R., K.F. Vanover and W.L. Woolverton (1993) Reinforcing effects of the D1 dopamine agonist SKF 81297 in rhesus monkeys. Psychopharmacology **113:** 51-52

Weinshank, R.L., N. Adham, M. Macchi, M.A. Olsen, T.A. Branchek and P.R. Hartig (1991) Molecular cloning and characterisation of a high affinity dopamine receptor (D1 beta) and its pseudogene. J Biol Chem **266:** 22427-22435

West, R. (1991) Psychobiological theories of addiction, In: The international handbook of addiction behaviour, I.B. Glass (ED) Tavistock/Routledge, London and New York

West, W.B., B.J. Van Groll and J.B. Appel (1995) Stimulus effects of d-amphetamine II: DA, NE and 5-HT Mechanisms. Pharmacology Biochemistry and Behaviour **51:** 69-76

White, N.M. and N. Hiroi (1998) Preferential localisation of self-stimulation sites in striosome/patch in the rat striatum. Proc.Natl.Acad.Sci. **95:** 6486-6499

Willner, P. and A. Towell (1982) Microstructural analysis of the involvement of beta-receptors in amphetamine anorexia. Pharmacology Biochemistry and Behaviour **17:** 255-262

Willner, P. (1991) Behavioural models in psychopharmacology In: P. Willner (ED) 'Behavioural models in psychopharmacology'. Theoretical, industrial and clinical perspectives. Cambridge, C.U.P

Willner, P. (1997) Animal models of addiction. Human Psychopharmacology **12:** S59-S68

Wilson, M.C. and C.R. Schuster (1972) The effect of chlorpromazine on psychostimulant self-administration in the rhesus monkeys. Psychopharmacology **266:** 115-126

Wilson, C., G.G. Nomikos, M. Collu and H.C. Fibiger (1995) Dopaminergic correlates of motivated behaviour: Importance of drive. Journal of Neuroscience **15:** 5169-5178

Wise, R.A., J. Spindler, H. deWit and G.J. Gerberg (1978) Neuroleptic-induced 'anhedonia' in rats: pimozide blocks reward quality of food. Science **201:** 262-264

Wise, R.A. (1982) Neuroleptics and operant behaviour: the anhedonia hypothesis. Behav Brain Sci **5:** 39-87

Wise, R.A. and M.A. Bozarth (1987) A psychomotor stimulant theory of addiction. Psychol Rev **94:** 469-492

Wise, R.A. (1993) In vivo estimates of extracellular dopamine and dopamine metabolite levels during intravenous cocaine or heroin self-administration. Semin Neurosci **5:** 337-342

Wise, R.A. and W.A. Carlezon JR (1994) Attenuation of the locomotor-sensitising effects of the D2 dopamine agonist bromocriptine by either the D1 antagonist SCH 23390 or the D2 antagonist Raclopride. Synapse **17:** 155-159

Witkin, J.M., Nichols, D.E., Terry, P. and J.L. Katz (1991) Behavioural effects of selective dopaminergic compounds in rats discriminating cocaine injections. Journal of Pharmacology and Experimental Therapeutics **257:** 706-713

Wolgin, D.L. and J.M. Hertz (1995) Effects of acute and chronic cocaine on milk intake, body weight, and activity in bottle-and cannula-fed rats. Behavioural Pharmacology **6:** 746-753

Wood, D.M. and E.M. Emmett-Oglesby (1986) Characteristics of tolerance, recovery from tolerance and cross-tolerance for cocaine used as a discriminative stimulus. Journal of Pharmacology and Experimental Therapeutics **237:** 120-125

Wood, D.M., K.C. Ritz and M.W. Emmett-Oglesby (1987) Evidence of a central mechanism mediating tolerance to the discriminative stimulus properties of cocaine. Pharmacology Biochemistry and Behaviour **28:** 401-406

Wood, D.M. and E.M. Emmett-Oglesby (1989) Mediation in the nucleus accumbens of the discriminative stimulus produced by cocaine. Pharmacology Biochemistry and Behaviour **33:** 453-457

Woolverton, W.L. and R.C. Trost (1978) Cocaine as a discriminative stimulus for responding maintained by food in squirrel monkeys. Pharmacology Biochemistry and Behaviour **8:** 627-630

Woolverton, W.L., D. Kandel and C.R. Schuster (1978) Tolerance and cross tolerance to cocaine and d-amphetamine. Journal of Pharmacology and Experimental Therapeutics **205:** 525-535

Woolverton, W.L., L.I. Goldberg and J.Z. Ginus (1984) Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. Journal of Pharmacology and Experimental Therapeutics **230:** 628-683

Woolverton, W.L. and L. Cervo (1986) Effects of central dopamine depletion on the d-amphetamine discriminative stimulus in rats. Psychopharmacology **88:** 196-200

Woolverton, W.L and K.M. Johnson (1992) Neurobiology of cocaine abuse. Trends In Pharmacological Sciences **13:** 193-200

Yamada, K. and T. Furukawa (1980) Direct evidence that for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology **67:** 39-43

Yorkel, R.A. and R.A. Wise (1978) Amphetamine reinforcement by dopamine agonists in the rat. Psychopharmacology **58:** 289-296

Yoshida, M., H. Yokoo, K. Mizoguchi, H. Kawahara, A. Tsuda, T. Nishikawa and M. Tonoka (1992) Eating and drinking cause increased dopamine release in the nucleus

accumbens and ventral tegmental area in the rat: Measurement by in vivo microdialysis. Neuroscience Letters **139:** 73-76

Zarrindast, M.R. and I. Elliasi (1991) Differential effects of dopamine agonists on locomotion in intact and reserpine-treated mice. General Pharmacology **22:** 1027-1031

Zarrindast, M.R., A.A. Owji and T. Hosseini-Nia (1991) Evaluation of dopamine receptor involvement in rat feeding behaviour. General Pharmacology **22:** 1011-1016

Zhang, S.P., L.W. Zhou and B. Weiss (1994) Oligonucleotides antisense to the D1 dopamine receptor mRNA inhibits D1 receptor-mediated behaviours in normal mice and in mice lesioned with 6-hydroxydopamine. Journal of Pharmacology and Experimental Therapeutics **271:** 1462-1470

Zhao, R.R., W.H. Farrell and F.L. Abel (1990) Effects of dopamine D1 and dopamine D2 receptor agonists on coronary and peripheral hemodynamics. European Journal of Pharmacology **190:** 193-202

Zhou, Q.Y., D.K. Grandy, L. Thambi, J.A. Kushner, T.H. Van, R. Cone, D. Pribnow, J. Salon, J.R. Bunzow and O. Civelli (1990) Cloning and expression of human and rat D1 dopamine receptors. Nature **347:** 76-80