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Dopamine Receptor Subtypes and Ingestive Behaviour

Rachel F. Genn

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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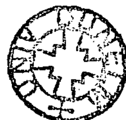
University of Durham
Department of Psychology
1999



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Rachel F. Genn

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Cooper, S.J. and Genn, R.F., (1997) Effects of the Dopamine Agonist 7-OH-DPAT on the Microstructure Of Licking in the Rat. Soc. Neurosci. Abstr., 23, 529.8.

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Thesis title: Dopamine receptor subtypes and ingestive behaviour

Author: Rachel F. Genn

Abstract: Both centrally and systemically administered dopamine agonists and antagonists decrease ingestive behaviour. The aim of this thesis was to examine whether drugs acting at different receptor subtypes decreased intake in different ways. A microstructural analysis was used to examine dopaminergic drug effects on licking behaviour. A dopamine D3 receptor agonist, 7-OH-DPAT, a dopamine D2 receptor antagonist raclopride and a mixed dopamine D2/D3 agonist quinpirole were compared in this paradigm. These drugs reduced the number of licks by differentially decreasing parameters which are thought to reflect the palatability of the stimulus such as mean bout duration of licking and the initial rate of licking. Follow-up experiments were conducted to further examine the possibility that motor deficits were underlying decreases in licking parameters. The effects of raclopride and 7-OH-DPAT were compared to the effects of a dopamine D1 antagonist SCH-23390 and were analysed using a brief contact licking test. Again, the behavioural expression of anorexia induced by these drugs seemed to rely on a differential decrease in mean bout duration. Results also revealed that the three drugs used differed in the extent to which they produced a motoric deficit. Attempts to block the effects of 7-OH-DPAT on licking parameters were made by using the putative D3 receptor antagonists PNU-99194A and amisulpride . In addition, the effects of these drugs alone on licking behaviour were examined. PNU-99194A failed to block the effects of 7-OH-DPAT and was relatively ineffective in producing changes in licking behaviour when administered alone. Amisulpride blocked the effects of 7-OH-DPAT only at high doses and when injected alone produced an increase in intake through an increase in mean bout duration of licking. Results from Chapters 4, 5 and 6 suggested that 7-OH-DPAT was having an effect on palatability. Therefore, Chapter 7 presents an experiment which examines the effect of 7-OH-DPAT on the licking behaviour of rats which encounter a devaluation of reward (successive negative contrast). 7-OH-DPAT reduced successive negative contrast leading to the proposal that D3 receptors may mediate relative as well as absolute reinforcer value. These results bear important implications for understanding the role of dopamine receptor subtypes in components of food reward and appetitive behaviour in general and may well have implications for the treatment of eating disorders.

Dedication

I dedicate this thesis to two people in particular; My mother, the natural philosopher and a perpetual source of inspiration, and my father who filled my life with love. Thankyou for the opportunities, support and guidance with which you have *always* provided me.

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The tribulations of writing a thesis were assuaged by the support and contributions of my friends and family. My sister understands more about dopamine than many psychopharmacologists and my Aunty Joan and Uncle Dennis, who have, over the course of this PhD, been made to realise that students can spend money more readily than any gambler.

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Chapter 1: The pharmacology of dopamine receptor subtypes

1.1 Chapter overview

There are two main aims of Chapter 1. The first is to provide a brief historical overview of the main milestones in the study of dopamine as a neurotransmitter in the central nervous system, and to describe the anatomy of dopamine-containing neuronal pathways, as well as the distinctions drawn between dopamine receptor subtypes. There will be a particular emphasis placed on the dopamine D3 receptor subtype, which has attracted a great deal of attention, having been implicated in the mediation of reward (Caine and Koob, 1993, 1995) and in the potential alleviation of psychotic symptoms (Sokoloff, 1990). Currently, the properties and functions of the dopamine D3 receptor have been extensively studied using a variety of approaches, including behavioural, physiological, neurochemical, pharmacological, and, more recently, molecular approaches both *in vivo* and *in vitro*.

The second aim of this chapter will therefore be to bring together the evidence derived from these several approaches to characterise the attributes of the D3 receptor subtype in comparison with those of other distinct dopamine receptor subtypes. In doing so, an introduction to the role(s) of the dopamine D3 receptor subtype in several types of behavioural response will be provided. In bringing together several lines of evidence it may prove possible to characterise better the role(s) of this important receptor subtype in more complex psychological functions, for example the motivational bases of behaviour (see Chapter 2).

1.2 A brief history of dopamine

Dopamine, is a major catecholamine in the central nervous system. The study of dopamine, from the molecular to the behavioural level, has provided an empirical and conceptual cornerstone in psychopharmacology. This widely distributed neurotransmitter has been the focus of much research during the past 40 years primarily because alterations in dopamine neurotransmission are involved, directly or indirectly, in several brain dysfunctions such as schizophrenia and Parkinson's disease. However, since dopamine has also been identified as having a role in the mediation of several behaviours, having a particular role to play in the regulation of emotion, motivation, cognition and reward, it is also of interest to those who wish to elucidate the role of dopamine in psychological functions.

Since the discovery of dopamine in the brain and its high degree of localisation within the basal ganglia (Carlsson, 1959), evidence has steadily accumulated to indicate that this catecholamine is a neurotransmitter in its own right and that it has consequential functions in the brain. This hypothesis has subsequently been reliably confirmed as evidence has accumulated from disciplines including biochemistry, pharmacology, histology and neurology. Prior to Carlsson's pioneering work, dopamine had been conceived of as an intermediate in the biosynthesis of the already acknowledged neurotransmitter noradrenaline.

The introduction of the histochemical fluorescence technique by Hillarp and colleagues (Falck, 1962; Falck et al., 1962) provided neuroscience with methods which facilitated the demonstration of the occurrence of monoamines, including dopamine, in nerve cell bodies and terminals within the central nervous system. This technique also helped to confirm dopamine's functional status as a neurotransmitter (Carlsson et al., 1962) since the concentration of dopamine in brain was approximately the same as noradrenaline, its distribution was different, suggesting that dopamine might have a neurotransmitter action of its own. Not only did this technique increase knowledge about the morphology and organisation of these neurons, it also outlined a system within which experimental data could be interpreted. As new evidence accrued, the fluorescence technique showed itself to be an adequate method of tracing the monoamine pathways in the central nervous system, (e.g.Anden et al., 1964a; Dahlstrom et al., 1964; Dahlstrom and Fuxe, 1964; Fuxe 1965; Ungerstedt 1971; for review see Dahlstrom and Carlsson, 1986).

In their classic mapping studies of central monoaminergic cell groups, Dahlstrom and Fuxe (1964) began to identify dopaminergic cells of origin and delineate ascending and descending projections from these cells. The early studies by Dahlstrom and Fuxe gave rise to the nomenclature used to distinguish dopaminergic cell groups from adrenergic and serotonergic cell groups. Groups were designated with letters and numbers. Current nomenclature relies heavily on these original findings, therefore, groups A1-A7 are noradrenergic, A8-15 are dopaminergic and C1-C3 are adrenergic. Owing to these original successes, histochemical methods were developed and refined, such as the glyoxilic acid fluorescence method (Lindvall and Bjorklund, 1974a), which allowed researchers to discover previously unknown dopamine-containing fibre systems. In the 1980s and 90s, histochemical fluorescence was largely supplanted by immunohistochemical staining for catecholamine-synthesising enzymes and more recently, the transmitters themselves (Hokfelt et al., 1984a; Decavel et al., 1987).

Originally, two distinct dopamine systems were identified, separating the telencephalic projections arising from dopamine containing neurons in the mesencephalon (Dahlstrom and Fuxe, 1964). The first was the nigrostriatal system originating in the dopamine cells of the pars compacta of the substantia nigra (SNC or A9). The second system was named the mesolimbic system to be found in areas medial to the substantia nigra in the ventral tegmental area of Tsai (VTA or A10), (see Figure 1.1 *a* and *d* repectively). These systems were collectively named the mesotelencephalic system reflecting all ascending telencephalic projections from the mesencephalic dopaminergic neurons (Bjorklund and Lindvall, 1978; Moore and Bloom, 1986). This general projection system could be further subdivided into the mesostriatal complex, which projects to the striatum proper and the ventral striatum, and the mesocorticolimbic system the pathways of which project to the limbic system, and specific regions of allocortex and neocortex.

Further neuroanatomical developments have suggested that previous distinctions between dopaminergic systems such as the meso-striatal, meso-limbic and meso-cortical pathways may no longer be valid. Although meso-limbic dopamine neurons had been defined as those which originated in the in the A10 region of the VTA, evidence was presented to suggest that the projections of the A10 neurons are not confined to structures such as olfactory tubercule or nucleus accumbens, but also innervate the ventromedial half of the striatum throughout its rostrocaudal extent; the latter region also being innervated by A9 Dopamine neurons in the SNC (substantia nigra), (Beckstead et al. 1979; Fallon and Moore,

1978). The discovery of such overlap blurred the prior distinction between the meso-striatal and meso-limbic systems.

More recently, spurred by the above inconsistencies, researchers such as Fuxe et al. (1985) and Bjorklund and Lindvall (1986) helped to describe the dopaminergic projections extensively. They distinguished between several different forebrain projections from mesencephalic (midbrain) dopamine cell groups, (see Figure 1.1 *a, b, c* and *d*).

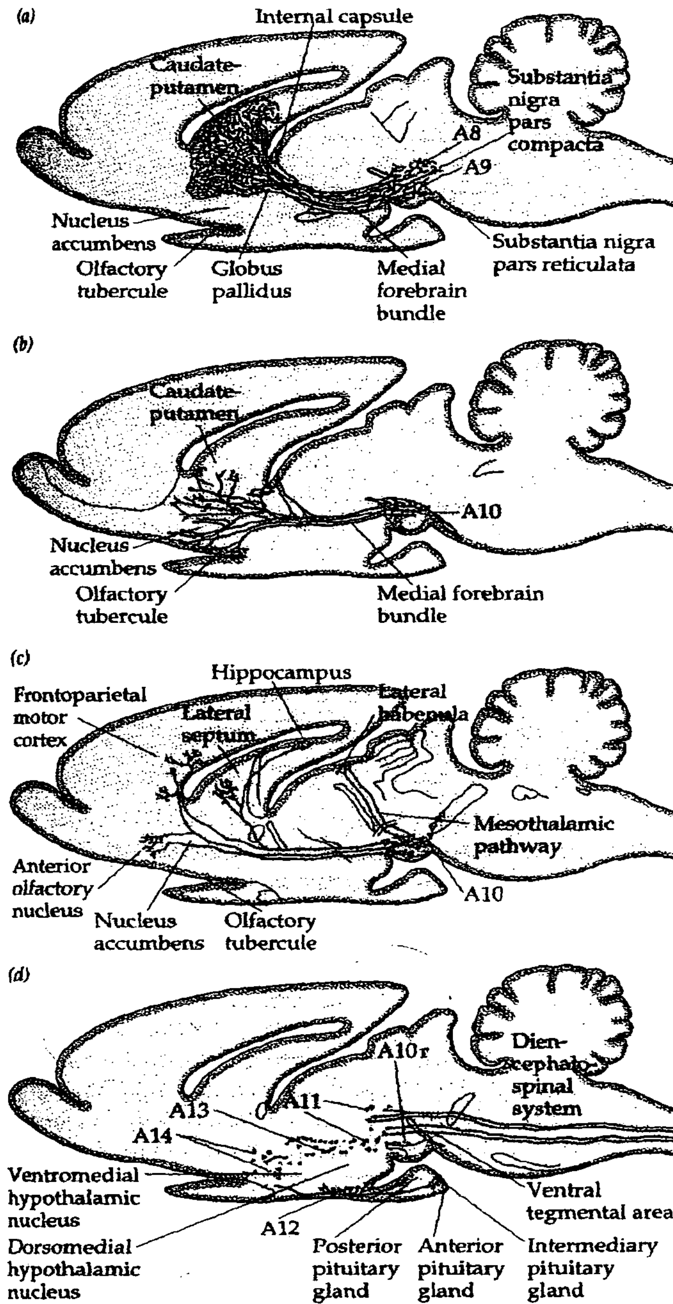


Figure 1.1 Dopaminergic pathways in the rat brain: Dopaminergic cell bodies are represented by small black triangles and axonal fibres by solid lines. (*a*) Dorsal mesostriatal (nigrostriatal) system. (*b*) Ventral mesostriatal (mesolimbic) system. (*c*) Mesolimbocortical and mesodiencephalic (mesothalamic) systems. (*d*) Periventricular, diencephalospinal, incertohypothalamic, and tuberohypophyseal systems. Groups A8-A15 represent dopaminergic cell groups. (Adapted from Fuxe et al., 1985).

Although it is beyond the scope of this thesis to discuss the functional significance of the separation of distinct dopaminergic projections, throughout this thesis there will be emphasis on a number of the structures to be found on these distinct pathways. The extent of this emphasis will be determined by the relevance of these structures to the mediation of several psychological functions which are central to the empirical thread of the thesis. Due to further technological refinements, dopaminergic receptor distribution is now appreciably more complex than originally believed. Therefore, more specific issues of distibution will be discussed in relation to dopamine receptor subtypes later in this chapter.

The original elucidation of such tracts led to rudimentary hypotheses as to their function, (Anden et al., 1966; Carlsson, 1959). Arising from the observation that dopamine was concentrated in the basal ganglia, a relationship between this catecholamine and extrapyramidal function was postulated, (Carlsson, 1959). The development of the selective neurotoxin 6-OHDA for catecholaminergic neurons (Ungerstedt, 1968) coincided with the histochemical analyses of catecholaminergic systems which conspired to provide a method of determining the functional correlates of such systems. A functional correlate of the ablation of catecholaminergic neurones by 6-OHDA was a Parkinson's-type syndrome. Earlier, Carlsson (1959) had also noted that the depletion of brain dopamine by reserpine led to Parkinson-like tremors. This led to clinical verification of the correlation between the degeneration of nigrostriatal pathways and the onset of Parkinson's disease (Hornykiewicz, 1975). Further work in this area revealed that connections between the nigrostriatal/mesolimbic systems appeared to play an important role in the regulation of several aspects of motor function such as locomotor activity, stereotyped behaviour, muscular tone and posture (for review see Iversen, 1977).

The study of stereotyped behavior became a research field in its own right largely based on the descriptions of stereotypy observed after the administration of amphetamine, in humans during the Second World War (Randrup et al., 1963). The stimulant effects of this drug in animals was well established but when administered at high doses (i.e. 3 mg/kg) behaviours were also noted that were abnormal in their invariant and repetitive nature (Randrup et al., 1963). Stereotyped sniffing, licking, mouth movements or biting had been observed in the absence of locomotor activity, grooming, eating and drinking. Pharmacological analysis of the behavioural effect produced by d-amphetamine seemed sensible and antagonists were postulated which might block the induction of amphetamine stereotypy (i.e. perphenazine) (Randrup and Munkvad, 1965). The success of this

drug to inhibit stereotypy created a theoretical link between drugs which were effective in treating symptoms of schizophrenia and those which antagonised amphetamine-induced stereotypy in rats. Therefore, one of the first clues to the cellular actions of antipsychotic agents provided the empirical links needed to establish what was later to be known as the dopamine hypothesis of schizophrenia (Snyder, 1974).

The study of schizophrenia is one of the most thoroughly researched areas of clinical psychopharmacology. Hypotheses which favoured an explanation of schizophrenia in terms of physiological mechanisms were parallelled by those which proposed a possible genetic aetiology of the disease. Until the 1950s there had been a lack of drug treatments which were specifically aimed at the treatment of schizophrenia. However, during the 1950s antipsychotic drugs were discovered which produced an alleviation of the psychotic symptoms of schizophrenia.

The first of these drugs was reserpine which was a forerunner of the group of antipsychotics known as the typical antipsychotics. This group constituted the phenothiazines (beginning with chlorpromazine), the butyrophenones (haloperidol) and the thioxanthenes. Developments in this area led to the synthesis of a further group of compounds named atypical antipsychotics of which clozapine was a prototypical example. These drugs were so-called as they were able to alleviate antipsychotic symptoms without producing the motor side-effects which afflicted patients treated by the more traditional antipsychotic compounds. Therefore, the search for novel antipsychotic agents with less marked extrapyramidal side effects was intensified and produced a large number of new dopamine antagonists. These drugs varied significantly in their chemical structure but shared the functional homogeneity of facilitating dopamine blockade. However, findings concerning the localisation and possible differential function of dopamine receptors made it difficult to construe dopamine receptors as a homogeneous group.

On the strength of such evidence, systems of classification under which dopamine receptors could be redefined as separate populations of neurons, began to emerge. Of the various classifications of dopamine receptors to have been proposed, the division into pre- and post-synaptic seems to have been one of the most consistent (Carlsson, 1975). The idea that dopaminergic receptors may play a part in regulating the release, metabolism and synthesis of dopamine was originally formulated in 1963 (Carlsson and Lindquist, 1963). The evidence for the existence of central presynaptic dopamine receptors is now based on a large body of electrophysiological and biochemical (see reviews by Seeman,1980; Horn, 1981)

and behavioural data (see later in this chapter and also Chapter 2), but was originally conceived in the light of studies which showed the ability of chlorpromazine and haloperidol to stimulate the metabolism and synthesis of brain catecholamines. Carlsson et al. (1963) speculated that the ability of these antipsychotic agents to block dopamine receptors might influence a negative feedback loop leading to *activation* of dopaminergic neurons.

However, it was studies in the behavioural field which championed a unique perspective on the functional significance of the localisation of dopaminergic receptors; a perspective which had heretofore been relatively obscured by the more reductionist neuroscience methods being used. Much of the evidence for the existence of self-inhibitory dopamine receptors has been gained from studies with apomorphine. This drug was identified as an agonist at dopamine receptors. This agonist generated a biphasic dose-response relationship with respect to behaviour with behavioural inhibition occuring in the lower dose-range. Higher doses were shown to stimulate behaviour and this was presumed to be a consequence of preferential post-synaptic action. The threshold doses for inhibition of behaviour and for inhibition of DOPA formation were approximately the same, which would fit the hypothesis of preferential activation of putative autoreceptors (Carlsson, 1975). Also, the firing rate of dopaminergic cell bodies was inhibited by apomorphine in very low dosage (Aghajanian and Bunney, 1974, 1977).

The functional term "autoreceptor " has been said to include: a) the presynaptic dopamine receptor, located on the dopaminergic nerve terminals, controlling the synthesis and release of the transmitter; b) somatic and dendritic receptors, whose stimulation results in decreased rates of firing of the dopaminergic neuron (Carlsson, 1975). The stimulation of these receptors lead to a decrease in dopaminergic function and therefore helped to provide a model for the interpretation of accrued behavioural data (i.e. from studies showing a differential effect of high and low doses of drugs such as apomorphine). Table 1.1 lists some of the original observations which could be attributed to either presynaptic or post-synaptic dopaminergic activity.

Table 1.1 Location of pre and postsynaptic dopamine receptors and their mediation of behaviours in the rat.

	RECEPTOR TYPE	
	PRESYNAPTIC	*POSTSYNAPTIC*
Location in brain	-thought to be located on the nerve terminals cell bodies and dendrites of the dopamine neurons	-thought to be on various parts of other neurons (i.e. cholinergic neurons)
Effects as a result of dopamine agonist action	-inhibition of dopamine release from tissue slices (Starke et al., 1978)	-induction of stereotyped behaviour (Ungerstedt, 1979).
	-the inhibition of dopamine synthesis in slices (Westfall et al., 1976)	-the reversal of reserpine induced hypomotility (Anden et al., 1973).
	-the reduction of electrical dopamine cell activity (Skirboll et al,. 1979).	-the induction of contralateral rotation after unilateral nigrostriatal lesions (Ungerstedt, 1979).
	-the inhibition of the increased dopamine synthesis in vivo after the reduction of impulse flow by administration of (GBL) (Walters and Roth, 1974)	-increase in striatal acetylcholine concentrations (Sethy et al., 1974)
	-by axotomy of nigrostriatal neurons (Carlsson, 1975). -decrease in locomotor activity (Strombom, 1975).	

More recently, studies on the functional dissociations within the extensive dopamine systems have highlighted ways in which this system can be further taxonomised. In ascribing functional roles to dopamine transmission much emphasis has been on the massive character of the dopamine projection to its major forebrain area, (the striatum and in particular the nucleus accumbens) (Bjorklund and Lindvall, 1978, 1984) not least because interference with dopamine transmission in this area disrupts behaviour motivated by both conventional and non-conventional reinforcers (see Chapter 2).

However, what must not be underemphasised are the differential spatial and temporal characteristics of dopaminergic transmission within this area, (Di Chiara et al., 1994, 1996b; DiChiara, 1995). For example, within the striatum, dopamine acts by two separate modalities, a tonic/non-synaptic mode and a phasic/synaptic mode (Di Chiara et al., 1996b; Di Chiara, 1995). More specifically, striatal dopamine is not only released in the synaptic cleft on junctional low-affinity receptors but diffuses to the extra-cellular fluid outside the synaptic compartment reaching concentrations sufficient to activate extra-junctional high affinity receptors.

By acting in the tonic mode, extracellular dopamine exerts modulatory influences that may be critical for the transmission of information by phasic ionotropic receptors (GABA A, AMPA, NMDA etc.) (Di Chiara et al., 1994). An impairment of tonic dopamine transmission in a given striatal area might ultimately result in an overall impaiment of the function of that area (Di Chiara, 1998). There are several techniques which allow the quantification of the tonic and phasic modes of dopamine transmission in animals. These *in vivo* monitoring techniques are brain microdialysis (Ungerstedt, 1984; Westerink et al., 1987; Di Chiara, 1990), voltammetry (Kissinger et al., 1973; Marsden et al., 1984; Stamford, 1986) and single unit recording (Miller et al., 1981; Steinfels et al., 1981; Schultz, 1992). By their nature, these methodologies constitute a direct approach to the issue of the behavioural relevance of differential dopamine transmission.

By virtue of the "online" characteristics of these methodologies, further taxonomisation of dopamine structures and pathways has arisen from studying the activity of dopamine neurons in several critical brain sites in response to differential rewarding stimuli. The findings from such studies are to be discussed in more detail in Chapter 2 (see Section 2.5) , therefore at this point it is sufficient to say that there is convergent evidence to suggest that the precise location of dopaminergic neurons bears important implications for their capacity for information coding (Schultz, 1998). More concisely, the functional capacity of dopaminergic neurons

is dependent upon their location along the dopamine projections in brain. Therefore, the possibility that dopaminergic neurons may represent dissociable aspects of behavioural processes probably has a definable neuraoanatomical basis.

Summary

The neuroscience methods and models which have evolved over the last 50 years have led to the establishment of well defined maps of dopaminergic projections. In turn, the functional role of dopamine has been further elucidated. However, a reliance upon strictly neuroanatomical data in the endeavour to discover a role for dopamine in behaviour has limitations. Pharmacological differences between dopamine receptor populations were beginning to emerge by the late 1970s due to further technological advances and these differences were beginning to be associated with differential physiological, biochemical and behavioural consequences. More contemporary methods such as radioligand binding and/or electron microscope autoradiography have furthered knowledge of the nature and location of dopaminergic neurons and most recently their receptor subtypes.

The next section of the chapter will introduce the notion of dopamine receptor subtypes which may be differentiated in terms of their pharmacological idiosyncracies and also their anatomical distribution. Most recent research which has associated particular dopamine receptor subtypes with certain aspects of behaviour, will also be discussed.

1.3 Dopamine receptor subtypes

Until recently, only two dopamine receptor subtypes had been definitively shown to exist, (Sibley and Monsma, 1992). Kebabian and Calne (1979) distinguished between these two receptor subtypes: these were the D1 receptors, which activate the enzyme adenylyl cyclase and increase intracellular levels of cAMP, and the D2 receptors, which exert an inhibitory influence on this enzyme. Both of these receptor subtypes belong to a large superfamily of neurotransmitter and hormone receptors that are coupled to their specific effector functions by means of guanine nucleotide regulatory proteins (G-proteins) (Dohlman et al., 1991). The way in which membership of these families has been organised bears important implications for the study of the functional role of dopamine receptors. Before the advent of molecular cloning techniques, receptor subtypes were defined primarily by their pharmacological profiles in eliciting a physiological or biochemical response (Sibley and Monsma, 1992).

Recent isolation of cDNAs and / or genes encoding novel receptor subtypes has confirmed that the dopamine receptor family is more diverse than previously imagined. Five pharmacologically distinct dopamine receptors have now been defined through molecular cloning techniques (Sokoloff et al., 1990; Sibley and Monsma, 1992). These are the dopamine D1, D2, D3, D4 and D5 receptor subtypes. These receptors have been conventionally split into two distinct families: the "D1-like" with D1 and D5 receptor subtypes as constituents, and the "D2-like" family with D2, D3 and D4 receptors as members (Sokoloff et al., 1990).

<u>Receptor taxonomy</u>

Based on amino acid sequence and gene organisation, the D3 receptor has been classified as a member of the family of the D2-like dopamine receptors (Sokoloff et al., 1990; Sibley et al., 1993). Based on these criteria, it has been shown that the rat D1 and D2 receptors possess only 41% homology with each other (Monsma et al., 1990), while, contrasting with this, the rat D3 receptor possesses 52% homology with the rat D2 receptor (Sokoloff et al., 1990). The D2 and D3 receptors exhibit 39% and 41% overall homology with the D4 receptor, respectively (Van Tol et al., 1991). Therefore, the evidence above goes some way to justifying the taxonomy employed in dividing the dopamine receptor subtypes. This system of classification facilitates the endeavour to discover similarities and differences between the D2 and the D3 receptor in terms of pharmacology,

transmission and function. Being of the same receptor family renders some potential physiological and functional distinctions less salient than they might be. One of the ways in which these distinctions can be understood is through the study of anatomical distribution of receptor subtype populations.

1.4 Localisation and distribution of dopamine receptor subtypes

A variety of approaches may be employed to study the localisation of the dopamine receptor subtypes. These have recently included molecular, pharmacological and immunological methods. Since selective radioligands capable of discriminating each member of the dopamine family are unavailable, the study of dopamine receptor distribution in the brain by classic autoradiography binding techniques has been difficult. Much of what is known of the regional distribution of the most novel receptor subtypes (D3, D4, D5) for which selective pharmacological tools have only recently been identified, is based on the localisation of receptor mRNA (Levant, 1997). However, it is well known that the distribution and relative abundance of mRNA does not necessarily covary exactly with the distribution and density of the receptor it encodes (Levant, 1997). Nevertheless, the detection of mRNA with appropriate probes is often presumed to be specific for the receptor of interest. Thus, *in situ* hybridisation has been used extensively as the method of choice to localise the various receptor mRNAs (Levant, 1997).

The dopamine D1-like receptor subtypes

The dopamine D1 receptor is the most widespread dopamine receptor and is expressed more abundantly than any other dopamine receptor subtype (Weiner et al., 1991). D1 mRNA is found in areas known to be under dopaminergic control such as the striatum, nucleus accumbens and olfactory tubercule. It is also found in the limbic system, hypothalamus and thalamus. In other areas with numerous binding sites for the D1 receptor, no mRNA is detected, suggesting that in these areas the D1 receptor is present in projections only. Examples of such areas are the entopenduncular nucleus, globus pallidus and in the substantia nigra pars reticulata, where D1 dopamine receptors originate from striatal GABAergic neurons co-expressing substance P (Le Moine et al., 1991).

The dopamine D5 receptor

The dopamine D5 receptor, as a member of the D1-like receptor family, is expressed at a much lower level than the D1 receptor, and shows a distribution

restricted to the hippocampus and to two sets of nuclei: the lateral mamillary nucleus and the parafascicular nucleus of the thalamus (Meador-Woodruff et al., 1992). However, the D1 receptor mRNA shows no significant expression in these nuclei. Moreover, these regions are not detected by radioligand binding, suggesting that the D5 receptor is either translocated axonally to other brain regions or that its level of expression is too low to be detected by standard binding procedures (Jaber, 1996).

The dopamine D2-like receptor subtypes

The dopamine D2 receptor has been found mainly in the striatum, olfactory tubercule and nucleus accumbens where it is expressed by dopaminoceptive GABAergic neurons co-expressing enkephalins (Le Moine and Bloch, 1995). It is also found in the substantia nigra pars compacta and in the ventral tegmental area, where it is expressed by dopaminergic neurons (Weiner et al., 1991). Thus, the D2 receptor is found at both presynaptic and postsynaptic levels in the brain. The D2 receptor has long been known to be expressed in the pituitary (Creese et al., 1977) where it regulates the production and secretion of prolactin.

The dopamine D4 receptor

The dopamine D4 receptor along with the D3 receptor seems to be restricted to the mesolimbic system where this system is thought to serve a role in modulating emotion and cognition. D4 receptor mRNA is abundant in frontal cortex, amygdala, thalamus, hypothalamus and pituitary with lower levels found in the hippocampus (Joyce and Meador-Woodruff, 1997).

The dopamine D3 receptor

The dopamine D3 receptor has received much attention, mainly because of its specific distribution around the limbic brain areas such as the shell of the nucleus accumbens, olfactory tubercule and Islands of Calleja, and its low expression in the striatum (Sokoloff et al., 1990, Bouthenet et al., 1991). Therefore, it seems that there is not a complete overlap between the D3 receptor and its closest homologue the D2 receptor in terms of neuroanatomical distribution.

In brief, D3 mRNA has been found mainly in the ventral striatal complex, the substantia nigra pars compacta the ventral tegmental area and the cerebellum (Diaz et al 1994). In the Islands of Calleja, both D3 receptor binding and

messenger mRNA are abundant in the entire population of granule cells, which are known to make sparse contacts with dopaminergic axons. A minor proportion of these granule cells appear to be in direct synaptic contact with dopamine axons, mainly those surrounding the islands (Fallon et al., 1993).

In the archicerebellum, Purkinje cell perikarya in lobules 9 and 10 express D3 mRNA, whereas binding sites were found only in the molecular layer. Surprisingly, no known dopaminergic projections are known to exist in this area, suggesting that the D3 receptor may mediate non-synaptic actions of dopamine (Diaz et al,1995).

Within the nucleus accumbens, D3 receptor markers are more extensively expressed in selected subterritories and subsets of neurons. Within this nucleus, three main areas can be distinguished: the rostral pole, the shell and the core (Landwehrmeyer et al., 1993). D3 receptor markers were both present in the rostral pole and parts of the shell (the ventromedial portion) but were almost absent from the core and from the septal pole of the shell (also known as the shell cone), (Griffon et al., 1995).

Griffon et al., (1995), futher showed a co-localisation of D3 receptor and neurotensin gene transcription the ventromedial shell with double in situ hybridisation studies. They also demonstrated the presence of dense D3 receptor binding accompanied by low mRNA levels in subcommisural ventral pallidium. These observations strongly suggest that a subpopulation of accumbopallidial neurotensin neurons (Sugimoto et al., 1987) expresses D3 receptors at somatodendritic level as well as at the terminal projections.

<u>Distribution of the D3 receptor in human brain</u>

The distribution of D3 receptors in human brain have recently been implicated in pathologies such as schizophrenia and drug addiction (see General Discussion). Therefore, a brief outline of their localisation will give some idea of the possible functional significance in such pathology in humans.

The overall pattern of distribution of the D3 receptor in human brain seems to be somewhat less restricted than the distribution of this receptor subtype in the rat brain (Herreolen et al., 1994). Highest densities of putative D3 sites are reported in the nucleus accumbens and the Islands of Calleja (Landwehrmeyer et al., 1993b; Murray et al., 1994). Moderate amounts of D3 binding have been observed in the

basal ganglia, parietal, temporal and occipital cortex, and cerebellar cortex, followed by substantia nigra , hippocampus, and the basolateral, lateral and basomedial amygdaloid nuclei (Herroelen et al., 1994; Murray et al., 1994; Lahti et al, 1995). D3 receptors have also been detected in moderate density in the pituitary, with somewhat greater labelling in the posterior lobe than the anterior (Herroelen et al., 1994).

Functional implications of the regional distribution of the D3 receptor

The evidence from in situ hybridisation studies reveals that there is uncertainty surrounding the possibility that the D3 receptor like the D2 receptor, is located presynaptically and/or postsynaptically. Therefore, the pre- or postsynaptic localisation of D3 receptors in the brain is a matter of controversy. A wealth of behavioural data has been brought to bear in order to address this question and will be discussed more fully later in this chapter. However, at the cellular level, evidence that the D3 receptor is located presynaptically comes from the detection of D3 mRNA in the substantia nigra and the ventral tegmental area. The detection of putative binding sites in dopaminergic terminal fields also suggests that a subset of D3 receptors may be presynaptic (Levant, 1997). Further corroboration comes from lesion studies. Unilateral dopaminergic lesions produced a marked decrease in D3 receptor density in the nucleus accumbens., suggesting the loss of presynaptic rather than postsynaptic sites (Levesque et al., 1995). Neurochemical studies also suggest a role for the D3 site as a synthesis and/or release-modulating autoreceptor (Waters et al., 1993).

Unlike the D2 receptor, which is abundant in the caudate/putamen or pituitary as well as limbic brain regions (Levant, 1996), very low levels of expression of the D3 receptor are detected in either caudate putamen or pituitary. The caudate putamen site is associated with negative side effects produced by most conventional antipsychotics, such as the extrapyramidal symptom, tardive dyskinesia. These observations suggest that neuroleptic drugs which have a high affinity for the D3 receptor could achieve their antipsychotic effects without encumbent extrapyramidal and neuroendocrine effects (Sokoloff et al., 1990).

The high densities of D3 receptors in lobules 9 and 10 of the cerebellum (coupled with the sparsity of D2 receptors) (Bouthenet et al., 1996) has led to investigations into a functional role for this cerebellar dopamine system. Recently, Barik and Beaurepaire (1996) showed that microinjections into the vestibulocerebellum produces alterations in locomotor activity in rats. This system

has not often been directly associated with the control of locomotion, rather it has been consistently related to the vestibular control of posture. Clinically, disorders involving these brain regions produce symptoms such as ataxia, whereas lesions of this brain region cause rigidity (Ghez and Fahn, 1981). These symptoms are obviously similar to those encountered in patients treated with antipsychotic drugs. Therefore, the substrate underlying these effects may not be exclusively striatal (Levant, 1997).

It has been also been suggested that the archicerebellum plays a part in the control of eye movements, abnormalities of which occur in a large proportion of schizophrenic patients. The participation of dopamine D3 receptors in this pathophysiology is partly supported by the observation of a genetic association between homozygosity at the D3 receptor gene and familial schizophrenia (Crocq et al 1992). However, this genetic linkage to schizophrenia remains inconclusive (see General Discussion.)

Dopaminergic receptor localisation studies around the nucleus accumbens are coupled with recent evidence for the functional and anatomical heterogeneity within this brain structure. The nucleus accumbens has been compartmentalised leading to a functional dichotomy between the more rostrally located core and the caudomedially located shell subregions (Zahm and Brog, 1992). Dissociations between these regions in terms of anatomical, behavioural, neurochemical and electrophysiological characteristics have been well documented. Of particular relevence here is the finding that D3 receptors are selectively distributed around the shell but are absent from the core (Landwehrmeyer, 1993). The shell has been shown to project primarily to limbic structures while the core preferentially sends efferent projections to motor related structures (Deutch and Cameron, 1992). Also, there is at least one report of two neurochemically distinct divisions in the human nucleus accumbens which display many of the characteristics of the rat nucleus accumbens, including a selective distribution of D3 receptor receptor density (Voorn et al., 1994).

Furthermore, several behavioural studies using animals have made use of the compartmentalisation of the nucleus accumbens in order to study the effects of stimulation of these discrete subregions some which are of particular interest here. For example, strong supporting evidence for the idea that the shell may play a more important role than the core in the mediation of feeding behaviour comes from a study which blockade of glutamate in the shell but not the core, induces a powerful feeding response (Maldonado-Irizarry et al., 1995); an effect which was partially

reversed by dopamine antagonists. Consequently, it was hypothesised that dopamine in the shell may have a modulatory effect on feeding behaviour. Further work from the Maldonado-Irizarry laboratory has shown that local microinjections of dopamine agonists into these subregions did not significantly affect feeding or drinking responses. Rather, using these compounds it seemed that the shell appeared to be relatively more sensitive to the motor activating effects of dopamine agonists than the core (Swanson et al., 1997).

The density of D3 receptors in the olfactory bulb has given rise to the targeting of this brain site in the search for the behavioural significance of this receptor subtype. The olfactory bulb is endowed with a strictly intrinsic source of dopamine and therefore this structure contrasts with the brain structures wherein dopamine is secreted from the terminals of projection neurons, like striatum, cerebral cortex, nucleus accumbens and olfactory tubercule (Lindvall and Bjorklund, 1983). Dopamine involvement in olfactory processing has been suggested recently by several lines of evidence. Single injection of a dopamine agonist in vivo reduces odour detection (Doty and Risser, 1989) and abolishes odour-induced metabolic activation pattern in olfactory bulb (Sallaz and Jourdan, 1992). Such data could indicate that dopamine could exert an inhibitory control on olfactory input. Dopamine may also be implied in olfactory memory since dopamine release increases during learning (Kendrick et al., 1988; Coopersmith et al., 1991; Keverne et al., 1993) and since injection of dopamine antagonists inhibits olfactory memory formation (Weldon et al., 1991).

In the light of the recent cloning of novel receptor subtypes, it is interesting to investigate the extent to which these subtypes may contribute to olfactory function by determining their locale and extent of distribution. For instance, Coronas et al. (1997) used a combined approach to characterise the formerly known population of D2-like binding sites in olfactory bulb as a major contingent of D2 receptors with a minor amount of D3 receptor but no D4 receptors. They discovered a striking overlap of subregional distributions of either mRNAs or specific binding sites of the two receptor subtypes. This could imply that D2 and D3 receptors might be co-expressed in the same neurons in olfactory bulb (Coronas et al., 1997).

Evidence of receptor colocalisation has led researchers such as Barik and Beaurepaire (1998) to hypothesise interactions which heretofore have been difficult to predict owing to a lack of refined techniques. The brain area under study was the Island of Calleja Magna which has the highest density of D3 receptors in the brain,

and which does not contain D2 receptors and therefore render this structure is an appropriate target for studies on the functional selectivity of D3 receptor stimulation. However, this brain structure does contain dopamine D1 receptors (Mengod et al., 1992). It is well established that D1 and D2 receptors interact in certain parts of the brain (i.e. D2 effectiveness being potentiated by concomitant D1 activation, Walters et al., 1987). Barik and Beaurepaire chose thermoregulation as their dependent variable as this was a response which had been related to D3 receptor function several times (see Table 1.3). Their results show that D3 receptor agonists microinjected into the island of Calleja Magna decreased body temperature and that this effect was potentiated by simultaneous injection of the D1 receptor agonist SKF 38393 (Barik and Beaurepaire, 1998). Therefore there is apparent synergy in the activation of these co-localised receptors despite their pharmacological contrariety.

1.5 Dopamine receptor pharmacology

Functional assays

As has already been mentioned, the original dissociation of D1 and D2 receptor subtypes was based on their opposing effects (stimulation and inhibition respectively) on adenylyl cyclase activity (Kebabian and Calne, 1979). Since these early discoveries, the use of functional or physiological assays has been invaluable in correctly classifying ligand specificity at the D1-like and D2-like receptors. However, the validity of this simple dissociative assay has been questioned (Downes and Waddington, 1993).

Since the cloning of novel dopamine receptor subtypes, much of the confirmation of agonist or antagonist activity of novel dopaminergic compounds at the D3 receptor comes from the use of functional assays. Levant (1997, 1998) reviews such data and highlights some of the most salient results with respect to the D3 receptor. D2 agonists, such as dopamine, quinpirole, and bromocriptine, have been shown to possess agonist activity at the D3 receptor as assessed by several physiological assays such as the induction of CHO (chinese hamster ovary) cell mitogenesis, melanocyte aggregation or extracellular acidification (Chio et al., 1994; Sautel et al., 1995a). The putatively D3 selective compounds 7-OH-DPAT and PD128907 also exhibit differential agonist activity in the mitogenesis test (Chio et al., 1994; Pilon et al., 1994; Potenza et al., 1994; Sautel et al., 1995a; Pugsley et al., 1995). In contrast, antagonists such as spiperone, sulpiride and nafadotride, block agonist induced activity in these tests (Sautel et al., 1995b). The D2/D3 ligand (+)-UH 232 has been shown to be a partial agonist at the D3 receptor in the

mitogenesis assay (Griffon et al., 1995). Therefore such assays have proven useful in determining the functional boundaries between novel and existing dopamine receptor subtypes.

Radioligand Binding Studies

One of the greatest breakthroughs in Neuroscience has been the emergence of a technique which provides the ability to localise and measure neurotransmitter receptors in homogenised brain tissue. The radioligand binding procedure represents a relatively simple method of achieving this aim. A ligand is selected which will bind to the receptor of interest. This ligand is then labelled with a radioactive substance (hence radioligand). The radioligand is incubated with the tissue (homogenate or tissue slice) under empirical conditions which have been shown to optimize binding. Radioligand which remains unbound after application is removed and the amount of radioligand bound to the tissue is then measured by a gamma counter. Several criteria are essential for concluding that receptor binding has occurred (i.e. specificity of binding to the receptor of interest alone; saturability, whereby binding steadily increases until all the receptors are occupied, reversibility of the ligand-receptor association and finally, the biological relevance of the binding in the assessment of the relationship between the ligand/receptor interaction and a consequent biological response). It is in this way that many of the dopamine receptor subtypes currently recognised have been located and measured since the 1960s and 70s.

At this stage of the thesis it is useful to provide a selection of ligands, which is by no means exhaustive but will contain some of those compounds which will be referred to throughout the text as having specificity for certain dopamine receptor subtypes. Table 1.2 provides some of the pharmacological tools used to study D1 and D2 receptors on the basis of their published specificity for these receptor subtypes.

Table 1.2 traditional functional classification of dopamine receptor subtypes and prototypical pharmacological compounds selective for D1 and D2 receptors

	D1	**D2**
Prototype receptor location	Parathyroid gland	Anterior and intermediate pituitary gland
Adenylate cyclase association	Stimulatory	Inhibitory or unlinked
Prototype selective agonists	SKF 38393 *[+/-]-1-Phenyl-2,3,4,5-tetrahydro-[1H]-3-benzazepine-7,8-diol*	bromocriptine *(+)-2-Bromo-12'-hydroxy-2'-(l-methyllethyl)-5'-(2-methyl-propyl)-ergotaman-3',6'-18-trione methanesulfonate*
	SKF-82958 *(±)-6-Chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide; 6-Chloro-N-allyl-SKF-38393 hydrobromide*	quinpirole *LY 171555*
Prototype selective antagonists	SCH-23390 *R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride*	raclopride *S(-)-Raclopride L-tartrate*
	SCH-39166 *(6aS,13bR)-11-chloro-6,6a,7,8,9,13b-hexahydro-7-methyl-5H-benzo [d]naphth[2,1-b]azepin-12-ol*	sulpiride *(+/-)-5-(Aminosulfonyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide*
	SKF-83566 *(±)-7-Bromo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride*	spiperone *N-Methyl-8-[4-(4-Fluorophenyl)-4-oxobutyl]-1-phenyl-1,3,8-triazaspiro-[4.5]decan-4-one hydrochloride*

(**Table 1.2** continued)

Location	Postsynaptic	Pre- and postsynaptic
Electrophysio-logical effect (Extracellular)	Inhibitory	Inhibitory

Table 1.2 adapted from Clark and White (1987)

Until recently, however, few pharmacological tools were available to properly discern an exclusive functional role for each of the dopamine receptor subtypes. Nevertheless, since the original cloning of the D3 receptor in 1990 (Sokoloff et al., 1990) several studies have examined the specificities and relative affinities of dopaminergic compounds for D2 and D3 receptors in various expression systems and in brain. The relatively extensive homology between the D2 and D3 sites leads to the prediction that the pharmacological profile of the D3 receptor should be generally similar to that of the D2 receptor. However, in her extensive review of such evidence (Levant, 1997) results of radioligand binding studies vary considerably. This variation seems to partially depend on the expression system or tissue, the radioligand used and the in vitro assay conditions used (Tang et al., 1994a; Burris et al., 1995; Levant et al., 1995).

However, some general conclusions seem to be currently accepted. The D3 receptor exhibits high affinity for non-selective and D2 selective agonists such as dopamine, quinpirole and apomorphine, and significantly lower affinity for the D1 selective agonist SKF 38393 (Sokoloff et al., 1990). The D3 site also possesses significantly higher affinity for D2 selective antagonists, such as spiperone and haloperidol, than the D1 selective antagonist SCH 23390 (Freedman et al., 1994b). Recently, racemic 7-hydroxy-2-(*N,N* - di -*n* - pro -pylamino)tetralin (7-OH-DPAT) was reported as a selective ligand for the dopamine D3 receptor: the Ki values for dopamine D2, D4 and D1 receptor subtypes were approximately 2, 3 and 4 orders of magnitude lower, respectively (Levesque et al., 1992). These findings led to speculation that this compound may be useful as the first limbic-selective dopamine agonist for probing the behavioural function of the dopamine D3 receptors in vivo (Caine and Koob, 1993; Daly and Waddington, 1993).

However, D2 receptors exist in multiple states having high and low affinities for D2 agonists (Burris et al., 1995). The G-protein coupled state of D2 receptors is believed to be the functional state of these receptors. More precisely, putative D3 receptor -selective agonists also interact with high-affinity, G-protein coupled state of D2 receptors. During labelling, by providing conditions which promote uncoupling of receptors from G proteins, it becomes possible to differentially affect affinities of D3 receptors in comparison to those of the D2 receptor, (Burris et al., 1995). The similarities in affinities of the agonist-preferring state of D2 and D3 receptors means that available agonists cannot be used to discriminate between behavioural effects mediated by D2 and D3 receptors.

Although it is beyond the scope of this thesis to list all of the radioligand binding data published to date (see Levant, 1997, for review), it is worthy of note that there are several ligands which are commonly used in determining the functional role of the D3 receptor. Therefore, several agonists and antagonists are listed below which have been shown to have equal or higher selectivity for D3 receptors over D2 receptors.

Table 1.3 Agonists and antagonists selective for the D3 receptor *in vivo* and *in vitro.*

D3 receptor agonists	*author*	D3 receptor antagonists	*author*
PD-128907	*Corbin et al. (1994)*	LU-201640	*Gross et al. (1997)*
	Pugsley et al. (1995)		
(+)- 7-OH-DPAT	*Damsma et al. (1993)*	(+)-S 14297	*Millan et al. (1995)*
		(+)-AJ76	*Svensson et al. (1986)*
(-)- Quinpirole	*Sokoloff et al. (1990)*	(+)-UH 232	*Svensson et al. (1986)*
S-14297	*Gobert et al. (1994)*	nafadotride	*Sautel et al. (1995)*
			Schwatrz et al. (1995)
		PNU 99194A	*Waters et al. (1993)*
		pirebidil	*Cagnotto et al. (1996)*
		PD-152255	*Corbin et al. (1998)*

Results from binding studies are inconclusive (Levant, 1997, 1998) and too numerous to mention here. However, in brief, PD 152255 seems to be the most potent antagonist available for the D3 receptor (Corbin et al., 1998) being 28- to 45-fold more potent at D3 receptors than at D2 receptors. This contrasts with the 5-20 fold selectivity for other antagonists available for the D3 receptor (Corbin et al., 1998). According to published literature, in terms of selectivity compared with D2 receptors, quinelorane is a more potent D3 receptor agonist than 7-OH-DPAT or quinpirole and 7-OH-DPAT is more potent at the D3 receptor than quinpirole (Sautel et al., 1995). Many agonist compounds have been claimed to have a higher selectivity for the D3 receptor but 7-OH-DPAT and quinpirole seem to have been adopted as the prototypical D3 receptor agonists. This is the case despite the considerably different reports on the selectivity of 7-OH-DPAT for the D3 receptor. While early reports claimed a 100-fold selectivity for the D3 receptor over the D2 receptor, (Levesque et al., 1992), more contemporary studies report, at best, a sevenfold selectivity for the D3 receptor, (Gonzalez and Sibley, 1995).

Also, it has recently been observed that 7-OH-DPAT binds with high affinity to the σ receptor site (Schoemaker, 1993; Wallace and Booze, 1995). The ratio of D3 receptor to σ binding sites in brain subregions were calculated by Wallace and Booze (1995). They showed that there was a 4 : 1 ratio of D3 receptor to σ binding sites in the core of the nucleus accumbens and a 1 : 1 ratio in the shell of the nucleus accumbens. Therefore, these authors acknowledge that behavioural effects of 7-OH-DPAT could feasibly be the result of an interaction between σ and D3 receptors. Sethy et al. (1996) conducted a neurochemical study which indicated that, at high doses, 7-OH-DPAT has both D-1 and D-2 agonist activities. 7-OH-DPAT-induced increases in Ach at high doses (0.3 to 30 mu mol/kg) and these effects were antagonised by the D2 receptor antagonist raclopride and also by the D1 receptor antagonist SCH-23390. Despite these caveats, many behaviours have been repeatedly associated with the effects of putative D3 receptor agonist compounds such as the above and a selection are listed in Table 1.3.

Table 1.3 Some of the simple behavioural and physiological effects of putatively D3 receptor-preferring compounds

Agonist induced behaviour	Reference
-induction of hypothermia -induction of yawning	*Damsma et al. (1993); Alhenius and Salmi (1994); Millan et al. (1994, 1995 a,b); Ferrari and Guiliani (1995); Khroyan et al. (1995); Kurashima et al. (1995)*
-decreased sniffing	*Daly and Waddington (1993)*
-decreased grooming	*Khroyan et al. (1995)*
-decreased climbing in mice	*Sautel et al. (1995 b)*
-increased penile erection and ejaculatory behaviour	*Ferrari and Guiliani (1995)*
-disruption of social behaviour	*Kagaya et al. (1996)*
-modulation of anxiety	*Rodgers et al. (1996)*
-inhibition of pilocarpine induced limbic seizures	*Alam and Starr (1994)*
-induction of emesis in the dog	*Yoshida et al. (1995)*
-depression of electroencephalogram patterns	*Popoli et al. (1996)*
-induction of catalepsy	*Millan et al. (1995 b); Sautel et al.(1995 b)*
-increased oxytocin secretion	*Uvnas Moberg et al. (1995)*
-decreased prepulse inhibition	*Caine et al. 1995*

(**Table 1.3** continued)

-increase in blood pressure	*Van den Buuse (1992)*

Is the D3 receptor an autoreceptor ?

Models of presynaptic and postsynaptic activity

The evidence for the existence of central presynaptic dopamine receptors is based on a large body of electrophysiological, biochemical and behavioural data. Autoreceptors on presynaptic nerve terminals participate in the local control of transmitter release. According to a review by Starke et al. (1989) dopamine release modulating autoreceptors exist in various brain areas of different animal species and belong to the D2 type. The high affinity of D3 receptors for dopamine supports the hypothesis that D3 receptors function as synthesis inhibiting autoreceptors in the striatum, (Meller et al 1993). However, there are several studies which cast doubt on an exclusively presynaptic location for the D3 receptor. For example, dopamine denervation downregulates D3 receptors (Levesque et al., 1995), a finding consistent with a post-synaptic localisation of the D3 receptor.

Most studies have been performed in the mesostriatal system, whereas few investigations have concerned dopamine autoreceptors in the terminal regions of the mesolimbic dopaminergic pathway. However, recent work by Barik and Beaurepaire (1998), using microinjections of D1 and D3 receptor agonists into the island of Calleja Magna, revealed that in dopamine-depleted animals reacted in the same way as non-depleted animals in a test for the D3 receptor mediated function of thermoregulation. They conclude that, at least in this brain area, D3 receptors are located post-synaptically. Therefore, evidence from a number of in vivo and in vitro assays will be reviewed to attempt to evaluate the controversial speculation surrounding the neuroanatomical location of the D3 receptor.

Evidence for a role of the D3 receptor in neuronal activity

As the "prototypical" D3 agonist, 7-OH-DPAT has been used to probe the effects of D3 receptor stimulation on neuronal activity. 7-OH-DPAT has been shown to inhibit firing of neurons in both the substantia nigra and ventral tegmental

areas, as well as in brain slice preparations (Bowery et al., 1994; Liu et al., 1994a; Devoto et al., 1995; Kreiss et al., 1995; Lejeune and Millan, 1995). Kreiis et al. (1995) found a strong relationship between the potency of ten dopamine agonists to inhibit neurons in the substantia nigra pars compacta and their affinities at D3 rather than D2 receptors. 7-OH-DPAT has also been reported to decrease firing of spontaneously active neurons in the nucleus accumbens, (Amano et al., 1994; Liu et al., 1994a).

Further electrophysiological studies seem to reinforce the idea that the effects of low doses of 7-OH-DPAT are mediated through D3 autoreceptors. The activation of these receptors decrease dopamine release / synthesis as is the case for the D2 autoreceptor. Lejeune and Millan, (1995) discovered that the firing rate of dopaminergic neurons in the VTA of anaesthetised rats was dose-dependently inhibited by 7-OH-DPAT (0.31-5.0 μg/kg IV). This effect was reversed by the selective D3 antagonist (+)-S14297. When administered alone, this antagonist did not modify firing rate. Haloperidol (16μg/kg IV) fully reversed the effects of 7-OH-DPAT and alone significantly increased the firing rate. Taken together these latter data suggest that the inhibitory (dendritic) dopamine D3 autoreceptors control the electrical activity of ventral tegmental area localised dopamine neurons.

<u>Evidence for a role of the D3 receptor in neurochemistry</u>

As autoreceptors on presynaptic nerve terminals participate in the local control of transmitter release, it is worthy of note that 7-OH-DPAT produced decreases in dopamine release as assessed by microdialysis or voltammetry as well as in accumbal slice preparations (Damsma et al., 1993; Rivet et al., 1994; Yamada et al., 1994; Devoto et al., 1995; Gilbert et al., 1995; Patel et al., 1995; Gainetdinov et al., 1996). Similar results have been demonstrated with the administration of the D3-selective agonist PD 128907 (Pugsley et al., 1995).

The D3 receptor has been implicated in the modulation of dopamine synthesis. In vivo, D3-selective drugs 7-OH-DPAT and PD 128907 have been reported to decrease dopamine synthesis, (Aretha et al., 1995; Gobert et al., 1995; Gainetdinov et al., 1996; Pugsley et al., 1995). There is evidence that this effect is presynaptic as it is observed in both normal and animals treated with gamma-butyrolactone which blocks impulse flow in nigrostriatal and mesolimbic dopamine neurons (Aretha et al., 1995; Pugsley et al., 1995). The involvement of the D3 receptor in such an effect is confirmed by the observation that 7-OH-DPAT

produced a greater decrease in dopamine synthesis in the nucleus accumbens (where D3 receptors are relatively abundant) than in the caudate nucleus (where D3 receptors are relatively sparse) (Aretha et al., 1995; Pugsley et al., 1995).

1.6 Evidence of a role for the D3 receptor in behaviour

There is anatomical, pharmacological, neurochemical and electrophysiological evidence to suggest both a presynaptic (Van Oene et al., 1984; Timmerman et al., 1991; Damsma et al., 1993; Meller et al., 1993) and also a postsynaptic (Waters et al., 1993; Svensson et al., 1994) preferential localisation for the dopamine D3 receptor. It has been suggested that the effects of putative D3 receptor agonists such as 7-OH-DPAT in the nucleus accumbens would act primarily on the postsynaptic D3 receptor and in the ventral tegmental area on the D3 autoreceptor (Freedman et al., 1994). These effects are manifest as changes in locomotion. Consequently, the D3 receptor has been widely cited in the modulation of locomotor activity.

At the behavioural level, attenuation of locomotor activities in response to low doses of dopamine agonists have been classically interpreted as resulting from selective stimulation of dopamine autoreceptors. On the other hand, potentiation of locomotor activities by intermediate dosages of dopamine agonists and the induction of stereotypies with high doses has been interpreted as resulting from postsynaptic effects, (Strombrom et al., 1976). The biphasic effect on locomotion, of compounds such as 7-OH-DPAT is described by Waddington and Daly (1993); Starr and Starr (1995); Franz et al. (1996) and is taken to reflect this differential stimulation of pre- and post-synaptic sites. However, as Meyer (1996) and Kling-Petersen et al. (1995) have demonstrated, the cause of this biphasic effect is not as obvious as the above assumptions imply.

Meyer (1996) questioned the hypothesis that the D3 receptor is both a presynaptic and a postsynaptic receptor. He found that microinjections of 7-OH-DPAT directly into the nucleus accumbens resulted in the potentiation of locomotion. These data support the hypothesis that the D3 receptors are postsynaptic receptors. However, microinjections of 7-OH-DPAT directly into the ventral tegmental area did not result in the attenuation of locomotor behaviours, but rather resulted in the potentiation of the behaviour. These behavioural data did not support the hypothesis that the D3 receptor in the VTA functions as an autoreceptor. Therefore, it seems that distinctions between the functional significance of a presynaptic and a postsynaptic D3 receptor is not clearly definable.

Also using intracerebral injections, Kling-Petersen et al., (1995) found that R-(+) 7-OH-DPAT inhibited spontaneous locomotor activity over a wide dose range after injection into the nucleus accumbens. Conversely, the D3 antagonist U99194A (30 x more selective for D3 than for D2 receptors) produced an increase in activity when injected into the nucleus accumbens. Local application of 7-OH-DPAT into the ventral tegmental area did not produce any significant behavioural effects. Using another D3 receptor antagonist *l*-Nafadotride (Sautel et al., 1995b) found that at low doses (0.1-1mg/kg) nafadotride, unlike haloperidol, increases spontaneous locomotion of habituated rats and climbing behaviour of mice, at doses that do not modify striatal HVA levels. At high doses (1-100 mg/kg) nafadotride, like haloperidol, produces catalepsy and antagonises apomorphine-induced climbing.

This clearly contrasts the behavioural effects of D2 and D3 antagonists suggesting that dopamine D3 receptor blockade results in motor activation while dopamine D2 receptor blockade gives rise to motor sedation. The above evidence lends weight to the idea that the D3 receptors, in contrast with D2 receptors are in the main, located postsynaptically where they display an inhibitory action on locomotor activity.

Neurochemical correlates of D3 receptor agonist-induced behaviours

Stahle (1992) presented compelling evidence that suppression of locomotor activity is mediated by a postsynaptic receptor rather than an autoreceptor as dopamine agonist-induced hypoactivity can be demonstrated even in the presence of substantially increased synaptic dopamine concentration. Svensson et al. (1994) also dispute the role of the D3 autoreceptor in hypolocomotion, as 7-OH-DPAT produced this phenomenon at doses which did not affect dopamine release or synthesis.

In manipulating the D3 antagonist U99194 (20 x more selective for D3 than for D2 receptors) Waters et al, (1993), have been able to demonstrate that the effects of this antagonist is most likely due to the blocking of a postsynaptic inhibitory dopamine D3 receptor . U99194 was able to increase locomotor activity in rats and to potentiate the stimulatory effects of amphetamine whereas the classical effects of the D2 receptor blockade is antagonism of amphetamine induced locomotion. Further confirmation of this comes from studies employing voltammetry (Gilbert et al., 1995). Their study revealed that 7-OH-DPAT had its effects at dopamine autoreceptors to decrease intracranial dopamine levels and to decrease behaviours

that are dopamine dependent such as self-stimulation to the ventral tegmental area. Systemic injections of 7-OH-DPAT (0.01-0.3 mg/kg IP) quickly and dose-dependently decreased responding for stimulation. Several in-vivo experiments support this finding by showing that 7-OH-DPAT decreases dopamine release using microdialysis (Mulder et al., 1987; Timmerman et al., 1991; Damsma et al. 1993). Similar results were reported for the potent D3 agonist PD 128,907 (Millan et al, 1996; Gobert et al.,1996) and the converse is reported for the D3 antagonist PD 156,586 (Bristow et al, 1996) which seems to be acting as an antagonist at D3 synthesis regulating autoreceptors.

Again, many of the results of the functional corollaries of D3 receptor stimulation are inconclusive. Without confirmation of the pharmacological specificity of novel compounds it is difficult to conclude whether the D3 receptor is indeed a pre-synaptic autoreceptor or a post-synaptic receptor. Indeed, as Levant points out, in vivo interaction of the putative D3 receptor agonists with the D3 receptor has yet to be demonstrated (Levant, 1997).

A role for postsynaptic D2 receptors in the biphasic effects of putative D3 receptor agonists?

Owing to reports of putative D3 receptor receptor selective compounds having a partial affinity at dopamine D2 receptors (Burris et al., 1995), it seems plausible that higher doses of D3 agonists such as 7-OH-DPAT may exert their effects at postsynaptic D2 rather than D3 receptors. Evidence underlying this assumption comes from observations of stereotyped behaviour at these higher doses. These stereotyped effects are classically attributed to postsynaptic D2 or mixed D1 and D2 receptor stimulation (Waddington and Daly, 1993) Waddington and Daly (1993) and Kurashima et al, (1995) attribute 7-OH-DPAT-induced stereotypy to a D2 receptor stimulation at high doses, (0.25, 5.0 mg/kg, SC). These researchers also observed that low doses of 7-OH-DPAT (10-250 μg/kg, SC) elicited yawning which is commonly attributed to the stimulation of dopaminergic autoreceptors (Strombom, 1976).

Therefore, behaviours which have been reliably elicited using relatively high doses of 7-OH-DPAT may be under the control of a post-synaptic population of D2 receptors. Koshikawa et al. (1996), show that this is the case for some of the sterotypy seen with the administration of 7-OH-DPAT. They showed that in the case of dopamine-dependent (nucleus accumbens shell specific) turning behaviour

and repetitive jaw movements, the effects of 7-OH-DPAT are solely due to its ability to activate D2 receptors.

However, this stereotypy whether mediated by the D2 receptor the D3 receptor or both, has not been universally observed with 7-OH-DPAT, even at high doses. For example, Waddington and Daly (1993) showed that higher doses of 7-OH-DPAT (0.1-10.0 mg/kg, SC) stimulated non-stereotyped sniffing, locomotion and chewing which were attenuated by the D1 antagonist BW 737C (without release of any atypical behaviours). Gilbert and Cooper (1995) did not note any signs of hyperlocomotion or stereotypical behaviours normally associated with mixed D1/D2 stimulation when systemically injecting 7-OH-DPAT (0.1-3.0, mg/kg IP), or when administering this compound intra accumbens (0.3-3.0 mg/kg, total dose).

Contrary to the above study, Smith et al. (1996) demonstrated the biphasic effects of 7-OH-DPAT on locomotor behaviour in the marmoset and found D2 antagonists to be effective in attenuating the stimulatory action of 7-OH-DPAT. However, these same antagonists failed to reverse the inhibitory effects of 7-OH-DPAT on locomotion. This supports previous work in the rat (Storey et al. 1995) and may, once more indicate a differential action of low and high doses of 7-OH-DPAT at D3 and D2 receptors respectively. Smith et al. (1996) do not consider that the D2 like effect that they seem to have isolated could be an inhibitory postsynaptic D3 effect, already identified by Svensson et al. (1994). It is plausible that the induction of hypolocomotion is mediated by the D3 postsynaptic receptor found in limbic structures (Depoortere et al, 1996) as well as/instead of the presynaptic D3 autoreceptor. The recruitment of such an explanation would not preclude contribution of behaviours mediated by non-D3-receptor subtypes.

It is worth mentioning here that the discrepancies in data that have been discussed herein may often be attributable to substantial differences in drug dosages and in methodological approaches (Storey et al., 1995). An observer rated behavioural check list used to study well habituated rats can produce drastically different data to that which is collected using unhabituated rats and an automated system (Storey et al. 1995).

Summary and Caveats

Progress in understanding the potential physiological and therapeutic significance of D3 receptors has long been hampered by the lack of a selective agonist at the D3 receptor rather than the D2 receptor. Thus, although (+)- 7-OH-DPAT is a preferential agonist at D3 receptors versus D2 receptors (Levesque et al., 1992) its use alone may be insufficient to unequivocally identify dopamine D3 receptor-mediated responses (Freedman et al., 1994a; Large and Stubbs, 1994; Millan et al., 1995).

Recent pharmacological characterisation of the D3 receptor suggests that D2/D3 -selectivity of many compounds varies depending on the in vitro assay conditions used (Burris et al., 1995; Levant et al.,1995) and such compounds may not exert their effects exclusively at dopamine receptor subtypes (Wallace and Booze, 1995). Accordingly, there has been concern over the selectivity of these drugs in vivo and the attribution of pharmacological effects to the D3 receptor (Freedman et al., 1994a; Large and Stubbs, 1994). In addition, the vast majority of studies have used 7-OH-DPAT as the "prototype" compound for D3 receptor agonism. Therefore, some of the observed effects may be idiosyncratic to this drug (Levant, 1997). However, inasmuch as agonists and antagonists are selective for the D3 receptor, this receptor has now been associated with many physiological and psychological functions, not least with the mediation of reward and reinforcement.

Chapter 2 provides a theoretical background to the association between dopamine and reward and continues to outline a role for dopamine receptor subtypes in particular aspects of reward, specifically components of food reward. Chapter 2 also contains descriptions and explanations of the various methodologies which have been utilised for the study of the neural bases of the control of ingestive behaviour, accompanied by the theoretical assumptions which are applicable to these empirical paradigms.

Chapter 2: The role of dopamine in motivated behaviour

2.1 Chapter overview

Dopamine projections from the substantia nigra and ventral tegmentum to forebrain structures such as the nucleus accumbens and neostriatum constitute some of the most thoroughly studied of all brain substrates for reward. Despite the established significance of these projections in sensorimotor function many authors have concluded that dopamine projections play a role in mediating the reward value of food, drink, sex, social reinforcers, drugs of abuse and brain stimulation above and beyond sensorimotor contributions (Wise, 1978, 1982).

Considerable evidence has shown that brain reward is dependent on the functional integrity of dopamine neurotransmission within the mesotelencephalic dopamine system in the brain. Among the many terminal loci of the mesotelencephalic dopamine system, the nucleus accumbens has been shown to be perhaps the most critically involved in brain reward (Salamone, 1993).

As early as 1954, Olds and Millner reported that rats would avidly self-deliver electrical stimulation through electrodes implanted in specific brain areas. This self-delivery was anatomically restricted to areas of the mesotelencephalic dopamine system implying that these brain areas were specialised for mediating brain reward. Indeed, laboratory animals will ignore a wide range of alternative reinforcers (i.e. food and water) in order to self-administer this rewarding electrical stimulation. The importance of discrete brain sites to intra-cranial self-stimulation (ICSS) was judged by measuring those which produced the highest rates of operant responding (i.e. lever pressing) for the lowest current levels. Drugs of abuse have been shown to lower brain stimulation reward thresholds in various mesotelencephalic loci and therefore drugs of abuse can be said to derive their rewarding properties by activating brain reward circuits.

Further confirmation of a "reward pathway" came from studies into intracranial self-microinjection of addictive drugs. These drugs of abuse will be readily self-administered into brain-reward loci within the mesotelencephalic dopamine system, but not into other brain loci. Examples include self-administration of amphetamine into the nucleus accumbens and prefrontal cortex (Hoebel et al., 1983; Phillips et al., 1981) cocaine into the prefrontal cortex (Goeders et al., 1983) and morphine into the ventral tegmental area, septum and nucleus accumbens (Bozarth and Wise, 1981), (see Figure 1.1 in Chapter 1).

"On-line" measurement of dopamine activation, quantified by electrophysiological, microdialysis or voltammetric techniques has also substantiated claims that mesolimbic and neostriatal dopamine projections serve as a "common neural currency" for rewards of most kinds sought by animals and humans (Wise 1978, 1982). By virtue of these techniques, it has been shown that dopamine activation is triggered by encounters with food, sexual partners, drugs of abuse, electrical brain stimulation at brain sites that support self-stimulation, and by the stimuli that have been paired with these incentives (Gilbert et al., 1995; Wilson et al., 1995; Fiorino et al., 1996; Schultz, 1996).

Much of the causal evidence that dopamine systems mediate reward comes from studies of pharmacological blockade dopamine receptors or the ablation of dopaminergic pathways in animals (Wise, 1994). The inhibition of mesotelencephalic dopamine function, produced either by lesions or by pharmacological blockade, also inhibits the rewarding properties of a number of reinforcers. For example, 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens and ventral tegmental area but not other brain loci, markedly inhibit self-administration of cocaine. Traditional neuroleptic drugs or dopamine blockers such as pimozide, (Wise, 1972) acting at these brain sites produce similar results with various reinforcers including food and sexual activity. More recently, evidence has accumulated to suggest that a number of pharmacologically distinct dopaminergic drugs have similar effects (Sanger, 1996).

To the degree that individual receptor subtypes can be separately manipulated by by selective drugs, D1, D2, D3 and D4 dopamine receptor subtypes have all been suggested to participate in at least some aspect of food, drug, or brain stimulation reward (Berridge and Robinson, 1998). Further, there has been much interest in the functional separation of the aforementioned dopamine projections in terms of the mediation of separable aspects of reward (Hitchcott and Phillips, 1997, 1998; Berridge, 1996). These discoveries have added to the complexity of empirical questions which attempt to redefine the nature of the contribution of mesolimbic and mesostriatal dopamine systems to reward. Therefore, the role of dopamine in the reward process been gradually redefined through empirical endeavour and this has led to a reciprocal reconceptualisation of the nature of reward. It is no longer useful or correct to conceptualise reward as a single psychological process. For some, a more correct interpretation of reward would be "a constellation of multiple processes, many of which can be separately identified in behaviour, especially after the component processes are dissociated by brain manipulations" (Berridge and Robinson, 1998, p. 4).

There remains a great deal of interest in the involvement of brain dopamine systems in processing information related to rewards. The application of neurobiological tools to behavioural questions has produced a number of working models of the mechanisms mediating the rewarding and aversive (or "motivational") properties of stimuli. Such models have elucidated how rewards function in order to influence behaviour and vice versa. A brief overview of the basic functions of rewards is provided in Figure 2.2. (below). This will serve to give a flavour of the assumptions governing how "rewards" might be manipulated and operationalsied in an experimental context.

The aim of this chapter is to present a basic model of the role of dopamine in reward in the form of the anhedonia hypothesis (Wise, 1978, 1982). Support for this model will be provided followed by the discussion of lines of evidence which constitute challenges to this hypothesis. Some of these challenges have, by virtue of their empirical weight, metamorphosed into alternative theories of the role of dopamine in reward (i.e. Salamone, 1996; Robinson and Berridge, 1993). Thus, through the provision of a general, yet varied, theoretical background it will be possible to critically evaluate studies on dopamine receptor subtype involvement in several reward paradigms which manipulate conventional reinforcers as well their "neurochemical homologues" (Di Chiara, 1998, p.55 and also see General Discussion) such as drugs of abuse. Finally, this chapter will serve to furnish the reader with specific theoretical and practical details which are assumed and employed in food reward research. The final aim of this chapter is to highlight current scientific thinking on the role of dopamine receptor subtypes in ingestive behaviour, specifically their putative contributions to separable components of ingestive behaviour.

The nature of rewards

Three basic functions of reward:-

-rewards elicit approach and consummatory behaviour due to reward objects being labelled with appetitive value through i) innate mechanisms or ii) following learning.

-rewards serve as goals of behaviour following associations between behavioural responses and outcomes (Dickinson and Balleine, 1994).

1

-rewards increase the frequency and intensity of behaviour leading to such objects (i.e. learning) and they maintain learned behaviour by preventing extinction.

-rewards serve as 'positive reinforcers' of behaviour in classical and instrumental conditioning procedures.

2

-rewards induce subjective feelings of pleasure (hedonia) and positive emotional states.

3

Figure 2.1 The nature of rewards as defined by cognitive and biological needs: Adapted from text presented by Schultz et al. (1998).

2.2 Dopamine directly mediates reward: The anhedonia hypothesis

Although many contemporary accounts of the neural substrate of reward and reinforcement postulate the involvement of several pathways in various aspects of the reinforcement process (Robbins and Everitt, 1987, 1996; Phillips et al., 1996; Harmer and Phillips, 1997; Berridge 1996), the idea of a general reinforcement mechanism or circuit subserving the behavioural effects of many different types of reinforcing stimuli (such as food, copulation and drugs of abuse) rests as the cardinal tenet in reward research.

The "anhedonia hypothesis" (Wise, 1978, 1982) was originally advanced in the context of food reward. It held that dopamine receptor antagonists (neuroleptic drugs) which block dopamine receptors block the positive reinforcement and positive affect that is associated with rewarding events such as feeding. Empirical evidence burgeoned to include other reinforcers such as psychomotor stimulants (cocaine and amphetamine), opiates and rewarding intra-cranial self-stimulation and drugs of abuse (Wise, 1994).

It was repeatedly reported by Wise and his colleagues that dopamine selective neuroleptics (such as the D2 antagonist pimozide) block or retard acquisition of food-rewarded operant habits and cause extinction of already learned operant habits despite continued food reward (Wise et al., 1978b). In behaviourist terms, when an operant habit undergoes extinction, initial responding is normal, but normal response rates are not maintained as they would be if the reward were given. In the neuroleptic treated rat, responding follows the same pattern (initially normal, then slowing, then fundamentally nil) despite the fact that the reward is still available to the animal. Wise and colleagues noted that behaviours induced by neuroleptic drugs were strikingly similar to the effects of normal instrumental extinction; a finding which was used to support Wise's original assertion.

However, concerns about the possible motor effects of dopamine antagonists were voiced soon after Wise's original papers. Due to dopamine's ubiquitous role in behaviours such as locomotion it was difficult to exclude a "motor" explanation of these results. It was at this level that the hypothesis was most vigourously challenged (i.e. Mason et al., 1980; Salamone 1994, 1997). The assumption was that dopamine blockers could be interfering with the ability to initiate the kinetic requirements necessary to access a rewarding stimulus (Wise, 1994). The neuroleptic induction of "anergia" rather than "anhedonia" was implied (Mogenson, 1980; Salamone, 1992).

Features of the pattern of responding reflecting instrumental "extinction" were pivotal in debating the motor versus motivational hypotheses. For example, it seemed that Wise saw within-session decline as *prima facie* evidence that a motor deficit could not be in operation in the operant situation (Salamone, 1996). Salamone points to Wise's assertion that if one was to appeal to the motor hypothesis then, "....there should be no period within a pimozide test when response rates are normal" (Wise, 1982, p.78). Initial normality of responding, it seemed, precluded the need to attribute these response patterns to any aspect of motor function. However, there are those who see the similarities between neuroleptic-induced within session decline in responding and "extinction" as incorrect and superficial (Salamone, 1996). Therefore, a great deal of research effort was exerted upon addressing this conceptual problem, with most of the studies conducted within an operant paradigm. Although it would not be feasible to present all of the experimental evidence available in addressing this problem, some of the most informative operant studies are presented below.

Dopamine receptor subtypes: Effects on operant responding

- for food and water

Since the original studies by Wise and colleagues, the availability of pharmacological compounds selective for specific receptor subtypes has helped to clarify the motor versus motivational debate to some extent. Fowler and colleagues examined the effects of neuroleptics such as haloperidol (a D2 antagonist) the microstructure of operant responding (i.e. on the duration of individual lever responses), and observed that pimozide increases (i.e. slows) lever press duration in a way that differs substantially from extinction (Fowler et al., 1986). They also noted that the D2 antagonist pimozide produces within session increases in the duration of lever press responses (Liao et al., 1990). Results such as these are difficult to explain by drawing a parallel with the effects of extinction.

In a study by Fowler and Liou (1994) of the effects of dopamine antagonists on operant responding for food, an across session decrease was seen over several days of testing with the D1-like antagonist SCH-23390 but not with the D2-like antagonist raclopride. A simple motor effect of these drugs would have been expected to produce a uniform decrease in responding within or across sessions. The authors, after extensive and detailed behavioural analysis suggest that the D1-receptor antagonist had a greater effect on reward than the D-2 like receptor. The D2-like antagonist was seen to have a greater role in motor function. These results

echoed previous operant studies by McDougall and colleagues (1991, 1992). These studies required rat pups to run for a nipple attachment reward. Results from both studies showed that SCH 23390 but not the D2-like antagonist sulpiride produced an extinction like decrease in running speed, although sulpiride augmented the effects of SCH 23390 when they were coadministered. The authors conclude that both D1 and D2 receptor are undoubtedly involved in reward-related learning. However, the differential effects of antagonists, when given alone, acting at their receptor subpopulations, suggest that D1 receptor may be more importantly involved (McDougall et al., 1991, 1992).

These and other results (Salamone et al., 1987) present the possibility that it is the maintenance of movement that is affected by dopamine-related manipulations. Such authors see the effects of neuroleptic drugs or nucleus accumbens ablations as response maintenance deficits or progressive motor dysfunctions rather than impairments in emotional processes (Fowler, 1990).

2.3 Nucleus accumbens dopamine as a "sensorimotor interface"

Therefore, it is clear that in parallel with the early and the contemporary investigations into the true nature of neuroleptic-induced response deficits was an appreciation that it was the *maintenance* of movement that was primarily affected by manipulations of the dopamine system (Gaddy and Neill, 1977; Salamone, 1987). A large body of evidence indicated that low doses of dopamine antagonists, or depletions of brain dopamine do not impair fundamental aspects of food motivation such as chow consumption and simple instrumental responses for food, (Salamone, 1992).

A further finding served to test the original anhedonia hypothesis. In its original form this hypothesis had little to say about the specific brain loci which might be critical to the mediation of dopamine related reinforcement. However, it gradually became apparent that the nucleus accumbens was the specific site at which natural and drug reinforcers preferentially activated dopamine systems (DiChiara and Imperato, 1986; Mark et al., 1991), and*ergo* this brain site seemed to be where dopamine mediated reward. Moreover, it became apparent, through anatomical and behavioural investigation that this site may be a "nodal point" for the integration of information necessary for both motivational and motor control. Mogenson (1980), proposed that the nucleus accumbens represented a functional interface between the limbic system and the motor system, thereby providing a link between motor and motivational processes.

Since this original assertion (Mogenson, 1980), considerable evidence has accrued showing that nucleus accumbens dopamine is involved in the *allocation* of responses in relation to various reinforcers (for review, see Salamone, 1997). According to Salamone, nucleus accumbens dopamine participates in the function of enabling organisms to overcome "response costs" or obstacles, in order to obtain access to stimuli such as food. Therefore, unlike the original anhedonia hypothesis, nucleus accumbens dopamine is not seen as directly mediating food reinforcement, but instead is seen as a type of higher-order sensorimotor integrator that is involved in modulating response output in relation to motivational factors and response constraints, (Salamone, 1997 and see General Discussion).

Responding for rewarding brain stimulation

As mentioned above, the earliest suggestions that brain dopamine might play an important role in reward function came from studies of brain stimulation reward (Olds and Millner, 1954). Results from this paradigm were instrumental in teasing apart the motor and motivational strands of neuroleptic-induced response deficits.

The curve shift paradigm

Confirmation that neuroleptics attenuated the rewarding effects of brain-stimulation came from the "curve-shift" paradigm of Edmonds and Gallistel (1974). This paradigm offers a dose-response analysis of brain stimulation reward (Liebman, 1983), with dose of stimulation manipulated through variations in the frequency, intensity or duration of the trains of rewarding stimulation pulses that the animal earns with each response (Wise, 1994). Using this methodology, it was possible to functionally uncouple the reward-attenuating and the performance-impairing effects of neuroleptics (Gallistel, 1987). Teasing apart these confounds diminished confidence in purely "motor" interpretations of the effects of neuroleptics (Fibiger et al., 1976).

In brief, low doses of dopamine antagonists cause parallel rightward shifts in the dose-response curve, increasing the amount of stimulation required for a given level of responding but not changing the maximum level of responding demonstrated when the animal is given maximal stimulation (Stellar et al., 1983). The fact that neuroleptic treatment can reduce the effectiveness of stimulation at doses that do not reduce the maximum response rate of the animals confirms for some that neuroleptics have a low-dose action above and beyond any performance-impairing effects of these drugs (Wise, 1994).

High doses of neuroleptics shift the curve to the right but also shift it down, reducing the maximal response rate of the animal as well as increasing the stimulation required to produce responding at maximal rates (Gallistel and Davis, 1983; Rompré and Wise, 1989; Stellar et al., 1983). A difference in curve-shift is seen after the administration of amphetamine. Alone, this drug causes parallel leftwards shifts and reverses rightwards shifts of the curve caused by dopamine antagonists (Gallistel and Freyd, 1987). That these shifts reflect independent mechanisms is seen to be confirmed when using a morphine pretreatment (Wise, 1994). For example, morphine injections that shift the curve to the left reverse the rightward shift but fail to reverse the downward shift caused when high doses of neuroleptics are given (Rompré and Wise, 1989).

Since dopamine receptor subtypes have been reclassified (Sokoloff, 1990) the effects of various dopamine receptor antagonists on reward have been assessed using brain stimulation as the rewarding stimulus. Hunt et al. (1994), showed that reward and performance effects could not be dissociated after the administration of the D2-like antagonist spiperone, but could be clearly uncoupled when animals had received the D1-like antagonist SCH-23390. Again, as with the results from Fowler and Liou (1994) this data could reflect a more important role for D1-like receptors in reward-related learning (Beninger and Miller, 1998).

Accordingly, a suggestion was made that reward-related learning may consist of a dopamine signal at D1 receptors (Beninger, 1992). A consequent prediction from this was that the administration of a D1 agonist would mask the potential reward signal and impair reward-related learning. Ranaldi and Beninger (1994) gave systemic and intra-cerebral injections of the D2/D3 agonist quinpirole and the D1 agonist A-77636 and found that both of these compounds, when injected systemically, reduced the rate-frequency functions to the left and reduced the threshold frequencies necessary to maintain responding for brain stimulation reward to the ventral tegmental area. Results after microinjection of these compounds were not clear cut but Ranaldi and Beninger concluded that the stimulation of D1 or D2 receptors by dopaminergic agonists enhanced the rewarding effects of brain stimulation.

Gilbert et al. (1995), in search of a role for the D3 autoreceptor in performance in this paradigm showed that systemically administered 7-OH-DPAT (0.01-0.3 mg/kg) dose-dependently reduced responding for electrical stimulation to the ventral tegmental area. Voltammetry was used in order to measure dopamine

levels in the nucleus accumbens and the authors observed that 7-OH-DPAT reduced the size of the dopamine-generated voltammetric signal. This effect of the putative D3 receptor agonist was not reversed by the D2 autoreceptor antagonist sulpiride. Hence, Gilbert et al. (1995), discussed the possibility that 7-OH-DPAT reduces the release of dopamine in the nucleus accumbens at D3, but not at D2 autoreceptors and that it is by this mechanism that the rewarding effect of ventral tegmental area stimulation is reduced. However, most recently, Nakajima and Patterson (1997) found that while intracerebral injections of raclopride and haloperidol (D2 receptor antagonists) reduced the rewarding effect of brain stimulation, neither (+)-UH 232, (a D3 antagonist) nor clozapine (a D4 antagonist) influence the rewarding effect of such stimulation.

Responding for drugs of abuse

Drug self administration procedures typically involve the implantation of long-term jugular catheters into animals who are then trained to self-adminster stimulants intravenously in daily sessions. This model has a high degree of face and construct validity (DiCiano et al., 1998) hence, the model is an excellent animal model of drug use.

Psychomotor stimulants

When given to trained animals that have the opportunity for intravenous psychomotor stimulant (such as amphetamine and cocaine) self-administration, neuroleptics give rise to a condition in which there is an initial compensatory increase in drug taking. Then, if neuroleptic doses are high enough, there is extinction of the lever-pressing habit (Yokel and Wise, 1975). More concisely, drug-self administration in animals exhibits a characteristic inverted U-shape dose-respose curve. Manipulations (such as neuroleptic treatment) that shift this curve to the left can be interpreted as increasing the reinforcing potency of the drug. This is usually manifested as a decrease in the frequency of stimulant self-injection. Since the animals respond more (not less) than usual following neuroleptic treatment, this can be construed as an effect of dopamine blockade on the reward value of the stimulant. Also, several dopamine agonists are self-administered in much the same way as psychomotor stimulants (Wise et al., 1976, 1990). These and other similar findings have led authors such as Wise, 1978; Wise and Rompré, 1989) to conclude that it is the ability of stimulants to activate the dopamine system that makes psychomotor stimulants habit-forming.

Further confirmation of a role for dopamine in the mediation of effects of psychomotor stimulants comes from lesion studies which have shown that cocaine self-administration in the rat depends upon an intact mesocorticolimbic dopamine system (Roberts et al., 1977). However, an important aside here is that a similar effect of these depletions on decreasing lever pressing for food reinforcement was not apparent (see General Discussion).

In order to map out a role for dopamine receptor subtypes in the formation and maintenance of such habitual drug use, researchers such as Caine and Koob (1993), have used relatively selective ligands for the dopamine D3 receptor in order to assess their ability to modulate cocaine self-administration. They found that dopamine agonists had differential affinities for the dopamine D3 receptor and that these affinities correlated highly with the agonists' relative potencies in decreasing cocaine self-administration. 7-OH-DPAT and quinpirole potently decreased cocaine self-administration at doses that were not by themselves reinforcing. All of the agonists, when self-administered in combination with cocaine, reduced cocaine intake by producing an increase in the interval between injections without disrupting self-administration. This is interpreted as an enhancement of cocaine's reinforcing effects because an increase in the dose of cocaine produces the same effect, (Hubner and Koob, 1990). These results point to the D3 receptor as a useful target for pharmacotherapies of cocaine abuse (Caine and Koob, 1993).

Refinements to the above study were carried out by the same authors. In a follow-up study, (Caine and Koob, 1995) tested the hypothesis that the administration of the D3 receptor agonist 7-OH-DPAT would shift the cocaine dose-effect function to the left (i.e. enhancing the reinforcing properties of cocaine). They favoured this prediction over those which stated that 7-OH-DPAT was producing non-specific effects or was exclusively enhancing the rate decreasing effects of high doses cocaine. Employing a number of reinforcement schedules, Caine and Koob again found that 7-OH-DPAT decreased cocaine (0.25 mg/infusion) self-administration by increasing the interval between reinforcers. In addition, pretreatments with subcutaneous 7-OH-DPAT not only decrease the self-administration of cocaine, but also lowered the minimum effective dose of cocaine to maintain self-administration under fixed-ratio schedules.

In order to elucidate the mechanism by which D3 receptor preferring agonists such as 7-OH-DPAT modulate cocaine reinforcement, Parsons et al., (1996) employed microdialysis in the nucleus accumbens to monitor dopamine

levels during the self-administration of cocaine and cocaine plus D3 receptor agonists. They discovered that both "7-OH-DPAT and quinelorane attenuated cocaine-induced elevations in nucleus accumbens dopamine concentrations at the same time that the reinforcing properties of cocaine were enhanced" (p. 1086). They postulate a post-synaptic nucleus accumbens receptor as the mechanism by which these D3 receptor agonists achieve their effects on nucleus accumbens dopamine levels. Sanger et al. (1996) used a wide range of dopaminergic drugs and measured their effects on operant responding in rats. Their aims were twofold: firstly, they investigated whether the potencies of dopamine agonists to decrease operant responding in rats would also correlate with published (i.e. Sautel et al., 1995) D3 receptor functional potencies. Additionally, they studied the ability of three antipsychotic drugs (haloperidol, amisulpride and remoxipride), to antagonise the effects of 7-OH-DPAT on rates of operant responding.

The potencies of the dopamine agonists to decrease operant responding correlated significantly with their published potencies to produce a functional D3 receptor but not a functional D2 response (i.e. stimulation of mitogenesis in transfected cells). Low doses of the antipsychotic amisulpride (1.0 and 3.0 mg/kg) antagonised the rate-decreasing effects of 7-OH-DPAT. At these low doses amisulpride is thought to exert preferential activity at presynaptic dopamine receptors. Their results were consistent with the view that D3 receptors may play an important role in mediating the behavioural effects of dopamine agonists and that these receptors have a presynaptic location.

Summary

It is clear that there is a substantial amount of empirical support for the anhedonia hypothesis. This has not constrained the reconceptualisation of the functional role of dopamine in reward. For example, the notion of nucleus accumbens dopamine as a sensorimotor interface can be seen to accomodate much of the data which originally bolstered the idea that dopamine directly mediated reward.

The discovery of novel dopamine receptor subtypes and the acknowledgement of the neuroanatomical heterogeneity of dopamine projections points towards a further reinterpretation of the functional role of dopamine. It remains contentious, not least with respect to the D3 receptor, whether agonists which are selective for these receptors exert their effects on behaviour pre- or post-synaptically. Moreover, it remains unclear which aspects or components of reward

or motor function are particularly associated with dopamine receptor subtype agonism or antagonism. The next section reviews further paradigms (i.e. conditioned place preference and conditioned place aversion) which have been used to address this issue. Technological advances have gone some way to clarifying this issue. The next section (Section 2.4) also discusses some of these methodologies, the employment of which have given rise to data which are not easily explained by the anhedonia hypothesis.

2.4 Problems for the anhedonia hypothesis: Involvement of dopamine in aversive and appetitive behaviours

Originally, Wise postulated that neuroleptics "take the pleasure out of normally rewarding brain stimulation, take the euphoria out of normally rewarding amphetamine and take the 'goodness' out of normally rewarding food" (Wise, 1982, p.263). Consequently, this hypothesis has had difficulty in accommodating results from studies which show that dopamine efflux (as measured by microdialysis) is elevated during aversive as well as appetitive conditions (for reviews see Blackburn, 1992; Salamone, 1994). Parallel to a corpus of empirical evidence showing that dopamine antagonists reliably reduce positively reinforced instrumental responding, runs a host of studies which reveal that neuroleptics also impair avoidance responding (Beninger et al., 1980; Blackburn and Phillips, 1990). Dopamine antagonists impair place aversion conditioning as well as interfering with place preference (Di Scala and Sandner, 1989). Several other characteristic features of neuroleptic treatment, such as the within-session decline in instrumental responding, have also been demonstrated to occur with neuroleptic-treated animals in avoidance tasks (Sanger et al., 1993). Again there are inconsistencies within the results. For example, there are those who have reported that there are no significant variations in striatal dopamine release in animals exposed to stimuli of either positive (appetitive) or negative (aversive) affective value (Mark et al., 1994).

These findings have prompted a reorganisation of assumptions underlying the correct interpretation of dopaminergic involvement in the rewarding situation and has led to questions such as: Do responses to environmental stimuli concern specific motivational attributes or reflect more general stimulus salience? (Robinson and Berridge, 1993; Schultz, 1992).

Nevertheless, other studies which measure on-line dopamine efflux or electrophysiological responses of dopaminergic neurons present evidence which is consistent with the anhedonia hypothesis. For example, Mirenowicz and Schultz (1996) compared dopamine impulse responses to motivationally opposing appetitive and aversive stimuli and found that these neurons "preferentially report environmental stimuli with appetitive rather than aversive motivational value", (p.449). Using microdialysis, Besson and Louilot (1997) have confirmed the capacity of dopaminergic neurons to code for affective directionality. Increases in striatal dopamine levels coincided with an attractive stimulus and decreases were observed on the presentation of an aversive stimulus.

Further evidence of a role for dopamine in associative processes comes from the place preference paradigm (see below for further details). This paradigm can reveal much about the possible subjective (both positive and negative) effects of agonists and antagonists which are selective for dopamine receptor subtypes.

Dopamine receptor subtypes and Pavlovian conditioning

Conditioned place preference and conditioned place aversion

The place conditioning paradigm measures the incentive-motivational effects of stimuli that become associated with drug effects through classical conditioning (or stimulus-stimulus associations). The environment becomes a cue eliciting approach (i.e. conditioned place preference) or avoidance (i.e. conditioned place aversion) depending on whether the rewarding or aversive properties of the drug become associated with the environment to which the animals have become familiar.

Drugs which are reported to exert their effects at the dopamine D3 receptor have been shown to produce both preference and aversion in this paradigm and the differential effects seem to be dependent on dose (Khroyan et al., 1997). For example, a low dose of 7-OH-DPAT (0.03 mg/kg) produces conditioned place aversion (Khroyan et al., 1995) whereas a higher dose (5 mg/kg) produces conditioned place preference (Mallet and Beninger, 1994; Kling-Petersen et al., 1995). Another mixed D2/D3 agonist quinpirole, if given at a high dose (1.0 mg/kg) also produces conditioned place preference (Hoffman and Beninger, 1988).

This differential dose effect was further validated by Chaperon and Thiebot (1996) who suggest that the aversive state experienced by rats given low doses of 7-OH-DPAT was probably accounted for by impaired dopamine transmission in mesolimbic dopamine pathways. This may be achieved through the preferential activation of inhibitory D3 postsynaptic receptors as has been suggested in the case of locomotor activity (Svensson et al., 1994). The emergence of conditioned place preference at higher doses of D3 receptor agonists might reflect the stimulation of postsynaptic D2 receptors. Therefore, Chaperon and Thiebot (1996) do not feel convinced that their results represent definite evidence of a preferential role for D3 receptors in 7-OH-DPAT induced learning.

Following the establishment of conditioned place preference with the D3 receptor antagonist PNU 99194A (Kling-Petersen et al., 1994). Chaperon and

Thiebot, (1996) tried to replicate this effect with another selective D3 receptor antagonist, *l*- nafadotride. This compound did not produce conditioned place preference and was deemed devoid of intrinsic reinforcing properties, at least at the doses studied. However, in a narrow range of low doses, *l*- nafadotride revealed food-induced conditioned place preference. Similarly, Guyon et al. (1993) noted a potentiation of the food-induced conditioned place preference achieved by administering low doses of the mixed D2/D3 receptor antagonists sulpiride (4mg/kg); amisulpride (0.5, 1 mg/kg) or pimozide (0.03; 0.06 mg/kg) before the food conditioning sessions. The positive effects of amisulpride were reversed by the dopamine D1-like receptor SCH-23390 (0.01 mg/kg). These effects were interpreted by these authors as a pro-hedonic effect of low doses of some D2/D3 receptor selective neuroleptics.

The neurobiological substratum of such effects is more difficult to pin-point. Both sets of authors who have identified a food-induced conditioned place preference with low doses of neuroleptics have invoked basal dopamine levels as an important connection to the observed food-induced conditioned place preferences. Since eating and/or the preparatory phase of appetitive behaviour have been shown to evoke the release of dopamine in mesolimbic structures (Phillips et al., 1991 and see Section 2.5) *l*- nafadotride may have favoured incentive learning only when basal dopamine function was high (i.e. when food was present) but not under standard conditions. As doses increased, these "pro-appetitive" effects of *l*-nafadotride were presumably cancelled out due to the blockade of post-synaptic D2 receptors by the drug, as suggested by the hypoactivity and catalepsy that has been observed after high doses (Sautel et al., 1995; Chaperon and Thiebot, 1996). The results of Guyon et al. (1993) were originally attributed to a D2 autoreceptor blockade, however, the more recent results obtained by Chaperon and Thiebot (1996) although analogous to the earlier results, are interpreted in terms of the ability of the D3/D2 receptor antagonists to remove the inhibitory influence of a subset of "D2-like" receptors on reward related activity. These possibilities may only be confirmed or rejected when more selective D3 receptor ligands become available and are used in conjunction with appropriate methodologies.

Therefore, it is suggested that low doses of D3 receptor agonists produce conditioned place aversion whereas high doses produce conditioned place preference. Khroyan et al. (1997) tested this general statement and used the selective D3 receptor agonists 7-OH-DPAT and PD 128907 in the place conditioning paradigm. Their hypothesis was not supported as only one dose of one of the agonists produced a conditioned place preference (PD 128907, 1mg/kg).

Khroyan et al. (1998) took advantage of the finding that low doses of the putative D3 receptor agonists such as 7-OH-DPAT produced a behavioural profile opposite to that produced by locomotor stimulants (i.e. low doses of 7-OH-DPAT produce conditioned place aversion compared to conditioned place preference produced by psychomotor stimulants). Therefore, they speculated that the investigation of interactions between low doses of 7-OH-DPAT and psychomotor stimulants could lead to important discoveries in the devlopment of treatments for psychomotor stimulant abuse. They determined that 7-OH-DPAT (0.1 mg/kg) when co-administered with *d*-amphetamine attenuated the previously established *d*-amphetamine conditioned place preference. They infer from these and other data that this dose of 7-OH-DPAT attenuates the reinforcing properties of *d*-amphetamine and is consistent with a whole host of psychomotor stimulant self-administration studies such as those of Caine and Koob (1993, 1995).

There are futher instances of a dissociation of behaviours which can be seen as characteristic of low and high doses of 7-OH-DPAT. In a study by DeFonseca et al. (1995) 7-OH-DPAT did not produce a conditioned place preference at any of the doses tested (0.01-5 mg/kg). However, the higher doses (0.25 and 5 mg/kg prevented the acquisition of a morphine-induced place preference. The lower doses (such as 0.01 mg/kg) did not have an effect on the place preference with morphine. The authors again nominate 7-OH-DPAT as a possible pre-clinical candidate for the treatment of opiate dependence and craving and attribute the differential dose effects in this paradigm to an interaction of 7-OH-DPAT with pre and post-synaptic D3 receptors.

Conditioned taste aversion

A further model with which to test the subjective effects of drugs is the conditioned taste aversion paradigm. This phenomenon is typically studied by allowing rats brief access to a novel taste solution and then administering an emetic agent such as lithium chloride. Consequently, previously poisoned rats consume less of the fluid relative to either their own baseline intake or relative to non-poisoned rats.

Although most studies employ peripheral emetics such as lithium chloride support a conditioned taste aversion it has been repeatedly demonstrated that drugs which act upon central dopamine systems also support this form of associative learning. For example, amphetamine (Cappell and LeBlanc, 1971) and cocaine (Foltin et al. 1981; Goudie et al., 1978) induce conditioned taste aversions.

However, original interpretations of the way in which drugs of abuse function as unconditioned stimuli in conditioned taste aversion have been extensively questioned (see Grigson, 1997).

The behavioural and neural mechanisms of conditioned taste aversion have been further elucidated by using specific dopamine agonists and antagonists as the unconditioned stimuli. Conditioned taste aversions have been reported with the direct dopamine agonist apomorphine, the dopamine D1 receptor agonist SKF 38393 and the D2/D3 receptor agonist quinpirole (Asin and Montana, 1989; Hoffman and Beninger, 1988). Not only do dopamine receptor subtypes seem to have a role to play in the initial acquisition of a conditioned taste aversion but also, antagonists at these sites attenuate the acqusition of amphetamine-induced taste aversion (Lin et al., 1994). Recently, Bevins et al. (1996) examined 7-OH-DPAT in this paradigm and found that this compound (0.1-10 mg/kg) produced a significant conditioned saccharin aversion suggesting a role for the D3 receptor in dopamine agonist induced conditioned taste aversions. They do, however, issue a caveat regarding the pharmacological specificity of such effects (see Section 7.4).

The D3 receptor and stimulus-reward learning

The D3 receptor has been selectively implicated in other forms of associative learning. In examining the role of the mesoamygdaloid dopamine projection in Pavlovian stimulus-reward learning, Hitchcott et al., (1997), investigated the effects of post-session intra-amygdala infused dopamine agonists SKF-38393 (D1), quinpirole (D2/D3) and 7-OH-DPAT (D3/D2) on the acqusition of a discriminative approach response. Only 7-OH-DPAT enhanced selectively CS+ (initially neutral stimulus paired with a 10% sucrose reward) approach in a dose-dependent fashion. Therefore, the authors infer that the D3 receptor receptor subtype is involved in the modulation of stimulus-reward learning by the mesoamygdaloid dopamine projection.

Summary

The data discussed in this section highlight some of the conceptual problems for the anhedonia hypothesis which have been borne out of the application of particular methodologies such as microdialysis. More precisely, dopaminergic activation is obviously integral to both appetitive and aversive motivation. This section also further outlines a role for dopamine receptor subtypes in some of the associative processes necessary for the operation of reward. A particular role for

the D3 receptor has been postulated in the acquisition of stimulus-reward learning. Again, with regards to the D3 receptor, the differential dosage effects of D3 receptor-preferring ligands on Pavlovian conditioning highlight the need for caution in interpretating data from these paradigms. The next section (Section 2.5) concentrates on the measurement of dopaminergic activity. In particular it discusses those aspects of motivated behaviour which have been thought to temporally covary with dopaminergic activity. Again, such evidence poses problems for theories which view dopamine as directly mediating reward.

2.5 Further problems for the anhedonia hypothesis: Does dopaminergic activity covary with the appetitive and/or the consummatory phase of behaviour?

Many studies have shown that appetitive stimulus conditions can be accompanied by an increase in accumbens dopamine release as measured by "on-line" techniques such as voltammetry, microdialysis or electrophysiology (Hoebel et al., 1983, 1992; Hernandez et al., 1987; Schultz et al., 1993; Gratton, 1994; Mark et al., 1994). In vivo procedures such as brain microdialysis have provided unique opportunities to investigate regional changes in dopamine release in freely moving animals during feeding or the performance of food motivated tasks. Although it has been shown rewards can both incite and reinforce behaviour, it remains unclear which of these effects reflect increased dopamine transmission (Richardson and Gratton, 1996).

There are many variables which influence electrochemical responses of dopamine neurones that are related to both the stimulus and the internal state of the animal. More concisely, the reward value associated with a stimulus is not a static, intrinsic property of that stimulus. Several microdialysis studies are testament to this assertion having manipulated the animals' motivational state in relation to appetitive stimuli such as food (Wilson et al., 1995; Pothos et al., 1995) and sexually receptive partners (Fiorino et al., 1997). Animals can assign different appetitive values to a stimulus as a function of their internal states at a) the time the stimulus is encountered, and b) as a function of their experience with the stimulus (Dickinson and Balleine, 1994).

Therefore, it is not surprising that many findings are opposed to an assumption implicit to much of the conventional thinking on meso- nucleus accumbens dopamine function, that is, increased dopamine transmission covaries specifically with the behavioural reinforcing action of rewards (Wise et al., 1978; Wise et al., 1982; Koob, 1992).

For example, there is considerable evidence to suggest that increases, specifically in nucleus accumbens dopamine transmission do not occur as a consequence of food consumption but rather occur during the anticipation of a meal, at which times the rat is engaged in preparatory feeding behaviours (Blackburn et al., 1989). Kiyatkin and Gratton (1994), reinforced the idea that dopamine transmission in the nucleus accumbens does not specifically covary with the consumption of food per se. In rats lever pressing for a food reward, nucleus

accumbens dopamine increased primarily in anticipation of earning food and that the immediate consequence of consuming food was a transient suppression of this increase, a result reminiscent of that of Salamone et al. (1994). More recently and in contrast with these results, Wilson et al., (1995) have found that consummatory rather than anticipatory aspects of feeding are most robustly associated with increases in nucleus accumbens dopamine release. To further complicate the issue, a number of studies show increases in nucleus accumbens dopamine efflux during appetitive *and* consummatory phases of male sexual behaviour (Damsma et al., 1992; Fumero et al., 1994; Mas et al., 1995b).

The "biphasic and time-locked" (Richardson and Gratton, 1996, p.8160) changes in nucleus accumbens dopamine transmission suggested by some of the microdialysis data are congruent with electrophysiological evidence that similar changes in ventral tegmental area dopamine cell activity are associated with food reinforcement (Schultz, 1986; Romo and Schultz, 1990; Schultz and Romo, 1990; Ljungberg et al., 1992; Schultz et al., 1993; Schultz et al., 1998)

Studies into the temporal characteristics of dopaminergic activity have shown that not only does dopamine efflux increase when an animal is engaging in "rewarding" behaviour but also increases when presented with a neutral stimulus (CS) which has been repeatedly paired with the primary reward such as food (US). This anticipatory aspect of dopaminergic activity has been elegantly displayed by Schultz and colleagues (1986, 1996, 1997, 1998). When recording from midbrain dopaminergic neurones of monkeys, they discovered that dopamine signals observed in response to the (CS) also diminished after extensive training. Dopamine neurones seemed only to fire if the event was worse (decreased spike production) or better (increased spike production) than that predicted by the (CS). Midbrain dopamine neurones could therefore be seen as "error predictors" which coded for the discrepancy between what was anticipated and what was actually encountered.

Dopamine neurons have therefore been seen to have a capacity to code for unpredictability or error. To test this prediction further, Fiorino et al. (1997) have measured dynamic changes in nucleus accumbens dopamine efflux during the appetitive and consummatory phases of copulation. Specifically, they have noted that, in behavioural terms, sexual satiety in male rats can be overcome if the original female is replaced with a novel and receptive alternative female. This is commonly known as the Coolidge Effect (Wilson et al., 1963). Therefore, in the light of the putative role of nucleus accumbens dopamine in the initiation and/or

maintenance of motivated behaviour, these researchers were interested in the dopaminergic correlates that may be associated with copulation, the apparent state of sexual satiation and the reinstatement of copulatory behaviour with the presentation of a novel female. They found that nucleus accumbens dopamine concentrations returned to baseline values at the onset of sexual satiety after having been enhanced by both the appetitive and consummatory phases of copulation. A novel receptive female was then put into close proximity to the male (behind a screen) which gave rise to a small increase in nucleus accumbens dopamine. This slight increase was interpreted by the authors as a potential spur to the reinstatement of sexual behaviour during which this slight signal was significantly augmented. Therefore, in this case, moderate dopamine activity coincides with the presentation of the novel female, only reaching significantly higher levels at the reinstatement of copulation and so the dynamics of dopamine activity may be more complicated than the dichotomy of "appetitive" and "consummatory" can reveal (see General Discussion).

Effects of motivational manipulations on nucleus accumbens dopamine release

-deprivation state

As dopamine obviously has *some* role to play in motivation, and motivation is defined with reference to both the internal and the external context of the organism, it is logical that motivational manipulations such as restricted eating and concomitant weight loss have been shown to have a selective effect on the neurochemistry of some critical brain structures involved in reinforcement. Pothos et al., (1995) observed that in rats reduced 20-30% below normal weight, basal extracellular dopamine in the nucleus accumbens decreased by 50%. However, no such change was observed in dorsal striatum or medial prefrontal cortex which could suggest that the ventral tegmental area- nucleus accumbens pathway mediates some unique aspect of motivation as it relates to food restriction and weight loss. They infer that the selective underactivity of nucleus accumbens dopamine systems in response to feeding resrictions and weight loss could underlie the increase in food and drug intake observed in underweight animals (and humans) when they attempt to restore extracellular dopamine levels by natural or artificial means.

A further example of manipulating levels of motivation is seen in work by Wilson et al. (1995). These researchers attempted to identify some of the dopaminergic correlates of motivated behaviour. Again, one of the variables which they manipulated was deprivation state. They discovered that the motivational state

of animals significantly influenced the magnitude of the neurochemical events associated with goal directed behaviours. The magnitude of increase during the consumption of a palatable meal was enhanced in rats which had been food-deprived for 20hr at the time of testing. Studies such as this highlight the importance of manipulating relative and absolute levels of motivational status (see Chapter 7 and General Discussion).

-incentive value

Examples of manipulating the value of the stimulus (or stimulus properties) in anticipation of a selective effects on dopamine levels are extremely important in delineating the involvement of brain dopamine systems in processing information related to rewards. In a particularly relevant study, Martel and Fantino (1996) examined the effects of the ingestion of two foods varying in palatability on the activity of the mesolimbic dopamine system. Rats have previously been shown to exhibit a preference for short cakes (high palatability) over regular chow (low palatability) (Cabanac, 1983, 1985). Therefore the study aimed to determine whether this preference was accompanied by an increase in extracellular levels of dopamine and its main metabolites (dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) measured by microdialysis in the nucleus accumbens. During feeding, dopamine rose significantly with a greater rise for the highly palatable food than for the low palatability food. Levels of DOPAC and HVA only reached significance during the ingestion of the highly palatable food. Moreover, dopamine release during the ingestion of the highly palatable food peaked during the first 25 minutes of food supply. This is consistent with an influence of the orosensory properties of foods rather than their postingestive effects (Mark et al., 1994; Schneider et al., 1986), and so, such neurochemical corellates of palatability manipulations could be extremely useful in redefining a role for dopamine in ingestive behaviour.

Richardson and Gratton (1996) manipulated the value of the stimulus in an attempt to determine how the apparent suppression of nucleus accumbens dopamine transmission previously observed during food consumption (Kiyatkin and Richardson, 1994) was affected by changes in the stimulus conditions such as: the rate of milk delivery; the duration of milk presentation; temporal contiguity between the operant response and milk presentation and the reinforcement schedule. Rats were trained to respond on a fixed ratio (FR1) schedule for 0.2ml condensed milk delivered over 30 sec. During this delivery period there was an accompanying cue light. Consumption of the initial milk rewards within the first session of the first

test day caused dopamine signal increases in the nucleus accumbens as measured by voltammetry. On subsequent days the most pronounced initial signal increases coincided with the presentation of conditioned stimuli that marked the start of the test session. Responses to each reinforced lever-press were preceded by increases and were followed (during milk consumption) by decreases in dopamine signal. If milk delivery was delayed there was a corresponding delay in dopamine signal decreases; the timing of which was bound by the duration of milk consumption. If the rate of milk delivery was increased there was a corresponding increase in the anticipatory dopamine signal. The authors take these results to be suggestive of an involvement of meso-nucleus accumbens dopamine neurons in coding for incentive rather than for the reinforcing properties of food.

Again such an anticipatory role for dopamine further reinforces the hypothesis that the disruption of autoshaped behaviour and instrumental behaviour by dopamine receptor antagonists is attributable to interference with the response-eliciting rather than the response-reinforcing properties of stimuli such as palatable food (Beninger, 1983).

Caveat and summary: Beyond the striatum

The data in this section go some way to identifying those aspects of internal and external states which may modify dopaminergic transmission. However, a further important consideration has to be outlined here. Not only are dopamine receptors pharmacologically diverse but the anatomical distribution of these receptors seem to have functional consequence in coding information concerning the nature of rewards. A very recent paper by Schultz and colleagues warns that target sites for electrophysiological measurement need to be chosen with care and in accordance with a very specific hypothesis. This is necessary when also acknowledging the considerable effects on dopamine activity that have been observed with changes in stimuli (both internal and external to the organism).

Hence, after extensive experimentation, Schultz et al. (1998) have postulated that different brain structures process reward information in very different ways. They claim "dopamine neurons emit a reward teaching signal without indicating the specific reward, striatal neurons adapt expectation activity to new reward situations, and orbitofrontal neurons process the specific nature of rewards". (P. 421). Therefore, many properties of the experimental situation must be closely controlled in order to elucidate the correct role for this neurotransmitter in motivated behaviour. The specific type of stimulation of dopamine (for example

tonic or phasic) (see DiChiara, 1998), the motivational impact of the stimulus (in terms of novelty, unpredictability, its perception by a specific sensory modality, deprivation state of the organism) and the particular brain areas targeted for observation are crucial variables to consider when interpreting empirical evidence. The next section (Section 2.6) deals with a theory which has, among other things, taken heed of data which reflects the "anticipatory" nature of dopamine which has been outlined in this section. A specifically motivational role for dopamine is put forward which differs significantly from the original anhedonia hypothesis.

2.6 Dopamine neurons code for generic motivational salience: Dopamine's role in incentive-sensitisation

Motivation is the process by which stimuli predictive of rewards and punishers becomes capable of controlling behaviour. Therefore, much of the evidence already viewed in this chapter clearly calls for a specifically motivational role for dopamine. However, motivation as a process might be most correctly viewed as having two parts: an acquisition phase and an expression phase. According to Robinson and Berridge (1993), dopamine is exclusively involved in the latter phase.

This association has been made in the light of many empirical studies which are reviewed as part of their thesis which postulates that firstly, addictive drugs share the ability to enhance mesotelencephalic dopamine neurotransmission and secondly, one psychological function of this neural system is to attribute "incentive salience" to the perception and mental representation of events associated with the activation of the system. Incentive salience is a psychological process that transforms the original perception of stimuli, investing them with importance, making them attractive "wanted" incentive stimuli. The repeated use of addictive drugs can sensitise this neural system to drugs *and* drug-associated stimuli (Robinson and Berridge, 1993). The sensitisation of dopamine systems is "gated by associative learning" hence it causes excessive incentive salience to be attributed to the act of drug taking and to stimuli associated with drug taking.

Therefore, at least for these authors, dopamine neurons code for generic motivational salience, a postulation which encapsulates much, but not all of the data presented thus far. There are many studies, especially using in vivo monitoring techniques such as brain microdialysis, which have shown that dopaminergic neurons are sensitive to the affective or hedonic properties of the stimulus as well as other motivational variables. Although theoretical points are to be considered more thoroughly in the General Discussion of this thesis, it is worth reiterating that many studies in the previous section serve as direct criticisms of theories, such as that of Robinson and Berridge (1993) which propose that mesolimbic dopaminergic activity is, in effect, insulated from hedonic and/or motivational manipulations (see Section 2.5, Section 2.11 and General discussion).

Interim summary: Theorising a role for dopamine in food reward

The anhedonia hypothesis has stimulated a massive amount of empirical research which in turn has formed the basis of several new models in which to interpret data on the the part dopamine has to play in reward. Empirical and conceptual problems for the anhedonia hypothesis or any model which postulates a "direct" role for dopamine in reward do not obviate a crucial role for dopamine in the acquisition and maintenance of motivated behaviour. Dopamine obviously has some part to play in the formation and maintenance of representations of Pavlovian and instrumental contingencies for a whole host of reinforcers.

The most obvious shift in theory is contained in the idea that brain dopamine activity is not necessarily directly involved in the mediation of "hedonia" but that it has an anticipatory or incentive quality which may have little to do with the perception, evaluation or actions taken as a consequence of, the subjective feelings of "pleasure".

What can be stated with security from the evidence presented up to this point, is that the process of reward is not a unitary and immutable phenomenon. The focus of this thesis is the way in which dopamine receptor subtypes might contribute to components of *food* reward which incorporates questions such as: What is the best way to measure food reward. Which critical brain structures/ dopamine receptor subtypes underlie dissociable aspects of food reinforcement? To what extent and under which circumstances do motivational processes rely on the dynamic nature of the reward value associated with a stimulus?

Therefore, in the following sections, the remit of the thesis is narrowed in order to attempt answers to these questions. Subsequent sections will serve to outline some of the common conceptions and methodologies employed in food reward research. It will also represent the forum for the presentation of empirical research in this area as well as new theories which attempt to account for such data.

Firstly there will be a brief explanation of the variables which are manipulated and measured in assessing the neural bases of food reward. Following this will be an overview of the effects of dopamine agonists and antagonists on feeding behaviour including preliminary data on the effect of such compounds on the microstructure of feeding and non-feeding behaviour. As total intake has been the most commonly determined dependent variable in food reward research, this measure is discussed leading to the presentation of alternatives to this measure in the

form of alternative methodologies which attempt to isolate components of ingestive behaviour and study the effects of dopaminergic compounds on these components. Data collected from alternative methodologies has led to the emergence of new theories of the role of dopamine in food reward which will also be described. Finally, there will be a discussion of the importance and utility of the fine-grained analysis of feeding behaviour in the form of the microstructural analysis of licking behaviour. The effects of dopaminergic compounds as applied to licking microstructure will be discussed and will form the basis of the rationale for the experiments presented in this thesis.

2.7 Important concepts in the study of ingestive behaviour

The discussion of dopamine's role in motivated behaviour at the beginning of this chapter serves to introduce both traditional and more contemporary concepts in reward research, as well as the specific role postulated for dopamine in those reward processes. Such discussion also highlights the need to explicate theoretical concepts such as reward and reinforcement in order to enhance their empirical utility. In much the same way, concepts central to feeding research are subject to scrutiny and need to be clarified here to be profitable in terms of understanding the later chapters. This is not a simple task since constructs which are central to research in this area, are hypothetical in that they cannot be directly observed.

Hence, concepts such as appetite, hunger, satiety and palatability are difficult to define and therefore not easily measurable (Blundell, 1979); this is especially the case in research using animals. Animal research into ingestive behaviour also cannot draw upon the evidence of subjective report of behavioural states which may be important in initiating or ceasing feeding (i.e. feelings of "fullness" or "pleasantness"). Nevertheless, many drugs tested preclinically on animals have been reported to have effects such as "appetite suppressant" or "satiety-inducing" ; in short these compounds are generally seen to reduce or remove the motivation to eat. However, there is debate as to whether the cues which lead to the cessation or reduction of feeding are best described as "drive - reducing" or are more correctly viewed as modulators of the value of incentive stimuli (Toates, 1986). Therefore the next section describes some of the minimal definitions or "skeletal properties of food reward" (Berridge, 1996) which have been necessary in order to make research into them productive.

Dopamine agonist- and antagonist-induced decreases in food intake are often interpreted as a loss of "appetite." However, this description does little to tell us why there has been such an apparent loss of appetite. A decrease in intake, drug induced or otherwise, could be reflecting one or more of several behavioural mechanisms which would be more or less obviously associated with a decrease in intake. Drug-induced decreases in feeding could reflect the promotion or cessation of central and/or peripheral satiety signals which mimic or block the inhibitory feedback signals produced by an accumulation of food in the gastrointestinal tract. Following drug administration, food may be also be underconsumed as a consequence of a perceived change in the palatability (i.e. taste, temperature, texture of food etc.). More generally, drugs could change feeding patterns by means of either a motoric deficit or the induction of stereotyped behaviour which

interferes with the actual act of feeding. One of the properties of a drug which apparently affects appetite may be to induce emesis or some other type of illness. Therefore, it is clear that the behavioural expressions of anorexia (or the antecedents thereof) are not always equivalent. It is not surprising then that the validity of empirical testing in feeding research has come under close scrutiny.

Many theories and empirical studies have relied on constructs such as hunger and satiety in trying to define the neural bases of the control of ingestion. Early theories conceived of hunger as "drive" which was reduced on presentation of "reward" in the form of food (i.e. Hull, 1943). Animals were often assumed to cease feeding as a consequence of a reduction of drive, possibly resulting in a state of satisfaction or fullness. Without subscribing to anthropomorphic definitions, at the most basic level, satiation might be described as being stimulated by ingested food resulting in an inhibition of feeding. As such, this process is an example of a negative-feedback action which is necessary for central neural integration (Smith, 1998). Nevertheless, definitions of satiety have ranged from those based on subjective feelings correlated with the tendency to eat or not, to those which have a grounding in an assumed internal or physiological state to those based on some consequence of ingestive behaviour (i.e. the amount of food consumed or the induction of a characteristic behavioural satiety sequence after feeding (Davis, 1998).

Some researchers have attempted to define the integration of the incentive properties (i.e. palatability factors) of food as well as accounting for physiological state (i.e. satiety factors). Cabanac (1990) encapsulates this interdependence of the two types of information in the concept of alliesthesia which is described as a change in pleasantness of a constant physical stimulus as a consequence of a change in physiological state. This definition incorporates much of what may now be said of the construct of palatability.

Palatability can be described as the hedonic component of food reward (Berridge, 1996). Defined in this way, palatability, does not merely substitute for aspects of the orosensory stimulus properties of food (i.e. taste, smell, flavour, texture and temperature) (Rogers, 1990). Rather, it results from a central integrative process that can incorporate aspects not only of the orosensory stimuli but also of the physiological state and of the animal's associative history (Grill and Berridge, 1985; Berridge, 1996). This type of definition of palatability supercedes definitions which were based on stimulus properties of tastants. These invariant properties of tastants (i.e. sucrose) were seen to lead to a predictable response in

consumers (i.e. increase in concentration led to increase in consumption).

However, it was soon realised that responses to prototypical tastants were not necessarily "stimulus bound". For example, the behavioural response to a sucrose solution depends at least in part on the physiological state of the animal and or the associative cues previously paired with the solution. Therefore, a definition of palatability evolved in appreciation of these factors. Models of the control of ingestion (i.e. Davis and Levine, 1977) incorporated a formalised definition of palatability in terms of the ability of palatability factors to exert excitatory control over feeding behaviour. These positive factors are seen by the authors to be countered by the inhibitory effects of food which has already been consumed (i.e. satiety signals) (for more detail see section 2.12). More formalised definitions offer the possibility of experimentally manipulating palatability factors, especially of prototypical tastants, as behavioural reactions to these tastants, all else being equal, have been shown to be relatively stable across several test situations.

Despite the obvious complexity of a construct such as palatability, there have been many attempts to operationalise this and other constructs central to feeding research. The following section (2.8) presents some of the experiments on dopamine receptor subtype agonists and antagonists on feeding and other behaviours that have been carried out in recent years. It provides a general background to those studies presented later in Chapter 2 which have specifically predicted dopamine receptor subtype involvement in *components* of ingestive behaviour. These latter type of experiments necessitate the operationalisation of constructs such as satiety and palatability and will therefore be described in detail in Sections 2.10-2.12).

2.8 The effects of dopamine receptor subtype selective compounds on feeding and other behaviours in the rat

It is well established that both dopamine agonists and antagonists reduce feeding. What is less well established is the way in which these compounds achieve these effects. As has already been mentioned in Chapters 1 and 2, dopamine has long been considered important in the regulation of motor behaviour, and so changes in motor control may be responsible for observed decreases in feeding. The motor systems involved in stereotyped and normal motoric behaviour may be separate from those required for normal ingestive behaviour. Alternatively, dopamine receptor stimulation or blockade may induce behaviours, such as stereotypy, which impede the execution of actions which are necessary for normal feeding (Terry, 1996).

If drug-induced changes in feeding behaviour are accompanied by the emergence of competing motor behaviours, it is difficult to ascribe a role for dopamine in food reward per se; hence, there is a need for a fine-grained analysis to the study of dopaminergic involvement in feeding and other behaviours which may be occurring between or instead of feeding. The following part of the Introduction will serve to provide some general information on the relative contribution of dopamine receptor subtypes to feeding in general and also to certain parameters of feeding as assessed by the microstructural analysis of ingestive behaviour (see also section 2.10 and 2.12).

2.8.1 Dopamine D1-like preferring compounds

Dopamine D1 agonists

Using non-deprived rats given access to a palatable wet mash diet, Gilbert and Cooper (1985) were the first to report that the D1 type agonist SKF 38393 produced a dose-dependent reduction in food intake. A decremental effect of SKF 38393 (4h after administration) was also shown in rats who were free-feeding (Martin-Iverson and Dourish 1988; Cooper and Al-Naser, 1993). This result was repeated in food-deprived rats and further confirmed by others (Zarrindast et al., 1991; Terry and Katz, 1992, 1994). SKF 38393 is a benzazepine compound with only partial agonist efficacy in the adenylyl cyclase assay of D1- type agonist efficacy (e.g. O'Boyle et al., 1989). Other benzazepines with differing efficacies also reduce food intake dose-dependently, such as SKF 75670 in non-deprived rats eating palatable mash (Rusk and Cooper, 1989 a), and both SKF 77434 and SKF

82958 in food deprived rats (Terry and Katz, 1992). Other D1 type agonists, although structurally dissimilar to the prototypical agonists such as SKF 38393 are also known to reduce feeding. The agonist A-77636 reduces intake in free feeding rats while and A-68930 a similar agonist decreases intake in food-deprived rats (Al-Naser and Cooper, 1994).

Dopamine D1 antagonists

The D1-type prototypical antagonist SCH-23390 reduced food intake in both food deprived (Gilbert and Cooper, 1985; Zarrindast et al, 1991; Terry and Katz, 1992) and free-feeding animals (Clifton et al., 1991; Naruse et al., 1991). An early study on the effects of a D1 antagonist on food intake (Koechling et al., 1988) showed that across a series of discrete meal segments, SCH-23390 increased the latency to eat and reduced the speed of eating.

Central mechanism of a dopamine D1-like effects on feeding?

Dopamine D1 agonists

There are studies which have attempted to establish central rather than peripheral sites as the basis of the effects of D1 agonists on ingestive behaviour. Dopamine D1 agonists which are peripherally rather than centrally active, such as SKF 82526 fail to reduce intake (Rusk and Cooper, 1989a). Somewhat counterintuitively, intracranial infusions of D1 agonists do not produce reductions in food intake. When SKF 38393 was infused into the nucleus accumbens there was no apparent associated anorectic effect (Phillips et al, 1995). Another brain area which has been shown to be important in feeding is the ventrolateral striatum (Inoue et al., 1992). When CY-208-243 was delivered to this brain site a reduction in feeding was not observed (Inoue et al., 1995). Therefore, it seems the brain sites critical to the action of D1 agonists remain to be mapped.

Dopamine D1 antagonists

When administered centrally, D1 antagonists have much the same effect on intake as systemic administration of the same drug. For instance, Inoue et al., (1995) found that in the absence of an hypophagic effect SCH-23390 severely disrupted water intake. The basis of this selective effect is unclear but may be related to the deprivation state of the animals used in the study.

Effects of D1-like preferring compounds on the microstructure of ingestive behaviour

Microstructural analysis of feeding and other behaviours offers a valuable insight into the mechanism by which drugs reduce intake. The theoretical assumptions underlying the use of this type of methodology will be discussed in detail in Section 2.10 and 2.12. For the purposes of this section of the thesis there will be a brief description of the studies which have found that dopamine receptor stimulation and blockade may affect particular *parameters* of feeding behaviour.

Dopamine D1 agonists

The microstructural analysis of the effects of the D1 agonist SKF 38393 on feeding and other behaviours showed this compound to share a behavioural profile (at least to some extent) with the non-selective agonists. For instance there was a significant reduction in time spent eating, but in the case of SKF 38393 this seemed directly attributable to a marked reduction in the frequency of bouts. The local rate of eating also declined, and was accompanied by a compensatory increase in the mean bout duration of the feeding bouts. Few other D1 agonists have been compared to the prototypical agonist SKF 38393 in the microstructural paradigm. However, Al-Naser and Cooper (1994) used this methodology to show that in common with the prototype, the D1 agonist A-68930 produced a reduction in intake which was accompanied by a dose-dependent decline in the total time spent feeding, and this decline in turn was primarily a product of a reduction in the number of meals consumed.

There were other common effects of SKF 38393 and A-68930 on microstructural parameters, for instance, eating rate decreased with dose while mean meal duration increased. Terry (1996) notes that, taken at face value, this commonality of results within the same class of compound could lead one to believe that there was a common mechanism underlying the actions of D1 agonists on feeding; namely a specific effect on the number of feeding bouts. Terry also notes that, interestingly, an effect on this parameter is also associated with reduced food intake as a consequence of pre-satiation (Cooper and Francis, 1993). This could be an indication that D1 agonists induce satiety. Further support for this idea comes from sham-feeding studies where some of these agonists (namely SKF 38393 and CY 208-243) effectively and dose-dependently reduced intake even in fistulated rats (see Section 2.10 on sham-feeding).

One way of further examining this assertion is to compare the D-1 agonists on their ability to produce competing behaviours in the feeding test situation. In the case of A-68930, there was significant enhancement of grooming at doses below the anorectic threshold dose (Cooper et al., 1990) which could have contributed to the reduction in meal frequency. The same authors observed an anorectic effect of SKF 38393 in the absence of competing motor effects. These differences in behavioural effects between the two D1 agonists can be explained in terms of their possible pharmacological characteristics but further research will need to be conducted to discern this.

Dopamine D1 antagonists

The anorectic effects of D1 antagonists have not yet been studied as thoroughly as those of other dopaminergic agents especially in terms of the microstructural variables that contribute to their effects. In terms of the ability of Dopamine D1 antagonists to produce competing motor responses, there is some evidence to suggest that certain doses of D1 antagonists can affect non-feeding behaviours without incurring a significant decrease in food intake. D1 receptor blockade has long been associated with the induction of stereotyped motor behaviour. In the case of SCH-23390 the systemic dose range which commonly reduces food intake (>0.01 mg/kg subcutaneously; >0.03 mg/kg intraperitoneally) (Terry, 1996) are those that are often associated with significant disruption of motor behaviour. Examples of this are the potentiation of the dorsal immobility response (Meyer et al., 1992); reduced locomotor activity (Meyer et al., 1993); and catalepsy (Morelli and DiChiara, 1985).

It is important therefore, to seek out a measure of behavioural specificity of the effects of such D1-type antagonists. Genn, (1994, unpublished dissertation B.Sc. dissertation report) showed that the D1- agonist SCH-39166 (0.03-0.3 mg/kg i.p.) significantly reduced motor behaviours such as locomotion, rearing and grooming but did not significantly decrease total intake of a sweetened mash. There was however a significant decrease in the number of meals consumed which was a product of a dose-dependent increase in the mean duration of feeding bouts.

Pharmacological specificity of D1-like effects on feeding: Blocking studies

Characterising the behavioural effects of D1 agonists on feeding has been made more difficult as attempts to demonstrate the receptor specificity of these behavioural effects are both few and inconclusive. Most researchers have chosen to demonstrate receptor-specific antagonism. Terry and Katz (1992) attempted to block the anorectic effects of SKF 38393 by pretreating food-deprived animals with the D1 antagonist SCH-23390. The antagonist failed to attenuate the anorectic effects of SKF 38393 but partially attenuated the reduction in feeding produced by another D1 agonist, namely SKF 77434. Conversely, SCH-23390 fully blocked the effects of the D1 agonist SKF 82958. These results call into question the prototypicality of compounds such as the D1 agonist SKF 38393 and have important implications for the interpretation of results which attribute reductions in feeding to the stimulation of D1 receptors (Terry, 1996).

Further obstacles to interpretation come from findings which imply that non-dopaminergic receptors could contribute to the anorectic effects of the prototypical D1 agonists (see also General Discussion). For instance, Zarrindast et al (1991) showed that the anorectic effect of SKF 38393 could be reversed by the non-specific 5-HT antagonist metergoline, a result which has been replicated in an food-rewarded operant paradigm (Terry and Katz, 1993). These studies follow findings which show the D1 agonist SKF 38393 to be partial agonists at the 5-HT2C receptor (Briggs et al, 1991). This complicates interpretation in that agonists acting on the 5-HT2C receptor are themselves anorectic agents (Kenneth and Curzon, 1988, 1991). It may also go some way to explaining the inability of SCH-23390 to attenuate the effects of SKF38393 as these two compounds could be having an additive effect at a common serotonergic site (Terry, 1996).

This problem was partially overcome by using the D1 antagonist SCH-39166 to attenuate the anorectic effects of SKF 38393. This antagonist has negligible affinity for the 5HT2 C receptor (Taylor et al., 1991). and so one might expect that it would be more efficacious in antagonising the effects of such a prototypical agonist such as SKF 38393 (Terry and Katz, 1994). However, the expected enhanced antagonism of SKF 38393 by SCH-39166 was not borne out of this study. More recently (Terry and Katz, 1994) have demonstrated the generality of the antagonist effect of SCH-39166 by showing that this compound inhibits deprivation induced food intake dose-dependently. Given the negligible affinity of

SCH-39166 at 5HT2 C receptors, this finding points towards D1-blockade as sufficient to reduce food intake.

2.8.2 Dopamine D2-like preferring compounds

Dopamine D2 agonists

Early reports of a role for the D2 receptor in feeding used D2- receptor agonists such as N-0437 (Rusk and Cooper, 1988) and RU 24213 (Martin-Iverson and Dourish, 1988). Both of these compounds produced significant reductions in palatable mash intake in non-deprived animals and N-0437 produced a comparable effect when powdered laboratory chow was used.

Contrary to these initial results, Martin-Iverson and Dourish (1988) found that food-intake was enhanced in free-feeding rats as a function of dose of PHNO, a D-2-type agonist. This effect could be attenuated by pretreartment with haloperidol, a D2 antagonist. This effect was found to be specific to a particular set of stimuli, namely laboratory food pellets as if the accessible diet was changed to sweetened milk or liquid chow, this effect was reversed producing the initially established hypophagia in response to a D2 agonist. The authors explained this discrepancy observed with solid pellets to the stimulation of chewing behaviour. As the effect was observed over a wide dose range it is unlikely that it could be attributed to the stimulation of D2 autoreceptors (see Chapter 1).

Nevertheless, the interaction of D2 agonists at autoreceptors to produce effects on feeding has become an interesting research area. However, studies which hypothesised this type of interaction have produced mixed results. For instance, there is some evidence to suggest that low doses of D2 agonists may act centrally upon autoreceptors to increase feeding (Morley et al., 1982). Opposition to this view comes from studies using systemic doses of the D2 agonist lisuride which purportedly act upon autoreceptors. In an early investigation, (Carruba et al., 1980) found that lisuride produced a hypophagic effect. However, in accordance with the possibility that dopamine D2 agonist might stimulate feeding at low doses (by autoreceptor activation) and inhibit feeding at higher doses (by postsynaptic receptor activation), Clifton et al., 1989, showed that N-0437 produced this type of biphasic dose-effect function in free-feeding rats. Ferrari et al.(1992a, 1992b) further scrutinised the effects of autoreceptor selective doses of D2 agonists. The agonist B-HT 920 decreased the latency to initiate feeding and increased food intake over a 6-h period in food-deprived rats. Two other D2

agonists (Lisuride and CQ 32-084) were tested within two different feeding paradigms (a modified X-maze and home-cage). The biphasic effects on feeding associated with differential receptor stimulation were apparent only in the stressful situation (i.e. hyperphagia at low doses of the D2 agonists when not in home cage). Ferrari et al., (1992b) suggest that this may reflect an anxiolytic action of D2 autoreceptor activation, rather than a specific interaction with mechanisms regulating food intake (see Chapter 7).

Systemic administration of quinpirole (mixed D2-D3 agonist with a similar profile of selectivity to 7-OH-DPAT Levesque et al., 1992) routinely inhibits food intake Zarrindast et al., 1991; Cooper and Al-Naser, 1993) and D2 agonist induced hyperphagia has not been reported elsewhere. The issue of presynaptic versus postsynaptic effects of dopaminergic compounds has already been reviewed in relation to locomotor behaviour in Chapter 1. However, these differential dose effects obviously have a bearing on feeding behaviour. A reconceptualisation of these effects might emerge from developments in dopamine receptor classification and the suggestion that certain behavioural effects previously attributed to D2 or D3 autoreceptor binding might instead be due to drug action at postsynaptic receptor subpopulations of the D3 receptor subtype (Waters et al., 1993).

D2- antagonists

Despite the widespread use of the D2 antagonist pimozide to demonstrate a reduction in food reward (i.e. a reduction in free-feeding,Wise and Colle, (1984) a reduction in feeding induced by deprivation; Wise and Raptis, (1986); feeding provoked by electrical stimulation of the lateral hypothalamus, Streather and Bozarth, (1987) and normal and sham intake of both saccharin and saccharin-sucrose solutions (Xenakis and Sclafani, 1981; Geary and Smith, 1985), there are many reports of failure to inhibit feeding by D2 antagonists leading to debate as to whether D2 antagonists can be said to consistently modify food intake (see Blackburn, 1992 and Chapter 2).

For example, an early study by Heffner et al., (1977) showed that while spiperone had a deleterious effect on food intake, another D2 antagonist, haloperidol, did not. Many others have reported no effect (see Blackburn et al., 1992 and Cooper et al., unpublished results). Nevertheless, Rusk and Cooper (1994) demonstrated that a high dose of the D2 antagonist YM 09151-2 (0.1mg/kg, i.p.) served to decrease consumption of palatable wet mash and also reduced deprivation induced feeding. A lower dose (0.01 mg/kg, i.p.) had the opposite

effect in food-deprived rats. A similar low-dose enhancement of feeding was not apparent after the administration of pimozide in this particular study but had previously been reported by Lawson et al. (1984). There are, however, a substantial number of reports of this low-dose effect of D2 antagonists such as spiperone (Cooper and Sweeney, 1980) and haloperidol (Hobbs et al., 1994).

Central mechanism of D-2 like effects on feeding?

Dopamine D2 agonists

Intraventricular bromocriptine, a D2 agonist, can stimulate feeding at low doses which supports the idea that D2 agonists acting on autoreceptors can increase intake. Also, infusion of quinpirole (mixed D2-D3 agonist) (Levesque et al. 1992) into the ventrolateral striatum has been shown to produce hyperphagia (Inoue et al., 1995). However, infusion of quinpirole into the nucleus accumbens is without effect (Phillips et al., 1995). These dissociable results highlight a possible site for the hyperphagic effect induced by D2 agonists.

Dopamine D3 agonists

There is only one study which specifically examines the effects of a D3 receptor agonist on feeding behaviour. Intake of a preferred 3% sucrose solution was significantly reduced by intra-accumbens infusions of the D3 receptor subtype-selective agonist 7-OH-DPAT (Gilbert and Cooper, 1995). Despite implications of an involvement of this particular receptor in reward (see Chapter 2), without further study, it is difficult to conclude that this receptor has a more or less important role to play in the reduction of intake when compared to other dopamine receptor subtypes.

D2 antagonists

Parada et al. (1988), have used sulpiride to investigate the neural bases of feeding behaviour induced by D2-antagonism. Injected into the lateral hypothalamus, sulpiride increased food intake. Other brain sites have also been identified in the mediation of D2-antagonist effects on feeding. Bakshi and Kelley (1991) showed that when the D2 antagonist haloperidol was injected into a number of striatal regions, there was a resultant dose-dependent increase in feeding duration without a significant increase in total intake (nucleus accumbens) or a reduction in feeding duration (ventrolateral striatum). These anorectic effects which occur as a

result of direct injection of a D2 agonist into the latter brain site have since been confirmed by Inoue et al., (1995).

Effects of D2-like preferring compounds on the microstructure of ingestive behaviour

Dopamine D2 agonists

Rusk and Cooper (1989b) have provided a microstructural analysis of ingestive behaviour after the administration of the D2 type agonist, N-0437. Despite early reports of hyperphagia in response to low doses of this agonist (Martin-Iverson and Dourish, 1988), the application of the microstructural approach revealed that N-0437 dose-dependently reduced food intake over a wide dose range (0.3-3.0 mg/kg). This drug effect on total intake seemed to be achieved primarily by a reduction in the local rate of eating without affecting the time-course of feeding. There was no effect of N-0437 on the total feeding duration nor on the frequency of feeding bouts. With regards the induction of non-feeding behaviours, N-0437 at the anorectic doses of 1.0 and 3.0 mg/kg significantly reduced the frequency of grooming whilst increasing sniffing behaviour. Locomotor activity was not affected. These same anorectic doses also increased the total duration of oral behaviours. This dose-dependent decrease in oral and other non-feeding behaviours are difficult to fully reconcile with the accompanying changes in feeding parameters.

More work is needed in this area as the only other report on the the microstructure of feeding in response to a D2 agonist (i.e. quinpirole) is by Al-Naser (1993). Results from this study revealed that quinpirole reduced intake in much the same way as N-0437 by decreasing the local rate of eating, suggesting a common mechanism of action. This result is in contrast to that of Clifton et al. (1989) who found a biphasic effect of N-0437 without the significant reduction in local rate of eating (although it was slightly reduced).

Dopamine D2 antagonists

Bakshi and Kelley (1991) used microstructural analysis of feeding responses after the central administration of the D-2 type antagonist haloperidol. Their main findings were dissociable effects on feeding parameters which were dependent on the site of injection (see also central mechanisms). With regards feeding enhancement, injection into the nucleus accumbens increased the mean

duration of feeding, rather than increasing feeding bout frequency. Central administration of haloperidol did not have a decremental effect on eating rate, although systemic injection of haloperidol has been shown to reduce feeding through alterations in this parameter accompanied by a decline in feeding duration (Salamone, 1988; Salamone et al., 1990).

Thus it seems that D2 agonists and antagonist reliably produce a decrease in the rate of eating regardless of the overall effect on intake (i.e. stimulation or inhibition). Terry et al. (unpublished results) observed that, after the administration of raclopride, feeding behaviours were affected in a biphasic manner. Eating rate did not change as a function of the dose of raclopride. Total duration of feeding decreased and latency to engage in feeding behaviour increased significantly as a function of increasing doses of raclopride. Latency seemed most profoundly affected by the highest dose of raclopride (1.0 mg/kg, i.p.). This effect, along with a reduction in the number of feeding bouts, seemed responsible for the high dose hypophagia which was observed. The low-dose hyperphagic effect of raclopride is the product of a combination of slight increases in both number of feeding bouts and mean bout duration. This enhancement occurs in the absence of competing behavioural responses, whereas the high-dose hypophagia was accompanied by disruption to non-feeding behaviours. Therefore, although this study shows that changes in eating rate are not necessarily characteristic of D2 antagonism, it is difficult to assume behavioural specificity of the D2 antagonist hypophagia.

Dopamine D3 antagonists

There is only one report of an effect of a specific D3 receptor antagonist on feeding. This finding was part of an "ethogram" collated by Clifford and Waddington (1998) in an attempt to further characterise the novel compound PNU-99194A. Eating counts were increased at 15-45 mg/kg PNU-99194A. However since microstructural parameters were not measured, it is difficult to propose a mechanism by which this compound achieved such an effect from this study alone.

Pharmacological specificity of D2-like effects on feeding: Blocking studies

As is the case for D-1 type agonists, the dependent variable in studies of D-2 type agonist -antagonist interaction studies has been the measure of total intake. However, receptor specificity of the anorectic effects of D2 agonists has been successfully demonstrated. Rusk and Cooper (1988) showed that pretreatment with the D2 antagonist YM 09151-2 abolished the decremental effect of N-0437 on

palatable mash consumption. Ladurelle and colleagues (1991) also showed reversal of the anorectic effects of RU 24926 by sulpiride in food-deprived mice. However, there are some anomolous results. For example, Zarrindast et al. (1991) found that whereas the anorectic effects of quinpirole could be reversed by pretreating animals with either sulpiride or pimozide, only pimozide was capable of reversing the decremental effects on feeding of another D2 agonist, bromocriptine. Specificity at other receptor subtypes remains possible as explanation of conflicting results.

Summary

There are obviously several inconsistencies in the evidence from studies which have tried to discover the relative contributions of dopamine receptor subtypes to ingestive behaviour. Such discordant evidence could be due to procedural differences (i.e. deprivation status, scheduling of food, type of diet and drug and test history, Terry, 1996), questionable pharmacological specificity of the dopaminergic compounds used, and most certainly differential effects of the doses used. In the light of such incomplete characterisation of dopaminergic drug effects on feeding, and also in the light of the reclassification of dopamine receptor subtypes, it is difficult to pull out dissociable receptor subtype contributions to the control of ingestive behaviour.

Nevertheless, in reviewing the literature, some authors have concluded that there may be some definable differences between those drugs which act at putative D2 receptors and those which act at D1 receptors. For example, much of the above evidence gave rise to the observation that D1 agonist and antagonist effects on ingestive behaviour very often occur in conjunction with competing motor behaviours. However, both agonists and antagonists in this class seem to produce a decremental effect on meal frequency, Terry (1996). The biphasic dose effects of both D2 agonists and antagonists render conclusions about the behavioural role of this receptor subtype difficult. While there is some evidence that low doses of this class of drug enhance intake and that high doses depress intake, this effect does not always occur. D2 preferring compounds have also been reported to primarily affect eating rate, but again, this effect does not always become manifest (Terry, 1996).

In a general sense, the disparity of the results presented above provide the perfect impetus for any study which seeks to tease out behavioural similarities and differences within and between pharmacological classes of dopamine receptors. The following section considers some of the methodologies which have been used in an attempt to further isolate specific effects of dopamine agonists and antagonists

on feeding behaviour. It also evaluates the utility of total intake measures in answering such questions. Further sections provide alternatives to total intake measures and the theoretical assumptions underlying the choice of optional dependent variables.

2.9 Total intake measures for liquid diets

Total intake measures are quick, efficient and cost effective approximations of an animals responsiveness to a given food or fluid (Grill, 1987) and the common use of this dependent measure is testament to these assertions. However, this measure is of limited value to those interested in the study of ingestive behaviour as it is a composite standard which reflects a variety of behavioural acts that occur during a time when an animal is feeding or drinking (Davis and Levine, 1977). Nevertheless, much of the evidence from studies of the neural bases of the control of feeding and drinking have used this measures. Therefore, it would seem sensible to outline some methods of procuring total intake measures and the effects of pharmacological manipulations on these dependent variables.

-two-bottle intake tests

In this test situation, animals are presented in their home cages with a choice between water and a taste solution, hence the common reference to this method as a preference test. Food is available ad libitum and test duration usually varies from 24-48hr. Over successive days the concentration of the taste stimulus is varied usually in an ascending fashion. The results are typically expressed as the ratio of taste to total fluid (water plus taste solution) intake as a function of the taste stimulus concentration. Control for bottle position is necessary as the preference may reflect this variable rather than solution choice.

-one-bottle intake tests

This test situation is usually short in duration (e.g. 15-30 min) and water is unavailable during this period. The taste solution presented to animals in their home cage is their sole fluid source. Interpretation of data from this situation is more difficult than data from a preference test, as in the absence of a water intake measurement it is difficult to exclude changes in physiological state and environmental fluctuations as explanations for changes in fluid consumption. To avoid this potential problem, water trials are often interspersed between other taste stimulus trials during the course of the experiment to provide a comparison. When this control has been implemented the data from one and two- bottled tests is found to be comparable (Grill et al., 1987).

Effects of dopaminergic agonists and antagonists in fluid intake tests

Towell et al. (1987), were among the first to report that the D2-type antagonist pimozide reduced the preference for sucrose over water, without altering total fluid intake. Using the single choice test, this antagonist was shown to selectively reduce intake at low sucrose concentrations, while sparing the intake of equal or higher volumes at higher concentrations. This finding confirmed the results of Bailey et al. (1986), who showed that pimozide supressed operant responding maintained by weak sucrose concentrations but that this finding did not hold when the stimulus was a higher sucrose concentration. It was difficult to explain such results in terms of a motoric deficit and so Muscat and Willner (1989) went on to further investigate the effects of D1-type and D2-type receptor blockade on sucrose consumption and preference.

In general, these authors concluded that antagonising either subtype of dopamine receptor appeared to be similar to those of reducing the concentration of sucrose. Both sulpiride (D2-type receptor antagonist) and SCH-23390 (D1-type receptor antagonist) decreased sucrose intake in single bottle tests when sucrose concentration was weak but neither drug had any effect when the sucrose concentration was made stronger. By manipulating drive or motivational level (i.e. reducing drive by using non-deprived animals) the effects were reversed for the single bottle test. In the two-bottle test, both drugs reduced preference for the weakest sucrose concentration but increased preference for the strongest concentration.

Therefore, it seemed that although neuroleptics usually suppress reinforced behaviour, if very sweet rewards are used, it is possible to demonstrate the opposite effect, an increase in performance following neuroleptic treatment (Phillips et al., 1991a). Studies of operant responding raise the possibility that the direction in which neuroleptics change responding may be dependent on the magnitude of reward available. In the light of such results, Phillips et al. (1991c) employed non-operant tests of sucrose solutions consumption and preference after the adminstration of the D2-type antagonist raclopride. These studies set out to determine the bases of the behavioural generality of the paradoxical effect of neuroleptics on behaviour maintained by a very sweet reward. A further line of investigation assessed the behavioural mechanism by which very sweet rewards alone suppress performance. Many authors attribute this finding to the highly satiating nature of high carbohydrate solutions, (Davis, 1977). Phillips et al. (1991c) examined the time course by which raclopride enhances intake of very

sweet solutions, to re-evaluate the potential contribution of satiety to the suppression of intake. Two further possibilities were also considered within the study; that very sweet solutions alone may have aversive effects and that raclopride may produce an anti-aversive effect in the face of the negative hedonic impact of those very sweet solutions. More concisely, Phillips et al. (1991c) wanted to elucidate the effect of choice upon preference and the overall (and within-session) effects of raclopride on these parameters.

Previously, Phillips et al. (1991a) reported that raclopride reduced intake of concentrations from the ascending limb and increased intake of concentrations from the descending limb of the concentration-intake curve (i.e. the concentration-intake function is shifted to the right). Further testing of these hypotheses involved the comparison of the effects of raclopride in two separate experimental paradigms. Animals were presented with either, 0.7, 7 or 34% sucrose solutions, each coupled with the presentation of water as an alternative (two-bottled test). In another experiment, separate groups were allowed access to all three of the above concentrations concurrently (three-bottle test).

In the two-bottled test, presentation of sucrose solutions yielded an inverted U-shaped concentration-intake function (similarly low intake measures for the high and low concentrations with highest intake measures in response to the intermediate sucrose concentration). However, the high sucrose concentration was preferred in the three-bottle test. Therefore, when comparing preference data from one two and three-bottle tests, it is difficult to attribute the suppressed intake measures on the descending limb of the single-bottle concentration-intake curve to the aversive properties of very sweet solutions. In both tests raclopride (100-400μg/kg) reduced intake of the intermediate sucrose concentration but increased the intake of the high sucrose concentration. These effects were apparent very early in the test session (within the first 5-min). Therefore, the potential "satiety" explanation seems unlikely.

In the two-bottle test, intake of the intermediate concentration showed both effects: an immediate decrease and a later increase. In the three-bottle test, sucrose-naive animals showed a gradual onset of preference for the high sucrose concentration. Immediate enhancements of intake by raclopride required three sessions of exposure. The authors suggest that both preference for sweeter solutions and concomitant concentration-dependent enhancements of intake induced by raclopride are acquired over time and involve some form of learning (also see

Pecina and Berridge, 1998, in Chapter 4). Raclopride also did not alter the consumption of a 0.001% solution of quinine. It was hypothesised that if the effects of raclopride upon reactivity were non-specific , then the drug might be expected to ameliorate the impact of quinine and increase the volume of quinine-adulterated water ingested. Raclopride had no effect on quinine or water intake but reduced the intake of the 0.7% sucrose solution in the same animals. This effectively negated the suggestion that the efficacy of raclopride may exend to a blunting of the direct impact of an aversive stimulus such as quinine. Although the exact mechanisms by which changes in responding to varying rewards are not yet clear, there are obviously many alternative aspects to the reward-dependent suppression or facilitation of consummatory behaviour by neuroleptics such as raclopride.

Using a very similar set of paradigms, Muscat et al. (1991) tested the hypothesis that the stimulation of high-concentration sucrose solution intake after the administration of raclopride could be dependent on calorie content of the solution used rather than its perceived sweetness. They found results which at face value could be in support of such an hypothesis. For example, in two-bottle tests sucrose and the non-nutritive sweetener saccharin produced comparable inverted U-shaped concentration-intake functions. The lack of effect of raclopride at the highest saccharin concentration, in contrast to the stimulation of intake observed at the highest sucrose concentration, is compatible with the hypothesis that the stimulation of sucrose intake is related to calorie content.

On further examination, the three-bottle test revealed that the highest concentrations of sucrose (34%) and saccharin (0.8%) differed in a way which was not related to caloric value but may rather be related to potential aversive properties of saccharin at high concentrations. Evidence for this comes from data which reflects differences between the preference and intake measures. For instance, in the case of sucrose, intermediate concentration solutions support the highest intake whilst high concentration solutions are shown to be preferred. This contrasts with the effects of saccharin solutions where the intermediate concentration solution not only supports the greatest intake but is also preferred in the three bottled test.

Calorie content was further excluded as an explanatory variable by rendering the high concentration sucrose solution aversive after the addition of the high concentration saccharin solution. Intake of this cocktail was not increased by raclopride despite its isocaloric relationship with the high concentration sucrose solution.

A possible change in palatability after the administration of raclopride becomes favourable after the exclusion of a postingestional explanation. If calories are to be effective they first have to be absorbed. The stimulatory effects of raclopride in these experiments were apparent within the first 5-min of the test and did not increase thereafter (Phillips et al., 1991a; Phillips et al., 1991C, Muscat et al., 1991).

Muscat et al. (1991), summarise by noting that the anomaly at the high concentration sucrose solution arises because at high concentrations there is a disjunction between reward and consumption: relative to 7% sucrose, 34% sucrose is preferred (three-bottle test) but underconsumed (two-bottle test). The consequence of this is that at high sucrose concentrations, the "quantity consumed is not monotonically related to reward". (pp. 213). In fact sucrose concentration seems to increase with reward (as measured by preference) while consumption decreases . These authors attempt to resolve the paradox by speculating that a decrease in reward might be reflected in an increase in consumption as is observed following the administration of several dopamine receptor antagonists.

There is a caveat to the above corpus of results. Cooper et al. have conducted many studies in which they tested the ability of dopamine antagonists to differentially affect various sucrose rewards. Although this work is largely unpublished, the general findings should be stated here. They found that dopamine antagonists decreased sucrose solution intake irrespective of sucrose concentration (1-30%). The studies by Willner, Muscat and Phillips above show that differential effects of dopamine antagonists may depend upon the value of the reinforcer, and the extent to which a motoric interpretation is favoured is often based upon the occurrence of such a differential effect. What should not be underemphasised is that this differential effect of dopamine antagonism does not always occur.

Central effects of raclopride?

Within their experimental design, Muscat et al. (1991), accommodated an hypothesis related to the pharmacological specificity of the effects produced by raclopride. In order to ensure that the stimulant effect of raclopride on consumption of 34% sucrose was centrally mediated, these researchers also tested the effects of domperidone (a D2-type antagonist that does not cross the blood brain barrier). Results showed that raclopride exerts its effects centrally rather than peripherally.

These authors followed results from Phillips et al., (1991b). Phillips and colleagues used intracerebral injections of the D2 antagonist sulpiride to investigate the neuroanatomical substrate of neuroleptic induced changes in sucrose intake. As with systemic raclopride, administration of the D2-type antagonist sulpiride to the nucleus accumbens or the anterodorsal striatum reduced intake of a sucrose solution from the ascending limb of the concentration-intake curve, but increased intake of 7% and 34% sucrose solutions. The only effect of sulpiride infusion into the basolateral amygdala was to increase intake of 34% sucrose.

Drug free preference tests

Hsaio and Smith (1995) tested the prediction of decreased preference in a different way. Using a two-bottle test, rats were tested for 5-min. The stimuli used were either a grape or an orange flavour mixed with 10% sucrose. these flavours were non-caloric and were equally preferred in an initial preference test. Conditions were then switched to a one-bottle test where animals had the chance to sample one of the flavoured sucrose solutions after pretreatment with raclopride (400µg/kg). Raclopride decreased 1-bottle intake by 55% under these conditions. On alternate test days, rats ingested the other flavoured 10% sucrose solution after vehicle injection. The volume ingested after vehicle injection was yoked to the volume ingested after raclopride treatment in the preceding test. Making the volumes equal insured that any change in preference was not due to different amounts of orosensory stimulation by sucrose. Rats were tested twice after raclopride treatment and twice after vehicle treatment. 24-hr after the last one-bottle test with raclopride pretreatment, rats were given a 2-bottle preference test for 5-min in the absence of drug pretreatment. In this test they had access to both flavours which had previously been associated with either vehicle or raclopride pretreatment.

Rats showed a significantly decreased preference for the flavour that had been paired with the raclopride pretreatment. The same reliable result was obtained after three repetitions of the protocol. The decrease preference was obviously not a direct effect of the drug, rather, it depended on prior experience of the drug-flavour pairing. (Smith, 1996) notes that this was not a result that could be explained in terms of a conditioned taste aversion as there was no progressive decrease of 1-bottle intakes across the sequence of raclopride treatments.

Summary

Inasmuch as decreases in total intake measures reflect a decrease in the efficacy of a reinforcer to maintain responding, both dopamine D1 and D2 antagonists clearly reduce reward. This decrease in responding is very often seen in the absence of a motoric deficit. Effects of dopamine antagonists on ingestive behaviour may vary as a consequence of reinforcer value but these results have not been observed universally (Cooper et al. unpublished results).

Total intake measure, although informative to a certain extent, are of limited use if the aim of an experiment is to elucidate a role for dopamine receptor subtypes in separable *components* of ingestive behaviour. The following section (Section 2.10) offers alternatives to total intake measures and the findings produced through the use of these optional methodologies. Findings using more refined technologies to examine ingestive behaviour have led to novel theories on the role of dopamine in food reward. These will be outlined in Section 2.11.

2.10 Alternatives to total intake measures

Meal pattern analysis

If the aim of a study is to describe and quantify the various types of behaviour that occur during a time when an animal is primarily engaged in ingesting food or fluid, it is necessary to adopt at least two strategies. The first is to catalogue all behaviours that an animal engages in during the test situation, the second, to determine which behaviours belong together as a functional unit (Smith, 1996). One paradigm which engages such strategies is that of meal pattern analysis which constitutes the observation and analysis of temporal patterns of feeding, typically over 24hr.

Theoretical assumptions underlying the temporal pattern of feeding drinking

The collection and analysis of temporal patterns of feeding and drinking relies heavily upon theoretical assumptions about the nature of the act of ingestion. Clifton (1987) outlines these assumptions clearly and so provides a theoretical background to a number of the experimental goals adopted by researchers into the temporal patterns which are integral to feeding.

Among the alternatives to measuring total intake, there are two types of analysis which are widely used for temporal patterns of ingestion. The first is simply to divide the time over which the recording was made into one or more intervals and calculate the number of items eaten, or time spent eating or drinking, in each of these intervals (Clifton, 1987). The second is to acknowledge that the behaviour is organised into a number of episodes or bouts. In the case of feeding these episodes are usually referred to as meals. With both these types of analysis there are assumptions as to the nature of the periods of non-ingestion. One of these assumptions rests on the fact that the non-ingestive periods must fall into two classes. These are short intervals between acts that do not characterise the end of a bout and there are long intervals that lie between bouts (Clifton, 1987).

Before proceeding with further analyses, a decision must be made as to the criterion which will separate these two classes of interval in an efficient way. Crucial to such a choice is a model of the expected distribution for the between act intervals. Traditionally, there was an assumption that two overlapping normal distributions might be expected (Baker, 1953). If these assumptions are formalised

this renders such a result unlikely as it requires complex changes in the probability of a fresh event with time since the last event (Clifton, 1987). The very simplest assumption would be that the time intervals between acts have a constant probability of ending. This assumption can be most easily tested by plotting the distribution of the interval data in the form of a survivorship curve (Slater, 1974). If the Y axis is logarithmic, then provided that a single negative exponential distribution fits the data the plot should be a straight line. However, this is not the case. The distribution has a typical "broken stick" shape which suggested that it might be better fitted by two overlapping negative exponential distributions (Clifton, 1987). One of these has a high probability generating the majority of short intervals and the second with a low probability generating the majority of long intervals.

As the two distributions overlap it would be impossible to achieve a perfect criterion, therefore, this type of analysis may not proceed without a choice of criterion that would best separate the two distributions. Slater, (1974) suggests that the best choice of criterion might be just to the right of the most obvious break in the survivorship function (see also Morris and Cooper, 1993). Misassignment seems to be unavoidable if the criterion chosen is too short (i.e. within-bout intervals are apportioned to the between-bout class). The number of misassignments increases rapidly as a shorter criterion is is taken but increases only slowly with a longer criterion. Therefore, care should be taken in deciding this separating criterion as the results of misassignment could have important consequences for interpretation (also see Section 2.12).

Once a suitable criterion has been chosen it is possible to produce a sequence of appropriately defined meals and intervals between them and then go on to calculate meal size, duration and intermeal interval. It is then feasible to ask specific questions as to the influence of pharmacological manipulations on these parameters thereby deconstructing the concept of ingestion and further elucidating neurotransmitter substrates which might underlie specific alterations in specified parameters.

The effect of dopamine agonists and antagonists on meal patterning

D1 type agonists

Clifton and Cooper (unpublished results in Cooper and Al-Naser, 1993) described the effects of the enantiomer of SKF 38393 (*R*(+)SKF 38393) on meal patterns at the onset of the night phase of the diurnal light cycle. This compound increased the latency to eat, with experimental animals taking approximately fours times as long as controls to begin eating. (*R*(+)SKF 38393) reduced the average meal size without affecting the frequency of eating bouts and this hypophagic effect lasted 2-3 hours. Meal patterns were also analysed in free-feeding rats after the administration of the D1-like agonist A-77636. In common with (*R*(+)SKF 38393) the anorectic effect of A-77636 seemed to be achieved by reducing the size of meals but the effect on meal bout frequency was quite different; A-77636 produced a slight increase in number of meals. This result brings the generality of the behavioural effects of D1-like agonists into question. To determine whether these differences are based on the compounds' respective pharmacological action, or could be better explained in terms of discrepant methodologies, needs further research.

D1-type antagonists

There have been few studies on the effects of D1 antagonists on the temporal patterns of feeding. By using meal pattern analysis Clifton et al. (1991) reported that SCH-23390 produced a small reduction in the number of meals consumed and in the feeding rate during a meal. There was no effect on meal size but there was a selective effect on water intake. This effect was substantially greater than the effect of SCH-23390 on food intake. Further support for this D1-type agonist effect on intake comes from a more recent study by Clifton (1995). Findings from this study show that SCH-39166 produces no anorectic effect at doses which severely disrupt water intake.

D2-type agonists

Again using a meal pattern analysis, Clifton et al., (1989) obtained a biphasic dose-response profile for the effects of N-0437 on feeding. At 0.3 mg/kg i.p. food intake was enhanced whereas 1.0 and 3.0 (mg/kg i.p.) significantly

reduced intake. Meal size was most profoundly decreased although meal duration increased at the lowest dose. These results reflect other observations of low dose hyperphagia induce by D2 agonists in other feeding paradigms (see Section 2.8).

D2-type antagonists

Clifton et al. (1991) used several D2-type antagonists to show that in general, mean meal bout duration, in addition to meal size increased after the administration of YM 09151-2, remoxipride and raclopride. This effect was significant for all but raclopride. However, in the short term, both raclopride and remoxipride increased food intake. Notwithstanding such differences, all the D2-type antagonists significantly slowed eating rate. Haloperidol has been shown to produce concordant results (Clifton, 1995). This result contrasts with the central administration paradigm in that eating rate was not affected by striatal infusion of haloperidol (Bakshi and Kelley, 1991).

These results have specific clinical significance as obesity is associated with typical neuroleptics when used as treatment for schizophrenia (Stanton, 1995). Atypical neuroleptics, such as clozapine and olanzapine, are preferred for clinical use owing to their negligible cataleptic effects. Unlike classical neuroleptics, these atypical neuroleptics do not exert their effects exclusively through their action at D2 receptors. When effects on meal patterning were compared, classical, but not atypical neuroleptics enhanced meal-size in free-feeding rats (Lee and Clifton, 1998). Such dissociations have been attributed to actions of atypical neuroleptics at alternative dopamine receptor subtype populations (Sanger et al., 1993).

Central mechanisms of effects of dopaminergic compounds on meal patterning?

As mentioned above, Bakshi and Kelley (1991) saw no effect on feeding rate over a 30 min test session after infusion of the D2 antagonist haloperidol into three striatal subregions, one of which was the nucleus accumbens. Clifton and Somerville (1994) examined the effects of electrolytic lesions of the nucleus accumbens which had no obvious effect on body weight or on the short term intake of solid food, sucrose and salt solutions. However, when recording of feeding and drinking was extended to 24hr periods, it became apparent that lesioned animals took many more meals of shorter duration and yet feeding rate within meals was not affected. These data highlights the importance of duration of test when trying to elucidate the neural mechanisms that underly the short- and long-term temporal structure of motivated behaviour.

Isolating components of ingestive behaviour

- test duration

There is a problem of interpretation in the study of ingestive behaviour which is most apparent (but by no means exclusive to) those studies using volume of intake as their dependent variable. Inherent in the design of such tests as the one and two bottle intake tests, is the need for a relatively long test duration (Grill et al., 1987). Tests of relatively long duration usually give rise to a greater cumulative intake, which in turn is likely to limit fluid intake. These effects, which appear over time, are no longer directly attributable to the effects of taste as they eminate from outside the oral cavity and are attributed to what are collectively named postingestive stimuli. Therefore, no matter how carefully the parameter of taste has been systematically varied, for instance by increasing sucrose concentration, there remains the probability that nongustatory (i.e. postingestive) factors also contribute to the results from these tests. More concisely, taste is but one of the dimensions along which solutions vary. Other dimensions are those such as caloric value or osmotic and mechanical impact.

An example of this can be seen in a study by Spector and Smith (1984) which shows that, over 24hr, rats' daily total intake of a sucrose solution increases with concentration up to a peak value at about 0.3M at which point the relationship between concentration and intake essentially reverses producing decreases in intake following further increases in sucrose concentration. They attribute this inverted U shape dose-response curve to the influence of postingestive stimuli. The prior dietary experience of the animal along with its motivational state can also influence the results from such tests (Davis and Levine, 1977).

One way to reduce postingestive stimuli is to reduce the duration of the intake tests (Grill and Berridge, 1985) therefore more effectively minimising the effects of such stimuli and consequently increasing the interpretive strength of the test with respect to taste (Grill et al., 1987). An example of the effects of sucrose concentration on intake in a short term test comes from Davis (1973). In contrast to the relationship between sucrose concentration and intake in the longer test of Spector and Smith, Davis (1973) revealed that if the test session is very brief (i.e. < 60 secs) total sucrose intake is a monotonically rising function of sucrose concentration. The same seems to be true of data collected during early portions of longer term drinking tests (i.e. Sclafani and Xenekis, 1987).

However, reducing test duration is not the only method which has been adopted in trying to isolate variables which may control ingestion. The following section will discuss conceptual considerations and empirical data which has been collected using in response to the identification of confounds in food reward research.

The sham-feeding preparation

The effects of dopamine agonists and antagonist on sham-intake

The advantage of sham-feeding is that it eliminates or minimises the post-ingestive satiating effects of sucrose and other stimuli. Therefore, the confound of the post-ingestive satiating effects of food interacting with dopamine antagonists that has been observed in some of the early lever-pressing and free-feeding experiments is effectively eliminated. When post-ingestive or satiating effects were abolished through gastric fistulation, sham feeding also eliminated the decrease in intake observed with sucrose solutions more concentrated than 10% (Mook, 1963; Sclafani and Nissenbaum, 1985; Weingarten and Watson, 1982). Thus, when rats sham-fed sucrose, there was a sigmoid-shaped relationship between sucrose concentration and 30-min intake (Bernz et al., 1983; Joyner et al., 1985). Furthermore, most concentrations elicited intake throughout the test session; thus any termination of intake within a test becomes significant (Smith, 1996). For these reasons, sham-feeding of sucrose is a sensitive assay for evaluating the hypothesised effect of dopamine antagonists to decrease the response-maintaining effect of food on ingestion. Hence, the application of this paradigm to drug studies can reveal much as to the neural controls of ingestion.

Dopamine D1 agonists

There is only one sham-feeding study which makes use of D1 agonists in order to examine their effects on the control of ingestion. It shows that SKF 38393 inhibits sucrose sham-feeding in gastric fistulated rats (Cooper et al, 1993), as does the ergoline D1 agonist CY 208-243.

Dopamine D1 antagonists

Schneider et al (1988) demonstrated that the D1 antagonist SCH-23390 inhibited sucrose sham feeding although its effects on corn-oil sham feeding were much weaker (Weatherford et al 1990). In this experiment no dose of SCH-23390

that did not produce obvious motor impairment, decreased the sham intake of 100% corn oil. These authors use another macronutrient solution in order to extrapolate their previous findings for sucrose. They allowed their sham-prepared animals access to corn oil as well as sucrose and discuss their results in terms of dopamine receptor subtype contributions to the mediation of the positive reinforcing effects of the orosensory stimulation produced by these different stimuli. They found that the inhibitory potency of SCH-23390 is inversely related to the hedonic potency of 100% corn oil and 6 and 10% sucrose as measured by 2-bottle prefernce tests.

Dopamine D2 agonists

Despite reducing intake in real feeding situations, the D2-type agonist N-0437 does not terminate sucrose sham-feeding, even though it reduces sucrose intake by non-fistulated rats (Cooper et al., 1989). Rusk and Cooper (1989b) suggest that N-0437 may only reduce intake in the presence of normal satiety cues. This profile of results in the sham-feeding preparation is in stark contrast to the actions of D1 type agonists in this preparation which produce strong dose-dependent reductions in intake (Cooper et al., 1993).

Dopamine D2 antagonists

Pimozide has been shown to reduce sham-feeding of both saccharin and saccharin-sucrose solutions (Geary and Smith, 1985). Schneider et al. (1986) linked the inhibitory potencies of four D2-type antagonists to their binding potencies at central D2 receptors. Central mediation of effects on sham-feeding have been verified by the administration of domperidone. This D2-type antagonist is excluded form the central nervous system and fails to reproduce the effects on feeding seen after the administration of pimozide (Duong and Weingarten, 1993). Along with a D1-type antagonist Weatherford et al., (1990) compared a the effects of a D2-antagonist raclopride on the sham feeding of sucrose and corn oil. Raclopride unlike SCH- 23390 did not only produce a decrease in sham-feeding which was secondary to a noticeable motoric deficit but did so at doses which did not produce motoric effects. They are conservative in their interpretation of these effects as they did not completely control for differences in the absolute hedonic potencies of the stimuli used. Therefore it is difficult to reinforce any discrete functional dissociations which may exist for D-1-type and D2-type in food reward (Waddington, 1989).

Roitman et al. (1997) determined that dopamine D2 antagonists haloperidol and raclopride also suppressed sham-drinking of NaCl solutions in the rat. Sodium ingestion in rats depleted of Na is a strong motivated behaviour that is enhanced further when depleted rats are sham-drinking. This suppression of sham drinking by D2-like antagonists was observed in the absence of any effect on real drinking of NaCl solutions. This led the authors to the conjecture that the positive motivating properties of NaCl stimulation in depleted, sham-drinking rats are mediated by central D2 receptors.

Oral Infusion Methods

When compared to intake tests, the benefits of the use of oral infusion methods lie in their brevity, and their elimination of the problem of stimulus sampling (e.g. when using an aversive taste stimulus). With such brief and direct methods there is no need to ensure stimulus sampling in the form of water deprivation or training with highly preferred taste stimuli (Grill et al., 1987). Rather, responses to a wide variety of tastants can be measured immediately (within seconds) without the need for animals to voluntarily initiate contact with the tastant. As well as ensuring stimulus sampling, this advantage is most apparent when assessing the role of taste in the ingestive behavior for a variety of neurological preparations that do not eat or drink (e.g. aphagic, adipsic, neonatal) (Grill and Berridge, 1985). Other impediments to eating and drinking, such as a loss or lack of motor control after the administration of certain drugs, do not pose insurmountable problems for this method. Hence, this method would be most seem to be most useful in assessing the effects of dopaminergic compounds on initial hedonic responses to tastants.

Intraoral intake tests

In intraoral intake tests animals are fitted with an intraoral cannula, the intraoral end of which is placed just rostral to the first maxillary molar (Grill and Norgren, 1978 a,b). Tubing is led out subcutaneously to the skull and secured to a short piece of stainless steel tubing and anchored to the skull with dental acrylic (Grill, 1987). In this test, the surgery and habituation procedures used are the same as those for the taste reactivity test (for full details see Grill and Norgren, 1978a).

On test days, the stimulus filled tubing is attatched to the rat's intraoral cannula and the rat is then placed in the test chamber. The delivery of the stimulus into the rat's mouth is controlled by an infusion pump and enters at a constant rate

(usually 0.5 to 1.0 ml/min). This rate of delivery was chosen as it was seen to be within the rat's capability to keep up with the accumulation of fluid in the oral cavity (Grill and Norgren, 1978 a,b). The test continues as long as the rat ingests the fluid stimulus. When fluid is seen to be expelled from the rat's mouth the pump is turned off. After a 30-sec pause the pump is turned back on. After this off-period the test only continues until two successive rejections of the stimulus occur. The volume ingested is computed by multiplying the infusion rate by the duration of ingestion. Again, as with the taste reactivity test, stimulus sampling is insured. In contrast to the taste reactivity test the oral infusion can be lengthy as the duration of the test is governed by the animal. This test procedure also makes use of a criterion for the termination of ingestion. Therefore, it is possible to measure total intraoral intake but due to the potentially longer duration, interpretive caution is needed due to the possibility of the influence of postingestive signals (Grill, 1987).

Recently, analogies have been made between intraoral infusion methods and spout-licking (Kaplan et al., 1995; Grill et al., 1996) inasmuch as rhythmic oral motor responses observed during intraoral infusions are organised in a burst/pause pattern and are emitted in the same frequency range and serve the function of intraoral transport of fluid into position for swallowing. This analogy indicates that there is potential value in analysing temporal patterns for various applications of the taste reactivity and intra-oral infusion paradigms.

The effects of dopaminergic compounds on intraoral intake tests

There are few studies which attempt to outline a role for dopamine receptor subtypes in the control of ingestion via an intraoral catheter. This is surprising as this paradigm eliminates the approach phase integral to the normal feeding situation, in favour of targeting consumption as the important dependent measurement. As has been mentioned, effects of dopaminergic compounds on feeding have often been interpreted as primarily motoric and so this method should be viewed as a highly appropriate paradigm in which to observe such effects.

Tyrka, Gayle and Smith (1992) compared the effects of raclopride on measures of independent ingestion (II) and also when animals were receiving sucrose via an oral catheter (OC). Pretreatment with raclopride resulted in decreased intake in the (II) tests but not in (OC) tests in rat pups. The authors conclude that this difference is due to raclopride decreasing the sensory control by sucrose of the appetitive behaviours required to maintain ingestion of sucrose during the (II) tests. As this function is effectively bypassed in the (OC) paradigm,

there is no reason to expect a decrease in intake after the administration of raclopride. Therefore, D2 receptor may be more involved in the appetitive phase of ingestion than the consummatory phase.

The work above was conducted with rat pups in an effort to uncover some of the dopaminergic influences on unconditioned responding for sucrose. Any differences after dopaminergic drug treatment of rats without prior experience of test conditions or stimuli cannot be explained in terms of incentive motivation or any other forms of learning about sucrose reinforcement (Tyrka and Smith, 1993).

In a further study, Tyrka and Smith (1993) compared the D1-like antagonist SCH-23390 and the D2-like antagonist raclopride in the (OC) test. They discovered that SCH-23390 but not raclopride decreased intake of intraorally infuse 10% sucrose in adult rats. Tyrka and Smith infer from these, and other results that D1 but not D2 receptors are necessary components of the neural network that processes the unconditioned gustatory stimulus of 10% sucrose into mouthing and swallowing movements that maintain ingestion.

The taste reactivity test

The taste reactivity test (Grill and Norgren, 1978), as its name implies, measures the initial oral motor behaviours resulting from the oral infusion of tastants. Brief oral infusions of taste stimuli elicit a number of discrete oral responses which can be easily identified and quantified and whose magnitude and distribution are taken to reflect differences in the discriminability and palatability of various taste stimuli. There are nine behaviours in the lexicon of taste reactivity responses which are categorised as either "ingestive" or "aversive" and are characterised by the removal (aversive) or acceptance (ingestive) of the taste stimulus from the oral cavity (see Grill and Berridge, 1985).

The effects of dopaminergic compounds on the taste reactivity test

The taste reactivity test has been used in conjunction with the administration of drugs which affect ingestive behaviour. As has been stated, drugs may achieve their anorectic effects by many central and peripheral mechanisms, therefore results from this paradigm can offer insight into whether a compound is changing the initial hedonic evaluation or palatability of a tastant. Many of the original conceptions of the role of dopamine in reward referred to the necessity of the integrity of dopaminergic systems for positive hedonic evaluation to take place. Since then,

dopamine has also been implicated in responding to aversive stimuli (see Section 2.4). The suggestion that blocking dopamine reduces the subjective enjoyment of food reward would appear on the surface to be amenable to test with the taste reactivity paradigm. However, many taste reactivity studies have lead to the absolution of dopamine in the process of attributing relative reward values to positive (Treit and Berridge, 1989; 1990) and negative hedonic stimuli (Pecina and Berridge, 1998) and indeed is seen by some to play a separate role from systems which effectively mediate hedonic evaluation (see Section 2.11). For example, Berridge et al., (1989) found no evidence that sweet solutions became less palatable or that bitter solutions became more aversive in animals with extensive damage to their brain dopamine systems, achieved by lesioning the nigrostriatal dopamine system with 6-OHDA. Even when dopamine was depleted by over 95% after combined lesions of the neostriatum and accumbens there did not appear to be any shift in the hedonic or aversive reaction patterns (Berridge and Robinson, in press, reported by Pecina and Berridge, 1998). Concordant with these findings are those of Treit and Berridge, (1990). They discovered that haloperidol pretreatment failed to reduce hedonic reactions or increase aversive reactions to a 1-min infusion of sucrose or quinine.

In contrast, Leeb et al., (1991) found decreased sensitivity to sucrose in the same paradigm using pimozide (the D2-type antagonist) treated rats. These animals showed progressively less appetitive or ingestive tongue movements and progressively more neutral mouth movements both within (an extinction-like effect) and across (an apparent decrease in resistance to extinction) daily test sessions. Conversely, from the same research group, Parker and Lopez (1990) reported that pimozide enhanced aversive reactions to to a 2-min infusion of concentrated quinine solution. Combined, these results suggest that pimozide serves to makes palatable tastes less pleasant and to make noxious tastes more unpleasant.

More recently and consistent with their previous reports, Pecina and Berridge (1997) reported that they could discern sensorimotor effects of pimozide on taste reactivity but not for a hedonic shift in palatability. These conclusions were bolstered by the observation that changes in reactivity patterns induced by pimozide did not appear until several minutes after oral infusion began. They explain the previous discrepant results from the Parker laboratory on differences in procedural factors. It has been due to these and related results that have led Berridge (1996) to conclude that dopamine systems do not mediate hedonic or aversive palatability and that dopamine suppression does not produce a condition of anhedonia.

2.11 New conceptions of the role of dopamine in food reward

Brain substrates of wanting and liking

In terms of food reward Berridge (1996) has gone further to propose separable neural substrates of components of food reward. These components represent distinguishable psychological functions which have heretofore been masked by the umbrella construct of food reward. Reward can be separated into "liking" (pleasure/palatability) and "wanting" (appetite/incentive motivation). Berridge believes these can be manipulated and measured separately. Liking and Wanting seem to have separable neural substrates. Mediation of "wanting" related to food reward has been attributed to mesotelencephalic dopamine systems and divisions of the nucleus accumbens and the amygdala. These systems and structures are not construed as critical to the mediation of liking related to food reward (Berridge, 1996). Although elegant, this theory derives much of its data from a single paradigm; The taste reactivity Paradigm (Grill and Norgren, 1978) which allows the study of initial affective or hedonic reactions to food. However, the theory does not fully acknowledge the advances in pharmacological techniques which have disclosed evidence as to the functional and neuroanatomical heterogeneity of dopamine receptor subtypes (see Chapters 4, 5 and General Discussion).

No role for dopamine in the ascription of relative reward?

The contrast effect paradigm

As has been made clear throughout the introduction, animals can assign different appetitive values to a stimulus as a function of their internal states at: a) the time the stimulus is encountered and b) as a function of their experience with the stimulus (Dickinson and Balleine, 1994). An example of this point can be taken from the food reward literature where a given concentration of sucrose solution may lead to an exaggerated increase or decrease in licking behaviour, depending upon the relative concentration of a previously presented sucrose solution. This exaggeration in responding is referred to as a contrast effect (Flaherty et al., 1982 and see Chapter 7). Incentive relativity or contrast effects may occur in many different paradigms and take several forms (see Flaherty, 1996).

Most pertinent to the theme of the thesis is the phenomenon of successive contrast which can be either negative or positive. In the case of negative contrast, this phenomenon is expressed behaviourally as a decrement in goal-directed behaviour (i.e. licking or runway behaviour) in response to a reward downshift, when compared to unshifted controls. Such a decrement in behaviour is often accompanied by increases in activity and indicators of a stress reponse (i.e. corticosterone, see Flaherty, 1986). Although this phenomenon appears simple, it is more correctly viewed as a chain of consequential psychological processes, such that, in the first place, a sensory detection of reward change is necessary followed by the evaluative comparison of the current reward with the memory of past rewards. At the next stage, a search for the missing reward is employed if the new reward is valued less than the previous one. This stage is followed by a stress/emotional response if the original reward is not obtained and eventually, recovery from contrast ensues (Flaherty, 1996). Selective drugs may selectively affect a link in this chain and a role for dopamine cannot easily be ignored due to the apparent plurality of participation of this neurotransmitter in aspects of reward and reinforcement.

Therefore, it is surprising that in the few studies which have attempted to influence contrast effects by blocking dopaminergic neurones with dopamine antagonists results have been negative, (Flaherty et al., 1992) and authors such as Flaherty have concluded that dopamine receptor blockers may affect the absolute but not the relative value of sucrose (but see Kentridge and Aggleton, 1993; Phillips and LePiane, 1986; Royall and Klemm, 1981 and Chapter 7).

Summary

It seems from the evidence above that there remain unanswered questions, at least with regard to the dopaminergic mediation of *relative* reward. Both Berridge and Flaherty see little noteworthy evidence to suggest that dopaminergic substrates are involved in the hedonic evaluation of stimuli but this is not a rigid conclusion as the work of Parker et al., (1990) illustrates. What gives a certain flexibility to the debate is an appreciation of the functional significance of the differences between dopamine receptor subtype populations in terms of anatomy, distribution and location. Flaherty, (1992), used antagonist compounds which were non-selective for dopamine receptor subtypes, hence, there is far more scope within such a paradigm to investigate the contribution of distinct receptor subtype populations to the mediation of incentive relativity.

2.12 Licking Microstructure: Models, methodology and applications

It is clear from the preceding sections of this Chapter that it has become possible to go beyond total intake as the basic metric in food reward research. By virtue of the many technological and methodological advances in this area, it is now feasible to examine the components of ingestive behaviours of animals at a very detailed level. Collectively, these techniques are called the microstructural analysis of ingestive behaviour. In the case of licking behaviour the data recorded are the onset times of each lick made by the rat during the test session. These data are obtained from an electronic lickometer which connects to a computer interface where the data are collected during the test session and are stored for later analysis.

For many years it has been appreciated that licking behaviour of rats ingesting a liquid diet consists of a series of rapidly occuring rhythmic tongue movements at a rate of 6-7 licks per second (Stellar and Hill, 1952). These licks occur in bursts and clusters of bursts (Davis and Levine, 1977; Davis, 1989), (see Figure 2.2) The size of bursts of licking was originally construed as number of licks that occur before a pause greater than 250msec occurs. The distribution of interlick intervals which fall into this range (constituting the within burst ili distribution) are fairly invariable with a small standard deviation and have led researchers to conclude that this reflects the operation of a motor pattern generator which may be found in the hypoglossal nucleus (Wiesenfield et al 1977). The size of these bursts are typically 3- over 100 licks (Davis, 1989).

These bursts of licking are separated by two types of pause each with its own characteristic frequency distribution and descriptive statistics (also see Section 2.10). The first type is brief, generating an ILI in the range of 250-500 msec. These ILIs have a symmetrical distribution, with an average of about 330 msec and are collectively named interburst intervals (IBIs) (Davis and Levine, 1977; Davis, 1989). Bursts which are separated by these types of pauses are combined by adding them together. Consequently, these groups of barely separated bursts may be called clusters. The second, longer types of pause separate these clusters and so are called (ICIs). These pauses are characterised by a skewed frequency distribution, ranging from 500msec to 100secs or more, with about half of them in the range of 0.5 to about 3 sec, (Davis and Levine, 1977; Davis, 1989).

Lick data are assembled into bouts depending on the assumptions underlying the definition of a bout. In early work, Davis and Levine, (1977) grouped licks into bursts (separating ILIs, </=250 ms) and clusters of bursts

(separating ILIs, </=500 ms) for the purposes of analysis. However, when the model was extended the primacy and utility of clusters in assessing palatability was highlighted (Davis and Smith, 1992). In the Experiments in this thesis we have chosen a value between the range of these ILI's as work from Morris and Cooper, (1993) has shown that an upper ILI specification of 400 ms as this interval was just longer than the break point in a log survivor plot of ILIs.

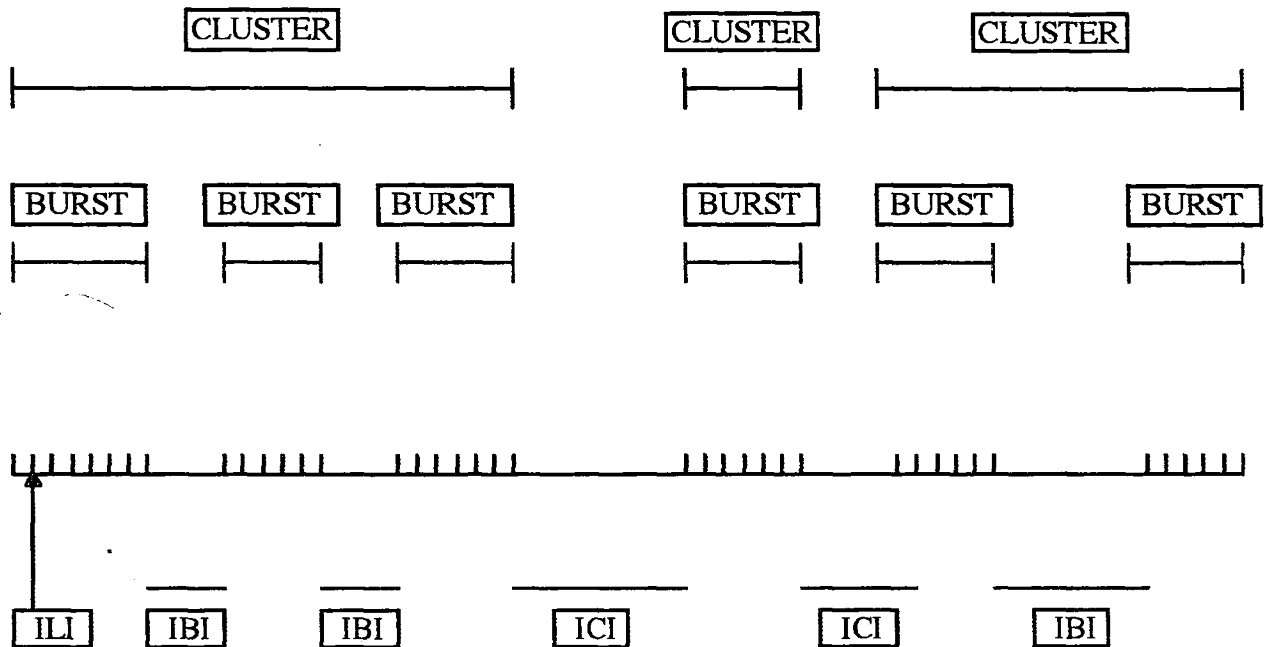


Figure 2.2 A schematic illustration of the temporal pattern of licking by a rat ingesting a liquid diet. The vertical lines represent individual licks and the intervals between them are interlick intervals (ILIs). The horizontal lines define graphically the terminology used within this paradigm.

Bursts are runs of licks separated by ILIs</= 250ms, Clusters are runs of licks separated by ILIs</= 500ms, Interburst intervals (IBIs) are the intervals between bursts (ILIs > 251ms and </=500ms), and Intercluster intervals are the intervals between clusters (ILIs > 500ms).

Adapted from Davis and Levine, (1977)

A model for the control of ingestion: Predictions and validations

In some cases the development of techniques which analyse ingestive behaviour at this level have been heavily influenced by models of the control of ingestive behaviour. One such model is that of Davis and Levine (1977) and has been developed more recently to accommodate a considerable corpus of empirical results on licking behaviour in several experimental contexts (Davis and Smith, 1992).

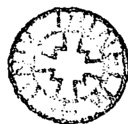
1) Predictions

It has been demonstrated repeatedly that taste, postingestive feedback, physiological state and prior experience interact to influence the amount consumed during a meal. What remains unclear is the extent to which these variables exert their influence during a meal. Also, what are the neural mechanisms underlying the modulatory effects of these variables? Davis and Levine (1977) formalised these questions and devised a predictive model of the control of ingestion of carbohydrates under real-feeding conditions. Results from their studies into the control of ingestive behaviour highlight at least two variables which are important to such control. One variable is the orosensory information which is proposed have a stimulatory effect on ingestion while the other is post-ingestive information which counteracts this stimulation and reduces intake. The model makes appropriate predictions about how manipulation of these two variables should affect the rate of ingestion.

The first prediction is that the initial rate of ingestion of a solution reflects the hedonic evaluation or palatability of that stimulus. Secondly, the model predicts that the rate of decline of licking over a period of time is a result of the development of a negative feedback signal arising from the postingestional cosequences of feeding. In an extension of their work, Davis and Smith (1992), have suggested that microstructural analysis of licking behaviour in rats may also provide relevant information concerning the precise role of orosensory and post-ingestive factors in influencing ingestive behaviour.

Rate measurements

However, before discussing these results it is necessary to clarify some of the major constructs of such models, such as the measurement of "rate". Many of



the references to licking or drinking rate are defined in part by the duration of tests used.

The first type of measure is rate of consumption within a bout or the local rate of eating/drinking/licking. As has already been mentioned, the consummatory behaviour within these licking bouts is quite stereotyped. A corollary of this is that the temporal separation of the onset of consecutive licks (or interlick interval), is relatively constant also. Hence, the local lick rate for each licking bout (which is the reciprocal of the interlick interval) is also relatively constant. In short-term experiments, local lick rate dose not seem to be influenced by the chemical nature of the stimulus fluid nor the physiological state of the animal (Davis, 1973). Even highly preferred mixtures (i.e. 3% glucose and 0.125% saccharin, for which it has been observed that, some rats drink amounts equal to their body weight during a 24hr period) does not alter average interlick interval as compared to water (Smith et al., 1980).

The second is the change in rate of licking over the test session (overall lick rate). This parameter can be influenced by the animal's physiological state (i.e. water deprivation) (Davis, 1973) and also by the chemical nature of the stimulus. Davis (1973) observed that overall licking rate increased monotonically with concentration when animals had access to sucrose, glucose and fructose solutions during 30s fluid presentations. If cumulative licking curves are derived, obtained by cumulating the number of licks in each successive minute during the test, they may be fitted by the least squares method to an exponential function. This provides an estimate of the initial rate of licking and of the rate of decline in the rate of licking (Breslin et al., 1996). This measure is often used in longer term tests as it offers the possibility of studying the interaction between orosensory stimulation and post-ingestive negative feedback which naturally occur during free-feeding situations.

2) Validations

In terms of validating the predictions for lick rate, there have been numerous experiments which have shown that initial lick rate increases as a function of increasing carbohydrate concentration (Davis, 1973; Davis and Smith, 1988). Conversely, an aversive stimulus such as quinine produces decreases in the initial lick rate, (Davis and Levine, 1977, Spector and St John, 1998).

The prediction for the decline of lick rate as a consequence of post-ingestive negative feedback signals has also been validated. By adding mannitol to the test

solution, the experimenter effectively slows the rate of clearance of ingested substances from the stomach. This is a result of the slow absorption of mannitol from the intestine and so creates a "false satiety" situation. Following from the prediction, mannitol has a selective effect on the decline of the rate of licking within the test session whilst having no effect on the initial rate of licking (Davis and Levine, 1977; Davis, Smith and Kung, 1995).

Other microstructural variables have been shown to reflect separable components and therefore have been incorporated into the model. For example, bout size (the number of consecutive licks before the criterion pause) has been suggested to reflect stimulus palatability (Davis and Smith, 1992; Davis and Perez, 1993). This is necessarily seen in the measurement of mean bout duration as a function of increasing sucrose concentration (Davis and Smith, 1992). This contrasts with the relationship between the much used measure of total intake and concentration which varies non-monotonically according to an inverted u-shaped function. This function is thought to reflect the accumulation of ingested material in the gastrointestinal tract (Spector and Smith, 1984). Spector and St. John (1998) have shown that when quinine was the stimulus, the substantial decrease in intake was entirely a function of a decrease in lick volume and bout size. Contrary to the intake-suppressing effects of quinine, pause duration decreased and bout number increased. Therefore, it seems that a number of parameters can be usefully examined in the extraction of information concerning variables affecting intake.

The generality of any principles derived from microstructural analyses of ingestive behaviour will ultimately depend on the number of different experimental contexts in which the licking data are collected. The more the predictions of a model are tested (using different macronutrient solutions) the more support may be mustered for the underlying theoretical assumptions. Therefore, there have been several experiments which have attempted to investigate whether the principles of the Davis and Levine model apply when the test stimulus is not a prototypical test solution such as sucrose.

For example, Davis, (1996), compared the results of a microstructural analysis when the test stimulus was Polycose (a complex mixture of mono-, di- and polysaccharides) to other mono- and disaccharides (such as sucrose). Both initial rate of licking and bout size were increasing linear functions of the concentration of Polycose. Similar results have been found with a fat emulsion, Intralipid (Higgs and Cooper, 1996) and the non-sweet tasting polysaccharide Maltodextrin (Higgs

and Cooper, 1998). However, Davis (1995) failed to find a similar result with increasing concentrations of corn oil.

Breslin et al. (1996) used the non-nutritive sweetener, saccharin, to determine whether orosensory stimulation contributed to the magnitude of the negative feedback signal during ingestion. Saccharin concentration was varied and each concentration was added to a standard 0.2M glucose solution. Initial rate of licking increased monotonically over the saccharin concentration range while estimates of the rate of decline of the rate of licking ramained constant over the test session. They concluded that there was no evidence for a orosensory contribution to the decline in the rate of licking. This further validates the discretion of the two separate variables, orosensory stimulation and post-ingestive inhibition, which are thought to exert control over feeding.

<u>Microstructural analysis of drug effects on licking behaviour</u>

The analysis of the temporal distribution of rhythmic tongue movements provides a behavioural description of the activity of the motor system which controls the ingestion of liquids. Variations in the characteristics of this distribution induced by experimental manipulations, such as drug treatment, provide a way of analysing how the drug influences the physiological and neurophysiological mechanisms that control this motor system (Davis, 1989; Davis and Smith, 1992; Asin et al., 1992). Therefore, not only does that model presented above make strong predictions about the variables which might control ingestive responses, but it can also elucidate more about possible motor disruptions thought to be induced by certain drug treatments.

Several drug studies have shown that there may be neural substrates which are particularly responsible for aspects of the control of ingestion. For example, drug effects on sham-feeding led to speculation that CCK and bombesin may be involved in the post-ingestive satiating effects of foods (Gibbs, Geary and Smith, 1993; Smith and Gibbs; 1992). This prediction was validated by microstructural analysis (Stratford, Gibbs and Smith, 1995) which revealed that Bombesin and/ or gastrin releasing peptide decreased intake by decreasing the rate of decay of lickingand decreasing the number of bouts without changing the initial rate of licking or the size of the bouts. In accordance with the hypothesis that drugs which act at benzodiazepine receptors achieve their appetite stimulant effects through an enhancement of palatability (Berridge and Pecina, 1995; Cooper and Higgs, 1996),

Higgs and Cooper, (1997) have shown that drugs which act as agonists at benzodiazepine receptors actually increase initial rate of licking and also increase mean bout duration in a fashion similar to that observed with increasing concentration.

Effects of dopaminergic drugs on the microstructure of licking behaviour: Can we exclude a motor deficit for licking?

There are few studies which have employed the microstructural methodology to study the effects of dopamine receptor subtype blockade or stimulation on components of ingestive behaviour. The first, (Schneider et al., 1990), used raclopride a dopamine D2 antagonist to investigate whether decreases in intake of sucrose solutions and water in sham-feeding tests could be ascribed to: i) a decrease in the positive reinforcing effect of sucrose or ii) to an impairment of licking movements. Raclopride-treated sham-feeding rats decrease their intake of sucrose solutions in the same manner as animals given a sucrose solution of reduced concentration. Schneider et al. (1990) point to their measures of average interlick interval (hence intrabout lick rate) in both groups: they interpret this measure as a reflection of motor capability and have clearly shown that average interlick interval is perfectly normal in both the neuroleptic-treated and reduced concentrations conditions. Indeed, Davis and Smith (1992) validated this measure by manipulating the accessibility of the spout and found that when an animal had to extend its tongue further to make contact with the tube there was a negative relationship with the intrabout lick rate.

The results of Schneider et al., (1990) are echoed in the work of Weatherford et al. (1990). These authors failed to find a difference in intrabout lick rate in neuroleptic-treated and normal rats. Therefore, motor deficit for licking does not seem to be "sufficient to explain the observed patterns of deteriorating commerce with rewarding stimuli", (Wise, 1994, pp.250). This work also reflects results from a number of tests on free feeding which suggest that neuroleptics have effects on the drinking of sweet solutions that parallel the effects of reward-dilution (Bailey et al., 1986; Geary and Smith, 1985; Schneider et al., 1986 a,b; Weatherford et al., 1990; Xenakis and Sclafani, 1981, 1982).

As has already been mentioned, the specification of a motor deficit in response to neuroleptics relies heavily on the doses used: high doses of dopamine antagonists are capable of causing significant motor deficits. There are however, paradigms which have revealed that the threshold doses for "reward" deficits seem

to be well below those for motor deficits. One such method; Reward summation function analysis was adapted from the curve shift paradigm of Edmonds and Gallistel (1974; see Chapter 2). In common with the curve shift paradigm, changes of the reinforcer response function on the horizontal axis are considered reward effects and changes on the vertical axis are considered motor effects. They observed that the D2-type antagonist pimozide reduced the reward value of sucrose without impairing response capacity (Bailey et al., 1986).

Nevertheless, a study by Asin et al. (1992) showed that the catecholamine agonists amphetamine and phenylpropanoline reduced intake primarily by reducing the number of bouts without affecting their size "suggesting a fractionation of the normal pattern of ingestion" (Asin et al., 1992, pp. 415). Therefore, the results did not represent an effect on the palatability of solutions per se. These researchers relied upon motor hypothesis to explain their results.

Among the many experiments which have tried to dissociate dopamine's involvement in motor versus motivational aspects of behaviour are those which have equated the response deficits apparent in operant situations (forelimb response force and duration) with those which are specific for licking (tongue extensions and lick durations). Original studies by Gramling and Fowler, (1984) showed a deficit in orolingual competence which was expressed in the inability of animals to reach fluid cups with their tongues after neuroleptic treatment. Rather than significantly slowing lick rate, neuroleptics influenced animals' licking behaviour so that they appeared only to make contact with the sucrose on approximately every other lick.

Drugs can now be studied in a task emphasising the fine motor details of rats' tongue movements during licking behaviour. Rats are allowed to lap drops of tap water from a force sensing disk. From this behaviour, at least four variables were derived: peak-force of tongue strikes; duration of tongue contact, number of separate tongue contacts in 2 min and the rhythm of the lapping behaviour as quantified by Fourier analysis (Fowler and Mortell, 1992).

Using this lingual motor task, Fowler et al. (1986, 1989, 1990) showed that subcataleptic doses of D2-like antagonist haloperidol produced disruption of forelimb response force and duration in an operant task while, at the same doses, decreasing number of licks, force of tongue protrusion and lick duration while increasing average interlick interval.

Davis and Smith (1992) have showed showed clearly that the mechanisms that control the cycles of extension and retraction of the tongue is influenced by sensory feedback and that these mechanisms can be influenced by experimental manipulations such as varying the accessibility of the drinking spout. In the same way, Fowler and Mortell (1992) note that while lick force was modulated by water drop delivery, this effect was not influenced by haloperidol and therefore suggests that there was no interference of the drug on sensory regulation of licking.

Studying a number of atypical neuroleptics, compounds which have been praised for their lack of encumbant extrapyramidal side effects, Fowler and colleagues have been able to compare the effects of these clinically efficacious compounds to the more traditional neuroleptics such as haloperidol. Lick rhythm was the dependent variable that most clearly distingushed haloperidol from clozapine (Fowler and Das, 1994): haloperidol had little effect on lick rhythm whilst clozapine reduced lick-rhythm in a dose-dependent manner (Das and Fowler, 1995). Also, within-session decrements in behaviour previously reported for haloperidol in the lick task were not produced by either clozapine or olanzapine (Das and Fowler, 1996) further driving the conceptual wedge between typical and atypical antipsychotics.

Summary

It is clear from the literature that it is both conceptually and empirically important to investigate the neural substrate for dopamine agonist and antagonist-induced changes in feeding behaviour. Evidence from many feeding paradigms; not least the microstructural methodology and taste reactivity test have been inconclusive. This is especially apparent in terms of a precise role for dopamine in motivational rather than exclusively motor apsects of behaviour. A specific role for dopamine receptor subtypes in licking behaviour has not yet been well established.

2.13 Aims of the thesis

As has been made clear throughout the introduction, both dopamine agonists and antagonists reduce the ingestion of several foods. What remains unclear is the specific way in which such compounds achieve these effects. The primary aims of this thesis are to compare a selection of drugs selective for dopamine receptor subtypes (both within and between pharmacological "super-families") on their ability to affect feeding parameters. By coupling pharmacological manipulation with the examination of licking behaviour at a very detailed level, it is possible to uncover some of the bases of the neural controls of ingestive behaviour. Further, extant models of the control of ingestion offer the possibilty of formulating hypotheses as to the effects of dopamine agonists and antagonists on particular licking parameters. Microstructural analysis of licking can therefore provide detailed information as to the components of ingestive behaviour which may be affected by these dopaminergic compounds.

The experiments reported in Chapter 4 (Experiments 1, 2 and 3) used microstructural analysis to examine whether a putative dopamine D3 receptor agonist, 7-OH-DPAT , a mixed D2/D3 receptor agonist, quinpirole and a D2 receptor antagonist, raclopride would differ in terms of the behavioural expression of anorexia in a 20-min test session. A further aim of the studies in Chapter 1 was to clarify whether these decreases in ingestive responses were due to decreases in licking parameters which most robustly reflect hedonic evaluation or palatability (i.e. mean bout duration and initial rate of licking).

Chapter 5 (Experiments 4, 5 and 6) formed a set of follow-up experiments to Chapter 1. The main aims of this set of experiments were firstly, to further compare a D1 antagonist (SCH- 23390) to the D3 receptor agonist 7-OH-DPAT and the D2 receptor antagonist raclopride in the microstructural paradigm using brief contact tests. The test session was shortened in order to increase its interpretive strength with regards to palatability. Secondly, an additional tastant was used in the form of Intralipid in order to further generalise the principles of the control of ingestion (Davis and Levine, 1977) to other macronutrient solutions. Finally, motoric effects of the compounds used in these experiments were considered and measures were taken in order to assess these (i.e. average interlick interval and intrabout lick rate).

The sixth chapter comprises a set of experiments (Experiments 7, 8 and 9) which aimed to test the pharmacological specificity of the effects of the D3 receptor agonist 7-OH-DPAT on licking parameters using microstructural analysis. In experiment 7, two doses of the selective D3 receptor agonist PNU 99194A were used, and in Experiment 8, low doses of the benzamide derivative amisulpride, were used to attempt to abolish the effects of 7-OH-DPAT on licking parameters. The results of Experiment 8 suggested a hyperphagic effect of higher doses of amisulpride. Therefore, a longer test (20-min) was used to test this prediction in drug-naive rats.

Chapter 7 contains one experiment (Experiment 9), the aims of which are related to the results from several preceding chapters (Experiments 1, 4, 7 and 8). The D3 receptor agonist 7-OH-DPAT seemed to be decreasing mean bout duration in both experimental contexts (short and long test sessions). This result can be interpreted as an effect of this compound on the palatability of solutions available to the animal. Therefore, 7-OH-DPAT may be seen to be affecting the initial hedonic evaluation of the stimulus. Hence, a contrast effect paradigm was used in order to further assess the ability of 7-OH-DPAT to affect the ascription of relative rather than absolute reinforcer value to a stimulus.

The general aims of this thesis are to futher outline a role for dopamine receptor subtypes in the mediation of ingestive behaviour and to present new evidence concerning the behavioural effects of the D3 receptor agonist 7-OH-DPAT in comparison to more traditional dopamine receptor agonists and antagonists. Further, the empirical evidence gathered for this thesis will be discussed in the light of contemporary theory in food reward research in order to assess the theoretical contribution of the experiments presented herein.

Chapter 3: General methods

3.1 Animals

The animals used in all of the experiments were adult male hooded Lister rats supplied by Charles River (Margate, UK). The body weights of animals at the time of testing were in the range 350-500g. Laboratory food pellets (SDS RMI (E), Essex,U.K.) were available for all animals on an ad libitum schedule, unless otherwise stated in particular experimental procedures. They also had access to water at all times. Animals were housed in pairs in plastic and metal cages and were tested in the experimental apparatus described below, to which they had been familiarised. Animals were maintained on a 12h light:12h dark cycle with lights on at 08.00h. Testing always took place between 09.00h and 18.00h (light phase). All experiments were carried out in the Life Sciences Support Unit, Durham University.

Caveat

The experiments contained in Chapters 4, 5 and 6 employ experimental designs which necessitate the re-use of animals for different drug doses (Experiments 1-6) or for different drugs (Experiments 7-9). This practice represents a conventional compromise in the field of behavioural pharmacology, whereby it is not unusual to re-use animals when training of animals is time-consuming and/or if the number of animals needed to avoid repeated drug administration becomes prohibitively great. The re-use of animals involves leaving a minimum period of 48 hr to allow the drug to be "washed-out" of the animal. However, the use of a wash-out period does not exclude the possibility of the development of tolerance or sensitisation to the drug. However, there is no evidence from this laboratory or from Professor Cooper's laboratory in Birmingham that baseline ingestive behaviour is altered and/or that drug-induced responses are diminished or enhanced by repeated drug treatments or repeated presentations of stimuli such as sucrose.

3.2 Drugs: Preparation and administration

Dopamine receptor agonists

(+-)7-OH-DPAT (7-hydroxy-2-(di-*n* -propylamino)tetralin) (Research Biochemicals International, UK and Sigma-Aldrich Co. Ltd, U.K.). 7-OH-DPAT

is a mixed dopamine D2/D3 receptor agonist which was dissolved in saline and injected (i.p.) 20 minutes before the start of testing. Details of preparation and administration, as well as an injection-test period of 20 minutes were chosen as previous work has shown this to be adequate time for a drug effect on intake to become apparent (Sokoloff, 1990; Sanger et al., 1993).

(-)-Quinpirole HCl (LY 171555) (Research Biochemicals International, UK) Quinpirole is also a mixed dopamine D2/D3 receptor agonist which was dissolved in distilled water and injected (s.c.) 20 min before the start of testing. Doses of quinpirole, injection-test times and route of administration were chosen based on previous feeding studies with this compound (Zarrindast et al., 1991; Cooper and Al-Naser, 1993).

Dopamine receptor antagonists

S(-)-Raclopride L-tartrate (Research Biochemicals International, UK). Raclopride is a dopamine D2 receptor antagonist which was dissolved in saline and injected (i.p.) 20 minutes before the start of testing. Doses of raclopride and injection-test time as well as route of administration were chosen on the basis of previous feeding studies (Phillips et al., 1991a, b) and from results from Professor Cooper's Birmingham laboratory.

R(+)-SCH-23390 ([R]-7-chloro-8-hydroxy-2,3,4,5-tetrahydro-3-methyl-1-phenyl-1H-3-benzazepine) (Research Biochemicals International, UK). SCH 23390 is a dopamine D1 receptor antagonist which was dissolved in sterile water and was injected (i.p.) 30 minutes before the start of testing. Injection-test time and route of administration were chosen on the basis of those used in previous studies (i.e. Gilbert and Cooper, 1985; Terry and Katz, 1992). Doses were chosen on the basis of previous studies on feeding and pilot studies conducted in our own laboratory.

PNU99194A (5,6-dimethoxy-2-(dipropulamine)indian-hydrochloride), was a gift from Dr. K. Svensson (Gothenburg, Sweden). PNU 99194A is a D3 receptor antagonist which was dissolved in saline and injected (i.p.) 30 minutes before the start of testing. Injection-test times were chosen as these had previously been shown to be adequate for a behavioural response to take effect (Waters et al., 1993). Choice of doses was limited as only a small amount of the drug was made available to our laboratory.

Amisulpride is a benzamide derivative and was a gift of Dr. David Sanger (Synthelabo, France). Amisulpride is a mixed dopamine D2/D3 receptor antagonist which was dissolved in ionised water with two drops of Tween (B.D.H Chemicals Ltd., Poole, UK) to aid suspension. This suspension was injected (i.p.) 60 minutes before the start of testing. Doses of amisulpride were chosen on the basis of its ability to block 7-OH-DPAT in an operant paradigm (Sanger et al., 1997). The route of administration and injection-test period was taken from those reported by Sanger et al., (1997).

In all cases, the injection volume was 1ml/kg.

It is of critical importance to note that the timing of drug injection-test is not an arbitrary matter, as the peak effect of a drug treatment can differ significantly depending on many physiological and pharmacological factors. Drug pretreatment times for the experiments presented in this thesis have been chosen on the basis of work from Professor Cooper's laboratory in Birmingham and also from reports cited above, wherein the time course of the behavioural effect of drugs used has previously been identified. These issues of the time course of drug actions are of particular importance when criterion test periods have theoretical implications (e.g. 1 min and 20 min licking tests- see Chapter 2) and therefore have a bearing on the interpretation of results.

3.3 Test Apparatus and Data Collection

In order to analyse licking behaviour, rats were trained to lick for fluids and the licks were individually logged and successive inter-lick intervals calculated. There were two types of test which differ in their duration and the apparatus used:

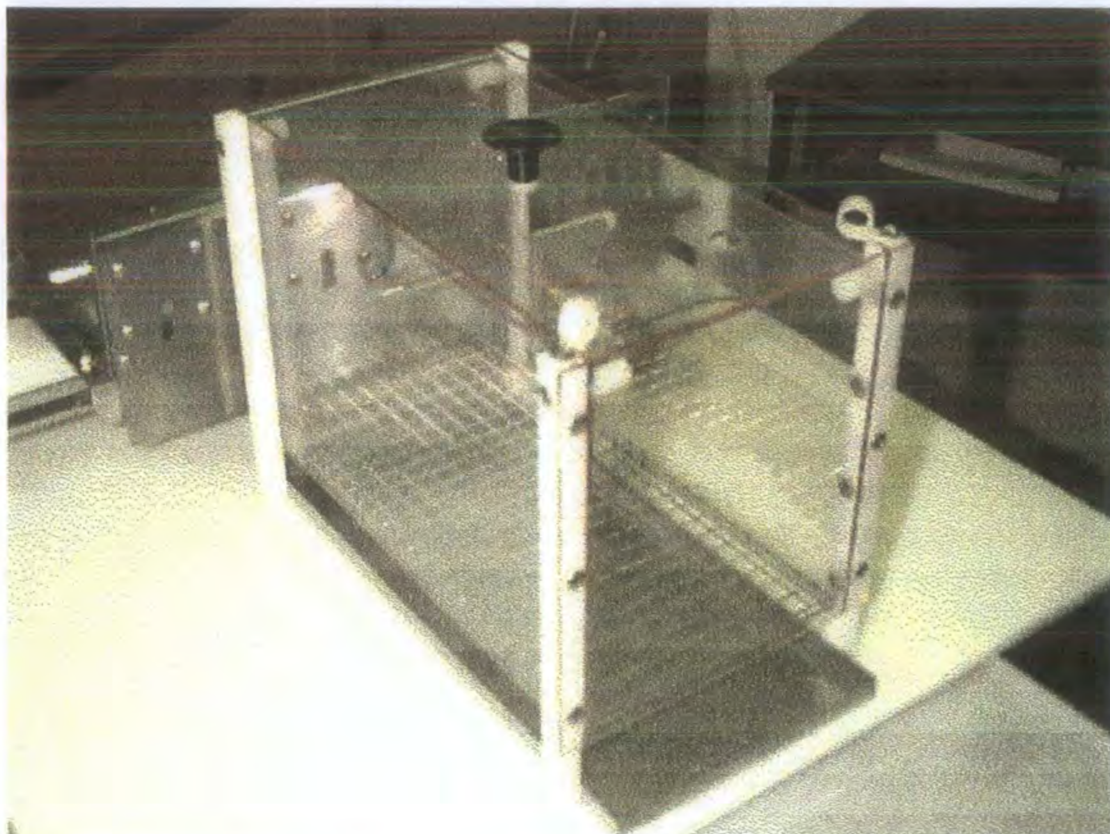


Figure 3.1 The MS80 lickometer

The brief contact tests (i.e. tests of 1-3 minutes) were carried out using the MS80 multistation lick analysis system (Dilog Instruments, Talahassee, Florida). Animals were placed in a perspex chamber which was approximately 16 cm wide, 30 cm long and 20 cm tall. A perspex lid with four small ventilation holes secured the chamber. The floor was a stainless steel grid made up of thin metal bars spaced 1 cm apart. An opening in the centre of the front face of the chamber allowed animals access to a drinking spout which was located 85mm from the grid floor of the chamber. The drinking spout was located 3mm behind the wall so that licking from the spout required a tongue protrusion through another small aperture. A motorised shutter could impede access, allowing drinking from the spout to be carefully controlled. This was to ensure the exclusive measurement of licks rather than contacts with the spout from paws or snout. Bottles containing test fluids were mounted on a motorised metal platform allowing the experimenter to determine whether the platform should move backward or forward. This allowed any bottle to be positioned in front of the drinking slot for a time allotted by the experimenter and controlled by the computer via a reversible motor. The experimenter could also manipulate the order and duration of presentations as well as the interpresentation intervals.

The lickometer was connected to an amplifier that passed less than 60 nA through the rat when tongue contact was made with the drinking spout. This current was fed to a computer which stored the time of each lick to the nearest millisecond. Data from brief contact sessions were initially collated using the (MS 80) (John D. Davis, Nyack, NY, U.S.A.) data collection program and analysis of the lick time data was performed using Dilog software (Ross Henderson, Dilog Instruments; Talahassee, FL, U.S.A., 1994) followed by further processing using a Microsoft Excel spreadsheet.

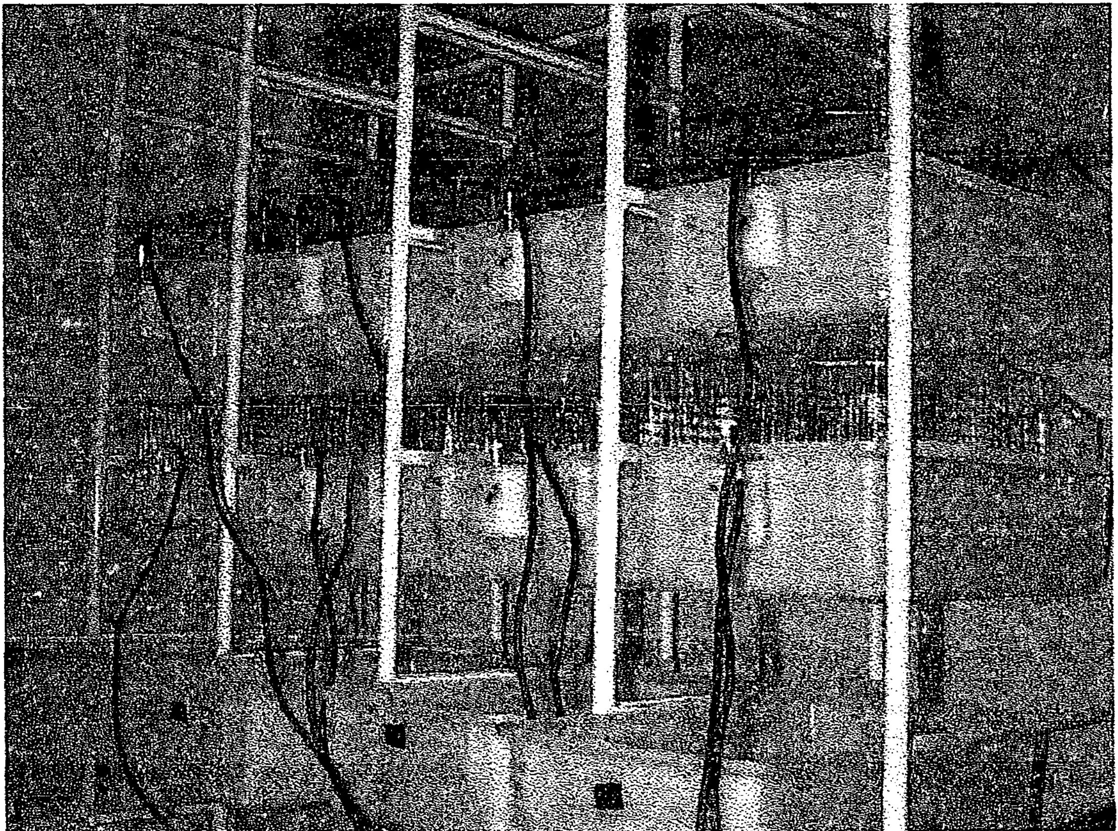


Figure 3.2 The contact 108 lickometer

Tests of longer duration (i.e. 10-20 minutes) took place in the Contact 108 lickometer apparatus (John D. Davis, Nyack, NY, U.S.A.). As this piece of apparatus had eight testing stations or lickometer boxes it was possible to test up to 8 animals simultaneously. The eight test chambers were modified home cages (i.e. plastic and metal cages) fitted at the front of the cage with a small aperture through which the animal had to protrude its tongue in order to access the drinking spout. Stainless steel drinking spouts were attatched to plastic calibrated tubes (50ml) through rubber bungs. These tubes containing test solutions were put in place by

the experimenter at the beginning of each test session rather than using computer controlled presentation. Again, each tongue contact with the spout completed a circuit and was recorded as a lick to the nearest millisecond.

Data recorded from the Contact 108 apparatus were first collated by the ILI8 (John D. Davis, Nyack, NY, U.S.A.) data collection program. Licking rate data was analysed by a collection of programs (Quicklick, John D. Davis, Nyack, NY,1990), or Dilog software (Ross Henderson, Dilog Instruments; Talahassee, FL, U.S.A., 1994) followed by further processing using a Microsoft Excel spreadsheet program. The creation of these programs was based on observations and assumptions about the nature of the licking behaviour of the rat ingesting a liquid diet (see Davis and Smith, 1992). Further analysis was performed using the Feed6 analysis program (David Barton, 1996) and a Microsoft Excel spreadsheet program.

3.4 Test fluids

Sucrose solutions

Sucrose solutions (granulated cane sugar, Tate and Lyle, London, U.K.) of varying concentrations (ranging between 1% and 32%) were made up weight/volume each day using tap water (during both training and testing).

Intralipid emulsions

Intralipid (Pharmacia; Milton Keynes, U.K.) was in the form of a commercially prepared 20% emulsion. This emulsion consists of fractionated soya bean oil, fractionated egg phospholipids, and glycerol. For a 20% solution it has a caloric value of 200 kcal per 100 ml. Several concentrations of Intralipid were made up by dilution with tap water (during both training and testing).

3.5 Experimental design and statistical analysis

Details of further statistical analysis and individual experimental designs are described in the Method sections of appropriate experimental Chapters, (i.e. Sections 4.2, 5.2, 6.2 and 7.2).

Chapter 4: Microstructural analysis of drinking for sucrose after the administration of 7-OH-DPAT, quinpirole and raclopride

4.1 Introduction

Results from preference tests have suggested that D-2 type antagonists such as pimozide reduce intake of sucrose solutions by decreasing the reinforcing potency of the solution without producing a motoric deficit (Bailey et al., 1986; Towell, 1987). However, alternative methodologies such as taste reactivity studies have revealed that the profile of initial responses to hedonic and aversive stimuli remain unchanged after the administration of several dopaminergic compounds (Treit and Berridge, 1990). In the light of such inconsistencies, considerable effort has been directed towards formulating hypotheses to explain neuroleptic-induced decreases in ingestion. In response to criticisms of total intake measures, many researchers have analysed the effects of dopaminergic drugs on *components* of ingestive behaviour.

For example, it has been shown that dopamine receptor agonists and antagonists of the D-1 and the D-2-type, reduce the ingestion of sweet solutions in gastric-fistulated rats (See Chapter 2). Therefore, it is inferred that the inhibition of sham intake is not due to the ability of dopamine receptor antagonists to increase the post-ingestive, negative feedback of the sucrose solutions. One hypothesis offers the possibility that dopaminergic compounds decrease the hedonic value or palatability of the stimulus (Smith, 1996). Another is that dopaminergic drugs induce motoric deficits which interfere with the ability to approach and/or consume accessible food sources (Salamone, 1992, 1994).

An appropriate model with which to test these hypotheses is the taste reactivity paradigm (see Chapter 2). Taste reactivity studies purport to measure the initial affective reactions to tastants (i.e. their palatability) while circumscribing the problems of possible motor deficits which may be preventing animals from approaching a drinking spout. Within this paradigm, a number of dopamine antagonist have been tested but results from such studies are far from consistent. For example, Berridge et al. (1989) found no evidence that sweet solutions became less palatable or that bitter solutions became more aversive in animals with extensive damage to their brain dopamine systems, achieved by lesioning the nigrostriatal

dopamine system with 6-OHDA. Even when dopamine was depleted by over 95% after combined lesions of the neostriatum and accumbens there did not appear to be any shift in the hedonic or aversive reaction patterns (Berridge and Robinson, in press, reported by Pecina and Berridge, 1997). Drugs which block dopamine receptors produced a similar result. Treit and Berridge (1990) discovered that haloperidol pretreatment failed to reduce hedonic reactions or increase aversive reactions to a 1-min infusion of sucrose or quinine.

In contrast, Leeb et al. (1991) found decreased sensitivity to sucrose in the same paradigm using pimozide (the D2-type antagonist) treated rats. Parker and Lopez (1990) reported that pimozide enhanced aversive reactions to a 2-min infusion of concentrated quinine solution. Combined, these results suggest that pimozide serves to makes palatable tastes less pleasant and to make noxious tastes more unpleasant. More recently and consistent with their previous reports, Pecina and Berridge (1997) reported that they could discern sensorimotor effects of pimozide on taste reactivity but they did not detect a hedonic shift in palatability.In the light of the conflicting results from taste reactivity studies, it is still unclear whether dopamine receptor subtypes have a specific contribution to make to the mediation of palatability.

Alternative methodologies may be employed in order to assess the palatability of tastants. It is clear fom the Chapter 2 that analysis of the rate and microstructure of ingestive behaviour may provide accurate "on-line" information concerning the factors responsible for controlling food and fluid intake (Davis and Levine, 1977; Davis and Smith, 1992). For example, manipulating concentration of ingested fluids produces quite specific effects on the rate and microstructure of licking for carbohydrates. The initial rate of licking and mean bout duration have been shown to vary monotonically with increasing concentration of carbohydrates (Davis et al., 1973; Davis and Levine, 1977; Davis and Smith, 1992), while the rate of decline of licking over the test session is thought to provide an estimate of the increasing influence of inhibitory post-ingestive behaviour (Davis et al., 1975; Davis and Levine, 1977). Therefore, microstructural analysis provides a comprehensive framework in which to study the effects of pharmacological compounds on several aspects of ingestive behaviour.

There are few studies which have applied these microstructural techniques to the study of the effects of dopamine agonists and antagonists on the control of ingestive behaviour. There are some reports on the microstructure of feeding in response to a D2 agonist (i.e. quinpirole). A study by Al-Naser, (1993) revealed

that quinpirole reduced intake in much the same way as another D2 receptor agonist N-0437 by decreasing the local rate of eating which indicated a common mechanism of action for the two compounds. This result is, however, in contrast to that of Clifton et al. (1989) who found a biphasic effect of N-0437 without the significant reduction in local rate of eating (although eating rate was slightly reduced).

Analysing the microstructure of licking, Asin et al. (1992) showed that the catecholamine agonists amphetamine and phenylpropanoline reduced intake primarily by reducing the number of bouts without affecting their size "suggesting a fractionation of the normal pattern of ingestion" (Asin et al., 1992, pp. 415). This investigation did not reveal an effect of dopaminergic drugs on the palatability of solutions per se. These researchers evinced a motor hypothesis in explaining their results and the similarities that were found between the effects of the two catecholaminergic drugs on licking behaviour were taken to reflect a common neurotransmitter mechanism.

Employing the sham-feeding preparation and a more specific D2-type antagonist, Schneider et al. (1990) showed that when the intake of a 10% sucrose solution was reduced by pretreatment with raclopride to the intake of a 5% sucrose solution (without raclopride treatment) microstructural licking patterns and the rate of licking observed under the two conditions were not significantly different. Therefore, the behavioural effects of raclopride were equated with the effects produced by a sucrose solution of a lower concentration. Further confirmation of a reduction in hedonic potency comes from the analysis of microstructural variables such as mean bout duration. Raclopride and dilution of a sucrose solution by 50% produced comparable decreases in mean bout duration which can be interpreted as a decrease in palatability. A deficit in oromotor movements was excluded through analysis of licking rate under the two conditions.

The results from Asin and Schneider go some way to highlighting the different behavioural effects on the same dependent variable which may be attributed to the mode of action of the drugs used. Thus, these studies highlight the importance of testing compounds which are selective for pharmacologically and neuroanatomically discrete dopamine receptor populations within the same paradigm. As the number of dopamine receptor subtypes has recently burgeoned (Sokoloff et al., 1990) and compounds which are specific for these subtypes have developed, it is possible to examine the contribution of these "new" receptor subtypes to the decrease in intake after the administration of both dopamine agonists

and antagonists. For example, much of what is known about the functional specificity of the D3 receptor has come from behavioural studies using 7-OH-DPAT. Indeed, it is with this compound that the D3 receptor has become implicated in the control of ingestive responses (Gilbert and Cooper, 1995).

However, there is much debate as to whether 7-OH-DPAT is acting at post-synaptic D2 receptors, D3 autoreceptors or post-synaptic D3 receptors (see Chapters 1 and 2). Quinpirole, although traditionally labelled as being a D2 selective agonist has recently been described as having affinity for the D3 receptor (Sokoloff et al., 1992). Therefore, one of the aims of this study was to determine whether the behavioural expression of the anorexia induced by dopamine based anorectics was the same or different depending upon the receptors they purport to stimulate (i.e. within the D2 family). Although raclopride has been studied using microstructural analysis (Schneider et al., 1990), the effects of quinpirole and 7-OH-DPAT on ingestive responses have not been examined in such detail. Nevertheless, these compounds have been shown to decrease ingestive behaviour. (Inoue et al., 1992, Gilbert and Cooper, 1995).

The aim of this study was to examine the similarity of effect on licking responses within this class of dopamine selective drugs. The prediction was that changes in microstructural variables induced by 7-OH-DPAT would be more similar to those induced by quinpirole than those induced by raclopride in this paradigm due to their similar modes of action (i.e. putative autoreceptor agonists vs. postsynaptic antagonist). The experiments reported in Chapter 1 are original studies of the effects of 7-OH-DPAT and quinpirole on licking microstructure (Experiments 1 and 2). The effects of raclopride on licking microstructure has already been conducted in other laboratories (Schneider et al., 1992) but is included in this chapter to serve as a comparison (Experiment 3).

Due to the conflicting evidence from taste reactivity studies, an integral purpose of the study was to examine microstructural variables in order to clarify whether observed changes after dopaminergic drug administration reflected variation in those parameters which most robustly reflect palatability (initial rate of licking and mean bout duration), a change in the rate of decay of licking (reflecting satiation) or a fractionation of the normal ingestive response (as reflected in the number of bouts).

4.2 Method

4.2.1 Animals

Seventy two non-deprived adult male hooded Lister rats weighing 350-400g at the time of testing were used. They were housed and maintained as described in Chapter 3. These animals constituted three groups of 24 subjects per experiment: differing in the type of drug treatment which was administered before testing (i.e. 7-OH-DPAT, quinpirole or raclopride).

4.2.2 Drugs

In Experiment 1, 7-OH-DPAT (Research Biochemicals International, UK), was dissolved in saline and injected i.p., 20 min before testing. Doses of 7-OH-DPAT were 0.1, 0.3 and 1 mg/kg or its vehicle. For the purposes of Experiment 2, quinpirole (Research Biochemicals International, UK) was dissolved in distilled water and injected s.c., 20 min before the start of testing at doses of 0.03, 0.1 and 0.3mg/kg or vehicle. In experiment 3, raclopride (Research Biochemicals International, UK), was dissolved in saline and injected i.p., 20 min before testing at doses of 0.03, 0.1 and 0.3mg/kg or vehicle. All details of drug preparation and administration for each drug can be found in Chapter 3.

4.2.3 Test Fluids

The three test solutions were 1, 3 or 10% sucrose solutions made up as described in Chapter 3. Groups were trained and tested on one of the sucrose solutions.

4.2.4 Apparatus

Testing was carried out using the 108 multistation lickometer described in detail in Chapter 3.

4.2.5 Procedure

Training

Each group of twenty four animals used in each of the first three experiments were randomly allocated to groups of eight. The allocation was based on the concentration of sucrose to which the group had access during training and testing (i.e. either 1, 3 or 10% sucrose). Each group was familiarised with the test apparatus and solutions for approximately 10 days or until baseline drinking levels were stable. Training consisted of 20-min access to the sucrose solution at the same time on each training day. Two days prior to testing animals received a sham injection of vehicle in order to familiarise them with the injection procedure.

Testing

Experiment 1: Microstructural analysis of drinking for sucrose after the administration of 7-OH-DPAT : Analyses of licking during a 20-min session and during first minute of 20-min session.

Following training, either 7-OH-DPAT (0.1, 0.3 and 1.0 mg/kg) or vehicle was administered i.p., 20-min before the rats were placed in the lickometer boxes where they had access to either 1, 3 or 10% sucrose solutions. Sucrose solutions were presented for a total test duration of 20-min. The study employed a mixed design with fluid concentration as the between-subjects variable and drug dose as the within-subjects variable. Injections were counterbalanced using a Latin Square design and there was a 48h time lapse between injections to avoid carry-over effects.

Experiment 2: Microstructural analysis of drinking for sucrose after the administration of quinpirole : Analyses of licking during a 20-min session and during first minute of 20-min session.

Following training, either quinpirole in doses of 0.03, 0.1 and 0.3 mg/kg or vehicle was administered s.c., 20-min before the rats were placed in the lickometer boxes where they had access to either 1, 3 or 10% sucrose solutions for a total of 20-min. The design of the study and the injection procedures are as described for Experiment 1.

Experiment 3: Microstructural analysis of drinking for sucrose after the administration of raclopride: Analyses of licking during a 20-min session and during first minute of 20-min session.

Following training, either raclopride in doses of 0.03, 0.1 and 0.3 mg/kg or vehicle was administered i.p., 20-min before the rats were placed in the lickometer boxes where they had access to either 1, 3 or 10% sucrose solutions for a total of 20-min. The design of the study and the injection procedures are as described for Experiments 1 and 2.

4.2.6 Design and statistical analysis

Initial analysis of the lick time data was carried out using programs as outlined in Chapter 3. In Experiments 1, 2 and 3 the change in lick rate across the session was also calculated and an exponential decay function of the form $y = Ae^{(-Bt)}$ was fitted by a least squares method to the rate of licking data from each experiment. This was done using the Sigma Plot graphics programme (Jandel Corp. Rafael, CA, 1986). Other measures derived from the raw data included total intake (ml) and a number of microstructural variables: Number of licks, mean bout duration (s) and number of bouts.

Experiments 1, 2 and 3 each employed a mixed design with fluid concentration as the between-subjects variable and drug dose as the within-subjects variable. Therefore, intake measures and microstructural variables from each experiment were analysed using a two-way ANOVA with a between-subjects factor of concentration and a within-factor of drug dose. If there was a statistical main effect of drug dose in the absence of a significant interaction between drug dose and fluid concentration, post hoc comparisons to determine any significant differences between doses and vehicle controls were made using Dunnet's t-test for each concentration. Statistical tests were performed using Super Anova and Statview SE+graphics (Abacus Concepts Increase., Berkeley, CA). A result was considered statistically significant if $p<0.05$.

4.3 Results

Experiment 1: Microstructural analysis of drinking for sucrose after the administration of 7-OH-DPAT : Analyses of licking during a 20-min session and during first minute of 20-min session.

a) for full 20 min test session

Total intake

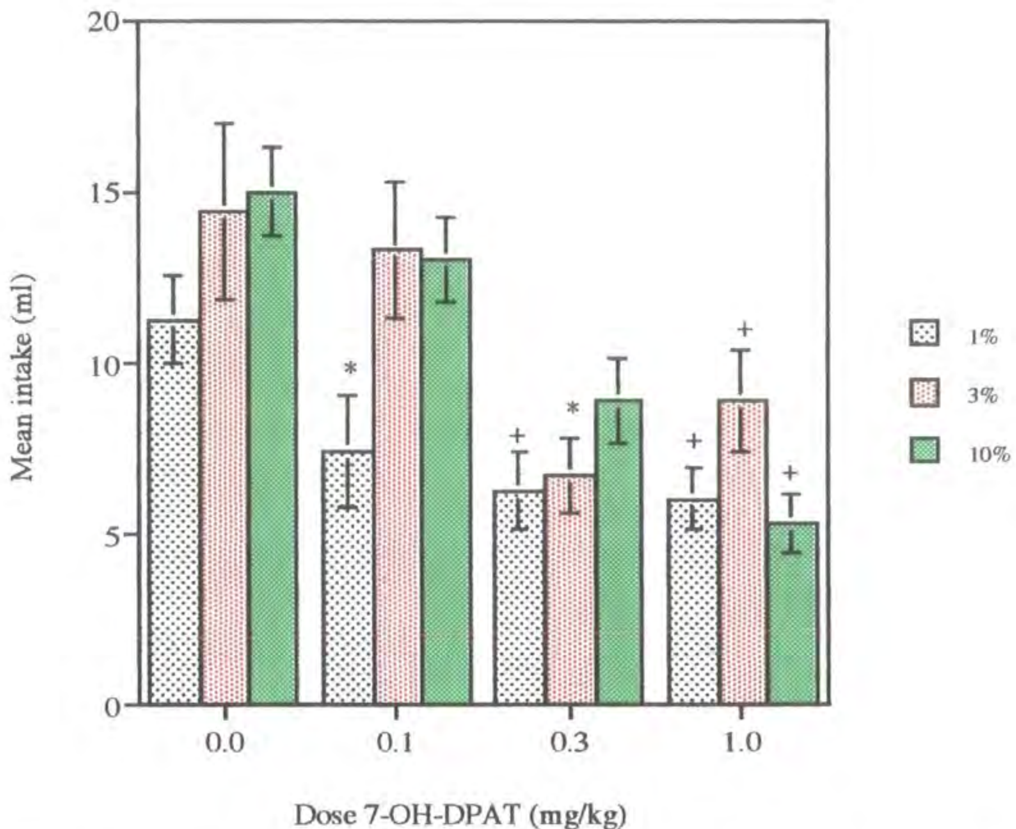


Figure 4.1 Intake of 1, 3 and 10% sucrose in a 20-min test as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$; Plus indicates significantly different from vehicle control group + $p < 0.01$ (Dunnett's t-test).

Figure 4.1 shows the mean intake of sucrose solutions as a function of drug dose. The volume consumed decreased significantly with drug dose giving a main effect of dose 7-OH-DPAT: ($F_{3, 21} = 16.96$, $p<0.0001$). There was also a significant effect of sucrose concentration: ($F_{2, 21}= 3.91$, $p<0.05$). However, there was not a significant interaction between drug dose and sucrose concentration: ($F_{6, 63} =1.62$, n.s.). Post hoc tests revealed that the 0.1 mg/kg dose of 7-OH-DPAT significantly decreased intake of the 1% solution alone

whilst the supression of intake produced by the 0.3 mg/kg of 7-OH-DPAT was significantly different from control in groups drinking the 1 or 3% solutions. At the highest dose (1.0 mg/kg), 7-OH-DPAT significantly decreased intake of all sucrose solutions when compared to the control group.

Rate of Licking

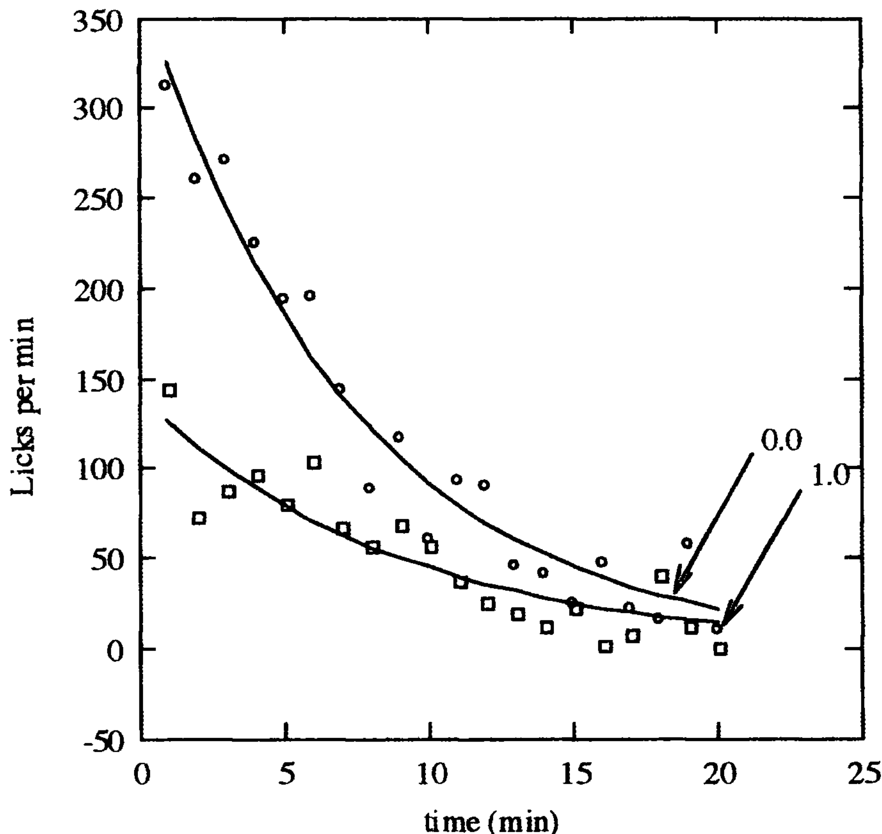


Figure 4.2 The effect of dose 7-OH-DPAT (mg/kg) on the rate of licking (licks per min) for a 10% sucrose solution for a) vehicle and b) high dose conditions. The lines represent the least squares fit of the function y=Ae (-Bt) to the data. Open circles (○) indicate the vehicle condition (0.0 mg/kg 7-OH-DPAT) while open squares (□) indicate the high dose condition (1.0 mg/kg 7-OH-DPAT).

The effect of the highest dose 7-OH-DPAT (1.0 mg/kg) on the rate of licking (licks per minute) at one minute intervals can be seen in Figure 4.2. The rate of licking in the vehicle condition is compared to that of the high dose condition (1.0 mg/kg). An exponential decay function (y=Ae (-Bt)) was fitted to the data. The parameters derived from the curve fitting process are shown in Table 4.1. A Spearman correlation between the actual data and predicted data revealed that this exponential decay function provided a good description of the data (see Table 4.1). At the 1.0 mg/kg dose, 7-OH-DPAT profoundly affected the initial rate of licking (A), reducing the licks per minute by over 50%. In

contrast, 7-OH-DPAT had little effect on the rate constant (B) serving only to decrease it slightly.

Table 4.1 Parameter estimates and standard errors for the least squares fit to the rate of licking of the function y=Ae (-Bt).

7-OH-DPAT	sucrose	A	B	r
Dose (mg/kg)	concentration (%)			
0	10			
estimate		374.52	0.14	0.955
S.E.		18.52	0.01	
1	10			
estimate		142.13	0.12	0.904
S.E.		13.94	0.012	

Parameters A and B refer to the intercept and rate constant respectively. r is the correlation coefficient resulting from the correlation of the actual and predicted values (Spearman correlation coefficient).

Microstructural Analysis

Number of licks

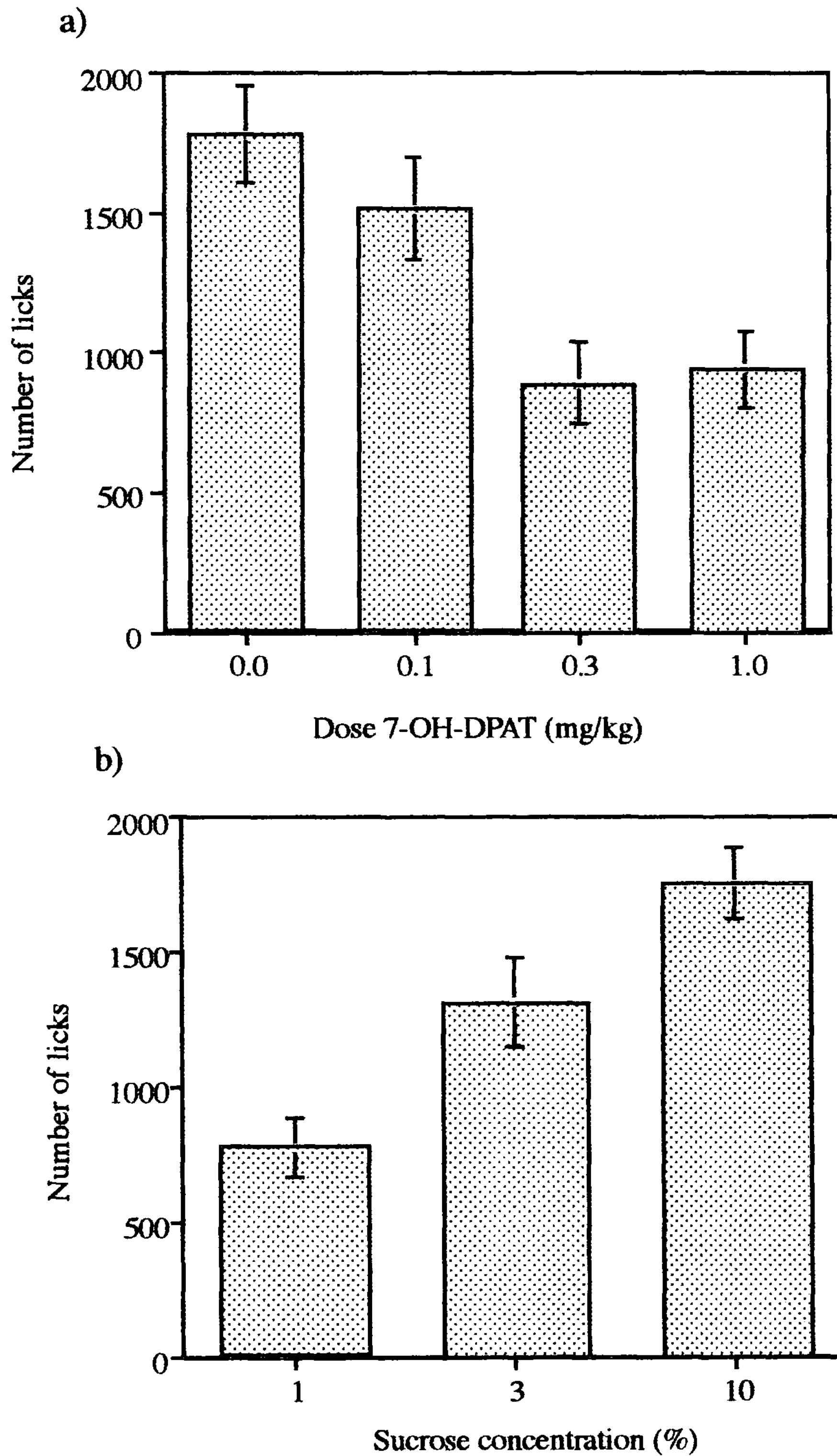


Figure 4.3 a) Number of licks collapsed across sucrose concentration in a 20-min test as a function of dose 7-OH-DPAT (0.1-1 mg/kg) + S.E.M.
b) Number of licks collapsed across doses 7-OH-DPAT in a 20-min test as a function of sucrose concentration (%) + S.E.M.

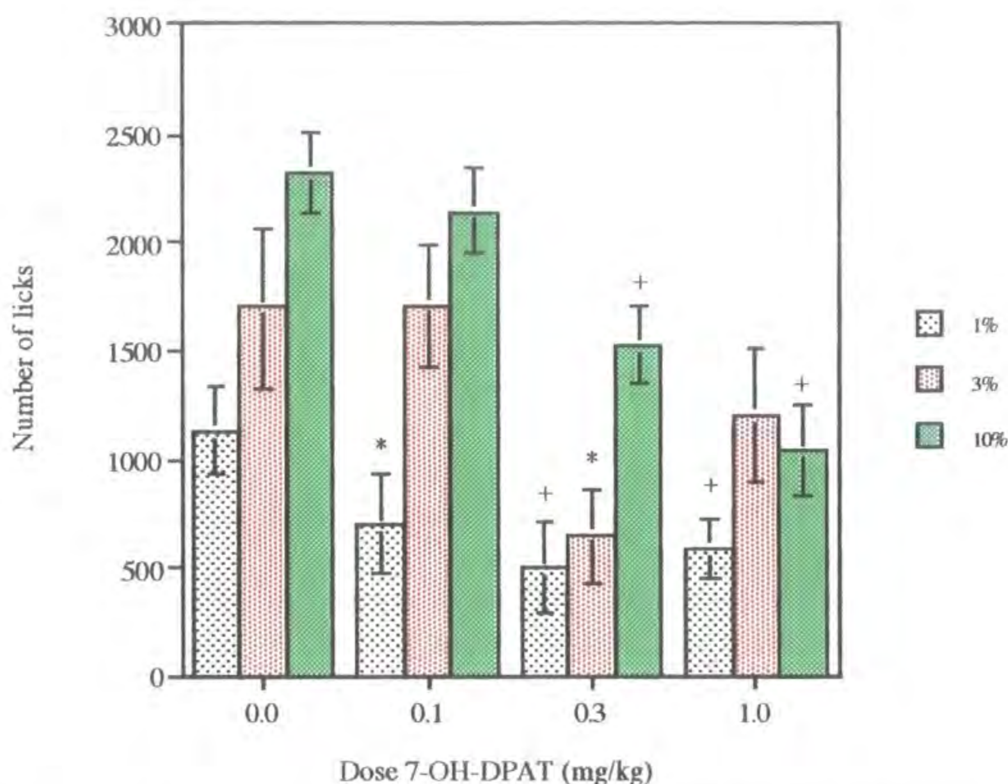


Figure 4.4 Number of licks for 1, 3 and 10% sucrose in a 20-min test as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p< 0.05$; Plus indicates significantly different from vehicle control group + $p< 0.01$ (Dunnett's t-test).

There was a main effect of drug dose on total number of licks: ($F_{3, 21} = 12.04$, $p<0.0001$) and also a main effect of concentration: ($F_{2, 21} = 12.38$, $p<0.0005$) (see Figures 4.3 a) and b) respectively). There was no significant interaction between dose and concentration: ($F_{6, 63} = 1.75$, n.s.). 7-OH-DPAT affected the total number of licks in response to differing sucrose solutions in much the same way as it affected total intake. As with mean intake, post hoc tests showed responses to the 1% solution to be most sensitive to drug treatment with all doses differing significantly from vehicle control. Only the medium dose of 7-OH-DPAT (0.3 mg/kg) affected those animals drinking the 3% solution compared to controls, while both medium (0.3 mg/kg) and high (1.0 mg/kg) doses of 7-OH-DPAT differed significantly from control in those drinking the 10% solution.

Mean bout duration

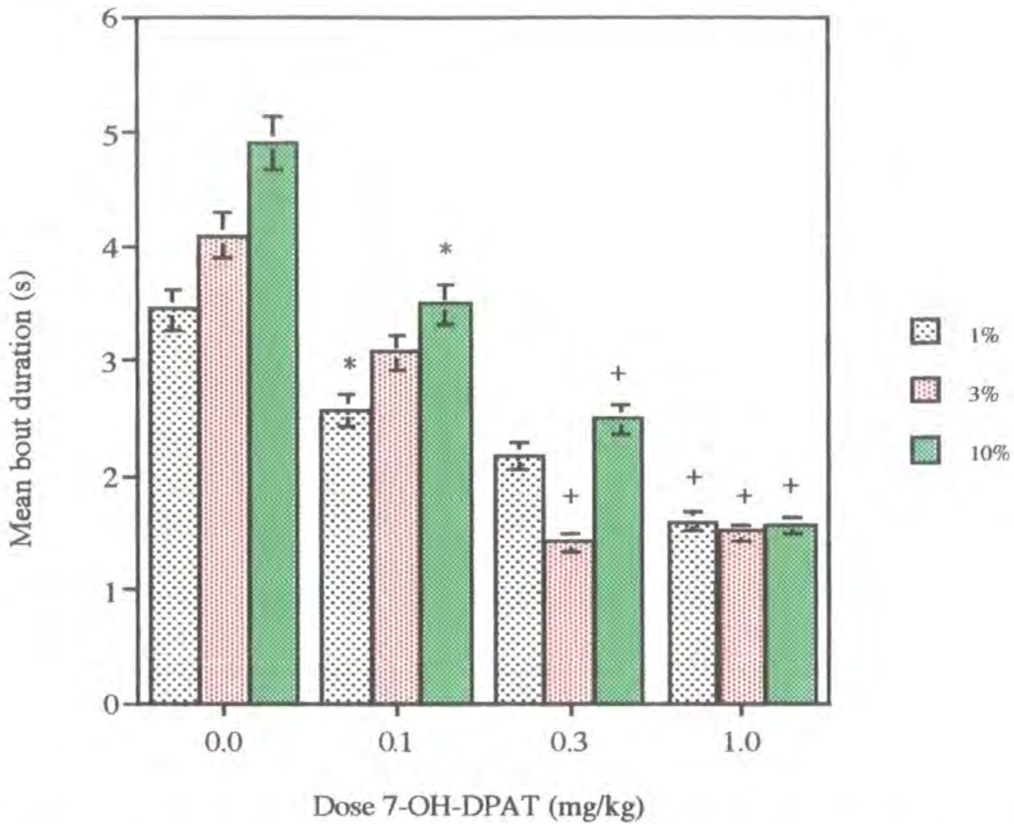


Figure 4.5 Mean bout duration of licking for 1, 3 and 10% sucrose in a 20-min test as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$; Plus indicates significantly different from vehicle control group + $p < 0.01$ (Dunnett's t-test).

Figure 4.5 shows that mean bout duration was significantly shortened as a function of drug dose. There was a main effect of drug dose: ($F_{3, 21} = 22.57$, $p<0.0001$) but not of concentration: ($F_{2, 21} = 1.86$, n.s.). There was no significant interaction between drug dose and sucrose concentration: ($F_{6, 63} = 0.98$, n.s.). Post-hoc tests showed that all doses of 7-OH-DPAT produced changes in mean bout duration that differed significantly from the vehicle control group when animals were drinking the 10% solution. For the 3% solution, significant differences from control are seen at the 0.3 and 1.0 mg/kg doses and for the 1% solution, the 0.1 and 1.0 mg/kg doses differ significantly from control.

Number of bouts

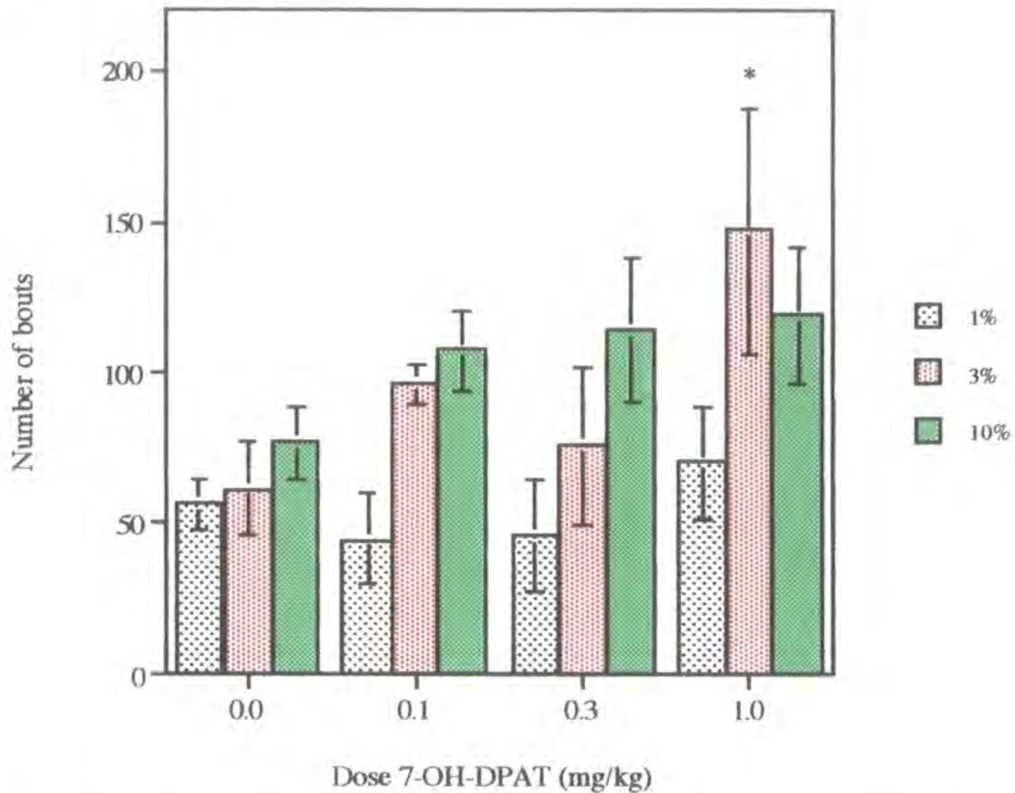


Figure 4.6 Mean licking bout frequency for 1, 3 and 10% sucrose in a 20-min test as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * p< 0.05; Plus indicates significantly different from vehicle control group + p< 0.01 (Dunnett's t-test).

As figure 4.6 shows, bout frequency generally increased as a function of dose of 7-OH-DPAT. There was a significant incremental effect of drug on mean bout frequency : ($F_{3, 21} = 3.33$, $p<0.005$) and also an effect of concentration following a similar pattern ($F_{2, 21} = 4.73$, $p<0.005$). Again, these two variables did not interact significantly : ($F_{6, 63} = 1.02$, n.s.). Only the highest dose of 7-OH-DPAT (1.0 mg/kg) produced a result which differed significantly from control and this was only in the case of the 3% group.

b) Microstructural analysis of data from the 1st min of a 20-min test session

Number of licks

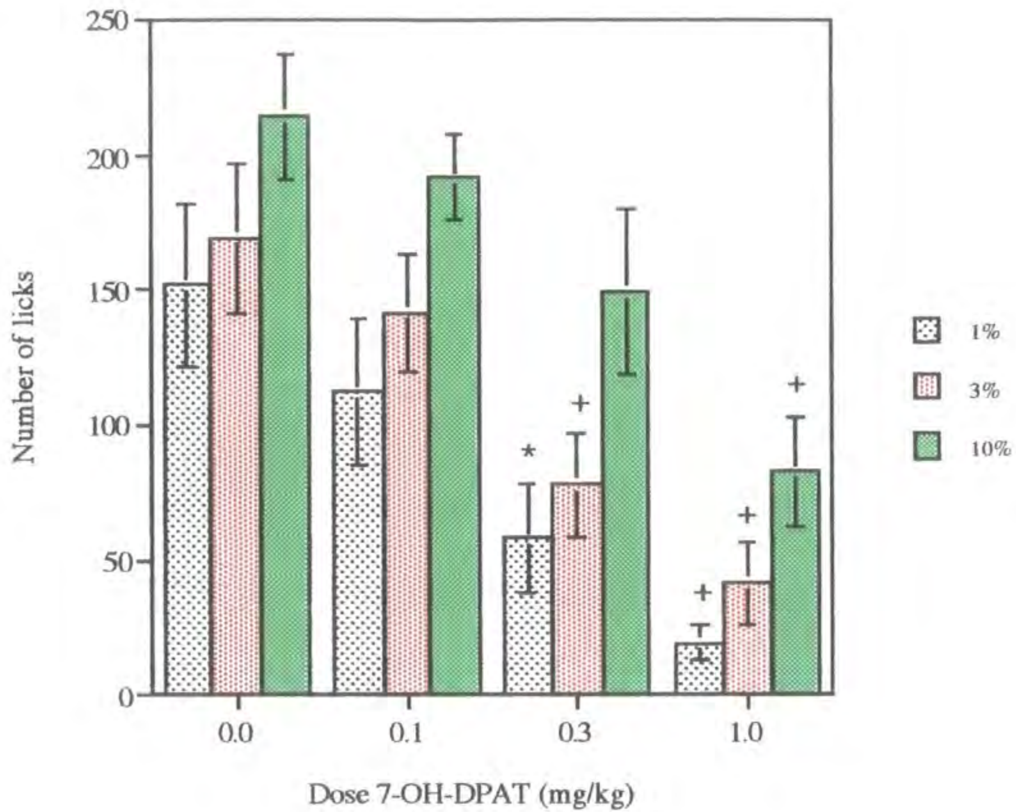


Figure 4.7 Number of licks for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$; Plus indicates significantly different from vehicle control group + $p < 0.01$ (Dunnett's t-test).

Figure 4.7 shows that during the first minute of a 20-min test, the effects of 7-OH-DPAT on the number of licks produced are more clear cut than the effects of the drug over the full 20-min. In this portion of the test there is a monotonic relationship between drug dose and number of licks: ($F_{3, 21} = 19.42$, $p<0.0001$). There is also an overall effect of concentration: ($F_{2, 21} = 12.241$, $p<0.0005$). There were no significant drug-concentration interactions: ($F_{6, 63} = 0.127$, n.s.) In general, the 1.0 (mg/kg) dose of 7-OH-DPAT seems to be the most effective in decreasing number of licks at this stage of the test. However, the 0.3 (mg/kg) dose has an early effect on all but one of the concentration groups (i.e. 1% and 3%).

Mean bout duration

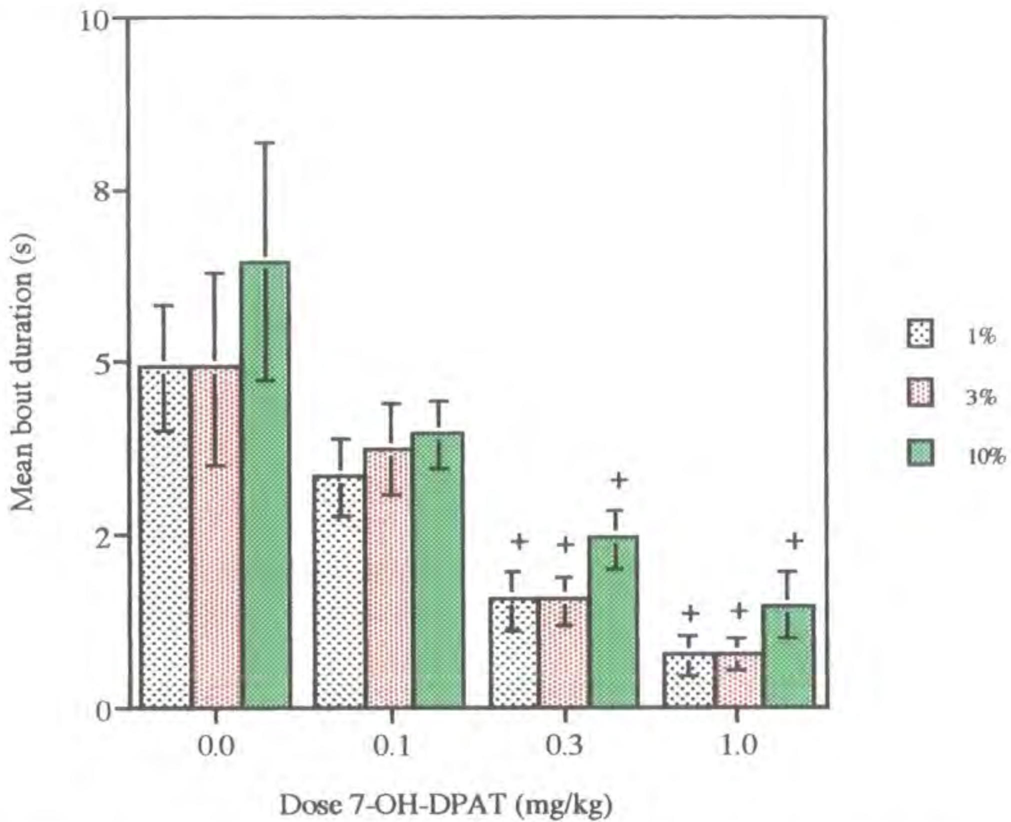


Figure 4.8 Mean bout duration of licking for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$; Plus indicates significantly different from vehicle control group + $p < 0.01$ (Dunnett's t-test).

Mean bout duration is also decreased significantly in the 1st min of the 20-min test. There remains a significant drug effect: ($F_{3,\ 21} = 19.06$, $p<0.0001$) yet, as is true of the 20-min data, there is no main effect of concentration: ($F_{2,\ 21} = 1.633$, n.s.) or any significant drug-concentration interactions: ($F_{6,\ 63} = 0.14$, n.s.). The 0.3 and 1.0 mg/kg doses of 7-OH-DPAT shorten licking mean bout duration significantly compared to controls at all concentrations. This differs slightly from the mean bout duration measures over 20-mins. Post-hoc tests show the 0.1 (mg/kg) dose of 7-OH-DPAT differs significantly from controls when data from the whole test session is analysed (see Figure 4.8).

Number of bouts

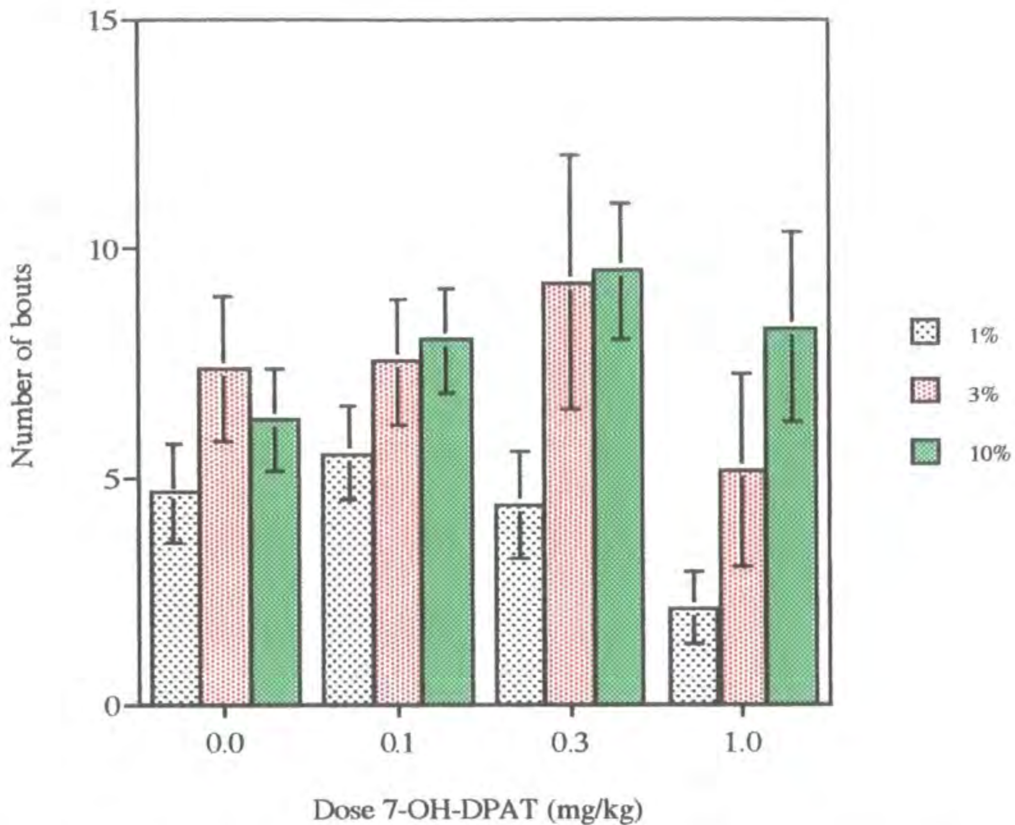


Figure 4.9 Mean bout frequency of licking for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M.

Figure 4.9 shows that in the 1st min of the 20-min test session there was no main effect of drug on mean bout frequency: ($F_{3, 21}$ = 1.46, n.s.). However, there was an effect of concentration: ($F_{2, 21}$ = 7.02, p<0.005). There was no significant interaction of dose-concentration: ($F_{6, 63}$ = .611, n.s.).

Experiment 2: Microstructural analysis of drinking for sucrose after the administration of quinpirole : Analyses of licking during a 20-min session and during first minute of 20-min session.

a) for full 20 min test session

Total intake

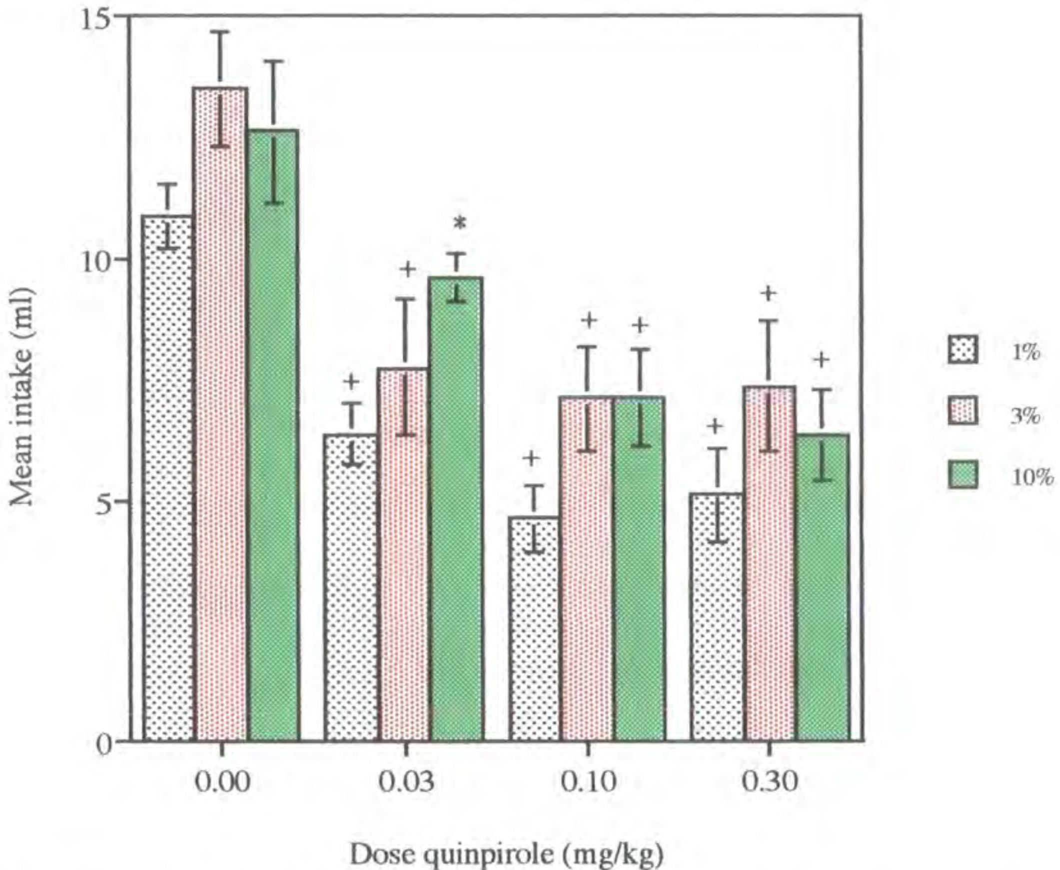


Figure 4.10 Intake of 1, 3 and 10% sucrose in a 20-min test as a function of dose quinpirole (0.03-0.3 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * p< 0.05; Plus indicates significantly different from vehicle control group + p< 0.01 (Dunnett's t-test).

Figure 4.10 shows that mean intake sharply decreases as a function of increasing dose of quinpirole. Measures of intake are similar at the medium dose (0.1 mg/kg) and the high dose (0.3 mg/kg) of quinpirole. There is a significant main effect of drug dose: ($F_{3, 21} = 22.734$, $p<0.0001$) and of sucrose concentration ($F_{2, 21} = 6.082$, $p<0.05$). There was no significant drug-concentration interaction: ($F_{6, 63} = 0.441$, n.s.). The results of post hoc tests showed all doses to be significantly different from vehicle control groups at each sucrose concentration.

Rate of Licking

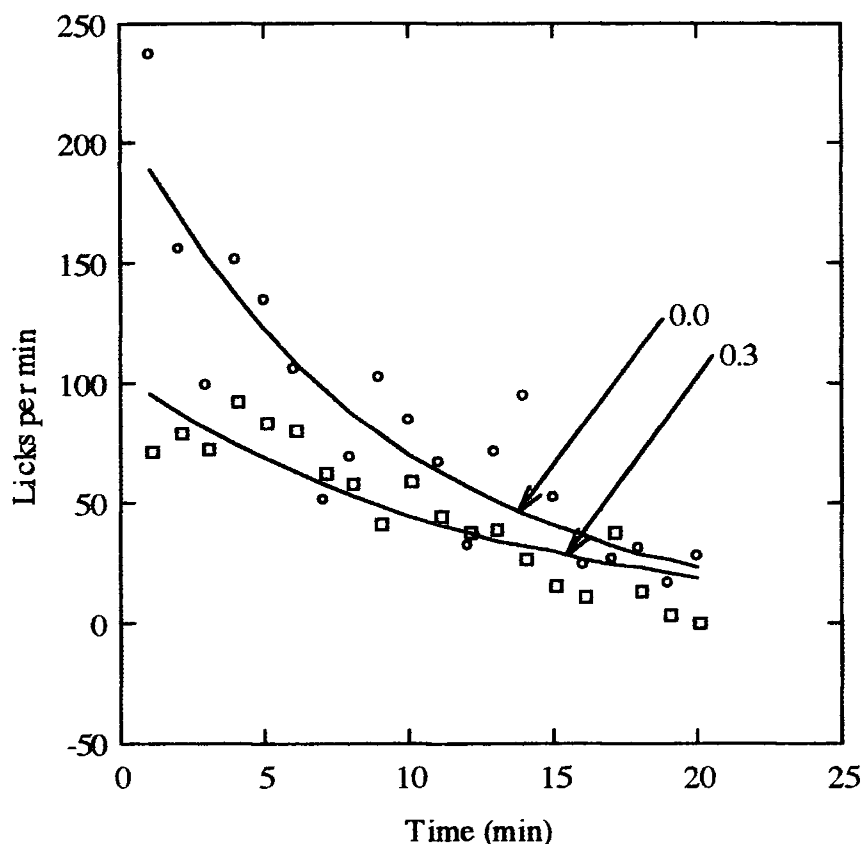


Figure 4.11 The effect of dose quinpirole (mg/kg) on the rate of licking (licks per min) for a 10% sucrose solution for a) vehicle and b) high dose conditions. The lines represent the least squares fit of the function y=Ae (-Bt) to the data. Open circles (**O**) indicate the vehicle condition (0.0 mg/kg quinpirole) while open squares (**□**) indicate the high dose condition (0.3 mg/kg quinpirole).

The effect of quinpirole on the rate of licking (licks per minute) at one minute intervals can be seen in figure 4.11. The rate of licking in the vehicle condition is compared to that of the high dose condition (0.3 mg/kg). An exponential decay function (y=Ae (-Bt)) was again fitted to the data. The parameters derived from the curve fitting process are shown in table 4.2. A Spearman correlation between the actual data and predicted data revealed that this exponential decay function provided a good description of the data (see table 4.2). At the 0.3 mg/kg dose, quinpirole roughly halved the initial rate of licking (A). In contrast, as was found with the high dose (1.0 mg/kg) of 7-OH-DPAT, quinpirole (0.3 mg/kg) had little effect on the rate constant (B) producing a marginal attenuation of this parameter.

Table 4.2 Parameter estimates and standard errors for the least squares fit to the rate of licking of the function y=Ae (-Bt).

quinpirole	concentration	A	B	r
Dose (mg/kg)	sucrose (%)			
0	10			
estimate		211.40	0.11	0.886
S.E.		21.0	0.02	
0.3	10			
estimate		104.10	0.08	0.891
S.E.		9.483	0.01	

Parameters A and B refer to the intercept and rate constant respectively. r is the correlation coefficient resulting from the correlation of the actual and predicted values (Spearman correlation coefficient).

Microstructural Analysis

Number of licks

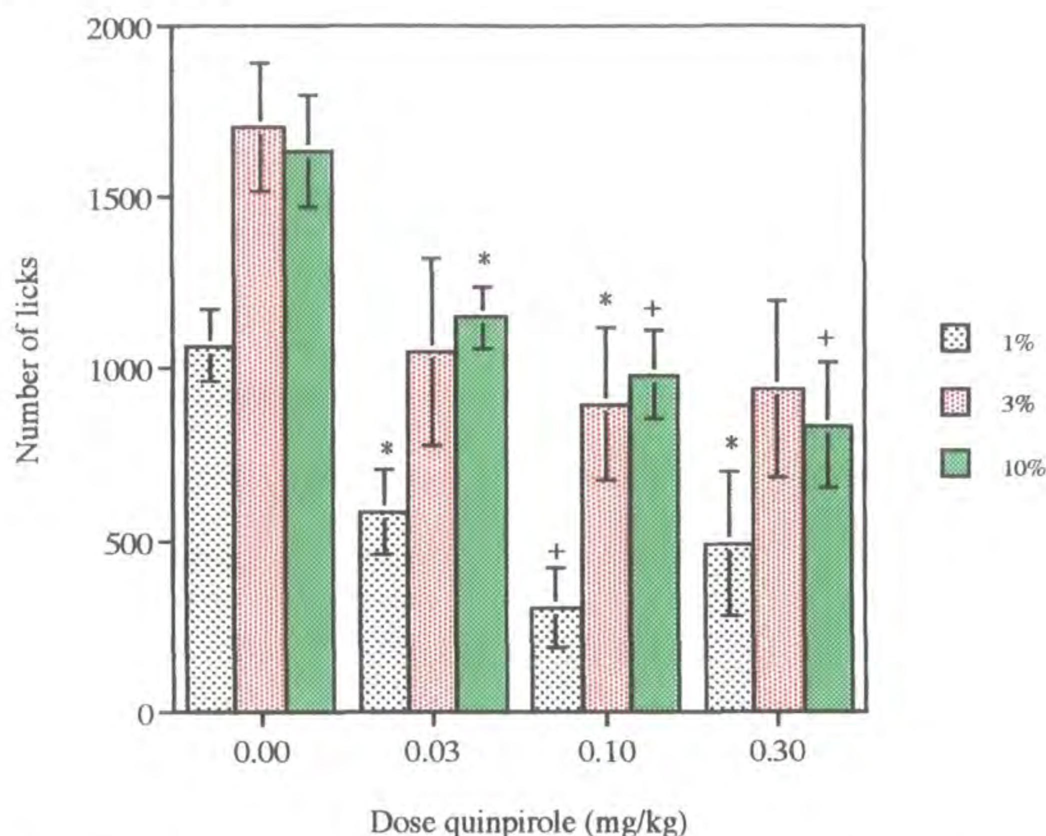


Figure 4.12 Number of licks for 1, 3 and 10% sucrose in a 20-min test as a function of dose quinpirole (0.03-0.3 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$; Plus indicates significantly different from vehicle control group + $p < 0.01$ (Dunnett's t-test).

The number of licks after the administration of quinpirole decreased significantly giving a main effect of drug dose: ($F_{3, 21} = 9.270$, $p<0.0001$). There was also a main effect of concentration, in that it increased number of licks monotonically: ($F_{2, 21} = 25.104$, $p<0.0001$). There was no interaction between drug dose and concentration: ($F_{6, 63} = 0.171$, n.s.). Post hoc means comparisons highlighted significant differences between all doses and vehicle control for those drinking 1% or 10% sucrose. Number of licks for those drinking 3% sucrose were only significantly different from vehicle at the 0.1 (mg/kg) dose of quinpirole (see Figure 4.12).

Mean bout duration

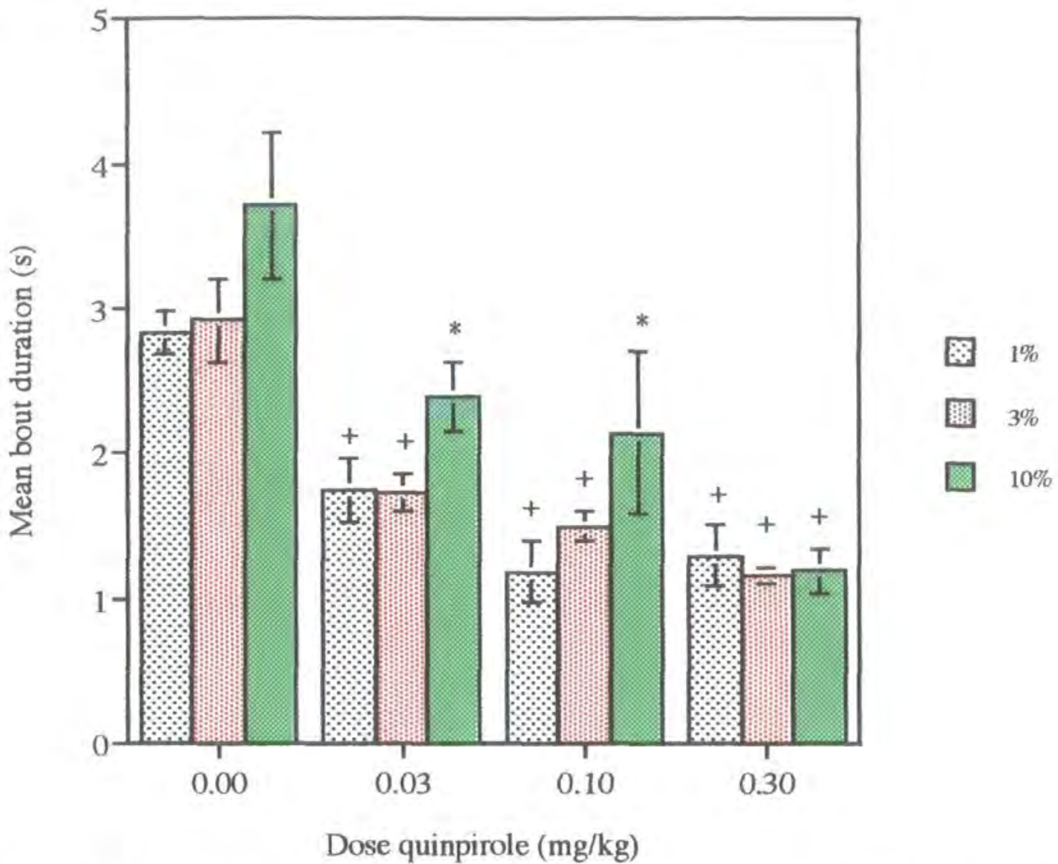


Figure 4.13 Mean bout duration of licking for 1, 3 and 10% sucrose in a 20-min test as a function of dose quinpirole (0.03-0.3 mg/kg)+ S.E.M. Asterisk indicates significantly different from vehicle control group * p< 0.05; Plus indicates significantly different from vehicle control group + p< 0.01 (Dunnett's t-test).

Figure 4.13 reveals that mean bout duration became shorter as the dose of quinpirole increased. This was found to be a highly significant main effect of drug: ($F_{3, 21} = 27.074$, $p<0.0001$). Concentration served to significantly increase mean bout duration: ($F_{2, 21} = 5.779$, $p<0.01$). There was no significant interaction between drug dose and concentration: ($F_{6, 63} = 0.826$, n.s.). Differences between dose groups and vehicle control groups were significant at each dose for each concentration.

Number of bouts

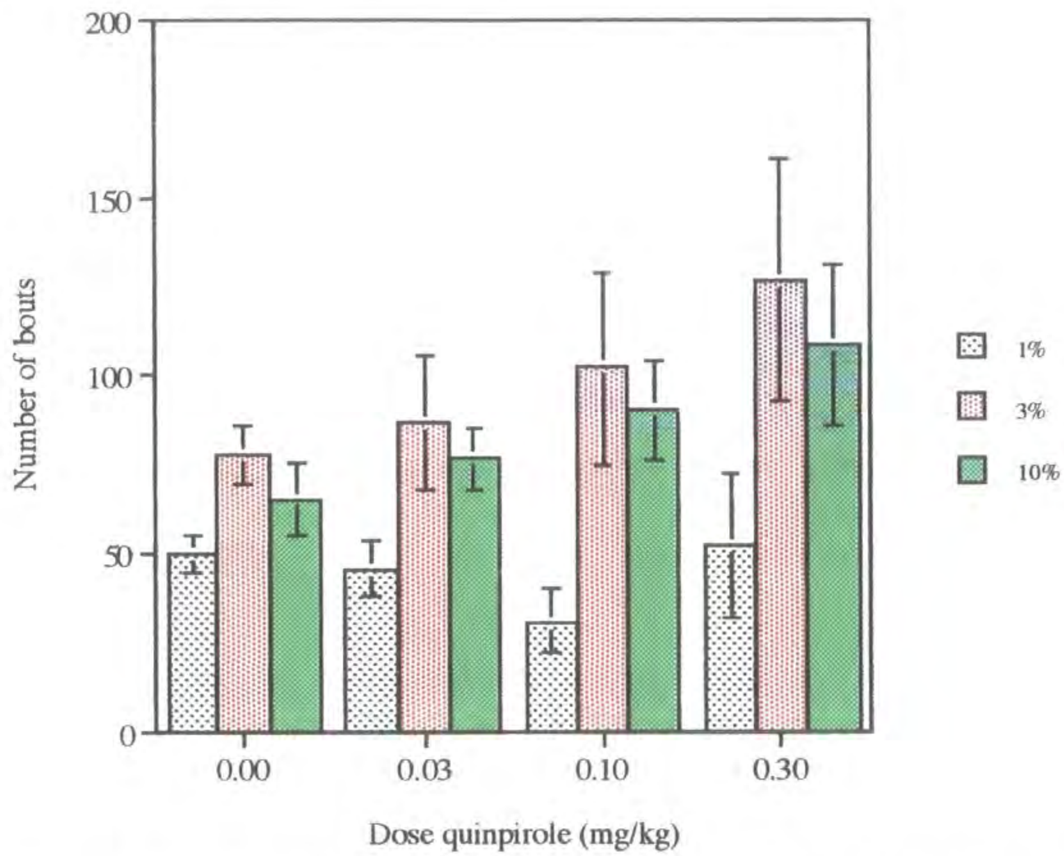


Figure 4.14 Mean licking bout frequency for 1, 3 and 10% sucrose in a 20-min test as a function of dose quinpirole (0.03-0.3) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$; Plus indicates significantly different from vehicle control group + $p < 0.01$ (Dunnett's t-test).

Figure 4.14 highlights that in the case of bout frequency, there was no significant main effect of drug: ($F_{3, 21} = 1.684$, n.s.). Therefore, post hoc tests were not conducted. Concentration significantly affected the number of bouts: ($F_{2, 21} = 12.878$, $p<0.001$) but there was no significant drug-concentration interaction: ($F_{6, 63} = .468$, n.s.).

b) Microstructural analysis of data from the 1st min of a 20-min test session

Number of licks

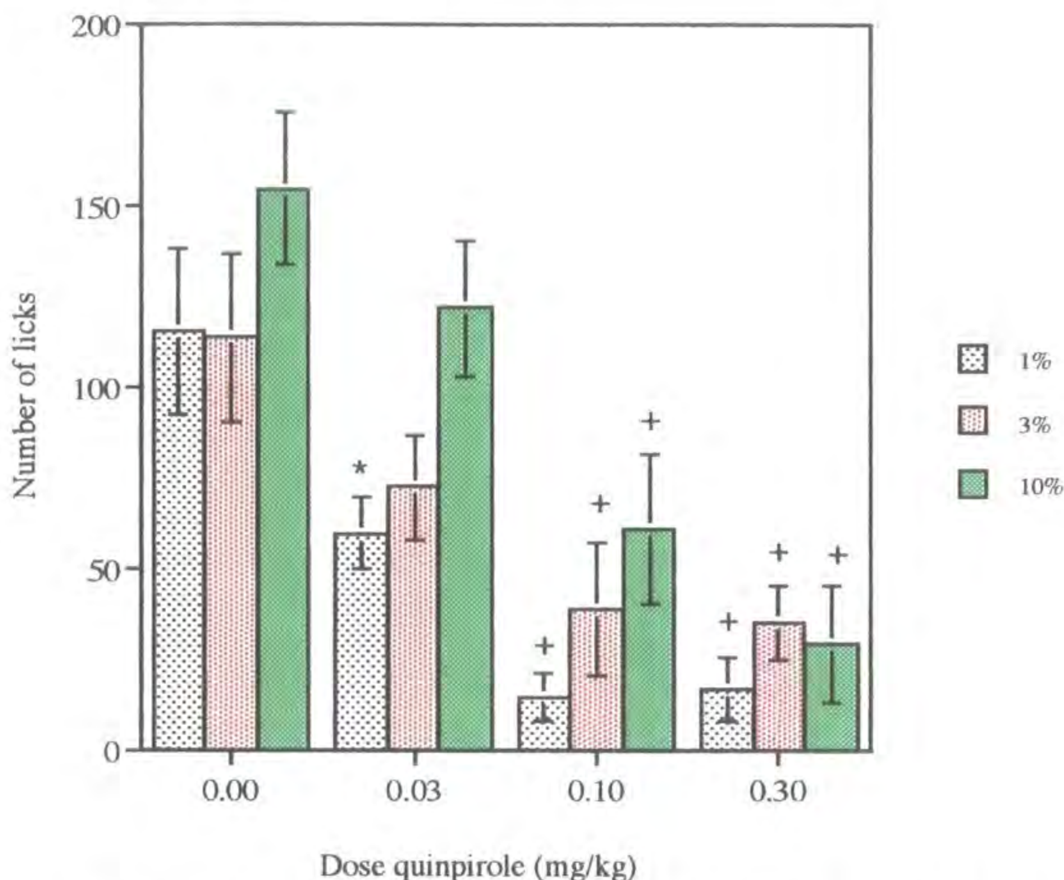


Figure 4.15 Number of licks for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose quinpirole (0.03-0.3 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p< 0.05$; Plus indicates significantly different from vehicle control group + $p< 0.01$ (Dunnett's t-test).

In the first minute of the 20-min test session number of licks decreased as a function of dose of quinpirole (see Figure. 4.15). This drug effect was statistically significant: ($F_{3, 21} = 28.156$, $p<0.0001$). Concentration also significantly affected the number of licks at this early stage of the test: ($F_{2, 21} = 3.679$, $p<0.05$). However, the drug-concentration interaction did not reach statistical significance: ($F_{6, 63} = .846$, n.s.). Following the significant main effect of drug, post hoc tests showed that the 0.1 and 0.3 (mg/kg) doses of quinpirole were significantly different from controls at all concentations. However, the low dose of quinpirole (0.03 mg/kg) was only different to controls in the group drinking 1% sucrose.

Mean bout duration

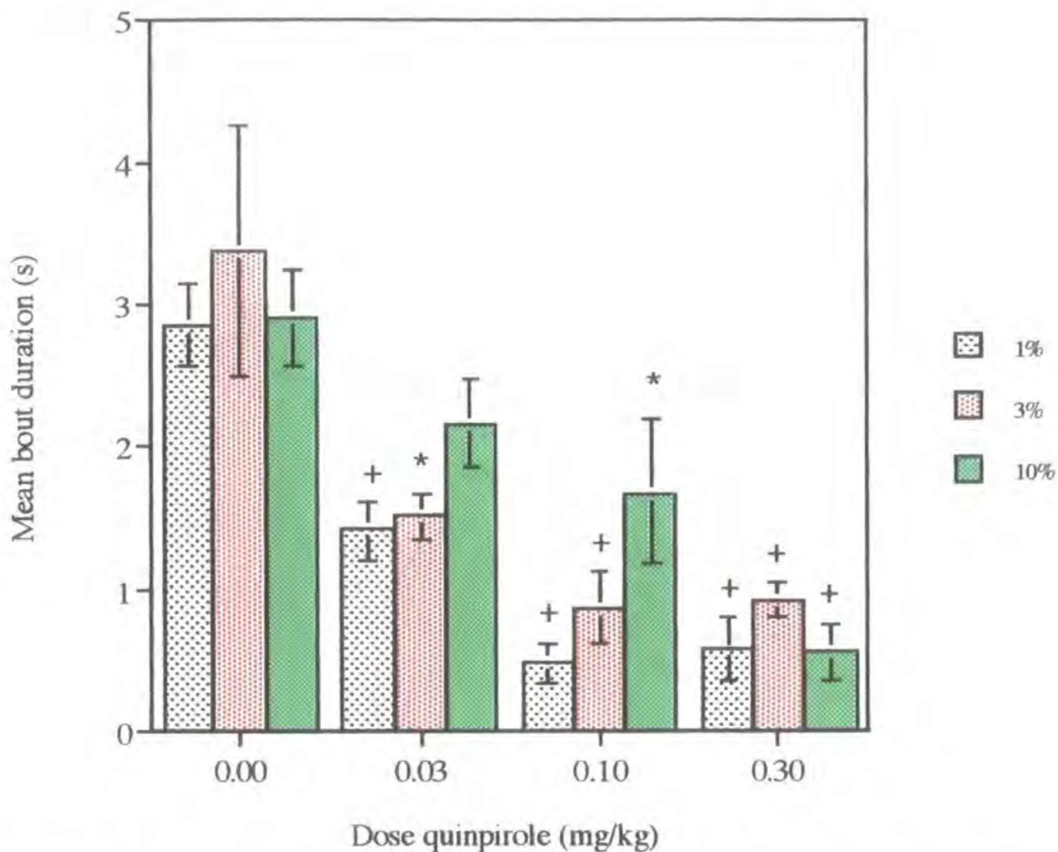


Figure 4.16 Mean bout duration of licking for 1, 3 and 10% sucrose from the first minute of a 20-min test as a function of dose quinpirole (0.03-0.3 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$; Plus indicates significantly different from vehicle control group + $p < 0.01$ (Dunnett's t-test).

Figure 4.16 reveals that drug dose also produced a significant decremental effect on the mean bout duration during the first minute of the 20-min test: ($F_{3, 21} = 24.523$, $p<0.0001$). The main effect of concentration during this first minute did not reach statistical significance: ($F_{2, 21} = 1.874$, n.s.); nor was there a significant drug-concentration interaction: ($F_{6, 63} = 1.021$, n.s.). Post hoc test showed that all doses were significantly different from controls at all levels of concentration with the exception of those animals in the 10% group which had received the low dose (0.03 mg/kg) of quinpirole.

Number of bouts

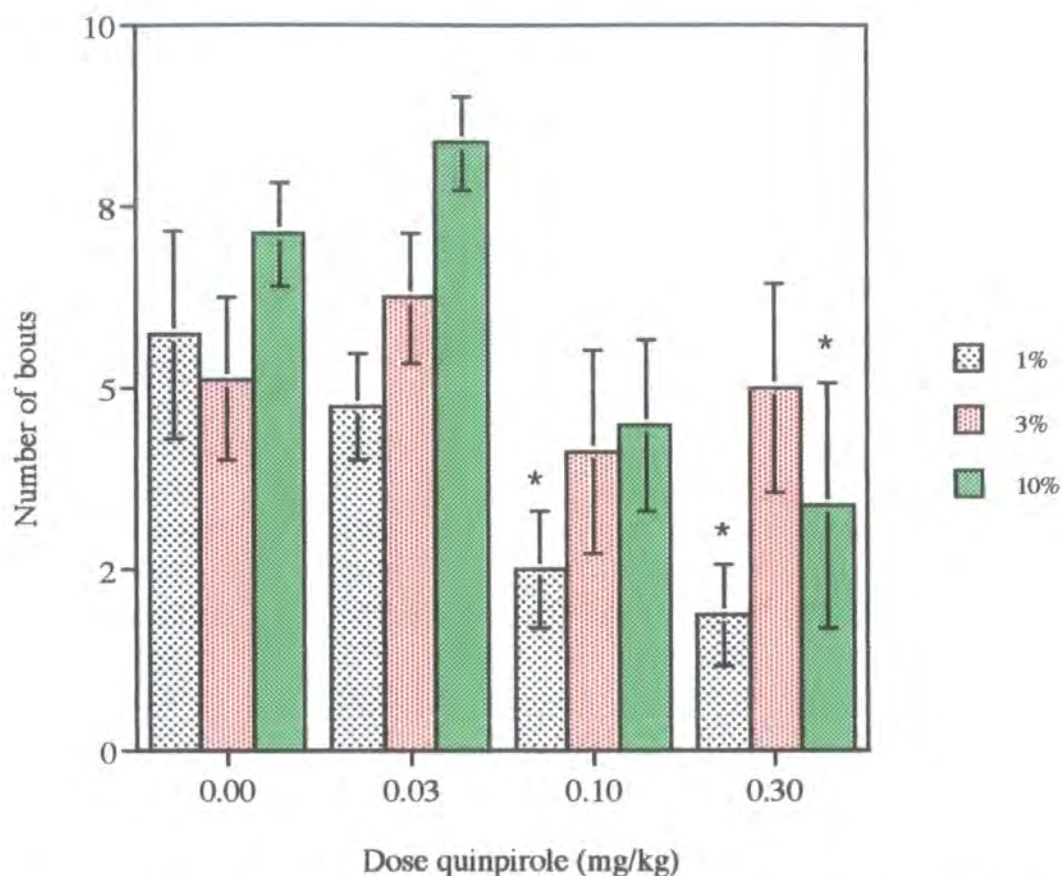


Figure 4.17 Mean bout frequency of licking for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose quinpirole (0.1-1.0 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p < 0.05$.

While mean bout frequency increased significantly as a function of drug dose over the 20-min test session, the opposite occurs within the first minute of the same test session (see figures 4.14 and 4.17). Figure 4.17 shows that mean bout frequency tended to decrease with increasing dose of quinpirole. This was found to be a significant main effect of drug dose: ($F_{3, 21} = 5.914$, $p<0.005$). Concentration had a similar and significant main effect on mean bout frequency when measured over 20 mins and also in the first minute of the test.

During the first minute, concentration served to significantly increase the number of bouts: ($F_{2, 21} = 3.615$, $p<0.05$). Again, there was no significant interaction of drug and concentration: ($F_{6, 63} = 0.887$, n.s.). Post hoc tests point to the higher doses as significantly different from vehicle controls (i.e. 0.1 and 0.3 mg/kg) for the 1% sucrose group; the 0.3 (mg/kg) is the only dose which is significantly different from vehicle in those drinking the 10% solution and in the 3% group, doses of quinpirole failed to significantly differ from vehicle controls.

Experiment 3: Microstructural analysis of drinking for sucrose after the administration of raclopride: Analyses of licking during a 20-min session and during first minute of 20-min session.

a) for full 20 min test session

Total intake

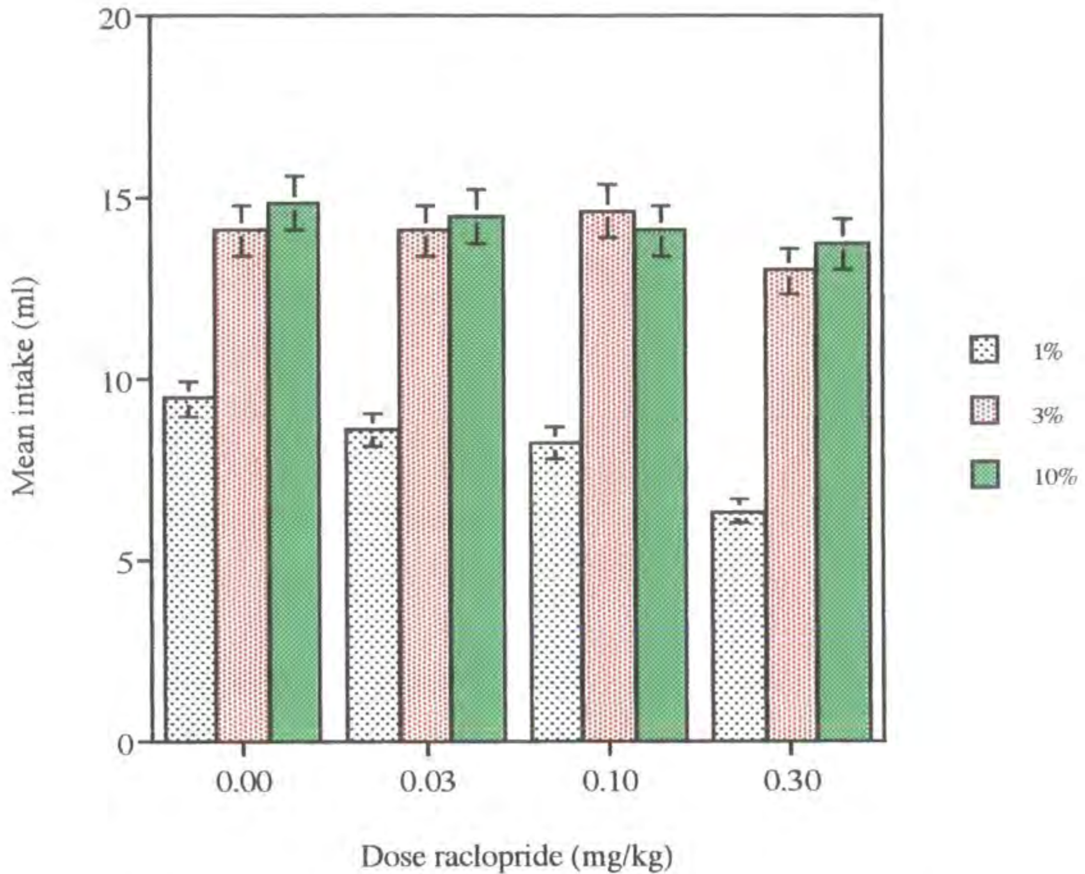


Figure 4.18 Intake of 1, 3 and 10% sucrose in a 20-min test as a function of dose raclopride (0.03-0.3 mg/kg) + S.E.M.

Figure 4.18 shows that intake decreased only slightly in the 3 and 10% sucrose groups and more noticably in the 1% group as a function of dose. Across concentrations the main effect of drug was not significant: ($F_{3, 21}$ = 1.291, n.s.). The main effect of concentration on mean intake was highly significant: ($F_{2, 21}$ = 25.716, p<0.0001). The drug-concentration interaction was non-significant: ($F_{6, 63}$ = 0.23, n.s.).

Rate of Licking

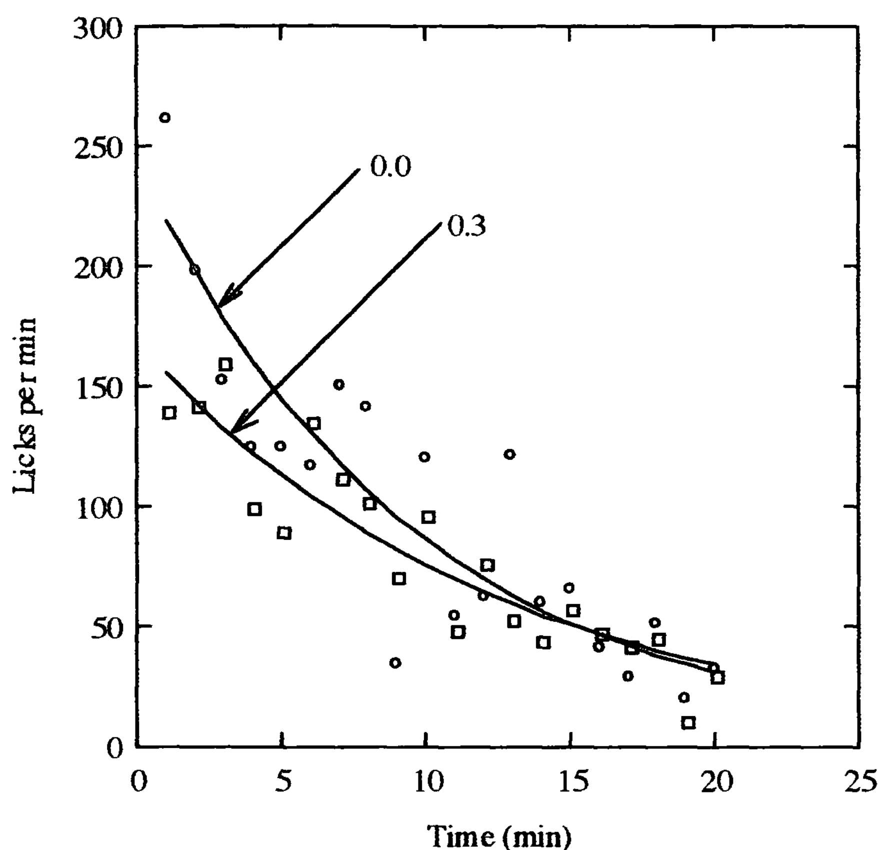


Figure 4.19 The effect of dose raclopride (mg/kg) on the rate of licking (licks per min) for a 10% sucrose solution for a) vehicle and b) high dose conditions. The lines represent the least squares fit of the function y=Ae (-Bt) to the data. Open circles (O) indicate the vehicle condition (0.0 mg/kg raclopride) while open squares (□) indicate the high dose condition (0.3 mg/kg raclopride).

The effect of raclopride on the rate of licking (licks per minute) at one minute intervals can be seen in figure 4.19. The rate of licking in the vehicle condition is compared to that of the high dose condition (0.3 mg/kg). An exponential decay function (y=Ae (-Bt)) was again fitted to the data. The parameters derived from the curve fitting process are shown in Table 4.3. A Spearman correlation between the actual data and predicted data revealed that this exponential decay function provided a good description of the data (see table 4.3). At the 0.3 mg/kg dose, raclopride decreased the initial rate of licking (A). This decrease was not as extreme as that produced by the highest doses of 7-OH-DPAT (1.0 mg/kg) or quinpirole (0.3 mg/kg). Raclopride (0.3 mg/kg) had little effect on the rate constant (B) producing a marginal attenuation of this parameter. In this way raclopride produced similar effects to 7-OH-DPAT and quinpirole (see results: Experiments 1 and 2).

Table 4.3 Parameter estimates and standard errors for the least squares fit to the rate of licking of the function y=Ae (-Bt).

raclopride	sucrose	A	B	r
Dose (mg/kg)	concentration (%)			
0	10			
estimate		242.63	0.10	0.889
S.E.		23.01	0.02	
0.3	10			
estimate		168.77	0.08	0.915
S.E.		12.13	0.01	

Parameters A and B refer to the intercept and rate constant respectively. r is the correlation coefficient resulting from the correlation of the actual and predicted values (Spearman correlation coefficient).

Microstructural Analysis

Number of licks

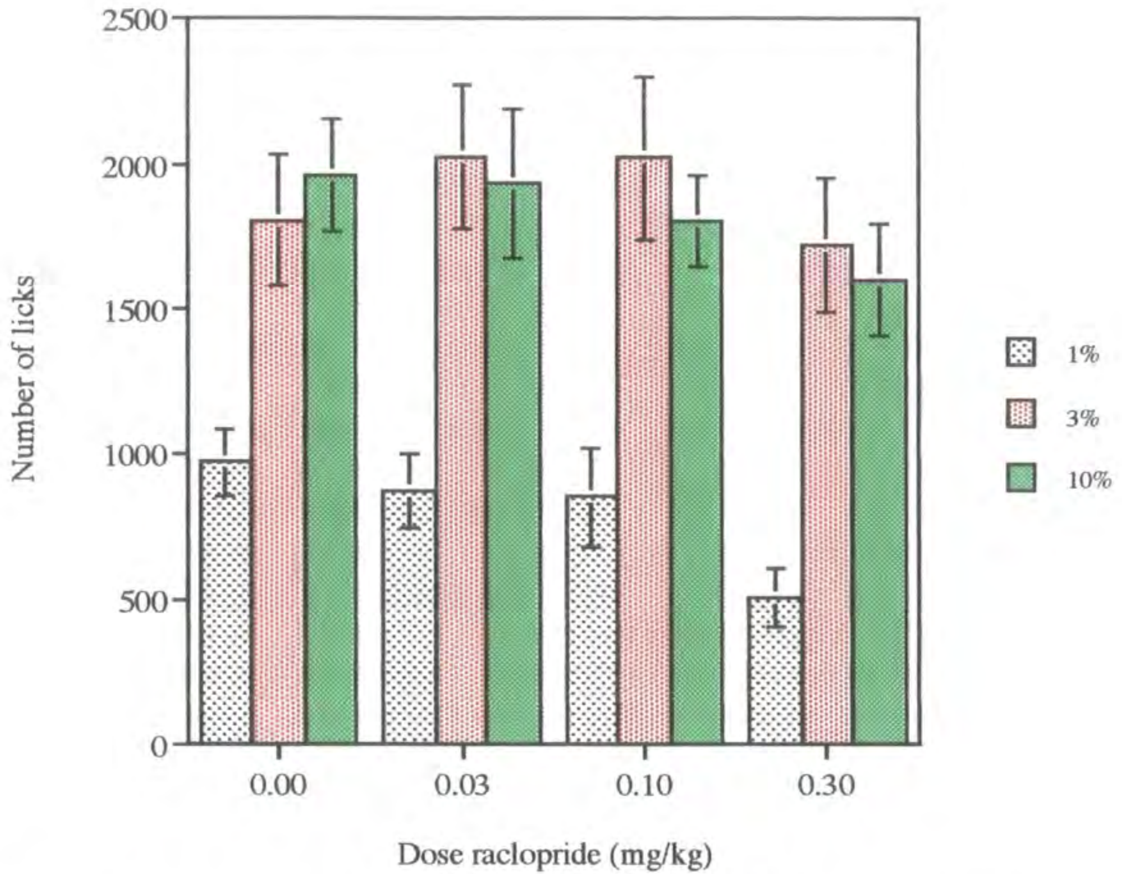


Figure 4.20 Number of licks for 1, 3 and 10% sucrose in a 20-min test as a function of dose raclopride (0.03-0.3 mg/kg) + S.E.M.

Figure 4.20 shows a similar decremental pattern to figure 4.18 with those in the 1% sucrose group licking less than animals in the 3 or 10% groups. Again, the main effect of drug on number of licks was not significant: ($F_{3, 21} = 2.109$, n.s.) and the main effect of concentration was highly significant: ($F_{2, 21} = 25.744$, $p<0.0001$). There was no significant drug-concentration interaction: ($F_{6, 63} = 0.289$, n.s.).

Mean bout duration

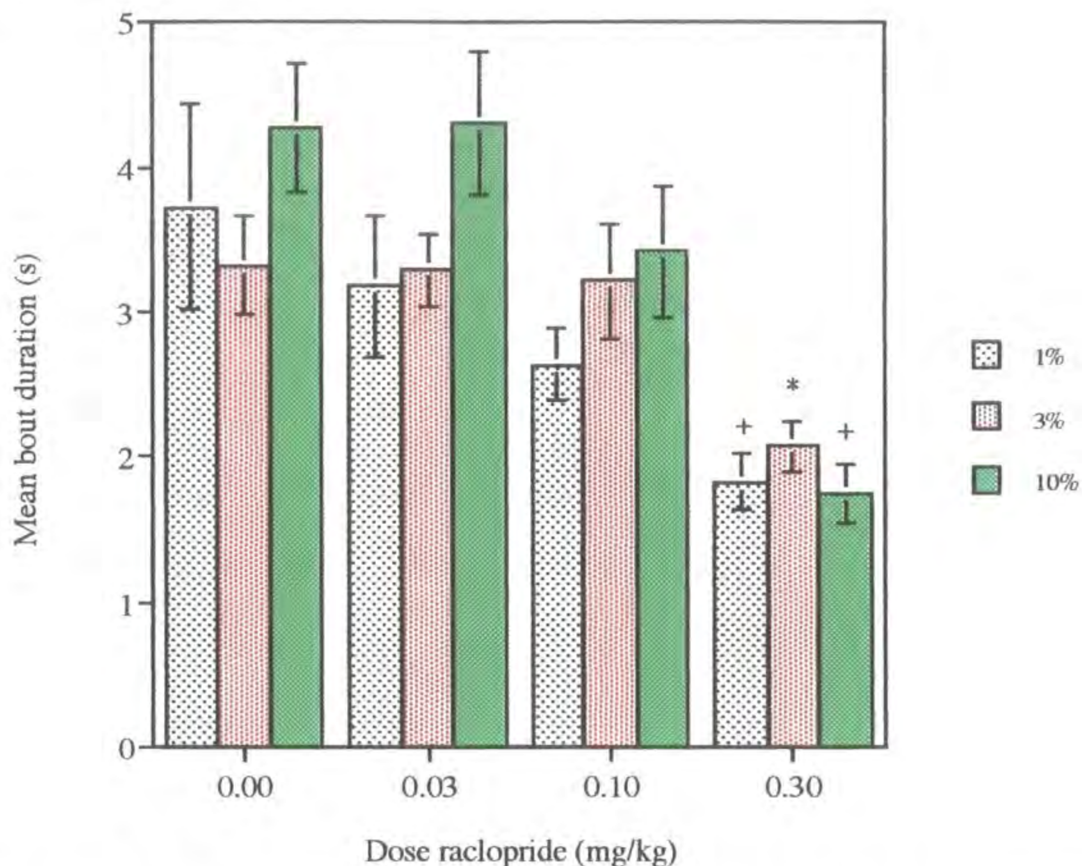


Figure 4.21 Mean bout duration of licking for 1, 3 and 10% sucrose in a 20-min test as a function of dose raclopride (0.03-0.3 mg/kg)+ S.E.M. Asterisk indicates significantly different from vehicle control group * p< 0.05; Plus indicates significantly different from vehicle control group + p< 0.01 (Dunnett's t-test).

Figure 4.21 shows an overall decrease in mean bout duration with increasing doses of raclopride. At the highest dose (0.3 mg/kg) mean bout duration is approximately halved compared to vehicle. For this parameter there was a significant main effect of drug: ($F_{3, 21} = 16.467$, $p<0.0001$) but no significant main effect of concentration: ($F_{2, 21} = 1.663$, n.s.). Neither was there a significant drug-concentration interaction: ($F_{6, 63} = 1.06$, n.s.). Post hoc tests revealed that the highest dose of raclopride (0.3 mg/kg), significantly attenuated mean bout duration when compared to vehicle control group. This was the case for each sucrose concentration group.

Number of bouts

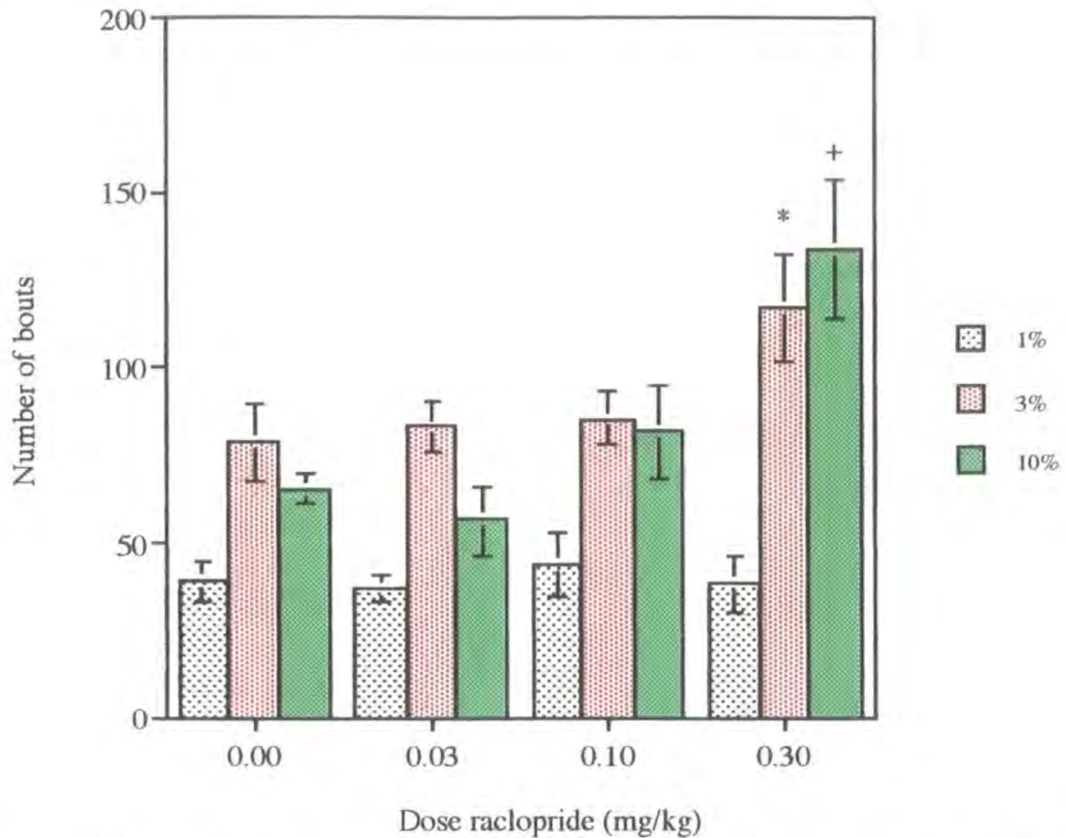


Figure 4.22 Mean licking bout frequency for 1, 3 and 10% sucrose in a 20-min test as a function of dose raclopride (0.03-0.3) + S.E.M. Asterisk indicates significantly different from vehicle control group * p< 0.05; Plus indicates significantly different from vehicle control group + p< 0.01 (Dunnett's t-test).

Number of bouts generally increased as a function of dose of raclopride (see figure 4.22). However, those animals drinking 1% sucrose did not increase their mean bout frequency to the same extent as animals in the 3 or the 10% groups. For this parameter there was a significant main effect of drug: ($F_{3, 21} = 8.674$, $p<0.0001$); concentration: ($F_{2, 21} = 20.58$, $p<0.0001$) and a significant drug-concentration interaction: ($F_{6, 63} = 16.467$, $p<0.05$). Post hoc tests showed the highest dose of raclopride (0.3 mg/kg) to be significantly different from vehicle controls for those animals drinking 3 or 10% sucrose.

b) Microstructural analysis of data from the 1st min of a 20-min test session

Number of licks

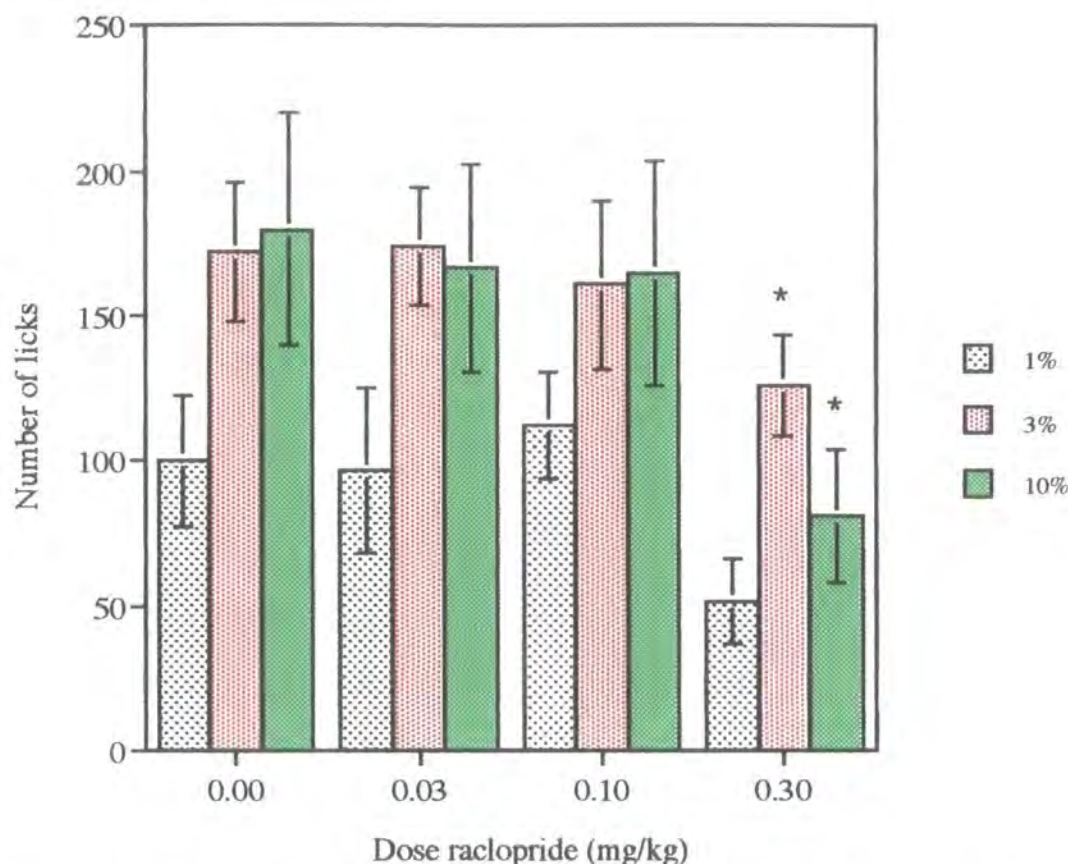


Figure 4.23 Number of licks for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose raclopride (0.03-0.3 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * p< 0.05 (Dunnett's t-test).

The decrease in the number of licks as a function of drug dose (see Figure 4.23) was only statistically significant at the highest dose of raclopride (0.3 mg/kg) where number of licks were reduced by approximately 40%. This main effect of drug was statistically significant: ($F_{3, 21} = 4.539$, $p<0.05$); as was the main effect of concentration: ($F_{2, 21} = 4.948$, $p<0.05$). However, there was no interaction between drug dose and sucrose concentration: ($F_{6, 63} = 0.339$, n.s.). Post hoc tests showed that during the first minute of the 20 min test session, the reduction in number of licks was most evident at the highest dose of raclopride (0.3 mg/kg) in the 3 and 10% sucrose groups.

Mean bout duration

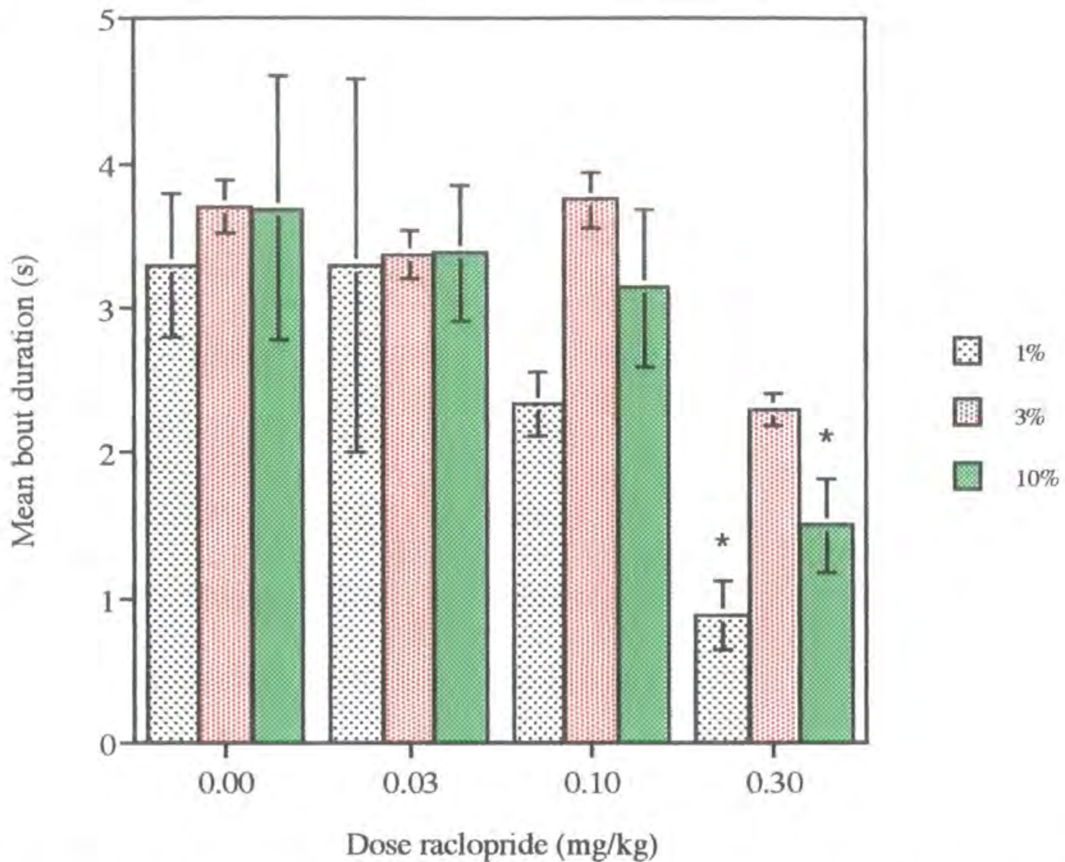


Figure 4.24 Mean bout duration of licking for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose raclopride (0.03-0.3 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle control group * $p< 0.05$ (Dunnett's t-test).

Consistent with the mean bout duration data from the full 20-min session, mean bout duration in the first minute was generally reduced by over 50% (see figure 4.24). The effects of drug dose on this parameter were significant: ($F_{3, 21} = 6.188$, $p<0.005$). However, there was no significant main effect of concentration: ($F_{2, 21} = 1.362$, n.s.) nor was there a significant drug-concentration interaction: ($F_{6, 63} = 0.306$, n.s.). The highest dose of raclopride (0.3 mg/kg) was most effective at reducing the mean bout duration at this early stage of the test. After a post hoc test the effects of this dose were shown to be significantly different from vehicle controls in the 1% and 10% sucrose groups.

Number of bouts

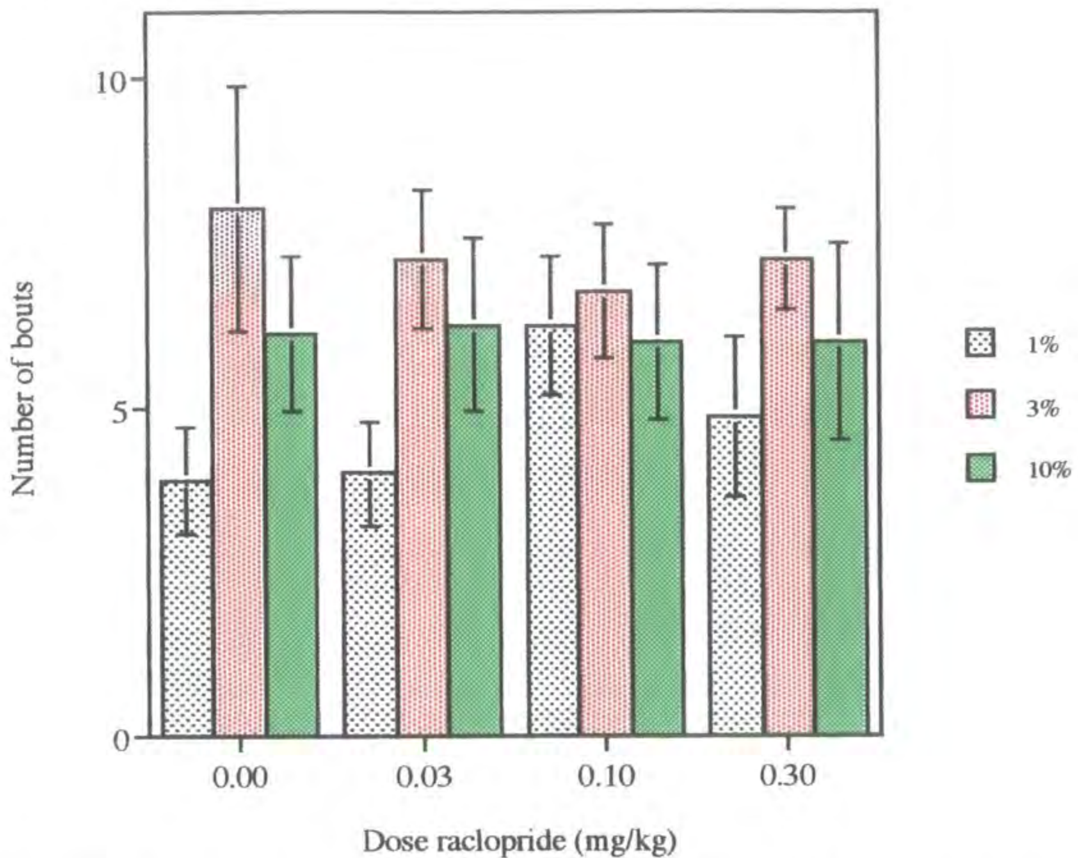


Figure 4.25 Mean bout frequency of licking for 1, 3 and 10% sucrose in the first minute of a 20-min test as a function of dose raclopride (0.03-0.3 mg/kg) + S.E.M.

Figure 4.25 shows that for the first minute of the 20-min test session there was no significant main effect of drug dose on mean bout frequency: ($F_{3, 21}$ = .092, n.s.). Concentration did show a main effect : ($F_{2, 21}$ = 4.977, $p<0.05$), however this relationship was non-monotonic. There was no significant drug-concentration interaction: ($F_{6, 63}$ = 0.477, n.s.).

Summary

Table 4.4 Drug effects on microstructural parameters when measured in the first minute of a 20-min test session and when measured over full 20-min of the test session.

	Data taken from:					
	20-min			1-min		
	7-OH	Quin	Rac	7-OH	Quin	Rac
Number of licks	⇩	⇩	▭	⇩	⇩	⇩
Mean bout duration (s)	⇩	⇩	⇩	⇩	⇩	⇩
Number of bouts	⇧	▭	⇧	▭	▭	▭

n=24 per drug group

⇩ = significant decrease ($p<0.05$)

▭ = n.s. effect of drug dose

⇧ = significant increase ($p<0.05$)

7-OH = 7-OH-DPAT (0.1-1.0 mg/kg)

Quin = quinpirole (0.03 - 0.3 mg/kg)

Rac = raclopride (0.03 - 0.3 mg/kg)

4.4 Discussion

Over the 20 minute test session, the three dopaminergic compounds did not reduce total intake to the same degree. Most profound decrements were found with increasing doses of 7-OH-DPAT which approximately halved the amount ingested at its highest dose. Quinpirole halved the volume of intake at both the medium and high doses (0.1 and 0.3 mg/kg) while raclopride did not significantly reduce intake overall (although a decreasing trend is evident for animals drinking 1% sucrose). These results suggest that these compounds may not be acting at the same receptor populations to achieve their differential effects on ingestive behaviour.

The pattern of results for total intake was reflected in the drug effects on total number of licks. When we examined the effects of the three drugs at the various level of sucrose concentration, differences emerged. The difference in number of licks between the vehicle and high dose conditions vary as a consequence of the value of the test solution (i.e. % sucrose), so that for 7-OH-DPAT there are differences between the percentage reduction between 1%, 3% and 10%. In the case of quinpirole number of licks are approximately halved at all concentrations and raclopride reduces the number of licks by 50% only at the 1% sucrose concentration, relatively sparing the intake of both the 3 and 10% solutions. Towell, (1987) observed, in accordance with the present results from raclopride, that in a one-bottle test, pimozide (a D2 antagonist) selectively reduced intake at low sucrose concentrations while sparing the intake of equal or higher volumes at higher concentrations.

The results from the administration of raclopride are reminiscent of studies by Phillips et al. (1991, a,b) who found that the extent to which the D2-receptor antagonist suppressed operant responding maintained by sucrose reinforcement was inversely related to the concentration of sucrose used. Indeed, facilitation of rewarded performance by pimozide (Phillips et al., 1991a) and raclopride (Phillips et al., 1991b) was observed when the stimulus was a high sucrose reward, even though the same compounds have been repeatedly shown to decrease the consumption of lower concentrations of sucrose (i.e. 1% and 10%) in several experimental situations (Phillips et al., 1991b).

The differential effects of a D2-antagonist (in the present experiment, raclopride) seem to be dependent upon the concentration of sucrose reward (Phillips et al., 1991b). The results from the present experiment suggest that it may not be the interaction between dopamine blockade and the absolute value of the reinforcer

that maintains or disrupts ingestive behaviour. For instance, in the present experiment responding (i.e. licking) for 3 and 10% solutions was spared relative to the amount ingested of the 1% solution. These findings also highlight the importance of considering the animal's prior experience with alternative values of reinforcer when interpreting drug effects on either conditioned or unconditioned responding (i.e. see Dickinson and Balleine, 1994 and General Discussion). Nevertheless there is evidence to the contrary. Cooper et al. have conducted many studies in which they tested the ability of dopamine antagonists to differentially affect various sucrose rewards. Although this work is largely unpublished, the general findings should be stated here. They found that dopamine antagonists such as raclopride and pimozide decreased sucrose solution intake irrespective of sucrose concentration (1-30%).

It is implied by the drug effects on the number of licks that the behavioural expression of the anorexia induced by these compounds differ significantly. As was mentioned in Chapter 2, the size of licking bouts reflect the persistance of the motor system which controls the tongue activity. Larger bouts reflect longer runs of uninterrupted licking and this variable has been reported to be directionally proportional to the concentration of the sucrose solution used as a tastant (Davis 1989; Schneider et al., 1990; Davis and Smith, 1992), and thus has been considered as a measure of palatability of the diet. As mean bout duration decreased significantly for all the compounds tested (over both the full 20-min session and the first minute of the test) this may be interpreted as an ability of each of these compounds, despite their pharmacological similarities and differences, to reduce the palatability of available sucrose solutions. For none of the drugs at any point of the test session could the decreases in number of licks observed be attributed to a decrease in the number of bouts (see Table 4.4). Therefore, the theoretical possibility that ingestion decreased as a result of a decrease in the number of bouts can be discounted.

Again, in the case of all three compounds, the reductions in ingestive responses were greater in the first minute than over the full twenty minute session. This was the case even for raclopride which had showed no significant effect on intake (or, consequently, number of licks) over the 20-min session This implies that these dopaminergic compounds are having their effects at the beginning of a meal. Changes in concentration in all three experiments were robustly reflected in the monotonic relationship between increasing concentration and increasing number of licks. This relationship seemed to be dependent on an increase in mean bout

duration rather than an increase in the mean bout frequency of licking. Therefore, such early effects on parameters thought to reflect hedonic evaluation suggest that these compounds are having an effect on palatability.

Further evidence to support the idea of dopaminergic involvement in early meal events comes from the exponential curve fits fitted to the licks per min data. In the case of 7-OH-DPAT the initial rate of licking (the intercept A) was severely dampened by the highest dose of 7-OH-DPAT (1.0 mg/kg) (over halved), while in the case of quinpirole the initial rate of licking was reduced by 50% (0.3 mg/kg). The estimate of A for raclopride at the highest dose (0.3 mg/kg) was almost double the estimate provided for the vehicle condition lick rate. Estimates of B (rate of decay of licking) were almost unaffected by each of these drugs. The decrease in the initial rate of licking found here after drug administration, is consistent with an inhibitory effect of dopamine receptor antagonism (or autoreceptor agonism) on the sensory/hedonic effect of sucrose, while the failure of the drugs to change the rate of decay of intake is evidence against the compounds decreasing intake by impairing ingestive movements necessary for sustained feeding (Davis and Levine, 1977). This profile of reduced initial rate of ingestion produced by dopamine receptor antagonists without a substantial change in the rate of decay of responding is equivalent to the profile of responding observed when sucrose solutions are devalued by dilution (Davis and Smith, 1988, 1992).

The results of Pecina and Berridge (1997) are useful in interpreting the present data. These authors identified certain characteristics of the data which led them to their interpretation of the results, one of which is the timing of the drug-induced effect. Conventional manipulators of hedonic or aversive taste palatability such as (hunger/satiety, aversion conditioning, brain lesions, opioid or benzodiazepine agents) typically produce taste reactivity patterns that can be detected in the first seconds or minute of a taste infusion (Berridge et al., 1996). In the hands of Pecina and Berridge, pimozide produced effects which only emerged gradually over successive minutes as the oral infusion was continued and that sometimes grew in strength as trials were repeated. They see these delayed effects as argument against an immediate and direct change in taste palatability in favour of the interpretation that the rats cannot maintain high response rates after an initial burst of of taste reactivity components.

Another feature of responding which Pecina and Berridge see as suggestive of impairment of sensorimotor function is the lack of selectivity of the drug effect (Pecina et al., 1997). They observed that behavioural suppression associated with

pimozide applied to hedonic, aversive and neutral categories of taste reactivity and to general activity. These authors see the results with pimozide as congruent with the "anergia" hypothesis advanced by Salamone and colleagues (McCullough and Salamone, 1992, Salamone, 1994; Salamone et al., 1990, Salamone et al., 1994) (see Chapter 2).

Therefore, the results of the present experiment could also be explained within this theoretical framework as reductions in mean bout duration over the 20-min test session were less profound than those data collected from the first minute of the test which could be construed as reflecting a gradual motor dysfuntion (Salamone, 1992, 1994) but could also reflect the induction of a post-ingestive negative feedback signal (Davis and Levine, 1977). Observations on the number of bouts, however, go some way to countering a motoric interpretation as bouts increased rather than decreased over the test session when animals were injected with raclopride or 7-OH-DPAT. This final observation is in contrast to the selective decrease in bouts after dopamine blockade found by both Asin et al. (1992) and Pecina and Berridge (1997).

The findings above are consistent with hypothesis which states that D2 antagonists such as pimozide reduce intake by decreasing the reinforcing potency of the sucrose solution without producing a motoric deficit (Towell, 1987; Bailey et al., 1986; Schneider et al., 1990). In this respect, the compounds used are similar to each other. However, because of the paucity of data on the effects of selective D3 receptor ligands on feeding, on the basis of these experiments alone it is difficult to tease out an effect on feeding parameters rather than idiosyncratic effects of the D3 receptor agonists used. In general, the dependent variables measured in this set of experiments (Experiments 1-3) reflect that the initial hedonic evaluation of tastants is impaired due to dopamine blockade.

The effects of quinpirole and 7-OH-DPAT have not, to date, been analysed at the level of individual licks. Both of these drugs seem to decrease ingestion by reducing those parameters which are thought to reflect palatability. This constitutes further confirmation of a role of D3 and D2 receptors in food reward. It is difficult to completely discount a motoric impairment induced by these pharmacological manipulations on the strength of these measures alone. Measures such as intrabout lick rate and latency could further illuminate possible motoric effects of these compounds. Therefore, Experiments 4, 5 and 6 aim to further investigate a motoric as well as a hedonic component to the effects of dopamine receptor subtype ligands on licking behaviour.

Chapter 5: Microstructural analysis using a brief contact test: The effects of 7-OH-DPAT, raclopride and SCH 23390 on drinking for sucrose and Intralipid

5.1 Introduction

Results from Experiments 1, 2 and 3 showed that the putative D3 receptor agonist, 7-OH-DPAT, the D2 receptor antagonist raclopride and the mixed D2/D3 receptor agonist quinpirole reduced the intake of sucrose solutions. Each of these compounds reduced the number of licks by decreasing the mean bout duration rather than the number of bouts. These reductions were most apparent in the first minute of the test sessions. Examination of the overall lick rate revealed that initial rate of licking was affected by these compounds rather than the rate of decay of licking over the test session. Taken together, these results imply that 7-OH-DPAT, raclopride and quinpirole reduced licking responses by reducing the palatability of the sucrose solutions rather than promoting satiety signals. An increase in number of bouts after the administration of 7-OH-DPAT, quinpirole (albeit non-significantly in the case of quinpirole) and raclopride in Chapter 4 represents preliminary evidence against the induction of a motoric deficit by these compounds.

As was mentioned in Chapter 2, a shorter test session helps to eliminate post-ingestive negative feedback and so gives the test more interpretive strength with regards the assessment of drug effects on palatability. Therefore, the set of experiments in this Chapter (Experiments 4, 5 and 6) used a 60 sec presentation of tastants in order to further eliminate these post-ingestive stimuli. However, there are mechanisms, other than those which are involved in the mediation of palatability and/or satiety, by which a reduction in intake may be realised. For example, drugs may affect some activational mechanism necessary to induce feeding. Therefore, along with microstructural variables, Experiments 4, 5 and 6 also measured the latency to begin drinking in the brief contact test.

A further possibility which was not fully resolved in Chapter 4 is that the decrease in the number of licks after the administration of dopaminergic antagonists (or putative dopamine autoreceptor agonists) could be partially due to a decrease in the rate of licking within a bout reflecting a motor impairment. Previously, Gramling and Fowler (1984) observed that neuroleptic treatment led to a decrease in lick frequency. On further analysis this result was attributed to an abnormal pattern of tongue protrusions aimed at the licking spout. Orolingual motor competence was

therefore said to be impaired by the neuroleptic treatment (but see General Discussion).

Nevertheless, Schneider et al. (1990) found that the D2 receptor antagonist raclopride did not affect the motor capability of rats as reflected by their interlick intervals. The administration of neuroleptics has been shown to affect intervals between bouts and the number of licks within a bout without affecting the rate of licking within a bout (Schneider et al., 1990; Weatherford et al., 1990). These results suggest neuroleptic treated animals were capable of making the rapid motoric responses necessary to sustain normal licking behaviour.

Due to this conflict in results, it is important that more investigation is directed towards the important distinction between the motor system which controls refined alterations in tongue protrusions (the actual act of licking) from that system which orchestrates licking behaviour into bouts of licking (Davis, 1989; Schneider et al., 1990). Specifically, the experiments in this Chapter serve to highlight such a distinction by testing three drugs which purportedly act at separable dopamine receptor subtypes using a microstructural analysis of licking behaviour in a brief contact test. In these shortened tests, microstructural measurements have been extended to include measures which may reflect a type of motoric impairment (i.e. latency, intrabout lick rate and average interlick interval).

The drugs used in Experiments 4 and 5 (7-OH-DPAT and raclopride) have already been used in conjunction with a microstructural analysis in Chapter 4 over a 20 min test session. The use of a shorter test session for Experiments 4, 5 and 6 along with the measurement of variables which further reflect motor competence will help to resolve questions regarding motoric effects of such drugs, which were only partially answered in Chapter 4. Experiment 6 uses a D1 receptor antagonist (SCH 23390) which works at post-synaptic receptors, to further broaden the comparison of the contribution of dopamine receptor subtypes to changes in licking microstructure.

More recent work has shown that SCH-23390 reduces food intake in both food deprived (Gilbert and Cooper, 1985; Zarrindast et al, 1991; Terry and Katz, 1992) and free-feeding animals (Clifton et al, 1991; Naruse et al, 1991). Further, it is fairly well established that the importance of differences between D1 and D2 receptor antagonists lies in the possibility that D1 antagonists may have lower extrapyramidal side effect liability (Coffin et al., 1989; Fowler and Liou, 1994). Due to its limbic selectivity, the same characteristic has been inferred for the

D3 receptor agonist 7-OH-DPAT (Sokoloff, 1990). However, an early study on the effects of a D1-type antagonist on food intake (Koechling et al., 1988) showed that across a series of discrete meal segments, SCH-23390 increased the latency to eat and reduced the speed of eating which may reflect a motoric effect of the drug. Therefore, it is unclear whether D1-like antagonists produce more of a motoric deficit than D2 receptor antagonists, thus, a further aim of this set of experiments was to examine in detail any changes in orolingual motor competence after the administration of SCH-23390 and to compare the results with lick data collected after the administration of raclopride and 7-OH-DPAT.

Chapter 2 also shows that the model for the control of ingestion formulated by Davis and Levine (1977) can incorporate lick data which has been collected in response to a variety of different macronutrient solutions. For example, Davis, (1996), compared the results of a microstructural analysis when the test stimulus was Polycose (a complex mixture of mono-, di- and polysaccharides) to other mono- and disaccharides (such as sucrose). Both initial rate of licking and bout size were increasing linear functions of the concentration of Polycose (Davis, 1996). This finding has been further confirmed by Higgs and Cooper, (1998). Similar results have been found with a fat emulsion, Intralipid (Higgs and Cooper, 1996). However, Davis (1995) failed to find a similar result with increasing concentrations of corn oil. Therefore, the final aim of this set of experiments was to attempt to replicate the effects of Intralipid concentration on mean bout duration, and to compare any concentration effects with those of sucrose solutions. At the same time, the effects of the dopaminergic agonist and antagonists on the two types of macronutrient fluid ingestion were investigated.

5.2 Method

5.2.1 Animals

Sixty non-deprived adult male hooded Lister rats (n=20 per Experiment 4, 5 and 6), weighing 350-400g at the time of testing were used. They were housed and maintained as described in Chapter 3.

5.2.2 Drugs

In Experiment 4, 7-OH-DPAT (Research Biochemicals International, U.K.) was dissolved in saline and injected i.p. 20 minutes before testing. Doses of 7-OH-DPAT were 0.1, 0.3 and 1 mg/kg or its vehicle. For the purposes of Experiment 5, raclopride (Research Biochemicals International, UK) was dissolved in distilled water and injected i.p. 20 mins before the start of testing at doses of 0.03; 0.1 and 0.3mg/kg or vehicle. In Experiment 6, SCH- 23390 (Research Biochemicals International, UK) was dissolved in sterile water and injected i.p. 20-min before testing at doses of 0.01, 0.03 and 0.1 mg/kg or vehicle. All details of drug preparation and administration for each drug can be found in Chapter 3.

5.2.3 Test Fluids

The two types of test fluid were sucrose solutions or Intralipid emulsions made up as described in Chapter 3. In each experiment (4, 5 and 6), 10 animals were trained and tested on 1, 3 and 10% sucrose while the remaining 10 animals had access to 1, 3 and 10% Intralipid. During both training and testing animals had experience of all concentrations of the fluid.

5.2.4 Apparatus

Testing was carried out using the MS80 lickometer described in detail in Chapter 3.

5.2.5 Procedure

Training

20 animals were assigned to each of Experiments 6, 7 and 8. These experiments differed in the type of drug treatment which was administered before testing (i.e. 7-OH-DPAT, raclopride or SCH 23390). The 20 animals in each experiment constituted 2 groups of 10 randomly assigned members. This split was based on the type of test solution they were to have access to during training and testing (i.e. sucrose solution or Intralipid emulsion). Each group was familiarised with the test apparatus and solutions for approximately 10 days or until baseline drinking levels were stable. Training consisted of 1 min access to each concentration of the test fluid (1, 3 and 10% sucrose solution or 1, 3 and 10% Intralipid emulsion) at the same time on each training day. Two days prior to testing animals received a sham injection of vehicle in order to familiarise them with the injection procedure.

Testing

Experiment 4: Early effects of 7-OH-DPAT on the microstructure of licking: a brief contact test.

Following training, 7-OH-DPAT (0.1, 0.3 and 1.0 mg/kg) or vehicle was administered i.p., 20-min before animals were placed in the lickometer chamber where they were presented with 1, 3 and 10% sucrose for 1 min per concentration, with an inter-presentation interval of 10s. The study employed a repeated measures design with all animals having access to all concentrations of their allotted test fluid after all doses of drug. Injections were counterbalanced using a Latin Square design as were the presentations of concentrations of test fluid. There was a 48h time lapse between injections to avoid carry-over effects.

Experiment 5: Early effects of raclopride on the microstructure of licking: a brief contact test.

Following training, raclopride was administered i.p. (0.03, 0.1 and 0.3 mg/kg) or vehicle. Presentation of test fluids, the design of the study and the injection procedures are as described for Experiment 4.

Experiment 6: Early effects of SCH-23390 on the microstructure of licking: a brief contact test.

Following training, SCH-23390 (0.01, 0.03 and 0.1 mg/kg) or vehicle was administered i.p. Presentation of test fluids, the design of the study and the injection procedures are as described for Experiment 5. The data from one animal in Experiment 6 (sucrose group) was excluded due to a swollen leg on the third injection day.

5.2.6 Design and statistical analysis

Initial analysis of the lick time data was carried out using the programs outlined in Chapter 3. Measures derived from the raw data included latency to engage in drinking (time from shutter opening to first lick) and a number of microstructural variables: number of licks, mean bout duration (s), number of bouts, intrabout lick rate (licks/s within bouts), and interlick interval (msec).

Data from Experiments 4, 5 and 6 were analysed using a two-way repeated measures ANOVA with drug dose and fluid concentration as factors. Where there was a significant main effect of drug but no significant interaction between drug dose and fluid concentration post hoc tests were employed. To do this, the main effect of drug was collapsed across concentration and Dunnett's t-test was used to determine any significant differences between animals who had received drug doses and vehicle controls. Statistical tests were performed using Super Anova and Statview SE+graphics (Abacus Concepts Inc., Berkeley, CA). A result was considered statistically significant if $p<0.05$).

5.3 Results

To avoid confusion and redundancy in the results section, the means and standard errors measured from Experiments 4, 5 and 6 are presented firstly in tabulated form without statistical information followed by graphs of the results from each parameter accompanied by statistical information.

Experiment 4: Early effects of 7-OH-DPAT on the microstructure of licking: a brief contact test.

A brief contact licking test involves the presentation of differing concentrations of selected fluids for 60 seconds. Below are the licking parameters measured after the administration of 7-OH-DPAT. The parameters measured were number of licks, mean bout duration, number of bouts, intrabout lick rate (and average interlick interval) as well as latency. Animals licking for all concentrations of Intralipid emulsions or sucrose solutions are represented in Table 5.1.

Table 5.1 The average (+ S.E.M.) of the feeding parameters at each dose of 7-OH-DPAT and at each sucrose / Intralipid concentration (1, 3 and 10%).

	Dose 7-OH-DPAT (mg/kg)							
	0.0	0.1	0.3	1.0	0.0	0.1	0.3	1.0
	Sucrose				Intralipid			
Latency (s)								
Fluid (%)								
1	5.5 (1.5)	2.9 (1.2)	5.0 (3.3)	27.3 (7.2)	21.7 (7.2)	26.6 (8.8)	25.1 (6.1)	44.9 (6.6)
3	10.0 (5.7)	3.3 (1.4)	3.8 (1.1)	21.5 (7.3)	25.6 (7.3)	19.4 (7.6)	22.4 (8.2)	42.4 (6.5)
10	7.2 (4.9)	7.9 (2.6)	8.7 (3.4)	18.8 (7.4)	12.2 (5.1)	8.8 (2.8)	17.3 (6.1)	31.1 (8.0)
Number of licks								
Fluid (%)								
1	84.7 (27.2)	66.6 (18.9)	67.1 (23.4)	43.5 (17.3)	93.0 (27.3)	60.3 (25.8)	67.1 (19.3)	9.3 (5.2)
3	159.4 (26.2)	196.4 (24.9)	127.2 (30.6)	88.5 (26.1)	119.4 (40.9)	123.4 (37.6)	77.4 (34.0)	18.0 (6.7)
10	332.6 (9.1)	316.6 (19.3)	241.6 (23.6)	159.9 (27.6)	258.0 (36.4)	247.7 (21.5)	248.8 (31.0)	81.7 (25.1)
Mean bout duration (s)								
Fluid (%)								
1	1.0 (0.2)	1.4 (0.2)	1.4 (0.4)	0.9 (0.3)	1.9 (0.3)	1.2 (0.4)	1.8 (0.5)	0.5 (0.2)
3	1.9 (0.3)	2.3 (0.2)	2.2 (0.3)	1.4 (0.3)	1.9 (0.5)	1.8 (0.4)	1.3 (0.5)	1.1 (0.4)
10	3.1 (0.2)	3.3 (0.3)	2.7 (0.2)	1.7 (0.2)	3.9 (0.6)	3.1 (0.3)	3.6 (0.6)	1.7 (0.7)

(**Table 5.1** continued)

Number of bouts								
Fluid (%)								
1	7.6 (1.9)	6.0 (1.2)	5.5 (1.1)	5.1 (1.2)	6.1 (1.6)	4.4 (1.9)	4.0 (0.8)	1.7 (0.9)
3	10.1 (1.3)	12.1 (1.5)	8.3 (1.4)	9.6 (1.7)	6.4 (2.1)	7.3 (1.5)	4.7 (2.0)	1.9 (0.5)
10	15.1 (0.7)	14.6 (1.0)	14.8 (0.6)	17.2 (2.6)	10.0 (1.5)	12.5 (0.9)	10.0 (1.5)	7.5 (2.3)
Intrabout lick rate (licks/s)								
Fluid (%)								
1	7.3 (0.2)	6.7 (0.1)	6.0 (0.3)	4.4 (0.7)	6.5 (0.7)	4.8 (1.1)	5.5 (0.9)	2.2 (0.8)
3	6.3 (0.7)	6.6 (0.1)	6.0 (0.2)	4.6 (0.6)	5.8 (0.9)	6.0 (0.7)	4.7 (1.1)	2.8 (0.8)
10	6.9 (0.01)	6.7 (0.1)	5.7 (0.2)	4.5 (0.5)	6.9 (0.2)	6.4 (0.1)	6.2 (0.2)	3.4 (0.8)
Interlick interval (msec)								
Fluid (%)								
1	140.1 (4.3)	157.1 (5.8)	165.5 (15.6)	177.7 (7.9)	143.9 (4.2)	127.6 (11.1)	141.8 (5.6)	178.6 (22.8)
3	142.1 (2.1)	154.4 (3.2)	169.3 (4.7)	198.9 (7.9)	137.1 (5.2)	155.3 (7.7)	128.3 (11.8)	200.1 (9.8)
10	145.0 (1.8)	150.9 (2.6)	176.7 (5.1)	201.8 (6.9)	147.3 (4.1)	159.4 (3.4)	163.6 (4.6)	190.5 (12.7)

n= 10 animals per test fluid group

Latency

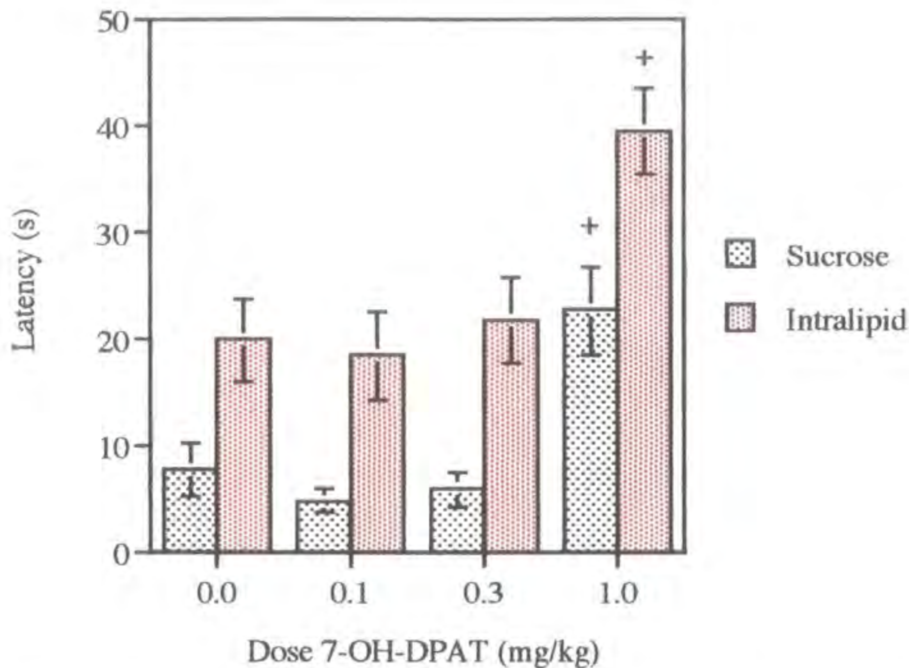


Figure 5.1 Latency to start drinking sucrose and Intralipid in a brief contact test as a function of increasing dose of 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Plus indicates significantly different from vehicle +p<0.01.

Figure 5.1 shows that the latency to begin drinking increased significantly with drug dose giving a main effect of dose 7-OH-DPAT: ($F_{3, 27} = 7.092$, $p<0.05$). However, there was no significant effect of sucrose concentration: ($F_{2, 18} = 0.069$, n.s.) on latency. There was not a significant interaction between drug dose and sucrose concentration: ($F_{6, 54} = 1.090$, n.s.). Post-hoc tests revealed that the 0.1 mg/kg dose of 7-OH-DPAT significantly increased latency. This effect of drug seemed to be most profound within the group drinking 1% sucrose where the increase in latency in the high dose group (0.1 mg/kg) was almost five times that of the vehicle controls.

In the case of Intralipid, there was also an incremental main effect of dose 7-OH-DPAT ($F_{3, 27} = 4.781$, $p<0.05$) but no effect of concentration: ($F_{2, 18} = 3.079$, n.s.) although latency tended to decrease with increasing concentration. Again, the interaction between drug dose and concentration was non significant: ($F_{6, 54} = 0.253$, n.s.). Post hoc tests revealed that the 1.0 mg/kg dose of 7-OH-DPAT significantly increased latency when compared to vehicle controls.

Microstructural analysis

Number of licks

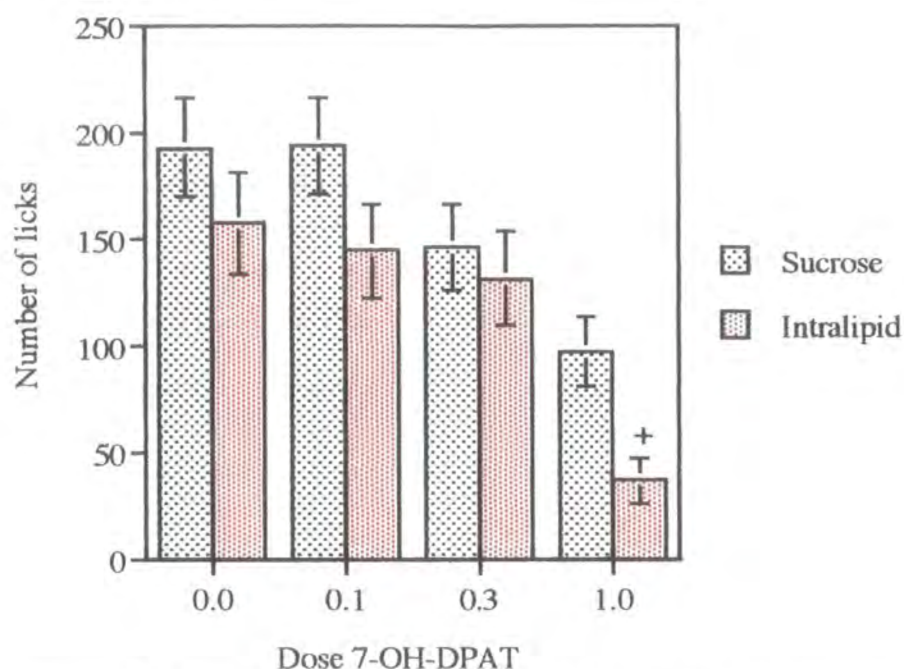


Figure 5.2 Number of licks for sucrose and Intralipid drinking in a brief contact test as a function of increasing dose of 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M. Plus indicates significantly different from vehicle +$p<0.01$.

Table 5.2 Main effect of fluid concentration on total number of licks .

Mean number of licks ± S.E.M.

Fluid (%)	1	3	10
Sucrose	68.5±10.8	142.9±14.5	262.7±14.9
Intralipid	57.4±11.3	84.6±17.1	209.1±18.3

n=10 animals per test fluid group

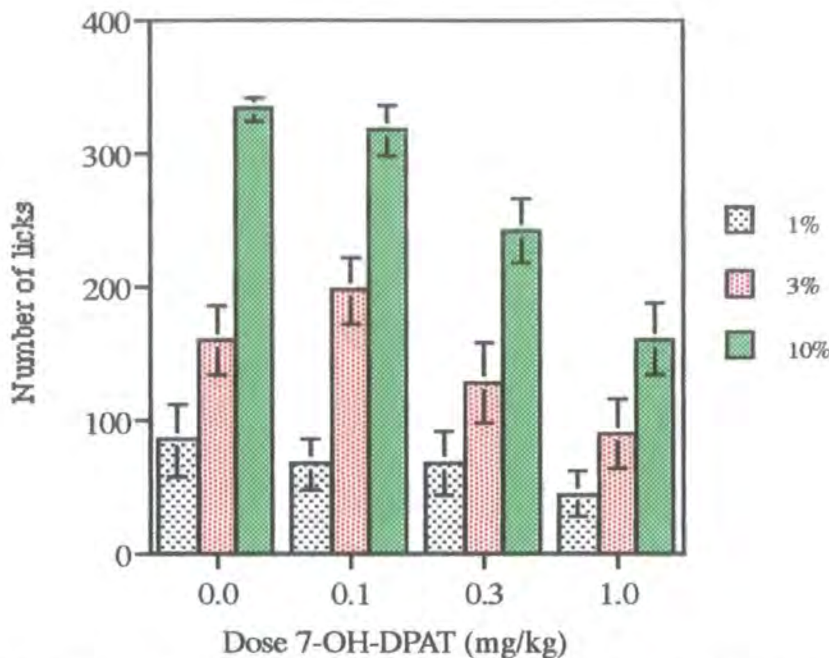


Figure 5.3 Total number of licks for 1, 3 and 10% sucrose as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M.

Number of licks for sucrose were approximately halved after the administration of the highest dose (1.0 mg/kg) of 7-OH-DPAT but the relationship between drug dose and number of licks was only monotonic in the case of those animals drinking 10% sucrose. Nevertheless, there was a significant main effect of dose 7-OH-DPAT: ($F_{3, 27} = 6.672$, $p<0.05$) and a highly significant main effect of concentration: ($F_{2, 18} = 113.430$, $p<0.0001$). There was also a significant interaction between drug dose and concentration: ($F_{6, 54} = 2.840$, $p<0.05$). Post hoc tests were not conducted owing to the significant interaction.

The number of licks for Intralipid were also decreased significantly as a function of drug dose: ($F_{3, 27} = 9.459$, $p<0.01$). These decreases seen after the high dose were more profound than those observed for sucrose. For instance, when animals were drinking 1 and 3% Intralipid, the mean number of licks after the highest dose of 7-OH-DPAT were approximately 10% of those observed in the vehicle control group, while those drinking 10%, under the same drug condition showed a decrease in the number of licks of approximately 60%. Increasing concentration gave rise to a significant increase in the number of licks: ($F_{2, 18} = 21.444$, $p<0.0001$). In this case there was no significant interaction of drug dose and concentration: ($F_{6, 54} = 1.725$, n.s.). Post hoc tests revealed that, as with sucrose, at the highest dose (1.0 mg/kg) of 7-OH-DPAT there was a significant difference in number of licks as compared to vehicle controls (see Figure 5.3).

Mean bout duration

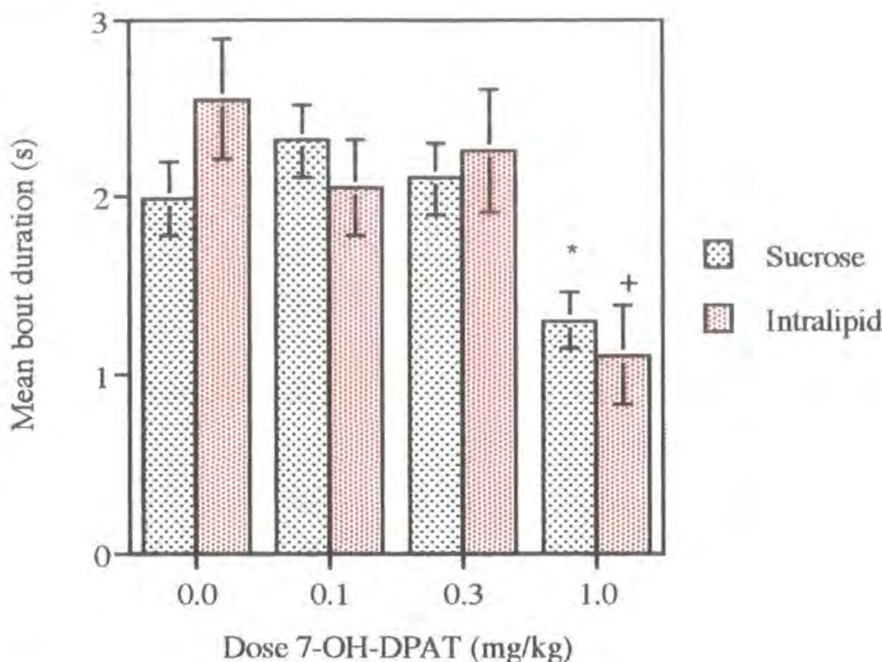


Figure 5.4 Mean bout duration for sucrose and Intralipid drinking in a brief contact test as a function of increasing dose of 7-OH-DPAT (0.1-1.0 mg/kg)+ S.E.M. Asterisk indicates significantly different from vehicle *p<0.05; Plus indicates significantly different from vehicle +p<0.01.

Table 5.3 Main effect of fluid concentration on mean bout duration .

	Mean bout duration ± S.E.M.		
Fluid (%)	1	3	10
Sucrose	1.2±0.1	2.0±0.2	2.7±0.2
Intralipid	1.3±0.2	1.5±0.2	3.1±0.3

n=10 animals per test fluid group

Figure 5.4 reveals that mean bout duration of licking for sucrose decreased significantly as a function of dose 7-OH-DPAT : ($F_{3, 27} = 4.512$, $p<0.05$). These decremental effects were most noticeable in those drinking 10% sucrose, where mean bout duration was shortened by approximately 45%. There was a highly significant effect of concentration on mean bout duration; as concentration increased so did mean bout duration and this relationship was monotonic: ($F_{2, 18}= 21.444$, $p<0.0001$), (see Table 5.3). There was no significant interaction between drug and concentration: ($F_{6, 54} = 1.910$, n.s.). Post hoc tests showed that at the

highest dose of 7-OH-DPAT (0.1 mg/kg) mean bout duration was significantly shorter than the mean bout duration measured for the vehicle controls.

As is clear from Figure 5.4, the mean bout duration for drinking of Intralipid was reduced non-monotonically as a function of dose 7-OH-DPAT: ($F_{3, 27} = 4.044$, $p<0.05$). In this case the most profound drug effects were observed within the 1% Intralipid group where mean bout duration was shortened by approximately 70% at the highest dose of 7-OH-DPAT (1.0 mg/kg) as compared to the vehicle control group. Increasing concentration led to a significant and monotonic increase in mean bout duration: ($F_{2, 18} = 12.844$, $p<0.01$). There was no significant interaction between drug dose and Intralipid concentration in the case of mean bout duration: ($F_{6, 54} = 1.085$, n.s.), (see Table 5.3). Post hoc tests showed that compared to vehicle controls, the highest dose of 7-OH-DPAT (1.0 mg/kg) significantly reduced mean bout duration.

Number of bouts

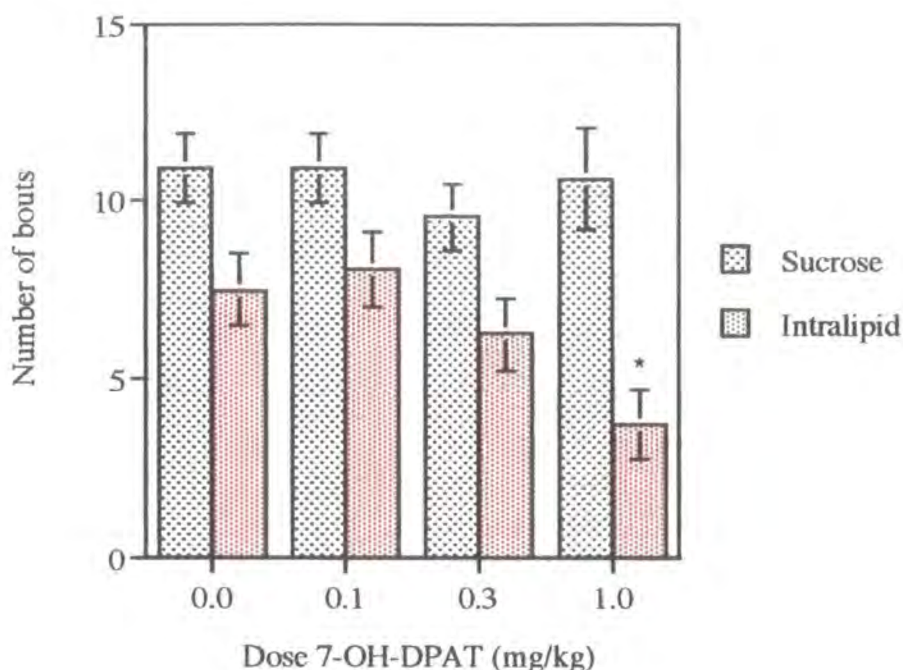


Figure 5.5 Mean number of bouts for sucrose and Intralipid drinking in a brief contact test as a function of increasing dose of 7-OH-DPAT (0.1-1.0 mg/kg)+ S.E.M. Asterisk indicates significantly different from vehicle *p<0.05.

Table 5.4 Main effect of fluid concentration on mean number of bouts .

	Mean number of bouts ± S.E.M.		
Fluid (%)	1	3	10
Sucrose	6.1±0.7	10.0±0.8	15.4±0.2
Intralipid	4.1±0.7	5.1±0.9	10.0±0.9

n=10 animals per test fluid group

There was no overall drug effect on mean bout frequency for those animals drinking sucrose: ($F_{3, 27} = 0.543$, n.s.). Number of bouts tended to decrease in the 1% and 3% drinkers but were slightly increased in the 10% sucrose group (see Figure 5.5). In contrast, there was a highly significant main effect of concentration on mean bout frequency: ($F_{2, 18} = 12.844$, $p<0.01$); whereby increasing concentration led to more bouts. There was no significant interaction of drug dose and concentration: ($F_{6, 54} = 0.995$, n.s.).

Figure 5.5 also shows that for those animals drinking Intralipid, there was a significant but non-monotonic decrease in mean bout frequency as a function of drug dose: ($F_{3, 27} = 2.965$, $p<0.05$). Similar to sucrose, increasing Intralipid concentrations gave rise to a significant increase in bouts: ($F_{2, 18} = 13.784$, $p<0.01$), (see table 5.4); however, there was no significant interaction between drug dose and Intralipid concentration: ($F_{6, 54} = 0.540$, n.s.). The highest dose of 7-OH-DPAT (0.1 mg/kg) was shown to be significantly different from vehicle controls in a post hoc test.

Intrabout lick rate

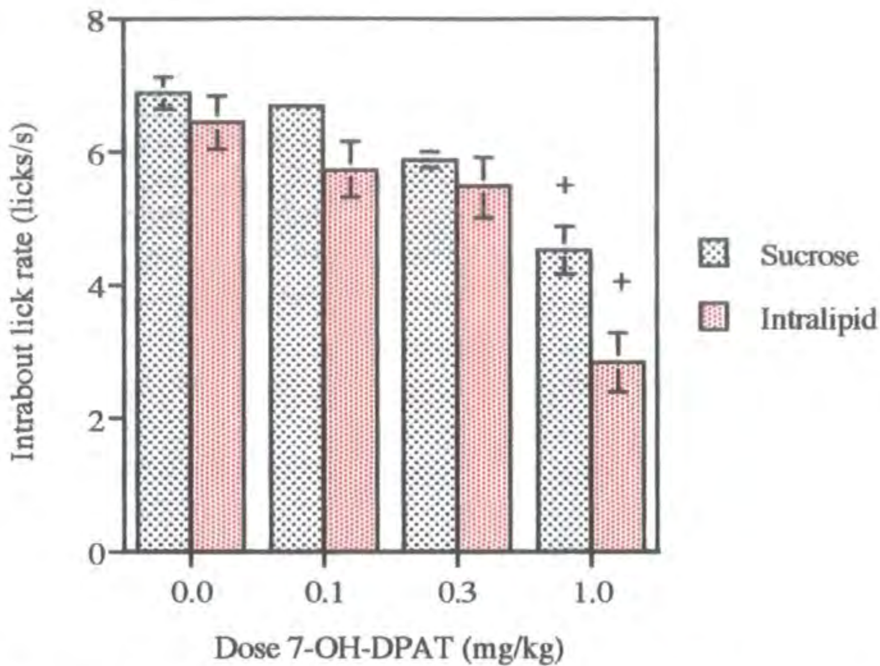


Figure 5.6 Intrabout lick rate for sucrose and Intralipid drinking in a brief contact test as a function of increasing dose of 7-OH-DPAT (0.1-1.0 mg/kg)+ S.E.M. Plus indicates significantly different from vehicle +$p<0.01$.

As can be seen in Figure 5.6, intrabout licking rate for sucrose decreased as a function of drug dose. Therefore, there was a significant and monotonic effect of dose 7-OH-DPAT on intrabout lick rate: ($F_{3, 27} = 22.650$, $p<0.0001$). Concentration did little to affect intrabout lick rate and so the effect of this variable was non-significant: ($F_{2, 18}= 0.507$, n.s.). Nor was there a significant interaction between drug dose and sucrose concentration for this parameter: ($F_{6, 54} = 0.507$, n.s.). The highest dose of 7-OH-DPAT (1.0 mg/kg) was shown to be significantly different from vehicle controls in a post hoc test.

Animals drinking Intralipid were affected similarly to those drinking sucrose within this feeding parameter. Intrabout licking rate for Intralipid decreased as a function of drug dose leading to a significant and monotonic effect of dose 7-OH-DPAT on intrabout lick rate: ($F_{3, 27} = 19.374$, $p<0.0001$). Concentration had a marginal effect on intrabout lick rate increasing it slightly but non-significantly in those animals drinking 10% Intralipid: ($F_{2, 18}= 1.996$, n.s.). There was no significant interaction between drug dose and Intralipid concentration for intrabout lick rate: ($F_{6, 54} = 0.415$, n.s.). Again the highest dose of 7-OH-DPAT (1.0 mg/kg) was shown to be significantly different from vehicle controls in a post hoc test.

Average interlick interval

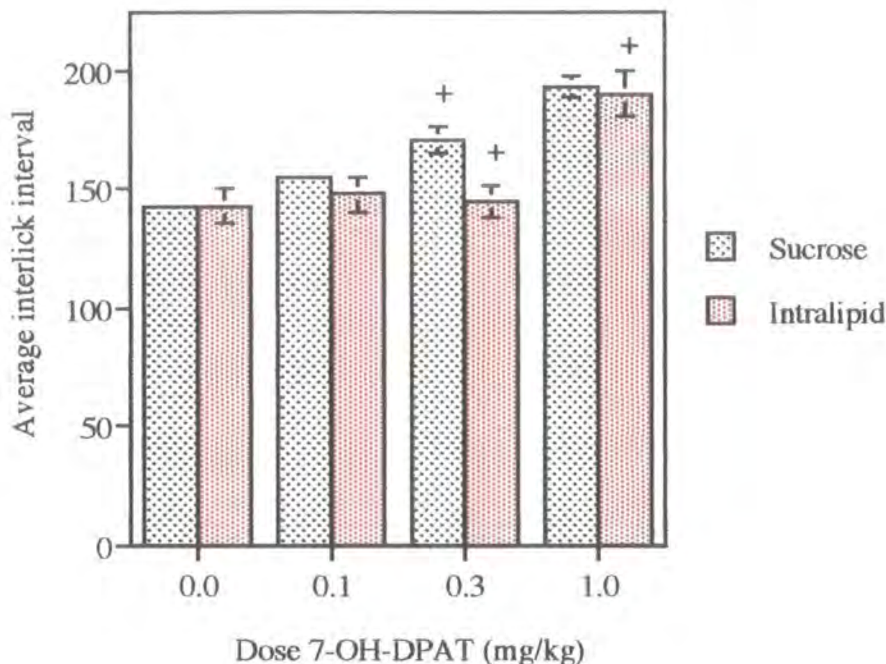


Figure 5.7 Average interlick interval for sucrose and Intralipid drinking in a brief contact test as a function of increasing dose of 7-OH-DPAT (0.1-1.0 mg/kg)+ S.E.M. Plus indicates significantly different from vehicle +$p<0.01$.

The average interlick interval for sucrose increased as a function of dose 7-OH-DPAT. This main effect was significant: ($F_{3, 27} = 18.703$, $p<0.0001$). Concentration did not significantly affect average interlick interval: ($F_{2, 18} = 3.446$, n.s.), nor was there any significant interaction between drug dose and sucrose concentration: ($F_{6, 54} = 1.450$, n.s.). Both medium (0.3 mg/kg) and high (1.0 mg/kg) doses of 7-OH-DPAT were shown to be significantly different from vehicle controls in a post hoc test (see Figure 5.7).

Again, the changes in average interlick interval induced by 7-OH-DPAT were similar for animals drinking sucrose and Intralipid. The average interlick interval for Intralipid increased as a function of dose 7-OH-DPAT but non-monotonically. This main effect was significant: ($F_{3, 27} = 12.378$, $p<0.0001$). Concentration, however significantly and monotonically increased average interlick interval for those drinking Intralipid: ($F_{2, 18} = 4.199$, $p<0.05$). As with sucrose, there was no significant interaction between drug dose and Intralipid concentration in this parameter: ($F_{6, 54} = 1.546$, n.s.). Only the highest dose of 7-OH-DPAT (1.0 mg/kg) was shown to be significantly different from vehicle controls in a post hoc test (see Figure 5.7).

Experiment 5: Early effects of raclopride on the microstructure of licking: a brief contact test.

A brief contact licking test involves the presentation of differing concentrations of selected fluids for 60 seconds. Below are the licking parameters measured after the administration of raclopride. A brief contact licking test involves the presentation of differing concentrations of selected fluids for 60 seconds. Below are the licking parameters measured after the administration of raclopride . The parameters measured were number of licks, mean bout duration, number of bouts, intrabout lick rate (and average interlick interval) as well as latency. Animals licking for all concentrations of Intralipid emulsions or sucrose solutions are represented in Table 5.5.

Table 5.5 The average (+ S.E.M.) of the feeding parameters at each dose of raclopride and at each sucrose / Intralipid concentration (1, 3 and 10%).

	Dose raclopride (mg/kg)							
	0.0	0.03	0.1	0.3	0.0	0.03	0.1	0.3
	Sucrose				Intralipid			
Latency (s)								
Fluid (%)								
1	10.2 (5.6)	8.7 (5.8)	11.8 (3.1)	9.7 (3.5)	17.4 (7.3)	17.7 (6.1)	12.3 (4.6)	23.4 (8.1)
3	11.4 (5.7)	6.5 (2.1)	14.1 (6.0)	11.5 (5.8)	11.6 (5.8)	24.0 (8.2)	29.6 (7.6)	25.1 (7.8)
10	3.0 (0.9)	9.4 (5.9)	7.5 (5.8)	9.8 (5.1)	15.1 (6.2)	17.3 (7.5)	20.2 (5.8)	15.9 (7.5)
Number of licks								
Fluid (%)								
1	96.6 (16.1)	54.4 (16.9)	86.1 (23.6)	27.9 (10.3)	77.1 (28.2)	48.7 (16.8)	93.3 (31.7)	18.0 (10.2)
3	189.5 (35.3)	167.5 (30.2)	192.2 (23.7)	94.8 (24.7)	122.5 (35.1)	103.3 (32.2)	75.1 (37.2)	34.7 (16.4)
10	312.6 (36.2)	312.7 (36.5)	300.4 (35.9)	234.8 (30.4)	190.0 (48.8)	245.9 (41.6)	183.0 (41.8)	146.4 (38.1)
Mean bout duration (s)								
Fluid (%)								
1	2.5 (0.5)	1.3 (0.4)	2.1 (0.4)	0.8 (0.2)	2.2 (0.6)	1.4 (0.3)	1.8 (0.3)	0.6 (0.1)
3	3.1 (0.7)	2.6 (0.3)	2.7 (0.5)	1.7 (0.4)	2.8 (0.6)	3.0 (0.5)	1.3 (0.3)	0.7 (0.2)
10	7.5 (2.7)	6.1 (1.8)	4.8 (1.3)	3.3 (0.6)	1.9 (0.4)	3.2 (0.3)	2.9 (0.4)	1.6 (0.1)

(**Table 5.5** continued)

Number of bouts								
Fluid (%)								
1	5.3 (1.0)	4.3 (0.7)	5.3 (1.0)	3.4 (0.9)	3.1 (0.8)	4.2 (1.2)	5.6 (1.7)	2.4 (1.1)
3	8.9 (1.7)	8.8 (1.2)	10.8 (2.2)	6.2 (1.4)	6.3 (1.7)	4.8 (1.8)	5.0 (2.1)	3.4 (1.3)
10	10.0 (2.3)	9.7 (1.8)	11.3 (2.1)	12.1 (1.9)	9.1 (2.5)	11.1 (2.0)	10.6 (2.8)	10.0 (2.4)
Intrabout lick rate (licks/s)								
Fluid (%)								
1	6.2 (0.7)	6.4 (0.8)	6.7 (0.1)	6.5 (0.4)	5.1 (1.2)	5.0 (1.1)	6.0 (0.8)	4.5 (1.3)
3	5.9 (0.7)	6.9 (0.1)	5.9 (0.6)	5.1 (0.8)	5.4 (0.9)	4.1 (1.1)	4.2 (1.1)	5.0 (1.1)
10	6.8 (0.2)	5.9 (0.6)	6.0 (0.6)	6.7 (0.2)	5.3 (0.9)	5.2 (0.9)	5.2 (0.9)	4.7 (1.0)
Interlick interval (msec)								
Fluid (%)								
1	131.6 (14.9)	130.3 (15.2)	154.4 (4.4)	163.8 (13.2)	105.4 (23.1)	105.0 (23.5)	144.2 (21.4)	86.7 (22.8)
3	149.9 (4.0)	145.9 (2.7)	139.5 (15.9)	129.8 (22.4)	123.1 (21.6)	89.8 (24.6)	90.4 (25.4)	102.1 (9.8)
10	134.7 (15.1)	137.3 (15.4)	135.7 (15.2)	152.5 (3.9)	121.4 (20.4)	123.0 (20.6)	125.5 (22.9)	108.3 (23.9)

n= 10 animals per test fluid group

Latency

Latency to drink sucrose did not change significantly with increasing doses of raclopride: ($F_{3, 27} = 0.112$, n.s.). Sucrose concentration did not significantly affect latency to start drinking: ($F_{2, 18} = 1.492$, n.s.) and there was no significant interaction between drug dose and sucrose concentration: ($F_{6, 54} = 0.719$, n.s.).

Intralipid drinkers were slower to approach the spout to drink as a function of dose raclopride and this relationship between dose and latency was monontonic. However, the result did not reach statistical significance: ($F_{3, 27} = 0.387$, n.s.). Neither did Intralipid concentration significantly affect the latency to begin drinking Intralipid: ($F_{2, 18} = 0.912$, n.s.). Again, as for sucrose, there was no significant interaction between drug dose and Intralipid concentration: ($F_{6, 54} = 0.700$, n.s.), (see Table 5.5).

Microstructural analysis

Number of licks

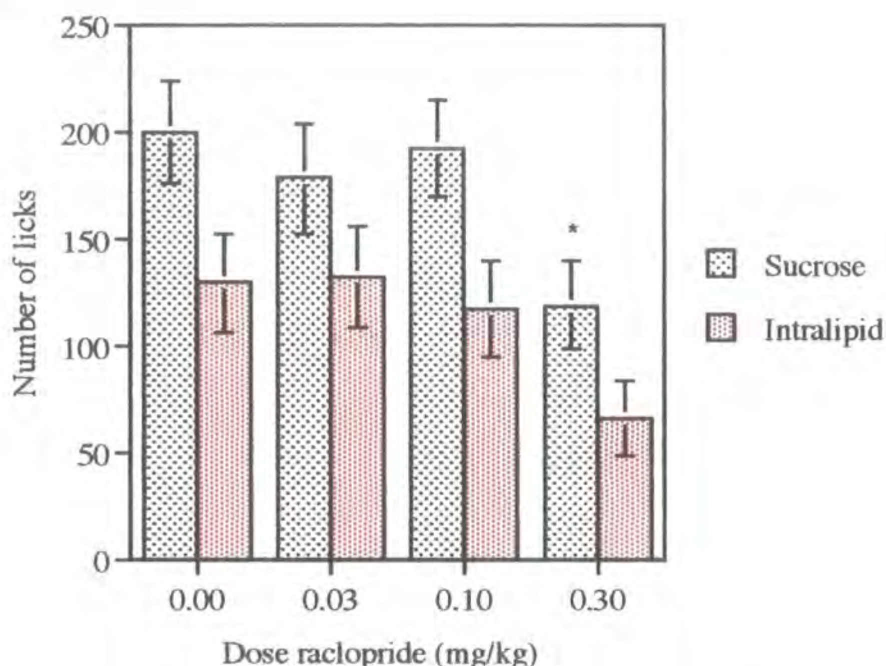


Figure 5.8 Number of licks for sucrose and intralipid drinking in a brief contact test as a function of increasing dose of raclopride (0.03-0.3 mg/kg) + S.E.M. Asterisk indicates significantly different from vehicle *p<0.05; Plus indicates significantly different from vehicle +p<0.01.

Table 5.6 Main effect of fluid concentration on total number of licks.

	Mean number of licks ± S.E.M.		
Fluid (%)	1	3	10
Sucrose	66.3±9.4	161.0±15.2	290.1±17.6
Intralipid	59.2±12.2	83.9±15.9	191.3±21.3

n=10 animals per test fluid group

Figure 5.8 shows that number of licks were reduced as a function of dose of raclopride. This result was statistically significant: ($F_{3, 27} = 3.041$, $p<0.05$). There was also a highly significant effect of sucrose concentration on the number of licks: ($F_{2, 18} = 181.408$, $p<0.0001$) and the relationship between concentration and number of licks was monotonic (see Table 5.6). However, there was no significant

interaction between drug dose and sucrose concentration: ($F_{6, 54}$ =0.442, n.s.). Post hoc tests revealed that the highest dose of raclopride (0.3 mg/kg) was significantly different to vehicle controls.

The number of licks for Intralipid were also reduced as a function of dose of raclopride. Unlike those for sucrose, number of licks for Intralipid were not decreased significantly: ($F_{3, 27}$ = 1.499, n.s.). As was the case for sucrose, concentration had a marked incremental effect on the number of licks for Intralipid: ($F_{2, 18}$= 19.794, $p<0.0001$), (see Table 5.6). Again, there was no significant interaction between drug dose and Intralipid concentration: ($F_{6, 54}$ =0.967, n.s.).

Mean bout duration

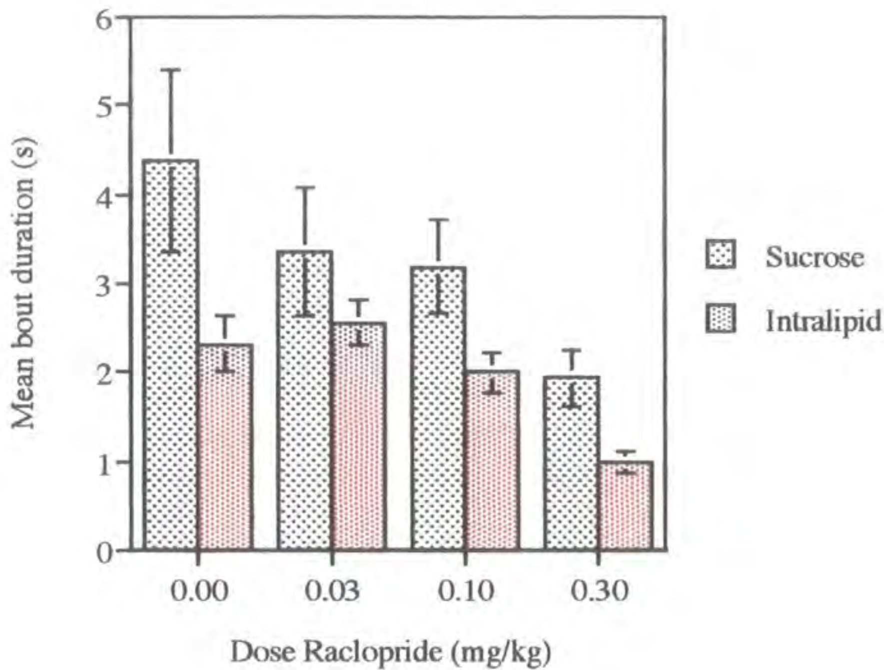


Figure 5.9 Mean bout duration for sucrose and intralipid drinking in a brief contact test as a function of increasing dose of raclopride (0.03-0.3 mg/kg) + S.E.M.

Table 5.7 Main effect of fluid concentration on mean bout duration .

	Mean bout duration ± S.E.M.		
Fluid (%)	1	3	10
Sucrose	1.7±0.2	2.5±0.3	5.4±0.9
Intralipid	1.5±0.2	2.0±0.3	2.4±0.2

n=10 animals per test fluid group

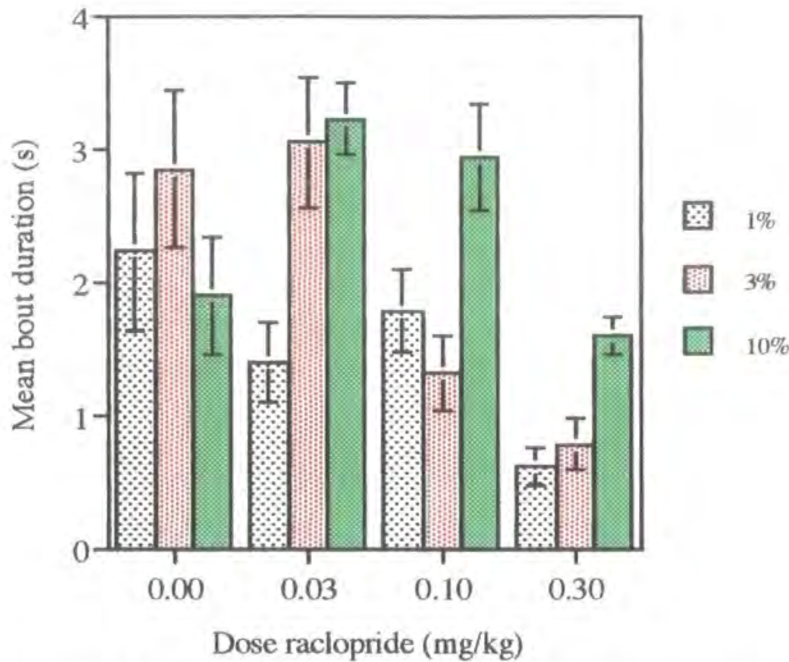


Figure 5.10 Mean bout duration of licking for 1, 3 and 10% Intralipid as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg) + S.E.M.

Mean bout duration of licking for sucrose was reduced overall by raclopride (see Figure 5.9). The reduction in mean bout duration by raclopride was non-significant: ($F_{3, 27} = 1.635$, n.s.). Changes in mean bout duration as a function of dose of raclopride were not uniform across all sucrose concentrations; mean bout duration was most noticeably attenuated when animals were drinking 10% sucrose solutions. There was a highly significant main effect of sucrose concentration on mean bout duration : ($F_{2, 18}= 29.167$, $p<0.0001$), (see Table 5.7); this was demonstrated as a linear increase in mean bout length as concentration increased. There was no significant interaction between drug dose and sucrose concentration: ($F_{6, 54} =0.520$, n.s.).

Figure 5.9 also shows that mean bout duration of licking for Intralipid was shortened overall as a function of dose of raclopride. Although this decrease was not linear, the main effect of drug was statistically significant: ($F_{3, 27} = 7.789$, $p<0.01$). Increasing Intralipid concentration led to an increase in mean bout duration which was also significant: ($F_{2, 18}= 10.041$, $p<0.01$), (see Table 5.7). Unlike sucrose, there was a significant interaction between drug dose and Intralipid concentration: ($F_{6, 54} =3.397$, $p<0.001$).

Number of bouts

Table 5.8 Main effect of fluid concentration on mean number of bouts .

Mean number of bouts ± S.E.M.

Fluid (%)	1	3	10
Sucrose	4.6±0.5	8.7±0.8	10.8±0.9
Intralipid	3.8±0.6	4.9±0.9	10.2±1.2

n=10 animals per test fluid group

Although there was no significant overall main effect of drug on mean bout frequency: ($F_{3, 27} = 0.391$, n.s.), the number of bouts were reduced by raclopride when animals were drinking 1 and 3% sucrose but were increased when these animals were drinking 10% sucrose. Increasing concentration of sucrose led to a monotonic increase in bouts that was statistically significant: ($F_{2, 18} = 31.557$, $p<0.0001$), (see Table 5.8). There was however, no significant interaction between drug dose and sucrose concentration: ($F_{6, 54} = 1.419$, n.s.).

A similar pattern of results was observed in animals licking for Intralipid. There was no main effect of drug on mean bout frequency: ($F_{3, 27} = 0.287$, n.s.). Again, for Intralipid, the number of bouts were reduced by raclopride when animals were drinking 1 and 3% Intralipid but were increased when these animals were drinking 10% Intralipid. As concentration of Intralipid increased so did the number of bouts and this effect of concentration was significant: ($F_{2, 18} = 32.623$, $p<0.0001$), (see Table 5.8). There was no significant interaction between drug dose and Intralipid concentration: ($F_{6, 54} = 0.396$, n.s.).

Intrabout lick rate

Licks within a bout for sucrose were not significantly affected by either drug: ($F_{3, 27} = 0.111$, n.s.) or sucrose concentration: ($F_{2, 18} = 0.787$, n.s.). Nor was there a significant interaction between drug dose and sucrose concentration: ($F_{6, 54} = 1.960$, n.s.).

In the case of Intralipid, the results are very similar to those of sucrose. There was no significant main effect of drug: ($F_{3, 27} = 0.088$, n.s.) or Intralipid concentration: ($F_{2, 18} = 0.518$, n.s.). There was no significant interaction between drug dose and Intralipid concentration: ($F_{6, 54} = 0.575$, n.s.).

<u>Average interlick interval</u>

The average interlick interval for sucrose increased slightly as a function of dose raclopride. This effect was non-significant: ($F_{3, 27} = 0.267$, n.s.). Concentration had no effect average interlick interval: ($F_{2, 18} = 0.438$, n.s.), nor was there any significant interaction between drug dose and sucrose concentration: ($F_{6, 54} = 1.629$, n.s.).

The average interlick interval for Intralipid decreased as a function of raclopride but non-monotonically. This effect was non-significant: ($F_{3, 27} = 0.214$, n.s.). Concentration, increased average interlick interval for those drinking Intralipid but this relationship was non-monotonic and the result was non-significant: ($F_{2, 18} = 0.982$, n.s.). As with sucrose, there was no significant interaction between drug dose and Intralipid concentration for this parameter: ($F_{6, 54} = 1.256$, n.s.).

Experiment 6: Early effects of SCH 23390 on the microstructure of licking: a brief contact test.

A brief contact licking test involves the presentation of differing concentrations of selected fluids for 60 seconds. Below are the licking parameters measured after the administration of SCH-23390. A brief contact licking test involves the presentation of differing concentrations of selected fluids for 60 seconds. Below are the licking parameters measured after the administration of SCH-23390. The parameters measured were number of licks, mean bout duration, number of bouts, intrabout lick rate (and average interlick interval) as well as latency. Animals licking for all concentrations of Intralipid emulsions or sucrose solutions are represented in Table 5.9.

Table 5.9 The average (+ S.E.M.) of the feeding parameters at each dose of SCH-23390 and at each sucrose / Intralipid concentration (1, 3 and 10%).

	Dose SCH-23390 (mg/kg)							
	0.0	0.01	0.03	0.1	0.0	0.01	0.03	0.1
	Sucrose				Intralipid			
Latency (s)								
Fluid (%)								
1	2.6 (0.7)	3.7 (2.1)	6.1 (2.1)	32.8 (10.1)	8.7 (4.2)	15.2 (6.4)	3.8 (1.2)	54.3 (5.7)
3	1.9 (0.6)	6.2 (3.9)	10.7 (6.6)	29.6 (9.2)	5.5 (1.6)	16.3 (6.8)	31.6 (8.5)	37.8 (7.9)
10	2.8 (1.4)	13.6 (7.0)	14.2 (7.3)	11.5 (7.0)	10.3 (4.1)	28.2 (8.0)	12.8 (5.5)	40.9 (7.5)
Number of licks								
Fluid (%)								
1	183.6 (43.5)	130.0 (28.7)	193.4 (36.6)	46.6 (24.4)	206.8 (32.4)	94.1 (25.1)	207.1 (31.8)	2.7 (2.7)
3	278.5 (20.6)	285.7 (36.5)	236.7 (45.6)	138.6 (49.3)	226.9 (37.9)	226.6 (31.8)	84.3 (33.9)	81.1 (37.1)
10	341.7 (9.0)	242.6 (46.6)	290.1 (36.6)	244.0 (46.0)	308.7 (15.9)	244.8 (44.4)	244.8 (42.7)	74.9 (34.8)
Mean bout duration (s)								
Fluid (%)								
1	3.4 (0.8)	2.1 (0.4)	3.4 (0.6)	0.8 (0.3)	3.0 (0.5)	1.6 (0.3)	4.0 (1.2)	0.08 (0.08)
3	3.2 (0.3)	6.3 (1.3)	5.8 (3.0)	3.6 (2.1)	3.4 (0.9)	3.1 (0.7)	1.5 (0.6)	1.4 (0.6)
10	6.6 (1.0)	7.2 (2.9)	7.2 (1.9)	12.5 (6.3)	3.6 (0.9)	4.5 (1.4)	4.0 (1.0)	1.1 (0.4)

(**Table 5.9** continued)

Number of bouts								
Fluid (%)								
1	7.5 (0.9)	8.5 (1.1)	8.5 (1.4)	3.8 (1.7)	10.5 (1.8)	7.1 (1.9)	10.6 (1.9)	0.5 (0.5)
3	13.4 (1.3)	11.0 (1.7)	9.3 (1.8)	5.7 (1.9)	11.0 (1.9)	12.0 (2.0)	5.4 (2.2)	4.7 (2.2)
10	9.1 (0.9)	9.2 (1.8)	7.6 (1.6)	5.5 (1.9)	16.1 (1.8)	8.5 (1.8)	9.6 (2.1)	5.4 (2.7)
Intrabout lick rate (licks/s)								
Fluid (%)								
1	6.5 (0.2)	6.3 (0.2)	5.6 (0.4)	3.1 (1.0)	6.7 (0.2)	6.0 (0.7)	6.1 (0.2)	0.6 (0.6)
3	6.3 (0.1)	5.9 (0.07)	5.5 (0.7)	3.6 (0.9)	6.8 (0.2)	5.6 (0.6)	3.8 (1.0)	3.1 (1.0)
10	6.3 (0.08)	5.3 (0.7)	5.3 (0.7)	4.8 (0.6)	6.5 (0.1)	5.0 (0.8)	6.2 (0.9)	4.0 (1.1)
Interlick interval (msec)								
Fluid (%)								
1	160.2 (7.8)	162.8 (5.1)	190.3 (21.0)	108.4 (36.6)	151.1 (5.1)	137.4 (15.9)	165.4 (3.9)	18.1 (18.1)
3	161.4 (3.7)	170.2 (2.1)	145.9 (18.6)	125.7 (31.8)	149.3 (4.0)	145.9 (16.5)	104.1 (24.1)	96.3 (26.6)
10	159.9 (2.1)	149.7 (18.9)	150.1 (21.0)	165.7 (21.6)	155.1 (2.6)	129.2 (21.7)	130.3 (21.9)	79.5 (26.9)

n= 9 animals per group (Sucrose)
n= 10 animals per group (Intralipid)

Latency

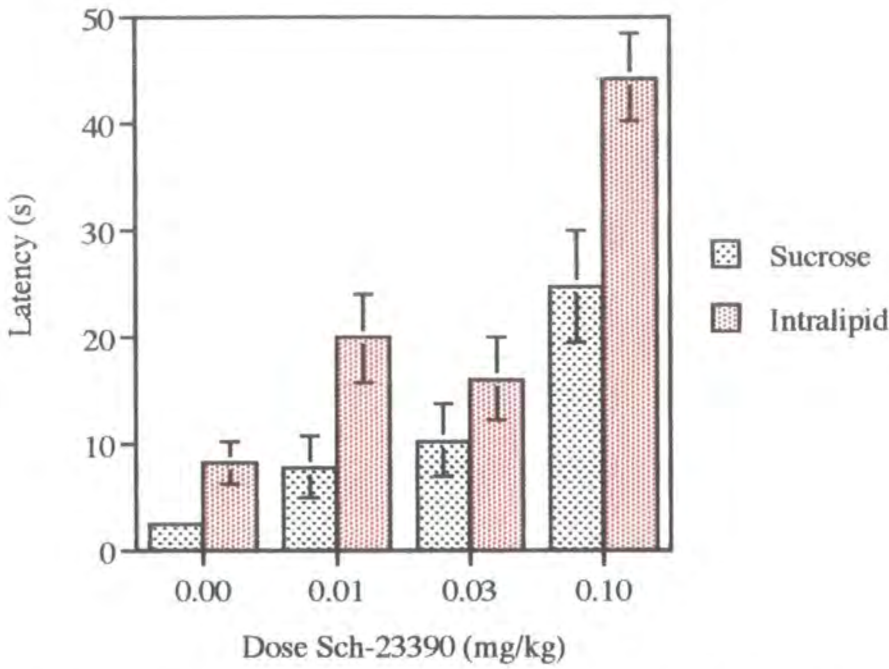


Figure 5.11 Latency to drink sucrose and Intralipid in a brief contact test as a function of increasing dose of SCH-23390 (0.01-0.1 mg/kg) + S.E.M.

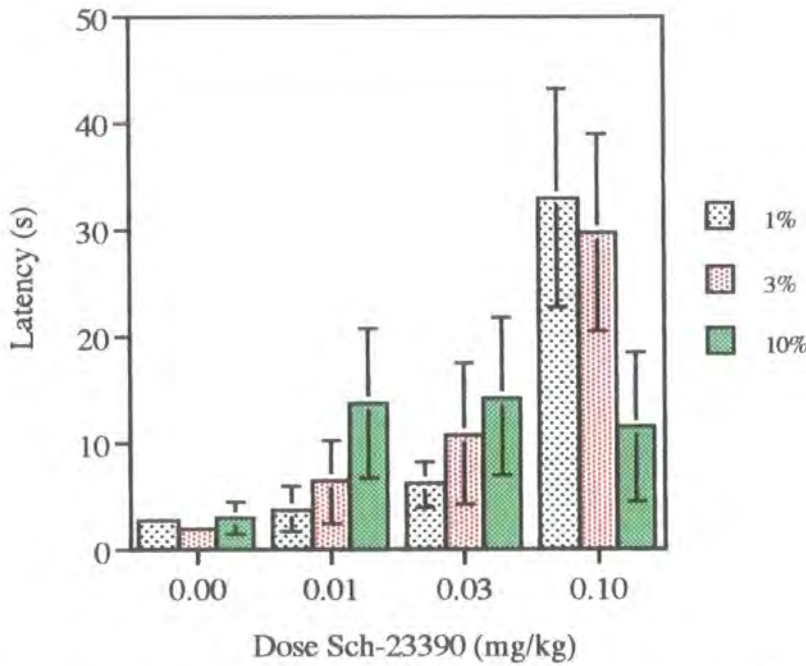


Figure 5.12 Latency to drink 1, 3 and 10% sucrose as a function of dose SCH-23390 (0.01-0.1 mg/kg) + S.E.M.

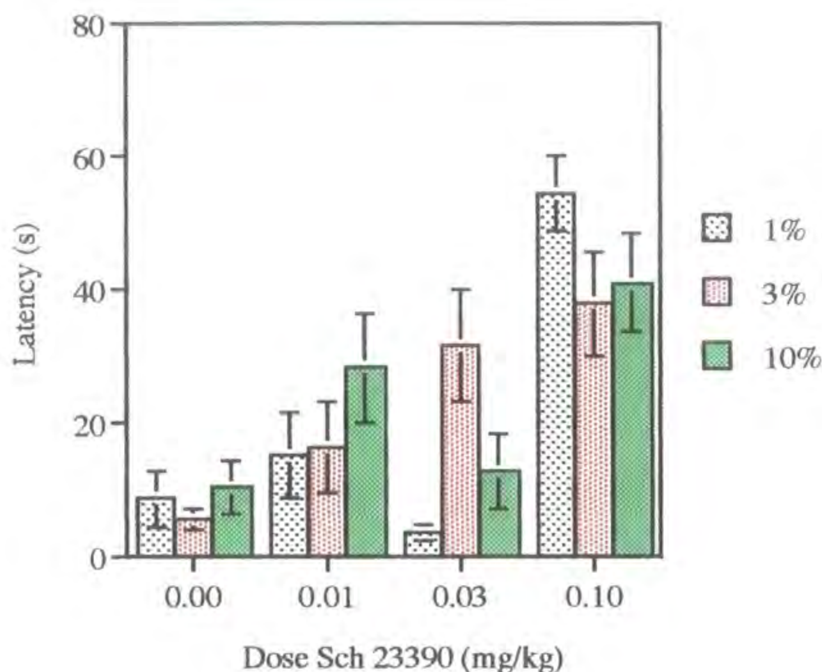


Figure 5.13 Latency to drink 1, 3 and 10% Intralipid as a function of dose SCH-23390 (0.01-0.1 mg/kg) + S.E.M.

Figure 5.11 illustrates that latency to start drinking sucrose was increased as a result of increasing doses of SCH-23390 . This result was statistically significant: ($F_{3, 24}$ = 4.922, $p<0.01$) and the relationship between drug dose and latency was monotonic. This effect of drug on latency was most prominent in animals drinking 1% sucrose, where at the highest dose (0.1 mg/kg) SCH-23390 produced latencies which were ten times those of animals who had been treated with vehicle (see Figure 5.12). There was no effect of sucrose concentration on the latency to start drinking: ($F_{2, 16}$ = 0.064, n.s.) and there was a significant interaction between drug dose and sucrose concentration: ($F_{6, 48}$ = 2.957, $p<0.05$.).

Similarly, as dose SCH-23390 increased so did latency to start drinking Intralipid (see Figure 5.11). This led to a significant main effect of drug: ($F_{3, 27}$ = 4.992, $p<0.01$). Intralipid concentration had a negligible effect on latency and so this result was non-significant: ($F_{2, 18}$ = 0.279, n.s.). Again, a highly significant interaction was found between drug dose and Intralipid concentration: ($F_{6, 54}$ = 5.694, $p<0.0001$), (see Figure 5.13).

Microstructural analysis

Number of licks

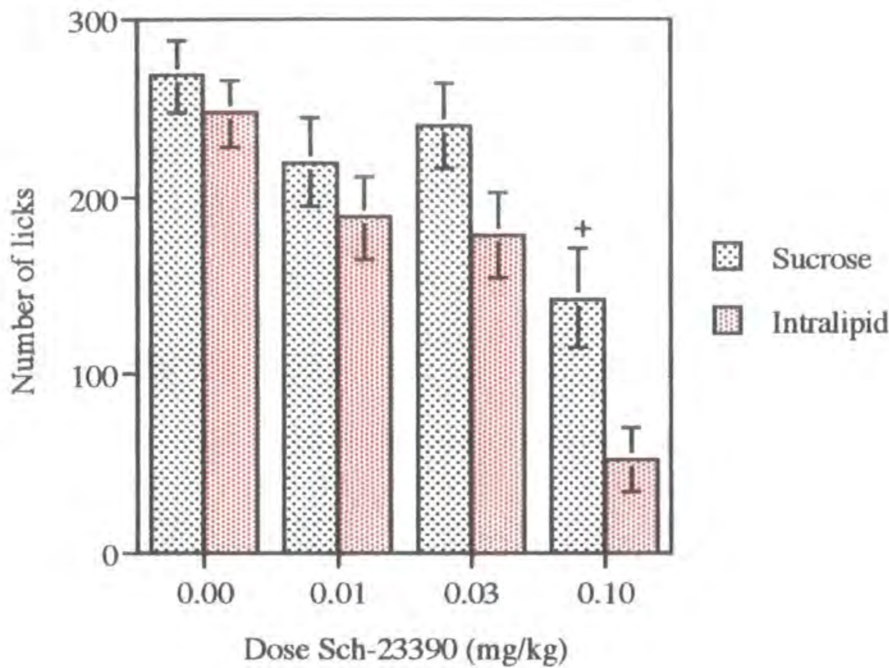


Figure 5.14 Number of licks for sucrose and Intralipid in a brief contact test as a function of increasing dose Sch-23390 (0.01-0.1 mg/kg) + S.E.M. Plus indicates significantly different from vehicle +p<0.01.

Table 5.10 Main effect of fluid concentration on total number of licks.

	Mean number of licks ± S.E.M.		
Fluid (%)	1	3	10
Sucrose	138.4±19.1	234.9±21.4	279.6±19.3
Intralipid	127.7±18.5	154.7±20.5	218.3±22.3

n= 9 animals per group (Sucrose)
n= 10 animals per group (Intralipid)

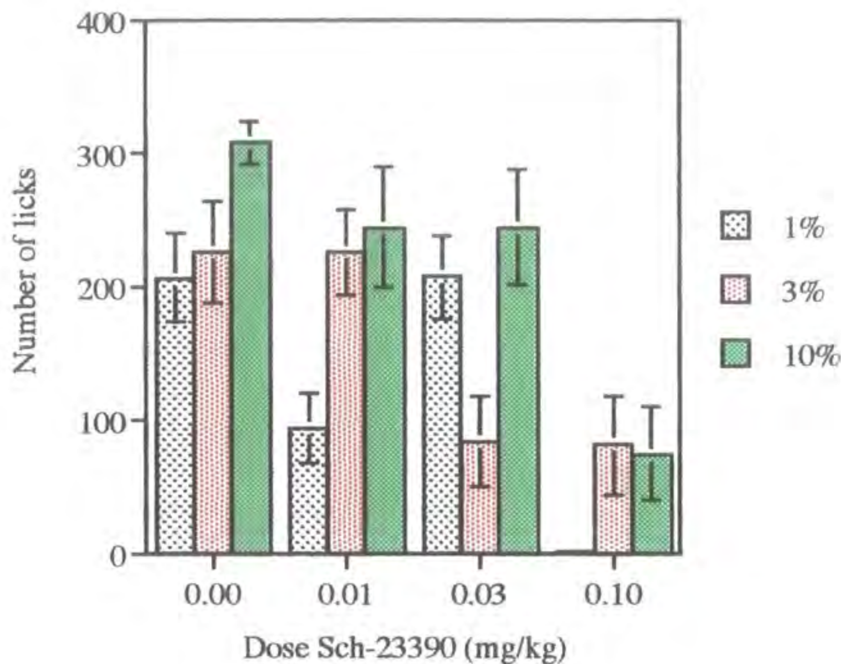


Figure 5.15 Total number of licks for 1, 3 and 10% Intralipid as a function of dose SCH 23390 (0.01-0.1 mg/kg) + S.E.M.

Figure 5.14 reveals that number of licks for sucrose were decreased as a function of dose SCH-23390. There was a significant main effect of drug dose on number of licks: ($F_{3, 24} = 3.993$, $p<0.05$). Conversely, as sucrose concentration increased so did the number of licks (see Table 5.10). This effect of concentration was highly significant: ($F_{2, 16} = 36.067$, $p<0.0001$). Drug dose and sucrose concentration did not interact significantly: ($F_{6, 48} = 1.362$, n.s.). Only the highest dose of SCH-23390 (0.1 mg/kg) was shown to have significantly different effects to vehicle controls.

The number of licks for Intralipid decreased overall after the administration of SCH-23390 (see Figure 5.14). This led to a highly significant main effect of drug: ($F_{3, 27} = 14.561$, $p<0.05$). Table 5.10 shows that number of licks increased as a function of Intralipid concentration. This led to a significant main effect of this variable: ($F_{2, 18} = 10.929$, $p<0.01$). There was also a significant interaction between drug dose and Intralipid concentration: ($F_{6, 54} = 4.082$, $p<0.01$) (see Figure 5.15).

Mean bout duration

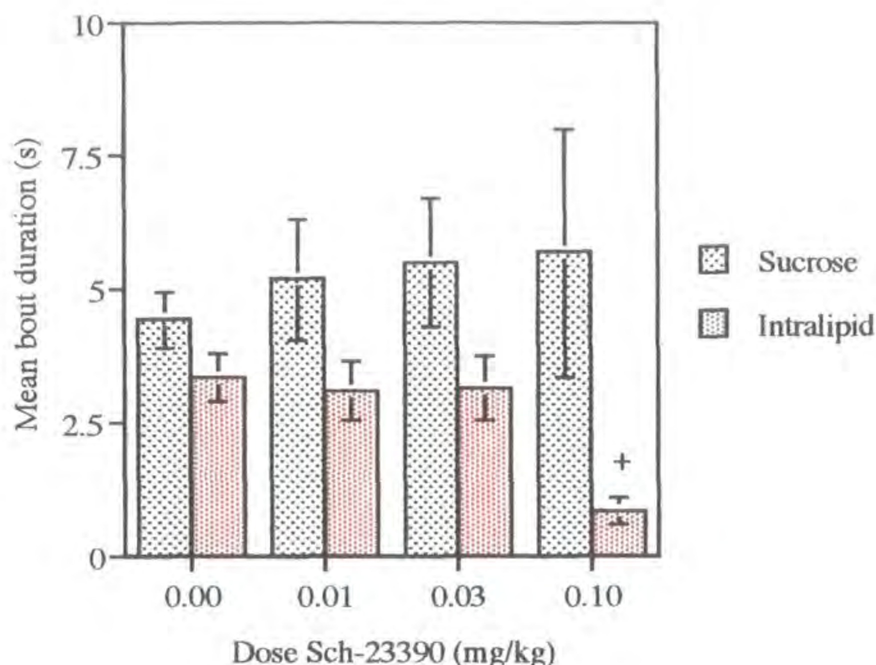


Figure 5.16 Mean bout duration for sucrose and intralipid drinking in a brief contact test as a function of increasing dose SCH-23390 (0.01-0.1 mg/kg) + S.E.M. Plus indicates significantly different from vehicle +$p<0.01$.

Table 5.11 Main effect of fluid concentration on mean bout duration .

Mean bout duration ± S.E.M.

Fluid (%)	1	3	10
Sucrose	2.5±0.3	4.8±1.0	8.4±1.8
Intralipid	2.2±0.4	2.3±0.3	3.3±0.5

n= 9 animals per group (Sucrose)
n=10 animals per group (Intralipid)

Results displayed in Figure 5.16 reveal that there was no significant effect of drug on the mean bout duration of animals drinking sucrose: ($F_{3, 24}$ =0.112, n.s.). Increasing concentration also augmented the length of bouts in a monotonic fashion and this result was significant: ($F_{2, 16}$ = 10.929, $p<0.05$), (see Table 5.11). There was no significant interaction between drug dose and sucrose concentration: ($F_{6, 48}$ = 1.524, n.s.).

In the case of Intralipid, mean bout duration did decrease as a function of drug dose and this effect was significant: ($F_{3, 27}$ =4.943, $p<0.01$). Length of bouts increased significantly as a function of Intralipid concentration: ($F_{2, 18}$ = 4.817, $p<0.05$), (see Table 5.11). As with sucrose, there was no significant interaction between drug dose and Intralipid concentration: ($F_{6, 54}$ = 1.934, n.s.). Post hoc tests revealed that at the highest dose (0.1 mg/kg) SCH-23390 reduced the length of bouts when compared to vehicle controls.

Number of bouts

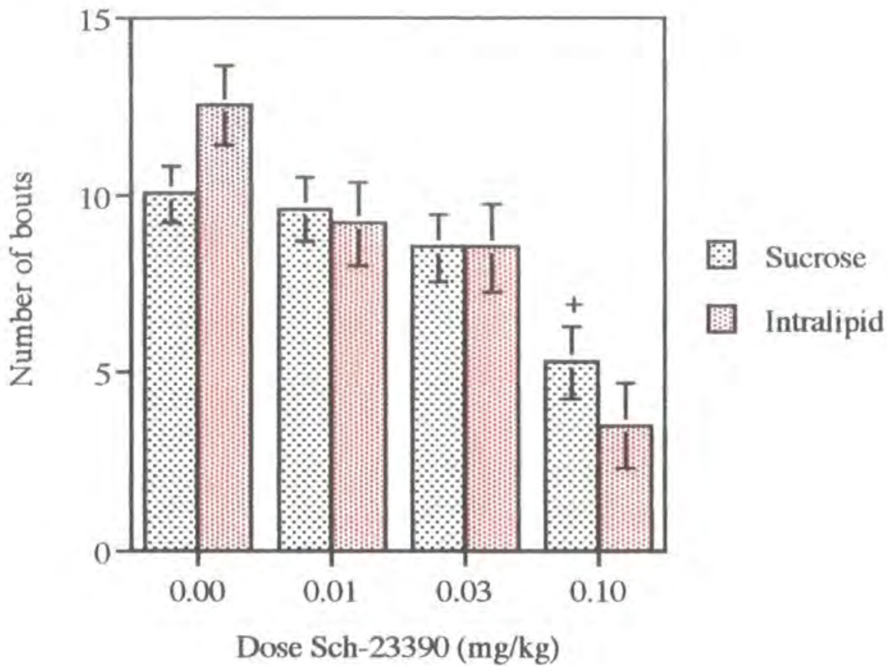


Figure 5.17 Number of bouts of sucrose and Intralipid drinking in a brief contact test as a function of increasing dose SCH-23390 (0.01-0.1 mg/kg) + S.E.M. Plus indicates significantly different from vehicle +p<0.01.

Table 5.12 Main effect of fluid concentration on mean number of bouts .

Mean number of bouts ± S.E.M.

Fluid (%)	1	3	10
Sucrose	7.1±0.7	9.9±1.0	8.1±0.8
Intralipid	2.2±0.4	2.3±0.4	3.3±0.5

n= 9 animals per group (Sucrose)
n= 10 animals per group (Intralipid)

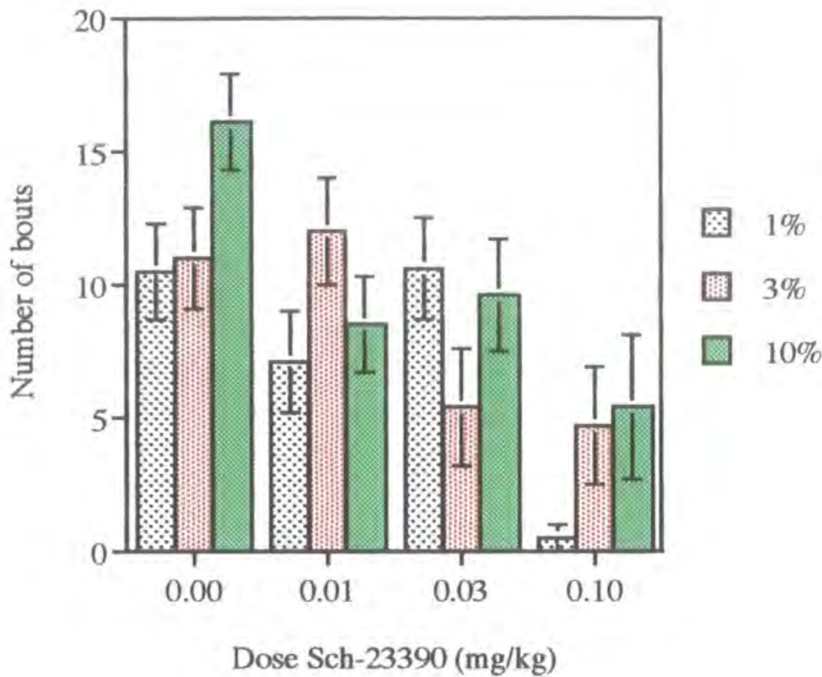


Figure 5.18 Number of bouts of licking for 1, 3 and 10% Intralipid as a function of dose SCH 23390 (0.01-0.1 mg/kg) + S.E.M.

Figure 5.17 illustrates that mean bout frequency for sucrose decreased dose-dependently as a function of increasing dose of SCH-23390 and this result was significant: ($F_{3, 24}$ =4.116, $p<0.05$). As sucrose concentration increased so did the number of bouts, however the relationship was non-monotonic (see table 5.12). This result was significant: ($F_{2, 16}$ = 5.827, $p<0.05$). There was no significant interaction between drug dose and sucrose concentration: ($F_{6, 48}$ = 0.992, n.s.). Post hoc tests showed that the highest dose of SCH-23390 (0.1 mg/kg) was significantly different to vehicle controls (results not shown).

Mean bout frequency for Intralipid decreased dose-dependently as a function of increasing dose of SCH-23390 and this result was highly significant: ($F_{3, 27}$ =8.183, $p<0.001$). The mean bout frequency for Intralipid increased monotonically as a function of concentration (see Table 5.12) but this result was not significant: ($F_{2, 18}$ = 2.825, n.s.). In the case of Intralipid there was a significant interaction between drug dose and Intralipid concentration: ($F_{6, 54}$ = 3.163, n.s.), (see Figure 5.18).

Intrabout lick rate

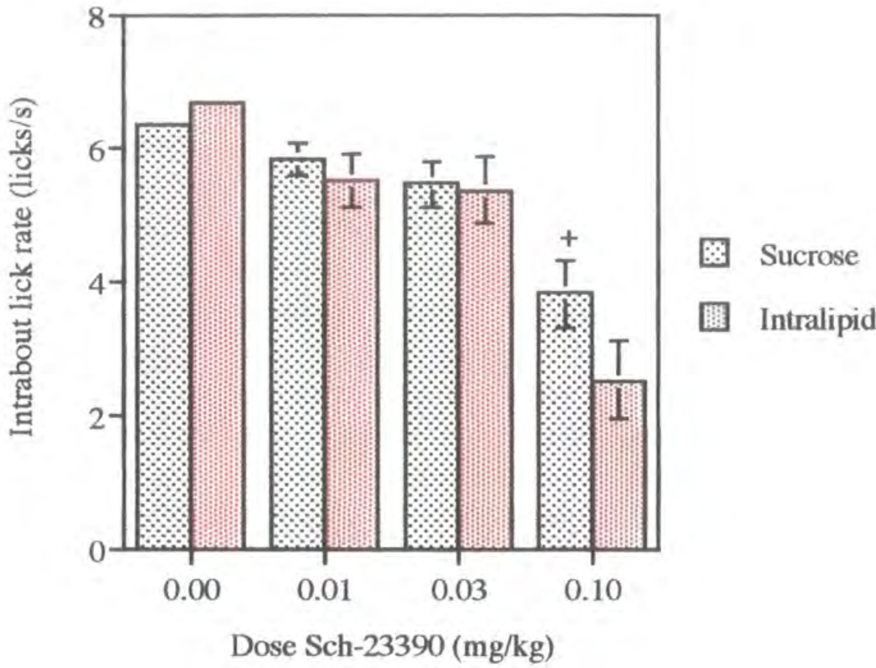


Figure 5.19 Intrabout lick rate for sucrose and Intralipid drinking in a brief contact test as a function of increasing dose SCH-23390 (0.01-0.1 mg/kg) + S.E.M. Plus indicates significantly different from vehicle +p<0.01.

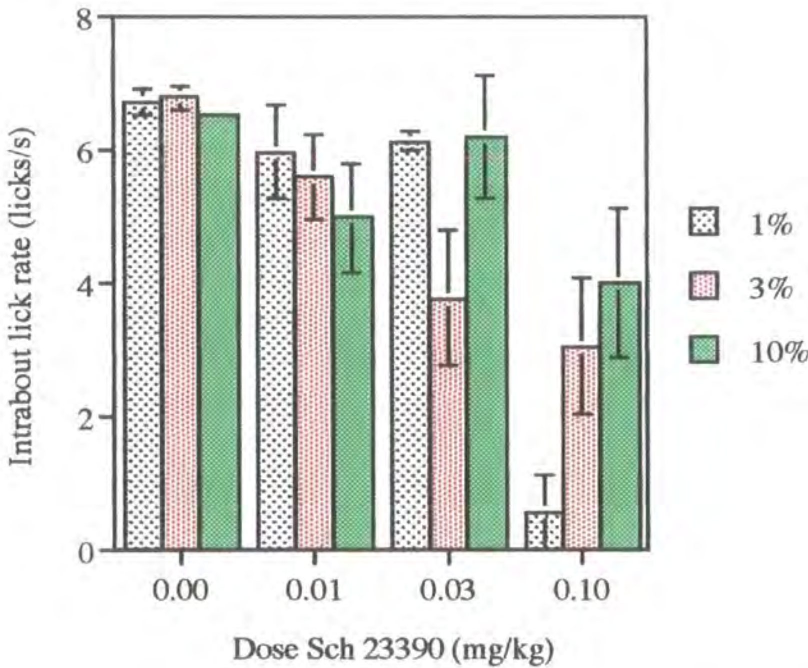


Figure 5.20 Intrabout lick rate for 1, 3 and 10% Intralipid drinking as a function of dose SCH 23390 (0.01-0.1 mg/kg) + S.E.M.

The rate of licking within a bout for sucrose decreased monotonically as the dose SCH-23390 increased (see Figure 5.19). This result was significant: ($F_{3, 24}$ =5.013, $p<0.01$) Drug dose had the most profound effect when animals were licking for 1% sucrose, (results not shown). The concentration of sucrose had no effect on this parameter: ($F_{2, 16}$ = 0.081, n.s.), nor was there a significant interaction between drug dose and sucrose concentration: ($F_{6, 48}$ = 2.161, n.s.). The highest dose of SCH-23390 (0.1 mg/kg) reduced intrabout lick rate significantly when compared to vehicle controls in a post hoc test (results not shown).

Intrabout lick rate for Intralipid also decreased as a function of dose SCH-23390 with the most dramatic reductions occurring when animals were drinking 1% Intralipid and had received the highest drug dose (see Figure 5.20). This result was highly significant: ($F_{3, 27}$ =11.925, $p<0.0001$). As with sucrose, intrabout lick rate was hardly affected by Intralipid concentration despite a slight increase in rate in response to 10% Intralipid. This result was non-significant: ($F_{6, 54}$ =1.808, n.s.). Unlike sucrose, there was a significant interaction between drug dose and Intralipid concentration: ($F_{6, 54}$ = 4.373, $p<0.01$).

Average interlick interval

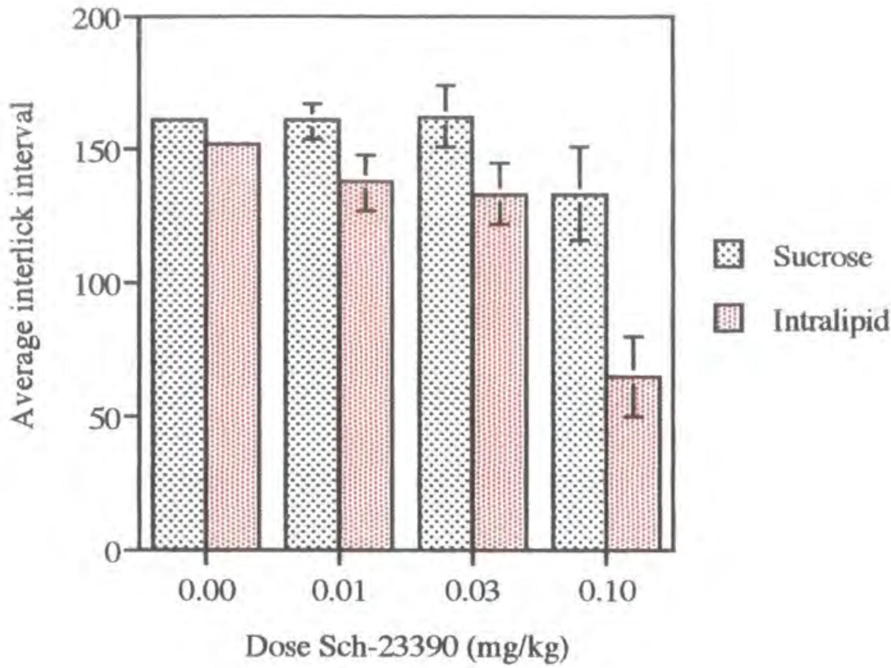


Figure 5.21 Average interlick interval for sucrose and Intralipid drinking in a brief contact test as a function of increasing dose SCH-23390 (0.01-0.1 mg/kg) + S.E.M.

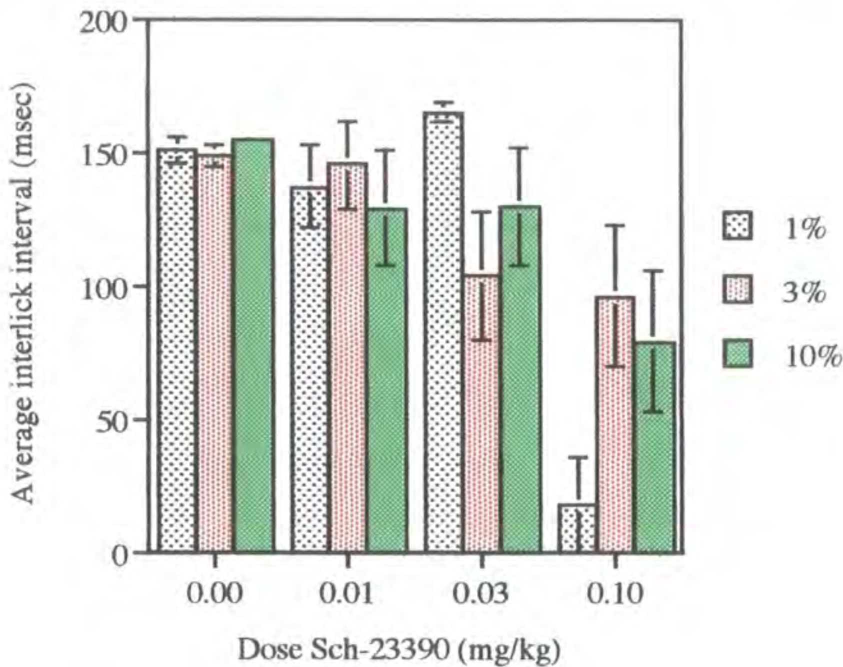


Figure 5.22 Average interlick interval for 1, 3 and 10% Intralipid drinking as a function of dose SCH 23390 (0.01-0.1 mg/kg) + S.E.M.

Figure 5.21 shows that the main effect of drug failed to reach statistical significance: ($F_{3, 24} = 1.420$, n.s.). Concentration did not significantly affect average interlick interval: ($F_{2, 16} = 0.1$, n.s.), nor was there any significant interaction between drug dose and sucrose concentration: ($F_{6, 48} = 1.461$, n.s.).

The changes in average interlick interval induced by SCH-23390 for Intralipid were dose-dependent. This main effect was significant: ($F_{3, 27} = 8.846$, $p<0.001$). Intralipid concentration, however, did not affect average interlick interval ($F_{2, 18} = 0.190$, n.s.). Unlike sucrose, there was a significant interaction between drug dose and Intralipid concentration for this parameter: ($F_{6, 54} = 4.699$, $p<0.001$), (see Figure 5.22).

Summary

Table 5.13 Effects of concentration on latency and microstructural parameters of licking for sucrose solutions or Intralipid emulsions in a brief contact test.

	Test Fluid					
	Sucrose			Intralipid		
	1%	3%	10%	1%	3%	10%
Latency	▭	▭	▭	▭	▭	▭
Number of licks	⇧	⇧	⇧	⇧	⇧	⇧
Mean bout duration (s)	⇧	⇧	⇧	⇧	⇧	⇧
Number of bouts	⇧	⇧	⇧	⇧	⇧	⇧
Intrabout lick rate (licks/s)	▭	▭	▭	▭	▭	▭
Average interlick interval (ms)	▭	▭	▭	⇧	▭	▭

n=10 per drug group per dose*

*n=9 for SCH-23390/Sucrose

⇩ = significant decrease ($p<0.05$)

▭ = n.s. effect of concentration

⇧ = significant increase ($p<0.05$)

7-OH = 7-OH-DPAT (0.1-1.0 mg/kg)

Rac = raclopride (0.03 - 0.3 mg/kg)

SCH = SCH 23390 (0.01 - 0.1 mg/kg)

Table 5.14 Drug effects on latency and microstructural parameters of licking for sucrose solutions or Intralipid emulsions in a brief contact test.

	Test Fluid					
	Sucrose			Intralipid		
	7-OH	Rac	SCH	7-OH	Rac	SCH
Latency	↑	▭	↑	↑	▭	↑
Number of licks	↓	↓	↓	↓	▭	↓
Mean bout duration (s)	↓	▭	▭	↓	↓	↓
Number of bouts	▭	▭	↓	↓	▭	↓
Intrabout lick rate (licks/s)	↓	▭	↓	↓	▭	↓
Average interlick interval (ms)	↑	▭	▭	↑	▭	↓

n=10 per drug group per dose*

*n=9 for SCH 23390/Sucrose

↓ = significant decrease ($p<0.05$)

▭ = n.s. effect of drug dose

↑ = significant increase ($p<0.05$)

7-OH = 7-OH-DPAT (0.1-1.0 mg/kg)

Rac = raclopride (0.03 - 0.3 mg/kg)

SCH = SCH 23390 (0.01 - 0.1 mg/kg)

5.4 Discussion

By shortening the test period to eliminate post-ingestive stimuli, it becomes possible to isolate the effects of drugs on the initial hedonic evaluation of the consumed fluid. It was predicted that increasing concentration of sucrose and Intralipid using a brief contact test would result in an increase in the mean bout duration of licking for either test fluid. As is clear from table 5.13, the effects of fluid concentration for sucrose and Intralipid on microstructural parameters were extremely similar, echoing the results of Higgs and Cooper (1996). Increasing concentrations of either sucrose or Intralipid gave rise to an increase in the number of licks, an increase in mean bout duration and an increase in the number of bouts.

Neither latency nor intrabout lick rate were affected by the concentration of the test fluid. The consistent observation that concentration has little or no effect on either intrabout lick rate and/or average interlick interval allows us to infer that any changes in these parameters are due to changes that are not related to the palatability of the diet. However, in Experiment 4, animals licking for Intralipid showed an increase in average interlick interval without an incumbent decrease in the reciprocal of this measure, intrabout lick rate. The effect of concentration on average interlick interval in Experiment 4 was monotonic yet small. In addition, this effect did not appear for either test fluid in Experiments 5 or 6. Therefore, this result does not seriously question the validity of the above interpretation of this measure (i.e. that intrabout lick rate is insulated from changes in concentration). Nevertheless, it has been suggested that when more viscous solutions are used there can be a concomitant increase in average interlick interval (Davis, 1996). Taken together, these results represent further confirmation of the model for the control of ingestion of Davis and Levine (1977).

The drug effects on microstructural parameters are less clear cut. Previously, in Chapter 1 it was found that dopaminergic drugs could affect those licking parameters which most robustly reflected palatability (i.e. initial rate of licking and mean bout duration). One of the aims of Experiments 4, 5 and 6 was to examine further the specificity of the effects of dopaminergic compounds on licking behaviour. For example, Davis and Smith (1992) showed that by making the drinking spout gradually more inaccessible to animals (so that they had to effectively "reach" for the spout) led to a gradual decrease in intrabout lick rate. Intrabout lick rate seems to measure motoric competence, and therefore any change in ingestion due to changes in intrabout lick rate (in the absence of any significant

decrease in mean bout duration) might indicate a motoric explanation to account for decreased ingestion.

In the case of sucrose ingestion, only 7-OH-DPAT decreased mean bout duration significantly, probably due to the highest dose (1.0 mg/kg). This compound also decreased intrabout lick rate by increasing the length of the average interlick interval. Latency was also increased by 7-OH-DPAT, again probably due to the highest dose. Latency is often seen as a measure of motor competence as increases in latency suggest difficulty in approaching the drinking spout (Smith, 1996). Hence, drug effects on this measure could reflect the operation (or not as the case may be) of a mechanism which is necessary to induce feeding. Therefore, 7-OH-DPAT, at the doses used, seems to affect those parameters which best reflect both palatability *and* also the motoric competence necessary to execute approach.

In the case of raclopride the number of licks for sucrose decreased significantly, as a result of small changes in several parameters. Statistical tests did not identify any specific way in which this decrease in the number of licks was achieved (i.e. through a decrease in mean bout duration or a decrease in intrabout lick rate). It may be inferred that raclopride produces a non-specific decrease in ingestion in this brief-contact test situation. SCH- 23390 increased the latency to lick and also decreased the intrabout lick rate and number of bouts without selectively affecting the mean bout duration. This profile is most probably characteristic of a motoric deficit. The decremental effect on number of bouts is suggestive of a fragmentation of normal feeding (Asin et al., 1992), as was the effect of SCH- 23390 on intrabout lick rate.

In the case of Intralipid ingestion, 7-OH-DPAT decreased the number of licks by increasing the latency to lick, shortened the bout length, decreased the number of bouts and decreased the intrabout lick rate by lengthening average interlick interval. Drug effects of SCH- 23390 and 7-OH-DPAT on licking for Intralipid were similar. The only parameter to be significantly affected in animals that received raclopride was mean bout duration which was significantly reduced, but not sufficiently to produce a significant decrease in number of licks. Therefore, there seems to be compensation for the decreases in mean bout duration by means of an increase in number of bouts (probably induced by the 0.03 and 0.1 dose of raclopride; see Figure 5.10) as this compound had very little effect on intrabout lick rate (see table 5.5).

In summary, the drug effects on licking microstructure show that there are differential effects on licking parameters dependent upon the dopamine receptor subtypes at which they exert their effects. From the overall results it seems that the ingestion of Intralipid may be slightly more sensitive to dopaminergic drug effects than that of sucrose. Moreover, the difference in behavioural effects go some way to
distinguishing neuroleptic-induced motoric and non-motoric effects on licking microstructure. It was predicted in the introduction that a D1 antagonist and a putative D3 receptor agonist might be less likely to induce motoric effects than a D2 receptor antagonist owing to the brain areas at which these drugs are purportedly acting (Coffin et al., 1989; Fowler and Liou, 1994). However, the opposite seems to be so, at least at the doses used in Experiments 4, 5 and 6. Thus, this hypothesis was not supported by the results from the brief contact tests used here. The motoric deficits induced by SCH-23390 and 7-OH-DPAT are more consistent with the results of Fowler and Liou (1994) than those of Schneider et al. (1990). This conclusion is bolstered by results from a pilot study carried out in our laboratory (results not included here). 7-OH-DPAT (0.1-1.0 mg/kg) was shown to reduce locomotion and rearing dose-dependently.

Nevertheless, the increases in latency and the decreases in intrabout lick rate induced by both SCH-23390 and 7-OH-DPAT do not preclude effects of these drugs on the palatability of the test fluids. As measured by mean bout duration, 7-OH-DPAT and SCH-23390 also decreased the palatability of sucrose solutions and Intralipid emulsions. Therefore, these compounds could be having early meal effects which are at once motoric and also related to the hedonic evaluation of the tastant, a view which is at odds with those studies which emphasise only motoric effects of dopaminergic compounds in a brief contact test (Pecina and Berridge, 1997, see Chapter 1). In general, the results from the brief contact tests (Experiments 4, 5 and 6) do not present a strong argument for a specific effect of these dopaminergic compounds on palatability.

Very recently, results have come to light which further implicate the D1 receptor in the mediation of orolingual/orophyrangeal motor control. Jia et al., (1998) observed that SCH- 23390 inhibits the swallowing reflex in guinea pigs which necessarily affected their drinking behaviour. This could account for the decrease in intrabout lick rate observed after SCH-23390 administration in Experiment 6. However, as is applicable to all the Experiments presented up to this point, caution must be used in ascribing particular behavioural roles to discrete receptor subtypes when the pharmacological specificity of compounds is

questionable. For example, SCH-23390, although often viewed as a prototypical D1 antagonist, has been shown, under certain conditions, to exert dopamine D1 agonist like effects. Chapter 1 has already discussed the debatable affinities reported for the dopamine D3 receptor. Therefore, the next chapter contains experiments which attempt to elucidate further the pharmacological specificity of behavioural effects induced by putative D3 receptor agonists such as 7-OH-DPAT.

Chapter 6: Attempts to block the effects of 7-OH-DPAT on licking microstructure using the dopamine D3 receptor antagonist PNU-99194A or the mixed dopamine D3/D2 antagonist amisulpride

6.1 Introduction

In an attempt to further outline a behavioural profile for D3 receptors, several D3 receptor antagonists have been developed and investigated preclinically (see Table 1.3, Chapter 1). For the purposes of the experiments in this Chapter, two D3 receptor antagonists were chosen in an endeavour to block the effects of 7-OH-DPAT on licking responses which have already been established in Chapters 1 and 2. Integral to the design of Experiments 7 and 8 were conditions where the effects of D3 receptor antagonists alone and in combination on licking microstructure could be assessed.

The first antagonist chosen was PNU-99194A and this compound was used in Experiment 7. There are several reports of the behavioural effects of this antagonist alone and in combination with putative D3 receptor agonists such as 7-OH-DPAT. Originally, PNU-99194A was identified and shown to have 25-fold selectivity for D3 receptor sites over D2 receptor sites and to induce behavioural stimulation in the absence of any concomitant change in dopamine release, consistent with blockade of postsynaptic D3 receptors (Waters et al., 1993). Further, PNU-99194A along with other D3 receptor-preferring antagonists, produce mild locomotor activation and have also been shown to establish a conditioned place preference in rats (Svensson et al., 1986, 1993; Waters et al., 1993; Mallet and Beninger, 1994; Kling-Petersen et al., 1995 a, b).

Drug discrimination procedures have often been employed to classify novel substances such as PNU-99194A and to examine the neural mechanisms involved in the discriminative stimulus effects (or subjective effects) of such drugs. These methods have revealed that PNU-99194A, despite its locomotor activating effects, does not have discriminative stimulus effects similar to those of the psychomotor stimulants (Baker et al., 1997).

Clifford and Waddington (1998) recently found the behavioural effects of PNU-99194A to be much broader than just stimulation of locomotion. They produced an "ethogram" which highlighted the behavioural effects of PNU-99194A

as inducing non-stereotyped sniffing, locomotion and some rearing, with both non-consummatory chewing and eating of faecal pellets and cage bedding. Eating was induced by PNU-99194A only at high doses of this compound (15 and 45 mg/kg) which renders the pharmacological specificity of this effect debatable.

Therefore, one of the aims of Experiment 7 was to further characterise PNU-99194A in terms of its low dose effects on feeding behaviour. In the light of results from other reward paradigms such as conditioned place preference it might be expected that PNU-99194A would have an effect on componenents of food reward. A brief contact test was employed to assess the effects of this D3 receptor antagonist on licking parameters. As 7-OH-DPAT has been shown, in previous chapters, to reliably reduce number of licks by means of decreasing mean bout duration of licking for tastants it was predicted that PNU-99194A would increase these salient parameters.

Despite its well documented status as a D3 receptor antagonist, PNU-99194A has only once been used in an attempt to abolish the behavioural effects of the putative D3 receptor agonist, 7-OH-DPAT. Using the discriminative stimulus method, Baker et al., (1998) conclude that PNU-99194A fails to block 7-OH-DPAT substitution for d-amphetamine or cocaine. Therefore, a further aim of Chapter 7 was to attempt to block the previously established effects of 7-OH-DPAT on licking microstructure.

Experiments 8 and 9 used another antagonist for the D3/D2 receptor, the atypical benzamide antipsychotic, amisulpride. Clinically, atypical neuroleptics are defined as drugs that are active in the treatment of schizophrenia but have a lesser propensity to induce extrapyramidal side effects than conventional neuroleptics (see Chapter 1). Within session response decrements which have been taken as characteristic of classical neuroleptics such as haloperidol and pimozide are not evident for a number of atypical neuroleptics (Sanger and Perrault, 1995). As these atypical compounds are claimed to produce fewer motor side effects than more traditional neuroleptics, this result confirms the view that within-session response decrements are probably the result of motor impairments.

Amisulpride also differs from most other antipsychotic drugs in showing similar affinities for D2 and D3 receptors (Schoemaker et al., 1997). In vivo, in rats, amisulpride blocks preferentially D2 and D3 receptors in limbic structures in comparison with its ability to block receptors found in the striatum (Schoemaker et al., 1997). Therefore, amisulpride has a combination of at least three

neurochemical properties which make it unique in the class of neuroleptics. The properties are a selectivity for D3 receptors (and to a slightly lesser extent D2 receptors) (Schoemaker et al., 1997), limbic selectivity and preferential affinity at low doses for presynaptic dopamine autoreceptors (Scatton et al., 1997).

For example, in rats, amisulpride preferentially inhibits effects produced by low doses of apomorphine (hypomotility and yawning) related to the stimulation of presynaptic D2/D3 autoreceptors (0.1 and 0.3 mg/kg). In contrast to this, at higher doses (i.e. 30 mg/kg) amisulpride antagonised apomorphine induced hypermotility, a postsynaptic dopamine receptor-mediated effect (Sanger et al., 1996). In addition, amisulpride does not induce catalepsy even at very high doses (i.e. 100 mg/kg) (Millan et al., 1995) but there is one report that it may induce catalepsy in mice at high doses (Navarro et al., 1997).

As is mentioned above, amisulpride, has been found (at low doses) to selectively antagonise the effects of dopamine agonists mediated by autoreceptors (Storey et al., 1995; Perrault et al., 1997). It has been shown that the rate decreasing effects of 7-OH-DPAT on operant responding can be antagonised by low doses (1 and 3 mg/kg) of amisulpride. As these doses of amisulpride have been shown to exert preferential activity at presynaptic dopamine receptors it is assumed that the rate-decreasing effects of 7-OH-DPAT are a result of this compound acting at presynaptic sites. This is further confirmed by the study of Gilbert et al. (1995). Their experiments were designed to test further the idea that 7-OH-DPAT had its effects at Dopamine autoreceptors to reduce intracranial dopamine levels and to reduce a behaviour that was dopamine-dependent (electrical stimulation to the ventral tegmental area). They found that 7-OH-DPAT quickly and dose-dependently reduced responding for brain stimulation reward delivered to this brain area.

It has also been shown that low doses of amisulpride (0.5 and 1 mg/kg) can also potentiate a food-induced conditioned place preference (Guyon et al., 1993). This effect is described as reflecting a "pro-hedonic" property of amisulpride which has a neural basis in the potentiation of the release of dopamine (which is unconditionally evoked by food but see Chapter 1 etc.), through a selective blockade of the release modulating D2-autoreceptors. This contrasts with the usual effect of neuroleptics to reduce food-induced conditioned place preference (see Chapter 2).

These results (i.e. prohedonic or hyperphagic effects) are not always found to be expressed in the same way for the classical and the atypical antipsychotics. For example, increase in meal size observed after the administration of classical neuroleptics (Clifton et al., 1991; Clifton, 1995) is absent in the case of the atypical neuroleptics (Clifton and Lee, 1998). Classical neuroleptics, when administered chronically, produce weight gain and often obesity (Stanton, 1995). In an animal model of neuroleptic induced obesity (female rats treated chronically with the atypical antipsychotic sulpiride for 21 days) it was found that weight gain was associated with accompanying hyperphagia which can be prevented in rats by the application of food restriction (Baptista et al., 1998). Therefore it is the induction of hyperphagia rather than metabolic changes (i.e. in adipose tissue) which seems to lead to neuroleptic induced obesity.

Therefore, the aims of the present experiments were to discover whether low doses of amisulpride, purportedly working at pre-synaptic D3 receptors, could abolish the effects of 7-OH-DPAT on selected feeding parameters (Experiment 8), which have been reliably demonstrated to be decrease by this drug in Chapters 4 and 5. Also, we tested whether the putative D3 receptor antagonist PNU-99194A could reverse the effects of 7-OH-DPAT on licking responses (Experiment 7). Integral to the design of these experiments were conditions where the effects of these antagonists alone on feeding could be assessed. Therefore, these antagonist were examined as to their effects on feeding behaviour and to further test the idea that admininstration of atypical antipsychotics could induce a "pro-hedonic" or hyperphagic response reflected in an increase in mean bout duration. Results from Experiment 8 suggested a "pro-hedonic" effect of higher doses of amisulpride which was further explored in a longer microstructural test in drug-naive animals (Experiment 9).

6.2 Method

6.2.1 Animals

Forty-eight non-deprived adult male hooded Lister rats weighing 350-450g at the time of testing were used (n=20 per Experiment 6 and 7, and n=8 in Experiment 9). They were housed and maintained as described in Chapter 3.

6.2.2 Drugs

In Experiment 7, PNU-99194A (gift from Dr. K Svensson, Gothenberg, Sweden) was dissolved in saline and administered in doses of 5.0 and 10.0 mg/kg, i.p. 30 min before testing. 7-OH-DPAT (Research Biochemicals International, U.K.) was prepared for injection by dissolving it in saline at a dose of 0.3 mg/kg and was then injected i.p. 20 min before testing. This dose of 7-OH-DPAT was chosen as it had been shown to reliably decrease parameters of feeding in Experiments 1 and 4.

In Experiment 8, amisulpride (Synthelabo, France) at doses of 1.0, 3.0, 10.0, 20.0 and 30.0 mg/kg was ultrasonically dispersed with ionised water and two drops of Tween 80 (see Chapter 3). This suspension was administered i.p., 1h before testing. 7-OH-DPAT (0.3 mg/kg) was prepared for injection by dissolving it in saline. This solution was then injected i.p. 20 min before testing. Only two of the pretreatment doses of amisulpride (1.0 and 3.0 mg/kg) were administered with 7-OH-DPAT (0.3 mg/kg) over the first four test days. Increasing doses of amisulpride (10.0, 20.0 and 30.0 mg/kg) were co-administered with 7-OH-DPAT in three further four-day test periods.

For the purposes of Experiment 9, one pretreatment dose of amisulpride (20.0 mg/kg) was used which was chosen due to its ability to block some of the effects of 7-OH-DPAT (0.3 mg/kg) in Experiment 8. Other details of amisulpride and 7-OH-DPAT preparation and administration are described for Experiment 8.

All details of drug preparation and administration for each drug can be found in Chapter 3.

6.2.3 Test Fluids

The sucrose solutions were made up as described in Chapter 3. In Experiments 7 and 8, ten animals were trained and tested on 1, 3 and 10% sucrose solutions. During both training and testing animals had experience of all concentrations of the fluid. In Experiment 9, only one concentration of sucrose solution was used (10%).

6.2.4 Apparatus

Testing in Experiments 7 and 8 was carried out using the MS80 multistation lickometer described in detail in Chapter 3. In Experiment 9, testing was carried out using the 108 multistation lickometer also described in Chapter 3.

6.2.5 Procedure

Experiment 7: Interactions between the D3 receptor antagonist PNU-91994A and the D3 receptor agonist 7-OH-DPAT determined by microstructural analysis of a brief contact test.

Training

Twenty rats were well familiarised with the test apparatus and experimental procedure. This involved placing each rat in the test chamber where they had access to a range of sucrose solutions (1, 3 and 10%) in a random order. Each concentration was presented for 60s and a 5s interval intervened btween subsequent presentations. This procedure continued at the same time each day until steady baselines of licking were observed (approximately 7-10 days). Two days prior to testing each rat received a sham injection of saline to familiarise it with the injection procedure.

Testing

Following this familiarisation period rats received i.p. injections of the antagonist/agonist combinations. A repeated measures design was used in which animals had injections of vehicle/vehicle; vehicle/0.3 mg/kg 7-OH-DPAT; vehicle/5.0 mg/kg PNU 91994A; vehicle/10.0 mg/kg PNU 91994A; 5.0 mg/kg PNU 91994A/0.3 mg/kg 7-OH-DPAT; 10.0 mg/kg PNU 91994A/0.3 mg/kg 7-OH-DPAT. After injections of drugs, the rats were then placed in the lickometer

chamber where they had access to all concentrations of sucrose (1, 3 and 10%). Each concentration was presented for a total duration of 60s (with an interpresentation interval of 10s) and the order of these presentations were randomised across test days. Injections were counterbalanced across animals and a period of at least 48h was allowed to elapse between each series of injections to ensure dispersal of drugs.

Experiment 8: Interactions between the D3 receptor antagonist amisulpride and the D3 receptor agonist 7-OH-DPAT determined by microstructural analysis of a brief contact test.

Training

Training procedures for Experiment 8 were the same as those described for Experiment 7.

Testing

Following the familiarisation period the rats received i.p. injections of drugs. Again, a repeated-measures design was used in which rats had injections of antagonist/agonist combinations. Combinations were as follows: vehicle/vehicle; vehicle/0.3 mg/kg 7-OH-DPAT; vehicle/1.0 mg/kg amisulpride; vehicle/3.0 mg/kg amisulpride; 1.0 mg/kg amisulpride/0.3 mg/kg 7-OH-DPAT; 3.0 mg/kg amisulpride/0.3 mg/kg 7-OH-DPAT. After injections of drugs, the rats were then placed in the lickometer chamber where they had access to all concentrations of sucrose (1, 3 and 10%). As for Experiment 7, each concentration was presented for a total duration of 60s and the order of these presentations were randomised across test days. Injections were counterbalanced across animals and a period of at least 48h was allowed to elapse between each series of injections to ensure dispersal of drugs.

Following preliminary data analysis it was decided that higher doses of amisulpride should be used in an effort to reverse the effects of 0.3 mg/kg 7-OH-DPAT. Therefore, three more doses of amisulpride were co-administered with 0.3 mg/kg 7-OH-DPAT in three subsequent sets of four injection test days. These subsequent tests were procedurally equivalent to the first, differing only in the doses of amisulpride used (see above). The first subsequent test used injection combinations as follows: vehicle/vehicle; vehicle/10.0 mg/kg amisulpride; 10.0 mg/kg amisulpride/0.3 mg/kg 7-OH-DPAT. In the second subsequent test rats were administered with vehicle/vehicle; vehicle/20.0 mg/kg amisulpride; 20.0 mg/kg amisulpride/0.3 mg/kg 7-OH-DPAT. Over the final four test days animals were injected with vehicle/vehicle; vehicle/30.0 mg/kg amisulpride; 30.0 mg/kg

amisulpride/0.3 mg/kg 7-OH-DPAT. A wash-out period of 1week was allowed between each series of injections as all drug tests were conducted using the same animals.

Experiment 9: Microstructural analysis of drinking for 10% sucrose after the administration of amisulpride : Analyses of licking during a 20-min session and during first minute of 20-min session.

Training

A group of 10 drug-naive rats was familiarised with the test apparatus and a 10% sucrose solution for approximately 10 days or until baseline drinking levels were stable. Training consisted of 20 mins access to the sucrose solution at the same time on each training day. Two days prior to testing animals received a sham injection of vehicle in order to familiarise them with the injection procedure.

Testing

Data from Experiment 8 suggested that at a dose of 20.0 mg/kg, amisulpride bagan to reverse many of the effects of 0.3 mg/kg 7-OH-DPAT on feeding parameters. Alone this dose of amisulpride seemed to produce a pro-hedonic effect (see Results for experiment 8). Therefore, the 20.0 mg/kg dose of amisulpride was administered alone and compared to vehicle. A repeated-measures design was used where animals had injections of either 20.0 mg/kg amisulpride or saline on two test days. After injection of drugs, the rats were placed in the lickometer boxes where they had access to a 10% sucrose solution for a duration of 20min. Injections were counterbalanced across rats and a period of 48h elapsed between each series of injections to ensure dispersal of drugs.

6.2.6 Design and statistical analysis

For Experiments 7 and 8, initial analysis of the lick time data was carried out using programs as outlined in Chapter 3. For Experiment 9, analysis of the lick time data was conducted using the feed 6 programme described in Chapter 3. For Experiments 7 and 8, measures derived from the raw data included a number of microstructural variables: number of licks, mean bout duration (s), number of bouts, intrabout lick rate (licks/s within bouts) and average interlick interval (ms). The measure of total intake (ml) was taken and analysed for Experiment 9 as were

the microstructural variables of number of licks, mean bout duration and number of bouts.

Data from Experiments 7 and 8 were analysed using a two-way repeated-measures ANOVA with drug dose and fluid concentration as factors. Where there was a significant main effect of drug post hoc tests were employed. To do this, the main effect of drug was collapsed across concentration and Dunnett's t-test was used to determine any significant differences between animals which had received drug doses and vehicle controls. As the agonist 7-OH-DPAT had a decremental effect on feeding parameters and the antagonist amisulpride seemed to have incremental effects on feeding parameters, a two-way repeated measures ANOVA revealed little as to where the significance of the differential drug effect might lie.

Therefore, post hoc tests were used even in the presence of a significant interaction between drug dose and fluid concentration as a means of separating out the total main effect of drug. All combinations were compared to a control in the post hoc tests but the control used depended on the type of antagonist /agonist combination to which it was to be compared. Thus in Experiments 7 and 8, the antagonist/agonist combinations were compared to vehicle/agonist combination, whereas the vehicle/antagonist combinations were compared to the vehicle/vehicle combination. In this way, it was possible to check whether i) the antagonist alone was having a significantly different effect when compared to vehicle/vehicle and ii) the antagonist was reversing the effects of the agonist as revealed by comparing antagonist/agonist combinations to vehicle/agonist.

For Experiment 9, both intake measures and microstructural variables such as number of licks, mean bout duration and number of bouts, were analysed using a one-way within-subjects ANOVA with drug dose as a factor.

All statistical tests were performed using Super Anova and Statview SE+graphics (Abacus Concepts Inc., Berkeley, CA). For Experiments 7, 8 and 9 a result was considered statistically significant if $p<0.05$.

6.3 Results

Experiment 7: Interactions between the D3 receptor antagonist PNU 91994A and the D3 receptor agonist 7-OH-DPAT determined by microstructural analysis of a brief contact test.

Number of licks

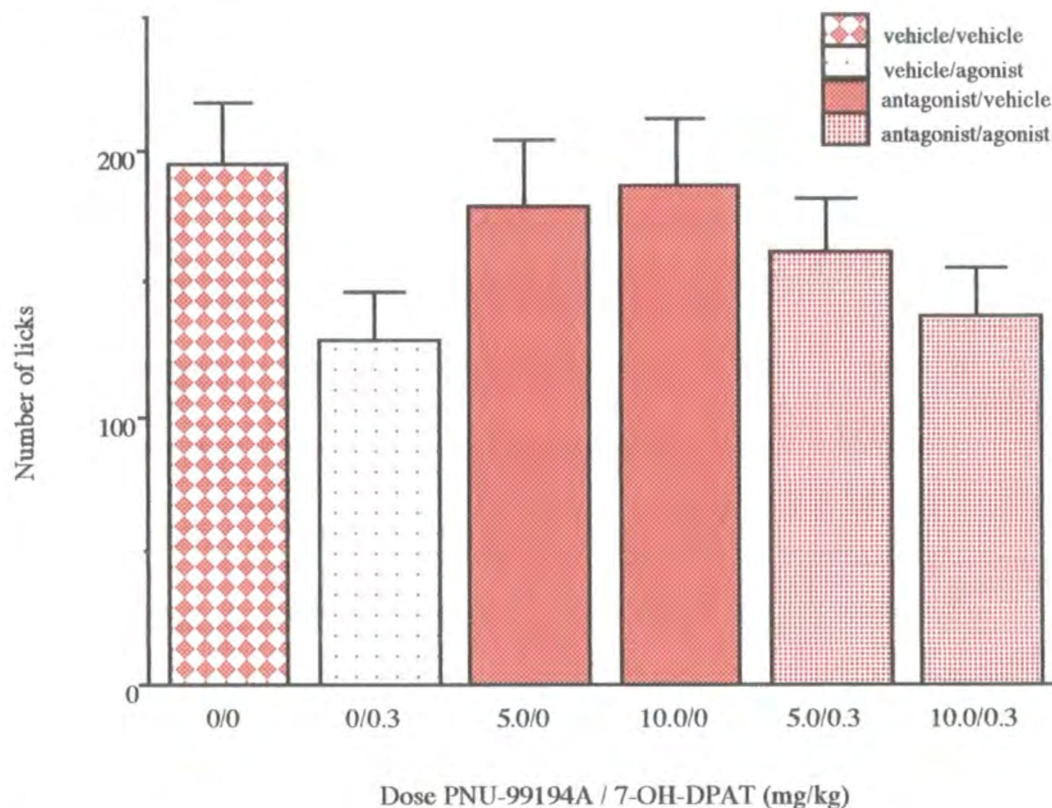


Figure 6.1 The effect of PNU-99194A (5.0 and 10.0 mg/kg) and 7-OH-DPAT (0.3 mg/kg) on the number of licks for sucrose in a brief contact test + S.E.M.

Table 6.1 Main effect of sucrose concentration total number of licks

Number of licks ± S.E.M.

Sucrose (%)		
1	3	10
63.7±9.0	148.1±10.4	284.0±9.7

n=10 animals per concentration

7-OH-DPAT alone (0.3 mg/kg) decreased the total number of licks for sucrose, by approximately one third, in a brief contact test (see Figure 6.1). A one-

way repeated measures ANOVA showed that for the total number of licks there was a significant main effect of the agonist 7-OH-DPAT ($F_{9,19} = 12.794$, $p<0.01$). A two-way repeated measures ANOVA revealed a main effect of drug ($F_{5, 45} = 4.479$, $p<0.01$) and a main effect of concentration ($F_{2, 18} = 116.676$, $p<0.0001$) on the number of licks (see Table 6.1). There was also a significant interaction between the effects of drug and concentration ($F_{10, 90} = 3.052$, $p<0.01$).

Post-hoc analyses showed that neither of the doses of PNU-99194A alone were significantly different from vehicle/vehicle condition and that pretreatment with PNU-99194A did not significantly attenuate the effects of 7-OH-DPAT when compared to the vehicle/7-OH-DPAT condition.

Mean bout duration

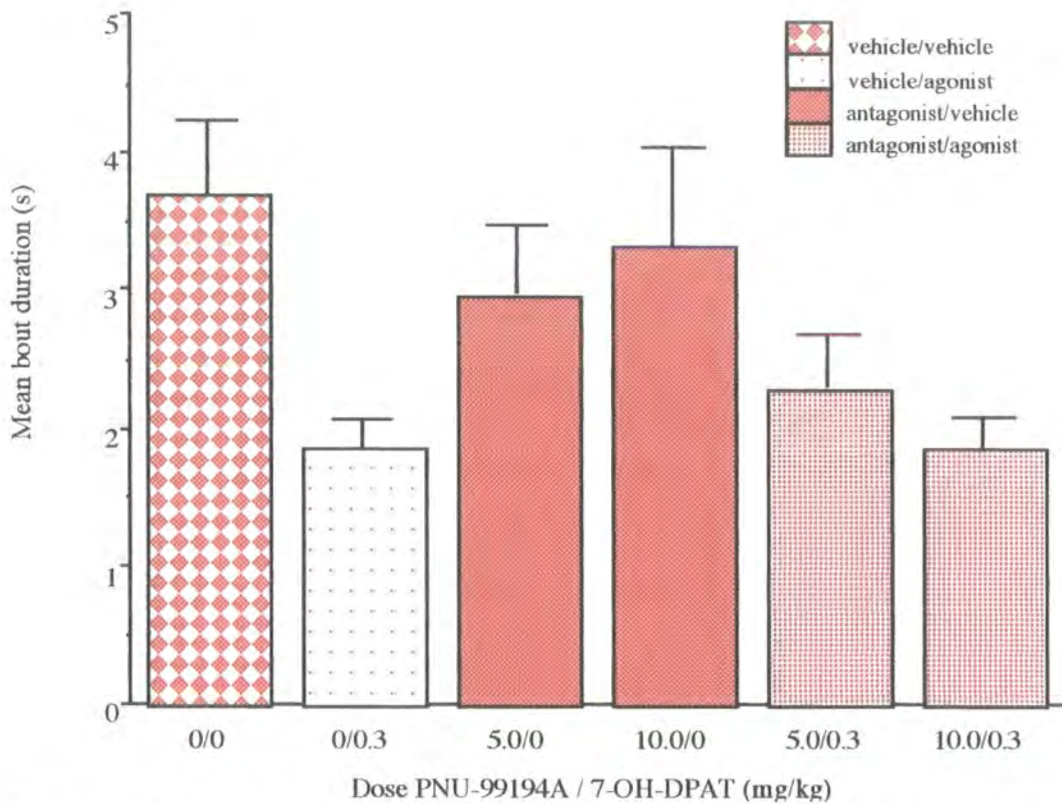


Figure 6.2 The effect of PNU-99194A (5.0 and 10.0 mg/kg) and 7-OH-DPAT (0.3 mg/kg) on mean bout duration of licking for sucrose in a brief contact test + S.E.M.

Table 6.2 Main effect of sucrose concentration on mean bout duration (s) .

Mean bout duration ± S.E.M.

Sucrose (%)		
1	3	10
1.5±0.2	2.3±0.2	4.3±0.4

n=10 animals per concentration

7-OH-DPAT alone (0.3 mg/kg) decreased the mean bout duration of licking for sucrose, approximately halving bout duration in a brief contact test (see Figure 6.2). A one-way repeated measures ANOVA showed that for mean bout duration there was a significant main effect of the agonist 7-OH-DPAT ($F_{9, 19} = 8.606$, $p<0.05$). A two-way repeated measures ANOVA revealed a main effect of drug ($F_{5, 45} = 3.368$, $p<0.05$) and a main effect of concentration ($F_{2, 18} = 20.778$, $p<0.0001$) on mean bout duration (see Table 6.2). As concentration increased so did mean bout duration. There was also a significant interaction between the effects of drug and concentration ($F_{10, 90} = 3.092$, $p<0.01$).

Post-hoc analyses showed that neither of the doses of PNU-99194A alone were significantly different from vehicle/vehicle condition and that pretreatment with PNU-99194A did not significantly attenuate the effects of 7-OH-DPAT when compared to the vehicle/7-OH-DPAT condition.

Number of bouts

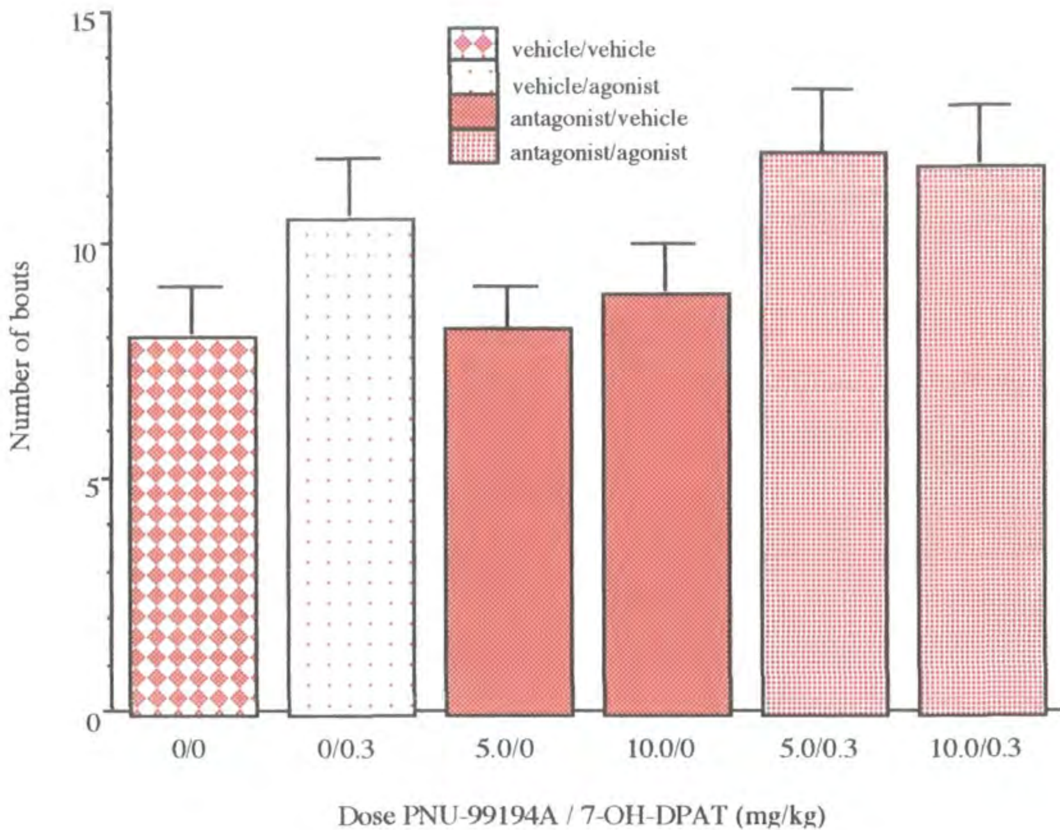


Figure 6.3 The effect of PNU-99194A (5.0 and 10.0 mg/kg) and 7-OH-DPAT (0.3 mg/kg) on bout frequency of licking for sucrose in a brief contact test + S.E.M.

Table 6.3 Main effect of sucrose concentration on number of bouts .

Number of bouts ± S.E.M.

Sucrose (%) 1	3	10
5.6±0.5	10.2±0.7	14.0±0.8

n=10 animals per concentration

Number of bouts were increased after the administration of 7-OH-DPAT (0.3 mg/kg) by approximately 25% (see Figure 6.3). A one-way repeated measures ANOVA showed that for mean bout frequency there was a significant main effect of the agonist 7-OH-DPAT ($F_{9, 19} = 6.661$, $p<0.05$). A two-way repeated measures

ANOVA revealed a main effect of drug ($F_{5, 45} = 4.995$, $p<0.001$). Table 6.3 shows that as concentration increased so did mean bout frequency. This was realised as a main effect of concentration ($F_{2, 18} = 27.352$, $p<0.0001$). There was no significant interaction between the effects of drug and concentration ($F_{10, 90} = 1.758$, n.s.).

Post-hoc analyses showed that neither of the doses of PNU-99194A alone were significantly different from vehicle/vehicle condition and that pretreatment with PNU-99194A did not significantly attenuate the effects of 7-OH-DPAT when compared to the vehicle/7-OH-DPAT condition.

Intrabout lick rate

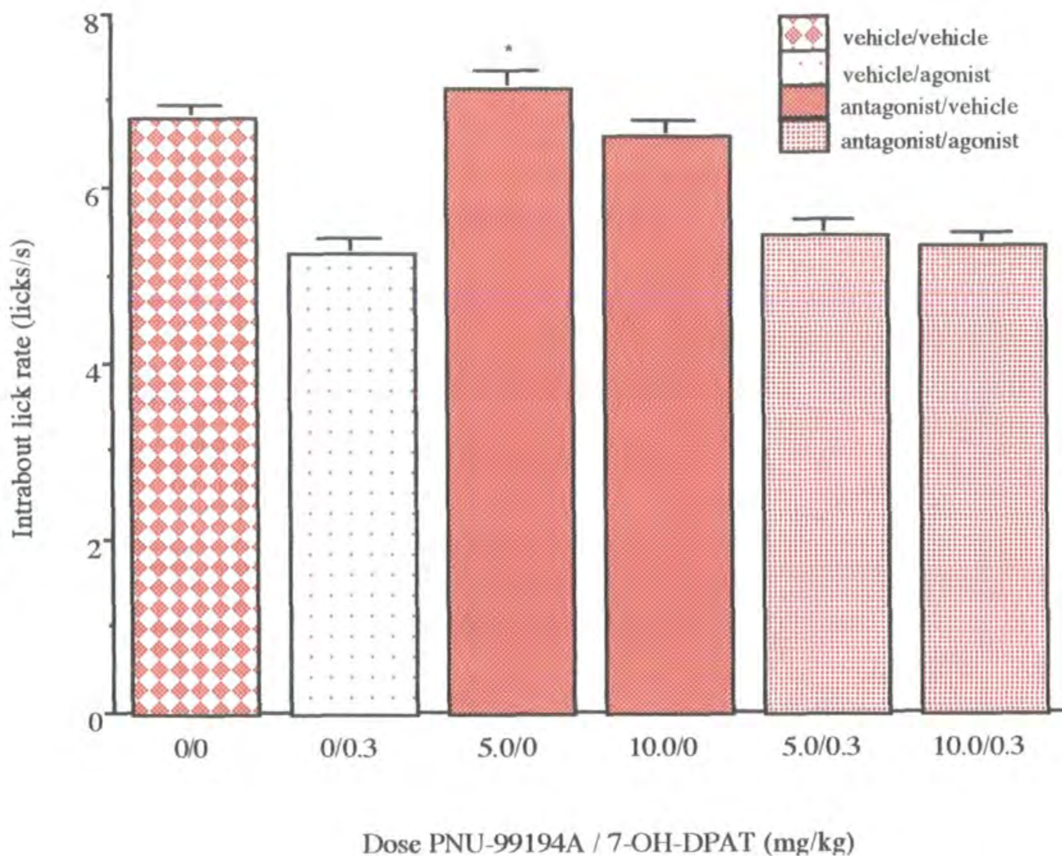


Figure 6.4 The effect of PNU-99194A (5.0 and 10.0 mg/kg) and 7-OH-DPAT (0.3 mg/kg) on intrabout lick rate for sucrose in a brief contact test + S.E.M. Asterisk indicates significantly different from vehicle / vehicle condition, *$p< 0.05$.

Table 6.4 Main effect of sucrose concentration on intrabout lick rate (lick/s) .

Intrabout lick rate ± S.E.M.

	Sucrose (%)	
1	3	10
6.3±0.2	6.1±0.1	6.0±0.1

n=10 animals per concentration

Intrabout lick rate was attenuated by 7-OH-DPAT (0.3 mg/kg) alone by approximately 2 licks/s (see Figure 6.4). A one-way repeated measures ANOVA showed that for intrabout lick rate there was a significant main effect of the agonist 7-OH-DPAT ($F_{9, 19} = 85.239$, $p<0.0001$). A two-way repeated measures ANOVA revealed a main effect of drug ($F_{5, 45} = 36.388$, $p<0.0001$) and a main effect of concentration ($F_{2, 18} = 6.26$, $p<0.01$) on licks within a bout. Table 6.4 shows that as concentration increased intrabout lick rate decreased. There was a significant interaction between the effects of drug and concentration ($F_{10, 90} = 2.247$, $p<0.05$).

Post-hoc analyses showed that the lowest dose of PNU-99194A alone (5.0 mg.kg) was significantly different from vehicle/ vehicle condition, ($p<0.05$). At this dose of PNU-99194A (5.0 mg.kg) animals licked faster within bouts than those animals who had received vehicle/vehicle doses. However, neither of the pretreatment doses of PNU-99194A were significantly different from the vehicle/7-OH-DPAT condition.

Average interlick interval

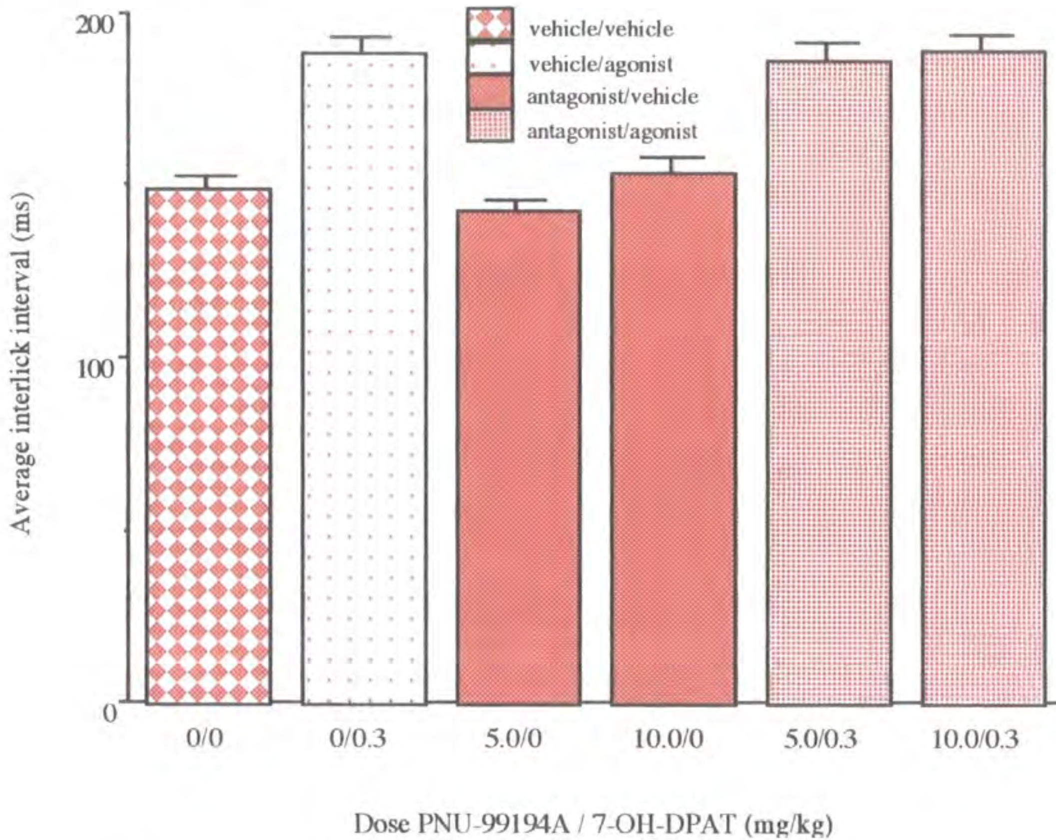


Figure 6.5 The effect of PNU-99194A (5.0 and 10.0 mg/kg) and 7-OH-DPAT (0.3 mg/kg) on average interlick interval for sucrose in a brief contact test + S.E.M.

Average interlick interval was lengthened by almost 50 ms after the administration of 7-OH-DPAT (0.3 mg/kg) alone (see Figure 6.5). A one-way repeated measures ANOVA showed that for average interlick interval there was a significant main effect of the agonist 7-OH-DPAT ($F_{9, 19} = 34.424$, $p<0.005$). A two-way repeated measures ANOVA revealed a main effect of drug ($F_{5, 45} = 22.392$, $p<0.0001$) but no main effect of concentration ($F_{2, 18} = 2.264$, n.s.) average interlick interval. There was no significant interaction between the effects of drug and concentration ($F_{10, 90} = 1.111$, n.s.).

Post-hoc analyses showed that neither of the doses of PNU-99194A alone were significantly different from vehicle/vehicle group and that neither of the pretreatment doses PNU-99194A were significantly different from the vehicle/7-OH-DPAT group.

Experiment 8: Interactions between the D3 receptor antagonist amisulpride and the D3 receptor agonist 7-OH-DPAT determined by microstructural analysis of a brief contact test.

Number of licks

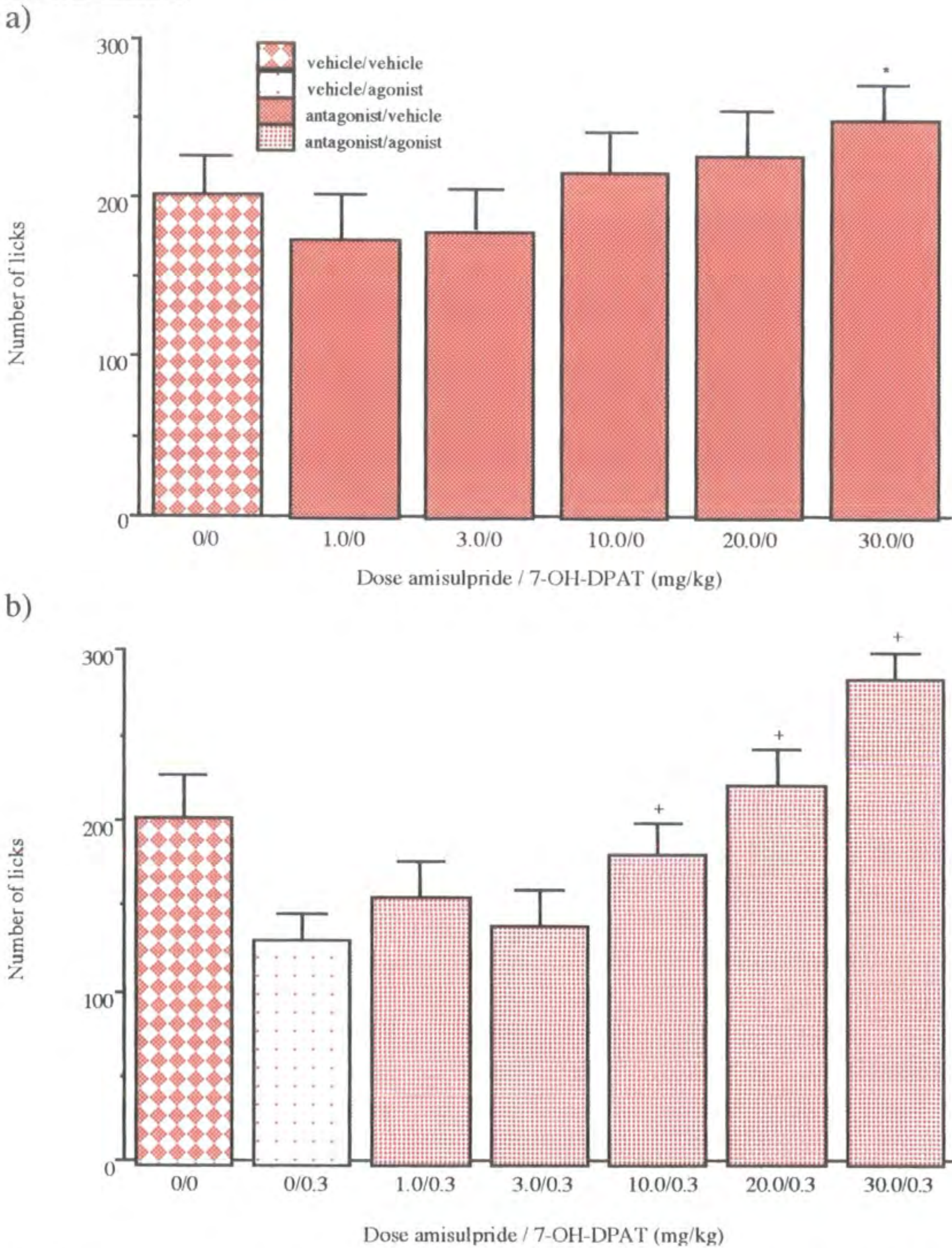


Fig 6.6 The effects of a) amisulpride (1.0, 3.0, 10.0, 20.0, 30.0 mg/kg) and b) 7-OH-DPAT (0.3 mg/kg) + amisulpride on total number of licks in a brief contact test + S.E.M. In a) asterisk indicates significantly different from vehicle / vehicle, $*p<0.05$. In b) Plus indicates significantly different from vehicle / 7-OH-DPAT; $+p<0.01$.

Table 6.5 Main effect of sucrose concentration on total number of licks .

Mean number of licks ± S.E.M.

Sucrose (%) 1	3	10
97.0±9.4	192.0±9.6	303.6±5.7

n=10 animals per concentration

7-OH-DPAT succeeded in decreasing number of licks by approximately one third, when compared to vehicle controls. A one-way repeated measures ANOVA showed this result to be significant: ($F_{9, 19} = 8.509$, $p<0.05$). A two-way repeated measures ANOVA showed that there was a significant main effect of drug dose: ($F_{11, 99} = 11.510$, $p<0.0001$) and also of sucrose concentration: ($F_{2, 18} = 166.698$, $p<0.0001$). As concentration increased so did number of licks (see Table 6.5). There was also a significant interaction between drug dose and concentration: ($F_{22, 198} = 4.479$, $p<0.01$). Number of licks increased monotonically as a function of amisulpride alone (1.0-30.0 mg/kg). Figure 6.6 shows that the highest dose of amisulpride alone (30.0 mg/kg) was significantly different from the vehicle/vehicle condition in a post hoc test ($p<0.05$).

In terms of agonist/antagonist interactions, pretreatment with some of the doses of amisulpride abolished the effect of 7-OH-DPAT (0.3 mg/kg) on number of licks. The elimination of the effects of 7-OH-DPAT began with the 10.0 mg/kg pretreatment dose of amisulpride and the effects of 7-OH-DPAT were fully abolished by the 10.0 and 20.0 mg/kg doses. In a post hoc test these three pretreatment doses (10.0, 20.0 and 30.0 mg/kg) were shown to be significantly different from the vehicle/7-OH-DPAT condition ($p<0.01$).

Mean bout duration

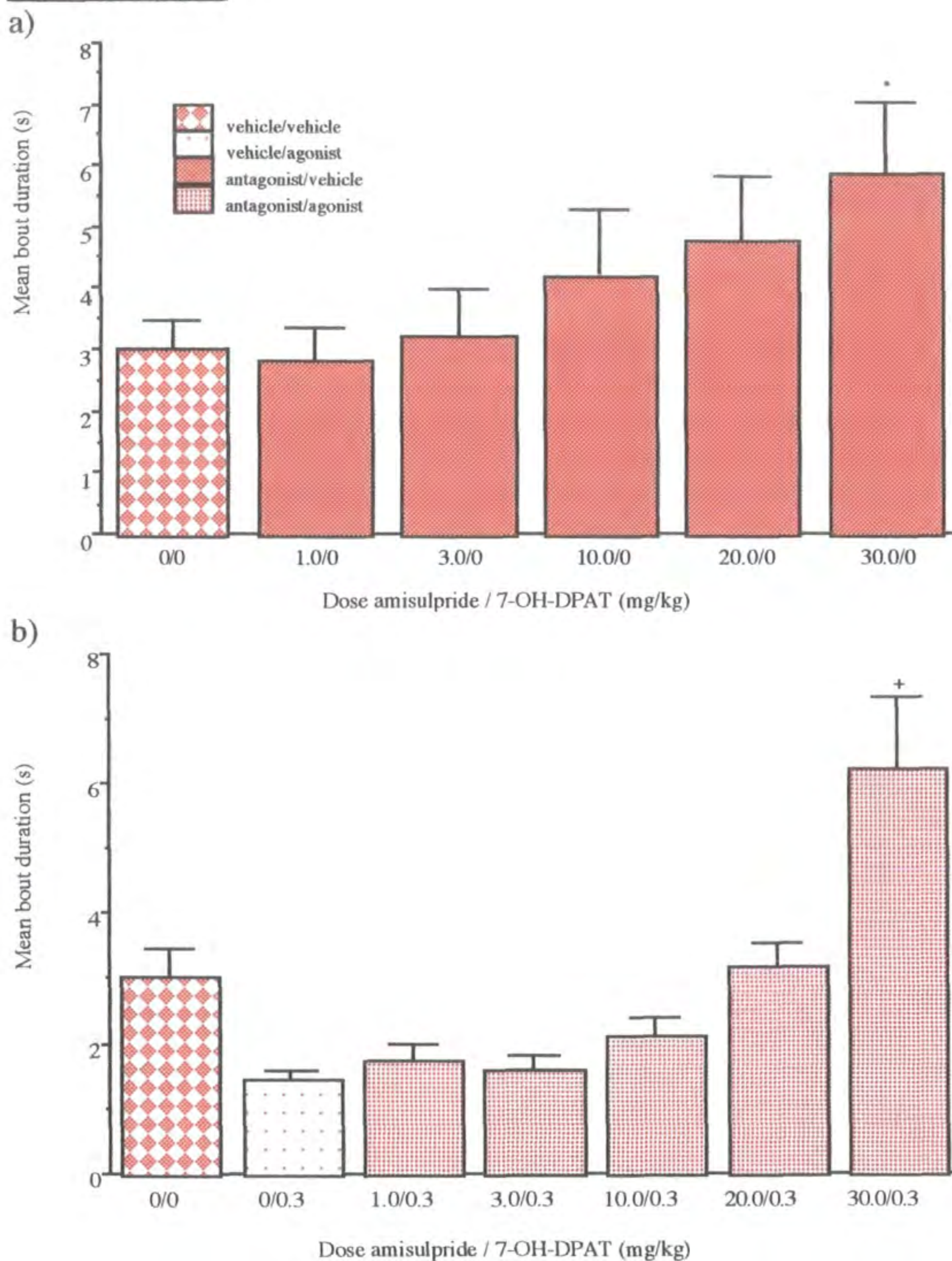


Figure 6.7 The effects of a) amisulpride (1.0, 3.0, 10.0, 20.0, 30.0 mg/kg) and b) 7-OH-DPAT (0.3 mg/kg) + amisulpride on mean bout duration of licking in a brief contact test + S.E.M. In a) asterisk indicates significantly different from vehicle / vehicle, $*p<0.05$. In b) Plus indicates significantly different from vehicle / 7-OH-DPAT; $+p<0.01$.

Table 6.6 Main effect of sucrose concentration on mean bout duration (s) .

Mean bout duration ± S.E.M.

Sucrose (%) 1	3	10
1.8±0.2	3.0±0.3	5.3±0.5

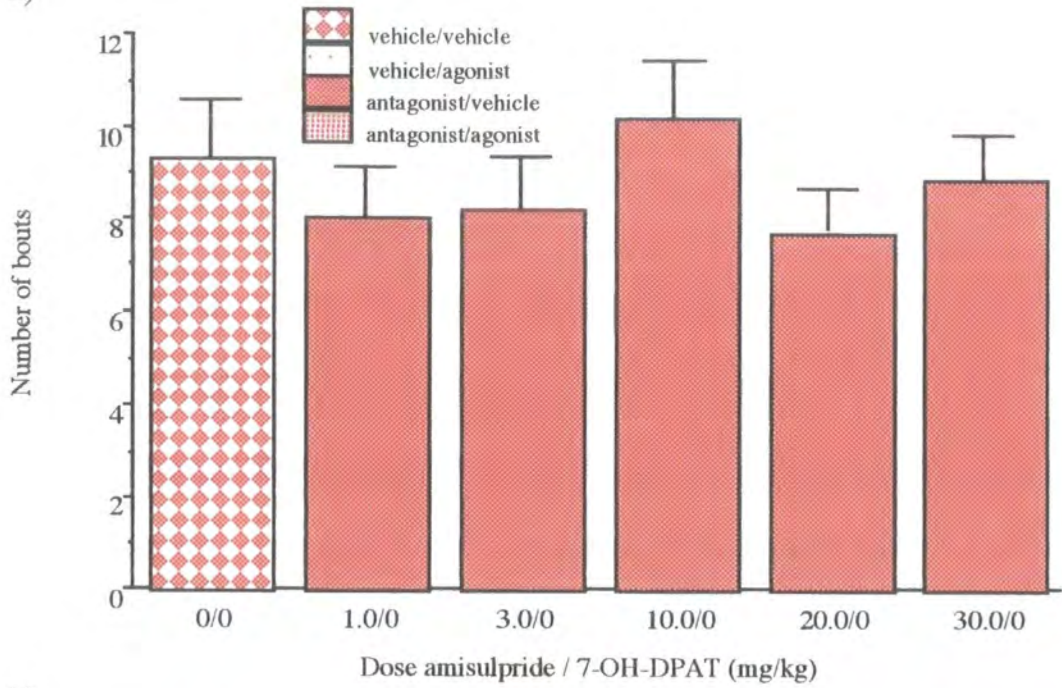
n=10 animals per concentration

7-OH-DPAT succeeded in approximately halving mean bout duration, when compared to vehicle controls. A one-way repeated measures ANOVA showed this result to be significant: ($F_{9, 19} = 11.426$, $p<0.01$). A two-way repeated measures ANOVA showed that there was a significant main effect of drug dose: ($F_{11, 99} = 5.523$, $p<0.0001$) and also of sucrose concentration: ($F_{2, 18} = 22.409$, $p<0.0001$) (see Table 6.6). There was also a significant interaction between drug dose and concentration: ($F_{22, 198} = 1.626$, $p<0.05$). Mean bout duration increased as a function of amisulpride alone (1.0-30.0 mg/kg). The highest dose of amisulpride alone (30.0 mg/kg) was significantly different from the vehicle/vehicle condition in a post hoc test ($p<0.05$) (see Figure 6.7 a).

The decremental effect of 7-OH-DPAT (0.3 mg/kg) on mean bout duration began to be attenuted by pretreatment with 10.0 mg/kg amisulpride and was fully abolished by 20.0 and 30.0 mg/kg. Not only did the highest dose of amisulpride abolish the decremental effects of 7-OH-DPAT (0.3 mg/kg) on mean bout duration, but this dose (30.0 mg/kg) increased mean bout duration over and above measures of mean bout duration for vehicle/vehicle controls. Pretreatment with the highest dose of amisulpride (30.0 mg/kg) was shown to be significantly different from the vehicle/7-OH-DPAT condition ($p<0.01$).

Number of bouts

a)



b)

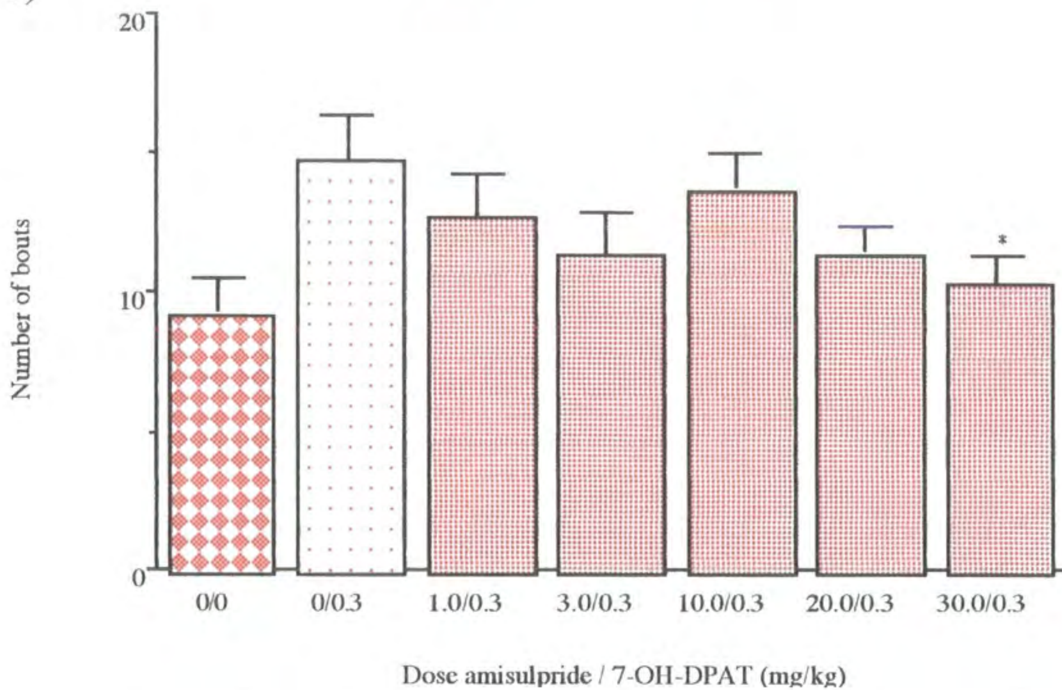


Figure 6.8 The effects of a) amisulpride (1.0, 3.0, 10.0, 20.0, 30.0 mg/kg) and b) 7-OH-DPAT (0.3 mg/kg) + amisulpride on number of bouts of licking in a brief contact test + S.E.M. In b) Asterisk indicates significantly different from vehicle / 7-OH-DPAT; *$p<0.05$.

Table 6.7 Main effect of sucrose concentration on number of bouts .

Number of bouts ± S.E.M.

Sucrose (%)		
1	3	10
6.7±0.4	11.4±0.5	13.8±0.6

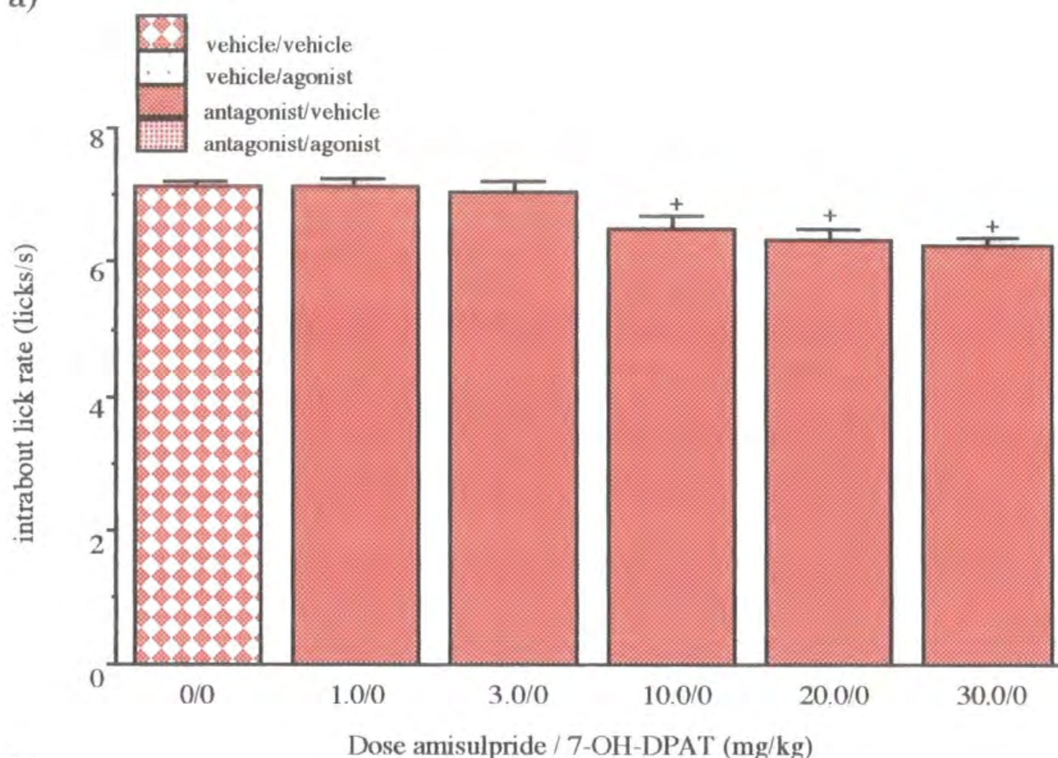
n=10 animals per concentration

7-OH-DPAT increased bout frequency by approximately 50%, when compared to vehicle controls. A one-way repeated measures ANOVA showed this result to be significant: ($F_{9, 19}$ = 10.496, $p<0.05$). A two-way repeated measures ANOVA showed that there was a significant main effect of drug dose: ($F_{11, 99}$ = 4.190, $p<0.0001$) and also of sucrose concentration: ($F_{2, 18}$ = 40.114, $p<0.0001$). As concentration increased so did number of bouts (see Table 6.7). There was also a significant interaction between drug dose and concentration: ($F_{22, 198}$ = 3.126, $p<0.01$). Number of bouts increased at the 10.0 mg/kg dose of amisulpride alone but higher and lower doses of amisulpride did little to change this parameter. None of the doses of amisulpride were significantly different from the vehicle/vehicle condition in a post hoc test .

In terms of agonist/antagonist interactions, pretreatment with doses of amisulpride partially attenuated the incremental effects of 7-OH-DPAT (0.3 mg/kg) on mean bout frequency. The highest dose of amisulpride almost abolished the effect of 7-OH-DPAT and this was shown to be significant in a post hoc test when compared to vehicle/7-OH-DPAT condition ($p<0.05$). (see Figure 6.8 b).

Intrabout lick rate

a)



b)

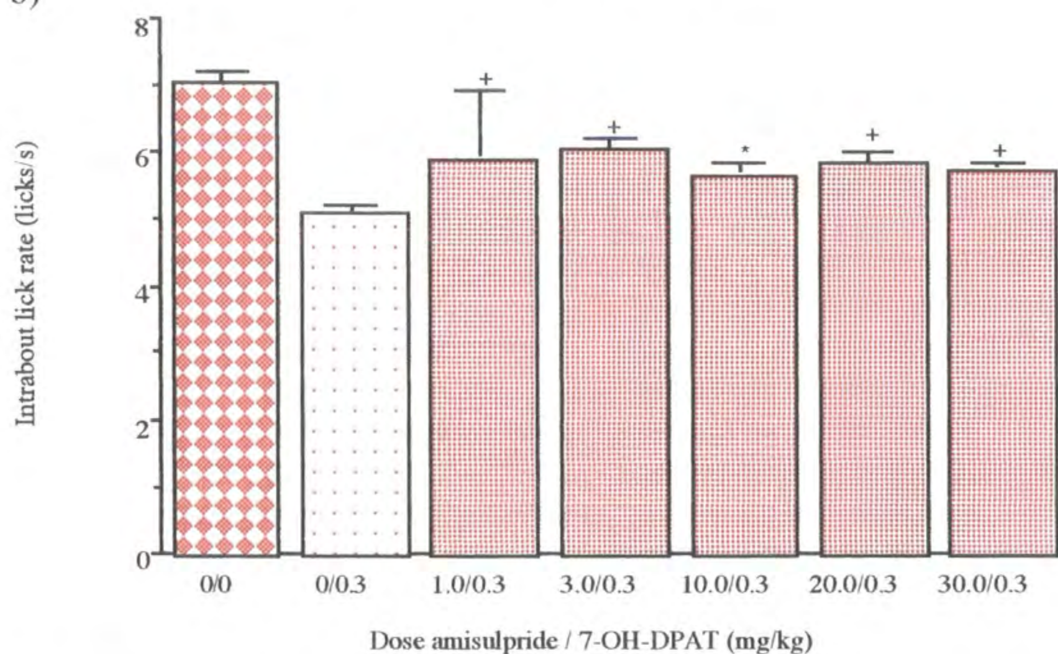


Figure 6.9 The effects of a) amisulpride (1.0, 3.0, 10.0, 20.0, 30.0 mg/kg) and b) 7-OH-DPAT (0.3 mg/kg) + amisulpride on intrabout lick rate in a brief contact test + S.E.M. In a) Plus indicates significantly different from vehicle / vehicle; +p<0.01. In b) asterisk indicates significantly different from vehicle / 7-OH-DPAT; *p<0.05; Plus indicates significantly different from vehicle / 7-OH-DPAT; +p<0.01.

Table 6.8 Main effect of sucrose concentration on intrabout lick rate (lick/s) .

Intrabout lick rate ± S.E.M.

Sucrose (%)		
1	3	10
6.4±0.09	6.3±0.07	6.0±0.05

n=10 animals per concentration

7-OH-DPAT succeeded in decreasing intrabout lick rate by approximately 2 licks/s when compared to vehicle controls. A one-way repeated measures ANOVA showed this result to be significant: ($F_{9, 19}$ = 313.061, $p<0.0001$). A two-way repeated measures ANOVA showed that there was a significant main effect of drug dose: ($F_{11, 99}$ = 30.430, $p<0.0001$) and also of sucrose concentration: ($F_{2, 18}$ = 5.906, $p<0.05$). As concentration increased intrabout lick rate decreased, (see Table 6.8). There was also a significant interaction between drug dose and concentration: ($F_{22, 198}$ = 1.768, $p<0.05$). Intrabout lick rate decreased as a function of amisulpride alone (1.0-30.0 mg/kg). Three doses of amisulpride alone (10.0, 20.0 and 30.0 mg/kg) were significantly different from the vehicle/vehicle condition in a post hoc test ($p<0.01$) (see Figure 6.9 a).

The decremental effect of 7-OH-DPAT (0.3 mg/kg) on intrabout lick rate was partially abolished by all pretreatment doses of amisulpride (1.0-30.0 mg/kg). Howerer, this abolition was not dose-dependent. A post hoc test showed that all pretreatment doses of amisulpride were significantly different when compared to the vehicle/7-OH-DPAT condition ($p<0.01$; $p<0.05$ for 10.0 mg/kg).

Average interlick interval

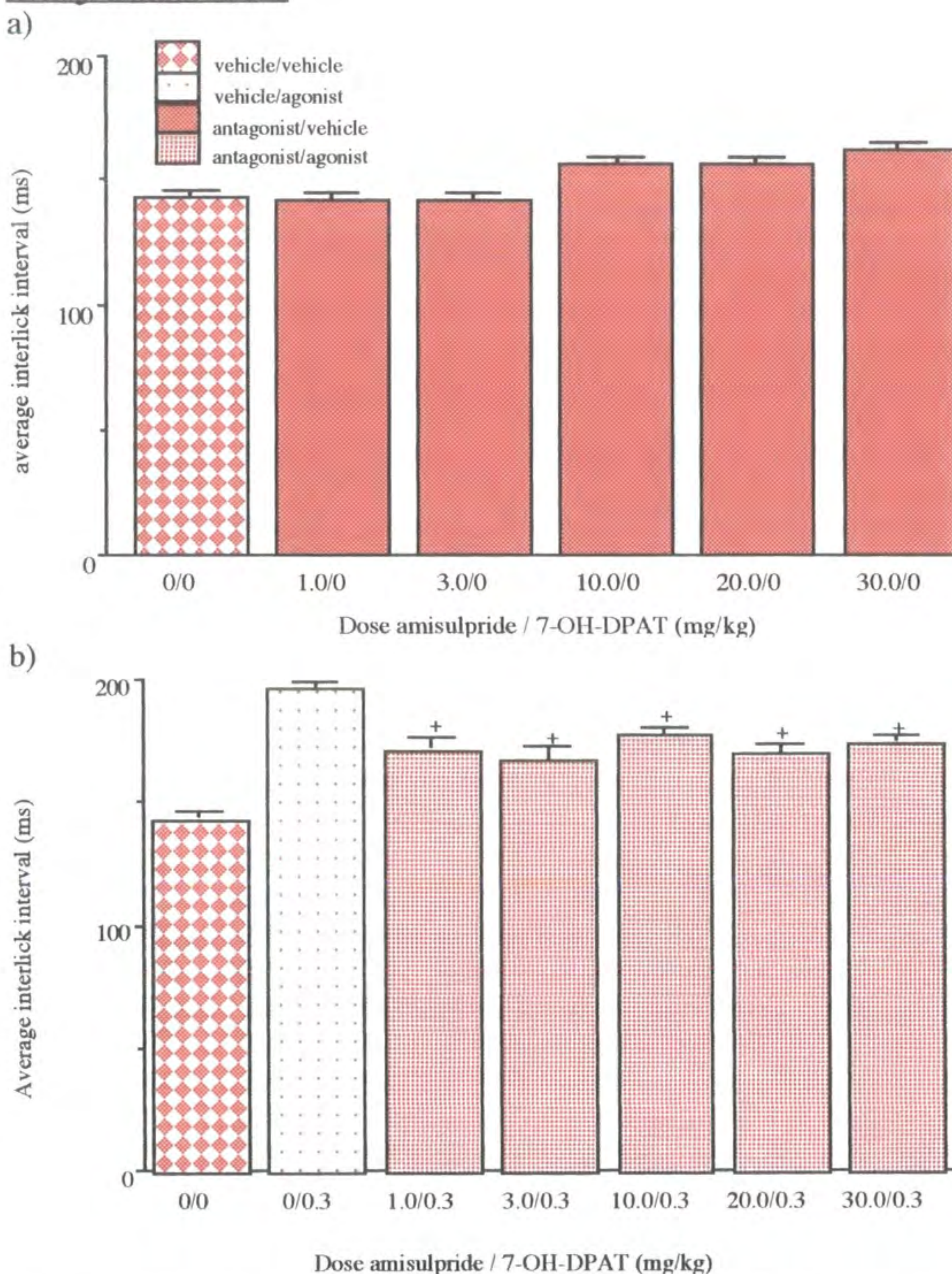


Figure 6.10 The effects of a) amisulpride (1.0, 3.0, 10.0, 20.0, 30.0 mg/kg) and b) 7-OH-DPAT (0.3 mg/kg) + amisulpride on average interlick interval in a brief contact test + S.E.M. In b) Plus indicates significantly different from vehicle / 7-OH-DPAT; +p<0.01.

7-OH-DPAT succeeded in increasing average interlick interval by approximately 50 ms, when compared to vehicle controls. A one-way repeated measures ANOVA showed this result to be significant: ($F_{9, 19} = 264.022$, $p<0.0001$). A two-way repeated measures ANOVA showed that there was a significant main effect of drug dose: ($F_{11, 99} = 23.250$, $p<0.0001$). Average interlick interval did not vary with concentration, therefore there was no main effect of concentration on this parameter: ($F_{2, 18} = 1.894$, n.s.). Nor was there a significant interaction between drug dose and concentration: ($F_{22, 198} = 1.071$, n.s.).

Average interlick interval only increased slightly as a function of the higher doses of amisulpride alone (10.0-30.0 mg/kg). Of these doses, none were found to be significantly different from vehicle/vehicle condition in a post hoc test (Figure 6.10 a). The incremental effect of 7-OH-DPAT (0.3 mg/kg) on average interlick interval was partially abolished by all pretreatment doses of amisulpride (1.0-30.0 mg/kg) but this effect was non-monotonic. A post hoc test showed that all pretreatment doses of amisulpride were significantly different when compared to the vehicle/7-OH-DPAT condition ($p<0.01$).

Experiment 9: Microstructural analysis of drinking for 10% sucrose after the administration of amisulpride : Analyses of licking during a 20-min session and during first minute of 20-min session.

The effects of one dose of amisulpride on total intake and licking microstructure was compared to the effects of vehicle in a 20 min test session.

a) for full 20 min test session

Table 6.9 Average (± S.E.M.) measures of total intake and microstructural licking parameters in a 20-min test session

	Dose amisulpride (mg/kg)	
	0.0	20.0
Amount intake (ml)	18.6±0.9	19.0±1.2
Number of licks	2069.5±103.6	1786.1±53.2
Mean bout duration (s)	3.6±0.4	3.4±0.5
Number of bouts	96.0±9.2	96.2±14.7

n= 8 animals per drug dose

Intake

Over a 20 min test session, amisulpride (20.0 mg/kg) did not affect total amount intake. Animals who received vehicle drank almost the same as those receiving the dose of amisulpride. There was no significant main effect of drug on this parameter: ($F_{1,7} = 0.051$, n.s.).

Microstructural analysis

Number of licks

Number of licks were decreased after the administration of amisulpride (20.0 mg/kg). This result reached statistical significance: ($F_{1,7} = 7.5$, $p<0.05$).

Mean bout duration

Mean bout duration also decreased after the dose of amisulpride (20.0 mg/kg). This result was slight and non-significant: ($F_{1,7} = 0.774$, n.s.).

Number of bouts

Number of bouts were very similar whether animals had amisulpride (20.0 mg/kg) or vehicle. Therefore there was no main effect of drug on bout frequency: ($F_{1,7}$ = 0.48, n.s.).

b) for the first min of a 20 min test session

Microstructural analysis

Number of licks

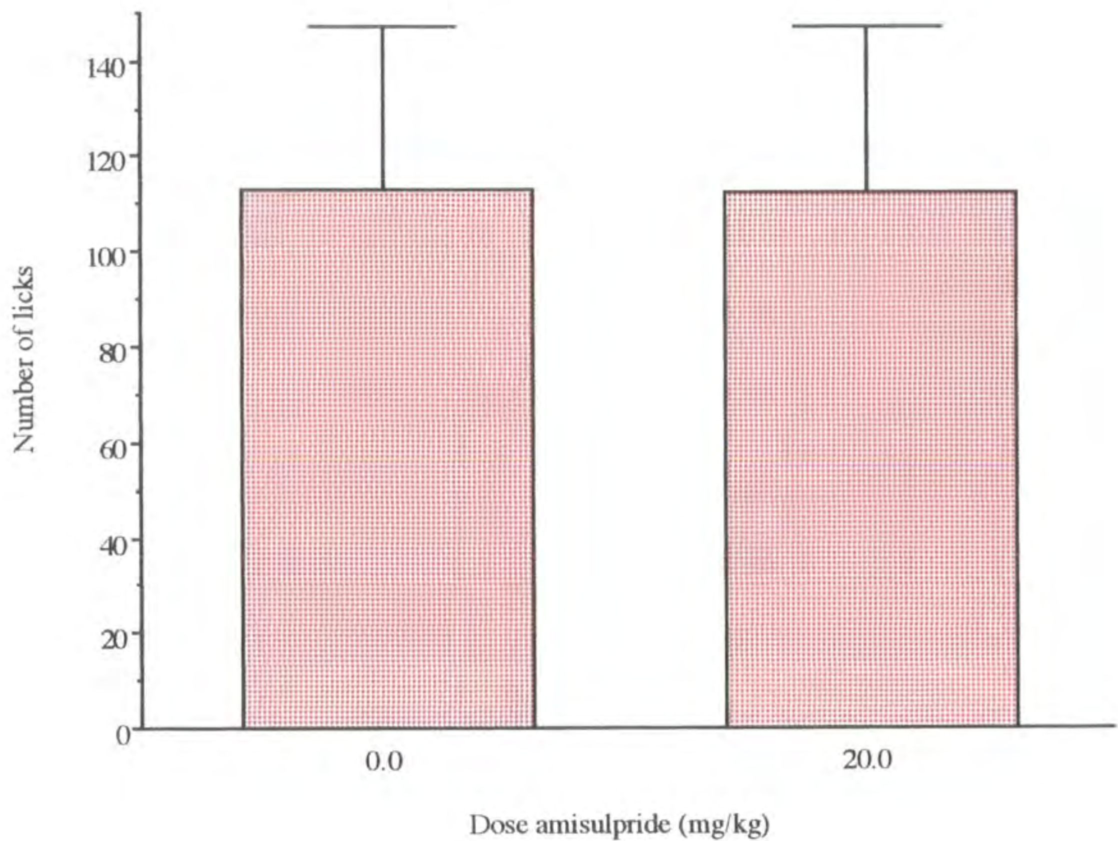


Figure 6.11 The effect of amisulpride (20.0 mg/kg) on the number of licks for 10% sucrose in the first min of a 20 min test session.

Illustrated in Figure 6.11 are data from the first min of a 20 min test session, where there was no effect of amisulpride (20.0 mg/kg) on the total number of licks. Therefore there was no significant main effect of drug on number of licks: ($F_{1,7}$ = 0.001, n.s.).

Mean bout duration

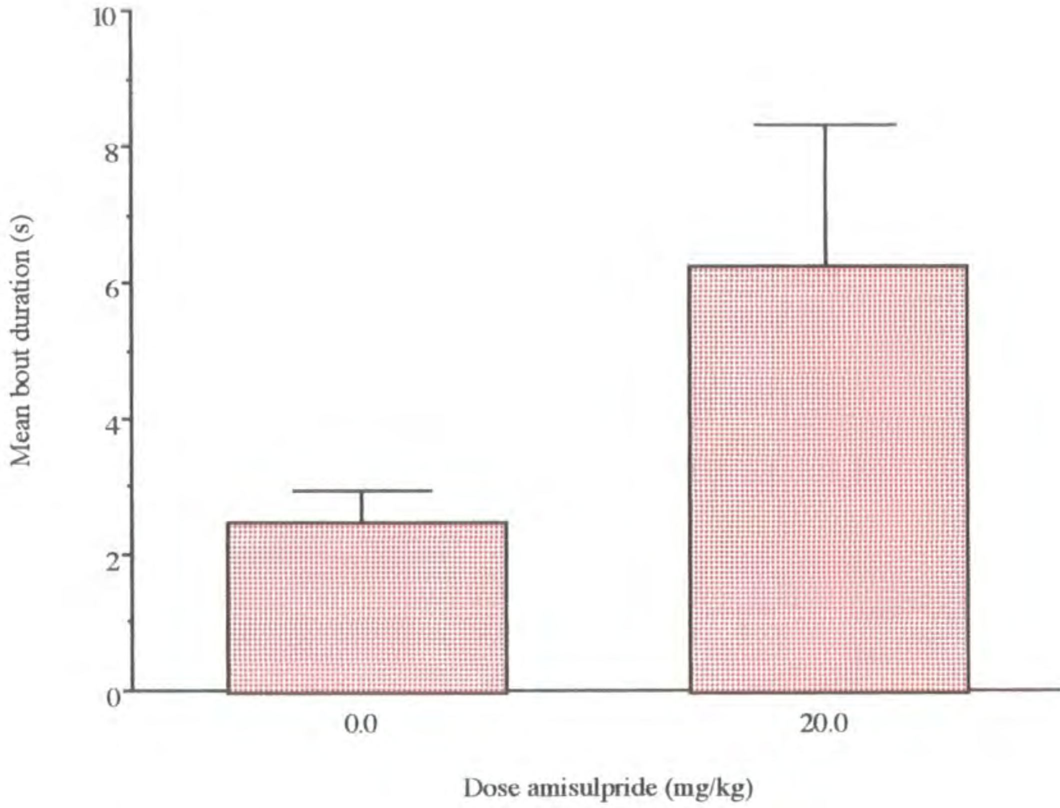


Figure 6.12 The effect of amisulpride (20.0 mg/kg) on the mean bout duration of licking for 10% sucrose in the first min of a 20 min test session.

Figure 6.12 shows that mean bout duration increased by approximately 4 seconds after the administration of amisulpride (20.0 mg/kg). However, this result did not reach statistical significance: ($F_{1,7} = 3.436$, n.s.).

Number of bouts

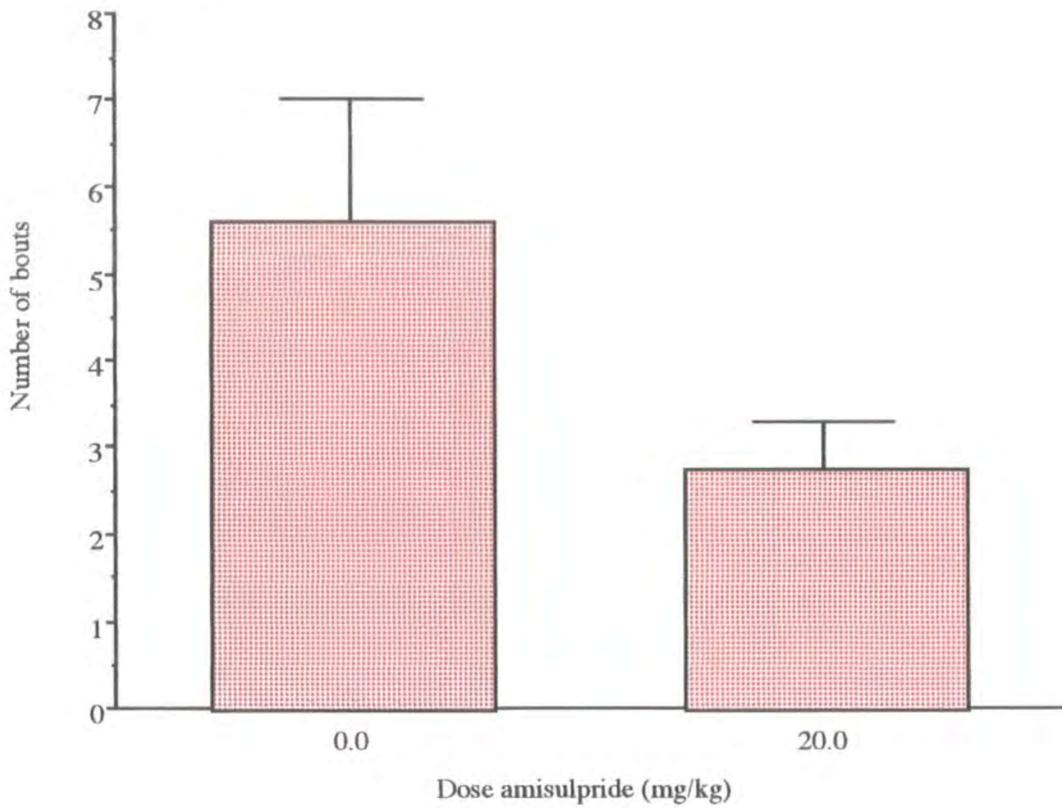


Figure 6.13 The effect of amisulpride (20.0 mg/kg) on number of bouts of licking for 10% sucrose in the first min of a 20 min test session.

From Figure 6.13 it is clear that there was a decremental effect of amisulpride (20.0 mg/kg) on mean bout frequency, the drug almost halved the number of bouts compared to the vehicle condition. This was shown to be a statistically significant main effect: ($F_{1,7} = 5.723$, $p<0.05$).

6.4 Discussion

In Experiment 7, there was a clear effect of 7-OH-DPAT to reduce the number of licks by shortening the mean bout duration. Bout frequency was increased by 7-OH-DPAT which further argues against a motoric impairment induced by this dose of 7-OH-DPAT. However, 7-OH-DPAT also significantly decreased intrabout lick rate which points *towards* some kind of orolingual motor deficit. Increasing sucrose concentration in Experiment 7 increases the number of licks, the mean bout durations and the number of bouts as has been determined in previous chapters. However, as concentration increased so did intrabout lick rate which, as was mentioned in Chapter 5, could be a result of the increasing viscosity of the higher sucrose concentrations rather than changes in palatability of the test fluid. Neither of the doses of PNU-99194A alone were significantly different from vehicle/vehicle conditions for number of licks, mean bout duration or mean bout frequency. However, at the 5.0 mg/kg dose, PNU-99194A did significantly increase intrabout lick rate which is in accordance with the previously reported stimulant effects of PNU-99194A (Waters et al., 1993).

In terms of the ability of PNU-99194A to attenuate or abolish the effects of 7-OH-DPAT on licking parameters it seemed that the lower dose of 5.0 mg/kg was most effective at eliminating 7-OH-DPAT's effects, although even at this dose the attenuation of 7-OH-DPAT's effects was not significant when compared to the vehicle/agonist condition in a post hoc test. For none of the parameters did PNU-99194A pretreatment produce effects that were easily distinguishable from 7-OH-DPAT when the agonist was co-administered with the antagonist. Therefore, it is reasonable to conclude that at the doses employed, PNU-99194A did not block the effects of 7-OH-DPAT in this test.

These results are generally consistent with the findings of Baker et al. (1998), who showed that PNU-99194A was unable to block 7-OH-DPAT substitution for D-amphetamine or cocaine in the drug discrimination paradigm. This result led these authors to question the pharmacological specificity of PNU-99194A. In a further experiment they confirmed this suspicion. By comparing the discriminative cue produced by PNU-99194A to several other dopamine agonists and antagonists they discovered that the subjective cue produced by PNU-99194A is probably based on D1 receptor antagonism (Franklin et al., 1998). Therefore, if PNU-99194A exerts its effects through D1 receptors it would not be surprising that this compound was unable to reverse the effects of 7-OH-DPAT in Experiment 7.

Alternatively, and most likely, the doses of PNU-99194A employed in Experiment 7 were too low to be effective. Support for this idea comes from the study by Clifford and Waddington (1998). Using doses comparable to the present experiment (5 and 10 mg/kg) PNU-99194A, the only behavioural count to decrease significantly was that of grooming. At 15mg/kg there was a significant increase in eating counts which were augmented further when the dose was raised to 45 mg/kg and therefore, the doses used in this experiment were probably too low to counter the significant decremental effects of 7-OH-DPAT on feeding parameters. Therefore, it is difficult to infer any common site of action of the two drugs (PNU-99194A and 7-OH-DPAT) from this experiment. Further experiments which assess higher doses of the antagonist on licking parameters, in conjunction with the assessment of other behavioural measures, need to be carried out. In combination with such studies, research is needed into the *concentrations* of such novel compounds that are selective for the D3 receptor. This information is not yet available but bears important implications for the interpretation of results from behavioural studies which use systemic routes of administration of novel drug compounds.

Again, in Experiment 8, main effects of concentration were observed. As concentration increased so did the number of licks, mean bout duration and the number of bouts. Increasing concentration led to a significant decrease in intrabout lick rate which probably reflected the increasing viscosity of the sucrose solutions. 7-OH-DPAT, on the other hand, decreased number of licks and mean bout duration significantly. It also increased mean bout frequency, decreased intrabout lick rate and increased average interlick interval significantly. These effects were differentially attenuated by doses of amisulpride.

The hypothesis tested in Experiment 8 was that low doses of amisulpride presumably act presynaptically and could block the effects of 7-OH-DPAT on licking parameters. This would have been consistent with the work by Sanger et al. (1996), who found that these low doses of amisulpride were sufficient to reverse the cueing and the rate-decreasing effects of 7-OH-DPAT on operant responding for food. Only at 10, 20 and 30 mg/kg did amisulpide reverse the effects of 7-OH-DPAT on number of licks. Therefore, the hypothesis was not confirmed. At 20 and 30 mg/kg, respectively, amisulpride reinstated mean bout duration to levels comparable to and greater than those observed in the vehicle condition. Number of bouts were increased by 7-OH-DPAT and were decreased by the pretreatment with amisulpride at 30 mg/kg. None of the doses of amisulpride totally abolished the

effects of 7-OH-DPAT on intrabout lick rate or average interlick interval. For these latter parameters, both low and high doses of amisulpride produced similar effects. This further suggests that the ability of amisulpride to affect components of food reward is separate from the ability of this drug to affect motor competence per significant effect.

However, there is a fundemental pharmacological issue which constraints the extent to which the action of amisulpride, in this case, can be described as antagonising the effects of 7-OH-DPAT. As is evident from the results, higher doses of amisulpride have a behavioural effect which is opposite to that of 7-OH-DPAT. Therefore, interactions between these two compounds is unlikely to represent a straightforward pharmacological antagonism. Further work on the pharmacological effects of a broad dose range of these two compounds in combination would help to solve this problem.

In Experiment 8, amisulpride alone had a dose-dependent incremental effect on number of licks and managed to increase significantly the number of licks at the highest dose (30 mg/kg). However, at doses which purportedly act at autoreceptors (1 and 3 mg/kg) there was a slight decrease in number of licks as compared to vehicle controls. This pattern was reflected also in the measures of mean bout duration. It therefore seems that at the higher doses, where mean bout duration was almost doubled by amisulpride, this compound increased ingestion by enhancing the palatability of the sucrose solution in a brief contact test. This may partially explain the induction of a food induced conditioned place preference by Guyon et al. (1993) in that, as the stimulus originally paired with salient features of the environment, food had inflated hedonic value which served to cement the associations necessary to establish a conditioned place preference. This is further supported by the non-monotonic relationship between dose of amisulpride and number of bouts. Amisulpride decreased the number of bouts at all doses (non-significantly), apart from 10mg/kg which slightly increased mean bout frequency.

Small doses (1 and 3 mg/kg) of amisulpride did not affect intrabout lick rate or the reciprocal of this (average interlick interval) at all. At the largest doses (10, 20 and 30 mg/kg) although amisulpride decreased licking within a bout significantly at these doses the effect was small. Therefore, it seems that this drug did not have any severe deleterious effects on the motor competence necessary for sustained licking responses. This is consistent with those reports which have observed a lack of catalepsy with amisulpride even with large doses (Millan et al., 1995).

Taken together, although it is usually said that large doses (>30.0 mg/kg) of amisulpride act at postsynaptic receptors, it is not clear whether the doses of amisulpride used in these experiments are selective for postsynaptic D2 receptors. Hence, a strong conclusion for the mode of action of 7-OH-DPAT cannot be drawn. What these results seem to suggest is that 7-OH-DPAT does not necessarily, or exclusively, exert its effects through autoreceptors of the D2 or the D3 type as low doses of amisulpride which preferentially act at these autoreceptor sites do not abolish the robust effects of 7-OH-DPAT on feeding parameters. A caveat is necessary at this level of explanation as the effects of amisulpride used may not be accurately reflected in this test situation. The same animals were used to test, at first the low doses but consequently the 3 higher doses of this drug. Therefore, what might be occurring is a tolerance to these higher doses of amisulpride. Although a wash out period of 1 week was used between injection cycles this possibility cannot be discounted in interpreting these results.

To partially circumvent this problem, we consequently tested the prediction that a dose of amisulpride which had been shown to effectively abolish the effects of 7-OH-DPAT on an important parameter such as mean bout duration in drug-experienced animals, would also induce a hyperphagic (i.e an increase in intake) effect in drug naive animals. If effects such as those reported by Guyon et al. (1993) are literally "pro-hedonic", then hyperphagia might be expected to be expressed as an increase in mean bout duration most prominently at the beginning of a test session.

In Experiment 9, a 20-min test session was used. The amount ingested of a 10% sucrose solution was not affected by a 20 mg/kg dose of amisulpride. Rather than being increased, the number of licks decreased with this dose of amisulpride but this significant decrease was not observed for mean bout duration. Again, the effect of amisulpride (20 mg/kg) on number of bouts was negligible. Contrary to measures taken over 20-min, results from the first minute of the test session highlight differential effects of amisulpride which are only evident at the beginning of the meal.

The number of licks after the administration of either vehicle or amisulpride were the same in the first minute. However, when looking at initial licking responses (within the first minute of the test) it was observed that amisulpride increased mean bout duration by a considerable amount (4s) which resulted in a significant reduction in bouts. Despite the considerable augmentation of bout length

after amisulpride in the first minute, this result is non-significant. Therefore, it is difficult to claim that amisulpride seems to be increasing the initial hedonic value of the sucrose solution as reflected in an increase in mean bout duration in this case. This study, however, further validates the idea that it is not due to a tolerance to amisulpride that we get the abolition of effects of 7-OH-DPAT in several parameters. It also highlights the fact that short tests or analysis of results from the beginning of a test are of great interpretive strength if one is concerned with initial hedonic reactions to tastants.

In summary, the results of Experiments 8 and 9 suggest that amisulpride affected feeding parameters which reflect palatability but these effects are only apparent when a test of short duration is used. The action of amisulpride alone suggests a specific action of this drug to enhance palatability. However, this occurs in the main when high doses of the drug are used and so cannot be definitely attributed to an antagonist action at D3 receptor autoreceptors. Furthermore, the results from Experiment 7 did not yield concrete conclusions as to the behavioural effects of PNU-99194A, owing to the small doses of the drug which were used.

Chapter 7: The effects of the putative D3 receptor agonist 7-OH-DPAT on successive negative contrast

7.1 Introduction

When an animal experiences one reward followed by a second reward of a different value (reward shift), "contrast" is said to occur if the animal exhibits an exaggerated response on presentation of the second reward (Dunham, 1968), (see Chapter 2). One example of a contrast effect can be observed in the licking behaviour of rats as licking behaviour is not always determined by a given concentration of sucrose alone, but often reflects an animal's prior experience with alternative concentrations of sucrose solutions (Flaherty, 1982). Thus, a given concentration of a sucrose solution may lead to an exaggerated increase or decrease in licking behaviour, depending on the relative concentration of the solution presented previously. If rats are trained in brief daily sessions to lick for a low concentration sucrose solution and are then shifted to a higher concentration there is an increase in the number of licks generated by these animals compared to animals who have had access to the higher concentration alone (Flaherty, 1982). This effect is known as a successive positive contrast. Alternatively, a successive negative contrast effect occurs when rats, previously given brief daily access to a high concentration of sucrose, are shifted to a low concentration. Under these circumstances, licking for the low concentration is suppressed relative to that generated by rats having experienced only the low concentration of sucrose.

If brain mechanisms exist which permit the comparison of rewards (i.e. behaviour is activated based on the *relative* value as well as the absolute value of rewards) then there is reason to assume that there are brain neurotransmitter systems which underlie the capacity to compare rewards (Flaherty, 1996). Therefore, the pharmacological bases of positive and negative contrast effects have been investigated by studying the effect of several classes of drugs on the development and maintenance of successive contrast. However, many of these studies have concentrated only on successive negative contrast.

In Chapter 2, evidence was reviewed which indicated that neuroleptics disrupt reinforced operant and consummatory behaviour - an outcome which has often been interpreted in terms of an "anhedonic effect" (Wise, 1978, 1982). However, conclusions based on parallels drawn between the effects of neuroleptics on reinforced behaviour and the withdrawal and/or reduction of reinforcement, remain debatable (see General Introduction and General Discussion). The majority

of studies of the effects of dopamine blockade on consummatory behaviour have been concerned with the behavioural effects associated with the *absolute* value of a reinforcer. Many of the studies which have tried to establish a role for dopaminergic receptor sites in the mediation of relative reward have found that drugs which act at these receptor sites are largely ineffective in modulating consummatory contrast. Flaherty and colleagues have conducted many studies examining drug effects on the behavioural effects of reward comparison which constitute a contrast effect. Briefly, they achieve this by administering drugs to animals a sub-group of which are "shifted" (i.e. 32-4% sucrose) and a subgroup which are unshifted (i.e. 4-4% sucrose). Drugs are administered to some drug dose subgroups on the first day of the shift. Animals which do not receive drug on this day receive a dose on the second post-shift day. The rationale behind using a second post-shift day comes from drug studies on negative contrast which show that animals must have some experience with the post-shift solution before the drug becomes effective on contrast (Flaherty et al., 1986). The differential effectiveness of the drug early and late in the post-shift period has several possible explanations and is, as yet, an incompletely understood phenomenon.

Since much of the literature has suggested that dopaminergic transmission mediates several aspects of reward (Wise, 1978, 1982; Flaherty et al., 1991b) have investigated the possibility that dopaminergic antagonists influence contrast. However, preliminary studies using a limited number of dopamine blockers have had negative results. For example, the neuroleptic chlorpromazine did not affect contrast when administered on either the first or the second post-shift day (Flaherty et al., 1992). The more specific dopamine antagonist, haloperidol, was also without effect on contrast when administered on the second post-shift day. This drug produced a dose-dependent decrease in consumption of the post-shift sucrose solution which was proportional in both shifted and unshifted rats. This result further suggested that haloperidol does not seem to affect the relative reward value of sucrose; rather, this drug may affect the absolute reward value of sucrose and/or have sedating or motor-impairing effects (Flaherty et al., 1992). Yet, it is important to note that drugs which act more selectively at specific dopamine receptor subtypes have not been thoroughly studied in this paradigm. It is difficult to eliminate a role for dopamine in the mediation of relative reward if these more extensive pharmacological studies are not carried out.

In addition, despite the results discussed above, there is some evidence to support the idea that dopamine is involved in the mediation of relative reward. By manipulating the more traditional contrast procedure, it has been shown that

blockade, and release from dopamine blockade may produce contrast (Kentridge and Aggleton, 1993; Phillips and LePiane, 1986; Royall and Klemm, 1981). The study by Kentridge and Aggleton (1993) used animals trained on a variable interval schedule. The magnitude of reinforcement used in each test session alternated daily between one and four 45-mg food pellets and this variation in magnitude was signalled by lights in the operant chamber. One group of rats were injected with a low dose of the dopamine blocker cis(Z)-flupentixol before testing, while another group received saline before the test session. A second phase saw the drug conditions reversed. The change in drug conditions between phases produced both positive and negative successive contrast effects, consistent with the hypothesis that dopamine blockade attenuated the hedonic impact of reinforcement. Using this novel experimental situation the authors propose a role for dopamine in the ascription of relative reward value to the stimulus (Kentridge and Aggleton, 1993).

It should also be noted that studies on contrast effects typically use outcome measures in the form of total licks or total intake. The emphasis on these total session variables is widespread within the literature, despite the established value of microstructural analysis in elucidating processes which may govern consummatory behaviour (Davis and Levine, 1977). There has been only one attempt to identify the patterns of licking behaviour associated with negative contrast (Grigson et al., 1993). Grigson and her colleagues identified patterns of licking behaviour which constituted a contrast effect in both deprived and free-feeding rats. Temporal analysis of the licking behaviour of deprived animals revealed that the contrast effect was, in the main, accomplished through a decrease in bout size and an increase in the number of bouts initiated.

An innovative way of further assessing the contribution of dopamine receptor subtypes to the control of ingestive behaviour would be to combine the temporal analysis of licking behaviour as a consequence of a reward down-shift with the administration of a selective pharmacological treatment. The aims of the present experiment were to adopt the microstructural analysis of successive negative contrast already assessed by Grigson et al. (1993) and also to examine the effect of the putative dopamine D3 receptor agonist, 7-OH-DPAT, on consummatory contrast. The dopamine D3 receptor has been implicated in a number of aspects of rewarded behaviour (see Chapter 2) including ingestive behaviour. Experiments 1, 3, 7 and 8 show that when using both 20 and 1 min presentations, 7-OH-DPAT decreases ingestive behaviour by reducing mean bout duration . This change in bout duration may be interpreted as a decrease in palatability (Davis and Smith,

1992), as this parameter varies monotonically with an increase in sucrose concentration.

Therefore, it is suggested that 7-OH-DPAT may have an impact on the initial hedonic evaluation of the test solution. If the behavioural expression of consummatory negative contrast relies, at least in part, on the hedonic evaluation of the stimulus, 7-OH-DPAT should affect the consummatory negative contrast which occurs as a consequence of reward downshift. The aim of the experiment was to test this prediction.

7.2 Method

7.2.1 Animals

Eighty-four adult male hooded Lister rats weighing 250-350g at the time of testing were used. They were housed and maintained as described in Chapter 3. Animals were food-deprived to approximately 80% of their free-feeding body weights and maintained at that level by once per day feeding. This level of deprivation was chosen in order that the experiment was comparable to that of Flaherty et al. (1992). Water was continuously available throughout the experiment.

7.2.2 Drugs

7-OH-DPAT (Research Biochemicals International, U.K.) was dissolved in saline and injected i.p. 20 minutes before testing. Doses of 7-OH-DPAT were 0.1, 0.3 and 1 mg/kg or its vehicle. An injection-test period of 20-min was used as previous work has shown this to be adequate time for a drug effect on intake to become apparent (see Experiments 1 and 4).

7.2.3 Test Fluids

The two test solutions were either 4% or 32% sucrose solutions and were made up as described in Chapter 3. Access to the two solutions during training or testing depended on whether animals had been assigned to either the shifted or unshifted group (see Section 7.2.5).

7.2.4 Apparatus

Testing was carried out using the 108 multistation lickometer described in detail in Chapter 3.

7.2.5 Procedure

The rats were randomly assigned to 14 groups, 7 of which received 4% sucrose throughout the experiment (unshifted group) and 7 of which received 32% sucrose for the first 8 days of the experiment and then were shifted to 4% for the remaining 2 days (shifted group). Access was given to the sucrose solutions for 10 min at the same time each day. When animals were well familiarised with the test

apparatus and solutions they received a sham injection of saline to familiarise them with the injection procedure (day 7).The seven subgroups of each shift condition differed in drug treatment. One subgroup was injected with isotonic saline on each of the first 2 post-shift days. The remaining six groups in each shift condition received injections of 0.1, 0.3 or 1.0 mg/kg 7-OH-DPAT on either the first or the second post-shift day. If an animal was not injected with drug on either of the post-shift days, they were injected with saline. Drugs were administered 20-min before each test session.

7.2.6 Design and statistical analysis

Initial analysis of the lick time data was carried out using programs as outlined in Chapter 3. Measures derived from the raw data included a number of microstructural variables: number of licks, mean bout duration (s) and number of bouts as these parameters have been shown to be sensitive to the effects of sucrose concentration and to the effects of 7-OH-DPAT treatments, (see Experiments 1 and 4).

Data were separated depending on whether they had been collected from the first min of the test session or whether it was taken over the full 10-min of the test session. This decision was made on the basis of previous observations concerning the interpretive strength of the test time (see Chapter 2). This applied to all analyses. Statistical tests were performed using Super Anova and Statview SE+graphics (Abacus Concepts Increase., Berkeley, CA). A result was considered statistically significant if $p<0.05$.

7.2.6.1 Terminal pre-shift period

Pre-shift measures were derived by averaging penultimate and preceding pre-shift day measures for each animal in each shift group. Effects of sucrose concentration (32 or 4%) before reward shift and before drug testing were compared using a one-way between-subjects ANOVA.

7.2.6.2 Negative contrast effects

Traditionally, a consummatory contrast effect post-shift is measured and analysed relative to the unshifted controls. For example, animals shifted to a lower sucrose concentration show suppressed licking *relative* to unshifted controls (Grigson et al., 1993, and see Introduction, Section 7.1). This conception of a

negative contrast effect assumes that in the pre-shift period, animals licking for a high sucrose solution will lick more than those licking for a lower solution. The effects of sucrose solutions were inversely related to the level of intake observed in the present experiment. Therefore, due to these unexpected baseline levels of drinking as reflected in terminal pre-shift measures (see Section 7.3) it was decided that a contrast effect was seen to be established if the pre-shift means of shifted animals were significantly greater than post-shift means for the same animals. This was achieved by employing a one-way within subjects ANOVA to analyse data from the shifted group.

7.2.6.2 Drug effects on negative contrast

On each of the first 2 post-shift days, the data for the groups that received the drug injection on that day were compared to that of one of the saline control groups injected on the same day. The saline control group used was chosen at random from the 4 control groups available for analysis on each post-shift day. The groups that received the drug injection on the alternative day were analysed separately. The effects of 7-OH-PAT on negative contrast were analysed using a two-way between subjects ANOVA with shift condition and dose of 7-OH-DPAT as factors. If there was a reliable dose effect on any of the microstructural variables which did not coincide with a significant interaction between dose and shift condition, post hoc tests were used to examine at which dose the drug effect had become effective. These were applied to each shift condition separately.

The drug effectiveness ratio (DER) (Flaherty, 1991b) was also computed for each level of the drug investigated (0.1, 0.3 and 3.0 mg/kg 7-OH-DPAT). The larger the ratio, the greater the effect on consummatory contrast. Below is the method by which it is computed:

$$\text{DER} = \frac{[\text{P (unshifted vehicle)}] - [\text{P (shifted vehicle)}]}{[\text{P (unshifted drug)}] - [\text{P (shifted drug)}]}$$

The greater the effect of the drug in reducing contrast, the larger the DER. (Flaherty, 1991b, 1996).

The computation of this ratio serves two purposes: firstly, it discounts pre-shift differences in lick frequency (in this case licks/min) by examining post-shift data in terms of proportions (P) of terminal pre-shift values. Thus, (P) may be derived by dividing (terminal pre-shift lick frequency) / (first post-shift day lick frequency plus terminal pre-shift lick frequency).

Secondly, the effectiveness of the drug is then examined by obtaining a ratio of the difference between vehicle-injected shifted and unshifted animals and drug-injected shifted and unshifted animals.

7.3 Results

Terminal preshift period

a) data from the full 10 min test session

Terminal pre-shift measures were calculated as described in section 7.2. Table 7.1 shows the effects of sucrose concentration (32 or 4%) on the microstructural variables in this phase of the experiment.

Table 7.1 Effects of sucrose concentration on average (±S.E.M.) parameters of licking over a 10-min test session.

	Sucrose concentration	
	32%	4%
Number of licks	589.4±24.9	1038.3±44.1
Mean bout duration (s)	2.962±0.1	4.4±0.2
Number of bouts	28.2±1.3	34.3±1.8

n= 42 per concentration group

Those animals drinking 32% sucrose licked approximately half as much as those that had access to 4% sucrose (see Table 7.1). The latter group were licking for longer within a bout and also initiated a higher number of bouts within the 10-min test session. Therefore, a one-way between subjects ANOVA showed that there was a main effect of sucrose concentration on number of licks: ($F_{1, 82}$ = 74.408, $p<0.0001$); mean bout duration: ($F_{1, 82}$ = 32.472, $p<0.0001$) and mean bout frequency: ($F_{1, 82}$ = 7.158, $p<0.05$).

b) in the first min of the 10-min test session

Table 7.2 Effects of sucrose concentration on average (±S.E.M.) parameters of licking in the first min of a 10-min test session.

	Sucrose concentration	
	32%	4%
Number of licks	119.0±11.672	167.6±6.5
Mean bout duration (s)	2.5±0.2	4.5±0.2
Number of bouts	6.5±0.7	5.5±0.3

n= 42 per concentration group

In the first minute of the 10-min test session the effects of sucrose concentration were less profound than the effects on microstructural variables over the full 10-min test session (see Tables 7.2 and 7.1 respectively). Number of licks and mean bout duration were greater for the group drinking 4% sucrose than for those drinking 32% sucrose and these results reached statistical significance. There was a main effect of sucrose concentration on number of licks: ($F_{1, 82} = 13.209$, $p<0.0005$) and on mean bout duration: ($F_{1, 82} = 53.207$, $p<0.0001$).

Negative contrast effects

Usually, a contrast effect post-shift is measured and analysed relative to the unshifted controls (see Section 7.2). However, due to unexpected baseline levels of drinking as reflected in terminal pre-shift measures (see above) a contrast effect was seen to be established if the pre-shift means of shifted animals were significantly greater than post-shift means. This was achieved by employing a one-way within subjects ANOVA to analyse data from the shifted group.

a) data from the full 10 min test session

Number of licks

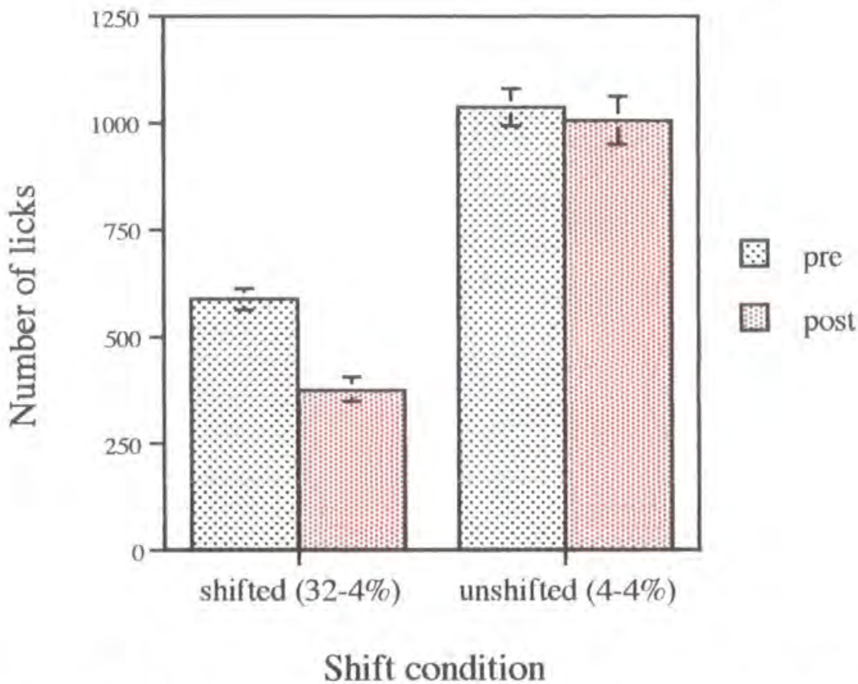


Figure 7.1 Average (+S.E.M) number of licks for sucrose in a 10-min licking test pre- and post-shift as a function of shift condition (32-4%) or (4-4%) on the first post-shift day.

In the post-shift period, half the animals (n=42) were shifted from 32-4% sucrose (shifted) while the other half continued to have access to a 4% sucrose solution (unshifted). Shifted animals generated fewer licks (approximately 250 licks) in the post-shift period relative to the pre-shift period whereas unshifted animals did not show such a profound difference despite a slight drop in the number of licks in the post-shift period (approximately 75 licks) (see Figure 7.1). A one-way within subjects ANOVA revealed that number of licks within the shifted group

was significantly affected by shift period (pre- and post-): ($F_{1, 46} = 15.358$, $p<0.0005$).

Mean bout duration

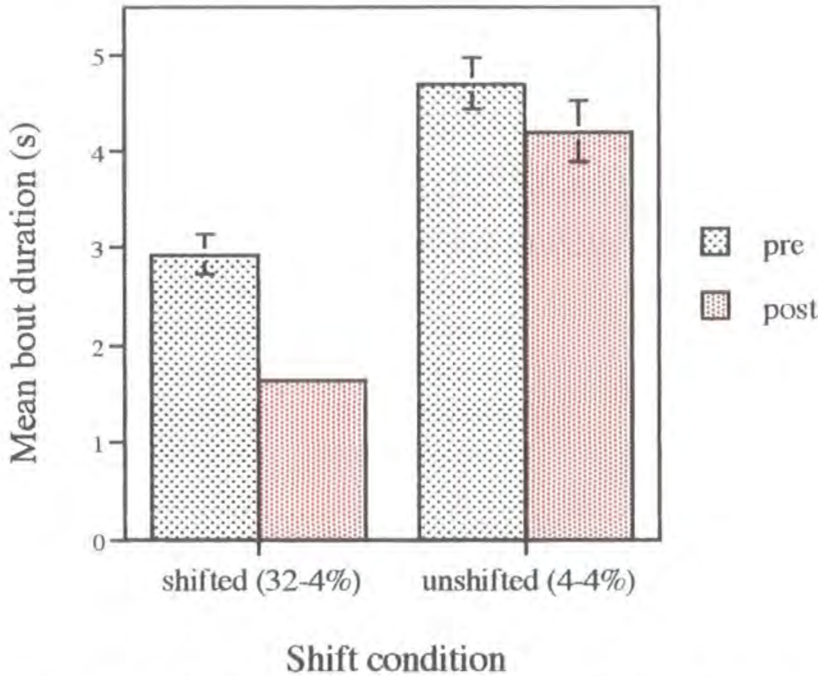


Figure 7.2 Mean (+S.E.M) bout duration of licking for sucrose in a 10-min licking test pre- and post-shift as a function of shift condition (32-4%) or (4-4%) on the first post-shift day.

Figure 7.2 shows that shifted animals licked with shorter mean bout durations in the post-shift period relative to the pre-shift period. This difference of almost 1.5 s was significant: ($F_{1, 46} = 58.207$, $p<0.0001$). Unshifted animals did not show such a profound decrease despite a slight reduction in mean bout duration of approximately 0.5s in the post-shift period.

Number of bouts

Number of bouts increased marginally in the unshifted group in the post-shift period. The number of bouts initiated by shifted animals were almost equivalent in the pre- and post- shift periods. Therefore, over the full 10-min session, there was no effect of shift on mean bout frequency. A one-way within subjects ANOVA on pre- and post- shift measures from the shifted group confirmed this observation: ($F_{1, 46} = 3.194$, n.s.). These results are not shown graphically.

b) in the first min of a 10-min test session

Number of licks

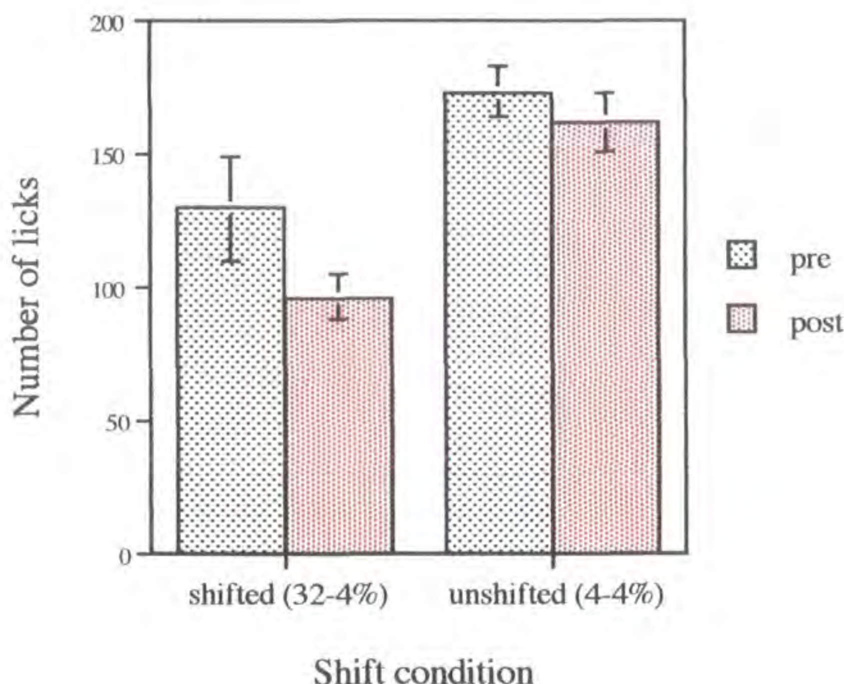


Figure 7.3 Average (+S.E.M) number of bouts of licking for sucrose in the first min of a 10-min licking test pre- and post-shift as a function of shift condition (32-4%) or (4-4%) on the first post-shift day.

Consistent with results over the full 10-min session, shifted animals generated fewer licks in the post-shift period relative to the pre-shift period whereas unshifted animals did not show such a profound difference despite a slight reduction in the number of licks in the post-shift period (see Figure 7.3). However, discordant with results collected over 10-min, the effect of devaluing sucrose to 4% did not produce a significant decrease in number of licks within the shifted group: ($F_{1, 46} = 0.691$, n.s.).

Mean bout duration

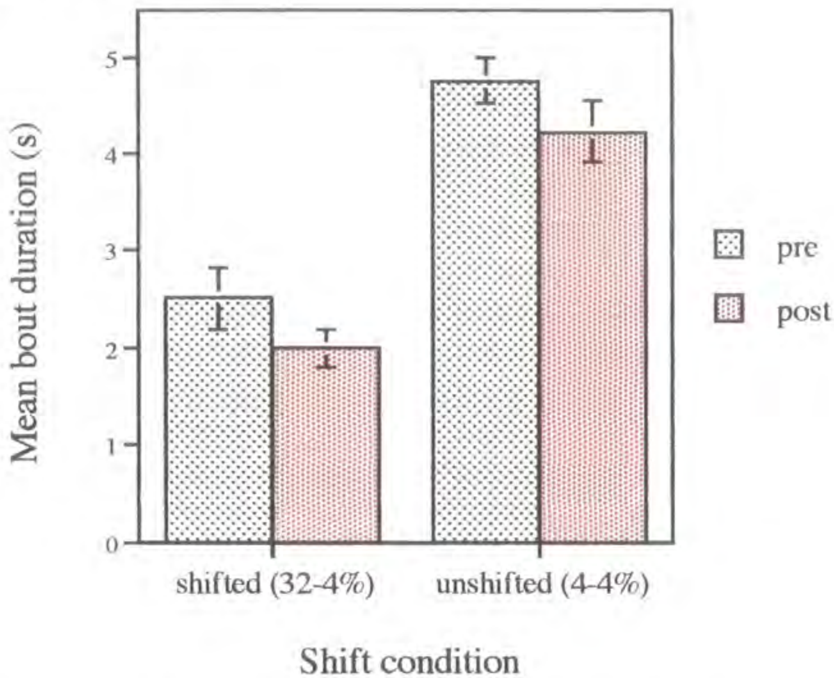


Figure 7.4 Mean (+S.E.M) bout duration of licking for sucrose in a 10-min licking test pre- and post-shift as a function of shift condition (32-4%) or (4-4%) on the first post-shift day.

Unlike measures of mean bout duration over the full 10-min test session, mean bout duration in the first min decreased to a similar extent in the post shift period irrespective of shift condition (see Figure 7.4). Within the shifted group, a one-way within subjects ANOVA showed that this decrease in mean bout duration did not reach statistical significance ($F_{1, 46} = 1.672$, n.s.).

Number of bouts

Measures from the first minute showed that mean bout frequency decreased slightly in the post-shift period for shifted animals but remained almost the same for unshifted animals. Consequently, within the shift group there was no main effect of shift on the number of bouts: ($F_{1, 46} = 0.691$, n.s.). These results are not shown graphically.

Summary

Table 7.3 The occurrence (+) or absence (-) of contrast in licking parameters shifted rats: data from the first min of a 10-min test session and from the full 10-min test session

	Data collected from:	
	First min	Full 10-min
Number of licks	-	+
Mean bout duration (s)	-	+
Number of bouts	-	-

n=42 in the shifted group

From the microstructural data it is clear that negative contrast effects were most apparent when data was analysed from the full 10-min session. These effects could be seen in measures of number of licks and mean bout duration but not in number of bouts.

Drug effects on negative contrast

In the third phase of the experiment, only one of the vehicle conditions (of four possible vehicle conditions per day / per group) was included in the analysis (see Section 7.2). A two-way between-subjects ANOVA was used to test for the main effects of the drug and shift conditions and for any interaction between the two.

a) over the full 10-min test session

Table 7.4 The average (± S.E.M.) of feeding parameters at each dose of 7-OH-DPAT collapsed over shift condition in a 10-min licking test on the first and second post-shift days.

		Dose 7-OH-DPAT (mg/kg)			
		0.0	0.1	0.3	1.0
Post-shift day					
	Number of licks				
1		598.4±76.1	536.5±54.0	409.0±79.7	394.1±43.8
2		725.1±71.8	540.0±104.3	496.8±76.0	494.5±114.3
	Mean bout duration (s)				
1		2.5±0.3	2.2±0.2	1.2±0.1	1.1±0.1
2		3.2±0.2	2.3±0.3	1.5±0.2	1.1±0.1
	Number of bouts				
1		32.8±3.0	37.8±2.9	53.5±9.9	58.9±5.8
2		30.8±2.2	24.3±3.6	54.6±6.7	44.9±11.5

n= 6 per dose group per day within each shift condition

Table 7.4 shows that both the number of licks and mean bout duration were dose-dependently decreased by 7-OH-DPAT on both the first and the second post-shift days. Number of bouts showed a dose-dependent increase on the first post-shift day but on the second post-shift day number of bouts did not increase in a dose-dependent manner. A two-way between-subjects ANOVA revealed that for number of licks there was a significant main effect of dose on post-shift day 1: ($F_{1, 40} = 5.394$, $p<0.005$) but not on post-shift day 2: ($F_{1, 40} = 2.774$, n.s.). There was a main effect of dose on mean bout duration on post-shift day 1: ($F_{1, 40} = 18.317$,

$p<0.0001$) and a significant and dose-dependent decrease was also revealed on post-shift day 2: ($F_{1, 40} = 19.858$, $p<0.0001$). The dose-dependent increase in number of bouts on post-shift day 1 was statistically significant: ($F_{1, 40} = 6.030$, $p<0.005$). There was also a main effect of dose on number of bouts on post-shift day 2: ($F_{1, 46} = 3.957$, $p<0.05$).

Table 7.5 The average (+ S.E.M.) of the feeding parameters in shifted and unshifted groups collapsed over dose conditions in a 10-min licking test on the first and second post-shift days.

		Sucrose condition	
		Shifted (32-4%)	Unshifted (4-4%)
Post-shift day			
	Number of licks		
1		325.3±32.4	643.7±38.0
2		341.3±37.6	786.9±58.2
	Mean bout duration (s)		
1		1.3±0.07	2.2±0.2
2		1.6±0.1	2.4±0.2
	Number of bouts		
1		36.8±3.2	54.6±5.3
2		32.8±3.6	44.5±6.5

n=24 per shift group

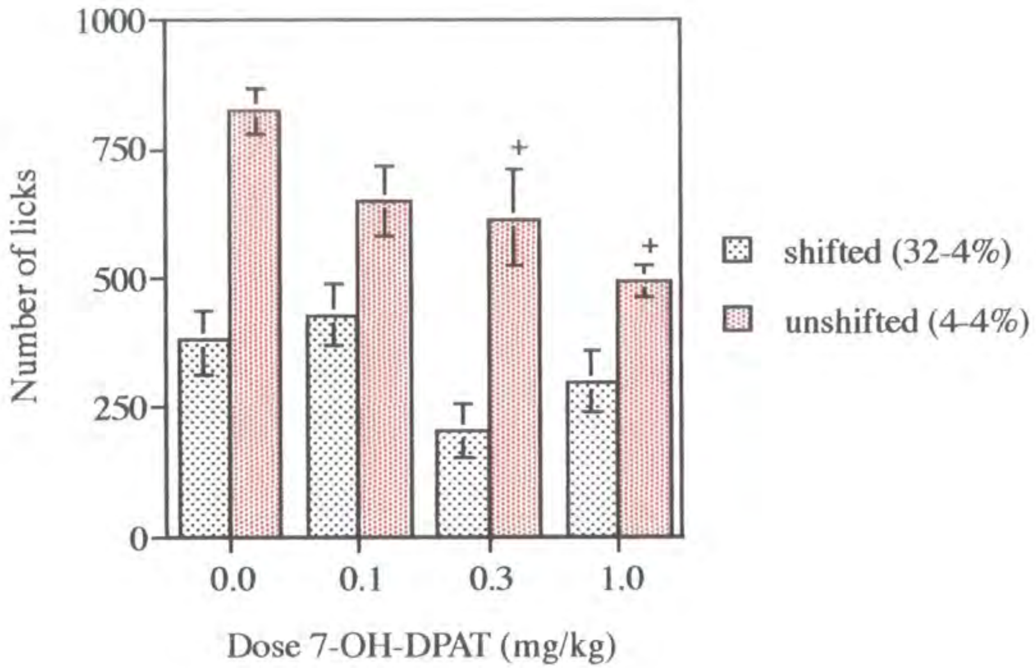
Table 7.5 shows the overall effect of shift on the parameters of licking. Shifted animals emitted approximately half the number of licks of unshifted animals on both the first and the second post-shift days. Unshifted animals engaged in longer bouts than shifted animals on days 1 and 2 and also initiated more bouts than the shifted animals on both post-shift days.

A two-way between subjects ANOVA revealed that for number of licks there was a significant main effect of shift on post-shift day 1: ($F_{1, 40} = 55.514$, $p<0.0001$) and on post-shift day 2: ($F_{1, 40} = 46.069$, $p<0.0001$). There was a similar result , in that mean bout duration also decreased on post-shift day 1: ($F_{1, 40} = 31.519$, $p<0.0001$) and 2: ($F_{1, 40} = 17.508$, $p<0.0005$). The increase in mean

bout frequency on day 1 was statistically significant: ($F_{1, 40} = 12.392$, $p<0.005$). However, there was no main effect of shift on number of bouts on post-shift day 2: ($F_{1, 46} = 2.931$, n.s.).

Number of licks

a) Post-shift day 1



b) Post-shift day 2

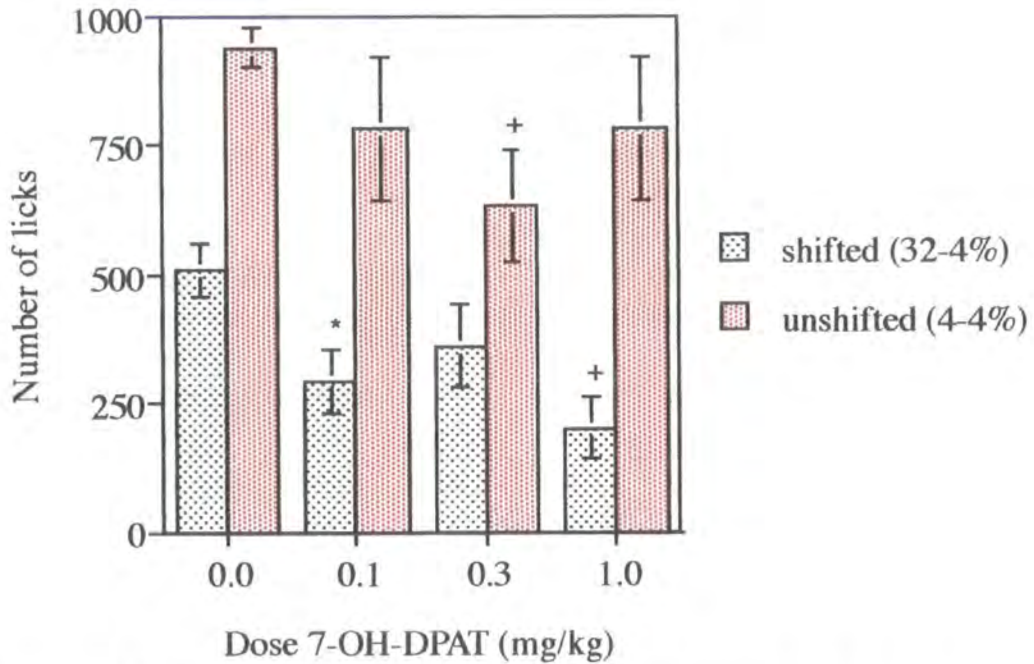


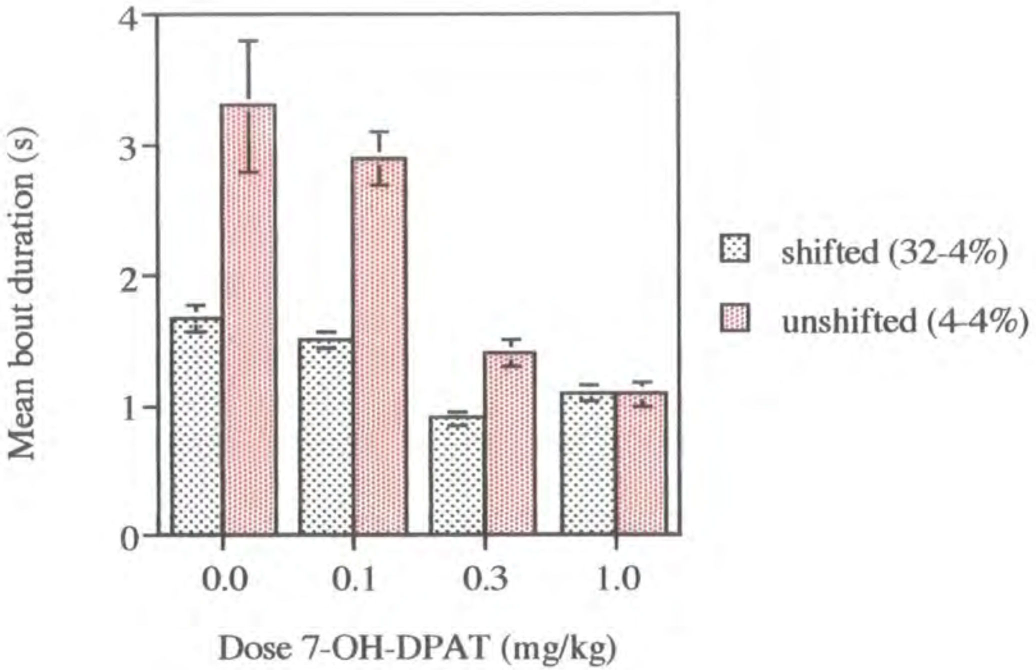
Figure 7.5 Average (+ S.E.M.) number of licks for sucrose over 10 min during the post-shift period as a function of drug condition in shifted (32-4%) and unshifted (4-4%) rats. a) Post-shift day 1- Plus indicates significantly different from vehicle +$p<0.01$; b) Post-shift day 2- Asterisk indicates significantly different from vehicle *$p<0.05$; Plus indicates significantly different from vehicle +$p<0.01$.

For number of licks, there was no significant interaction between shift and drug dose on the first: ($F_{3, 40} = 2.257$, n.s.), or the second post-shift day: ($F_{3, 40} = 2.774$, n.s.). In the absence of significant interactions, post-hoc tests revealed that within the unshifted group number of licks were decreased significantly by the medium and high doses of 7-OH-DPAT (0.3 and 1.0 mg/kg) on post-shift day 1 when compared to vehicle controls ($p<0.01$). On post-shift day 2 a post hoc test showed that the number of licks emitted by unshifted animals was significantly decreased by the 0.3 mg/kg dose of 7-OH-DPAT, ($p<0.01$).

In the shifted group, post hoc tests showed that none of the doses of 7-OH-DPAT decreased number of licks significantly on post-shift day 1, while on post-shift day 2 both the low and the high (0.1 and 1.0) doses achieved a significant decremental effect when compared to vehicle controls, ($p<0.05$ and $p<0.01$ respectively), (see Figure 7.5).

Mean bout duration

a) Post-shift day 1



b) Post-shift day 2

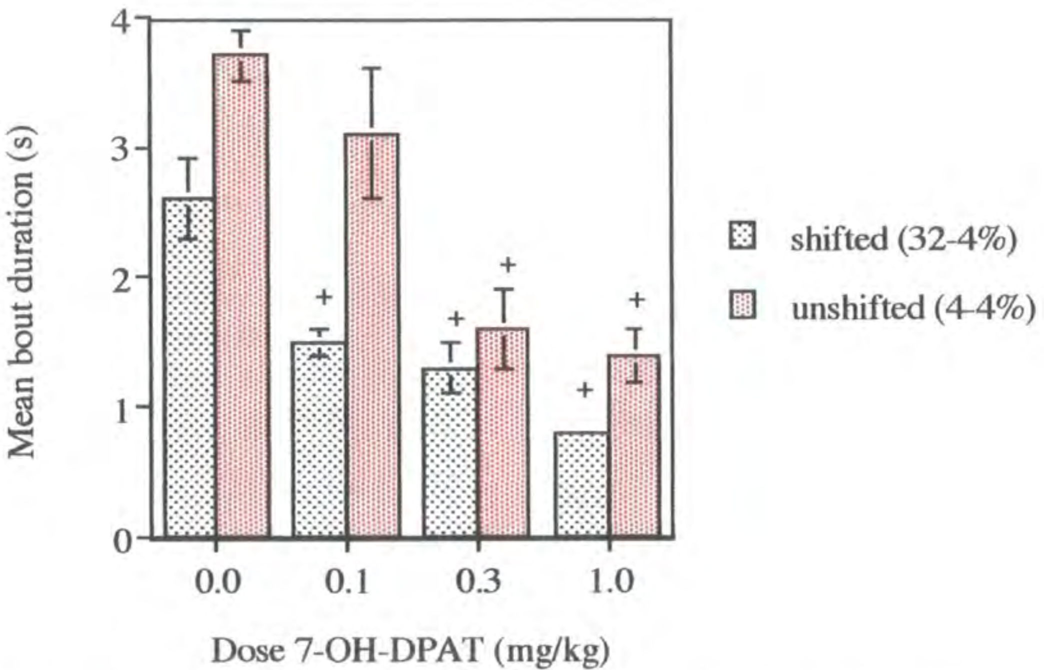
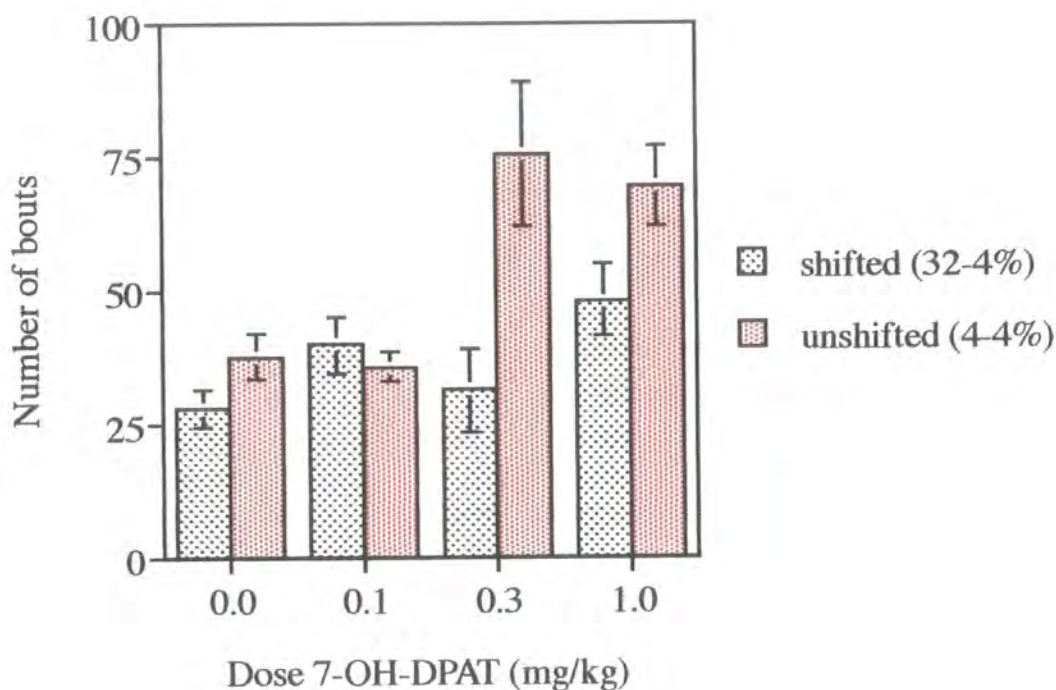


Figure 7.6 Mean (+ S.E.M.) bout duration of licking for sucrose over 10 min during the post-shift period as a function of drug condition in shifted (32-4%) and unshifted (4-4%) rats. a) post-shift day 1; b) post-shift day 2 -Plus indicates significantly different from vehicle +$p<0.01$.

The data represented in Figure 7.6 reveals that on post-shift day 1, there was a highly significant interaction between drug dose and shift condition for mean bout duration: ($F_{3, 40} = 5.365$, $p<0.005$). Differences between the shifted and unshifted groups in mean bout duration became smaller as doses of 7-OH-DPAT increased. At the highest dose (1.0 mg/kg) mean bout duration for both groups were almost equal. A similar trend existed for this parameter on post-shift day 2. However, there was no significant interaction between shift condition and drug dose for mean bout duration on the second post-shift day: ($F_{3, 40} = 1.812$, n.s.).

Number of bouts

a) Post-shift day 1



b) Post-shift day 2

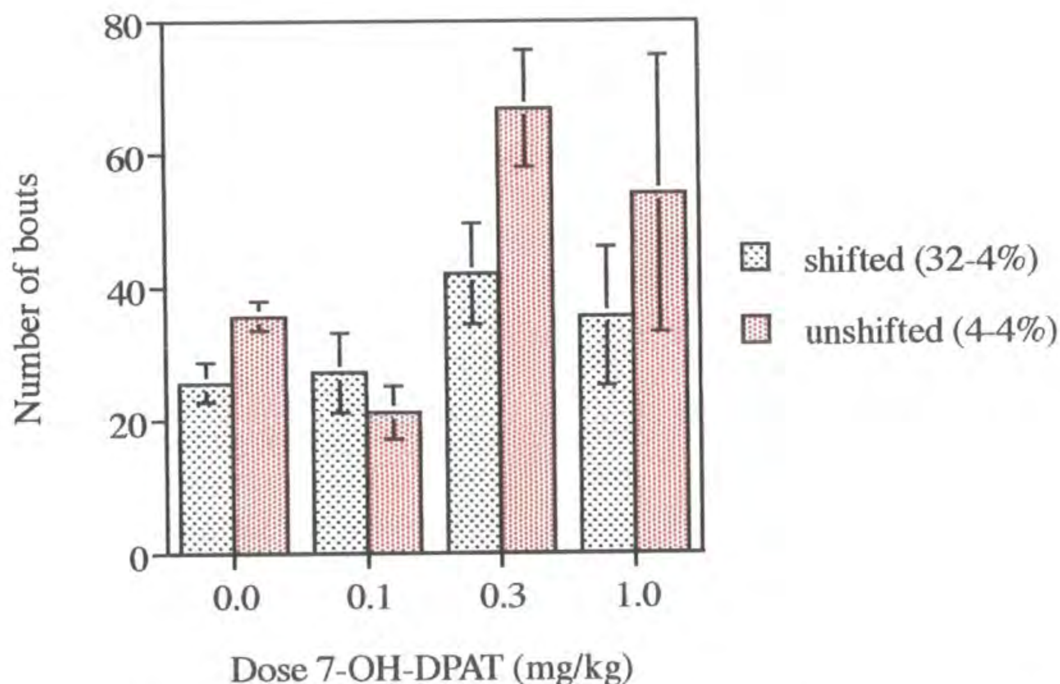


Figure 7.7 Average (+ S.E.M.) number of bouts of licking for sucrose over 10 min during the post-shift period as a function of drug condition in shifted (32-4%) and unshifted (4-4%) rats. a) post-shift day 1; b) post-shift day 2.

Differences in mean bout frequency between the shifted and unshifted groups did not vary systematically as a function of dose 7-OH-DPAT. Nevertheless, there was a significant interaction between drug dose and shift condition on post-shift day 1: ($F_{3, 40} = 4.112$, $p<0.05$). There was no such interaction between dose and shift on post-shift day 2, (see Figure 7.7).

Drug effectiveness ratios

Table 7.6 Drug effectiveness ratios (DERs) obtained from data from a 10-min of licking test on the first and second post-shift days as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg).

	Dose 7-OH-DPAT (mg/kg)		
	0.1	0.3	1.0
Post-shift day			
1	1.90	1.07	2.20
2	0.87	1.59	0.72

n= 6 per dose group per day

The method of computing drug effectiveness ratios derived for shifted animals are described in section 7.2. These ratios give a measure of the ability of a drug to reduce contrast in shifted rats. Therefore, Table 7.6 shows that on post shift day 1 the highest dose of 7-OH-DPAT (1.0 mg/kg) was most effective in reducing contrast overall, while on post-shift day 2 contrast was most reliably reduced by the medium dose (0.3 mg/kg 7-OH-DPAT).

<u>b) in the first min of a 10-min test session</u>

Table 7.7 The average (+ S.E.M.) of feeding parameters at each dose of 7-OH-DPAT collapsed over shift condition in the first min of a 10-min licking test on the first and second post-shift days.

		Dose 7-OH-DPAT (mg/kg)			
		0.0	0.1	0.3	1.0
Post-shift day					
	Number of licks				
1		123.5±15.8	86.4±9.7	46.1±7.1	41.2±5.9
2		114.0±17.0	85.1±19.7	64.3±16.0	28.4±7.9
	Mean bout duration (s)				
1		2.8±0.4	2.3±0.3	1.3±0.2	1.0±0.1
2		4.0±0.7	2.0±0.4	1.6±0.5	0.8±0.2
	Number of bouts				
1		6.3±0.7	5.9±0.7	5.9±1.1	5.3±0.9
2		4.3±0.5	5.4±0.9	5.9±1.2	3.3±0.8

n= 12 per dose group per day

Table 7.7 shows that, in the first minute, both number of licks and mean bout duration were dose-dependently decreased by 7-OH-DPAT and this was apparent on both post-shift days. On the first post-shift day number of licks were significantly and dose-dependently decreased: ($F_{3, 40} = 17.972$, $p<0.0001$). The same trend was observed on the second post-shift day: ($F_{3, 40} = 11.039$, $p<0.0001$). Mean bout duration was also significantly and monotonically reduced in the first minute on the first post-shift day: ($F_{3, 40} = 23.763$, $p<0.0001$) and the second post-shift day: ($F_{3, 40} = 9.429$, $p<0.0001$). Number of bouts in the first minute hardly varied at all as a function of 7-OH-DPAT on the first post-shift day: ($F_{3, 40} = 0.184$, n.s.). On the second post-shift day, mean bout frequency in the first min increased systematically as a function of the low and medium doses of 7-OH-DPAT (0.1 and 0.3 mg/kg) but this trend was not borne out in relation to the highest dose (1.0 mg/kg). Therefore, this result did not reach statistical significance: ($F_{3, 40} = 2.167$, n.s.).

Table 7.8 The average (+ S.E.M.) of feeding parameters in shifted and unshifted groups collapsed over dose conditions in the first min of a 10-min licking test on the first and second post-shift days.

		Sucrose condition	
		Shifted (32-4%)	Unshifted (4-4%)
Post-shift day			
	Number of licks		
1		59.6±7.7	91.9±10.6
2		37.5±7.3	108.5±12.7
	Mean bout duration (s)		
1		1.3±0.1	2.4±0.2
2		1.2±0.3	2.9±0.4
	Number of bouts		
1		5.5±0.6	6.3±0.6
2		3.2±0.5	6.2±0.6

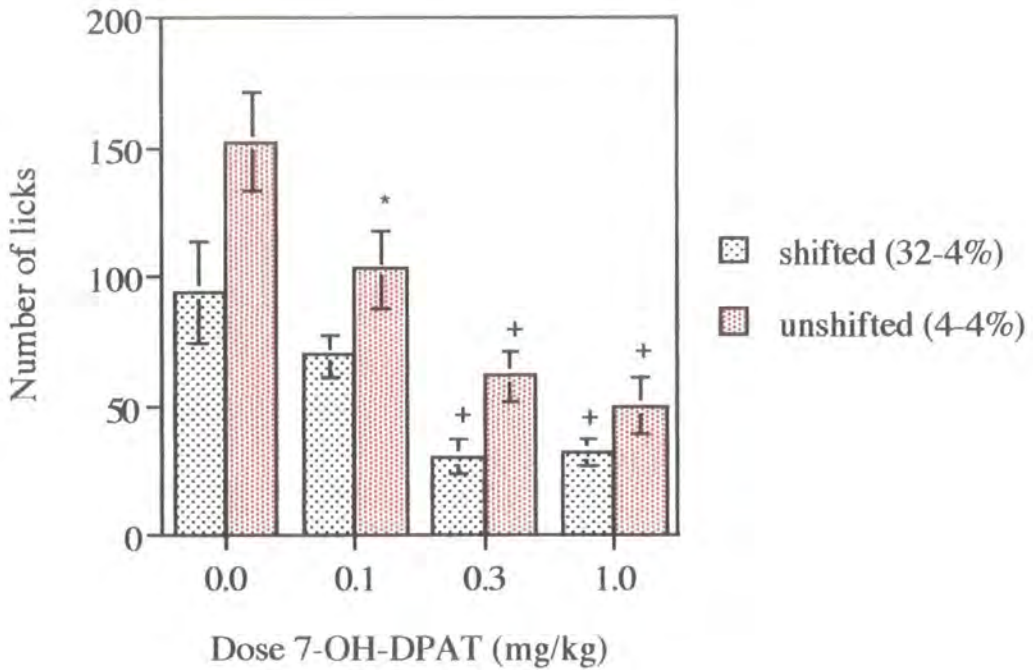
n=24 per shift group per day

Unshifted animals generated more licks and licked with longer bout durations than those which were shifted. This trend was observed on both post-shift days. Unshifted animals also initiated more bouts than unshifted animals on both post-shift days. However, the difference in mean bout frequency for shifted and unshifted animals was more profound on the second post-shift day.

The effect of shift condition on number of licks in the first minute was significant on post-shift day 1: ($F_{1, 40} = 15.071$, $p<0.0005$) and also on post-shift day 2: ($F_{1, 40} = 42.919$, $p<0.0001$). There was a highly significant main effect of shift on mean bout duration in the first minute on post-shift day 1: ($F_{1, 40} = 44.046$, $p<0.0001$) and on post-shift day 2: ($F_{1, 40} = 14.287$, $p<0.005$). In the case of mean bout frequency, there was no significant main effect of shift condition on the first post-shift day: ($F_{1, 40} =0.793$, n.s.) but mean bout frequency was significantly affected by shift on the second post-shift day: ($F_{1, 40} = 13.613$, $p<0.005$).

Number of licks

a) Post-shift day 1



b) Post-shift day 2

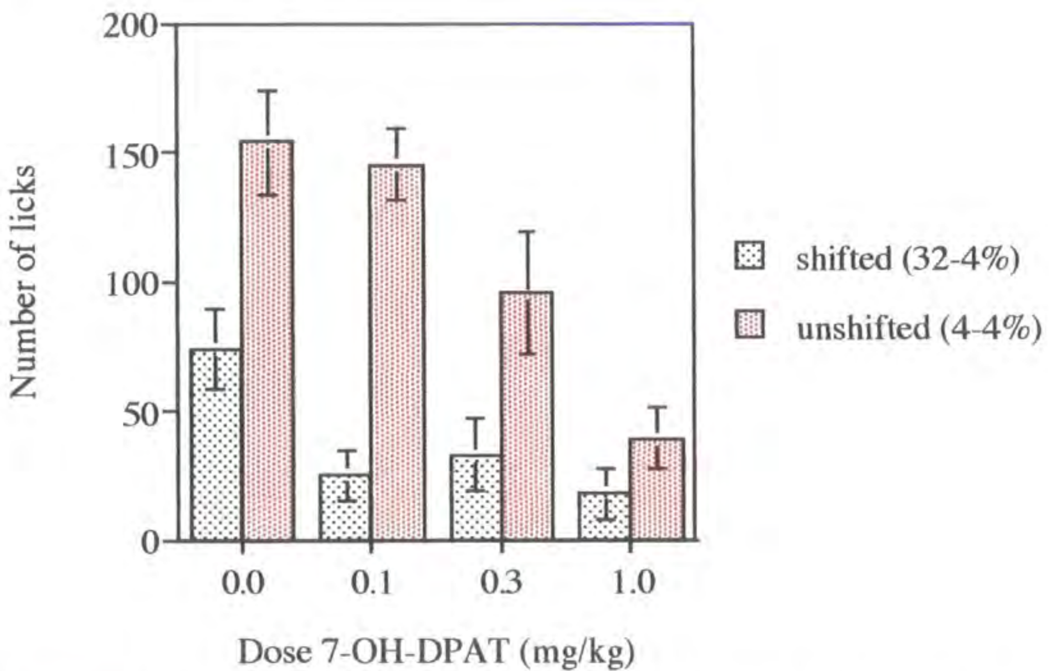
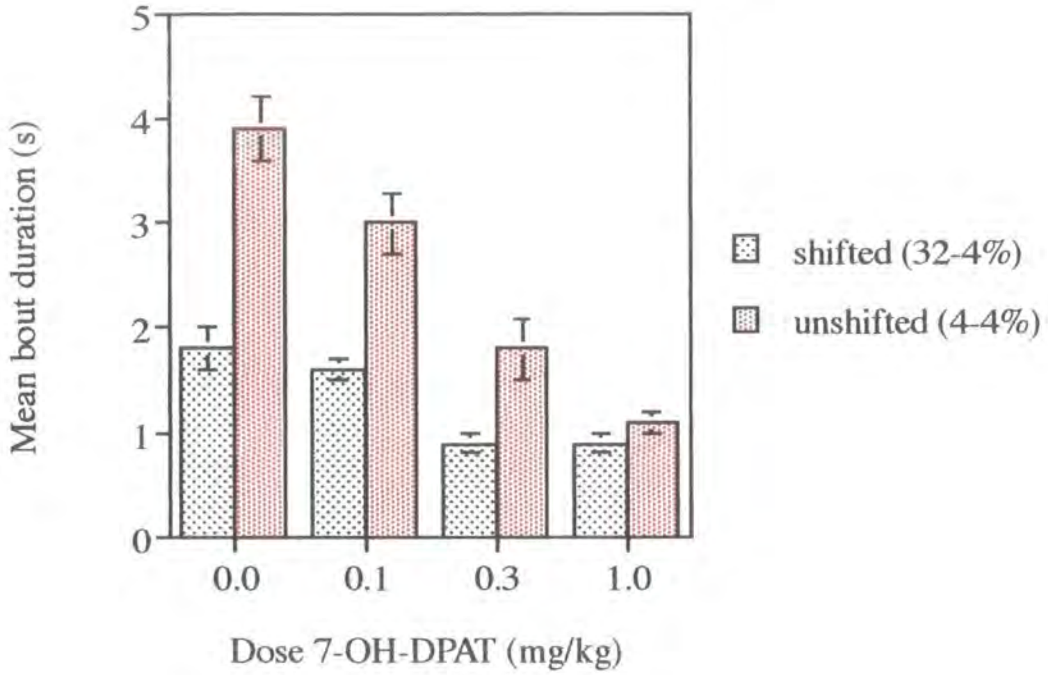


Figure 7.8 Average (+ S.E.M.) number of licks for sucrose in the first min during the post-shift period as a function of drug condition in shifted (32-4%) and unshifted (4-4%) rats. a) post-shift day 1- Asterisk indicates significantly different from vehicle $*p<0.05$; Plus indicates significantly different from vehicle $+p<0.01$; b) post-shift day 2.

Figure 7.8 shows that for number of licks, there was no significant interaction between shift and drug dose on the first post-shift day: ($F_{3, 40} = 2.257$, n.s). On the first post-shift day dose-response curves for shifted and unshifted groups were parallel (see Figure 7.8 a). In the absence of significant interactions, post-hoc tests revealed that within the unshifted group number of licks were decreased significantly by all doses of 7-OH-DPAT (0.1-1.0 mg/kg) on post-shift day 1 when compared to vehicle controls ($p<0.01$ for 0.3 and 1.0 mg/kg; $p<0.05$ for 0.1 mg/kg). On the second post-shift day there was a significant interaction between shift and drug dose for this parameter: ($F_{3, 40} = 3.537$, $p<0.05$) (see Figure 7.8 a or b).

Mean bout duration

a) Post-shift day 1



b) Post-shift day 2

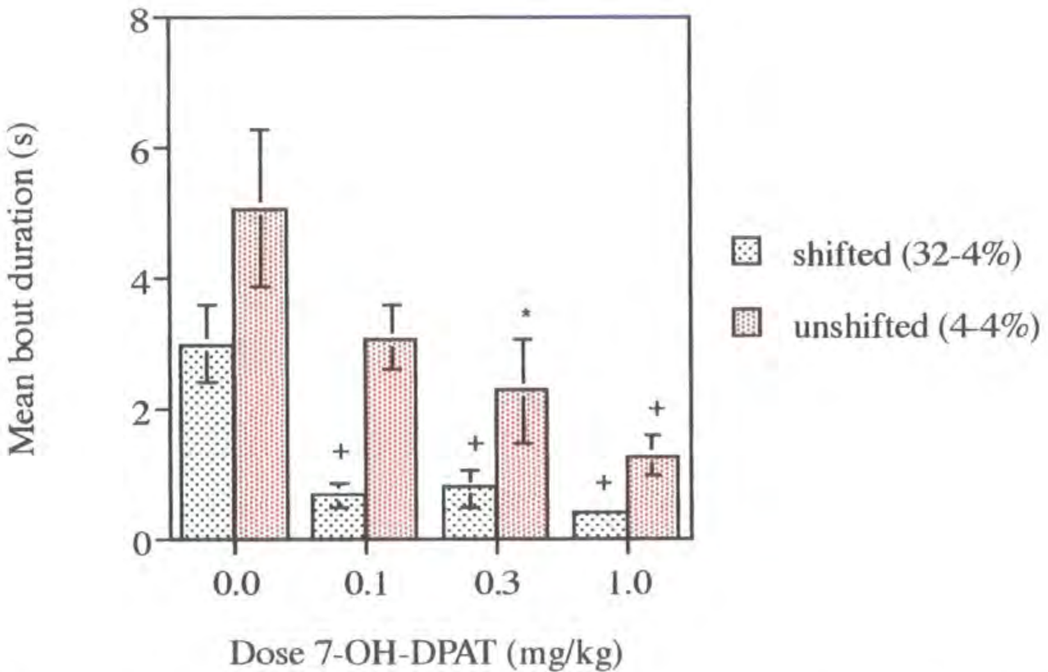


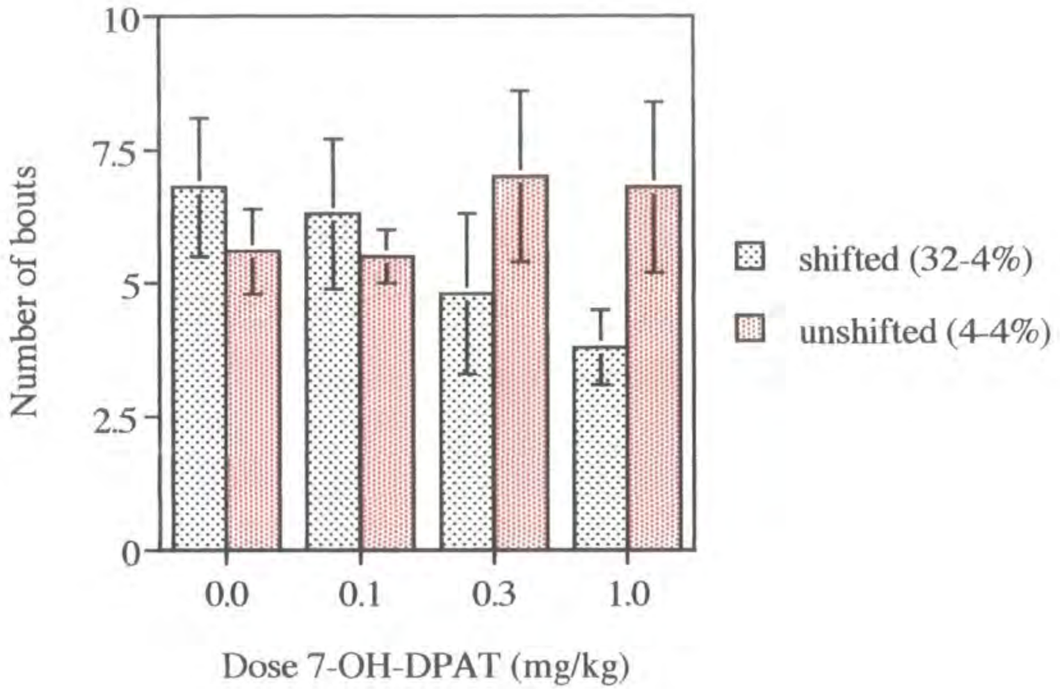
Figure 7.9 Mean (+ S.E.M.) bout duration of licking for sucrose over 10 min during the post-shift period as a function of drug condition in shifted (32-4%) and unshifted (4-4%) rats. a) post-shift day 1; b) post-shift day 2-Asterisk indicates significantly different from vehicle *$p<0.05$; Plus indicates significantly different from vehicle +$p<0.01$.

On post-shift day 1, there was a highly significant interaction between drug dose and shift condition for mean bout duration: ($F_{3, 40} = 5.995$, $p<0.0001$) which was probably due to the equivalence of responding after the highest dose of 7-OH-DPAT (1.0 mg/kg) in the shifted and the unshifted groups (see Figure 7.9 a). As with the 10-min results, differences between the shifted and unshifted groups in mean bout duration became smaller as doses of 7-OH-DPAT increased. There was no significant interaction between shift condition and drug dose for mean bout duration on the second post-shift day: ($F_{3, 40} = 0.621$, n.s.).

In the absence of a significant interaction, post-hoc tests were used and revealed that in the unshifted group, both medium and high doses (0.3 and 1.0 mg/kg) of 7-OH-DPAT decrease mean bout duration significantly when compared to vehicle controls, ($p<0.05$ and $p<0.01$ respectively). In the unshifted group it appeared that in the first minute, all doses of 7-OH-DPAT (0.1-1.0 mg/kg) decreased mean bout duration to a similar extent when compared to vehicle controls, ($p<0.01$).

Number of bouts

a) Post-shift day 1



b) Post-shift day 2

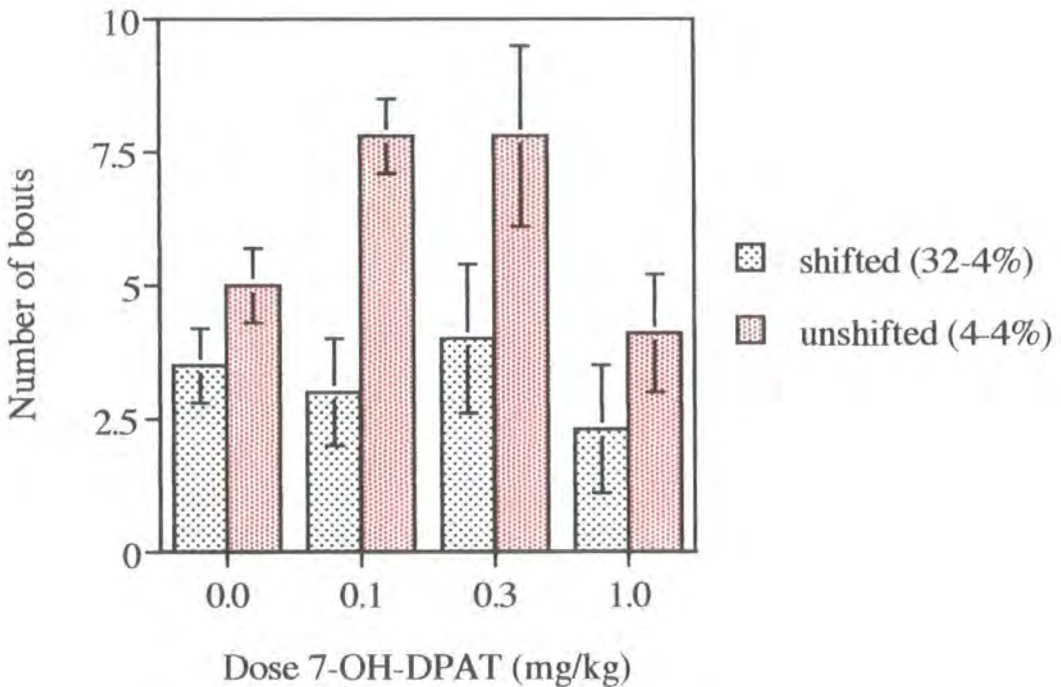


Figure 7.10 Average (+ S.E.M.) number of bouts of licking for sucrose in the first min during the post-shift period as a function of drug condition in shifted (32-4%) and unshifted (4-4%) rats. a) post-shift day 1; b) post-shift day 2.

There was no significant interaction between drug dose and shift condition on post-shift day 1 : ($F_{3, 40} = 1.396$, n.s.). There was also no significant interaction between dose and shift on post-shift day 2: ($F_{3, 40} = 0.966$, n.s.).

Drug effectiveness ratios

Table 7.9 Drug effectiveness ratios (DERs) obtained from data from the first min of a 10-min licking test on the first and second post-shift days as a function of dose 7-OH-DPAT (0.1-1.0 mg/kg).

	Dose 7-OH-DPAT (mg/kg)		
	0.1	0.3	1.0
Post-shift day			
1	1.70	1.90	3.00
2	0.67	1.30	3.50

n= 6 per dose group per day

The drug effectiveness ratios derived for shifted animals are described in Section 7.2. On post-shift day 1there was a dose-dependent increase in the effectiveness of 7-OH-DPAT to reduce contrast in shifted rats. This dose-dependent trend also existed on post-shift day 2 and at the highest dose (1.0 mg/kg) 7-OH-DPAT was more effective at reducing contrast than it was on post-shift day 1.

Summary

Table 7.10 The occurrence (+) or absence (-) of a significant interaction ($p<0.05$) between drug dose and shift condition on licking parameters: Data from the first min of a 10-min test session and from the full 10-min test session over post-shift day 1 and post-shift day 2.

	Data collected from:	
	First min	Full 10-min
Number of licks		
day 1	-	-
day 2	+	-
Mean bout duration (s)		
day 1	+	+
day 2	-	-
Number of bouts		
day 1	-	+
day 2	-	-

n=48 per post-shift day

Table 7.10 illustrates that significant interactions between drug dose and shift condition were found in the first minute of the 10-min test session for number of licks on post-shift day 2 and mean bout duration on post-shift day 1. When data was analysed from the full 10-min test session, there were significant interactions for mean bout duration and number of bouts, both of these findings from post-shift day 1.

7.4 Discussion

Overall, the results of this experiment show that shifting reward from 32% sucrose to 4% sucrose produced a successive negative contrast effect. This was reflected in the microstructure of licking as a decrease in mean bout duration rather than a decrease in mean bout frequency. This result is consistent with findings by Grigson et al. (1993) who originally characterised successive negative contrast effects in terms of licking microstructure. Contrast effects rely on the comparison of post-shift rewards with the memory of pre-shift reward. 7-OH-DPAT reduced the successive negative contrast effect. More concisely, 7-OH-DPAT succeeds in reducing successive negative contrast. This is contrary to previous findings such as Flaherty et al. (1992) whereby contrast effects remained unimpaired under the influence of haloperidol and chlorpromazine. Haloperidol and chlorpromazine are non-selective dopamine antagonists whereas 7-OH-DPAT has at least some specificity for the D3 receptor. Therefore, D3 receptors may be involved in the mediation of relative reward value as well as absolute reward value of both pre- and post-shift solutions.

The effects of sucrose concentration on licking, as reflected in the terminal pre-shift measures were somewhat unexpected. In general, before the shift, animals licking for the high sucrose concentration (32%) were licking less and with shorter bouts than those animals who had access to the lower sucrose concentration (4%). Although unexpected, this result is not unprecedented; nor does it preclude the possibility of a contrast effect post-shift. Grigson and colleagues noted that during the preshift period of numerous experiments investigating contrast, rats generally make more licks for higher concentrations. They insert the caveat that this effect does not always develop and is not necessary for the occurrence of a contrast effect when the sucrose concentration is reduced (Grigson et al., 1992, 1994).

One explanation of the pre-shift measures may be in terms of procedural detail such as test duration. In the present experiment, animals were trained and tested for 10-min daily. This relatively long training duration coupled with the eight day training regime could have led to a conditioned satiating effect of the higher concentration of sucrose (as described by Smith, 1996) towards the latter end of the training period. It is from this portion of the training period that terminal pre-shift measures are necessarily derived. A shorter training and testing duration could help to eliminate this explanation. Also, analyses of licking microstructure have shown that more concentrated fluids are not always more palatable or reinforcing (Davis and Levine, 1977 and see Chapter 2).

Successive negative contrast effects were apparent in the changes in total number of licks over the 10-min test session. Animals that were shifted from 32% to 4% sucrose licked significantly less post-shift than they did pre-shift. The licking patterns associated with successive negative contrast were manifest as significant decreases in mean bout duration rather than any change in bout number. As the size of bouts can be interpreted as a measure of palatability (Davis and Smith, 1992), it is inferred that negative contrast effects are, at least in part, based on the "hedonic inferiority" of the post-shift solution as compared with the pre-shift solution (Flaherty et al., 1990, pp.88). Results from the first minute of the 10-min test session are not so well defined. The decreases seen in feeding parameters in this early portion of the test session were similar to data from the full test session but did not reach significance. It may be that a minute is not long enough for a contrast effect to be expressed behaviourally.

These temporal differences in expression of behaviour related to reward contrast also impinge upon the interpretation of drug effects on successive negative contrast. It has been observed frequently that when drugs are adminstered on the first post-shift day, effects on contrast are not apparent. However, attenuation of contrast does appear if drugs are administered on the second post-shift day. For example, the benzodiazepine, chlordiazepoxide, effectively eliminates negative contrast when the drug is administered for the first time on the second post-shift day (Flaherty et al., 1980). This led Flaherty (1990, 1991) to speculate that different processes may be involved in the initial occurrence of contrast and the subsequent recovery from contrast. Moreover, drug treatments may be differentially effective in these dissociable circumstances.

One hypothesis which has been offered to explain these temporal differences in drug effectiveness post-shift is the following: it is possible that the process of comparing the post-shift solution with the memory of the pre-shift solution, (thus determining that the post-shift solution is hedonically inferior) requires a certain amount of time and experience with the post-shift solution (Flaherty et al., 1980). Chlordiazepoxide does not become effective in reducing contrast until the reward difference is detected and responded to (Flaherty et al., 1980). To test this hypothesis, Flaherty et al., (1986) allowed animals access to the post-shift solution of 4% sucrose for 20-min rather than the usual 5-min. Before access, animals were injected with chlordiazepoxide and the drug became effective during the second five minutes of the extended test period. This period would normally correspond to the

second post-shift day, therefore, this hypothesis was supported in the case of chlordiazepoxide.

In the present experiment, there were significant interactions between shift and drug dose on feeding parameters on both post-shift days and these were, in the case of mean bout duration, apparent even within the first minute of the first post-shift day. Therefore, it seems that recovery from contrast as produced by 7-OH-DPAT was almost immediate.

Other hypotheses exist to explain the differential effectiveness of drugs early and late in the post-shift period. One explores the possibility that negative contrast is not stressful on the first post-shift day, but is stressful on the second post-shift day (Flaherty et al., 1985). This hypothesis was tested by measuring corticosterone levels on post-shift days as this chemical signal correlates highly with a stress response. Flaherty found that corticosterone is elevated on the second post-shift day and postulates that the rise in corticosterone is reflecting an anticipatory frustration response and/or uncertainty and anxiety based on the experience of the previous day (Flaherty et al., 1985).

It is therefore not surprising that many of the drug treatments which have been found to reduce contrast are anxiolytics which produce their best effects on contrast on the second post-shift day (see Figure 7.11). Further, it is possible that it is this anxiolytic property of 7-OH-DPAT which makes it effective in reducing contrast, as the D3 receptor receptor has been recently implicated in the regulation of anxiety (Rodgers et al., 1996, Steiner et al., 1997, Bartoszyk, 1998, also see Chapter 1).

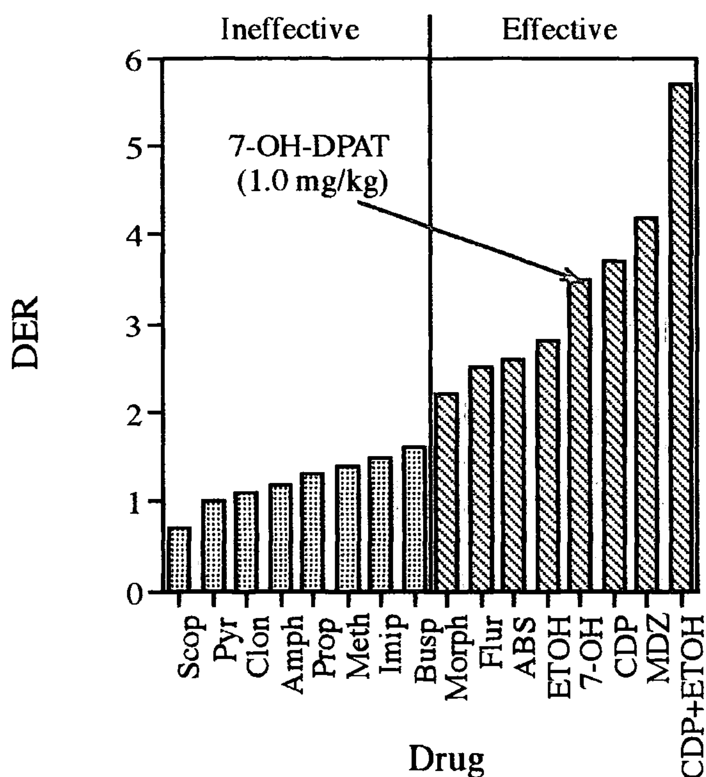


Figure 7.11 Drug effectiveness ratios (DERs) obtained in a series of dose/reponse studies utilizing the consummatory negative contrast procedure. The values plotted represent the results obtained with the most effective dose of each drug. The larger the DER value, the greater the contrast reduction. Scop = scopolamine; Pyr = pyrilamine; Clon = clonidine; Amph = amphetamine; Prop = propanolol; Meth = methysergide; Imip = imipramine; Busp = buspirone; Morph = morphine; Flur = flurazepam; ABS = amobarbital sodium; ETOH = ethanol; 7-OH = 7-OH-DPAT; CDP = chlordiazepoxide; MDZ = midazolam, (Figure 7.11 adapted from Flaherty, 1991, with the inclusion of data from the present experiment).

From Figure 7.11 it is interesting to compare the effectiveness of 7-OH-DPAT in reducing successive negative contrast to other drugs which have been tested within the same paradigm. 7-OH-DPAT is more effective than ethanol at reducing contrast and is marginally less effective than chlordiazepoxide at achieving this result. Of the ineffective drugs tested, many are dopaminergic compounds (either indirect or non-selective). Therefore, it may be 7-OH-DPAT's status as a putative D3 receptor agonist and/or anxiolytic which sets it apart from this class of drugs in this paradigm.

The DER gives a convincing picture of the ability of 7-OH-DPAT to reduce contrast. If the interpretation of the apparent reduction in contrast relied solely on the measurement of microstructural parameters (see pages 255-272) then an

alternative explanation might be in terms of the emergence of a "floor" effect after the administration of 7-OH-DPAT post-shift.

However, there is a potential confound in many drug studies in the contrast paradigm in that a number of the drugs which are effective in reducing contrast also have appetite stimulating effects. For example, it is well-established that the benzodiazepines induce hyperphagia in virtually all experimental settings (Cooper and Estall, 1985). Analysis of the microstructure of hyperphagic effects of benzodiazepines show that these drugs achieve their effect by increasing mean bout duration (Higgs and Cooper, 1996; 1997). Therefore it is difficult to eliminate appetite-stimulating effects when explaining the ability of drugs such as benzodiazepines and barbiturates to reduce contrast. A further benefit of applying a microstructural analysis to recovery from contrast would be to eliminate the hyperphagic effects of drugs such as benzodiazepines. Hence, what the figure (above) cannot reveal is whether these effective compounds reduce negative contrast in the same way as it was achieved here. To answer this question it is necessary to appeal to the drug-induced changes in microstructural variables during contrast and the subsequent recovery from contrast. As yet there is no evidence that the microstructural changes associated with benzodiazepine-induced recovery from contrast are not manifest as an increases in mean bout duration. Consequently, it may be the case that drugs which have hyperphagic effects are reducing contrast by reinstating pre-shift levels of ingestion through increasing bout duration; a basic behavioural effect of benzodiazepines in the absence of any change in shift. Further experimentation, using microstructural methodology needs to be undertaken to solve this problem.

Flaherty has reason to believe that recovery from contrast is not simply related to appetite stimulation because although drugs such as chlordiazepoxide is relatively ineffective in reducing contrast on the first post-shift day, even though it often has an appetite-stimulating effect on that day (Flaherty et al., 1986, 1990b). However, microstructural analysis should help to elucidate the underlying changes in feeding parameters associated with benzodiazepine-induced recovery from contrast. In the present experiment, the confound of appetite-stimulation does not exist. 7-OH-DPAT has been repeatedly shown to reduce ingestion by shortening mean bout duration (see Experiments 1 and 4) and therefore reducing the palatability of solutions. In this experiment it is decreases in mean bout duration which are most strongly associated with successive negative contrast. However, although there are significant interactions between shift condition and drug dose for mean bout duration over 10-min and in the first min of the test session on post-shift day

one it does not seem that it is on this parameter that 7-OH-DPAT is exclusively having its contrast reducing effects.

Further evidence of this assertion comes from the examination of mean bout frequency data. Although this parameter is virtually unchanged as a function of shift there is a significant interaction between shift condition and drug dose on number of bouts. This is apparent on the first post-shift day over the full 10-min test session. If a successive negative contrast can be achieved (as revealed by number of licks) without changes in mean bout frequency then 7-OH-DPAT - induced recovery from contrast might be expected to be successful without any changes in this parameter. The interactions for mean bout frequency are evidence that this may not be the case.

Another possibility offered by Flaherty as to the differential effectiveness of anxiolytics in the post-shift period is that the differences reflect the operation of different psychological processes underlying contrast at these discrete stages of the post-shift period. For example, the initial response to reward reduction may involve the activation of a search process to locate the "missing" reward (Flaherty et al., 1978, 1979). Subsequently, as the missing reward is not located, the animals may enter a conflict period triggered by the drive to consume the post-shift reward (especially in food deprived animals) balanced against the preference for the remembered pre-shift reward. It may be that this conflict stage is stressful and during this stage that anxiolytics such as CDP and ethanol (Becker and Flaherty, 1982) become effective in reducing contrast.

The idea of a search process might well be reflected by an increase in bout frequency on the first post shift day where animals are repeatedly "trying out" the solution in order to match it with the memory of the pre-shift solution. This seems to be the case for shifted animals in this experiment but only in the first min of the test and on the first post-shift day. On post-shift day 2 this effect is no longer apparent.

Inasmuch as the drug-induced recovery from contrast may be an artefact of appetite-stimulation, the results of this experiment constitute the first evidence that a drug which reduces contrast is not stimulating appetite and may not be promoting recovery from contrast in the same way as it was achieved. 7-OH-DPAT is effective in reducing contrast on both the first and the second post-shift day, though, overall, it is marginally better on the first post-shift day and more especially

when data is analysed from the first minute. Therefore, this experiment also provides evidence of a reduction in contrast by 7-OH-DPAT in animals which do not seem to need a great deal of time to recognise and act upon the presentation of a contrasting stimulus. This is a result which may have been obscured had dependent variables been limited to a more composite measure such as total intake or total licks. However, more work needs to be done to discover the exact pharmacological properties of putative D3 receptor agonists such as 7-OH-DPAT.

Chapter 8: General discussion

8.1 Aims of the General discussion

This final chapter is subdivided into the five following sections. The empirical findings of the experimental chapters are summarised in Section 8.2, and are discussed in relation to the aims of the thesis (Chapter 2, Section 2.13). They are also juxtaposed with additional experiments which would help to address still unresolved issues (i.e. a motor versus a motivational role for dopamine). In Section 8.3, the present data are discussed within the framework of Berridge's (1996) theory of dopamine's involvement in food reward. The validity of his theory will also be evaluated briefly. Section 8.4 has two purposes: firstly, it deals witht the clinical implications of the work; secondly, it examines the extent to which findings on the bases of food reward per se are relevant to broader aspects of reward and addiction. Section 8.5 briefly proposes future directions for research into the functional role of dopamine in information-processing related to rewards.

8.2 Summary of empirical findings and future experiments

Microstructural analysis of licking can provide detailed information as to the components of ingestive behaviour which may be affected by either pharmacological manipulation and/or manipulation of the properties of the ingested diet as well as the motivational state of the animal. These observations have been incorporated in a model for the control of ingestion (Davis and Levine, 1977; Davis and Smith, 1992). As has been repeatedly stated throughout the thesis, this model predicts that increasing carbohydrate solution concentration leads to the selective lengthening of bouts of licking and also leads to an increase in the initial rate of licking. Changes in these parameters, as a result of changing concentration or drug treatment may best reflect changes in palatability of the liquid diet (Davis and Levine, 1977; Davis and Smith, 1992).

Throughout the thesis, many of the results showed that increasing concentration led to an increase in the mean bout duration of licking which helps to confirm the notion that this parameter represents a measure of palatability. However, for both sucrose and the fat emulsion Intralipid, a linear relationship between concentration and mean bout duration was not always evident and so doubt remains as to the validity of this parameter as a measure of palatability. Also, in previous reports not all types of tastant produce a robust and linear relationship

between increasing concentration and mean bout duration. Davis (1995) could not replicate these effects with corn oil and so speculated that it may be a property of this particular stimulus other than "taste" palatability (e.g. such as texture or temperature) which leads to these anomolous results. Further microstructural analyses using a wide range of macronutrient solutions need to be conducted in order to further verify the model. Also, the range of concentrations used in the experiments in Chapters 4, 5 and 6 is narrow (1-10%) and so the full behavioural implications of a stimulus' properties cannot be fully explored or explained as a consequence of the results from these chapters.

In Chapter 7, terminal preshift measures of mean bout duration did not reflect the "absolute reinforcer value" of the sucrose solutions used even when these durations were measured from the first minute of the test session. More precisely, animals licking for a 32% sucrose solution did not engage in longer bouts of licking than those animals which had access to a 4% sucrose solution. This affirms the idea that behavioural responses are far from stimulus bound (Grill and Berridge, 1985) and that previous experience and motivational state as well as the animals' representation of the stimulus response relationship (i.e. what leads to what) (Dickinson and Balleine, 1994) are important factors in the control of licking and therefore ingestion. Concept definitions and operationalisable measures of palatability must reflect these considerations.

To further explore the limits to which a given stimulus value might reliably affect ingestive behaviour it would be informative to microstructurally analyse the development of responses to tastants of differing reinforcer value. In this way, from the initial introduction of the tastant, parametric changes in licking could be measured longitudinally (i.e. over days). For example, licking patterns during training in the lickometer are assumed to reach stable levels at a rate which is not significantly affected by the stimulus properties per se. However there has been little effort to systematically investigate the contribution of absolute or relative reinforcer value to the establishment of this asymptotic behaviour.

Experiments which try to uncover this relationship could go further in addressing the original Hullian question of "does more pleasure equal more learning?". The extent to which a stimulus can be construed as a "goad" with a relatively stable value, would be more apparent when the behavioural responses to it were examined at such a detailed level. The "goad" of course could be manipulated not only in terms of the physical stimulus properties but also by motivational state (see Dickinson and Balleine, 1994). By using this type of

paradigm it would be possible to examine what learning about the stimulus-response relationship "looks like" microstructurally.

One of the primary aims of this thesis was to further examine whether dopamine agonists and antagonists, selective for different dopamine receptor subtypes decrease the intake of liquid diets in the same or in different ways. By adopting a microstructural analysis, this was investigated in Experiments 1, 2 and 3.

The experiments reported in Chapter 4 constituted preliminary evidence that a D3 receptor agonist (7-OH-DPAT), a mixed D2/D3 receptor agonist (quinpirole) and a D2 receptor antagonist (raclopride) were decreasing the palatability of the diet as reflected by a decrease in the mean bout duration of licking and the initial rate of licking, rather than significantly decreasing the number of bouts. This is the first set of experiments to compare 7-OH-DPAT, quinpirole and raclopride at such a detailed ingestive behavioural level. Nevertheless, these compounds did not affect these parameters to the same extent; 7-OH-DPAT seemed to be most potent in this test, followed by quinpirole then raclopride.

These experiments clearly showed that drugs which putatively exert their effects through separate dopamine receptor subtypes (i.e. D2 and D3 receptor subtypes) are capable of decreasing the initial hedonic evaluation of the tastant. This conclusion is further confirmed by the observation that drug-induced changes in parameters which reflect palatability were most profound in the first min of the 20-minute test session used for the experiments in Chapter 1. Results from experiments 9 and 10 also support the idea that licking responses in the first minute of the test session are of greater interpretative strength as drug and concentration effects are more profound when data are analysed from this portion of a longer test.

Measures of motoric competence such as intrabout lick rate/average interlick interval and latency were included as dependent variables for the experiments in Chapter 5. This reflected the specific aims of the experiments in this chapter which were to further examine the possibility of the primacy of a motor deficit in explaining the decreases in licking parameters that had been observed in Chapter 4. A dopamine D1 antagonist was employed in Experiment 6 providing a further comparison of the effects of drugs acting at D2 and D3 receptors on licking microstructure. To increase the interpretive strength of the test with regards to palatability, a brief contact test was used. Also a different type of macronutrient fluid was used in the form of an Intralipid emulsion.

Again increasing concentration led to an increase in mean bout duration of licking in Experiments 4, 5 and 6 which further validates the model of Davis and Levine. These conclusions extended also to increasing concentrations of Intralipid which adds to the generalisability of this model and follows further work in the same vein reported in Chapter 2. The consistent observation that concentration has little or no effect on either intrabout lick rate and/or average interlick interval provides us with the inference that any changes in these parameters are due to changes that are not related to the palatability of the diet.

However, in Experiment 4, there was an effect of concentration on average interlick interval which was monotonic yet small. It has been suggested that when more viscous solutions are used there can be a concomitant increase in average interlick interval (Davis, 1996). Higgs (PhD Thesis, 1996) suggested an appropriate experiment with which to test whether viscosity was having an effect on intrabout lick rate (and therefore average interlick interval). The addition of texture enhancing constituent to the tastant (such as guar gum) would increase viscosity without affecting the taste palatability of the diet. The effect of concentration on intrabout lick rate did not appear for either test fluid in Experiments 5 or 6. Therefore, the increase in average interlick interval as a result of increase concentration observed in Experiment 4 does not serve to seriously question the validity of the above interpretation of this measure (i.e. that intrabout lick rate is insulated from changes in concentration). Taken together these results represent further confirmation of the validity of the model for the control of ingestion of Davis and Levine (1977).

There were no reliable effects of test fluid concentration on latency from the experiments in Chapters 5, 7 and 8. This suggests that this measure is not sensitive to factors associated with changes in tastant concentration (i.e. palatability) and so may best be construed as a more valid measure of motoric competence. However, this is not always reflected in the literature as there are many researchers who define latency in motivational terms (e.g. Wilson et al., 1995). Indeed, approach behaviour has become a meaningful dependent variable in reward research and one which has been shown to covary with dopaminergic activity (see Chapter 1).

Ill-defined measures such as latency reflect the ambiguity which surrounds the "motor versus motivation" hypotheses which proliferate throughout reward research. The point at which dopamine involvement in "motor" evolves into an involvement in "motivational" remains contentious. For some, such as Salamone, this dichotomy is false and it is in the functional and anatomical overlap between

these two aspects of behaviour where we may find answers to the precise role of dopamine in rewarded behaviour (Salamone, 1996). Therefore, it is difficult to make any strong claims as to the effects of the drugs used in these experiments on latency measures.

While the findings presented in Chapter 5 further validate the effects of concentration within the framework of the model of the control of ingestion, drug effects in this set of experiments are less clear cut than those presented in Chapter 4. In general, Experiments 4, 5 and 6 do not present a strong argument for a *specific* effect of these compounds on palatability when a short test session is used. Although 7-OH-DPAT decreased mean bout duration of licking for both test solutions and SCH-23390 decreased this parameter for Intralipid, these compounds also produced changes in licking parameters and latency measures which may be seen as being consistent with a motoric deficit (i.e. increases in latency and decreases in intrabout lick rate). Raclopride seems, at the doses used, to be having a non-specific effect on licking parameters in the shorter test session. The data presented in Chapter 5 cannot be used to resolve the issue of whether the dose of 7-OH-DPAT which is most effective in reducing mean bout duration is reflecting a separate motor impairment or a more selective effect on palatability.

One explanation of neuroleptic-induced decreases in intrabout lick rate has been to invoke an explanation in terms of an orolingual motor deficit (Gramling and Fowler, 1985; Fowler, 1990). They noted that the neuroleptic-induced deficit was manifest as "missed licks" which effectively doubled the length of the interlick intervals in their experiment. Further examination of the interlick interval after the administration of 7-OH-DPAT, SCH-23390 and raclopride revealed that this explanation does not apply to the data in Chapter 5 (results not shown). Nor did any of the drugs used in Chapter 5 shift the distribution of the average interlick intervals to the left or to the right. These data could suggest that these drugs may be affecting licking behaviour at the level of inter*bout* intervals which would be consistent with the effects of 7-OH-DPAT on cocaine self-administration (i.e. Caine and Koob, 1995). Further work needs to be conducted with an analytical focus at the level of interbout intervals to further confirm this hypothesis.

As was referred to in Chapter 4, Pecina and Berridge (1997) explained the effects of pimozide on taste reactivity measures in terms of producing a sensorimotor deficit which was primarily characterised by temporal characteristics of responding (i.e. a *progressive* motor dysfuction emerged rather than an *immediate* effect on taste reactivity). However, results from Chapter 5 show that

raclopride SCH-23390 and 7-OH-DPAT had their most profound effects on the initial hedonic evaluation of the stimulus.

In order to further assess the extent to which the data presented in this thesis fits with the "sensorimotor" hypothesis let us recapitiluate the basic predictions of this hypothesis. The idea that nucleus accumbens dopamine represents a sensorimotor interface incorporates the idea that interference with nucleus accumbens dopamine (i.e. via phamacological blockade) alters the allocation of responses away from more vigourous or effortful responses towards the selection of less effortful responses. The most compelling evidence that the drugs used in Chapter 5 produce a motor deficit is from the slowing of intrabout lick rate. The slowing of intrabout lick rate could feasibly represent "the selection of a less effortful response". However, in order to operationalise a concept such as effort or vigour, there must be some distinction between rate and, for instance "force" of licking as is examined by Fowler and colleagues (see Chapter 2). One way in which novel dopaminergic agonists and antagonists (especially those with an atypical profile) have been characterised behaviourally is by the measurement of lick force or vigour. These measurements would be especially informative in examining the extent to which such compounds induce a specifically "sensorimotor" deficit.

Another way to test this hypothesis can be seen in the most recent work by Salamone (1997) who has employed experimental "cost-benefit" strategies after the manipulation of dopamine transmission. In this way, it is possible to carefully control and manipulate the "size" of energy barriers to be crossed as well as the size of rewards to be gained. With the help of more specific pharmacological tools it would be possible to manipulate specific dopamine receptor subtype populations in distinct regions of the nucleus accumbens, thereby postulating a role for these subtypes in the mediation of specifically "sensorimotor" function. These conceptualisations of the function of dopamine in reward veer research away from ill-defined dichotomies of motor and reward capacity. Rather they help to focus on the overlap between what are traditionally seen as discrete functions in which dopamine had a specific part to play.

The Experimental aims of Chapter 6 were to elucidate the pharmacological specificity of the effects of 7-OH-DPAT on licking microstructure. This aim was achieved by using two drugs which purportedly blocked the action of 7-OH-DPAT in other behavioural paradigms. These were PNU-99194A and amisulpride. A further aim of Chapter 6 was to examine these putative D3 receptor antagonists alone using microstructural analysis. PNU-99194A was ineffective in blocking the effects of 7-OH-DPAT in this paradigm but due to recent evidence (Clifford and

Waddington, 1998) we had reason to believe that the doses employed in Experiment 7 were too low to be effective. We favoured this interpretation in the stead of one which might question the extent to which PNU-99194A or 7-OH-DPAT exert their effects through an a action at D3 receptors. Amisulpride produced most interesting results in that not only did it completely attenuate the effects of 7-OH-DPAT on licking behaviour at higher doses but also produced a hyperphagic or "prohedonic" effect (Experiments 8 and 9). In terms of the pharmacological specificity of the attenuation of the effects of 7-OH-DPAT by amisulpride, it seems that amisulpride achieved this effect only at those doses which purportedly act postsynaptically at D2 receptors (Sanger, 1996). Therefore, any claim that the results of 7-OH-DPAT's effects on feeding are mediated solely by D3 receptors would not be justified on the basis of the results presented in Chapter 6.

Central injections into putative presynaptic and postsynaptic sites of D3 receptor using a wide range of doses of 7-OH-DPAT would go some way to clarifying this issue. For example, Meyer (1996) used such a method in order to establish the pharmacological specificity of the effects of 7-OH-DPAT on locomotor behaviour. He also questioned the hypothesis that the D3 receptor is both a presynaptic and a postsynaptic receptor. He found that microinjections of 7-OH-DPAT directly into the nucleus accumbens resulted in the potentiation of locomotion. These data supported the hypothesis that the D3 receptors are postsynaptic receptors. However, microinjections of 7-OH-DPAT directly into the ventral tegmental area did not result in the attenuation of locomotor behaviours, but rather resulted in the potentiation of the behaviour. These behavioural data did not support the hypothesis that the D3 receptor in the VTA functioned as an autoreceptor. Therefore, it seems that distinctions between the functional significance of a presynaptic and a postsynaptic D3 receptor is not clearly definable.

Chapter 1 presented a host of data which is testament to the last statement. The issue of whether the D3 receptor functions as an autoreceptor remains contentious but may be further clarified by centrally measuring dopamine synthesis and release in conjunction with the central administration of D3 receptor specific compounds and the measurement of simple physiological and/or behavioural correlates.

Chapter 7 represented the first report of a drug which is specific for a dopamine receptor subtype, successfully reducing successive negative contrast; an effect which was initially brought about by a reward downshift. What remains enigmatic is that those drugs which most effectively reduce contrast (such as the

benzodiazepines) also increase palatability whereas, 7-OH-DPAT reliably reduces palatability and yet still reduces contrast. It could be that 7-OH-DPAT reduces contrast by virtue of its anxiolytic properties. This is an explanation which cannot be fully confirmed or disputed by the results in Chapter 7. Nevertheless, 7-OH-DPAT reduces contrast significantly and therefore, inasmuch as it stimulates D3 receptors and/or D2 receptors, these results constitute evidence for dopamine receptor subtypes in relative reward.

Another possibility is that 7-OH-DPAT is having effects elsewhere. Indeed, some of the behavioural effects of 7-OH-DPAT have been attributed to this compound's interaction at σ binding sites (Wallace and Booze, 1995). Bevins (1997) recruits an action of 7-OH-DPAT on σ binding sites in explanation of the capability of 7-OH-DPAT to induce a conditioned taste aversion. Selective σ ligands have been shown to induce taste aversions expressed as a selective σ-mediated emetic response in pigeons (Hudzig et al., 1993). Therefore, it could be the anxiolytic effects of σ receptor stimulation which give this putatively dopaminergic compound its unique characteristic in the contrast paradigm. Until these details of pharmacological specificity are clarified it is difficult to ascribe a certain role for the dopamine D3 receptor subtype in the reduction of contrast and more broadly, in the mediation of relative reward. Further employment of the contrast paradigm in conjunction with more specific receptor subtype ligands could help to resolve this enigma.

Finally, an interesting finding from Chapter 6 was that higher doses of amisulpride gave rise to licking patterns (i.e. increased the number of licks, increased mean bout duration but had no effect on the number of bouts) which were exactly like generated after the administration of benzodiazepines (Higgs and Cooper, 1998). Therefore, it might be predicted that this compound would also reliably reduce contrast.

Summary

A general point warranted here is that the pharmacological specificity of compounds such as 7-OH-DPAT is of immense importance, not least because of the prototypical status that this compound has gained in the search for a behavioural effect of D3 receptor stimulation. The development and application of novel

selective compounds for dopamine receptor subtypes is imperative. A wide range of behavioural tests and dose ranges will help to elucidate the role for individual receptor subtypes in food reward. Nevertheless, inasmuch as dopamine receptor ligands are specific for individual receptor subtypes, from the summaries above it is clear that the experiments presented in this thesis have gone some way to fulfilling the experimental objectives delineated in Chapter 2.

8.3 Implications of results for Berridge's (1996) theory of food reward

Berridge (1996) and also Berridge and Robinson (1993, 1998) nominate mesolimbic and mesostriatal dopamine systems to play a part in mediating the incentive salience of rewards. They also claim that these dopamine systems modulate the motivational value of rewards in a manner separable from hedonia and reward learning. Also, it should be noted that the various kinds of theoretical formulation, including that of Berridge (1996) and Berridge and Robinson (1993, 1998), that have emerged to address the actions of neuroleptics have not yet effectively integrated the findings associated with the now many categories of dopamine receptor ligands.

Berridge and Robinson (1998) focus on the functional role of the mesostriatal dopamine projections in general rather than addressing the question of individual subtype contribution to components of reward. This emphasis may reflect an underestimation by these authors of the heterogeneity of dopamine systems and functional disparity of these systems and their constituent receptor subtypes. The experiments presented in this thesis aimed to help to redress this balance. Perhaps central injections of a wide dose range of selective dopamine agonists and antagonists would produce taste reactivity patterns which would be incompatible with their current model.

Berridge (1996) criticises many of the manipulations of behaviour which have been deemed as revealing dopamine's function in reward. He sees much of the data representing support for a role for dopamine in hedonia as, at best, correlational evidence and at worst compatible with several of the theories outlined in the Chapter 2 which postulate dopamine's involvement in hedonic evaluation of stimuli. He criticises many of the traditional measures of reward (such as those of instrumental behaviour or choice paradigms) claiming that they are confounded in that conclusions about whether an incentive stimulus is liked is inferred based on

behavioural evidence that it is wanted (i.e. whether an animal will choose it, consume it, or work to acquire it). Berridge claims that this inference is grounded in the assumption that rewards are always "wanted" to the same degree that they are "liked" (Berridge and Robinson, 1998).

This point of criticism is seen by Berridge to be justifiably levelled at the licking microstructure paradigm. In a personal communication, Berridge states: "... licking can be (perhaps always is) an instrumental act when its directed at something. Palatability affects licking, I imagine, much as it affects instrumental performance in general: liking controls wanting. Our conclusion is only that dopamine doesn't mediate the liking part itself. That it mediates 'liking related wanting' I would have no problem with." (Kent Berridge, 1997, personal communication) In the light of this statement, Berridge seems to believe that measures of palatability such as mean bout duration and initial rate of licking confound the two psychological functions. In using licking microstructure as a methodology and in assuming the validity of models for the control of ingestion such as that of Davis and Levine we presume to separate parameters of behaviour which are directly associated with discrete psychological functions such as liking (i.e. we assume that we can manipulate and measure hedonic value). The effects of test fluid concentration presented in the experimental chapters have gone some way to validating the model of Davis and Levine (1977). However, that initial rate of licking and/or mean bout duration are pure measures of palatability cannot be fully confirmed until more experimental work has been conducted.

As was mentioned in Chapter 2, Berridge relies almost exclusively on the taste reactivity paradigm for data collection. As is appreciated in science as whole, the generality of any functional principles derived from a paradigm will ultimately depend on the number of different experimental contexts in which the data are collected. Further, there are assumptions underlying the conceptual validity of the taste reactivity paradigm which are contentious. Briefly, Wise (1992) has noted that this methodology was originally viewed as a measure of taste-elicited fixed action patterns of ingestion (Grill and Norgren, 1978; Grill and Berridge, 1985) but has increasingly become to be construed as a measure of subjective hedonic responses to food (Berridge and Grill, 1984; Berridge and Valenstein, 1991). Indeed, in their recent paper assessing the effects of pimozide on taste reactivity measures, Pecina and Berridge, (1997) refer to these responses as "affective reaction measures" (pp. 801); a reference which confirms Wise's earlier criticism.

Studies using human subjects can give insight to the general applicability of the theoretical assumptions underlying the taste reactivity paradigm. Consistent with previous reports of taste reactivity in human newborns (Blass et al., 1989) Graillon et al. (1997) have recently shown that crying newborns respond differentially to intraoral orogustatory stimuli. Increased mouthing has been shown to be a robust response to positive hedonic stimuli, such as intraoral sucrose, as has initial crying reduction. However, by extending the range of stimuli used to quinine water and corn-oil as well as sucrose solutions, these authors showed that "taste salience" rather than positive hedonic valence account for increased mouthing and initial crying reduction. Therefore, the interpretation of taste reactivity measures in humans may constitute problematic data for theories which are built upon data from taste reactivity measures in animals.

8.4 Clinical Implications

Chapter 1 illustrates that, traditionally, antipsychotic drugs were thought to achieve their effects through their action at postsynaptic D2 receptors. Since the reclassification of dopamine receptor subtypes, compounds which were found to be selective for the D3 receptor were also nominated as potential antipsychotic agents with low extrapyramidal side-effects owing to their limbic selectivity (Sokoloff et al., 1990). Since these early claims, there have been many attempts to include the D3 receptor in the genetic aetiology of schizophrenia however, these results are inconsistent. A discussion of the involvement of dopamine receptor subtypes in the aetiology and treatment of schizophrenia is beyond the scope of this thesis. However, there are other clinical implications which are more germaine to the thesis as a whole.

8.4.1 Dopamine receptor subtypes and feeding behaviour in humans

There is little definitive evidence of the precise role of dopamine receptor subtypes in the regulation of human ingestion. However, based on predictions from the animal reward literature, the D3 receptor was recently postulated as a candidate in abnormal eating behaviours such as anorexia nervosa. Despite a fairly well established role in the mediation of reward, Bruinslot et al. (1998) have recently found a lack of association between the D3 receptor gene and anorexia nervosa. Despite the fact that the number of patients tested was small, they believe that there is good evidence that the Bal I DRD3 polymorphism does not play a major role in the genetic component of anorexia nervosa.

Despite the paucity of data on the effects of specific dopamine agonism and antagonism on feeding in human subjects, there are a few reports on the effects of chronic administration of classical antipsychotic compounds in the clinical population. However, Stanton (1995) has found a relationship between chronic classical antipsychotic treatment and a tendency toward obesity. Traditionally, typical and atypical antipsychotics were differentiated in terms of their extrapyramidal side effect liability. However, there is reason to believe that these classes of drugs may be dissociable in terms of their effects on feeding behaviour (see Lee and Clifton, 1998; Baptista et al., 1997, Chapter 6). Atypical antipsychotics have now been differentiated from typical antipsychotics in several reward paradigms. Therefore, it would be interesting to compare the feeding patterns and weights of clinical populations who are undergoing chemotherapy in the form of these two types of dopaminergic compound.

8.4.2 From "appetite" to "hyper-appetite": A similar role of dopamine in responding for conventional and non-conventional reinforcers?

Dopamine receptor subtypes have been nominated to have, to varying extents a part to play in the mediation of reward for food, but have also been heaviliy implicated in the acquisition and maintenance of drug addiction. Habit forming drugs of various pharmacological classes increase extracellular dopamine in the dorsal and ventral striatum as indicated by brain microdialysis (DiChiara, 1995). As such, various dopaminergic compounds have been suggested to be potential treatments for drug abuse (i.e. 7-OH-DPAT, Caine and Koob, 1995). In the case of the D3 receptor the relationship between its function and the modulation of drugs of abuse has been further strengthened due to observations such as those of Staley and Mash (1996). Their *post mortem* analyses revealed that there were adaptive increases in dopamine D3 receptors in the mesolimbic "reward circuits" of human cocaine fatalities.

This remainder of this section deals with how far the "ingestion" of foods and drugs of abuse are analogous in terms of behaviour and of the neurochemical precursors and consequences of commerce with both types of reinforcer. More concisely, the clinical implications of the experiments reported in this thesis depend at least, in part, upon the neurochemical homology of both conventional and non-conventional reinforcers.

Self administration studies provided much of the support for the anhedonia hypothesis (see Chapter 2) and therefore, traditionally, the effects of food and drugs on nucleus accumbens dopamine were hypothesised as being functionally equivalent. This equivalence has been seen to be, by some, over-emphasised, (Salamone, 1996) and by others as incorrect (Roberts, 1977). For example, cocaine self-administration is severely disrupted by nucleus accumbens dopamine depletions. The same depletions have little effect on lever pressing for food reinforcement (Roberts et al., 1977). Nevertheless, Wise (1997) and others (Berridge and Robinson, 1993, 1998) make use of many tenets in their theories which are equally applicable to food and drugs of abuse.

In particular, Wise (1997) has recently attempted to assess the reliability of this analogy. Comparing instrumental responding for the two types of reinforcer, Wise notes that animals regulate the intake of drugs of abuse (psychomotor stimulants and opiates) much as they regulate food intake. Although Wise points to several obvious differences between the ingestion of food and drugs of abuse, he also draws many parallels between the two behaviours. For example, he notes that just as it has often been hypothesised that feeding is deficit driven, so has it been hypothesised that addiction is deficit driven. Parameters of ingestion are also compared. Wise asks us to consider self-administered injections as a drug "meal" and notes that meal size and frequency control the intake of the drug just as they control the intake of foodstuffs. In response to the alteration of meal size by the experimenter (dose per injection) the animal adjusts meal frequency to compensate. He also claims that it is obvious that the presence of exteroceptive food stimuli plays an important role in food-seeking that is similar to the role played in drug-seeking by interoceptive drug stimuli. Wise also specifies a similar role for dopamine in aspects of these analogous behaviours (see Wise, 1997).

If, as Wise suggests, dopamine can be construed as common reward currency, this would obviate the need for "currency exchange" despite crossing into new stimulus territory, as it were. Such claims are now being examined by researchers such as DiChiara (1998). DiChiara (1998) uses the similarities and differences in the neurochemical correlates of the two types of reinforcer to formulate a motivational learning hypothesis of the role of the mesolimbic dopamine in drug addiction and compulsive drug use. This hypothesis differs from that of Berridge and Robinson, (1993) in several ways. This thesis is not the correct forum from which to provide an exhaustive analysis of these theories but some points are pertinent to the General discussion in that they dictate the extent to which one might

extrapolate present conclusions from food reward research to wider clinical theory on drug abuse and addiction. The following section evalutes some of the most recent evidence to suggest that drugs of abuse may *not* act as neurochemical surrogates for conventional reinforcers.

Di Chiara (1998) fully acknowledges that while there is much evidence that does not preclude the subservance of the dopamine system in responding to both types of reinforcer, there are differences in the specific portion of the dopamine system which is affected by their "ingestion" and the type of dopamine activity which may be most closely associated with them. Since the compartmentalisation of the nucleus accumbens (see Chapter 1 and 2), it has been repeatedly demonstrated that drugs of abuse such as cocaine, morphine, nicotine and amphetamine, produce selective (or depending on the dose, preferential) increases in extracellular dopamine in the nucleus accumbens "shell" (Alheid and Heimer, 1988; Heimer et al., 1991).

Assuming that drugs of abuse act as neurochemical surrogates for conventional reinforcers, a prediction from the above results would be that conventional reinforcers should also activate specifically dopamine transmission in the portion of the mesolimbic dopamine system that projects to the nucleus accumbens "shell" as opposed to the "core". This hypothesis was supported by results from a study by Tanda and Di Chiara (1998). They showed that a palatable snack food (Fonzies) increased extracellular dopamine selectively in the "shell" of the nucleus accumbens as opposed to the core of the nucleus accumbens in rats fed ad libitum with standard food. However, the consumption of Fonzies has also been shown to increase extracellular dopamine in the prefrontal cortex (Bassareo and Di Chiara, 1997). This feature of responding by dopamine neurons is not a property of non-psychostimulant drugs of abuse (Bassareo et al., 1996).

Another way in which DiChiara and colleagues have empirically differentiated between the two types of reinforcer has been by thoroughly examining the conditions under which dopamine neurons adaptively respond to conventional reinforcers. DiChiara states "..although it is commonly assumed that mesolimbic dopamine neurons are responsive to conventional reinforcers, this responsiveness is the exception rather than the rule, being related to particular experimental conditions" (1998, pp. 57). He notes that only modest increases in extracellular dopamine in the nucleus accumbens are observed when animals are non-deprived (Wilson et al., 1995) and that in undeprived conditions it seems that novelty appears to be an important factor in determining the responsiveness of dopamine transmission. An example of this last point came from experiments

wherein animals, fed *ad libitum* with standard laboratory food are presented with a novel snack food (Fonzies, as above). Feeding of Fonzies led to an immediate increase in extracellular dopamine in the nucleus accumbens and prefrontal cortex. The neurochemical effects of eating a novel, palatable food were selectively blunted in the nucleus accumbens if animals had eaten a previous fonzies meal (Bassareo and DiChiara, 1997). In addition, DiChiara (1998) reviews a wealth of data which suggest that conditional stimuli can also activate mesolimbic dopamine transmission, again depending upon the type of stimulus used and the motivational state of the animal.

Drawing upon the above evidence, the claim that mesolimbic dopamine neurons code for generic motivational salience (Robinson and Berridge, 1993) irrespective of the class of reinforcer, may be too strong. DiChiara (1998) notes that even within a class of reinforcer (i.e. conventional reinforcers) mesolimbic dopamine neurons seem to code for specific stimuli which are of high motivational impact associated with at least several factors (i.e. novelty, unpredictability, deprivation state). These and other results (see Di Chiara, 1998) show that there is at least preliminary evidence to suggest a more topographically heterogenous profile of responding for mesolimbic dopamine neurons than previously thought. As dopamine receptor subtypes become more fully understood, the factors affecting the responsiveness of mesolimbic dopamine will burgeon.

8.5 Future directions

It was outlined in Chapter 2 that recent evidence gathered from studies using single-unit recording, have clarified and extended theorising on the aspects of reward processing which might be best associated with discrete dopaminergic substrates. In the light of the recent functional segregation of dopamine systems by Schultz (1998) (see Chapter 2), it becomes possible to hypothesise as to the effects of absolute and relative reward values on aspects of reward processing in fronto-basal ganglia systems.

According to Schultz, dopamine neurons do not discriminate well between different rewards, rather they "apparently signal a scalar value that does not provide information about the reward other than its goodness relative to prediction" (pp. 427). Reward prediction is also a capacity of striatal neurons but of a different form. Neurons in this area seem to produce sustained activations reflecting the expectations of rewards established through experience in a particular behavioural

situation. A further capacity of striatal neurons is apparent in their ability to adapt the "reward expectation-related activity" in response to changes in the environmental situation lead to modified reward expectations. Neurons of the orbitofrontal cortex produce differential responses to stimuli predicting rewards which are different (i.e. in terms of reinforcer value). Schultz (1998) speculates that neurons in the orbitofrontal cortex may be involved in the detailed analysis of the particular reward objects.

Therefore manipulations which impair dopamine transmission at these discrete stages of reward-related information processing would lead to very different behavioural consequences. These behavioural consequences would best be studied at a detailed level of analysis. Behavioural differences predicted using Schultz's framework would be amenable to testing using microstructural analysis of licking. For example, significant effects of changing concentration on mean bout duration of licking might not be expected if midbrain dopamine neurons are ablated but may be predicted if selective lesions of the orbitofrontal cortex are performed.

A way in which such functional dissociations could be further validated would be by measuring dopamine efflux in the relevant brain sites in response to absolute or relative variation in reinforcer value. Not only would this methodology further test the specific predictions of Schultz (1997) but it would also represent a shift from the reliance upon a single paradigm which may limit the utility of the data collected. Examples of evidence using this paradigm do not always fit neatly with Schultz's basic predictions and significant increases in striatal dopamine efflux have been both associated with (Wilson et al., 1995) and dissociated from (e.g.) the changing hedonic value of the stimulus.

Therefore, brain microdialysis is an appropriate methodology to apply to this problem in an attempt to reveal the extent to which dopaminergic neurons in the ventral striatum code for relative incentive value. Below are a selection of particular experiments which I propose to conduct in the light of these findings.
An appropriate experimental question might be: Does dopamine efflux in the ventral striatum change in response to an unpredicted up or downshift in incentive magnitude (i.e. sucrose concentration)? In the case of a reward "downshift" an appropriate procedure would be as follows. Animals would be trained to ingest a 30% sucrose solution and then implanted with dialysis probes. After training they would be assigned to 4 groups: No shift, shift to 10%, to 3% or to 1% sucrose respectively. Dopamine efflux would be measured prior to and after the shift.

As mentioned earlier, conditioned stimuli repeatedly associated with primary rewards, can when presented alone, also produce an increase in dopamine efflux (Phillips et al., 1991; Schultz, 1997). Therefore, the aim of the following experiment would be to provide preliminary data on the ability of conditioned stimuli repeatedly paired with differing sucrose concentrations to produce a differential change in ventral striatal dopamine efflux. Animals would be assigned to 4 groups and trained to drink 1%, 3% 10% and 30% sucrose respectively. At the same time, they would be trained to associate a tone (CS) with the primary reward (US) of the respective sucrose solution. After training each group will be presented with the appropriate (CS) alone and dopamine efflux would be measured and compared between sucrose concentration groups.

After having tested the ability of the (CS) to produce absolute changes in dopamine efflux dependent upon the reward value of the (US) (i.e. sucrose solution) it would be sensible to investigate whether dopamine efflux is differentially altered by relative shifts in reward which are not predicted by the (CS). In doing this we may test Schultz's (1997) prediction that responses of dopamine neurones depend entirely on the unpredictability of stimuli. Further, it might be suggested that these neurons should code for the magnitude of discrepancy (error) between actual and anticipated incentive value. We are able to vary the (USs) (decreased or increased sucrose concentration) therefore manipulating their similarity to a constant (CS). Experiments such as those above would provide further evidence as to the functional capacity of anatomically discrete dopaminergic substrates. The outcomes of the above studies would have relevance to a range of important issues, including neural and behavioural mechanisms of drug addiction and craving.

Caveat

A caveat which is applicable to the entire thesis is best laid out here. Schultz emphasises the need to model, not only reward information from the various dopaminergic nodal points of the brain but also reward relevant information from other neurotransmitter systems. It would be naive to discount the contributions of other neurotransmitter systems to the mediation of food and other reward. In fact the previously well established relationship between dopamine and both food reward and drug addiction is now being teased apart, de-emphasised and indeed has been stongly questioned recently due to evidence gathered using genetic modification techniques (Caine, 1998). Therefore, although this thesis focuses

strongly on the functional significance of dopamine in the mediation of food reward, this does not preclude an appreciation of the contributions of distributed non-dopaminergic signals in the negotiation of reward.

8.5.1 Towards explicit models of reward

Despite the above caveat, Schultz's framework, due to its explicit nature, provides a parsimonious computational account of the roles of different dopamine receptor populations. Schultz has been able to replicate the "teaching signal" emitted by dopamine neurons, by applying an algorithm, formulated in the light of his empirical work, to a neural network. Neural networks are obviously useful in falsifying empirical data collected from on-line measures of neuronal activity and constitute a major new direction in reward research. It is clearly necessary to delineate the nature of dopamine activity in several functionally important brain sites. One way in which the computational approach might benefit this research area is in helping to specify the realtionships among dopamine release in these various sites (Smith, 1996). In doing this, it may become possible to pinpoint the stage of processing by dopamine neurons, at which the affective sign becomes adhered to stimuli and in turn how this affect laden stimuli modifies behaviour in various experimental situations.

The recent proliferation of dopamine receptor subtypes and new information regarding molecular and genetic aspects of cellular function will lead to the metamorphosis of many psychological constructs such as "reward". As Smith (1996) so elegantly predicts, words such as "network" and "processing" "will begin to pass from their current vague, logical status to a precise description of dopamine receptor-mediated effects" (pp. 135).

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