Central mechanisms in the perception of reward

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Central Mechanisms in the Perception of Reward.

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Statement of Copyright.

Declaration.

The work contained in this thesis was carried out by the author between 1983 and 1988 while a postgraduate student in the Department of Psychology at the University of Durham. None of the work contained in this thesis has been submitted in candidature for any other degree.

Abstract.

The perception of reward is crucial in many psychological processes. Wise (1982) has suggested that the neurotransmitter dopamine is involved in producing 'hedonic' responses to rewards. Dopamine is also involved in the control of movement and hunger. Testing dopamine's rôle in reward perception is therefore complicated by these other actions; the effects of manipulations can often be interpreted as actions on hunger or motor control, rather than on reward. Two methods are described in this thesis which isolate reward effects.

The effects of dopaminergic drugs on the rewarding impact of exploration were investigated. The use of exploration eliminates the influence of hunger, while the impact of motor deficits was reduced by using choice measures. Control experiments assessed effects on activity and emotionality. Results indicated that dopamine was involved in exploratory reinforcement independently of its rôles in hunger or simple motor control.

Tests of whether dopamine is involved in hedonia, or merely in engaging responses to reinforcers, used the 'behavioural contrast' paradigm. Contrast occurs when animals over-react to unexpected changes in reinforcement and allow response elicitation effects to be dissociated from effects on hedonia. Although the dopamine anatagonist α-flupenthixol did not affect contrast induced by changes in reinforcement value, the introduction or withdrawal of the drug could, itself, induce contrast effects. It is concluded that dopamine is involved in hedonia independently of any involvement in response elicitation. A speculative model of the nature of dopamine's involvement in hedonia is proposed and its implications for our understanding of Parkinson's disease and schizophrenia, in which dopamine dysfunctions are implicated, are discussed.

Dedication.

For Mum and Dad, with love and thanks.

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Contents.

Chapter 1

Introduction.

The aim of this thesis is to investigate the involvement of the neurotransmitter dopamine in central processes associated with reinforcement.

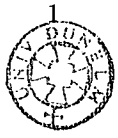
The use of the term 'reinforcement' may imply adherence to a particular class of learning theory, but it is not intended to. The notion that stimuli in the environment (i.e. distal stimuli) may act as predictors of biologically important events (and hence have the power to modify behaviour) has utility for all classes of learning theory. Reinforcing stimuli, whether they are intrinsically linked to significant events (e.g. the sight or smell of food) or whether they are associated with such events by conditioning (e.g. a light acting as a discriminative stimulus in a Skinner box), can be assumed to elicit specialised processing in the brain on the basis of their adaptive value.

Recent evidence which casts light on the rôle of dopamine systems in the reinforcement processes will be reviewed after a brief discussion of anatomical and biochemical classifications of dopamine systems in the brain.

1.1 Dopamine Systems in the Brain.

The monoamine dopamine was identified as a potential neurotransmitter in the brain in 1958 by Carlsson, Lindquist, Magnusson and Waldeck (1958). A variety of chemicals have effects on dopamine systems. Dopamine antagonists (otherwise referred to as neuroleptics or dopamine blockers) block dopamine receptors, decreasing the functional effects of dopaminergic neurons on the cells they synapse on. Examples of neuroleptics commonly used are haloperidol, pimozide, flupenthixol, spiroperidol and chlorpromazine (which also has noradrenergic action). The drug reserpine depletes stores of dopamine (and noradrenaline) in dopaminergic neurons. Dopamine agonists such as apomorphine directly stimulate dopamine receptors. The stimulant amphetamine has a number of actions, all of which exaggerate the effectiveness of dopaminergic neurotransmission. Cocaine also has a stimulant effect on dopamine systems due to blockade of the pre-synaptic re-uptake of released dopamine. The selective neurotoxin 6-hydroxydopamine can permanently destroy dopaminergic cells. It also attacks noradrenergic cells. In order to protect these, a noradrenaline reuptake blocker is used before 6-hydroxydopamine injection if only dopaminergic cells are to be destroyed. Pre-treatment of this kind can be assumed whenever 6-hydroxydopamine lesions are discussed in this thesis unless there is a statement to the contrary.

The anatomical distribution of dopaminergic neurons in the brain has been described by a number of authors (Dahlstrom and Fuxe, 1964; Lindvall and Björklund, 1977; Loughlin and Fallon, 1984; Moore and Bloom, 1978; Ungerstedt, 1971; Wang, 1981). For the purposes of discussion in this thesis the distributions of dopaminergic projections from midbrain areas to telencephalic and cortical terminal fields will be briefly noted (other dopaminergic systems are unlikely to be involved in reinforcement processes). These projections can conveniently be described as the nigro-striatal, meso-limbic and meso-cortical systems. The nigro-striatal system consists of projections from the substantia nigra pars compacta to the neostriatum



(caudate-putamen) and the globus pallidus. The meso-limbic system consists of projections from the ventral tegmental area to the nucleus accumbens, amygdaloid complex and septum. The meso-cortical system consists of projections from the substantia nigra and ventral tegmentum to a range of cortical areas (frontal, enterorhinal, piriform etc.) together with the olfactory bulb, anterior olfactory nucleus and olfactory tubercle. It is important to note that the distinction between these systems is somewhat artificial. The substantia nigra and the ventral tegmentum are adjacent and may be thought of as a continuous system. For example, although more cells project from the ventral tegmentum to the nucleus accumbens there are also some nigral projections to the nucleus accumbens. The tripartite division of midbrain dopamine systems is useful in describing the targets of lesion and central injection studies, but when possible neural circuits are considered more precise anatomical specification is required.

In addition to the anatomical classification discussed above there is also evidence for a number of distinct dopamine receptor types. Seeman (1981) provides an extremely comprehensive review of the evidence for different dopamine binding sites and the likelihood that they may represent distinct biochemically active receptors. At present little is known of any functional differences between the rôles of different receptor types in reinforced behaviour, although there do appear to be differences some other types of behaviour resulting from receptor stimulation (e.g. stimulant induced grooming, Molloy and Waddington, 1984).

Another division of receptors is based on functional rôle at the neuronal level and receptor location relative to synapses. This is the distinction between post- and pre-synaptic receptors, (or autoreceptors, Carlsson, 1975). Post-synaptic dopamine receptors are located on neurons to which dopaminergic cells synapse. Activation of post-synaptic receptors by dopamine release from pre-synaptic neurons results in changes in the activity of the post-synaptic neuron. Autoreceptors, on the other hand, cause changes in the activity of the pre-synpatic neuron after it releases dopamine. In effect autoreceptors appear to 'turn off' the pre-synaptic cell by a local negative feedback process after it releases dopamine. Stimulation of autoreceptors inhibits firing, dopamine release and dopamine synthesis in the pre-synaptic neuron (Aghajanian and Bunney, 1977; Carlsson, 1977). Although the different locations and functional rôles of post-synaptic receptors and autoreceptors do not necessarily require that autoreceptors are of a distinct biochemical type, it does appear that autoreceptors differ from post-synaptic receptors in their response characteristics to dopamine agonists, being much more sensitive to dopamine and to agonists such as apomorphine (Carlsson, 1975; Seeman, 1981). A consequence of this is that autoreceptors can be stimulated by very low doses of dopamine agonists which are ineffective at post-synaptic sites. Activation of autoreceptors leads to a reduction in dopaminergic activity, hence low doses of agonists have a paradoxically depressant effect on dopamine systems.

Manipulations of autoreceptors have been shown to have notable behavioural consequences. Some characteristics of the behavioural response to autoreceptors can be conditioned (Möller, Nowak and Kuchisnsky, 1987). Autoreceptor stimulating doses of apomorphine appear to attenuate the responding for food reinforcement delivered on a fixed ratio operant schedule (Carnoy, Soubrie, Peuch and Simon, 1986). This effect does not appear to be a consequence of a motor deficit. Autoreceptor activation has an effect on exploration similar to habituation (Höglund and Meyerson, 1985). A final result which may have valuable potential clinical implications

is that doses of post-synaptic antagonists and pre-synaptic autoreceptor agonists which have similar effects on apparently limbic-forebrain behaviours such as exploration differ in their motor effects (Ahlenius and Hillegaat, 1986). Post-synaptic antagonists, which are used in the treatment of schizophrenia, (which one may speculate as being related to limbic-forebrain dysfunctions), have much greater effects on motor systems than do autoreceptor antagonists. This may be a consequence of a non-uniform distribution of autoreceptors over dopamine systems (see Shepard and German, 1984). It is also of interest since changes in autoreceptor sensitivity may be implicated in habituation to dopaminergic drugs (White and Wang, 1984).

The experiments presented in this thesis do not attempt to specifically manipulate different dopamine systems on the basis of anatomy or receptor type, nevertheless consideration of the division of dopamine systems is necessary in discussing the implication of these experiments in the light of data from other studies.

1.2 Dopamine and Reinforcement.

Historically the hypothesis that the neurotransmitter dopamine is involved in the processing of reinforcement originated with the finding that dopaminergic drugs altered animals' rates of self-administering rewarding electrical brain stimulation (Olds and Travis, 1960; Stein, 1962). Self-stimulation rates were increased by stimulants and decreased by antagonists and depleting agents, indicating that dopaminergic activity may correlate with reinforcement. The drugs used in these studies (chlorpromazine, reserpine, amphetamine) act at both dopaminergic and noradrenergic sites, however, the finding that effective self-stimulation sites and the locations of dopaminergic cell bodies correlated lead to the proposal that dopamine systems may be involved in reinforcement (Crow, 1972, 1973). It has subsequently been found that rates of intracranial self-stimulation can be reduced by drugs which specifically antagonise dopamine receptors (e.g. Fouriezos and Wise, 1976).

Although normal dopamine function appears to be necessary for intracranial self-stimulation at some sites, it cannot be clearly inferred from this that dopamine is involved in reinforcement processing. One problem is the artificiality of electrical brain stimulation. Electrical brain stimulation may not be an analogue of the occurrence of natural reinforcers. For example, it has been proposed that electrical brain stimulation reduces drive (e.g. Routenberg and Lindy, 1965), simultaneously activates both motivational and reinforcement mechanisms (Deutsch and Howarth, 1963) or activates response patterns which are reinforcing in themselves (Glickman and Schiff, 1967).

One approach to the solution of these problems is to investigate the effects of manipulations of dopamine systems on behaviour reinforced with natural rewards. Although the parameters of drive and reinforcement can be more easily identified and manipulated in such experiments there are still serious problems in attributing changes in behaviour produced by manipulations of dopamine systems to effects on reinforcement processes.

First, there is strong evidence that dopaminergic neurons in the hypothalamus are involved in the control of hunger and satiety (e.g. Leibowitz and Rossakis, 1979a, 1979b). Dopamine is involved in a satiety mechanism. Dopamine's action in this satiety mechanism is inhibitory, the predicted effect of dopamine blockade in this mechanism is therefore to inhibit satiety, i.e. prolong feeding. This action is in the opposite direction to a putative reinforcement attenuating effect of dopamine

blockade, so it would not necessarily confound the interpretation of results in which dopamine blockade reduced response to reinforcement. It is, nevertheless, a factor which must be taken into account in developing and testing dopamine theories of reinforcement.

Second, it has been proposed that dopamine systems may be involved in selective attention (e.g. Matthyse, 1977). Apparent sensory neglect has been found in animals which had dopaminergic neurons destroyed by injection of the selective neurotoxin 6-hydroxydopamine into dopamine rich regions on one side of the brain only (e.g. Ljungberg and Ungerstedt, 1976; Marshall, Berrios and Sawyer, 1980). Attentional deficits were assessed by comparing animals' accuracy and latency to respond to tactile or visual stimuli presented on either side of the body. Failure or inaccuracy of response to stimuli presented to the side opposite the lesion was assumed to be indicative of a sensory deficit. Carli, Evenden and Robbins (1985) have shown, however, that these results may be due to motor, rather than sensory, deficits. When animals were conditioned to make different responses to visual stimuli presented to their left and right visual fields no accuracy deficits were found with unilateral 6-hydroxydopamine lesions. When the required responses were head movements towards or away from the visual stimulus response latencies were increased when the movement was towards the side contralateral to the lesion, regardless of the side on which the stimulus was presented. When the response involved pressing one of two levers at the opposite end of the test chamber, no deficits were found, however, accuracy was significantly reduced when the stimulus light was dimmed, again regardless of lesion side.

Carli, Evenden and Robbins' (1985) results indicate that attentional deficits are unlikely to account for reductions in response to reinforcers after dopamine blockade. They are, however, a demonstration of the most problematic known function of dopamine systems for testing dopamine reinforcement theories, namely the involvement of dopamine in motor control. Dopamine systems are closely associated with motor control. Degeneration of dopaminergic cells in the substantia nigra was identified as a major factor in Parkinson's disease, a primarily motor disorder, by Ehringer and Hornykeiwicz (1960). High doses of even peripherally administered dopamine antagonists cause catalepsy (Janssen, 1970). Bilateral 6-hydroxydopamine lesions produce extremely profound akinesia (Ungerstedt, 1971), while unilateral lesions cause motor deficits on one side of the body, animals therefore tend to move in circles (Ungerstedt and Arbuthnott, 1970). Direct injection of dopamine into the neostriatum on one side of the brain causes rotation in the opposite direction (Ungerstedt, Butcher, Butcher, Andén and Fuxe, 1969). Injections of stimulants such as amphetamine or apomorphine cause significant increases in locomotor activity until animals begin to exhibit stereotypy (repeated stereotyped motor sequences, see e.g. Robbins and Sahakian, 1983). At least one dopamine system, the nigro-striatal, therefore appears to be involved in motor control.

The involvement of dopamine in motor control makes testing for additional involvement in reinforcement processing difficult. If treatment with a dopamine blocker reduces an animal's response rate for a reinforcer, that reduction can be attributed to an effect on reinforcement systems or to a motor deficit. Given the well documented involvement of dopamine in motor control, additional evidence or a more sophisticated experimental design is required if effects on reinforcement processes are to be inferred.

Studies of the involvement of dopamine in reinforcement have recently been reviewed by Beninger (1983) and Wise (1978, 1982, 1985). A literature survey of the studies cited in these reviews will not therefore be presented, rather the lines of argument and the conclusions drawn by these authors will be outlined. A selection of pertinent papers published since these reviews appeared will then be discussed.

Wise's position is that dopamine blockers (neuroleptics) attenuate the hedonic impact of reinforcers. His 'anhedonia hypothesis', has been modified a number of times. As initially presented (Wise, Spindler, deWit and Gerber, 1978) the anhedonia hypothesis held that neuroleptics blocked the pleasurable consequences of primary reinforcement. Reductions in response to reinforcement were expected to be exhibited as deficits in response maintenance but not response initiation.

Wise (1982) modified his original hypothesis to take account of the results of Gray and Wise (1980) which showed attenuations of response rates in a partial reinforcement schedule. As a consequence, neuroleptics were now held to block both primary and secondary reinforcers. It was also noted that this blockade was not necessarily total. Wise admitted that neuroleptics may effect non-reinforcement functions (e.g. motor systems), but maintained that anhedonia accounted for most neuroleptic effects at reasonable dose levels. Anhedonia was held to apply to the majority of positive reinforcers (including opiates). Wise noted that he suspected dopamine to be involved in negative reinforcement, but that negative reinforcers were not included in the anhedonia hypothesis due to a lack of evidence.

In his most recent statement of the anhedonia hypothesis (Wise, 1985) the attenuating, rather than blocking, effect of neuroleptics on hedonic impact was emphasised and claims of generality over reinforcement classes were reduced. Wise still held strongly to the position that neuroleptic effects were not attributable to a deficit in initiating responses to reinforcers.

The anhedonia hypothesis can be summed up as follows; neuroleptics attenuate the hedonic impact of primary and secondary reinforcers independently of any effects they may have on performance, even when those effects are on response elicitation by reinforcers. The consequences of anhedonia include failures of response maintenance towards reinforcers and failures to learn about new associations with reinforcers. Wise does not commit himself to any particular learning theory, or to any particular model of reinforcement processing at an anatomical level, the anhedonia hypothesis is therefore very general. This generality can make interpretation of tests of the anhedonia hypothesis difficult. For example, it has been shown that the substrate of medial forebrain bundle intracranial self-stimulation is not transmitted in dopaminergic fibres (Bielajew and Shizgal, 1982; Gallistel, Shizgal and Yeomans, 1981; Shizgal, Kiss and Bielajew, 1982). Wise proposes, quite reasonably, that these non-dopaminergic fibres synapse with dopaminergic cells in the ventral tegmentum which themselves project to sites which will support self-stimulation unaffected by dopamine blockade (Wise, 1982, p.80). He does not speculate on the specific rôle of the dopaminergic stage in this partitioned reinforcement signalling system.

Wise's primary line of argument in support of his hypothesis is that the pattern of response obtained when rats work for reinforcement under neuroleptics is basically similar to that produced when they undergo extinction. The fundamental point is that it can be shown that animals under neuroleptics have the capacity to respond normally, however, after experiencing a number of reinforcers when in an

anhedonic state, they cease to maintain responding. Wise has shown, for example, that when animals trained to respond on a continuous reinforcement schedule are then tested under the neuroleptic pimozide their response rates at the start of the test session are normal; response rate only declines after a number of reinforcers have been obtained (Wise, Spindler, deWit and Gerber, 1978). He has also shown that once rates have declined they can be reinstated to normal levels by a disinhibiting stimulus such as re-presentation of a temporarily unavailable response lever (Fouriezos and Wise, 1976). Wise also claims that the decline in responding over repeated days of testing under pimozide is similar to that found in extinction (Wise, Spindler, deWit and Gerber, 1978) and that the lower acquisition rates and asymptotic response rates found when animals are trained to lever press for food under low doses of pimozide reflect an attenuation of the reinforcing impact of the food. Evidence was also presented for transfer of the effects of extinction on responding to subsequent testing under pimozide (e.g. Wise, Spindler, deWit and Gerber 1981). This transfer does not operate in the opposite direction (i.e. from pimozide to extinction, Mason, Beninger, Fibiger and Phillips, 1980) and is not reliably found with all reinforcers (Gerber, Sing and Wise, 1981), it must therefore be treated as only limited support for the anhedonia hypothesis.

Beninger (1983) comes to a different conclusion from Wise in his review of the rôle of dopamine in locomotor activity and learning. First, Beninger holds that comparatively low doses of neuroleptics may have significant effects on motor systems. He then goes on to discuss the rôle of dopamine in two types of learning, stimulus-stimulus associative learning and incentive-motivational learning. He concludes that dopamine is not involved in the association of reinforcers with neutral stimuli, but that it is necessary if neutral stimuli are to acquire the power to elicit goal directed responses (i.e. become conditioned incentive stimuli).

The stimulus-stimulus association studies discussed by Beninger differ very significantly from the operant tasks used by Wise. In these stimulus-stimulus association studies a reinforcer is presented in conjunction with a neutral stimulus in a classical conditioning paradigm (i.e. no response is required from the animal) to neuroleptic treated animals. When these animals are subsequently tested drug-free, the neutral stimulus elicits behaviour appropriate to the reinforcer it was paired with during training. Results of this type had been found by Hunt (1956) with tone-shock pairings tested in a conditioned emotional response paradigm (in which the conditioned response is largely autonomic). Beninger also describes studies in which association was demonstrated by effects on operant responses in the drug-free test, e.g. defensive burying of a previously electrified prod (Beninger, MacLennan and Pinel, 1980) or transfer of a classically conditioned tone-food pairing to an operant discrimination task (Beninger and Phillips, 1981). On the basis of findings of this type Beninger concludes that neuroleptics do not disrupt the association of reinforcers with neutral stimuli and hence that neuroleptics do not disrupt perception of reinforcement. This conclusion is at odds with Wise's notion that neuroleptics block the reinforcing impact of reward.

In contrast to the results obtained with stimulus-stimulus learning, Beninger reviews a range of studies which show that dopamine blockade disrupts the acquisition and maintenance of incentive-motivational learning, i.e. the ability of a neutral stimulus, when previously paired with a reinforcer, to elicit operant responses. For example, Beninger and Phillips (1980) showed that a tone paired with food under neuroleptic treatment subsequently failed to elicit lever pressing. The

difference between this study and the effect of tone-food conditioning in Beninger and Phillips (1981) must be stressed. In Beninger and Phillips (1981) the tone aided the acquisition of discrimination in a test where responding was maintained by food reinforcement. In the 1980 study the tone alone failed to elicit responding. It can be concluded that the tone-food association formed under drug aided discrimination of the reinforcing qualities of food but did not allow the tone in itself to act as an incentive stimulus.

Beninger cites evidence from studies of the acquisition and expression of conditioned stimulant drug responses as further evidence for the blockade of incentive-motivational learning. Beninger and Hahn (1983) showed that the heightened locomotor activity which occurs after amphetamine could be conditioned to a specific environment. If an animal was repeatedly placed in a particular environment after amphetamine injection then that environment could induce elevated activity levels even in the absence of amphetamine. The establishment of amphetamine-environment conditioning could be blocked by pimozide, however, pimozide did not reduce the heightened activity shown by animals in which amphetamine-environment conditioning had taken place without pimozide.

Studies such as those cited by Wise show that neuroleptics also interfere with the maintenance of learned incentive motivation. In addition a number of studies with dopaminergic stimulants have shown enhancements of previously established incentive motivational stimuli (e.g. Robbins, Watson, Gaskin and Ennis, 1983).

Beninger acknowledges that, although the evidence supports his hypothesis that dopamine is required for incentive learning but not stimulus-stimulus learning in the case of positive reinforcers, dopamine blockade does not appear to block incentive learning for negative reinforcers. The defensive burying study mentioned above (Beninger, MacLennan and Pinel, 1980) shows that a prod paired with electric shock under pimozide can elicit a goal directed operant response, defensive burying, in drug-free conditions. Similar results have been found in transfers from conditioned avoidance to escape responses. Rats fail to learn to avoid shock when dopamine systems are disrupted, although the intensity of the shock stimulus does provoke escape even in 6-hydroxydopamine lesioned animals (e.g. Fibiger, Phillips and Zis, 1974). Beninger, Mason, Phillips and Fibiger (1980) have shown that animals which failed to learn an avoidance response to a tone when trained under dopamine blockade, nevertheless, showed an avoidance response in later drug-free testing. In this situation the tone is acting as an incentive stimulus during testing, not simply an aid to discrimination. It therefore appears that dopamine blockade does not disrupt the acquisition of negative incentive stimuli.

Beninger concludes his review with a model of the processes that may underlie dopamine's involvement in incentive motivational learning. Essentially Beninger believes that dopamine is involved in the formation of associations between environmental stimuli and motor responses in the striatum. The rôle of dopaminergic neurons is to signal the occurrence of reinforcement, this leads to alteration of the synaptic strength between selected neurons carrying sensory and motor information. Dopamine is therefore held to be involved in the elicitation of responses by reinforcing stimuli.

The differences between Beninger's and Wise's hypotheses can be summarised as follows. Wise holds that dopamine is involved in the occurrence of the hedonic response to reinforcers while Beninger holds that it is only involved in the elicita-

tion of responses by reinforcers. A consequence of this is that Beninger's model predicts deficits in the initiation of responses to reinforcers while Wise holds that deficits should only occur in response maintenance, and not in initiation. Wise holds that both primary and secondary reinforcement can be affected by neuroleptics; Beninger's model implies that response to primary reinforcers should be unaffected by neuroleptics. Both models leave the question of the generality of dopaminergic effects over types of reinforcement open. Some more recent studies which are pertinent to these differences and to the generality of dopaminergic effects on reinforcement will now be discussed.

Wise has shown that pimozide can induce decreased response to food as a primary reinforcer. Wise and Colle (1984) found that pimozide increased latencies to start eating and decreased the number of pellets eaten, in a design where food was presented in a feeding platter at intervals during each test session. Wise and Colle (1984) argue that the fact that pimozide treated rats sometimes showed latencies as short, or even shorter than, rats in the control condition ruled against an interpretation of the results in terms of a motor deficit. A replication of this study by Wise and Raptis (1986) showed that the effects of pimozide on latency to begin eating and the amount of food consumed was minimal on the first test day. By the third test day, however, mean latency had become much greater under pimozide (although 'best score' latencies were as short as those of control animals) and a large number of pellets were left uneaten. It may be argued that the apparatus provided a number of secondary reinforcer cues which may have contributed to the pimozide induced deficit. Jenck, Gratton and Wise (1986) have shown that eating induced by hypothalamic stimulation can be inhibited even when food pellets are simply distributed all over the floor of the test cage, a situation, (albeit using an artificial source of 'motivation'), where secondary cues are even less likely to be used than in Wise and Colle (1984).

Neuroleptics have also been found to depress free consumption of sucrose solutions (Geary and Smith, 1985) and water (Ljungberg, 1987). Although it has been shown that neuroleptics have greater effects on learned responses controlled by secondary reinforcers than they do on reflexive response for primary reinforcement (Gramling and Fowler, 1985), these results clearly present a problem for Beninger's model.

A number of studies have demonstrated effects which cannot be easily explained by reductions in the response eliciting abilities of reinforcers. Two studies have shown changes in the partial reinforcement extinction effect (PREE) with dopaminergic drugs. The implication is that the frustrative effects of non-reward were modified, in other words, the effect was on the negative hedonic impact of non-reward. Ettenberg and Camp (1986) showed that administration of pimozide on some daily discrete food reinforced runway trials could produce the PREE during extinction. Weiner, Feldon and Bercowitz (1987) found that amphetamine abolished the PREE if given on non-reinforced trials indicating that it may have compensated for non-reward.

Heyman and Seiden (1985) investigated the effect of amphetamine on response distribution between concurrent variable interval schedules using Herrnstein's (1970, 1974) matching law. They found that the parameter 'Re', which is an inverse measure of reinforcement efficacy, was decreased by amphetamine, while the parameter 'k', which is a measure of response cost, was unaffected. This implies, albeit indi-

rectly, that amphetamine had accentuated the reinforcing impact of food without changing response topography or cost. Similar studies of neuroleptics have shown a decrease in rewarding efficacy accompanied by an increase in response cost (Heyman, 1983; Heyman, Kinzie and Seiden, 1986). Some studies have produced results indicating motor deficits, but not changes in reward efficacy, using neuroleptics in this paradigm. Morley, Bradshaw and Szabadi (1984) based their estimates of effects on 'k' and 'Re' on response rates from only two schedule values, although they did use a range of pimozide doses. Strictly speaking the hyperbolic curve fitting required for parameter estimation cannot be performed with this data. Morley, Bradshaw and Szabadi's conclusion that pimozide effects motor systems but not reinforcement efficacy must therefore be treated with caution. Heyman, Monaghan and Clody (1987) used five component schedules, their parameter estimates can therefore be treated as valid. They found that low doses of flupenthixol affected the motor parameter 'k', but not the reinforcement efficacy parameter 'Re'. Examination of their data shows extremely large variability between subjects in estimated 'Re'. At reasonable dose levels (i.e. 0.02 and 0.03 mg/kg) rewarding efficacy appeared to be slightly attenuated, although the data were very noisy. It also appears that the statistical analyses in this study were based on ratios of parameters obtained under drug to parameters obtained in a baseline condition without injection rather than to scores from the vehicle injection condition. Data from the vehicle injection condition indicate that this more appropriate comparison would have yielded a greater apparent decrease in rewarding efficacy. The conclusions of this study should also therefore be treated with caution.

Studies involving the parameterisation of response rate curves from varied intensities of intracranial self-stimulation also show effects of dopaminergic drugs consistent with changes in rewarding efficacy over and above any motor effects. A characteristic curve (the reward summation function) is produced if rates of self-stimulation are plotted against stimulation intensity. This curve is shifted to the right if the efficacy of stimulation is decreased (e.g. by reducing frequency), but the curve is flattened if responding is made more difficult (e.g. by sub-paralytic curare injection, Edmonds and Gallistel, 1974). Gallistel and Freyd (1987) found that pimozide produced a dose related right-shift in the reward summation function with medial forebrain bundle stimulation, while amphetamine produced a left-shift. Miliaressis, Malette and Coulombe (1986) found similar effects with pimozide when stimulating central periaqueductal grey. Both studies found that responding broke down to such an extent under larger doses of pimozide that no manipulation of stimulation parameters could maintain responding sufficiently well to generate reward summation functions. One consequent hypothesis is that dopamine may have a gating function in hedonic reinforcement systems.

The curve-shift paradigm described above is one of a number of 'rate-free' measures of stimulation efficacy. Another 'rate-free' test, the current resetting paradigm, has been used by Zarevics and Setler (1979) to assess the effects of pimozide on the rewarding impact of medial forebrain bundle intracranial self-stimulation. In the 'resetting' paradigm the stimulation current obtained by lever pressing is gradually decreased as responding proceeds, however, a single response on a second lever will reset the current to its original intensity (although pressing this second lever will not, itself, produce stimulation). The intensity at which animals stop responding on the lever which produces stimulation and make a response on the reset lever gives a measure of the reinforcing impact of stimulation. Zarevics

and Setler (1979) found that pimozide raised the intensity at which animals would reset the current to maximum intensity without markedly disrupting response rates on the stimulation lever. The implication of this is that pimozide decreased the rewarding impact of stimulation (a higher current intensity was necessary to maintain responding) without disrupting motor systems.

The studies described above lend support to Wise's hypothesis because they indicate that manipulation of dopamine systems appears to alter the rewarding impact of reinforcers. Nevertheless, it has been shown that neuroleptics do not effect animals' ability to discriminate reinforcement from non-reinforcement. Corradini, Tombaugh and Anisman (1984) showed that pimozide did not effect the ability of mice to learn position or cue discriminations in a water escape task. As Wise does not hold that neuroleptics necessarily disrupt the impact of negative reinforcers (such as water immersion) Corradini, Tombaugh and Anisman's finding does not directly challenge Wise's hypothesis. Bowers, Hamilton, Zacharo and Anisman (1985) have shown that pimozide fails to effect choice accuracy in an intracranial stimulation reinforced discrimination task, a result which is more problematic for Wise.

Martin-Iverson, Wilkie and Fibiger (1987) used a psychophysical discrimination technique to show that haloperidol does not cause changes in animals' average estimates of quantities of reinforcement (although it does decrease accuracy of discrimination) at doses which increased response latencies. They therefore argue that haloperidol is effecting response elicitation by reinforcers without having any hedonic effect. They do, however, report an apparent hedonic effect with amphetamine. Drawing inferences from their data is very complicated as hedonic and motivational effects on stimulus generalisation performance may interact with similar effects on the reinforcing value of the reward. Their findings that amphetamine, increased food pellet sweetness and increased levels of food deprivation all lead to an apparent increase in the perceived number of food pellets, (rather than the decrease one may intuitively expect if fewer, hedonically more stimulating, pellets were perceived as equal to more of the less satisfying pellets received in training), underline the problems of interpretation. Explanations for these effects can be constructed in terms of the effects of incentive motivation and satiation on hypothetical generalisation gradients underlying discrimination performance. One consequence of this type of analysis is that the lack of effect found with haloperidol does not necessarily reflect a lack of hedonic effect. Although Martin-Iverson, Wilkie and Fibiger's paper raises important issues, it cannot be treated as evidence for a lack of hedonic effect without appropriate data from a number of necessary control experiments.

The discrimination experiments described above are all presented as tests of the anhedonia hypothesis. It is not clear that this is the case. The ability to discriminate reward from non-reward, or even the number of food pellets presented, does not necessarily imply that the hedonic effects of reinforcers are unaffected. In anthropomorphic terms, although the haloperidol treated rat may not particularly enjoy the food pellets in the Martin-Iverson experiment, it still knows whether it has just eaten one of them or four of them. Choice measures which do not include some measure of the amount of behaviour directed to each of the goal stimuli do not necessarily provide information about the hedonic effects of dopamine manipulations.

Other studies provide evidence in favour of Beninger's contention that neurolep-

tics cause deficits in the initiation of responses. Deficits of this nature are typical of patients suffering from Parkinson's disease who have to make great efforts to initiate movements but are capable of sustaining them once begun.

Blackburn, Phillips and Fibiger (1987) report that pimozide caused disruption of preparatory responses to food consumption, but not the food consumption itself. A buzzer and light were paired with presentation of food. These stimuli were activated 150 seconds before food presentation and stayed on throughout the 60 seconds when the liquid diet was available. When animals were undrugged they entered the feeding niche at the onset of these stimuli and then consumed the food when it was presented. After pimozide, niche entry during stimulus presentation was significantly decreased, however, when the food was presented animals entered the niche and consumed it. Blackburn, Phillips and Fibiger also showed that the doses of pimozide used in this experiment did not cause reductions in consumption of the liquid diet when it was presented in a free feeding situation. Blackburn, Phillips and Fibiger conclude that these findings imply a disruption of the incentive value of preparatory stimuli by dopamine blockade. These results could also be explained in terms of anhedonia which had greater effect on weaker reinforcers (a version of Clody and Carlton's (1980) stimulus efficacy hypothesis), if it is assumed that the tone-light stimulus compound is less well associated with reinforcement than the sight and smell of the food or the sound of the food pump operating. Blackburn, Phillips and Fibiger note, however, that dopamine blockers differentially effect tasks using the same reinforcer and discriminative stimuli but different responses (e.g. Ettenberg, Koob and Bloom, 1981; Waquier and Niemegeers, 1979) and hold that such effects cannot be explained in terms of a stimulus efficacy hypothesis, but are consistent with dopaminergic involvement in the response eliciting qualities of incentive stimuli.

More direct evidence for dopaminergic involvement in reinforcement related behaviour is reviewed by Mogenson (1987). Mogenson and his associates have traced a neural circuit from limbic areas to the mesencephalic locomotor region, dopaminergic projections from the ventral tegmentum to the nucleus accumbens form one stage of this circuit. Mogenson proposes that dopamine is involved in modulating the effectiveness of emotional signals from limbic structures in evoking movement via the mesencephalic locomotor region. Sawaguchi, Matsumura and Kubota (1986) provide evidence showing that dopaminergic cells in the frontal cortex may also be involved in motor processes, rather than in response to reinforcement related stimuli per se.

Fowler, Lacerra and Ettenberg (1986) have investigated the effects of haloperidol on the detailed biophysical characteristics of operant responding. They report that while haloperidol treated rats respond with equal or greater force than controls and with equal latency they are slower to remove their paws from the response manipulandum having made a response. This is interpreted as being similar to a Parkinsonian deficit. It certainly provides evidence for a response initiation deficit (the response being paw removal), however, in this case the deficit is not in initiation of a reinforcement directed behaviour.

Salamone (1986) also argues that neuroleptics reduce incentive-related motor activity but not hedonic impact. He did not test drug effects on response initiation directly, but compared the effects of haloperidol and extinction in tasks which required active and passive behaviour in rats in order to obtain reinforcement. The

'active' task was lever pressing on a fixed ratio 20 schedule. A dose of 0.1 mg/kg haloperidol produced decreases in response rate comparable to those produced by extinction, however, the time course of the deficit differed between drug and extinction conditions. Haloperidol produced a larger deficit at the start of the test session, but by the end of the session haloperidol treated animals were responding faster than those in extinction. The 'non-active' task was essentially a conditioned place preference test. Animals were rewarded at intervals if they were in one particular part of an open field (near a food dispenser). In addition to measuring the effects of haloperidol and extinction on conditioned place preference, effects on activity throughout the test field were measured. A much higher 0.4 mg/kg dose of haloperidol was used in the place preference experiment. This high dose reduced activity to levels significantly below that of animals in extinction or in the control condition, but did not reduced the proportion of time spent in the 'reinforcing' area of the test cage, although extinction did. Salamone concludes that haloperidol's effects on the fixed ratio task were due to a deficit in response elicitation, and that the failure to effect place preference demonstrates that reinforcement, even mediated by secondary place cues, still had value for the haloperidol treated animals. Salamone (personal communication) characterises the deficit he found as generally motivation, rather than reinforcement, attenuating, in that animals still orient to sources of reinforcement but are less likely to respond to them. These results are also consistent with an attenuation (rather than a complete blockade) of reinforcing impact in conjunction with a failure of haloperidol to alter animals' ability to discriminate sources of reinforcement.

It can be seen from this short survey that the rôle of dopamine in reinforcement is still far from clear. Many of the 'rate-free' studies purporting to support Wise can be criticised on the grounds that some responses are still required in order to measure rewarding efficacy, the elicitation of these responses may be disrupted. Curve parametrisation techniques may show results which differ in pattern from those obtained with explicitly induced motor deficits (e.g. with curare), but these curves may still reflect a deficit in the elicitation of responses by reinforcers rather than an attenuation of reinforcing impact or a simple motor capacity deficit. Studies which show normal discrimination after neuroleptics do not directly address Wise's hypothesis, and the lack of effect on discrimination complicates the interpretation of studies such as Salamone's (1986). The evidence reviewed by Mogenson (1987) provides strong support for an involvement of dopamine in the elicitation of responses by reinforcement. There is, however, no reason why this should preclude a parallel involvement in the mediation of the hedonic impact of reinforcers.

1.3 The Generality of Dopamine's Effects on Reinforcement.

Although it is not clear what the precise nature of the deficit produced by neuroleptics in reinforcement processing is, there has been some progress in assessing its generality. The conditioned place preference (CPP) paradigm has been widely used in the assessment of the effects of dopamine blockade on the reinforcing properties of various drugs. The ability of neuroleptics to block the acquisition of CPP with another drug being a measure of dopamine's involvement in the reinforcement produced by that other drug.

The finding that the acquisition of amphetamine CPP could be blocked by neuroleptics (e.g. Spyraki, Phillips and Fibiger, 1982a) was criticised as evidence for reinforcement attenuation by Swerdlow and Koob (1984). They suggested that the

reinforcing property of amphetamine responsible for place preference conditioning may be amphetamine induced hyperactivity. Neuroleptic induced reductions in activity could therefore account for the blockade of CPP without any involvement of putative dopaminergic reinforcement mechanisms. Carr, Phillips and Fibiger (1988) have shown that the restraint used by Swerdlow and Koob could itself produce CPP by affecting the perceived novelty of the environment. When this novelty effect was minimised by habituating animals to the test apparatus, CPP could be induced by amphetamine in restrained animals. This suggests that Swerdlow and Koob's criticism is unfounded and that neuroleptics do disrupt the reinforcing properties of amphetamine.

Spyraki, Fibiger and Phillips (1983) have also demonstrated attenuations of heroin induced CPP with neuroleptics. This result conflicts with Pettit, Ettenberg, Bloom and Koob's (1984) finding that 6-hydroxydopamine lesions of the nucleus accumbens failed to attenuate heroin self administration, however, Bozarth and Wise (1986) review a range of studies implicating ventral tegmental dopamine in opioid reinforcement, (see also Mackey and van der Kooy, 1985 who discuss a number of methodological problems in assessing morphine and cocaine CPP). It appears that the results of self-administration studies such as Pettit, Ettenberg, Koob and Bloom (1984) must be treated with care. Reward attenuation normally results in decreased rates of operant behaviour, however, in drug self-administration studies increased rates of self administration are found when the drugs reinforcing value is decreased. It is likely that this increase serves to maintain a given effective level of the receptor agonism by the drug (see e.g. Pickens, Meisch and Thompson, 1978). One may therefore expect different patterns of results when manipulations directly effect the receptors which the reinforcing drug agonises, as opposed to effecting systems with reinforcing consequences which may be mediated through systems having different neurotransmitters. The results of Pettit, Ettenberg, Bloom and Koob (1984) and Ettenberg, Pettit, Bloom and Koob (1982), who demonstrated increases in self administration of heroin with naltrexone (an opiate receptor antagonist) but not flupenthixol and increases in self administration of cocaine with flupenthixol but not naltrexone, do not necessarily imply that dopamine is not involved in some stage of the processing of opioid reinforcement.

Meso-limbic dopamine has been implicated in the reinforcing properties of diazepam. Spyraki and Fibiger (1988) found that 6-hydroxydopamine lesions of the nucleus accumbens or haloperidol injections both blocked the acquisition of diazepam induced CPP. Dopamine therefore appears to be involved in the reinforcing properties of a number of drugs; psychomotor stimulants (amphetamine, cocaine), opioids (heroin, morphine) and benzodiazepines (diazepam). It is also of interest to note that the CPP paradigm has been used to demonstrate blockade of food reinforced place preference with haloperidol (Spyraki, Fibiger and Phillips, 1982b). (Note that Salamone's (1986) study failed to find an effect on the expression of CPP, but did not assess neuroleptic effects on CPP acquisition).

Dopamine systems appear to be implicated in reinforcement produced by a range of drugs. Natural reinforcers will now be considered. Many of the studies cited above used food as a reinforcer. Gerber, Sing and Wise (1981) and Ljungberg (1987) have found reductions in operant response rate for water reinforcement. Ljungberg (1987) has also found reductions in consumption of water in water deprived rats. White and Major (1978) found that pimozide impaired latency to initiate drinking, this effect was shown to reflect a learning, rather than performance, deficit. In

addition a number of studies have found deficits in responding and consumption of sweet solutions (e.g. sucrose solutions, Geary and Smith, 1985; or saccharin solutions, Gramling, Fowler and Collins, 1984).

Ettenberg and Carlisle (1985) assessed the effects of flupenthixol on operant responding for temperature and on preferred temperature. Temperature differs from most reinforcers (and is similar to self-administered drugs) in that reductions of temperature cause increases in response rates as animals attempt to maintain body temperature. Ettenberg and Carlisle found no evidence of increased response rates after flupenthixol or any change in animals preferred temperature (assessed in a temperature gradient tube).

Everitt reports the results of some preliminary experiments which suggest that dopamine blockade can reduce response rates of male rats for secondary reinforcers associated with presentation of sexually receptive females (Everitt and Stacey, 1987). There is therefore a possibility that sexual reinforcement may be mediated by dopamine systems.

Papp and Bal (1986) investigated the effects of haloperidol and 6-hydroxydopamine lesions on dominance relations in pairs of rats. Effects on dominance were assessed by noting changes in the winners of competition tests between pairs of water deprived rats competing for water reinforcement (i.e. success in fighting). They found that haloperidol or tegmental 6-hydroxydopamine lesions in rats found to be dominant in control tests reversed dominance. Although this reversal is likely to be partially attributable to effects on the value of water reinforcement, it may also provide some evidence of effects on the motivational and emotional concomitants of fighting.

The results of studies on effects of dopamine manipulations on negative reinforcers such as shock have been equivocal. Shock can elicit escape even in animals with 6-hydroxydopamine lesions (e.g. Fibiger, Phillips and Zis, 1974), however, animals fail to make avoidance responses to stimuli preceding shock when treated with neuroleptics (e.g. Fibiger, Zis and Phillips, 1975). Similar deficits can be produced by combined meso-limbic and nigro-striatal 6-hydroxydopamine lesions (Koob, Simon, Herman and Le Moal, 1984). Beninger, Mason, Phillips and Fibiger (1980) have shown, however, that when animals which had experienced stimulus-shock pairings under neuroleptics and failed to make avoidance responses were subsequently tested drug-free they showed an avoidance response. The conclusion drawn by the experimenters and by Beninger (1983) was that neuroleptics failed to block the acquisition of incentive responses to stimuli paired with negative reinforcement. The negative reinforcer must not only be discriminable under dopamine blockade, but also also be capable of supporting incentive-motivational learning.

A recent study by Carey and Kenney (1987) suggests that the insensitivity of response to aversive reinforcers to dopamine blockade is transitory. Carey and Kenney studied the effects of haloperidol on a learned, rather than reflexive escape task. Animals were trained to run down a 100 cm electrified alley to reach a goal box. A short delay between placing the animal in the alley and the onset of shock allowed avoidance attempts to be measured. Carey and Kenney found that haloperidol severely attenuated acquisition of this learned escape response and reduced the number of escape attempts animals made before shock onset. When drug treatments were reversed (the control condition in this experiment was haloperidol injection after testing) the group now receiving haloperidol before testing initially

escaped with latencies similar to control condition performance, indicating that long latencies in the experimental condition were due to a learning, rather than motor, impairment. As testing proceeded, however, the performance of the 'haloperidol before' group was gradually impaired, while the group switched to haloperidol after testing improved their escape performance and increased their number of avoidance attempts. Significant latency differences between the groups had only occurred four days after drug conditions were reversed. Similar effects were found after a second reversal of drug conditions. It can be concluded that neuroleptics can disrupt learned responses to aversive stimuli, but that the attenuation of negative reinforcers requires some time before it produces significant behavioural effects.

The effect of dopamine blockade on the reinforcing properties of exploration is particularly hard to assess. The consummatory response to exploratory reinforcement is dependent on locomotor activity, the dissociation of motor and reinforcement effects is therefore very difficult. Some studies have made use of test arenas with holes drilled into their floors ('hole boards'), the number of times animals place their heads in these holes is held to be a measure of exploratory behaviour (e.g. File and Wardill, 1975). When used alone, this type of measure cannot dissociate reductions in 'nose pokes' into the holes due to reduced exploratory activity from reductions due to decreased locomotion around the apparatus (an animal may make less nose pokes because it encounters fewer holes if it moves around the test cage less). The use of a separate measure of locomotor activity can ameliorate this problem to some extent (see Sanberg, Hagenmeyer and Henault, 1985 for a discussion of multivariate approaches to the measurement of locomotor behaviour). Ungerstedt and Ljungberg (1977; Ljungberg and Ungerstedt, 1978) developed apparatus which could concurrently measure locomotion and nose-pokes, however, they express doubts as to the validity of nose pokes as a measure of exploratory behaviour. They found that both apomorphine and amphetamine increased locomotor activity, but that amphetamine increased nose-poking while apomorphine decreased it. No assessments were made of the effects of neuroleptics.

Ahlenius, Engel and Zöller (1977) examined the effects of haloperidol and apomorphine on exploration and latent learning. A four arm (square) maze was used with blind alley extensions at each corner. Exploration was defined as the number of arms entered during the first and last five minutes of a test session. This measure too is confounded by possible locomotor effects. Haloperidol and apomorphine were both found to decrease arm entries. In the subsequent latent learning test (one part of the maze now being baited with food) haloperidol was shown to have had no effect on latent learning, lower doses of apomorphine (which had probably reduced activity to due to autoreceptor effects) also failed to effect latent learning, but higher doses produced some disruption.

The effects of dopaminergic drugs and 6-hydroxydopamine lesions of the mesocortical and mesolimbic dopamine systems (combined) on animal's investigation of novel objects were assessed by Fink and Smith (1980). Investigative activity was measured by counting the number of times an animal put its head into an alcove containing a novel object. This method appears less susceptible to confounding by motor deficits than hole board counts, although severe motor impairments may still effect it. The 6-hydroxydopamine lesion was found to decrease investigation of novel objects. Treatment of lesioned animals with apomorphine increased investigation of novel, but not familiar objects. This effect of apomorphine could be reversed by haloperidol.

Spontaneous alternation in Y-mazes may also be an index of exploratory reinforcement (Montgomery, 1951b). Oades, Taghzouti, Simon and Le Moal (1985) found that amphetamine eliminated alteration due to increases of collateral behaviours at the choice point of the maze. This effect could be reversed by haloperidol.

None of the studies described above provide conclusive evidence that dopamine is involved in the reinforcing aspects of exploratory behaviour, although Fink and Smith's (1980) results are suggestive of such a rôle. It is of interest to not that, using a pulse-collision technique, Durivage and Miliaressis (1987) have shown that exploration induced by medial forebrain bundle electrical brain stimulation and medial forebrain bundle intracranial self-stimulation may be mediated by different sets of fibres.

There is then evidence of considerable generality of dopamine's involvement in responses to a wide range of drug reinforcers and a number of natural reinforcers. One study failed to find any effects on temperature reinforcement (a methodologically difficult problem). The evidence for involvement in exploratory reinforcement is equivocal. The dissociation of motor and reinforcement effects in the study of exploration also requires methodological ingenuity.

1.4 Conclusions.

This brief review has highlighted two issues which need to be addressed. First, the question of whether dopamine systems are necessary for stimuli to have hedonic impact, or whether they rôle is primarily at an 'output stage', gating the elicitation of responses by incentive stimuli, and hence the acquisition of conditioned incentives. Second, the question of whether the generality of dopaminergic involvement in reinforcement extends to exploratory behaviour.

Chapter 2

Dopamine and the Reinforcing Properties of Exploration.

2.0 Introduction.

It has been suggested that the neurotransmitter dopamine may be involved in a number of brain functions; the control of voluntary movements, the central signalling of hunger and hedonic or response eliciting aspects of reinforcement. Although these rôles are not mutually exclusive, the dissociation of evidence for involvement in any specific function is complicated by these possible multiple rôles. The series of experiments described in this chapter attempt to test the hypothesis that dopamine is in involved in reinforcement processing independently of any simple rôle in motor control. The effects of dopaminergic manipulations on a measure of behaviour allocation for a choice of levels of non-food reinforcement will be assessed in a manner which minimises the effect of disruption of motor control.

Dopamine's involvement in motor control systems is forcefully demonstrated by Parkinson's disease which Hornykiewicz (Ehringer and Hornykiewicz, 1960) showed to be caused by abnormally low levels of dopamine in the substantia nigra. Similar motor deficits can be induced in animals by dopamine specific neurotoxic lesions (Ungerstedt, 1979) or by high doses of systemically administered dopamine receptor blockers (Ettenberg, Cinsavich and White, 1979). These disruptions of motor control confound tests in which the hedonic impact of reinforcement is assessed by simply measuring response rates in an operant situation. Any decrease in response rates produced by dopamine blockade could be attributed equally well to a reduction of the hedonic impact of the reinforcer or to a motor deficit.

One approach to the solution of this problem is to examine the effects of dopaminergic manipulations on tasks which measure animals' relative preference between two reinforcers. The underlying argument is that disruptions to motor systems and changes in the impact of reinforcers need not effect measures of preference in the same way. A motor impairment will produce a proportional reduction in response rate no matter what reinforcer is controlling responding. In a test of preference between two reinforcers of different values we would expect a motor impairment to reduce response rates for both reinforcers by equal proportions (e.g. both rates may be halved). The motor impairment therefore reduces overall response rates, but does not alter the ratio of responses allotted between the two reinforcers.

On the other hand, a general reduction of the impact of reinforcers need not effect responding between two reinforcers proportionately. Such a reduction may decrease the difference in relative value of the two levels of reinforcement and hence the difference in responding between them. Although an animal may still discriminate relative value in ordinal terms, response distribution may, nevertheless, be altered.

A possible model for this type of effect of the attenuation of reinforcement impact is to posit that the manipulation proportionately decreases the objective values of reinforcers, but that the subjective effectiveness of, and response rates produced by, reinforcers are dependant on a logarithmic function of objective value

(as is the case for response rates maintained by varying concentrations of sucrose solution (Pfaffmann, 1982)). Since the predicted effects of motor impairments and attenuation of reinforcement impact are different, measuring the effects of dopamine manipulations on preference between a choice of reinforcers offers the chance of dissociating a rôle for dopamine in reinforcement processing from its rôle in motor control.

Dopamine's rôle in motivation may also confound the assessment of effects on reinforcement systems. It has been demonstrated that dopamine is involved in mediating metabolic state controlled hunger mechanisms (e.g. Biggio, Porceddu, Fratta and Gessa, 1977; Liebowitz and Rossakis 1979a, 1979b). Dopaminergic inputs to the perifornical hypothalamus inhibit hunger. An implication of this is that dopamine blockade should interfere with this hunger inhibition, resulting in dopamine blocked animals remaining hungry when control animals are becoming sated.

This motivational effect operates in the opposite direction to the hypothesised anhedonic or response elicitation inhibiting effects of dopamine blockade and so may obscure such reinforcement effects. The effective increase in drive may not only effect animals' response rates when working for food reinforcers in an operant situation, but also accentuate the extent to which they discriminate between reinforcers of different values.

In order to take account of the factors outlined above, the experiments described in this chapter investigate the effects of dopamine manipulations on choice behaviour for a non-nutritive reinforcer – exploration. The use of such a reinforcer not only avoids the potential problems of dopamine's effects on hunger, but also provides a test of the generality of a reinforcement mediating rôle for dopamine. Freed and Zec (1982) in a commentary on Wise's (1982) review, specifically suggest that a range of natural reinforcers such as handling, exploration and sex need to be used in investigating dopamine's rôle in hedonia.

Experiment 2.1 – Assessing the Behaviour of Normal Animals in the Exploration Choice Apparatus.

2.1.1 Introduction.

The experiments described in this chapter are concerned with measuring the effects of various dopamine manipulations on animals' choice between exploratory reinforcers of different values. It is necessary to assess the behaviour of normal animals in the apparatus used in these experiments in order to confirm that the various stimuli used do indeed have different reinforcing values.

The apparatus used in these experiments is based on that of Carlsson (1972). His apparatus consisted of a large box, one side of the floor of this box was covered with a variety of objects whilst the other side was plain. This apparatus was used to test the effect of a single dose of apomorphine (1.0 mg/kg) on exploration in a between groups design. In the drug experiments presented later in this chapter the effects of low doses of dopamine blockers and stimulants will be assessed. As these manipulations are likely to be weaker than that produced by the comparatively

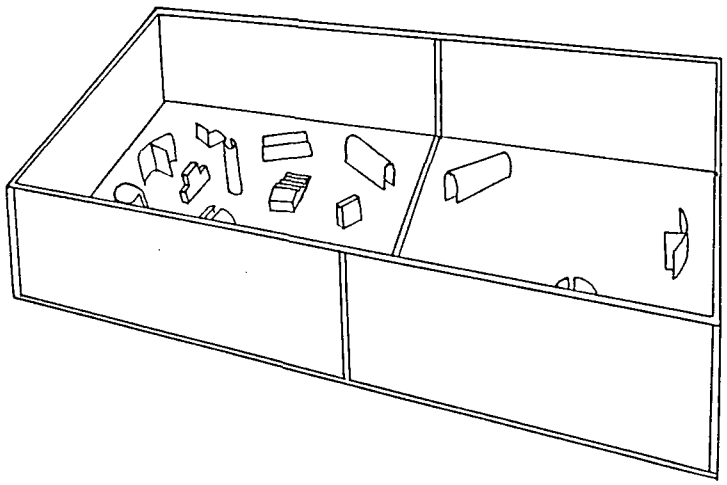
high dose of apomorphine used by Carlsson, a more sensitive repeated measures design is employed. In addition, preference will be measured not only between an object covered area and a plain one, but also between areas containing differing numbers of objects. The apparatus used in these experiments therefore consisted of a box similar to Carlsson's into which a range of different floors could be inserted. Each floor covered only half of the base of the box, thus a range of choices could be provided by using differing combinations of floors in either side of the box.

A number of assumptions made about the apparatus in the drug experiments are presented later in this chapter. These involve the reinforcing values of exploring the various floor types and the relative values of various pairs of floor types. The current experiment tests these assumptions using normal animals. In addition, the effect of habituation to the apparatus (which can be seen as an analogue of a blunting of reinforcing impact) on exploration preference was assessed.

2.1.2 Method.

Subjects.

The subjects were 12 experimentally naive male DA strain rats (Bantin and Kingman, Hull) weighing approximately 200 gm at the start of the experiment.

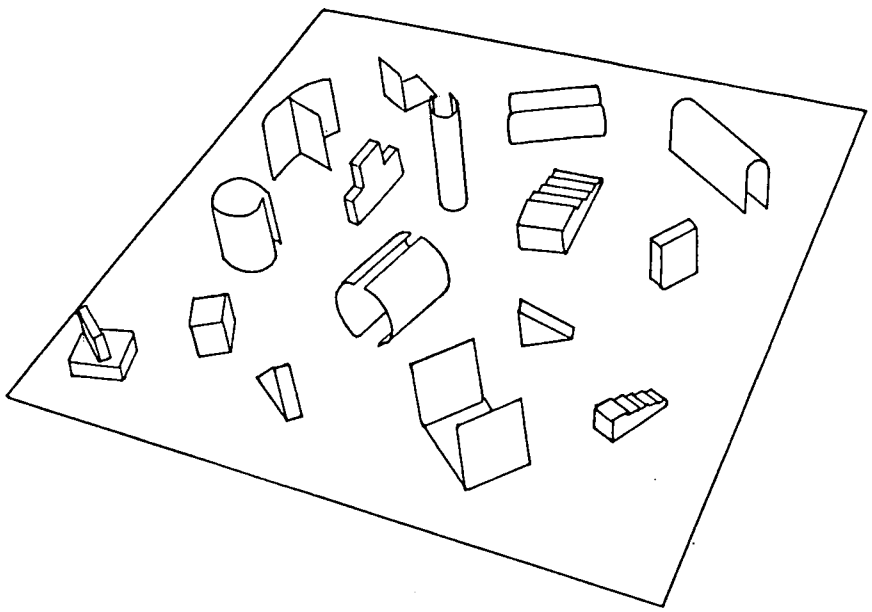


2.1 The exploration preference apparatus.

Apparatus.

The testing box measured approximately 120 cm x 60 cm x 30 cm. It is shown in figure 2.1. The sides were made of clear perspex backed with plain grey card and the top was open. The base was constructed so that two removable 60 cm x 60 cm floor panels could be inserted to form the floor of the box. The floor panels used in the experiment proper were made of black or white perspex, some of them had perspex objects glued to them. In addition, two clear perspex floor panels, backed with the same grey card as used on the side walls, were used for pretraining.

The following floor panels were used. One black floor panel with sixteen objects, also in black perspex, glued to it. Two black panels each with eight objects glued to them. The eight objects used were a subset of those used on the sixteen object panel. The two eight object panels were mirror images of one another. A black panel with four objects glued to it. The four objects were a subset of those used in the eight object panels. A black panel with no objects. A white panel with no objects. Corresponding objects were positioned in similar positions on all floors, so that when two object covered floors were inserted in the test box corresponding objects on the two panels would be in mirror image positions. The sixteen object floor is shown in figure 2.2.



2.2 The sixteen object exploration apparatus floor panel.

A large mirror was positioned at a 45 degree angle approximately 100 cm above the test box. This allowed the experimenter to observe animals in the test box without being directly visible to the animals. The only illumination in the test room was a 30 watt 25 cm long incandescent tube attached to the bottom edge of the mirror above the test box.

Procedure.

For four days prior to the start of the experiment proper each animal was placed in the test box fitted with the grey backed floor for five minutes a day to familiarise them with the test room and test box.

The experiment proper lasted fourteen days. On each day each rat was placed in the test box for five minutes with floors fitted according to the design described below. The floors were cleaned before each rat was tested to remove urine and faeces which may have attracted the animal being tested. Every ten seconds the experimenter noted down which of the two floors the animal's head was currently over and whether the animal was engaged in exploratory behaviour. An animal was defined to be exploring if it was visibly sniffing while standing rearing or walking. Non-exploratory behaviours included standing, sitting, lying and running without

sniffing, and grooming. Half an exploration was given to each floor when an animal was exploring over the joint between the two floors. These observations were used to calculate scores of the amount of time each animal spent exploring each side of the test box each session.

Design and Analysis.

It is assumed in the counterbalanced design of the drug experiments that the ratio of reinforcing values between floors for various pairings of floors is approximately equivalent. These pairings are divided into four groups within which reinforcing ratios are assumed to be similar. Since the preference which an animal shows for exploring one floor over another is dependent on the ratio of their reinforcing values, it is assumed that the preferences produced within each group of floor pairs will be approximately equivalent. That is, for all pairs of floors in a group, it is assumed that the degree of preference shown to exploring the more over the less preferred floor will be similar. These groups are based on a notional ordering of the reinforcing values of exploring the different floors. It is assumed that the exploratory value of the floors increases from the white floor to the black floor to the four object, eight object and finally sixteen object floors. The pairs of floors constituting each group are derived from the *difference* between the positions of the two floors making up a pair in this notional ordering. The pairs of floors in the *difference* 1 group are those which are adjacent in the notional ordering (e.g. the white floor and the plain black floor, or the four and eight object floors). The pairs in the *difference* 2 group differ by 2 in the value ordering (e.g. the four and sixteen object floors), or by 3 in the *difference* 3 group (e.g. the white and eight object floors). There can only be one pair in the *difference* 4 group (the white and sixteen object floors).

The assumptions specifically tested are as follows.

1. That animals do indeed show progressively more exploration of the floors higher in the notional ordering. The sixteen object floor is generally explored more that the eight object floor which in turn is explored more that the four object floor and so on.

2. That the relative amounts of exploration shown to the two sides of each pair of floors in a group is the same. For the *difference* 1 group the relative value of the black floor compared to the white floor is the same as that of the four object floor to the black floor, the eight object floor to the four object floor and the sixteen object floor to the eight object floor. Similarly for the *difference* 2 group the relative preferences for white and four objects, black and eight objects, four objects and sixteen objects is the same, as is that for pairs with a *difference* of three (white and eight objects, black and sixteen objects). There is only one pair with a *difference* of four (white and sixteen objects) so a test for the homogeneity of this group is unnecessary.

3. That the degree of preference shown to the more over the less preferred sides in each pair of floors is greatest in the *difference* 4 group, getting progressively less in the *difference* 3, 2 and 1 groups.

Finally the effect of habituation to the floors over the experiment is assessed on preferences for pairs of floors from all four *difference* groups and on the total number of explorations each animal made during the first and last four trials.

The following design was used to perform these tests. As there are five floor types there are ten possible pairings of floors. In the first ten sessions each animal was tested with every possible pair of floors. As each floor was presented in combination with every other floor, the total amount of exploration made in each floor type over these ten trials was used to measure the absolute reinforcing value of exploring each floor, and hence test the ordering assumption. Preference scores for each pair of floors collected over these ten sessions were used to assess the homogeneity of the *difference* groups and the degrees of preference shown for the more preferred sides in each *difference* group. The first four sessions were arranged so that each animal experienced one pair of floors from each *difference* group during these trials. In a final block of four sessions (days 11 to 14) each animal again experienced one pair from each *difference* group. Exploration preferences and totals from this last block and the first four trials were used to assess the effects of habituation.

A number of rules and counterbalancing procedures were used to determine the specific floors used for each animal on each day.

The floors used to form a pair for any specific *difference* were counterbalanced across animals. If one animal was tested with a *difference* 1 pair made up of white and black plain floors another animal was tested on the same day with a *difference* 1 pair made up of floors from the opposite end of the ordering scale (i.e. sixteen and eight object floors for this example). This also ensured that on any day the notionally more preferred floors were on the left hand side of the test box for half the animals and the right for the others. The side of the box on which the notionally more preferred floor was placed was alternated for each animal each day. Wherever possible no animal was presented with the same floor on consecutive days.

The order in which each animal was presented with pairs of floors from the four *difference* groups was the same for the first four sessions and for the final block of sessions, however the floors which made up these pairs were selected from opposite ends of the ordering for the initial and final blocks. This ensured that the floors experienced by half the animals in the first block were experienced by the other half in the same order in the final block and vice versa.

A variety of methods are available by which the observed number of explorations an animal makes on either floor on a particular trial may be combined to form a preference score. The simplest is to take a ratio between the number of explorations on the notionally more preferred side and that on the notionally less preferred side. Although this method is simple, it is likely to lead to statistical problems as small variations in the number of explorations on the less preferred side will have very large effects on high preference ratios and only make small differences to low preference ratios. The variance of ratio scores is therefore likely to be large in the high *difference* groups and small in the low *difference* groups.

Ferguson (1976, p.236) suggests that the use of the arc sine transformation may reduce the inhomogeneity of variance caused by proportional data. An alternative method which reduces the variance problems encountered with extreme values is to use a form of savings score. This is the method which will be used in the current experiment and the following drug studies. Preference is defined as follows:

$$\text{Preference} = \frac{E_p - E_n}{E_p + E_n}$$

where E_p is the exploration score for the notionally more preferred side and E_n that for the notionally less preferred side.

The differences these three methods make to compliance with the assumptions of the analysis of variance will be highlighted by examples of their use in the analysis of the habituation experiment. This experiment is similar in form to the analyses required by the drug studies which follow.

Huynh and Mandeville (1979) describe the tests required to assess the validity of F tests with repeated measures data. Tests of the Box M maximum likelihood ratio on all between subjects factors should be followed by Mauchly sphericity tests on within subjects factors. As there are no between subjects factors in the habituation experiment or the drug studies it follows that we need only use the Mauchly sphericity test of covariance matrices for each repeated factor. It should also be noted that this test is not required for factors having only two levels. An F test on only two levels of repeated measures data essentially reduces to a test of the deviation of the differences between scores at the two levels from zero. As such it cannot be subject to problems of inhomogeneity of variance. No tests were made for normality, as deviations from this assumption have little effect on the validity of analysis of variance (Keppel, 1973) or the Mauchly sphericity test (Huynh and Mandeville, 1979).

Plots of cell means and variances from the habituation experiment scores are also presented to indicate the effects of large preference values on variance using the three methods of calculating preference.

2.1.3 Results.

It can clearly be seen from figure 2.3 that the simple ratio method of calculating preference scores produces disproportionately large variances at high preference values. This is reflected in the severe deviations from the sphericity assumption shown for both the *difference* and *difference* by block factors in table 2.1. An arc sine transformation of these scores barely improves the situation. The savings-like method, however, shows little relationship between mean and variance and does not break the sphericity assumption for either the *difference* or *difference* by block factors, as shown in table 2.1.

The total amounts of exploration animals allotted to each of the side types during the first ten sessions are shown in figure 2.4. It can be seen that the various side types elicited differing amounts of exploration in accordance with the notional ordering of their reinforcing values. An analysis of variance showed that side type significantly affected exploration ($F = 144.1$; df $4, 44$; $p < 0.001$). An orthogonal polynomial trend analysis (Keppel, 1973) confirmed the evidence of figure 2.4 that total exploration was predicted by the notional ranking of sides in a strongly linear manner ($F_{linear} = 237.46$, df 1,44, $p < 0.001$, all other F's non-significant).

The exploration preference scores for each possible side type grouped by *difference* are shown in figure 2.5. A series of analyses of variance indicated that preference scores within the *difference* 1, 2 and 3 groups were homogeneous (all F's non-significant). As the *difference* 4 group only includes one floor pair the question

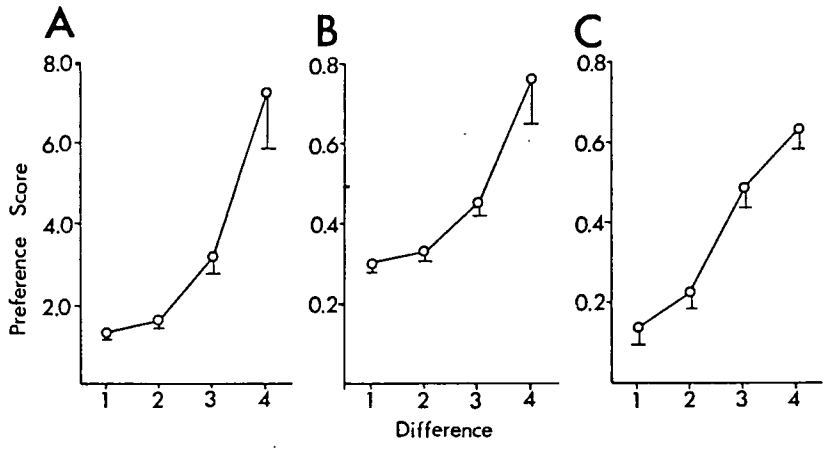
Difference.

Calculation Method	Mauchly W	χ^2	Significance
Ratio	0.0044	57.724	$p < 0.001$
Arc Sine Ratio	0.0103	44.516	$p < 0.001$
Savings	0.5667	5.517	n.s.

Block by Difference Interaction.

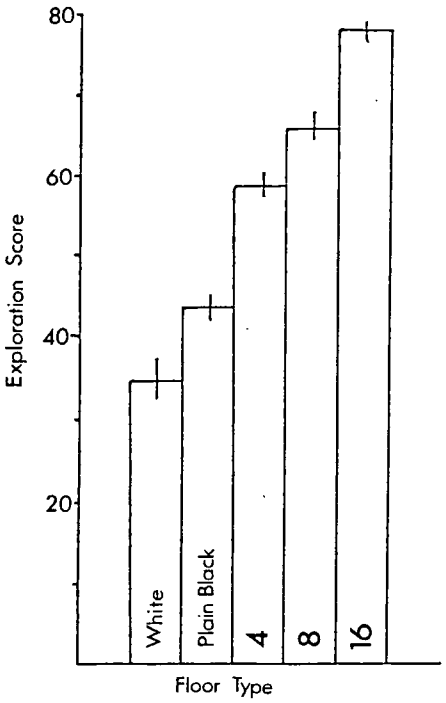
Calculation Method	Mauchly W	χ^2	Significance
Ratio	0.0146	41.073	$p < 0.001$
Arc Sine Ratio	0.0270	35.100	$p < 0.001$
Savings	0.5022	6.697	n.s.

Table 2.1 The effects of three methods of calculating preference on deviations from the assumptions underlying the analysis of variance. Preference scores were calculated from exploration data from the habituation experiment and analysed in two way repeated measures analyses of variance with the factors *difference* and block. The χ^2 test of Mauchly's W has 5 degrees of freedom in all cases.

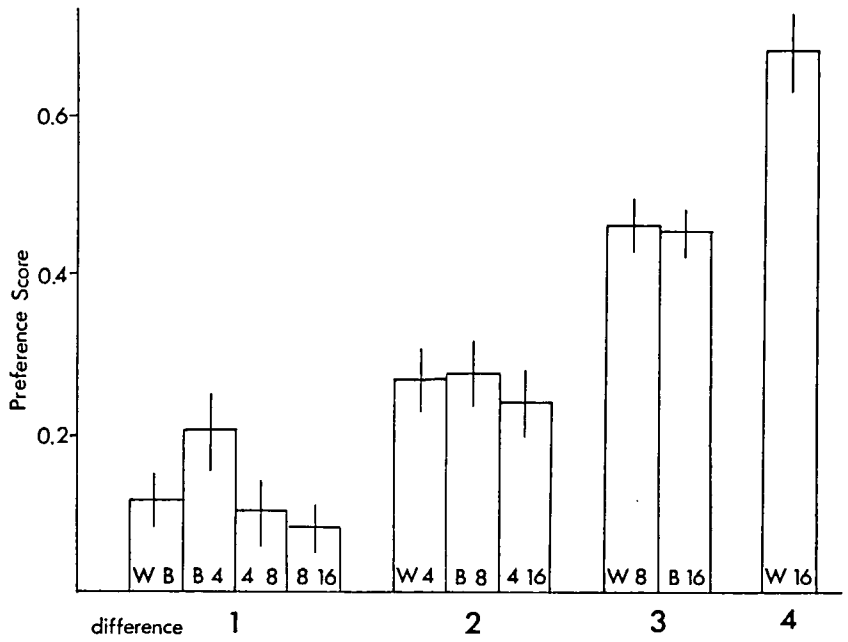


2.3 Means and standard errors of preference scores calculated using a simple ratio method (**2.3a**), an arc-sine transformation of this ratio (**2.3b**), or using a savings-like method (**2.3c**) for the four levels of *difference*. The raw scores were taken from the first four trials of experiment 2.1. The resulting mean scores and standard errors are shown.

of preference homogeneity within this group does not arise.

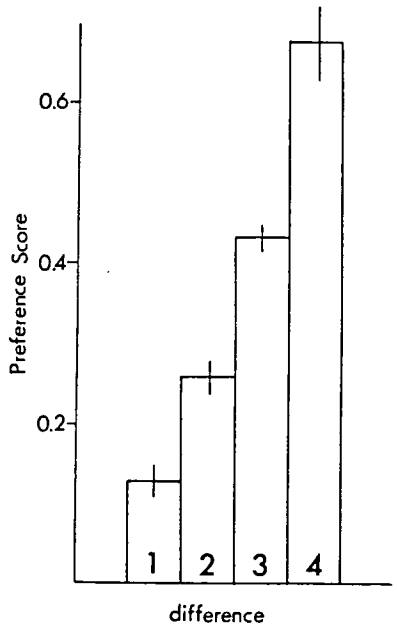


2.4 Means and standard errors of total explorations of each side type during the first ten trials of experiment 2.1.

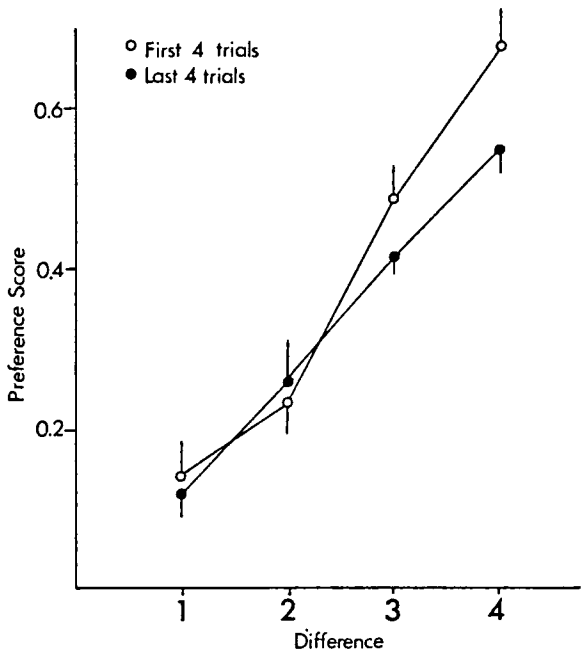


2.5 Means and standard errors of preference scores, calculated using the savings method, for all combinations of floor types. Floor combinations are grouped according to notional *differences* in reinforcing value. Scores are taken from the first ten trials of experiment 2.1.

Figure 2.6 shows the means of these score for each difference group. An analysis of variance shows that pairs of floors from the four *difference* groups produced differing preferences ($F = 98.21$, df 3,33, $p < 0.001$). A trend analysis shows that these preference are linearly related to the predicted ordering of *difference* groups ($F_{linear} = 95.90$, df 1,33, $p < 0.001$, all other F's non-significant).



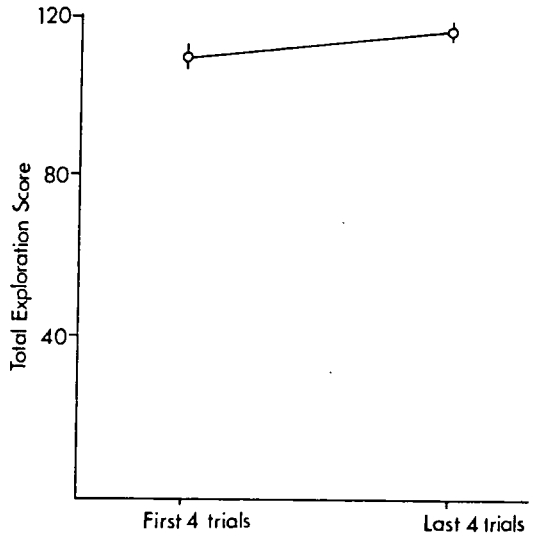
2.6 Mean preference scores, and their standard errors, produced by each *difference* group over the first ten trials of experiment 2.1.



2.7 Preference scores produced in each *difference* group during the first and last four trial of experiment 2.1. Comparison of these scores can be used to assess the effects of habituation on exploration preference. Means and standard errors are shown.

The preference scores produced by pairs of floors from each *difference* group during the first and last four sessions of the experiment were used to assess the effects of habituation. They are shown in figure 2.7. It can be seen that preferences were little changed by repeated exposure to the apparatus. A two way analysis of variance confirmed the effects of *difference* on preference scores (*difference* $F = 106.28$, *df* 3,33, $p < 0.001$). Any effects of habituation would be revealed in the trial block

factor or the trial block by *difference* interaction. Neither of these were significant, however the trial block by *difference* interaction approached significance ($F = 2.38$, df 3,33, $p = 0.087$). The test of total explorations over the first and last four trials did indicate that experience had a small but significant effect of total time spent exploring (see figure 2.8; $t = 2.77$, df 11, $p < 0.05$ two-tailed). Total exploration was higher during the final block, however, and not lower as may have been expected.



2.8 Mean total exploration (exploration scores from all floors combined) and standard errors produced during the first and last four trial of experiment 2.1. Comparison of these scores can be used to assess the effects of habituation on total exploration.

2.1.4 Discussion.

The results of the experiment are in agreement with the assumed values of the various side types and *difference* groups.

No effect of habituation on exploration preference was found. Whilst a significant effect of habituation would have been of interest as it may have indicated the form that any reward attenuating manipulation may have on exploration preference (i.e. as a simple main effect or as an interaction with *difference*), the lack of any effect does not detract from the major results of the experiment. The significant increase in total exploration between the initial and final four trial blocks indicates that, if anything, the animals may have been habituating to the various extraneous sources of distraction such as the test box walls and the testing room, rather than the exploration floors. It may be concluded that there was no habituation to the apparatus over the experiment. This, in itself, is encouraging as it indicates that there will be little attenuation of preference due to habituation, rather than to the experimental manipulations, in the drug studies presented later in the chapter.

Two features of the design call for additional discussion. The inclusion of the white floor in the range of floors was potentially problematic. The other four floors range from the fairly neutral plain black floor to rich environment of the sixteen object floor. These floors form part of a continuum of environments which are assumed to be positively reinforcing to explore. It was felt, however, that the plain white floor may have been slightly aversive to explore as the dark animals were clearly exposed whilst on the white floor. The white floor was nevertheless included in the range of floors in order to increase the range reinforcing values available (floors with 32 or more objects would have been impractical; being composed almost entirely of objects with very little 'floor' left they may have been physically difficult to explore). The results, however, indicate that the white floor still elicited some exploration and did not produce exceptionally divergent absolute exploration or preference scores relative to those obtained with the black floors.

The method used to assess absolute exploration scores for each floor type involved some compromise. Although these scores were generated from a set of trials in which every floor was paired with every other floor, this design does bias results. If the notional ordering was correct, then low value floors such as the plain white floor are paired with floors of predominantly higher value (and hence may be explored less because of the alternatives they are paired with) whilst high value floors are paired with floors of predominantly lower value. Another approach to testing the ordering hypothesis would have been to present each floor in conjunction with a 'control' floor such as the grey backed floors used in pre-training. As the drug experiments use preference scores rather than absolute amounts of exploration, however, the absolute exploration results are not crucial to the validity of the drug experiments. An extra experiment specifically testing absolute exploration does not seem warranted. It is still possible to obtain partial data on absolute exploration which is not confounded. The absolute amount of exploration of four of the floor types can be compared when each is paired with the fifth, and hence absolute preferences can be obtained for four of the floors. This procedure can be carried out using each of the floors as the 'control' floor in turn. Such analyses have been performed and their results are in full agreement with the analysis of variance and trend analysis of absolute exploration of floor types presented.

In conclusion, this experiment fully confirms the assumptions made in the design of the apparatus for use in the following drug experiments.

Experiment 2.2 – A Test Battery Assessment of the Effects of the Dopamine Blocker α-Flupenthixol on Exploration.

2.2.0 General Introduction.

The first experiment of this series (2.2.1) examines the effect of dopamine blockade on exploration preference using the apparatus which was tested in experiment 2.1. It is hypothesised that if dopamine plays a rôle in the mediation of reinforcement, then dopamine blockade produced by an antagonist drug may reduce the extent to which environments differing in complexity are preferred over one another. The choice based measure of the preference test should greatly reduce the

extent to which motor impairments produced by dopamine blockade can influence the results.

The drug selected for use in this experiment is α-flupenthixol. It is a selective dopamine receptor antagonist having no effect on other catecholamine receptors, is readily soluble in water and reaches peak effectiveness two and a half to three hours after intra-peritoneal injection (Møller-Neilsen, Pedersen, Nymark, Franck, Boeck, Fjalland and Christensen, 1973).

Additional experiments in this series investigate other mechanisms which may bring about a reduction in exploration preference.

A measure of the motor effects of the doses of flupenthixol used was obtained by assessing the drug's effects on activity in cages which were very similar to the animals' home cages. The environment in such cages does not contain any objects which may elicit exploration and is familiar to the rats, as such, any drug induced reduction in activity in these cages is more likely to be due to motor impairments than to anhedonic effects. This experiment is described in section 2.2.2.

One further possible confounding factor is the effect of flupenthixol on the aversive properties of exposure in the centre of an open space. Animals initially tend to stay near the walls of an open field and avoid spending much time in the centre of the field (Williams and Russell, 1972). This tendency may exaggerate the differences in exploration between floors with many objects and those with few objects in which the rat is more likely to be in an exposed position. Although we may hypothesise that flupenthixol attenuates the negatively reinforcing consequences of exposure, such an effect could also be produced by more general anxiolytic properties of the drug. The experiment is aimed at examining the attenuation of positive reinforcement derived from exploration, rather than anxiolytic effects. A test was therefore made of the drug's effects on animal's tendency to avoid exposure in a large open space using an emergence procedure (Archer, 1973) in order to assess the contribution of drug induced attenuation of the aversive properties of exposure to any changes in exploration preference. This experiment is described in section 2.2.3.

Finally, a hole board test (File and Wardill, 1975) was carried out as an alternative measure of drug effects on exploratory behaviour, it is described in section 2.2.4.

Experiment 2.2.1. – The Effect of α-Flupenthixol on Exploration Preference.

2.2.1.1. Introduction.

In this experiment the effect of the dopamine antagonist α-flupenthixol on exploration is investigated using the apparatus assessed in the previous experiment. If dopamine plays a part in reinforcement processing it may be hypothesised that flupenthixol will attenuate exploration preference. An embedded experiment assessed the effect of flupenthixol on the absolute amount of exploration alloted to two of the floors when they are paired with a third 'control' floor. It also provides a measure of the drug's effects on the total amounts of exploratory and non-exploratory

behaviour performed by the animals.

Although the experimental design is based on a choice measure, and so is less susceptible to confounding by motor impairments, potential motor effects are reduced further by the use of very low doses of the drug (0.01 and 0.03 mg/kg). The higher of these doses has, however, been shown to effect response rates for food reinforcers (Robbins and Watson, 1981). A dose of 0.025 mg/kg has been shown to significantly effect dopamine release and metabolism in vivo (Imperato and Di Chiara, 1985).

2.2.1.2 Method.

Subjects.

The subjects were 12 experimentally naive male DA strain rats (Bantin and Kingman, Hull) weighing approximately 200 gm at the start of the experiment.

Drugs.

Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at doses of 0.01 and 0.03 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The exploration apparatus described in the previous experiment was used.

Procedure.

For four days prior to the start of the experiment proper each of the animals was placed in the test box fitted with the grey backed floor for five minutes a day in order to familiarise them with the test room and test box.

The experiment proper lasted fifteen days. Two and a half hours before testing each animal was injected intra-peritoneally with either 0.01 or 0.03 mg/kg flupenthixol or an equivalent volume of saline. The experiment was run as a 'blind' study in order to avoid unintentional biases in the rating of animals' behaviour. The bottles containing the saline and drugs were labelled 'A', 'B' and 'C' by a technician according to a code not known by the experimenter, the code only being revealed to the experimenter at the end of the experiment. On each day each rat was placed in the test box for five minutes with floors fitted according to the design described below. The floors were cleaned before each rat was tested to remove urine and faeces which may have attracted the animal being tested. Every ten seconds the experimenter noted down which of the two floors the animal's head was currently over and whether the animal was engaged in exploratory behaviour. An animal was defined to be exploring if it was visibly sniffing while standing rearing or walking. Non-exploratory behaviours included standing, sitting, lying and running without sniffing, and grooming. These observations were used to calculate scores of the amount of time each animal spent exploring each side of the test box each session.

The design accommodates two embedded experiments. The first assesses the effect of two doses of flupenthixol and saline on exploration preference at each of the four side *difference* levels. The second examines the effects of the drug on the absolute amount of exploration alloted to the plain black and eight object floors, when both are presented in conjunction with the four object floor. Data from this second experiment can also be used to assess the effects of the drug on the total amount of exploration animals make in the apparatus, rather than on its distribution between sides.

In the preference experiment a number of different sequences of *differences* and sets of floors were used to present the four side *differences* three times. These sequences were counterbalanced within three groups of four rats for presentation order and for the floors used to construct any particular *difference*. If one animal was tested with a *difference* 1 pair made up of white and black plain floors another animal was tested on the same day with a *difference* 1 pair made up of floors from the opposite end of the ordering scale (i.e. sixteen and eight object floors for this example). Drug presentation order was counterbalanced across the three groups of rats. No animal was presented with the same drug or the same floor type on consecutive days within the embedded preference experiment.

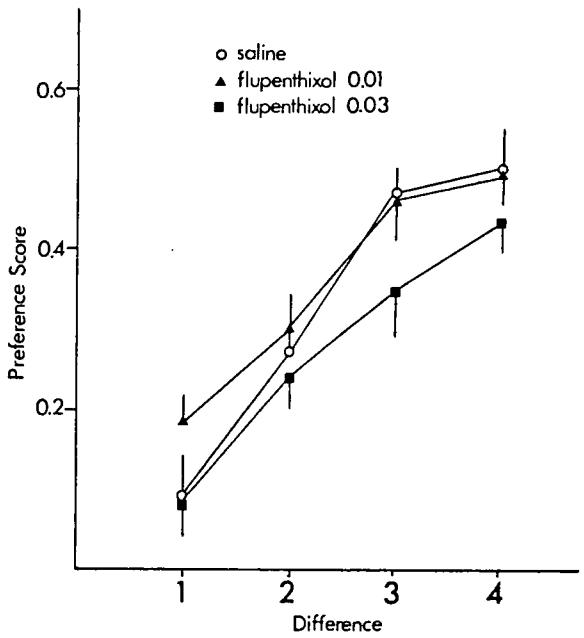
The absolute exploration experiment required the pairs plain black vs. four objects and eight vs. four objects to be presented in each of the three drug conditions (0.01 and 0.03 mg/kg flupenthixol and saline). For each animal three of these trials were carried out as part of the preference experiment. The three additional trials required were inserted into the design on the fifth, tenth and fifteenth days of the experiment. Unfortunately some repetition of floors or drug conditions from the previous day's testing was unavoidable in these inserted trials.

Throughout the entire experiment the side of the box on which the notionally more preferred floor was placed was alternated for each animal each day.

2.2.1.3 Results.

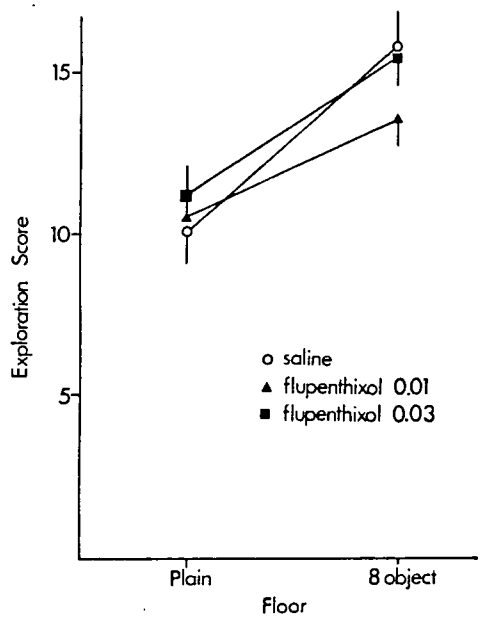
The results of the preference experiment are shown in figure 2.9. A two-way repeated measures analysis of variance of preference scores showed that preference was significantly affected by floor *difference* ($F = 31.12$, *df* 3,33, $p < 0.001$) and drug level ($F = 5.86$, *df* 2,22, $p < 0.01$), but that no drug by *difference* interaction occurred ($F < 1$). However, a test of the sphericity of covariance matrices in the repeated measures analysis of variance design indicated that there was significant deviation from the statistical model in the factor drug, which could lead to an overestimation of the significance of the drug effect (Mauchly's $W = 0.347$, χ^2 (with $2df$) $= 10.565$, $p < 0.005$).

Although the use of the savings-like preference score eliminates the systematic relationship between the means and variances of cells in the analysis, repeated measures analysis of variance also requires assumptions about the covariances between scores at different levels of a factor. A sufficient, but not necessary condition for valid F ratios in repeated measures analysis of variance is that the covariances should be approximately equal. In conjunction with the assumption of equality of variances this condition implies an assumption of compound symmetry of the covariance matrices. Huynh and Feldt (1970) have shown that the necessary and sufficient condition is the equality of variances of *differences* for all pairs of treatment measures assumed to be correlated. This condition is tested by the sphericity



2.9 The effect of α-flupenthixol on exploration preference at each level of side *difference*. Means and standard errors are shown.

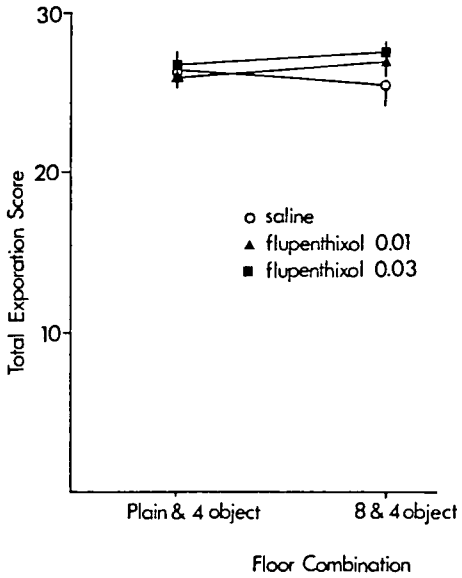
test. If this condition is not met the size of the error term in the analysis will be underestimated and hence the significance of the F ratio will be overestimated. The extent of this overestimation can, however, be calculated and a correction can be made. Greenhouse and Geisser's epsilon provides a means of correcting for the overestimation of significance caused by deviations from compound symmetry of the covariance matrix by making a conservative adjustment to the degrees of freedom associated with the offending factor (Howell, 1982).



2.10 The effect of α-flupenthixol on exploration of the plain and eight object floors when presented paired with a four object 'control' floor.

The Greenhouse-Geisser epsilon for the factor drug was found to be 0.60521, yielding corrected degrees of freedom of 1.2, 13.3. This correction still indicated that there was a significant effect of drug on preference ($F = 5.86$, df 1,13, $p < 0.05$).

Figure 2.10 shows the effects of flupenthixol on means (with standard errors) of the absolute exploration scores produced by the plain black and eight object floors when both were presented in conjunction with the four object floor. No significant effects of drug or a drug by floor type interaction were found. Floor type significantly affected amount of exploration ($F = 30.58$, df 1,11, $p < 0.001$).



2.11 The effect of α-flupenthixol on total exploration of the plain plus four object and eight object plus four object floor combinations. Means and standard errors are shown.

The total scores from these sessions (all explorations made regardless of side) are shown in figure 2.11. An analysis of variance showed that there were no significant differences in the total amount of exploration due to either floor combination, drug or a floor combination by drug interaction.

2.2.1.4 Discussion.

The results of the preference experiment show that flupenthixol does effect exploration preference. In addition, flupenthixol was not shown to decrease the total amount of exploratory behaviour performed by animals in the apparatus. This indicates that the drugs effect on preference cannot be attributed to a motor deficit which effects exploration on different floors disproportionately. Such a deficit should produce a decrease in total exploration, and no such decrease was found. It can therefore be concluded that flupenthixol affected preference through an action not related to motor systems. No evidence was found for an effect on the absolute amount of exploration made in the plain and eight object floors.

It may be argued that the analysis of the total explorations was of lower power than the analysis of preferences and thus less likely to reflect any underlying drug effect. An analysis of total explorations was performed on data from the full pref-

erence experiment and it too found no evidence of drug effects, floor combination effects or interactions. In fact, throughout the experiment animals appear to spend most of their time exploring, regardless of drug conditions or floor types. The only aspect of their behaviour which varied significantly was the distribution of these explorations between floors.

Figure 2.9 shows the effect flupenthixol had on exploration preference. The high dose of flupenthixol clearly attenuates preferences at *differences* 3 and 4. At all *differences* apart from 1 the low dose of flupenthixol appears to have little effect on preference. At *difference* 1 the low dose of flupenthixol appears to accentuate preference, however, an analysis of simple main effects at *difference* 1 indicated that there was no significant drug effect. This pattern of results is consistent with an attenuation of the rewarding impact by the high dose of flupenthixol.

Although an attenuation of preference by flupenthixol has been demonstrated it need not necessarily have been caused by interference with reinforcement mediating mechanisms. However, the lack of a drug effect on total exploration score makes a motor explanation of the preference effect implausible. At this point we may conclude that flupenthixol has an effect on exploration preference which is consistent with a reinforcement mediating rôle for dopamine, and which is unlikely to be explained by any motor effect of the drug.

Experiment 2.2.2. – The Effect of α-Flupenthixol on Non-Goal Directed Activity.

2.2.2.1. Introduction.

In the previous experiment the effect of flupenthixol on exploration preference was investigated. Although the design of the experiment limited the extent to which drug induced motor impairments could influence the results of the preference experiments, low doses of flupenthixol, which were unlikely to produce such motor deficits, were nevertheless used. The present experiment provides a more direct assessment of the effects of flupenthixol on motor systems at the dose levels used in the previous experiment. Evidence that these doses of flupenthixol do not produce significant motor impairments would confirm the hypothesis that the preference attenuation obtained in the previous experiment cannot be attributed to a motor deficit.

Any measure of a manipulation's effect on behaviour must, to some extent, be a combination of a measure of the manipulation's effects on the ease of performance of behaviour and on the reinforcer which elicits that behaviour. The aim of the present experiment is to assess flupenthixol's effect on motor systems, it is therefore desirable that the behaviour studied should as little reinforcer controlled as possible. A measure of animals' spontaneous activity in a familiar environment containing no explicit sources of reinforcement, such as objects or food, meets this requirement. Testing was therefore conducted in cages very similar to the animals' home cages. An additional dose of 0.09 mg/kg flupenthixol was also tested.

The higher dose of flupenthixol was used in addition to the doses used in the

previous experiment in order to confirm that the apparatus could provide a sensitive measure of a drug induced reduction in activity.

2.2.2.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at doses of 0.01, 0.03 and 0.09 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The apparatus consisted of four identical testing cages. Each of these cages was identical to the animals home cages (NKP Plastics, Dartford, Kent) with sawdust spread on the floors, except for the following modifications. Three 4 mm diameter photocells were mounted at equal distances along one of the long sides of the cage at a height of 3 cm and three 10 mm diameter focussed infra-red emitters were mounted in corresponding positions along the other long side of the cage. The photocells and emitters were mounted flush with the inner wall of the cage. Each photocell was connected to circuitry which produced a 5 volt output when the beam between the emitter and the photocell was unbroken, and 0 volt output when it was interrupted. The outputs from the photocells on all four boxes were connected to a BBC model B microcomputer (Acorn Computers, Cambridge) via a Control Universal (Cambridge) optically isolated lab interface. The computer was programmed in a combination of BBC Basic and 6502 assembly language, all potentially time critical response detection being handled in assembler. Each change in state of each cell was logged and time binned totals of these changes were recorded for each test cage.

The test cages were kept in the same room as the animals' home cages, being housed at the bottom of the home cage rack. The computer and interface were kept outside the room.

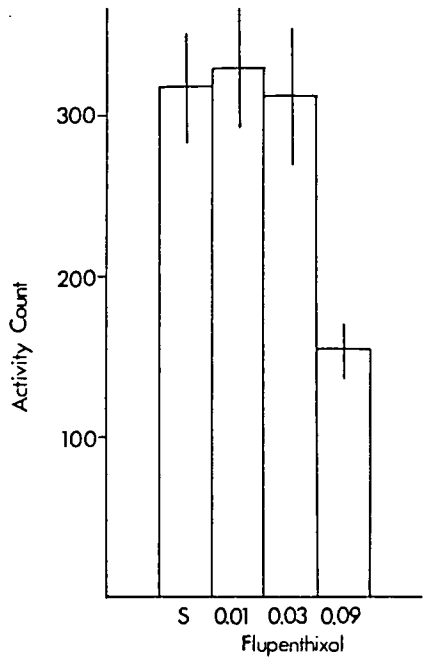
Procedure.

The experiment started directly after the end of the previous experiment and lasted four days. On each day each animal was injected with saline or flupenthixol approximately two and a half hours before it was tested. Testing simply consisted of transferring the animal from its home cage to a testing cage in the same room. Each animal was tested in the same testing cage at the same time each day. There was no food in the test cages' hoppers, nor any water bottles. Recording of activity on the computer outside continued for 30 minutes. After testing the animals were returned to their home cages and the sawdust in the test cages was replaced with clean sawdust in readiness for the next batch of animals.

The order of drug treatments was counterbalanced across animals.

2.2.2.3 Results.

The results of the experiment are presented in figure 2.12. Time binning of the activity counts did not effect the results so only total activity counts over the 30 minute sessions are presented. A one-way repeated measures analysis of variance showed that drug dose significantly effect activity ($F = 7.28$, df 3,33, $p < 0.001$). As can be seen from the figure, this effect can be entirely attributed to the decrease in activity brought about by the 0.09 mg/kg dose of flupenthixol. The lower doses used in the previous experiment (0.01 and 0.03 mg/kg) did not effect activity.



2.12 The effect of α-flupenthixol on activity monitored in the 'home-cage' activity boxes. Mean counts and standard errors are shown.

2.2.2.4 Discussion.

The experiment clearly shows that the doses of flupenthixol used in the previous experiment did not effect spontaneous locomotor activity. It may therefore be inferred that these doses do not produce any significant motor impairment. The large drop in activity produced by the higher dose shows that flupenthixol can reduce activity and that the apparatus used was capable of detecting such an effect. It may be concluded that the effect on exploration preference found in the previous experiment cannot be attributed to non-specific locomotor impairment.

Experiment 2.2.3. – The Effect of α-Flupenthixol on Emotionality as Measured by an Emergence Test.

2.2.3.1. Introduction.

Having determined that the attenuation of exploration preference demonstrated in experiment 2.2.1 cannot be attributed to a motor impairment, this experiment evaluates another mechanism which could compete with the hypothesised reinforcement attenuating effect of flupenthixol in explaining the exploration preference results.

The model of dopamine's action in which the impact of reinforcement is held to be attenuated by flupenthixol explains the attenuation of preference by assuming that the reinforcing effect of exploring floors in the exploration apparatus is decreased by flupenthixol. This drop in reinforcement decreases the extent to which one floor is preferred over another. An alternative explanation is to assume that flupenthixol attenuates the negative consequences of staying in the less preferred floors. Rats initially avoid the centres of large open spaces, preferring to stay near the walls of open fields (Williams and Russell, 1972). This can be interpreted as an avoidance of exposure to potential predators. Floors with many objects on them have little exposed floor area, while those with few or no objects consist predominantly of exposed area.

An attenuation of the negative consequences of open field exposure can be couched in terms of a reduction in the impact of negative reinforcement, terms very similar to those used in the reinforcement hypotheses being tested. They may also be interpreted in terms of a more generalised reduction in emotionality. Indeed, the effects of dopamine blocking drugs in humans have been reported as a sensation of "emotional blunting" (Belmaker and Wald, 1977). In the context of this series of experiments it is not possible to distinguish behaviourally between these two mechanisms. We cannot distinguish between an animal which subjectively perceives the threat of exposure correctly but is less 'worried' by it, and one which simply fails to perceive the threat correctly.

Emotionality may be measured in a number of different ways, however, many methods use a level of discomfort which is incompatible with exposure in an open field. The emergence test, however, uses open field exposure itself as the stressor and is hence highly suitable as a measure of the effects of flupenthixol on emotionality for the current purposes.

2.2.3.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The apparatus consisted of an emergence tube and an open area into which the animal must emerge. The emergence tube was a section of circular plastic pipe 22 cm long and 5 cm in diameter closed off at one end. A piece of hardboard card was used to cover the open end of the pipe. The experimental area was designed to be as exposed as possible. A white melamine surface 50 cm *times* 150 cm mounted on a trestle 80 cm above the floor was used. The surface was illuminated by a bright fluorescent tube 100 cm above it and a 150 watt photo-flood mounted on a 50 cm stand at the opposite end of the surface from the emergence tube.

Procedure.

The experiment took place four days after completion of the previous experiment.

The animals were randomly assigned to two groups of six. Approximately two and a half hours prior to testing the rats were injected with either 0.03 mg/kg flupenthixol or saline depending on their group.

Animals were transferred to the testing room, which they had never previously entered, individually. The animal was then placed inside the emergence tube with its head pointing towards the open end, and the open end was covered over. The tube was placed at one end of the emergence area so that when the cover on the open end of the pipe was removed the animal could walk out into the emergence area. The experimenter stood behind the emergence tube and kept silent from this point. After 30 seconds the cover was removed and a stop-watch was started. The times taken for the animal to place both of its front feet outside the tube and to place all four feet outside the tube were recorded. The experiment ceased after five minutes if the animal had not emerged from the tube by then, uncompleted emergences being scored as taking five minutes.

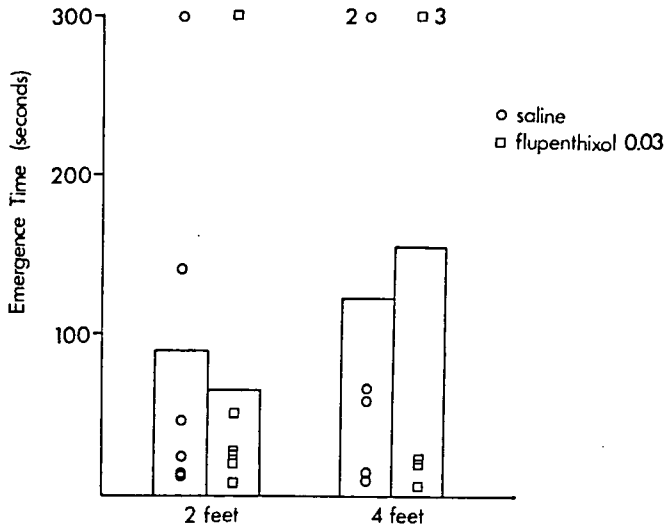
2.2.3.3 Results.

The two and four feet emergence times are presented in figure 2.13. As the group sizes are small a non-parametric test is appropriate. Mann-Whitney U's were calculated for the effects of flupenthixol on both two and four foot emergence, neither the effect on two or four foot emergence was significant (two feet, $U = 15.6$, n 6,6, non-significant; four feet, $U = 17.0$, n 6,6, non-significant).

2.2.3.4 Discussion.

The lack of a drug effect on emergence indicates that the exploration preference results of experiment 2.2.1 cannot be plausibly explained by a flupenthixol-induced decrease in animals' responsiveness to the threat of open field exposure. Having ruled out both motor effects and changes in the perception of, or emotional response to, open field exposure in the dose range of flupenthixol used, we may conclude that the mechanism underlying experiment 2.2.1's results was due to a change in reinforcement associated with exploration.

It may be argued that the between groups emergence test used in this experiment lacked statistical power compared to the repeated measures design of the exploration preference experiment. It should be noted, however, that this emergence design is capable of detecting significant drug effects (see experiment 2.3.1). Furthermore, the open field conditions used in the emergence experiment were far



2.13 The effect of α-flupenthixol on two- and four-foot emergence times. Individual times and group means are shown.

more extreme than any potentially aversive open field conditions which may have occurred during the exploration experiment. One might therefore expect the emergence experiment to detect any change in emotionality or the impact of negative reinforcement which could have affected the results of the exploration preference experiment.

Experiment 2.2.4. – The Effect of α-Flupenthixol on a Standard Measure of Exploratory Behaviour – the Hole Board Test.

2.2.4.1. Introduction.

Having established that flupenthixol effects the distribution of exploration between environments of differing complexity, and that this effect cannot be attributed to changes in locomotor ability or responsiveness to the negative consequences of open field exposure, this experiment investigates the drug's effect on a standard measure of exploratory behaviour. It is of interest to compare the results of the preference experiment with those obtained using a standard test. Such a comparison may indicate the sensitivity of the preference design. Although standard tests do not attempt to control for motor impairments in the way that the preference design does, in conjunction with tests for motor impairments and emotionality, they too may be used in the assessment of the effects of manipulations on the hedonic aspects of reinforcement if they are sufficiently sensitive.

The standard test chosen is the 'hole board' or 'nose poke' test (File and Wardill, 1975). It has clear practical advantages when compared to the exploration preference experiment in that it may potentially be automated. As used here, it is much quicker to administer than the exploration preference procedure, although it is often used in multiple session, repeated measures, designs.

2.2.4.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at doses of 0.03 and 0.09 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The hole board was designed to fit into the testing box used in the exploration experiment. As it was the same size as a single exploration floor a grey card backed perspex sheet was fitted into the testing box in order to divide it in half. The hole board itself was made of black perspex (30 cm $\times$ 30 cm) with nine 2 cm diameter holes drilled in it in a regular grid pattern. Electronic counters connected to photocells fitted underneath the board allowed objective measurement of the number of times an animal placed its head in the holes. The test room illumination was the same as that used in the exploration preference experiment.

Procedure.

The experiment was carried out twice, once using saline and 0.03 mg/kg flupenthixol and once using saline and 0.09 mg/kg flupenthixol. The first run took place eight days after the completion of the emergence experiment and the second run five days after that.

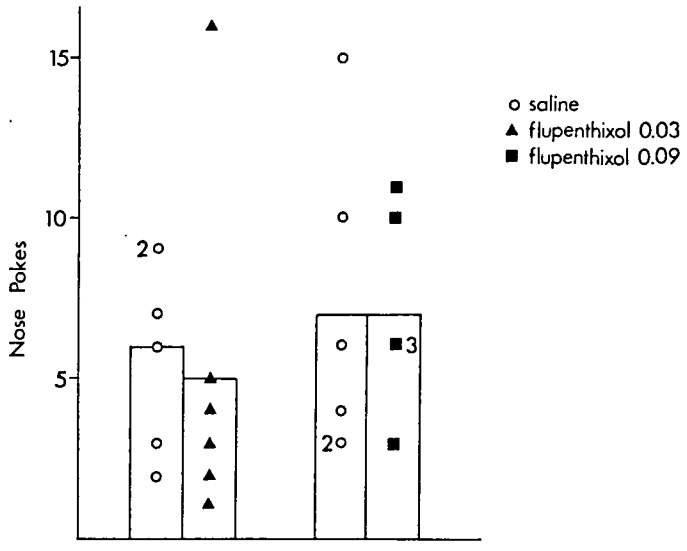
For the first run (0.03 mg/kg flupenthixol) animals which had been assigned to the saline group in the emergence experiment were assigned to the drug group and those which had been in the drug group were assigned to the saline group.

For the second run (0.09 mg/kg flupenthixol) half of the animals from the first run's drug group were assigned to the saline group and half to the drug group.

The procedure for both runs was identical. Animals were injected with saline or drug, according to group, two and a half hours before testing. Testing consisted of placing the animal in the test box fitted with the hole board floor for five minutes. As the electronic counters could be triggered by animals feet and tails entering the holes in addition to nose pokes, the experimenter observed animals during testing and noted any 'spurious' counts not triggered by the animals heads entering the holes.

2.2.4.3 Results.

The results of both the 0.03 and 0.09 mg/kg experiments are presented in figure 2.14. The data used were the electronic counter scores less any observed 'spurious' counts. Mann-Whitney U tests failed to show any significant effect of flupenthixol on nose poke behaviour at either dose level (0.03 mg/kg U = 12.0, n 6,6, non-significant; 0.09 mg/kg U = 15.5, n 6,6 non-significant). These tests were conducted as one-tailed tests since the results of the exploration preference experiment provide a clear hypothesis of the expected direction of any drug effect.



2.14 The effect of α-flupenthixol on exploration measured by 'nose-pokes' in the hole board test. Individual scores and group means are shown.

2.2.4.4 Discussion.

It may be concluded that the nose poke test, as used here, is a less sensitive measure than the exploration preference procedure. This is quite understandable as only short single trials were used. Nevertheless, the nose poke test may not be ideally suited to long repeated measures designs since animals are likely to habituate to the relatively simple test environment over prolonged testing. In order to obtain similar power to the exploration preference experiment the nose poke design would require a much larger subject cohort. It is worth noting, however, that the nose poke test conducted exactly in the manner used here can detect significant drug effects on exploratory behaviour (see experiment 3.2.4).

2.2.5 General Discussion.

The results of this series of experiments support the hypothesis that flupenthixol interfered with dopamine mediated reinforcement processes. The 0.03 mg/kg dose of flupenthixol attenuated exploration preference without reducing animals' total amounts of exploratory behaviour. This indicated that the effect could not be attributed to a motor deficit. The possibility of a motor based mechanism for the effect was eliminated when it was shown that 0.03 mg/kg of flupenthixol did not reduce home cage activity. The emergence experiment indicated that this dose level of flupenthixol did not reduce responsiveness to open field exposure, another reasonable explanation of flupenthixol's effect on exploration preference.

Strong evidence has therefore been produced that dopamine plays a rôle in responses to non-food reinforcers. Further studies will attempt to consolidate this finding by examining the effects of dopamine stimulants on exploratory behaviour in similar series of experiments.

The experiments in this chapter have not explicitly attempted to dissociate hedonic and response eliciting effects of dopamine on reinforcement processes. Nevertheless, it may be conjectured that a decrease in the ability of reinforcers (in this case, objects to be explored) to elicit preparatory behaviour may lead to an overall reduction in exploration. As no overall reduction was found one may be tempted to infer that flupenthixol attenuated hedonic impact rather than response elicitation. It is, however, difficult to say whether approach behaviours are indeed preparatory in the context of exploration. No conclusions will therefore be drawn as to whether the effect on reinforcement found in these experiments, and those that follow, are due to hedonic or response elicitation effects. Experiments which attempt explicitly to dissociate these mechanisms are presented in chapter 5.

The method of assessing exploration used here succeeded in detecting a change of behaviour induced by a relatively weak drug dose (in comparison to Carlsson's 1.0 ml/kg apomorphine for example). It is worthwhile considering the features of this method which allow such sensitivity in comparison to other methods of measuring exploration, in particular Carlsson's (1972) design on which the current method was based.

Exploration may be defined as behaviour which primarily provides an animal information about its environment. Berlyne (1960) divided into exploration into 'inquisitive exploration' which "brings the animal into contact with objects that are not already represented in the stimulus field", and 'inspective exploration' which "yields further stimulation from objects already acting on receptors". As such it can be seen that exploration can involve a wide range of behavioural phenomena, and hence potential measures. In common with all other behavioural measures of an underlying phenomenon, the measurement of exploration is potentially confounded by unrelated variations in the ability or propensity of animals to behave in general. The problem is in many ways more severe for exploration since it is so closely linked to levels of locomotor activity. Two methods are used to minimise locomotor effects in measures of exploration; tests can be designed which reduce the contribution of locomotion to the measure (e.g. choice measures or tests which require very little movement from the animal), alternatively the experimenter may distinguish between explicitly exploratory behaviour and other activities. These two methods can, of course, be combined.

The tendency of animals to enter less recently explored arms of a maze (spontaneous alternation) has been investigated as a measure of exploratory behaviour (e.g. Montgomery, 1951). Although alternation is a choice measure, and thus less susceptible to locomotor effects than absolute measures of behaviour, it is questionable whether alternation in a standard maze is necessarily dependent on the reinforcing properties of exploration. Halliday (1968) argues that although the novelty which leads to initial choices of maze arms is reinforcing (as an opportunity for inquisitive exploration), alternation is subsequently dependent on rates of habituation to this novelty, and not on reinforcement per se. This situation can, however, be remedied by changing the environments in the maze arms after each choice (see chapter 4). Problems also arise because of marked individual differences in animals' baseline tendency to alternate. Repeated measures designs can reduce the effects of these differences, however, habituation between test sessions is likely to reduce the sensitivity of the measure if more than a few trials are used. In fact, as Halliday notes, maze based measures of exploration generally tend to be rather insensitive. This lack of sensitivity limits maze based measures applicability to assessing the effects

of low drug doses on exploration.

Novelty can also be used to provide reinforcement in a runway task. It has been shown that animals will run faster down a runway to a goal box which contains novel objects than to one in which objects are unchanged between trials (Schneider and Gross, 1964). This task is very dependant on locomotor ability, and hence problematic for assessing the effects of dopamine blockade on exploration.

Berlyne (1955) developed an apparatus in which the reinforcing value of approaching novel objects can be measured independently of rates of locomotion and which does not involve contributions from the decreased value of stimuli to which animals have habituated (as in alternation tasks). The apparatus consists of a test box with an alcove at one end in which objects can be placed. Exploratory behaviour can be measured as the number of times, or length of time, an animal places its head in the alcove during a test session. This apparatus can be automated by the use of a photocell across the alcove entrance. The objects used can be varied across sessions. Although this test is independent of rates of behaviour, and hence distinguishes between exploration and non goal directed locomotion, motor impairments could nevertheless effect the measure. In cases where such impairments are likely a choice measure is preferable, however, Fink and Smith (1980) have used apparatus of this type to investigate the effects of various dopamine manipulations on exploration.

A method which is related in some ways to these tests is light contingent bar pressing. Here an animal obtains the opportunity to gain additional information about its environment not by moving into a novel space, but by illuminating a darkened test chamber for a short period. The animal controls this access to information through bar pressing in an operant schedule where illumination is the reinforcer. Such operant techniques are not confounded by variations in non-goal directed locomotion and allow automated measurement of exploratory reinforcement. Light is, however, a very weak reinforcer, and the bar press response is subject to drug induced motor impairments. Light contingent bar pressing dose not therefore seem a suitable measure of drug effects on exploratory reinforcement.

Non-goal directed locomotion can also be discriminated from exploration by explicitly measuring both types of behaviour in time sampled observational studies. For example, an animal may be defined to be exploring only if it is visibly sniffing when it is observed. Such observational methods have been used to assess exploratory behaviour in simple open fields (Russell, 1973; Russell and Chalkly-Maber, 1979). The plain open field is more often used as a measure of emotionality than exploration (Archer, 1973); in such a sparse environment the effects of manipulations on emotionality may outweigh those on exploration. As has been noted, when the hypothesis being tested is concerned with hedonia, discrimination between effects on the positive hedonic consequences of exploration and the negative ones of open field exposure are difficult to distinguish.

Open fields can, however, be modified so that they elicit more exploratory behaviour and hence probably reduce (but do not eliminate) the negative contribution of emotionality effects to measures of exploration. The hole board consists of a plain arena with holes drilled in the floor. The tendency of animals to explore these holes (by poking their noses into them) is a measure of exploratory behaviour (File and Wardill, 1975). Nose pokes are in effect defined as exploratory behaviour and all other behaviour is discounted from the measure. Once again, the exploratory

measure may be confounded by motor effects.

Carlsson's (1972) apparatus is basically an open field containing richly structured and sparse environments. As exploration is assessed by time sampled observation, non goal directed locomotion is not included in exploration scores. The apparatus also allows preferences for the structured over the sparse environments to be measured. As has been argued previously, a choice measure such as this is less likely to be influenced by motor impairments than an absolute measure of exploration.

Carlsson's study was specifically concerned with the effects of apomorphine on exploration and habituation, he therefore used a single comparatively long test session in a between groups design (a repeated measures design being inapplicable to a test of within session habituation). The exploration studies described in the current and following chapters are concerned with exploratory reinforcement but not habituation, in fact habituation should be minimised if possible. The current design therefore used a number of much shorter test sessions within which little habituation will occur. These multiple sessions allowed a repeated measures design to be used which has the advantage of reducing the effects of individual differences in the analysis of drug effects. The use of multiple floor types should also decrease between sessions habituation. Although the floors used objects which were similar, being subsets of a common pool, animals experienced different overall floor configurations each day, very few of which were repeated within the experiment. The use of multiple floor types served two purposes, in addition to reducing between sessions habituation it also allowed drug effects to be examined at various levels of *difference* in complexity between sides.

In summary, Carlsson's apparatus, which is already suitable for assessing the effects of dopaminergic manipulations on exploration, was improved for purposes of the current experiments by minimising both within and between sessions habituation. The low levels of habituation produced consistently high levels of exploration throughout the experiment and therefore limited the possibility of floor effects on overall exploration when dopamine blockers were administered. The high overall levels of exploration also allowed reasonable ranges of preference scores to occur. The effects of a number of drug levels could be assessed on preference in a repeated measures design which is more sensitive than the between groups design originally used by Carlsson.

Application of this method to the assessment of flupenthixol's effects on exploration indicated an involvement of dopamine in processing the reinforcing qualities of exploration. The series of control experiments ruled out motoric and emotional interpretations of flupenthixol's effect on exploration preference. Dopamine's involvement in reinforcement processing therefore appears to generalise to exploratory reinforcement. This conclusion will be tested further by assessing the effects of dopamine stimulants on exploratory behaviour.

Chapter 3

Dopamine Stimulants and the Reinforcing Properties of Exploration.

3.0 General Introduction.

The experiments presented in the previous chapter showed that flupenthixol, a dopamine receptor blocker, decreased the degree to which environments of differing complexity were preferred over one another at dose levels which did not effect locomotor activity or reactivity to exposed environments. These results were interpreted as evidence for dopaminergic mediation of reinforcement processes. An implication of this hypothesis is that stimulation of dopamine systems should produce opposite effects, that is they should increase exploration preference. The experiments presented in this chapter test this hypothesis.

Drugs that stimulate, rather than block, neurochemical systems can operate by a variety of mechanisms (see e.g. Iversen and Iversen, 1981). They can directly stimulate post-synaptic receptors (e.g. apomorphine), increase the availability of the neurotransmitter in the pre-synaptic terminal (e.g. amphetamine), inhibit re-uptake of the transmitter into the pre-synaptic terminal (e.g. amphetamine) or inhibit the breakdown of the transmitter in the synaptic cleft (e.g. deprenyl and other monoamine oxidase inhibitors). These mechanisms are not functionally equivalent and therefore may not all be functional 'opposites' of post-synaptic receptor blockade.

Post-synaptic receptor blockade reduces the effectiveness of released transmitter on post-synaptic neurons, however, it has no effect on synapses where no transmitter is being released from the pre-synaptic terminal. Post-synaptic blockade therefore reduces the effects of ongoing activity in pre-synaptic neurons on the post-synaptic neurons to which they synapse, but has no functional effect at synapses where the pre-synaptic neuron is inactive. Drugs which increase the efficacy of transmitter release from the pre-synaptic neuron, either by increasing the amount of transmitter released or by increasing the length of time that the transmitter remains in the synaptic cleft, also functionally only effect ongoing activity (e.g. Von Voigtlander and Moore, 1973). On the other hand, drugs which directly stimulate the post-synaptic receptors increase activity in post-synaptic neurons regardless of pre-synaptic activity.

Both of these forms of stimulation can be seen as 'opposites' of blockade depending on how blockade is characterised. If the effects of blockade are interpreted as a general decrease in post-synaptic activity (given, of course, that a decrease in no activity is still no activity) then direct agonism is the opposite. If blockade is thought of as specifically decreasing the effectivness of ongoing activity then drugs which primarily effect the pre-synaptic neuron (e.g. amphetamine) produce the 'opposite' functional effect to blockade.

The effects of both apomorphine, which is a dopamine receptor agonist, and d-amphetamine, which facilitates transmitter release and inhibits re-uptake, will be assessed using the test battery described in the previous chapter.

Experiment 3.1 – A Test Battery Assessment of the Effects of Apomorphine on Exploration.

3.1.0 General Introduction.

Apomorphine is a direct dopamine receptor agonist (Andén, Rubenson, Fuxe and Hökfeldt, 1967), however its behavioural effects and its neuropharmacological actions can be both stimulatory and inhibitory (Bradbury, Cannon, Costall and Naylor, 1984). At high doses apomorphine stimulates post-synaptic neurons by binding to their dopamine receptors. At low concentrations, however, it binds to autoreceptors on pre- synaptic neurons which inhibit the release of dopamine (e.g. Carlsson, 1977) and hence leads to a decrease in post-synaptic activity. Although post-synaptic dose levels of apomorphine can produce increases in locomotor activity and other stimulant effects, as the dose increases more 'stereotyped' behaviour (e.g. floor licking, gnawing etc.) is produced which competes with locomotor and other behaviours resulting in a general reduction of all non-stereotyped behaviours at high doses (Isaacson, Yongue and McClearn, 1978).

Mason (1984) suggests that doses in the range 0.05 to 0.1 mg/kg act on autoreceptors. As the aim of this experiment is to investigate the effects of the stimulation of dopaminergic systems on exploration doses higher than these were used. Very high doses were avoided in an attempt to minimise stereotypy, nevertheless, experimental procedures were modified to take account of potential stereotypy.

Experiment 3.1.1 – The Effect of Apomorphine on on Exploration Preference.

3.1.1.1 Introduction.

This experiment assesses the effect of apomorphine on exploration with the same preference test used in the assessment of flupenthixol in the previous chapter. The apparatus used is identical, however the procedure has been modified slightly. The hypothesis being tested is that apomorphine will increase exploration preference. This hypothesis assumes a dose of apomorphine which does not produce marked autoreceptor effects or stereotypy.

As has been noted, apomorphine may produce stimulant or depressive effects. Carlsson (1972) demonstrated an increase in exploration preference at a dose of 1.0 ml/kg using male Wistar rats. Isaacson, Yongue and McClearn (1978) demonstrated a reduction in exploration assessed in a hole board at the same dose level, but using hooded Long-Evans rats; they also found a significant sex-difference in apomorphine's effect on exploration. As the DA strain rats used in this experiment have been found to be sensitive at comparatively low doses of other drugs in our laboratory (e.g. scopolamine, J.P. Aggleton, personal communication) the possibility that the doses used in this experiment (0.4 and 0.8 mg/kg) may produce stereotypy cannot be ruled out.

Although the dose range of apomorphine used in this experiment was chosen to minimise stereotypy and pre-synaptic effects, it is likely that some isolated instances of stereotyped behaviour may occur. The aim of the experiment, however, is to assess the stimulant effects of apomorphine at doses below those which produce stereotypy, and hence behavioural competition with exploration. As stereotyped behaviour was assessed as 'non-exploratory', very low total exploration scores are an indication of stereotypy. If any animal exceeded a pre-defined criterion of ten or more non-exploratory scores on any session it was retested at the end of the experiment. It should be noted that retesting should produce conservative results, stereotypy is most likely in the high dose condition where high preference scores are expected, however preferences at towards the end of the experiment will, if anything, be lower than those produced earlier on due to habituation (notwithstanding the lack of habituation found in the experiment 2.1). Data are presented both with and without these retest sessions.

3.1.1.2 Method.

Subjects.

The subjects were 12 experimentally naive male DA strain rats (Bantin and Kingman, Hull) weighing approximately 200 gm at the start of the experiment.

Drugs.

Apomorphine hydrochloride (Sigma, Poole) dissolved in a 0.2 mg/ml ascorbic acid solution vehicle at doses of 0.4 and 0.8 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The exploration apparatus described in chapter 2 was used.

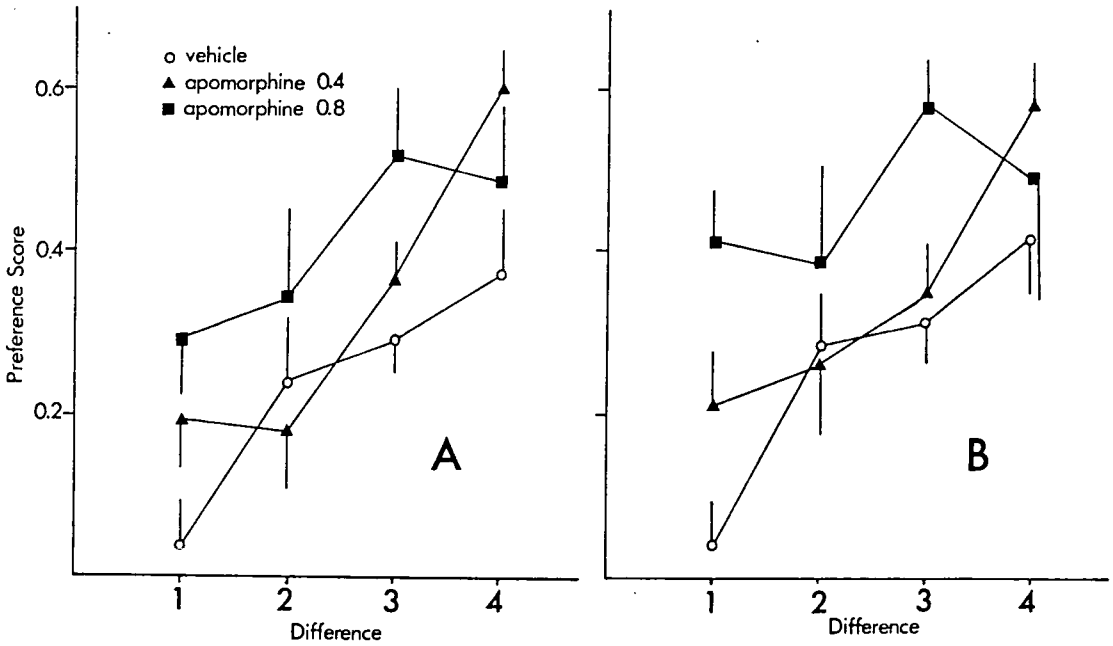
Procedure.

The experimental design was identical to that used in experiment 2.2.1, however, if an animal was observed to be exploring on ten or less time samples (out of thirty) it was retested using the same floor types and drug level in additional sessions at the end of the experiment. These retests continued day by day until total exploration scores of more than ten were available for all sessions which fell below the criterion. The experimental procedure was identical to that used in experiment 2.2.1 except that animals were injected fifteen minutes (rather than two and a half hours) prior to testing.

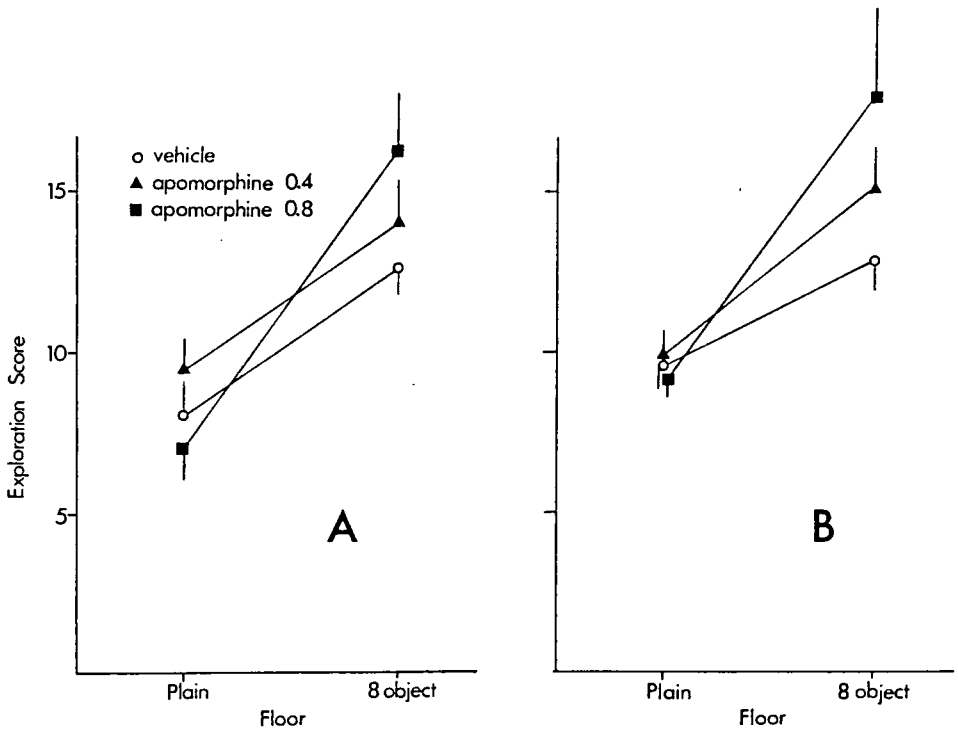
3.1.1.3 Results.

Although a total of 20 retest sessions were required from the 180 sessions of the experiment, the results of analyses using these retest scores in place of scores from under criterion sessions and those using only the original scores did not differ notably.

The results of the preference experiment are shown in figures 3.1a and 3.1b (using original and retest scores respectively). Two way repeated measures analyses of preference scores show that preference was significantly affected by floor *difference* ($F = 10.42$ original scores; $F = 12.52$ with retests; df $3, 33$; $p < 0.001$ both analyses) and by drug level ($F = 9.80$ original scores; $F = 15.84$ with retests; df $2, 22$; $p < 0.001$ both analyses). Drug by *difference* interactions were non-significant



3.1 The effect of apomorphine on exploration preference. **3.1a** Original scores, **3.1b** with retest scores substituted for sub-criterion scores. Mean scores and standard errors are shown.

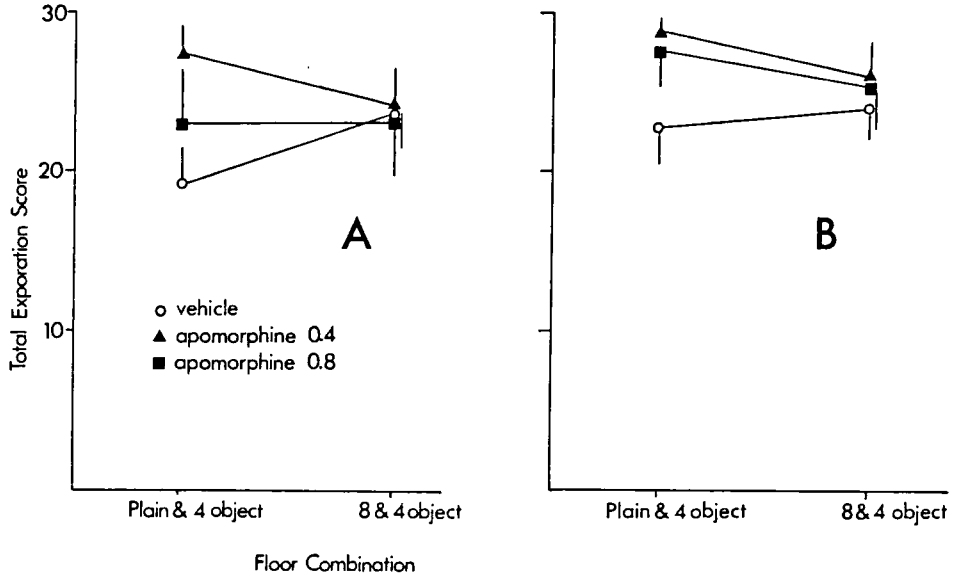


3.2 The effect of apomorphine on exploration of the plain and eight object floors when presented paired with a four object 'control' floor. **3.2a** Original scores, **3.2b** with retest scores substituted for sub-criterion scores.

($F \leq 1.0$) in both analyses.

Figures 3.2a and 3.2b show the effects of apomorphine on mean absolute exploration scores (and standard errors) produced by the plain black and eight object floors when presented in conjunction with the four object floor. No significant effects of drug or a drug by floor interaction were found in either analysis (drug

$F < 1.0$ both analyses; interaction $F = 2.01$ original scores, $F = 2.19$ with retests, $df 2, 22$). Exploration was significantly affected by Floor type ($F = 28.53$ original scores; $F = 34.94$ with retests; df $1, 11$; $p < 0.001$ both analyses).



3.3 The effect of apomorphine on mean total exploration of the plain plus four object and eight object plus four object floor combinations. **3.3a** Original scores, **3.3b** with retest scores substituted for sub-criterion scores.

The total scores from these sessions (all explorations made regardless of side) are shown in figures 3.3a and 3.3b (original scores and scores including retests). Analyses of variance show no significant effects of floor types, drug or floor by drug interactions in either analysis.

3.1.1.4 Discussion.

The results of the preference experiment show that apomorphine increased exploration preference, replicating Carlsson's (1972) result.

Examination of figures 3.1a and 3.1b shows little difference in preference scores between control and 0.4 mg/kg apomorphine in all but the *difference* 4 condition. At the *difference* 4 level there is a marked difference between the preference scores in the 0.4 mg/kg and control conditions. The preference scores in the 0.8 mg/kg condition are considerably higher than those of the 0.4 mg/kg and control conditions at the first three *difference* levels. When the original scores are used there appears to some decline in preference at *difference* 4 in the 0.8 mg/kg condition. The standard error of these scores is, however, large, and given a certain amount of stereotyping at this dose, preference has probably reached a ceiling for this drug dose at the *difference* 3 and 4 levels. Examination of figure 3.1b, which shows data in which retest scores replace sub-criterion session scores (and which therefore should be less affected by low preferences due to stereotypy), shows a slight increase in preference between *difference* 3 and 4 in the 0.8 mg/kg condition, but again a ceiling has probably been reached.

Apomorphine was shown not to effect the total amount of exploration made in the apparatus in the embedded experiment. Separate analyses carried out on total scores in the preference experiment do, however, show significant increases in total exploration with apomorphine ($F = 7.75$ original scores; $F = 10.88$ with retests; df $2, 22$; both data sets showed significant sphericity, Greenhouse-Geisser epsilons yielded a corrected df $1, 14$ for both analyses; $p < 0.05$ original scores, $p < 0.01$ with retests), but no effects of *difference* or drug by *difference* interactions.

Carlsson's (1972) results are from a single thirty minute testing session, however a figure is provided showing exploration scores broken down for successive five minute intervals. He showed that apomorphine attenuated the habituation of total exploratory behaviour over the thirty minute session, however, the exploration scores he obtained during the first five minutes of the session were similar for apomorphine and control treated animals (apomorphine appeared to produce slightly higher scores even during this interval). The results obtained here are largely consistent with those of Carlsson, although differences in total exploration scores were not large, (as in the first five minute interval of Carlsson's data), significant differences are shown in the statistically more powerful twelve trial preference experiment, although not in the six trial embedded experiment. The restricted range of floors used in the embedded experiment may also have affected the extent to which apomorphine increased exploration. No evidence was found there for an effect on the absolute amount of exploration made in the plain and eight object floors.

Although these total exploration effects were found, the issue of drug induced changes in motor capacity are not as critical in the assessment of apomorphine as they were for flupenthixol. In the flupenthixol experiment the lack of a drug effect on total exploration was seen as evidence against motor impairment. This was important as it could be argued that a drug induced motor impairment could produce a reduction in exploration preference not attributable to a change in reinforcement. In the case of stimulants it is hard to argue that an increase in behaviour per se will lead to increased preference. The fact that an increase in exploration was found with apomorphine does not effect the validity of interpreting the increase in preference found in terms of a change in reinforcement impact.

Experiment 3.1.2 – The Effect of Apomorphine on Non-Goal Directed Activity.

3.1.2.1 Introduction.

The previous experiment showed an increase in exploration preference which may be interpreted as evidence for an apomorphine induced enhancement of reinforcement. A small increase in overall exploration was also shown. Stimulation of dopamine systems has been shown to effect locomotion and exploration independently. Robbins and Iversen (1973) found decreases in exploratory behaviour but increases in locomotion using a Berlyne box in amphetamine treated rats. Isaacson, Yongue and McClearn (1978) obtained similar results in apomorphine and ET495 (perebidil) treated rats tested in a hole-board. In the Isaacson, Yongue and McClearn (1978) study the behaviourally effective doses used may have produced

stereotyping (the effective apomorphine doses were 1.0 and 5.0 mg/kg) which one may conjecture increased locomotion but decreased goal-directed exploration. This is less likely in the Robbins and Iversen study in which a comparatively low dose of 1.5 mg/kg d-amphetamine was used.

The present experiment investigates whether the increase in exploration preference and the small increase in overall exploration occurred independently of changes in locomotor behaviour using the same procedure as experiment 2.2.2.

3.1.2.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

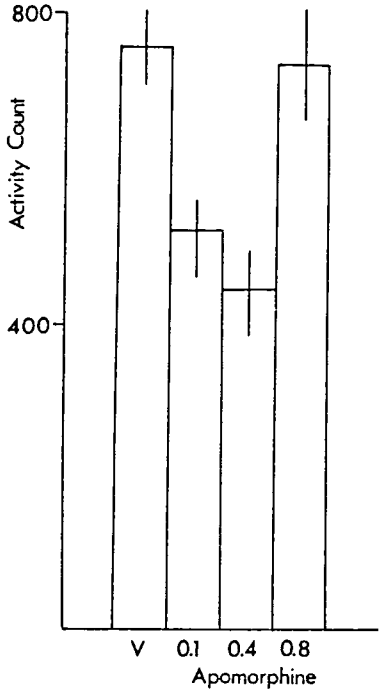
Apomorphine hydrochloride (Sigma, Poole) dissolved in a 0.2 mg/ml ascorbic acid solution vehicle at doses of 0.1, 0.4 and 0.8 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The activity monitoring apparatus described in experiment 2.2.2 was used.

Procedure.

The experimental design and procedure was identical to that used in experiment 2.2.2, except that animals were injected fifteen minutes (rather than two and a half hours) prior to testing.



3.4 The effect of apomorphine on activity monitored in the 'home-cage' activity boxes. Mean counts and their standard errors are shown.

3.1.2.3 Results.

The results of the experiment are presented in figure 3.4. A one way repeated measures analysis of variance showed that drug dose significantly affected activity ($F = 7.72$; df $3, 33$; $p < 0.001$). It can, however, be seen from figure 3.4 that this effect is entirely due to a reduction in activity at the two lower doses of apomorphine. The 0.8 mg/kg dose, which produced changes in total exploration and preference in the previous experiment, has little effect on activity.

3.1.2.4 Discussion.

The results of this experiment indicate a dissociation between apomorphine's locomotor effects and the increases in total exploration and exploration preference shown in the previous experiment. The results of the previous experiment cannot be attributed to any non-specific change in activity level produced by apomorphine.

The form of the dose response curve suggests that the 0.4 mg/kg dose is having a pre-synaptic sedative effect on the animals which is compensated for by post-synaptic stimulation at the 0.8 mg/kg dose (some post-synaptic stimulation may, nevertheless be occurring at the 0.4 mg/kg dose). If the suppression of activity shown at the 0.1 and 0.4 mg/kg doses was due to stereotypy, a reduction in activity would be expected at the higher 0.8 mg/kg dose. If this is the case the exploration effects in the previous experiment may be attributed to post-synaptic stimulation by apomorphine. Investigation of the effects of an extended dose range of apomorphine on activity may clarify the question of whether the 0.8 mg/kg dose is primarily effecting post-synaptic receptors.

Experiment 3.1.3 – The Effects of an Extended Dose Range of Apomorphine on Non-Goal Directed Activity.

3.1.3.1 Introduction.

This experiment aims to extend the dose response curve of apomorphine's effects on activity in order to clarify the primary site of action of the 0.8 mg/kg dose of apomorphine which produced changes in exploration in experiment 3.1.1. Suppression of activity by doses below 0.4 mg/kg and normal or elevated activity produced by higher doses would indicate that 0.8 mg/kg was approximately the lowest dose of apomorphine which acted primarily on post-synaptic receptors without producing excessive stereotypy in DA strain rats.

3.1.3.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

Apomorphine hydrochloride (Sigma, Poole) dissolved in a 0.2 mg/ml ascorbic acid solution vehicle at doses of 0.0125, 0.025, 0.2, 1.6 and 3.2 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

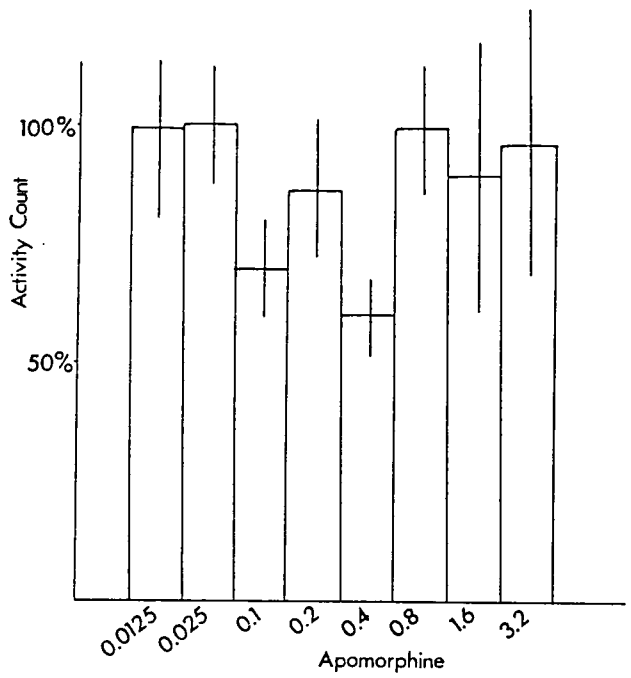
The activity monitoring apparatus described in experiment 2.2.2 was used.

Procedure.

The procedure was identical to that used in the previous experiment. The experimental design was modified to allow a counterbalanced order of drug treatment at six dose levels across animals.

3.1.3.3 Results.

The results of this experiment, combined with those of the previous experiment, are presented in figure 3.5. In order to reduce differences between the two experiments the data are presented in terms of percentages of activity shown in vehicle control conditions. Scores for the doses 0.1, 0.4 and 0.8 mg/kg were calculated using activity scores for those doses and for the control vehicle injection from the previous experiment. Scores for the other doses use control and drug activity scores from the current experiment. No statistical analyses were made.



3.5 The effect of an extended range of apomorphine doses on activity monitored in the 'home-cage' activity boxes. Activity is presented as a percentage of activity in the vehicle control conditions of the two experiments, **3.1.2** and **3.1.3** in which this data was obtained. Means and standard errors are shown.

3.1.3.4 Discussion.

As the error bars in figure 3.5 show, the activity data collected is very noisy, particularly at the higher doses where some animals became very active whilst others were nearly immobilised. However, the general pattern of results confirms

that the 0.8 mg/kg dose appears to have post-synaptic activity which outweighs the pre-synaptic effects visible between 0.1 and 0.4 mg/kg. Informal observation of the animals suggest that the two high doses produced pronounced behavioural effects, either as excessive locomotion or as stereotypy.

Experiment 3.1.4 – The Effect of Apomorphine on Emotionality as Measured by an Emergence Test.

3.1.4.1 Introduction.

Changes in locomotor activity do not form a reasonable explanation for the increase in preference found with apomorphine in the first experiment. An increase in the tendency to avoid exposure in the centre of open spaces is, however, consistent with an increased tendency to remain in the more complex of a pair of floors in the exploration preference apparatus. Coupled with a general increase in exploration this could account for the increased preference found with apomorphine.

The effect of apomorphine on animal's response to open field exposure was assessed using an emergence test. As was noted in experiment 2.2.3, a change in an animal's response to open field exposure can be explained in terms of a change in the impact of its negatively reinforcing consequences, or in terms of some general change in emotionality. In the context of this series of experiments the two explanations cannot be dissociated.

3.1.4.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

Apomorphine hydrochloride (Sigma, Poole) dissolved in a 0.2 mg/ml ascorbic acid solution vehicle at a dose of 0.8 mg/kg body weight and injected intraperitoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

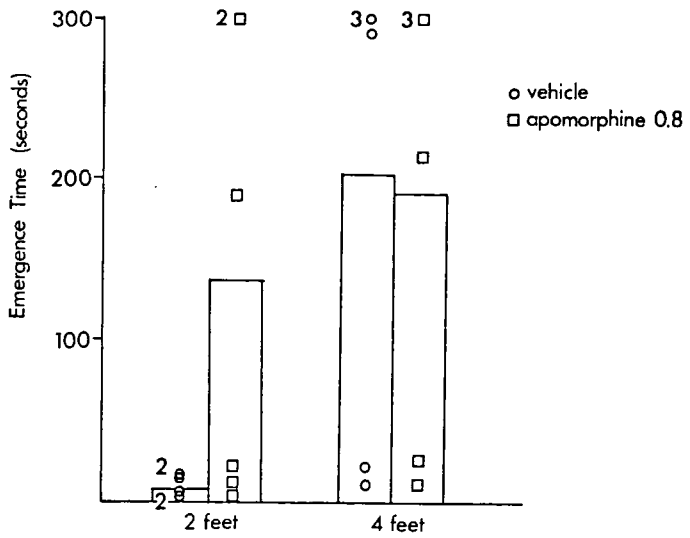
The emergence apparatus described in experiment 2.2.3 was used.

Procedure.

The experimental procedure was identical to that used in experiment 2.2.3 save that injections were made fifteen minutes, rather than two and a half hours, prior to testing.

3.1.4.3 Results.

The two and four foot emergence times are presented in figure 3.6. It can be seen that although times are similar between groups for four foot emergence, apomorphine treated animals took longer to place two feet out of the emergence tube than the vehicle treated controls. A Mann-Whitney U test confirms this (two



3.6 The effect of apomorphine on two- and four-foot emergence times. Individual times and group means are shown.

feet, $U = 4.0$; n 6, 6; $p < 0.05$).

3.1.4.4 Discussion.

The increase in two foot emergence time produced by apomorphine can be explained in a number of ways. It could be due to a heightened impact of the negative consequences of open field exposure. This explanation is consistent with the hypothesis that dopamine mediates reinforcement, the reinforcement being negative in this case. On the other hand, the increased emergence time may be the result of some general 'over emotionality'. If this excess emotionality is held to be due to a central effect of apomorphine then this explanation may also reduce to a mechanism consistent with a reinforcement mediation hypothesis. In any case, if 'emotionality' can be conceptually distinguished from hedonic impact, it is unlikely that it could be behaviourally dissociated in this context.

A further explanation, which is not compatible with the reinforcement mediation hypothesis, is based on reports that apomorphine produces nausea in humans (Mason, 1984). The same is probably true in rats as apomorphine can be used to induce conditioned taste aversions (Green, 1969). It may be conjectured that an apomorphine treated, and hence nauseous, rat may be less likely to venture into an open space simply because its sickness makes it less likely to act. By the same token, however, we may expect a nauseous rat to explore less. Experiment 3.1.1 shows that the doses of apomorphine used increased total exploration, so the argument that changes in emergence are in some way due to nausea seems of limited validity.

Other explanations related to the 'nausea' hypothesis are possible. For instance it is conceivable that apomorphine may induce supersensitivity to bright light (dopamine is the major catecholamine transmitter in the retina, Moore and Bloom, 1978; Osborne, 1984), and hence increase emergence time into the brightly lit emergence area. If this was the case, however, the effect would also be expected

in the four foot emergence, whereas if the apomorphine is effecting the impact of negative reinforcement, and hence 'timidity', a two foot effect, yet no four foot effect, is not hard to deal with.

It can be concluded that apomorphine has an effect on the impact of the negatively reinforcing aspect of open field exposure. This effect may be due to a change in emotionality or a direct effect on reinforcement (the relationship between emotion and reinforcement will be discussed in the final chapter). It seems unlikely that any parsimonious non-reinforcement based explanation of the results of both this experiment and experiment 3.1.1 is possible.

Experiment 3.1.5 – The Effect of Apomorphine on a Standard Measure of Exploratory Behaviour – the Hole Board Test.

3.1.5.1 Introduction.

The hole-board has been used as a measure of the effects of apomorphine on exploration in a number of studies (e.g. Isaacson, Yongue and McClearn, 1978; Ljungberg and Ungerstedt, 1976). Decreases in exploratory activity have been found over a wide range of doses (e.g. 0.01 to 1.0 mg/kg in the Ungerstedt and Ljungberg study). Given the increase in exploratory behaviour found in experiment 3.1.1 and by Carlsson (1972), the possibility that changes in exploratory behaviour are affected by the nature of the testing apparatus will be investigated (the preference apparatus provides a much 'richer' environment than a hole-board).

3.1.5.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

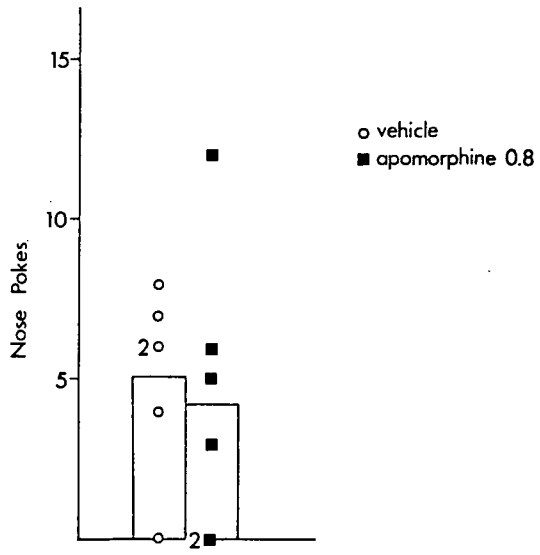
Apomorphine hydrochloride (Sigma, Poole) dissolved in a 0.2 mg/ml ascorbic acid solution vehicle at a dose of 0.8 mg/kg body weight and in a volume of 1.0 ml/kg body weight.

Apparatus.

The hole-board apparatus described in experiment 2.2.4 was used.

Procedure.

The experimental procedure was identical to that used in experiment 2.2.4, save that injections were made fifteen minutes, rather than two and a half hours, prior to testing. Only one dose of apomorphine (0.8 mg/kg) was assessed.



3.7 The effect of apomorphine on exploration measured by 'nose-pokes' in the hole-board test. Individual scores and group means are shown.

3.1.5.3 Results.

The results are presented in figure 3.7. The nose-poke score (corrected electronic counts) of the apomorphine group was slightly less than that of the control group, however this difference was not statistically significant (Mann-Whitney $U = 13.0$; n 6, 6; non-significant).

3.1.5.4 Discussion.

A single test session using a between groups design may not be statistically powerful enough to detect a small drug effect on exploration in a hole-board (Isaacson, Yongue and McClearn (1978) used a repeated measures design over 14 days). It is, however, interesting to note that the trend detected in the current experiment was of a decrease in exploration with apomorphine, in accordance with other findings. It may be speculated that the rich environment of the exploration preference apparatus engages more exploratory behaviour than the hole-board, and hence in the hole board the effects of stereotypy dominate the stimulating effects of apomorphine on exploration.

The results of the entire series of apomorphine experiments support the hypothesis that dopamine plays a rôle in the mediation of reinforcement, however, the emergence experiment shows that this mediation may be due to a change in emotionality when dopamine systems are manipulated with apomorphine. The experiments also show the difficulties of using apomorphine as a manipulation, great care must be taken in selecting doses of the drug as its low dose pre-synaptic effects and the production of stereotypy at higher doses can both mask its stimulating effects on non-stereotyped behaviour. The problems associated with stimulation of dopamine systems may be reduced by using amphetamine, rather than apomorphine, as the stimulant manipulation. The effects of amphetamine on exploration are assessed in the following series of experiments.

Experiment 3.2 – A Test Battery Assessment of the Effects of d-Amphetamine and α-Flupenthixol on Exploration.

3.2.0 General Introduction.

Amphetamine has a number of advantages over apomorphine as a stimulant manipulation of dopamine systems. It does not have any behaviourally significant sedative autoreceptor activity, and, although amphetamine can induce stereotypy, it has been shown to have effects on reinforcement governed behaviour at doses well below those which induce stereotypy. For example, Phillips and Fibiger (1973) demonstrated large increases in hypothalamic and nigral intracranial self-stimulation rates at amphetamine doses of less than 0.5 mg/kg, while doses of 5.0 mg/kg are needed to produce serious stereotypy (Scheel-Kruger and Jones, 1973). There are, however, difficulties in that amphetamine is not a specific dopamine stimulant.

Amphetamine acts on catecholaminergic neurons (dopaminergic and noradrenergic systems) via a range of mechanisms (see Groves and Tepper (1983) and Kuczenski (1983) for reviews). It may have some direct agonist action, perhaps via a metabolite, however its functional effects are largely dependent on actions which increase transmitter availability and block re-uptake of released transmitter. These actions may be mediated via an effect of autoreceptor stimulation, however, any direct autoreceptor effects inhibiting dopamine synthesis and release are outweighed by increased release of dopamine from cytoplasmic pools (Kuczenski, 1983). This release appears to induce a compensating increase in dopamine synthesis. Amphetamine induced release is probably primarily of dopamine and not of noradrenaline (Kuczenski, 1983). As was noted earlier, one important difference between amphetamine and apomorphine is that amphetamine's dopamine releasing properties depend on ongoing activity (Von Voigtlander and Moore, 1973).

Robbins, Watson, Gaskin and Ennis (1983) have shown differences in the effects of apomorphine and amphetamine in a task which differentiates between reinforcer directed and other responses. They showed that, in the acquisition of a continuous reinforcement schedule in a Skinner box, amphetamine increased response rates on the lever which provided reinforcement, but not on a second lever where pressing had no reinforcing consequences. In contrast, apomorphine increased responding on both levers. Robbins and Sahakian (1983) conjecture that this difference may be due to the lack of pre-synaptic modulation of apomorphine's effects. Amphetamine may therefore be a more sensitive manipulation in the exploration preference experiment. Increases in responsiveness which are closely tied to reinforcing value should result in larger increases in exploration preference than the more general increase shown by apomorphine in the Robbins, Watson, Gaskin and Ennis study.

The action of amphetamine on both dopaminergic and noradrenergic systems presents a problem if it to be used to assess the role of dopamine alone. Although the catecholamine releasing properties of amphetamine act mainly on dopamine, amphetamine blocks both dopamine and noradrenaline re-uptake. It is likely that only noradrenaline re-uptake is functionally effective at physiological dose levels (Heikkila, Orlansk and Cohen, 1975). There is evidence that the d- and l- isomers

of amphetamine have different actions on dopamine and noradrenaline systems. Unfortunately there is some confusion over the nature of this difference. Taylor and Snyder (1970) and other early investigators held that d-amphetamine produced far greater noradrenaline uptake blockade than the l- isomer while both isomers were equipotent in their dopamine releasing action. More recently this story has reversed, the evidence being that the d- isomer is more effective than the l- isomer in releasing dopamine (Church and Moore, 1974) while both are equipotent in blocking noradrenergic uptake (Svensson, 1971). One solution to the problem of teasing out dopaminergic effects from the dual action of amphetamine may be to use the l-isomer as a noradrenergic control condition for dually acting d-amphetamine. The solution chosen here, however, is to assess a challenge of d-amphetamine's effects with flupenthixol. As flupenthixol does not have any noradrenaline blocking activity (Møller-Nielsen, Pedersen, Nymark, Franck, Boeck, Fjalland and Christensen, 1973) any difference between the effects of d-amphetamine alone and amphetamine challenged with flupenthixol may be attributed to the dopaminergic component of amphetamine's action.

Experiment 3.2.1 – The Effects of d-Amphetamine and α-Flupenthixol on Exploration Preference.

3.2.1.1 Introduction.

This experiment assesses the effects of d-amphetamine on exploration with the same preference test used in the assessment of flupenthixol in the previous chapter. The dopaminergic basis of any stimulant effect of amphetamine on exploration preference is tested by challenging amphetamine with flupenthixol. There are, therefore, the following three drug conditions; saline control, amphetamine and amphetamine plus flupenthixol. If the hypothesis of dopaminergic mediation of reinforcement is supported, an increase of exploration preference would be expected with amphetamine relative to saline control. This increase should be reduced in the amphetamine plus flupenthixol condition.

The use of drug combinations necessitates a minor change in method. As flupenthixol reaches peak effectiveness two to three hours after injection (Corbet, Stellar, Stinus, Kelley and Fouriezos, 1983) but amphetamine takes only a few minutes to act, drugs were injected separately two and a half hours and fifteen minutes prior to testing. Saline injections were given two and a half hours prior to testing in the amphetamine alone and control conditions. It is particularly important to match injections across conditions as Riffee, Wilcox and Smith (1979) have shown that saline pre-injection one hour prior to drug injection potentiates the effects of both amphetamine and apomorphine.

As the design of the preference experiment only allows three levels of drug treatment, and the amphetamine manipulation requires an amphetamine plus flupenthixol control in addition to a vehicle control, each experiment is limited to testing a single dose of amphetamine. In addition to making dose selection more critical, the likelihood of obtaining a significant result (i.e. the 'effective' power) in an omnibus analysis of variance is reduced. If the experimental results are consis-

tent with the hypotheses then we may expect very similar preference scores from the saline and amphetamine plus flupenthixol conditions. The experimental hypotheses quite clearly call for two specific comparisons, a one tailed test of the hypothesis that amphetamine scores are higher than saline scores and a one-tailed test of the hypotheses that amphetamine plus flupenthixol scores are lower than amphetamine alone scores. These two tests will not be subject to the same problem of similarity between control groups as the omnibus analysis of variance.

3.2.1.2 Method.

Subjects.

The subjects were 12 experimentally naive male DA strain rats (Bantin and Kingman, Hull) weighing approximately 200 gm at the start of the experiment.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at a dose of 0.1 mg/kg body weight. Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight. All drugs were injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The exploration apparatus described in chapter 2 was used.

Procedure.

The experimental design was identical to that of experiment 3.1.1. The procedure was identical to that used in experiment 3.1.1 except that animals were injected two and a half hours prior to testing with either flupenthixol or saline and fifteen minutes prior to testing with either amphetamine or saline.

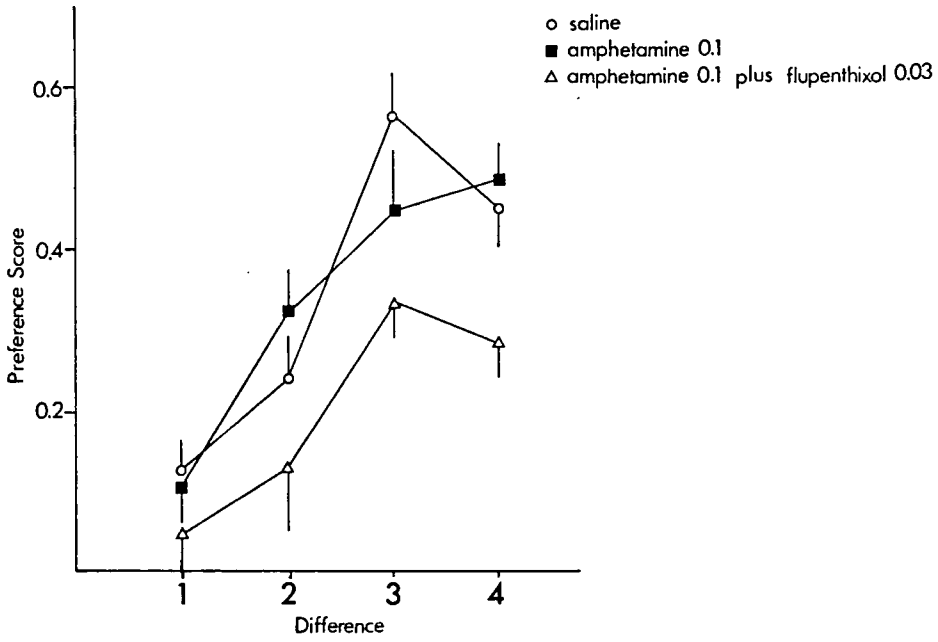
3.2.1.3 Results.

There were no trials in which the total exploration score fell below the retest criterion of ten or less (see experiment 3.1.1), hence no retesting was required.

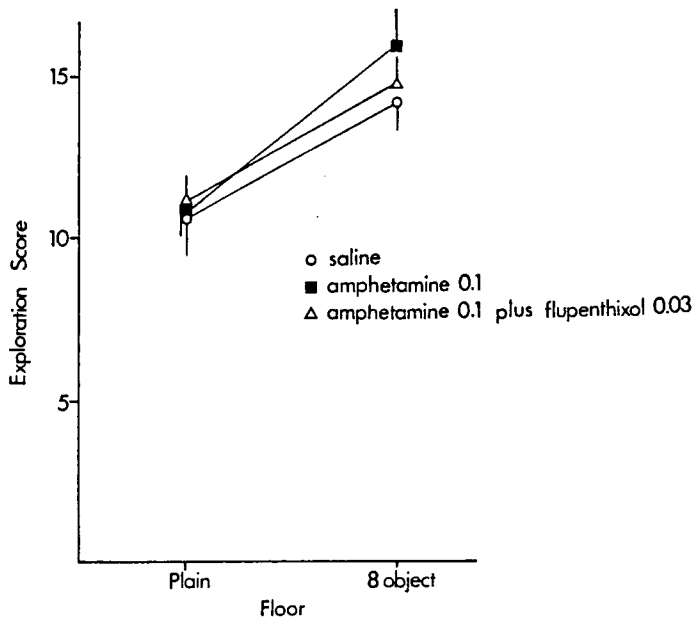
The results of the preference experiment are shown in figure 3.8. A two way repeated analysis of variance of preference scores showed that preference was significantly affected by floor *difference* ($F = 41.34$, *df* $3, 33$, $p < 0.001$) and by drug ($F = 5.86$, *df* $2, 22$, $p < 0.01$) but that no drug by *difference* interaction occurred. Examination of figure 3.8 indicates that this effect was not due to a stimulant effect of amphetamine relative to the saline control condition, but rather to reductions of preference caused by flupenthixol in the amphetamine plus flupenthixol condition relative to both the saline and amphetamine conditions.

The specific hypothesis tests comparing amphetamine with saline, and amphetamine with amphetamine plus flupenthixol, were carried out using the procedure described in Keppel (1973) for comparing marginal means within the factor drug. As there are clear hypotheses concerning the order of difference between pairs one-tailed tests are appropriate. The results confirm the impression from figure 3.8 that the amphetamine dose was ineffective (Amphetamine vs. Saline, $F < 1$). The effect of flupenthixol was, however, highly significant (Amphetamine plus Flupenthixol vs. Amphetamine, $F = 10.758$, *df* $1, 11$, $p < 0.005$ one-tailed).

Figure 3.9 shows drug effects on absolute exploration scores produced by the



3.8 The effect of α-flupenthixol and d-amphetamine on exploration preference. Means and standard errors are shown.

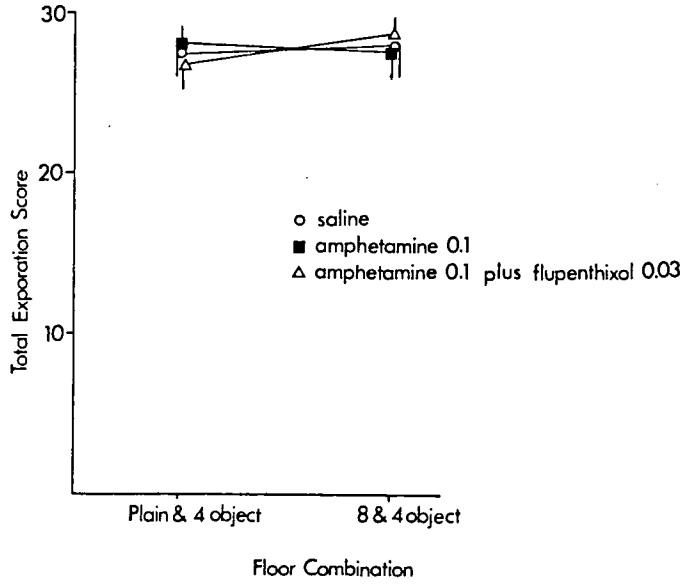


3.9 The effect of α-flupenthixol and d-amphetamine on exploration of the plain and eight object floors when presented paired with a four object floor. Means and standard errors are shown.

plain black and eight object floors when both were presented in conjunction with the four object floor. No significant effects of drug or a drug by floor type interaction were found. Floor type significantly affected amount of exploration ($F = 29.94$, df $1, 11$, $p < 0.001$).

The total exploration scores from these sessions (all explorations made regardless of side) are shown in figure 3.10. An analysis of variance of these scores showed

no significant effects of drug, floor combination or a drug by floor combination interaction.



3.10 The effect of α-flupenthixol and d-amphetamine on mean total exploration scores (and their standard errors) of the plain plus four objects and eight object plus four object floor combinations.

3.2.1.4 Discussion.

The amphetamine dose used appeared to be too low to produce any effect on preference (see for comparison experiment 3.3.1), although an increase in activity was found with this drug dose in a pilot study. The results of the experiment do, however, replicate the effect of flupenthixol on exploration preference found in experiment 2.2.1. Once again, total exploration was unaffected, both in the embedded absolute exploration experiment and in a separate analysis of total exploration scores in the preference experiment.

Experiment 3.2.2 – The Effects of d-Amphetamine and α-Flupenthixol on Non-Goal Directed Activity.

3.2.2.1 Introduction.

The previous experiment did not show any effect of a 0.1 mg/kg dose of d-amphetamine on exploration preference. An attenuation of preference was shown when this dose of amphetamine was administered in conjunction with 0.03 mg/kg flupenthixol. The current experiment assesses the effects of these manipulations and that of a higher does of 0.3 mg/kg of d-amphetamine on activity in the apparatus described in experiment 2.2.2.

Minor modifications were made to the computer software used in this experiment on the basis of experience gained during the apomorphine experiment. Consecutive counts on the same infra red beam were differentiated from actions which resulted in consecutive breaks of different beams. Sanberg, Hagenmeyer and Henault (1985) suggest that such a technique allows automated measurement of stereotypy (characterised by repetitious activities which should result in repeated breaks of the same beam) and gross movements (which break one beam after another). Both types of scores were collected and analysed separately.

3.2.2.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at doses of 0.1 and 0.3 mg/kg body weight. Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight. Drugs were injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

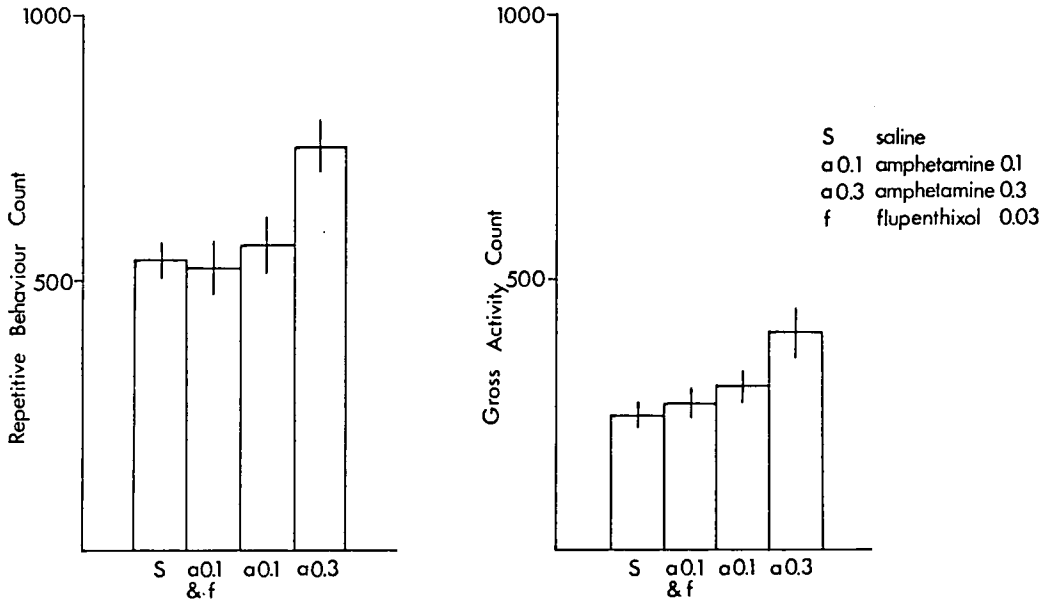
The activity monitoring apparatus described in experiment 2.2.2 was used.

Procedure.

The experimental design and procedure was identical to that used in experiment 2.2.2, except that animals were injected two and a half hours prior to testing with either flupenthixol or saline, and fifteen minutes prior to testing with either amphetamine or saline.

3.2.2.3 Results.

Drug effects on both 'gross movement' scores and 'repetitive behaviour' scores are presented in figure 3.11. Analyses of variance show significant drug effects for both measures (Gross movement, $F = 5.11$, $df\ 3, 33$, $p < 0.01$; Repetitive behaviour, $F = 5.49$, $df\ 3, 33$, $p < 0.005$). Examination of figure 3.11 shows that these effects appear to be primarily due to a strong stimulant effect of the 0.3 mg/kg dose of amphetamine, shown particularly clearly in the repetitive movements data. The low dose of amphetamine and the amphetamine plus flupenthixol combination have



3.11 The effect of α-flupenthixol and d-amphetamine on gross activity and repetitive behaviour in the 'home-cage' activty boxes. Means and standard errors are shown.

little effect on gross activity or repetitive behaviour.

3.2.2.4 Discussion.

The lack of any effect of the flupenthixol amphetamine combination, in the light of the ineffectiveness of 0.1 mg/kg alone, replicates the findings of experiment 2.2.2. The clear behavioural effects shown by 0.3 mg/kg d-amphetamine indicate it may be a more suitable dose level to use in an exploration preference experiment.

Experiment 3.2.3 – The Effects of d-Amphetamine and α-Flupenthixol on Emotionality as Measured by an Emergence Test.

3.2.3.1 Introduction.

The exploration preference showed no amphetamine effect but did replicate the effect obtained in experiment 2.2.1 with the flupenthixol plus amphetamine combination. This experiment therefore assesses the effects of this drug combination, in which the dose of amphetamine is assumed to be virtually inactive, as an attempt to replicate experiment 2.2.3.

3.2.3.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at a dose of 0.1 mg/kg body weight. Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight. Drugs were injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

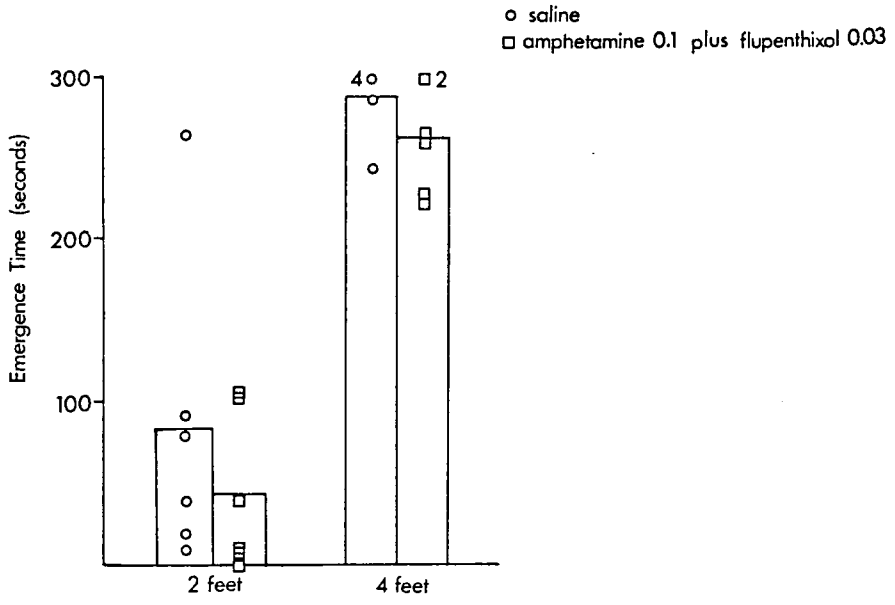
The emergence apparatus described in experiment 2.2.3 was used.

Procedure.

The experimental procedure was identical to that used in experiment 2.2.3 save that animals were injected two and a half hours prior to testing with either flupenthixol or saline, and fifteen minutes prior to testing with either amphetamine or saline. The drug conditions tested were doubly injected saline control and 0.1 mg/kg amphetamine plus 0.03 mg/kg flupenthixol.

3.2.3.3 Results.

The two and four foot emergence times are presented in figure 3.12. Mann-Whitney U tests did not show a significant drug effect on either two or four foot emergence (two foot, $U = 12$, n 6,6, non-significant; four foot, $U = 10$, n 6,6, non-significant).



3.12 The effect of α-flupenthixol and d-amphetamine on two- and four-foot emergence times. Individual times and group means are shown.

3.2.3.4 Discussion.

The results of this experiment replicate those of experiment 2.2.3, indicating that the attenuation of exploration preference shown in experiment 3.2.1 (and in experiment 2.2.1) were unlikely to be due to changes in animals' emotionality.

Experiment 3.2.4 – The Effects of d-Amphetamine and α-Flupenthixol on a Standard Measure of Exploratory Behaviour – The Hole Board Test.

3.2.4.1 Introduction.

Experiment 3.2.1 failed to detect any stimulant effect of amphetamine on exploration preference but did show an attenuation with the amphetamine plus flupenthixol combination. This experiment examines the effects of the same manipulations, compared to saline injected controls, on exploration in hole board tests.

3.2.4.2 Method.

Subjects.

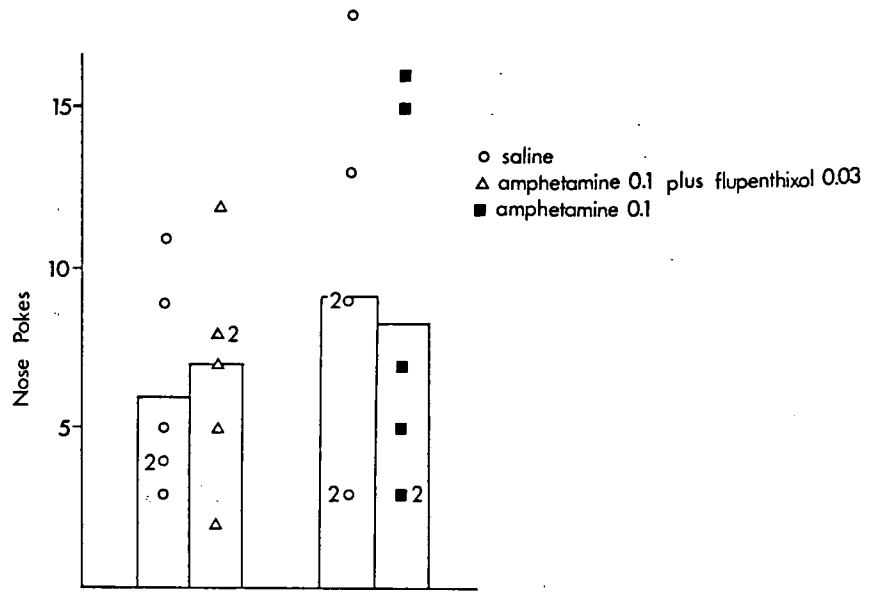
The same twelve rats used in the previous experiment served as subjects.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at a dose of 0.1 mg/kg body weight. Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight. Drugs were injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The hole-board apparatus described in experiment 2.2.4 was used.



3.13 The effect of α-flupenthixol and d-amphetamine on exploration measured by 'nose-pokes' in the hole board test. Individual scores and group means are shown.

Procedure.

The experimental procedure was identical to that used in experiment 2.2.4 save that animals were injected two and a half hours prior to testing with either flupenthixol or saline, and fifteen minutes prior to testing with either amphetamine or saline. The experiment was carried out first comparing amphetamine plus flupenthixol with double saline injected controls, and then, five days later, comparing amphetamine plus saline pre-injection with doubly injected controls.

3.2.4.3 Results.

The results are presented in figure 3.13. Analyses of the nose-poke scores (corrected electronic counts) from both the amphetamine plus flupenthixol and amphetamine alone trials did not show statistically significant drug effects (Amphetamine plus Flupenthixol, $U = 14.5$, n 6, 6, non-significant; Amphetamine alone, $U = 16.0$, n 6, 6, non-significant).

3.2.4.4 Discussion.

No drug effects on exploration were detected using the hole board procedure. This is consistent with the results of experiment 2.2.4 which showed no effect of flupenthixol on this test and with the results of other experiments in this series which indicate that the 0.1 mg/kg dose of amphetamine is too low to be functionally effective.

This series of experiments failed to demonstrate the hypothesised accentuation of exploration preference with amphetamine. The dose of amphetamine was, however, very low and behaviourally ineffective. This series of experiments can, however, be seen as a successful replication of the experiment 2.2 series. Flupenthixol has been shown to attenuate exploration preference while leaving locomotor activity and emotionality unaffected.

Experiment 3.3 – A Test Battery Assessment of the Effects of a Higher Dose of d-Amphetamine and α-Flupenthixol on Exploration.

3.3.0 General Introduction.

The series of experiments using a 0.1 mg/kg d-amphetamine failed to demonstrate any amphetamine effects, however, when a dose of 0.3 mg/kg was used in the activity experiment (3.2.2) an effect on behaviour was found. The current series of experiments uses this higher dose, which should nevertheless be low enough to avoid inducing stereotypy, in a second test of the hypothesis that amphetamine has a potentiating effect on the reinforcing properties of exploration which can be challenged by flupenthixol. The dose of flupenthixol remains at 0.03 mg/kg.

Experiment 3.3.1 – The Effects of a Higher Dose of d-Amphetamine and α-Flupenthixol on Exploration Preference.

3.3.1.1 Introduction.

This experiment assesses the effects of the higher 0.3 mg/kg dose of d-amphetamine on exploration with the same preference test used in the previous experiment. It is hypothesised that amphetamine will increase exploration preference and that this increase can be reversed by flupenthixol.

The retest procedure used in experiment 3.1.1 will be used if necessary.

3.3.1.2 Method.

Subjects.

The subjects were 12 experimentally naive male DA strain rats (bred in the University of Durham Psychology Department) weighing approximately 200 gm at the start of the experiment.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at a dose of 0.3 mg/kg body weight. Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight. Drugs were injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The exploration apparatus described in chapter 2 was used.

Procedure.

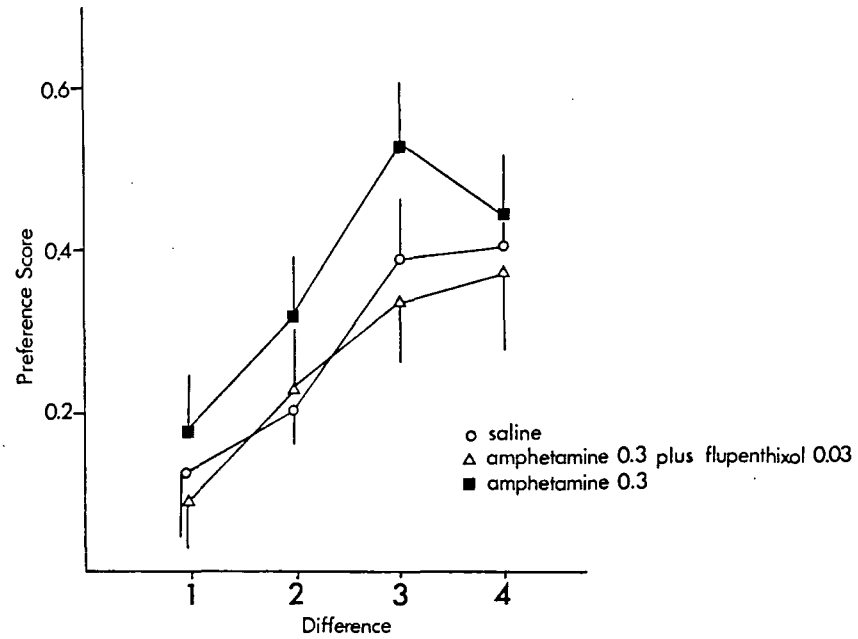
The experimental design and procedure was identical to that used in experiment 3.2.1.

3.3.1.3 Results.

Only one retest session was required, there are no differences between the results of analyses which include this score rather than the original sub-criterion score.

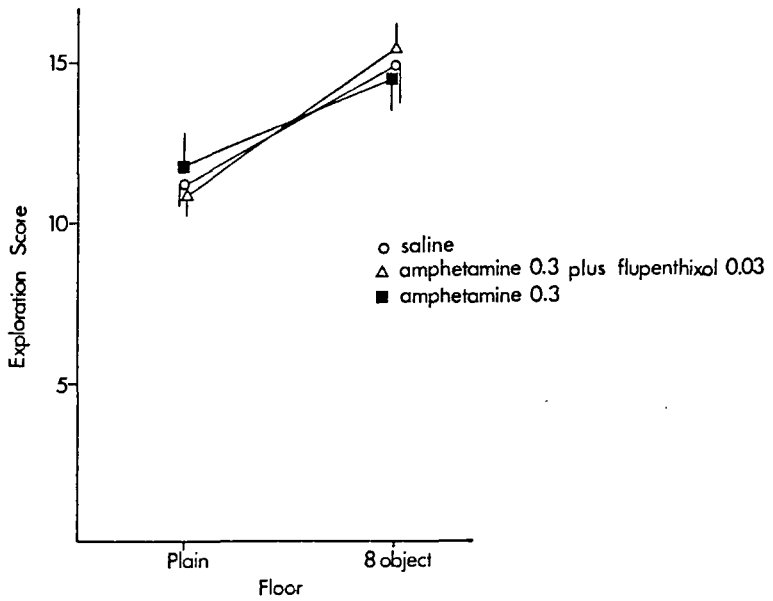
The results of the preference experiment are shown in figure 3.14. It can be seen that the pattern of results is consistent with the experimental hypotheses that amphetamine should increase preference, and that this effect should be reversed by challenge with flupenthixol. Analysis of variance does not, however, show a statistically significant drug effect ($F = 2.30$, df $2, 22$, non-significant). Floor type significantly effects preference ($F = 13.58$, df $3, 33$, $p < 0.001$). There was no floor by drug interaction ($F < 1$).

Test of the specific hypotheses comparing amphetamine with saline and amphetamine with amphetamine plus flupenthixol were carried out using the procedure described in Keppel (1973) for comparing marginal means within a factor. As there are clear hypotheses concerning the order of difference between pairs one-tailed tests were considered appropriate. Although the overall analysis was not significant, the increase in preference with amphetamine shown in figure 3.14 is reflected



3.14 The effect of α-flupenthixol and a higher dose of d-amphetamine on exploration preference. Mean scores and their standard errors are shown.

in the comparison of marginal means (Amphetamine vs Saline, $F = 3.95$, df $1, 11$, $p < 0.05$ one-tailed), however, the attenuation of this effect by flupenthixol fails to reach significance (Amphetamine vs Amphetamine plus Flupenthixol, $F = 2.93$; df $1, 11$; $p = 0.0575$ one-tailed).

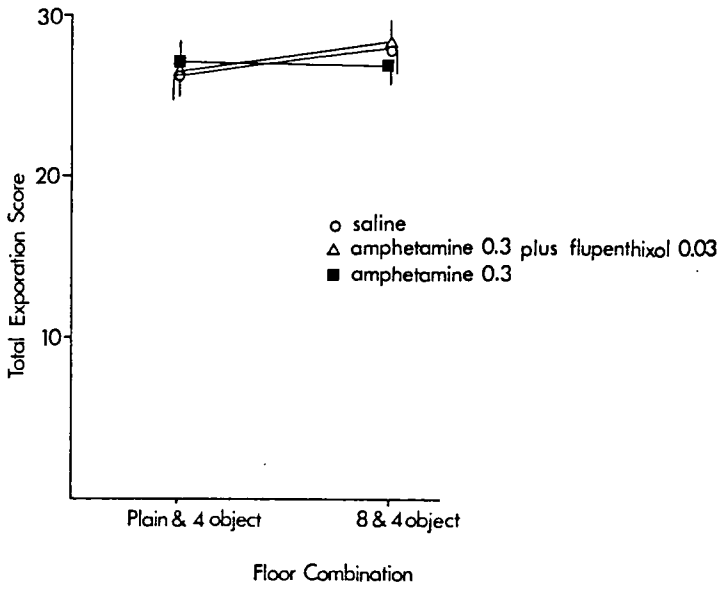


3.15 The effect of α-flupenthixol and a higher does of d-amphetamine on exploration of the plain and eight object floors when presented paired with a four object floor. Means and standard errors are shown.

Figure 3.15 shows drug effects on absolute exploration scores produced by the

plain black and eight object floors when both were presented in conjunction with the four object floor. No significant effects of drug or a drug by floor type interaction were found. Floor type significantly affected amount of exploration ($F = 28.07$; df $1, 11$; $p < 0.001$).

The total exploration scores from these sessions (all explorations made regardless of side) are shown in figure 3.16. An analysis of variance of these scores showed no significant effects of drug, floor combination or a drug by floor combination interaction.



3.16 The effect of α-flupenthixol and a higher does of d-amphetamine on total exploration of the plain plus four objects and eight object plus four object floor combinations. Means and standard errors are shown.

3.3.1.4 Discussion.

Although the analysis of variance of the preference experiment results did not yield a significant overall drug effect, the consistency of the pattern of results with the experimental hypotheses is striking. Specific tests of these hypotheses confirmed that amphetamine increased preference relative to the saline control condition. Figure 3.14 shows that this increase in preference is challenged by flupenthixol. This challenge was not, however, found to be statistically significant. Unfortunately the variation of scores within conditions is large, as can be seen from the standard error bars in figure 3.14.

Informal observation of animals receiving 0.3 mg/kg amphetamine indicated that higher doses were unlikely to produce notably higher preference scores. Although there was little sign of stereotypy, some animals appeared to become so active that their exploration seemed quite undirected. They ran about a great deal, sniffing objects as the came upon them, rather than appearing to explore one object and then become attracted to another which they then explored. It is likely that at higher doses of amphetamine this high activity will mask any further increases in exploration preference. This less directed exploration may not preclude the occurrence of high exploration scores in non-choice situations.

It is worthwhile comparing figure 3.14 with figure 3.8, which shows the results of experiment 3.2.1. Both show the results of exploration preference experiments using amphetamine, amphetamine plus 0.03 mg/kg flupenthixol, and a saline control condition. The only difference in manipulations was the dose of amphetamine. In figure 3.8 flupenthixol plus 0.1 mg/kg amphetamine attenuated exploration preference. In figure 3.14 the higher 0.3 mg/kg dose of amphetamine eliminated this attenuation in the amphetamine plus flupenthixol condition. A similar comparison can be made of the effects of amphetamine alone in the two experiments. The low dose, which failed to eliminate flupenthixol attenuation of exploration preference, did not differ from the saline control in figure 3.8, while the higher dose, which eliminated flupenthixol attenuation in figure 3.14, produced preferences higher than the saline control.

The challenge of flupenthixol's attenuating effect on preference by the high, but not the low, dose of amphetamine can, in itself be seen as evidence for an effect of amphetamine on exploratory reinforcement.

It has been shown that amphetamine increased exploration preference, however, the attenuation of that effect by flupenthixol was not statistically significant. A comparison with the results of experiment 3.2.1 clearly shows that the attenuation of preference with flupenthixol and the low (0.1 mg/kg) dose of amphetamine was eliminated in the current experiment with the higher dose of amphetamine. Statistical comparison across experiments is not possible, however, this can be seen as evidence for the hypothesis that amphetamine's effects on preference are dopaminergically mediated.

It must be conceded that the effect of amphetamine found was weak compared to that of flupenthixol or apomorphine. Although the pattern of results was consistent with the hypotheses, scores were highly variable. This variability may be due to competing behavioural effects not connected with reinforcement. Some of these effects are investigated in the following experiments, others are discussed later in the chapter.

Experiment 3.3.2 – The Effects of a Higher Dose of d-Amphetamine and α-Flupenthixol on Non-Goal Directed Activity.

3.3.2.1 Introduction.

This experiment examines the effects of higher doses of amphetamine on activity. It was conjectured in the previous experiment that doses of amphetamine higher than the 0.3 mg/kg used would probably fail to increase exploration preference as increases in general activity would mask such increases. In addition to assessing the effects of the drug treatments used in experiment 3.2.1, this experiment also assesses the effects of a higher 0.9 mg/kg dose of amphetamine on gross activity and repetitious behaviour (which can be construed as a measure of stereotypy) in a sparse environment.

3.3.2.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at doses of 0.3 and 0.9 mg/kg body weight. Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight. Drugs were injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

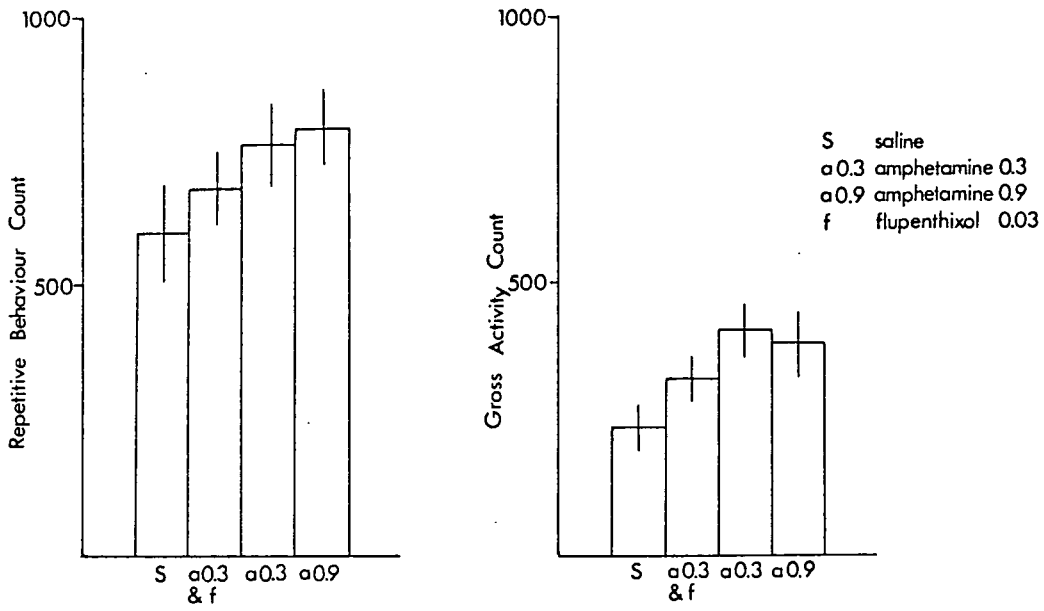
The activity monitoring apparatus described in experiment 2.2.2 was used.

Procedure.

The experimental design and procedure was identical to that used in experiment 3.2.2. The drug conditions tested were double saline injection control, 0.3 mg/kg amphetamine plus 0.03 mg/kg flupenthixol, 0.3 mg/kg amphetamine (plus saline pre-injection) and 0.9 mg/kg amphetamine (plus saline pre-injection).

3.3.2.3 Results.

Drug effects on both 'gross movement' scores and 'repetitive behaviour' scores are presented in figure 3.17. Analyses of variance showed significant drug effects for both measures (Gross movement, $F = 8.44$, $df\ 3, 33$, $p < 0.001$; Repetitive behaviour, $F = 3.88$, $df\ 3, 33$, $p < 0.05$). It can be seen that all drug treatments, even the amphetamine flupenthixol combined condition, produced increases of gross and repetitive activity. The high 0.9 mg/kg dose of amphetamine did not appear to produce notably larger effects than the 0.3 mg/kg dose.



3.17 The effect of α-flupenthixol and higher doses of d-amphetamine on gross activity and repetitive behaviour in the 'home-cage' activty boxes. Mean counts and their standard errors are shown.

3.3.2.4 Discussion.

All the drug treatments increased activity. In particular the combined amphetamine flupenthixol treatment produced increases in activity while having no effect on exploration preference. The question of the relationship between activity and preference in the exploration experiment is complex. Discussion of that relationship is required here as this is the first instance of an effect on activity by a drug dose having an effect on exploration preference in the experiments reported in this thesis.

The test battery employed throughout this chapter was designed primarily to test the effects of dopamine blockers on exploration preference, and, in addition, provide control experiments to assess the validity of explanations of preference attenuation based on drug effects on processes other than reinforcement.

A common theme in the discussion of the effects of dopamine blockers on responses to reinforcement is that their effects may be due to motor impairments rather than changes in reinforcement processes. When the exploration preference experiment is considered, however, it is not clear that a motor impairment will lead to an attenuation of exploration preference (the predicted result of reduced sensitivity to reinforcement).

If a motor impaired animal which processes reinforcement normally can move at all, we may assume it is more likely to move towards a reinforcer (e.g. an environment containing many objects). Once it is in that environment, if it is capable of exploring, we may assume it will sniff at the objects it comes across. However, the response cost, for this motor impaired animal, of moving away from the more complex half of the test box and exploring the less reinforcing side is greater than that for an unimpaired animal. As the cost of switching sides, or indeed of moving anywhere, is increased, but reinforcement is held to be normal in this purely motor impaired animal, the difference in net reinforcing value (reinforcing value less response cost) between sides in the exploration apparatus may well be increased. The implication is that the exploration preference score of the motor impaired animal will be higher than that of normal animals.

Of course, it may be argued that the response cost of exploring an area covered with many objects is greater than that of exploring an area with only a few objects. If this were so, then a drug-induced increase in response cost would effect the net reinforcing values of exploring more and less complex areas roughly proportionately to their values for normal animals. No change in exploration preference with motor impairment would be expected.

The point of these arguments is not really to suggest that increases in preference should be expected with dopamine blockers if dopamine's rôle is confined to motor systems, but rather to point out that there is little reason to expect a decrease in preference if this is the case. The doses of flupenthixol used are unlikely to have significantly affected the response cost of moving about in the exploration apparatus in any case. The most certain method of avoiding problems with motor explanations of the effects of dopamine blockade is to show that no detectable motor impairment exists in a situation unlikely to provide strong reinforcers, as was done in experiment 2.2.

In the current experiment an effect of amphetamine on exploration preference has been demonstrated, and the active dose of amphetamine has also been shown

to effect activity levels. The question that must be addressed is whether an effect of amphetamine which simply increases activity, without altering reinforcement, could account for an increased exploration preference produced by amphetamine. It has been argued that a motor impairment is unlikely to produce an attenuation of exploration preference; a corollary of this argument is that an increase in the tendency of animals to move about is unlikely to cause an increase in preference. In terms of a response cost argument it may be assumed that an increased tendency for locomotion implies a reduction in the response cost of exploration, and hence an decrease in the effective difference of net reinforcement between more and less complex sides in the activity box. A decrease in exploration preference would therefore be expected. More informally, it may be argued that an animal which runs around more is more likely to explore the less preferred side of the test box because it has arrived there often as a result of its activity, rather than as a result of the attraction of exploring the less-preferred side per se.

It can be concluded that the increases in activity produced by the 0.3 mg/kg dose of amphetamine do not provide a good explanation of amphetamine's effects on exploration preference. Robbins and Sahakian (1983) discuss a range of amphetamine's behavioural effects. These will be considered in more detail at the end of the chapter.

Finally it should be noted that the 0.9 mg/kg dose of amphetamine did not produce an increase in gross activity over the 0.3 mg/kg dose, although a small increase in repetitive behaviour was recorded. This may indicate the beginnings of a dominance of stereotyped behaviours over other behaviours. If this is so the use of a 0.9 mg/kg dose in the exploration apparatus is unlikely to produce any additional increases in exploration preference over those found with the 0.3 mg/kg dose.

Experiment 3.3.3 – The Effect of a Higher Dose of d-Amphetamine on Emotionality as Measured by an Emergence Test.

3.3.3.1 Introduction.

Experiments 3.3.1 and 3.3.2 have demonstrated behavioural effects of the 0.3 mg/kg dose of amphetamine. This experiment assesses the effect of this dose on emergence in order to establish whether the effects of amphetamine on exploration preference can be attributed to a change in animals' response to open field exposure.

3.3.3.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at a dose of 0.3 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

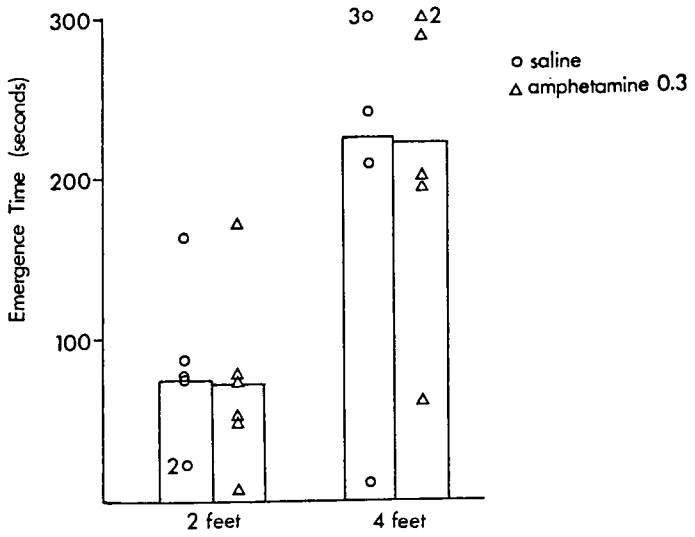
The emergence apparatus described in experiment 2.2.3 was used.

Procedure.

The experimental procedure was identical to that used in experiment 3.2.3. The drug conditions tested were doubly injected saline control and 0.3 mg/kg d-amphetamine (plus saline pre-injection).

3.3.3.3 Results.

The two and four foot emergence times are presented in figure 3.18. Mann-Whitney U tests did not show a significant drug effect on either two or four foot emergence (two foot, $U = 15.5$; n 6, 6, non-significant; four foot, $U = 15$; n 6, 6, non-significant).



3.18 The effect of a higher dose of d-amphetamine on two- and four-foot emergence times. Individual times and group means are shown.

3.3.3.4 Discussion.

The results indicate that the effects of amphetamine on exploration preference cannot be attributed to a change in animals' emotionality. This adds weight to the argument that amphetamine affected preference through a change in reinforcement processes similar to those which flupenthixol is assumed to have disrupted. Amphetamine does, however, have a range of behavioural effects which may also account for changes in exploration preference, these will be discussed later in the chapter.

Experiment 3.3.4 – The Effects of a Higher Dose of d-Amphetamine and α-Flupenthixol on a Standard Measure of Exploratory Behaviour – The Hole Board Test.

3.3.4.1 Introduction.

Although the amphetamine induced increases in exploration preference above saline control found in experiment 3.3.1 were weak, it is possible that more substantial increases in exploration may be detected in the nose poke apparatus. Increases in the impact of reinforcement produced by amphetamine may have been masked by concurrent increases in activity or the tendency of animals to switch between behaviours (Robbins and Watson, 1981). Both of these effects could cause behaviour to be distributed more evenly between sides of the exploration apparatus, and hence mask any increase in preference. These effects would, however, be less likely to reduce a non-choice measure of exploration such as the hole board test.

The drug conditions chosen for testing were 0.3 mg/kg amphetamine plus 0.03 mg/kg flupenthixol and 0.9 mg/kg amphetamine plus saline pre-injection. The amphetamine plus flupenthixol condition eliminated preference attenuation in experiment 3.3.1 and a similar condition using the lower 0.1 mg/kg dose of amphetamine in experiment 3.2.4 did not reduce exploration in a hole board test. If the conjecture that amphetamine's reinforcement enhancing effects are masked to some extent in the preference design, this drug condition can be predicted to produce an increase in exploration in the hole board test. The 0.9 mg/kg condition was selected as being likely to increase exploration and is essentially a test of the hole board procedure's sensitivity.

3.3.4.2 Method.

Subjects.

The same twelve rats used in the previous experiment served as subjects.

Drugs.

D-Amphetamine sulphate (Sigma, Poole) dissolved in 0.9% saline at doses of 0.3 and 0.9 mg/kg body weight. Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.03 mg/kg body weight. Drugs were injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

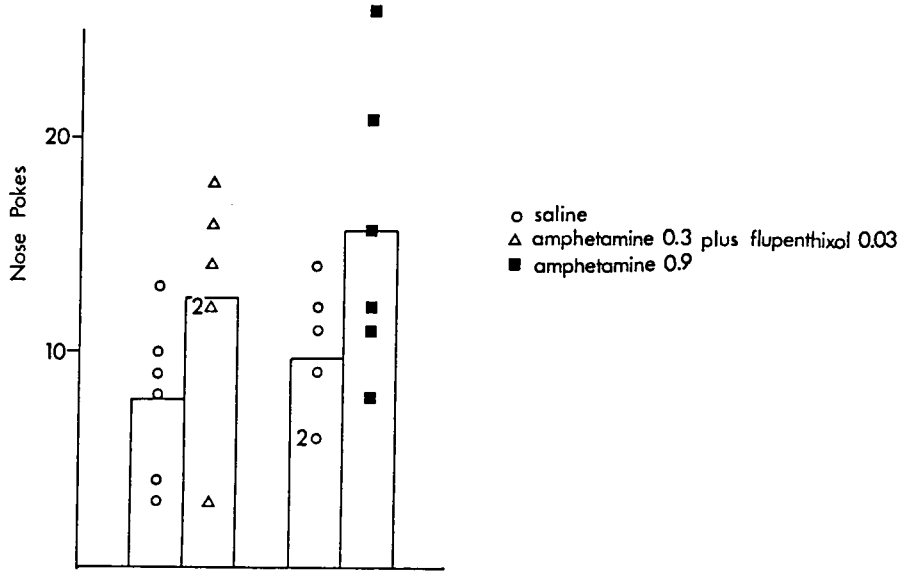
The hole board apparatus described in experiment 2.2.4 was used.

Procedure.

The experimental procedure was identical to that used in experiment 2.2.4. The experiment was carried out first comparing 0.3 mg/kg amphetamine plus flupenthixol with double saline injected controls, and then, five days later, comparing the 0.9 mg/kg dose of amphetamine plus a saline pre-injection with doubly injected controls.

3.3.4.3 Results.

The results are presented in figure 3.19. Both drug conditions appeared to increase exploration. Analysis of the nose-poke scores (corrected electronic counts) from the amphetamine plus flupenthixol test showed a statistically significant drug effect ($U = 7.5$, n 6, 6, significance corrected for ties, $p < 0.05$ one-tailed test). A test of the effect of the 0.9 mg/kg dose just fails to reach significance ($U = 8.0$, n 6, 6, significance corrected for ties, $p = 0.054$ one-tailed test).



3.19 The effect of α-flupenthixol and higher doses of d-amphetamine on exploration measured by 'nose-pokes' in the hole board test. Individual scores and group means are shown.

3.3.4.4 Discussion.

The increases in exploration produced by the two drug treatments can be seen in figure 3.19. The fact that the difference between the first two groups is significant while that between the second two is not points out the limited power of this small subject number, between groups, design. Longer test sessions in a repeated measures design would certainly have increased the sensitivity of the test.

Given the limited power of the design, the significant increase in exploration with the 0.3 mg/kg amphetamine plus flupenthixol treatment is particularly striking. It was argued in the introduction that behavioural effects of amphetamine not directly connected with reinforcement could mask the drug's hypothesised reinforcement sensitizing effects on exploration preference. The results of this experiment show that this may well be the case, however, the possibility that the same effects caused an apparent increase in exploration in the hole board test cannot be ruled out. Increased locomotion may lead to elevated scores, it is also possible that an increase in behavioural switching between exploration of holes and locomotion may increase the number of holes investigated.

Although the effects of the 0.9 mg/kg dose of amphetamine just missed statistical significance, some animals were quite clearly stimulated to increased exploration. The wide range of scores obtained in this drug condition also indicate the potential problems of inter-subject variability which limit the usefulness of higher doses of amphetamine in the exploration preference design.

3.4 General Discussion.

The results of the experiments reported in this chapter are generally consistent with the hypothesis that dopamine is involved in reinforcement processing. However, neither the apomorphine or amphetamine experiments provide completely clear results alone.

Carlsson's (1972) finding that apomorphine increased exploration preference was replicated in experiment 3.1.1. However, the longer emergence times found in experiment 3.1.5 raise the possibility that this increase was not due to an accentuation of reinforcement. It has been argued that increased emergence time is unlikely to have been due to apomorphine induced nausea. The two other likely explanations of these results, 'emotionality' and accentuated sensitivity to negative reinforcement, cannot be distinguished in these experiments.

Theoretical differences between emotion and reinforcement are discussed in some detail in the final chapter. The model of emotion used is that of Rolls (1986). Briefly, it is assumed that emotional reactions to reinforcement may come about in two ways. First, an animal's response to reinforcement may be characterised as 'emotional' if the reinforcer is particularly strong. Second, reactions may be emotional when reinforcement, (or its omission), is unexpected. In the emergence, (and maybe to a lesser extent in the exploration), experiments, animals are encountering moderately strong negative reinforcers. There is no reason for them to have expectations about these reinforcers. In this context then, emotion is simply part of a response to negative reinforcement. Reinforcement is antecedent to emotion, any change in the hedonic impact of reinforcement must also alter emotion. A change in emotionality is unlikely to be dissociable from a change in hedonia. A change in emotionality may, however, differ from a change in the response eliciting power of reinforcement. This difference is only likely to become evident in experiments which involve recall of the impact of previous reinforcing events (i.e. subsequent expectations). In the experiments presented in this chapter, hedonic, emotional and response eliciting effects are not dissociable.

In conclusion, the results obtained with apomorphine imply that dopamine is involved in reinforcement at least to the extent of modulating behavioural output according to motivational or emotional state. A straightforward reinforcement model is tenable if it assumed that increased emergence is due to accentuated impact of negative reinforcement.

Some difficulties were encountered in the apomorphine experiments because of stereotypy. These difficulties prompted an investigation of the effects of amphetamine, which is less likely to produce stereotypy at effective stimulant doses than apomorphine. It was suggested that as amphetamine's action is dependent on ongoing neural activity it may have provided a more powerful manipulation than apomorphine of the putative dopaminergic systems involved in reinforcement processing. This was not the case.

The first series of experiments using amphetamine (3.2) failed to demonstrate any behavioural effects of the dose used. They are best regarded as a replication of the results of the experiment 2.2 series which demonstrated motor independent exploration preference attenuation with flupenthixol.

The higher (0.3 mg/kg) dose of amphetamine used in experiment 3.3 produced an increase in preference over saline control. This effect was statistically significant in the specific one-tailed test of the hypothesis that amphetamine would increase preference. There was considerable variability in the effects of amphetamine. This was reflected in part by the non- significant drug effect in the overall analysis of experiment 3.3.1's results.

The second necessary comparison between amphetamine and amphetamine plus flupenthixol failed to reach significance, it is not therefore certain that the increase in preference found with amphetamine alone can be attributed purely to its dopaminergic action, a noradrenergic contribution is possible. Comparison of the preferences shown by the amphetamine plus flupenthixol group, relative to saline control, with the equivalent groups in experiment 3.2.1 shows that the higher dose of amphetamine eliminated flupenthixol induced attenuation of exploration. This lends credence to the hypothesis that amphetamine's effects were dopamine dependent, but, as statistical comparisons between the two experiments are not possible, the conclusion that amphetamine's preference accentuating action was dopaminergically mediated must be tentative.

The results of further experiments show that the 0.3 mg/kg amphetamine dose is capable of producing strong behavioural effects. The weak effects shown in experiment 3.3.1 cannot be attributed simply to a weak drug dose. The 0.3 mg/kg dose produced a marked increase in activity in experiment 3.3.2. Perhaps more strikingly, even in conjunction with flupenthixol, the dose produced an increased exploration score in the hole board test.

In summary a consistent explanation is needed of the following results from this chapter, in conjunction with the effects of flupenthixol found in chapter 2 and replicated in experiment 3.2.

1) The apomorphine induced accentuation of exploration preference at a dose which did not effect activity levels, but which did produce an increase in emergence time.

2) The weak accentuation of preference found with amphetamine at a dose which significantly increased activity and exploration score in the low powered hole board test even though challenged by flupenthixol, yet did not effect emergence.

3.4.1 The Dual Actions of Apomorphine and Dissociations Between Effects on Exploration Preference and Activity.

In experiment 2.2 flupenthixol attenuated exploration preference without effecting activity or emergence. These results can be interpreted in terms of differential sensitivities of the task used to a comparatively weak drug dose. If dopamine has a rôle in reinforcement processing, (perhaps in conjunction with some motor rôle), it may be assumed that situations involving reinforcement and behaviour will be more

sensitive to dopamine manipulations that those in which reinforcement plays little part. Specifically, it is assumed that the effect of the 0.03 mg/kg dose on dopamine systems was too weak to effect non-reinforcer directed locomotor behaviour or the statistically low-powered emergence experiment, but still strong enough to influence response to the reinforcing qualities of exploration.

A similar assumption about weak doses is not tenable in the case of apomorphine as lower doses of the drug were found to significantly decrease activity. In addition to explaining the effects of the higher preference accentuating dose of apomorphine, the lower 0.4 mg/kg dose which had no effect on preference, but decreased activity must be considered. This dissociation is the opposite of that found with flupenthixol. It is not credible to use contradictory arguments of the 'task demands' type to explain both the flupenthixol and 0.4 mg/kg dissociations.

The effects of lower doses of apomorphine, together with the pattern of results obtained in experiment 3.3.3, indicate that at the 0.8 mg/kg dose there is a balance between pre- and post-synaptic effects of apomorphine on locomotion. Although there is no change in activity at this dose, there are significant reductions in activity at lower doses which must be due to pre-synaptic action. At the 0.8 mg/kg dose it can be inferred that apomorphine is having significant pre-synaptic action, but this action is compensated for, but not yet dominated, by the effects of post-synaptic stimulation on locomotor behaviour. This changeover dose is in accord with the intra-peritoneal dose ranges for pre- and post-synaptic apomorphine activity quoted in Seeman (1981).

The implication of this balanced action is that although locomotor activity levels are unchanged by the 0.8 mg/kg dose, the underlying behaviour of dopamine systems is significantly affected. Pre-synaptic effects which inhibit dopamine release and synthesis reduce the effectiveness of ongoing neuronal activity in pre-synaptic neurons on post-synaptic receptors. Direct post-synaptic agonism is independent of ongoing neuronal activity. The net result is that although there must be considerable post-synaptic activity with the 0.8 mg/kg dose this activity is not as dependent on pre-synaptic activity as it would be in untreated animals.

At the 0.4 mg/kg dose it is likely that pre-synaptic receptors are saturated and reasonable levels of post-synaptic stimulation are beginning to occur (Seeman, 1981). The same maximal level of pre-synaptic action can be assumed to occurring at the 0.4 and 0.8 mg/kg doses. There is evidence that apomorphine's pre-synaptic action inhibits exploration at low doses (e.g. 0.1 mg/kg, Höglund and Meyerson, 1985). It therefore seems likely that there is a balance between pre- and post-synaptic effects on the exploration preference task at the 0.4 mg/kg dose. On the other hand, at the same 0.4 mg/kg dose, pre-synaptic effects are dominating post-synaptic effects in the activity monitoring experiment. The 'task demand' argument used in explaining flupenthixol's effects assumed that exploration preference was more susceptible to dopaminergic manipulations than activity. A similar argument can be made here for apomorphine's post-synaptic action. If post-synaptic effects on exploration are more powerful than those on activity, but both exploration and activity are being potentially suppressed by pre-synaptic action, then results of the type obtained with the 0.4 mg/kg dose can be explained (see e.g. Ahlenius and Hillegaart, 1986). Exploration preference is normal because post-synaptic effects on the reinforcing qualities of exploration are strong enough to compensate for pre-synaptic effects, but the post-synaptic stimulation of locomotion is not strong

enough to compensate for the pre-synaptic inhibitory effects.

It may be argued that the application of a similar argument to pre-synaptic effects would nullify the explanation given above. Two counter-arguments are available. First, it may be argued that the pre-synaptic effects of apomorphine will be weaker than post-synaptic effects in general, if only because pre-synaptic action only functionally effects ongoing neuronal activity, while post-synaptic action effects all cells regardless of activity. It should also be noted that Aghajanian and Bunney (1977) report that microiontophoretic injection of apomorphine can only produce a maximum of 75% inhibition of pre-synaptic cells, whilst dopamine itself can totally inhibit firing. Hence, even at maximally effective pre-synaptic doses, ongoing neuronal activity is still likely to be producing some post-synaptic effects.

The second counter-argument is more thought provoking. It is possible that not all dopaminergic systems possess autoreceptors (Bannon, Chiodo, Bunney and Roth, 1982), pre-synaptic actions of apomorphine will not effect these systems at all. This may imply that pre-synaptic actions will generally be outweighed by post-synaptic effects, but, more interestingly, it is possible to argue that the results imply that systems lacking autoreceptors are involved more directly in reinforcement processing than systems with autoreceptors. It was originally thought that all cells in the meso-cortical dopamine system lacked autoreceptors (Bannon, Chiodo, Bunney and Roth, 1982). This result was not replicated by Wang (1981) who identified autoreceptors in some meso-cortical system cells. Shepard and German (1984) have shown that the meso-cortical system contains two subpopulations of cells, a fast firing group originating in medial A10 which do not possess autoreceptors and a lateral A10 slow firing group which do. Nevertheless, this result must be treated with caution as Fadda, Gessa, Marcou, Mosca and Rossetti (1984) found evidence suggestive of dopamine autoreceptors in the medial prefrontal cortex.

The experiments described in this thesis do not set out to answer questions concerning the division of functions between anatomically distinct dopamine systems. It is therefore interesting to note the convergence of this argument, intended to explain the results of the effects parenteral injections of apomorphine in experiment 3.1.1, with the conclusions drawn from studies which have directly assessed different anatomical areas. For example Speciale, Miller, McMillen and German (1986) show that locomotion is accompanied by elevation of dopamine metabolism in the striatum (and in nucleus accumbens with animals running at high speed) but not in the prefrontal cortex, whereas footshock stress increased dopamine metabolism in the frontal cortex (and in accumbens at high shock intensities) but not in the striatum. This pattern of results is interpreted as evidence for cortical involvement in emotional processes, nigro-striatal involvement in motor processes, and a mixed rôle for the nucleus accumbens which shares features of the 'sensory-motor' basal ganglia and the 'emotional' limbic system (see e.g. Thierry, Tassin, Blanc, Stinus, Scatton and Glowinski, 1977). Anatomical studies suggest that lateral 'meso-cortical' projections identified as lacking autoreceptors project both to cortex and to limbic structures such as the nucleus accumbens and the amygdala, but not to the striatum (Loughlin and Fallon, 1984).

A hypothesis may be derived from the studies discussed above that the 0.4 mg/kg dose of apomorphine is inhibiting locomotion through its action on autoreceptors, and that this inhibition is to some extent compensated for by post-synaptic activity. However, effects on exploration preference are less affected by pre-synaptic action as at least some of the dopamine neurons involved in the mediation of reinforcement or emotionality lack autoreceptors. Post-synaptic action is therefore capable of completely counteracting pre-synaptic effects on preference. Although the systems lacking autoreceptors have been described as being involved in 'emotional' processes (presumably largely because the manipulation most often used in dopamine turnover studies is footshock) it is not clear whether these systems are strictly involved in emotion, or whether they could also be involved in other processes connected with the perception of, or response to, reinforcers.

The results of experiment 3.1 have been explained in terms of the dual action of apomorphine on pre- and post-synaptic dopamine receptors. The effects of apomorphine on exploration preference and emergence may be due to indirect changes in the impact of reinforcers, for example through changes in emotion or motivation, although an explanation in terms of direct changes in reinforcement perception is still possible. Detailed consideration of the pattern of results at the 0.4 and 0.8 mg/kg doses of apomorphine on activity and exploration preference lead to a speculative hypothesis that reinforcement processes are mediated to a greater extent by dopamine systems lacking autoreceptors, while dopaminergic systems possessing autoreceptors are more involved in motor functions.

3.4.2 The Multiple Behavioural Actions of Amphetamine and its Weak Effect on Exploration Preference, but Strong Effects on Activity and Hole Board Test Results.

The detailed interpretation of apomorphine's effects was complicated by its dual pharmacological actions. There are no pharmacological difficulties of this type in the interpretation of amphetamine's effects. Although amphetamine effects noradrenaline receptors in addition to dopamine ones, and evidence was tentative that the effects obtained were due solely to dopaminergic action, in this section the behavioural effects of amphetamine, regardless of their noradrenergic or dopaminergic basis, will be discussed.

Stronger effects than those obtained with apomorphine had been expected from amphetamine because of its wider non-stereotypy inducing stimulant dose range and the functional dependence of its action on ongoing neuronal activity. The question to be addressed is 'why was amphetamine's effect on exploration preference weak, particularly given its strong effect on activity and the increase in exploration in the hole board even when administered in conjunction with flupenthixol ?'.

Amphetamine has been implicated in reinforcement processes (for example though self administration studies (Pickens and Harris, 1968), increased response rates for food reinforcement at sub-anoretic doses (Blundell and Latham, 1978) and potentiation of intracranial self-stimulation (Stephens and Herberg, 1975)), however, it has also been shown to have a number of other behavioural effects which may explain the results obtained in experiment 3.3.

Robbins and Watson (1981) report that in a task where reinforcement was distributed randomly across two levers in a Skinner box, amphetamine increased the probability of switching responding from one lever to another, even at doses which had little or no effect on response rates. Evenden (1986) has shown that this effect is not simply due to a general increase in response rate produced by amphetamine, and that a corresponding decrease in behavioural switching can be produced by the dopamine antagonist chlorpromazine. Evenden and Robbins (1985) have also shown that this effect is not due to an amphetamine-induced breakdown in stimulus control. It may therefore be concluded that amphetamine increases the tendency of animals to switch between behaviours.

The weak effects of amphetamine on exploration preference may be due in part to increased behavioural switching. It has already been argued that increases in locomotor behaviour are likely to reduce exploration preference scores. This effect would be potentiated by increases in behavioural switching. It may be hypothesised that animals will spend less time exploring individual objects, switching to another behaviour such as locomotion. Whilst walking or running it is assumed that animals are only minimally concerned with the objects surrounding them, and hence are likely to spend more time in the less complex floor of the exploration apparatus than would normally be expected. If they come into contact with an object they may revert to exploration. The net result is a tendency for preference to attenuate.

The result obtained was, however, an increase in preference, albeit a weak one. It is therefore proposed that a reinforcement potentiating effect of amphetamine was acting in opposition to switching and locomotor effects. These multiple behavioural effects may also have contributed to the large variations in preference found with amphetamine.

The same effects may also explain the increase in exploration found in the amphetamine plus flupenthixol condition of experiment 3.3.4. Increased behavioural switching may result in less total time spent exploring holes in the hole board experiment, however, only nose entries into holes were counted. More individual nose-pokes would occur if animals increased the rate at which they switched between exploration of holes and other behaviours such as locomotion and 'non-hole' exploration.

Robbins and Sahakian (1983) discuss other behavioural effects of amphetamine, in particular the emergence of stereotypy. Models have been proposed which can explain the pattern of results obtained in this chapter which do not require detailed consideration of such issues.

3.4.3 Conclusions.

The experiments reported in this chapter add weight to the hypothesis that dopamine is involved in reinforcement processes, and not simply motor control. The possibility that this involvement is not direct, but is a consequence of effects on motivational or emotional systems cannot be ruled out. When the results of the experiments presented in this chapter and those presented in chapter 2 are considered together, however, the weight of evidence indicates that it can be concluded that dopamine is involved in the mediation of exploratory reinforcement.

Chapter 4

The Effect of Dopamine Blockade on Preference for Novelty.

4.0 General Introduction.

The experiments described in chapters 2 and 3 demonstrated a dissociation between the effects of dopamine manipulations on exploration and locomotion. These results were interpreted as evidence of a rôle for central dopamine systems in reinforcement processing over and above any rôle they may play in motor control. The precise nature of dopamine's involvement in reinforcement is not clear. It may be directly involved in the evaluation or signalling of reinforcement or it may play a rôle in motivational or emotional systems, either directly or in signalling such states to other systems (e.g. in the elicitation of responses by reinforcers).

In this chapter the rôle of dopamine in reinforcement is further investigated by assessing the effects of dopamine manipulations on rats' response to novelty, a reinforcer closely related to exploration.

Montgomery's theory of exploratory drive holds that exploration is, in fact, induced by novel stimuli (Montgomery, 1951a,1951b,1952,1953). Montgomery (1953) showed that animals' tendency to explore was dependent on the novelty of the environments (in this case maze arms) with which they were presented.

In the current experiment novel and familiar stimuli are presented to animals in two arms of a Y-maze and their choice of arm (containing a novel or familiar stimulus) is then recorded. This method is similar to the exploration preference test used previously, in that is it a choice measure, there are, however, important differences in the implications of the simple measure of choice, as opposed to the measure of behaviour allocation.

Although the choice nature of the exploration preference design controls for simple motor deficits, the behaviour being measured does involve motor systems. Exploration in the preference experiments can be seen as 'consummatory' behaviour. No distinction is possible in this design between drug effects on such consummatory behaviour and those on the perception of the stimuli to which that behaviour is subsequently directed or the elicitation of responses by those stimuli. By measuring choice in the Y-maze, rather than actual exploratory behaviour, perceptual effects may be dissociated from those on consummatory behaviour. This form of design is, of course an example of a discrimination task. As changes in the hedonic impact of reinforcement consumption do not necessarily predict changes in discrimination performance it is possible that drug-induced changes in reinforcement impact will not produce changes in novelty selection. It is, however, possible that novelty in distal stimuli is in itself reinforcing, in which case anhedonia may produce an attenuation of novelty selection.

The retention of exploratory behaviour as the reinforcer has distinct advantages in this type of experiment. The use of a primary reinforcer whose value (i.e. novelty) is clearly assessable from a distance obviates the need to train animals to perform the task. Dopaminergic drugs can influence learning, perhaps as a consequence of their involvement in reinforcement processes (Wise and Schwartz, 1981). Although this is

an interesting phenomenon in its own right as a method of assessing dopamine's rôle in reinforcement, differential learning rates between drug conditions would merely complicate the present experiment.

Experiment 4.1 – The Effect of α-Flupenthixol on Rats' Selection of Novel Stimuli in a Y-Maze.

4.1.1 Introduction.

The experimental design used to assess flupenthixol's effects on response to novelty in this experiment is based on a test previously used to assess recognition memory in this laboratory (e.g. Aggleton, 1985). When used to assess recognition memory, animals are differentially reinforced for selecting novel stimuli, however, it has been found that animals select the novel stimulus with a probability significantly greater than chance even in early trials before learning can have taken place (J.P. Aggleton, personal communication). The effect of flupenthixol on this phenomenon will be studied. Animals will not be differentially reinforced for selecting novel stimuli, but rather will receive food reinforcement no matter which stimulus they select. The use of food reinforcement in addition to the reinforcing properties of novelty is necessary in order to maintain running over the number of trials required to reduce the effects of random behaviour on measures of stimulus selection.

As the reinforcing value of selecting the novel stimulus is likely to be quite low, stimulus choice is liable to be disturbed by many other factors. For this reason animals were given very extensive pre-exposure to the maze and pre-training in the general procedure. The action of the guillotine door used in the maze was particularly likely to disturb the rats and perhaps induce random stimulus choice.

The effects of the drug doses used, 0.03 and 0.09 mg/kg flupenthixol, have previously been assessed on non-reinforcer directed activity (experiment 2.2.1). The lower dose was not found to have any effect on locomotion while the higher dose significantly decreased it. The lower dose was also found to attenuate exploration preference in experiment 2.2.1.

If dopamine plays a part directly in the identification of reinforcing stimuli, a reduction of novel stimulus selection towards chance is predicted in the drug conditions. However, other influences on reinforcement processing do not necessarily imply effects on stimulus choice. An anhedonic effect does not necessarily predict a disruption in discrimination performance, although one may be found if the distal presentation of novel stimuli is reinforcing. An effect on the elicitation of responses by reinforcers is also unlikely to alter discrimination performance. The concurrent presentation of food reinforcers, may, however, have stronger response eliciting power than novelty. An attenuation of response elicitation by reinforcers may therefore lead to a reduced tendency to select novel stimuli.

4.1.2 Method.

Subjects.

The subjects were 12 experimentally naive male DA strain rats (Bantin and Kingman, Hull) weighing approximately 190 gm at the start of the experiment. One animal ceased running during the course of the experiment, its responses were therefore dropped from the results.

Drugs.

Cis-Z-flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at doses of 0.03 and 0.09 mg/kg and injected intra-pertoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

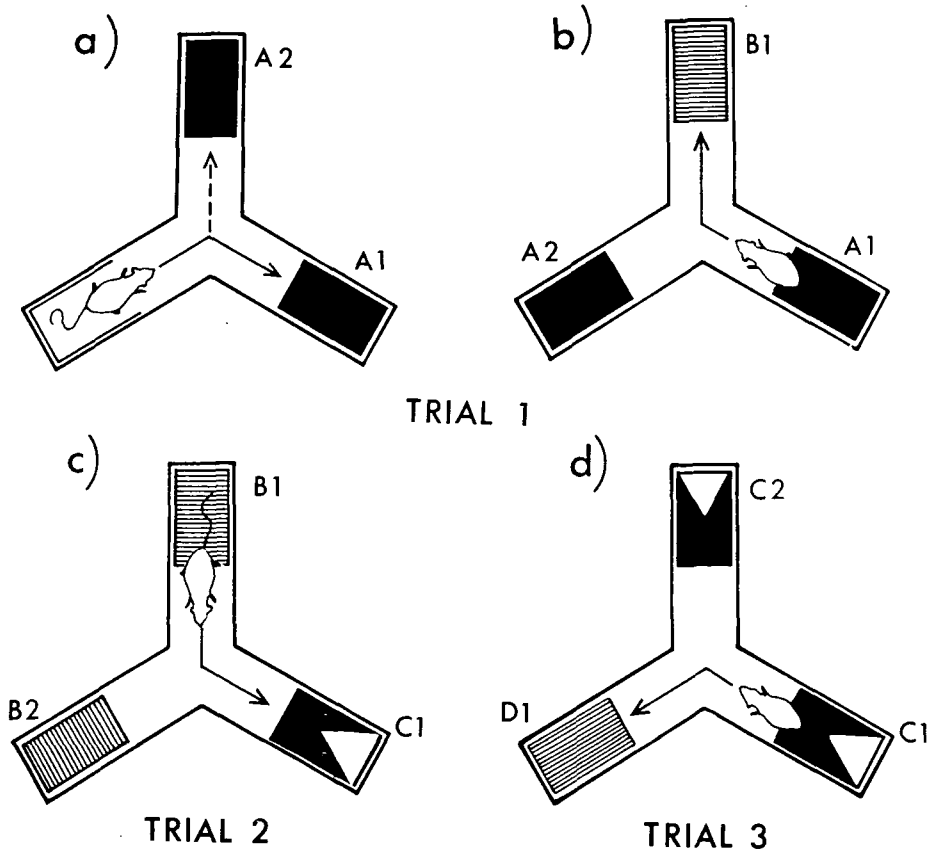
The animals were tested in an aluminium Y-maze. Each arm was 13 cm wide, 20 cm high, and covered with a wire grid. Forty five pairs of hardboard boxes served as both start boxes and goal boxes. These boxes could be fitted into the end of each arm of the maze, forming a total arm length of 26 cm. The boxes in each pair were made as similar as possible, but each pair was distinctive. To this end the walls and floors of the boxes were painted in different colours and patterns, and the floors were lined with a variety of materials such as sandpaper, wooden strips, metal, perspex and cloth. In addition, each pair of boxes contained an identical object, such as a plastic cup, a metal bracket, or a wooden block, although no two pairs contained the same object. The boxes were either 16 or 19 cm high; they were 12 cm wide, and 9 cm deep. The floors protruded an additional 9 cm from the box, so that the floor of each box began 8 cm from the centre of the Y maze. An aluminium guillotine door was set in the centre of the maze, blocking the three arms. Metal tubes, which dispensed the reward pellets (45 mg, Campden Instruments Ltd., London), ran to the back wall of each box, the back of which had been cut away to allow the animal access to food. The Y-maze was illuminated by a fluorescent ceiling light 215 cm above the apparatus.

Procedure.

The rats were handled daily over a week before pre-training began. Pre-training took place over thirteen consecutive days during which the rats were taken off ad lib food and put on daily feedings of 5 gm per day, which was gradually increased to 12 gm per day by the start of the experimental period. During pre-training rats were weighed each day and then familiarised with the Y-maze.

For the first four days of pre-training each rat was placed in one arm of the maze, the guillotine door was kept closed and a blank goal box, with food pellets towards its back, was positioned at the end of the arm. The rat was allowed to explore the arm until it had eaten the food pellets, this took between five and ten minutes. For the next two days the rats were allowed to explore the entire maze for ten minutes per day. The guillotine door remained raised and blank goal boxes were positioned at the ends of all three maze arms. On the first day each arm was baited with three food pellets, on the second and subsequent days only two were given. Pellets in each arm were replaced every time an animal ate the pellets and left the arm. This training continued for a further two days, however, now the guillotine door was raised and lowered as the rats moved around the maze.

In the final stage of pre-training, lasting five days, the raising and lowering of the guillotine corresponded to the conditions in the experiment proper. The door was only raised 30 seconds after the rat had consumed the pellets in an arm, and was lowered as soon as the rat had entered another arm. On the final three days the rats were given intra-peritoneal saline injections two and a half hours before running in the maze. The nine day experimental period immediately followed pre-training.



4.1 Diagrammatic representation of the testing procedure.

Each test session consisted of fourteen test trials and one starting trial. Animals were injected with either saline or flupenthixol two and a half hours before testing. For the starting trial the rat was placed in an arm of the maze containing a blank start box and no reward pellets. The guillotine door was raised, allowing the rat to select between two arms containing a matching pair of goal boxes, (A1, A2, figure 4.1a), both baited with two food pellets each. After the rat had made an arm selection (both of its hind feet had been placed in the chosen arm) the guillotine door was closed and the rat was allowed to eat the food pellets and examine the goal box it had selected (A1). The guillotine door remained closed for 30 seconds after the rat had eaten the reward pellets. During this time the boxes in the other two arms were quietly removed. The box that matched the one the rat was presently occupying (A2) was put in one arm while a novel box (B1), which the rat had not previously seen, was put in the other arm. Both of these goal boxes were baited with two pellets each. The arms which the two boxes were placed in (left or right relative to the current position of the rat) were predetermined by a pseudo-random

sequence. The opening of the guillotine door constituted the end of the starting run and the beginning of the first test trial proper.

Each test trial was conducted as follows. After the rat had eaten the food pellets and examined the goal box (A1) for 30 seconds the guillotine door was raised to allow the rat to select between novel and familiar goal boxes (figure 4.1b). Once the rat had entered one of the goal boxes the guillotine door was closed behind it. If the rat selected the novel box the test trial was completed. The boxes in the two other arms were quietly removed and a box (B2) matching the current start box (B1) was placed in one arm, while a new novel box (C1) was placed in the other arm, according to the predetermined pseudo-random sequence (figure 4.1c). Although one of the goal box arms still contained pellets, while the other was empty, box arms were re-baited with two pellets each in order to avoid giving aural cues which may have influenced the rats subsequent choice. The process was then repeated using new boxes for each of the remaining test trials.

If the rat did not select the novel box and, instead entered the familiar one, then a correction trial was run before starting the next test trial. A correction trial involved placing the box matching the unselected novel box in the original start arm and baiting both arms with two reward pellets. Once the rat had eaten the pellets and examined the box the guillotine door was opened and the rat was allowed to select one of the two matching boxes. After the rat had made a selection, the two remaining boxes were removed and the now familiar box was placed in one arm while the new novel box was placed in the other arm according to the pseudo-random sequence. Both boxes were baited with two pellets each. The next test trial was therefore ready to begin, the situation now being identical to that at the end of a successful test trial (figure 4.1c).

These procedures were used throughout the fourteen trials in each testing session.

Design and Analysis.

The experimental design consisted of three blocks of three sessions. During each block each animal was tested under each drug condition. Order of drug administration was counterbalanced across animals and between blocks (with minor imperfections due to the loss of one animal from the experiment).

As there were forty five pairs of boxes, boxes were only presented once in each block of three trials. The order of box presentation was different in each block.

The pseudo-random sequences determined which arms, relative to the current position of the rat, the novel and familiar boxes were placed in. These sequences were constructed so that during each day the novel box was to the left of the rat on seven of the fourteen trials and to the right on the other seven. In addition, the novel box was not on the same side more than three times in succession. This design controlled for the effects of response preferences and minimised the likelihood of such side preferences developing. New sequences were used on each day.

As the aim of the experiment was to assess the effects of flupenthixol on the rewarding quality of novelty, it was necessary to determine that novelty was reinforcing in this experimental design. That is, that boxes were selected with more than chance probability. One sample t-tests were conducted on the proportion of novel choices made in the saline condition during each three session block. Only

those blocks in which the novel boxes were selected significantly above chance were used in the analysis of drug effects, as testing for a drug-induced reduction of novelty selection is meaningless when novel objects have ceased to be selected at a level above chance in the control condition.

Drug effects were analysed in a 2-way analysis of variance of the factors 'trial block' and 'drug' on the number of choices each animal made of the novel stimuli on each test day in each drug condition. Only those trial blocks in which novelty selection was significantly above chance in the saline condition were entered into this analysis.

Although the experiment aims to investigate drug effects on novelty selection, a number of other behavioural tendencies which may compete with novelty selection can be assessed. Two measures are obtainable, spatial alternation scores and response bias scores. Spatial alternation scores are measures of the number of times an animal changes its spatial choice in any session. Response bias scores are measures of the extent to which an animal chooses one particular dominant spatial response in any session. These measures may prove important in the analysis of the object novelty choice responses. For example, Ryder and Watson (reported in Halliday, 1968) found that an experiment performed by Berlyne and Slater (1957) which had failed to detect any difference between 'novelty' and 'complexity' could be made more sensitive by grouping subjects according to their response alternation tendencies.

It may appear that alternation and response bias scores are not independent. To a limited extent this is true, if an animal has a total response bias it alternation score must be zero. Likewise an animal with a maximum alternation score must have close to zero response bias (the first trial cannot effect alternation score, but does contribute to the response bias score, so response bias score is not necessarily zero). However, apart from these extreme cases, the two measures are not fully dependent. For example, an animal which has no response bias may alternate its side choice throughout a session, obtaining a maximum alternation score, or it may choose left seven times followed by right seven times, scoring close to minimum on alternation. As response bias increases, however, the range of alternation scores attainable falls towards the minimum. A negative correlation between response bias and alternation may therefore be expected on logical grounds. Likewise, as novel boxes are presented on both sides equally, a negative correlation between response bias and novelty selection may be expected. This is not the case with spatial alternation. Neither pure alternation or non-alternation will necessarily produce particularly high or low object novelty selection scores.

Analyses of drug effects on both response bias and spatial alternation will be carried out. One may be tempted to perform an analysis of covariance of novelty selection with bias and alternation scores, however, the likelihood of correlations between bias and novelty scores indicates that this is unwise (see e.g. Howell, 1982), at least in the case of bias. The division of rats into high and low groups of bias and the treatment of these groups as separate dependent variables is to be preferred. A similar form of analysis will be carried out with alternation scores for the sake of consistency.

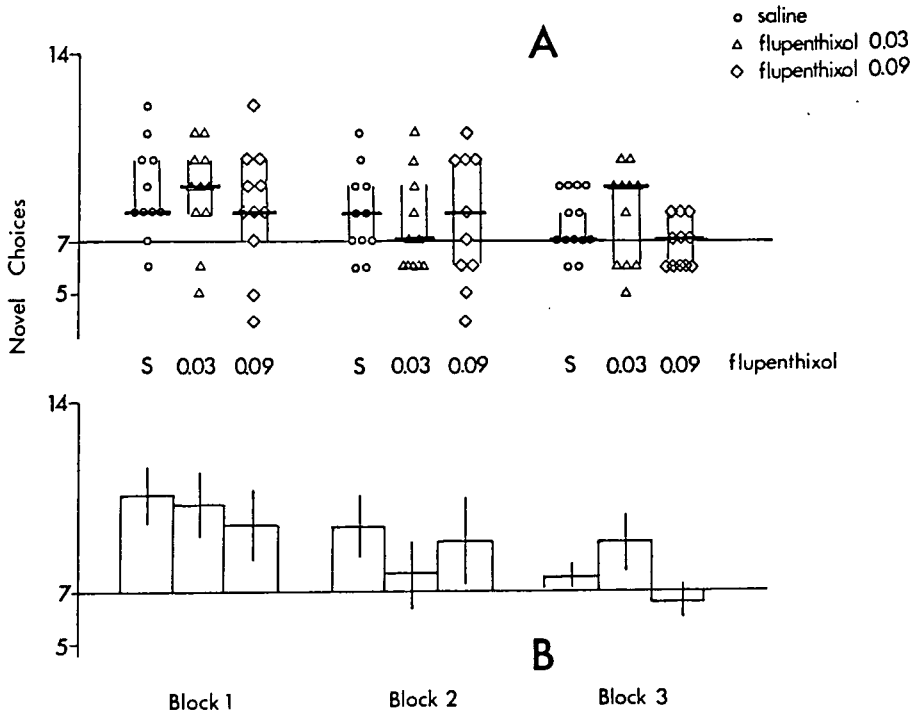
4.1.3 Results.

One sample t-tests showed that novel boxes were selected in the saline condition

significantly above chance during the first two trial blocks, but that this effect had disappeared in the third trial block (Block 1, $t = 3.392$, df 10, $p < 0.005$; Block 2, $t = 2.213$, df 10, $p < 0.05$; Block 3, $t = 1.174$, df 10, non-significant).

Similar analyses were performed on choices in the two drug conditions, they showed that novel choice was significantly above chance in both drug conditions during the first trial block (0.03 mg/kg flupenthixol, $t = 3.003$, df 10, $p < 0.01$; 0.09 mg/kg flupenthixol, $t = 1.965$, df 10, $p < 0.05$) but that choice was not significantly different from chance in the second or third trial blocks.

Results from all three blocks are presented in figures 4.2a (raw data, medians and inter-quartile ranges) and 4.2b (means and standard errors). It is important to note that neither of these figures can truly represent the repeated measures nature of the data.

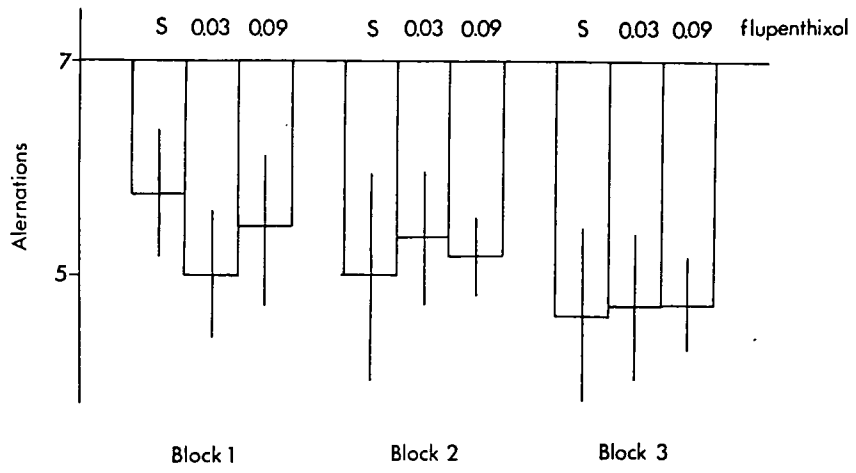


4.2 Selection of the novel stimulus choice over all three trial blocks and drug conditions. **4.2a** Medians, inter-quartile ranges and individual scores. **4.2b** Means and standard errors. Random choice would produce a score of 7 on average.

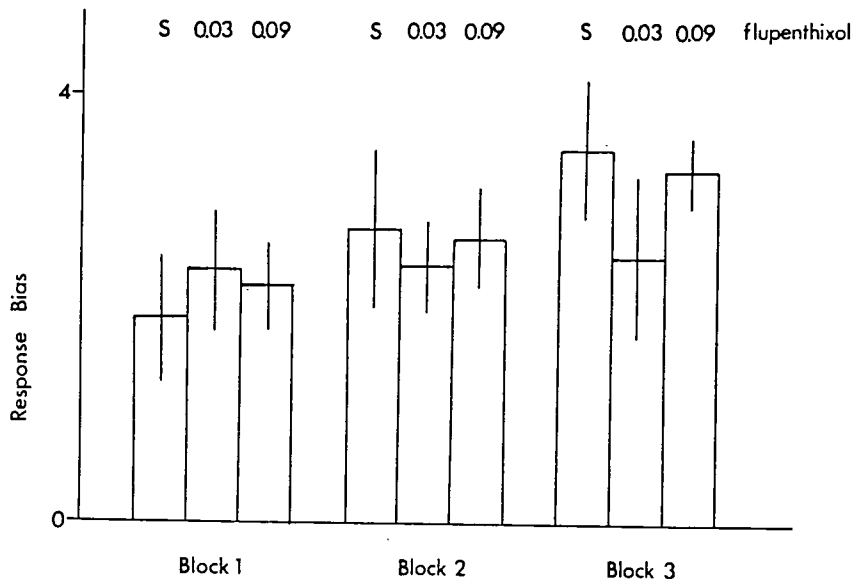
An analysis of variance of novelty selection during the first two trial blocks failed to show any effect of drug ($F < 1$), block ($F = 1.41$, df 1, 10, non-significant) or drug by block interaction ($F < 1$).

Alternation scores in both drug conditions in all blocks were below chance (figure 4.3). One sample t-tests show these differences to be significant for flupenthixol 0.03 mg/kg in trial block 1 ($t = 2.270$, df 10, $p < 0.05$), for 0.03 and 0.09 mg/kg in block 2 (0.03 mg/kg flupenthixol, $t = 3.496$, df 10, $p < 0.005$; 0.09 mg/kg flupenthixol, $t = 1.871$, df 10, $p < 0.05$) and for all three conditions in block 3 (saline, $t = 2.996$, df 10, $p < 0.05$; 0.03 mg/kg flupenthixol, $t = 3.757$, df 10, $p < 0.005$; 0.09 mg/kg flupenthixol, $t = 6.331$, df 10, $p < 0.001$).

Significant within sessions response bias was shown in all drug conditions in all trial blocks (see figure 4.4). All significance levels were at $p < 0.01$ or less (one



4.3 Spatial alternation scores over all three trial blocks and drug conditions. Random choice would produce a score of 7 on average. Means and standard errors are shown.



4.4 Spatial response bias scores over all three trial blocks and drug conditions. Means and standard errors are shown.

at $p < 0.01$, three at $p < 0.005$, five at $p < 0.001$). It is important to note the method of calculating response bias. Bias is defined as the absolute deviation of side selection from chance within a session. A score of zero therefore represents no bias, while higher scores represent both left and right side biases within a session.

Analyses of variance on response bias scores and alternation scores did not show significant drug, trial block or interaction effects for either measure.

Analyses of variance which took bias or alternation tendencies into account as independent variables (groups being divided according to whether animals mean bias scores over the first six trials were above or below the median) did not yield any significant results.

4.1.4 Discussion.

The failure to detect any effect of flupenthixol on novelty selection, even when measures were taken to account for other behavioural phenomena which may influence behaviour, may be explained in two ways. Either the result genuinely reflects a lack of effect of flupenthixol on novelty selection, or the test is too insensitive to detect such an effect.

Tests of deviations of novel object choice from chance in the saline condition clearly indicate that in the first two trial blocks novelty was attractive. It is tempting to cite the disappearance of this effect in the two drug groups during block two as evidence for a flupenthixol induced attenuation of the discrimination of the reinforcing properties of novelty. This attenuation is not reflected in the repeated measures analysis of drug effects, furthermore, the disappearance of significant novelty selection in the 0.9 mg/kg condition appears to be due as much to high inter-subject variability as to reductions in mean novelty selection. Mean novelty selection scores in the first two trial blocks are, however, higher for the saline condition than the drug conditions which is at least suggestive of an undetected effect.

The large variability within conditions lends some credence to the argument that the test was too insensitive to detect drug effects, however, the repeated measures nature of the design should reduce the effects of inter-subject variability on the analysis.

An additional factor that is likely to contribute to test insensitivity is the decline in novelty selection over days. Although some of this decline is accounted for in the analysis by the Blocks factor, the use of a counterbalanced repeated measures design means that some variance due to day to day decline in novelty score becomes included within cells. For example, some animals receive saline injections on the first day of a trial block while others receive it on the second or third days. If flupenthixol is attenuating novelty selection this attenuation is likely to be masked in animals receiving saline on the last day of a block and accentuated in animals receiving saline on the first day. Counterbalancing between animals eliminates the appearance of such biases in mean scores, they nevertheless increase error variance. An analysis of covariance of novelty selection scores by the factors drug and block, with drug presentation days within blocks as covariates, showed a comparatively high (but non-significant, $F = 2.94$, df $1, 19$, $p = 0.102$) contribution of presentation day to drug effects (drug effects were nevertheless non-significant in this analysis).

The superimposition of non-differential food reinforcement may also reduce the sensitivity of the test. As has been noted, object novelty is competing with a number of other factors in governing animals' response choice. Response biases towards making turns to one particular side in the Y-maze are particularly likely to overshadow the effects of novel stimuli. Figure 4.4 shows the large and increasing extent of these biases. All of these bias scores differ significantly from chance.

The acquisition of superstitious response biases reinforced by the food pellets may well accentuate pre-existing response biases. Response biases effectively reduce the extent to which choice is influenced by stimulus novelty. As the range of scores

that may be expected in any session is already very limited (from a fairly small novelty selection effect in the saline condition to chance if flupenthixol completely blocks the effect of novelty) further reductions in this range are likely to increase the likelihood that random variations in choice will obscure any systematic drug effect on novelty selection.

It is therefore concluded that, although the counterbalanced repeated measures design initially appears to offer a reasonably powerful test of flupenthixol's effects on novelty selection in small subject group, when account is taken of other influences on response choice the design is flawed. A simple between groups design may well have been more powerful in practice as confounding effects would be more easily separable from the drug effects under test, and more data could be gathered before novelty selection tendencies began to wane.

No significant drug effects on choice were found. As the power of the experiment appears limited, little inference can be drawn from these results. It is nevertheless worthwhile considering what this form of experiment can reveal about the rôle of dopamine in reinforcement processes.

Essentially the Y-maze experiment is a discrimination task. A number of studies have failed to find effects of dopamine blockade on discrimination in tasks similar to the Y-maze experiment (e.g. Bowers, Hamilton, Zacharko and Anisman, 1985; Tombaugh, Szostak, Voorneveld and Tombaugh, 1982). These studies found no reductions in choice accuracy with doses of dopamine blockers which significantly reduced response or running rates required to obtain the reinforcers in the choice tasks. The Y-maze experiment did not measure running rate towards selected stimuli, however, the failure to detect a choice impairment is consistent with the results of these other studies (although there are reasons to question the power of the design). Motor deficits have been proposed as the most parsimonious explanation of these type of results (e.g. Tombaugh, 1982). If the results of the current experiment are accepted as truly reflecting a failure of flupenthixol to influence choice, then this explanation is called into question. The 0.03 mg/kg drug dose used did not reduce home cage activity, but did effect the exploration preference experiment. These results can, however, be reconciled if it is assumed that dopamine blockade produces a disruption of the reinforcing effects of exploratory behaviour, but not in the identification of stimuli which may be reinforcing to explore. A flupenthixol-treated animal may perceive novelty correctly, and recognise it as more reinforcing to explore a novel rather than familiar stimulus, however, exploration of that stimulus may be reduced if the hedonic component of reinforcement is attenuated, or if reinforcement no longer continues to elicit further exploratory behaviour.

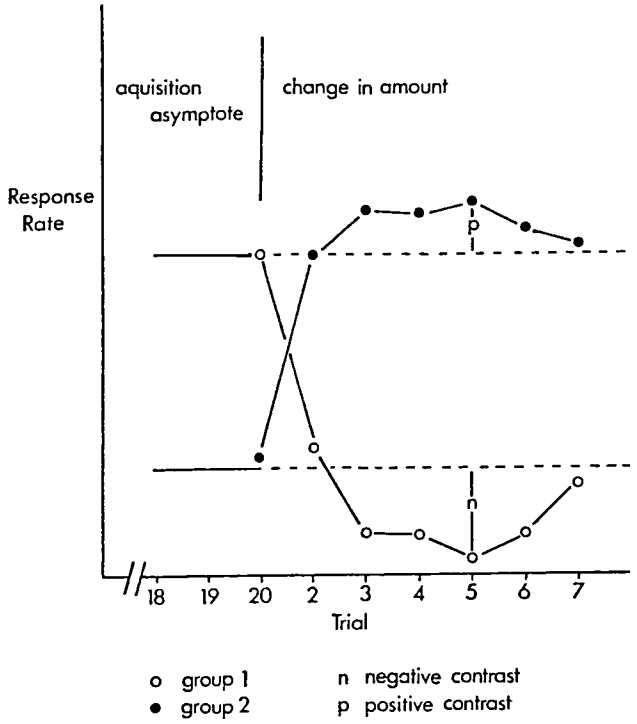
In summary, this experiment failed to show any effects of dopamine blockade on discrimination between stimuli which had differing exploratory reinforcing values. This result does not, however, imply that dopamine systems are not involved in the subsequent processing of reinforcement.

Chapter 5

The Effects of Dopamine Blockade on Behavioural Contrast.

5.0 General Introduction.

The major difficulty in assessing the rôle of dopamine in reinforcement processing is the possible confounding influence of dopamine's involvement in motor control. Any simple effect that dopamine blockade may have in reducing an animal's response to reinforcers can be attributed to a disruption of motor control systems, rather than to a reduction in the animal's responsiveness to reinforcement. Earlier experiments in this thesis used choice measures as a method of dissociating reinforcement from motor functions. The successive contrast paradigm (Crespi, 1942, 1944) is an alternative tool which will be used to examine the dissociation of dopamine's motor and hedonic rôles in this chapter.



5.1 Idealised successive contrast effect. Data after Zeaman (1949).

Successive contrast is the phenomenon whereby an animal 'over-reacts' to a sudden unexpected change in reinforcement value. Figure 5.1 shows idealised results from a contrast experiment (after Zeaman, 1949). Two groups of animals have been trained to respond for reinforcement. Group 1 initially worked for a high value reinforcer, while group 2 worked for a low value reinforcer. After a number of trials both groups have learned the task and their response rates have stabilised ('acquisition asymptote' in figure 5.1). On the 20th trial, (the first contrast trial). the amount of reinforcement was changed, group 1 now received the low reward and group 2 the high reward. On this first 'changeover' trial the animals could not know that the amount of reinforcement had changed until they obtained their reinforcement. Response rates on the first contrast trial were therefore still at acquisition levels. Contrast effects were shown over the next few trials. The response

rate of animals in group 1 fell to the low reinforcer acquisition rate on the next trial, however, on the following trials it dropped well below the acquisition rate. That is, response rates for the low reward fell below the rate that would be expected if the animals had not previously experienced the higher value reward. This excess in the decrease of response rate following reinforcer devaluation, or 'undershoot', is a negative contrast effect, labelled 'n' in figure 5.1. Group 2 showed a corresponding excess increase in responding, or 'overshoot'. Their response rate rose above the high reward acquisition rate after a few trials. The positive contrast effect is labelled 'p' in figure 5.1. The typically transitory nature of runway contrast effects and the larger size of negative contrast are reflected in figure 5.1.

It should be noted that there are a number of other phenomena which are also referred to as contrast effects, e.g. simultaneous and transient contrast, which are not relevant to he experiments presented in this chapter.

Successive contrast effects may be used to investigate dopamine's rôle in reinforcement in two ways. First, if dopaminergic drugs affect the perceived values of reinforcers, (and hence the recalled and expected values of those reinforcers), they may be used to induce contrast effects. Negative contrast should be induced by the introduction of dopamine blocking drug treatment after prolonged baseline training under drug-free conditions. Positive contrast may be induced if animals are under drug treatment during the baseline training, and then that drug treatment is withdrawn. Although motor effects could be used to explain negative contrast, if both negative and positive contrast can be induced by the introduction and withdrawal of drug treatment, then a drug effect on the perceived impact of reinforcement must be inferred.

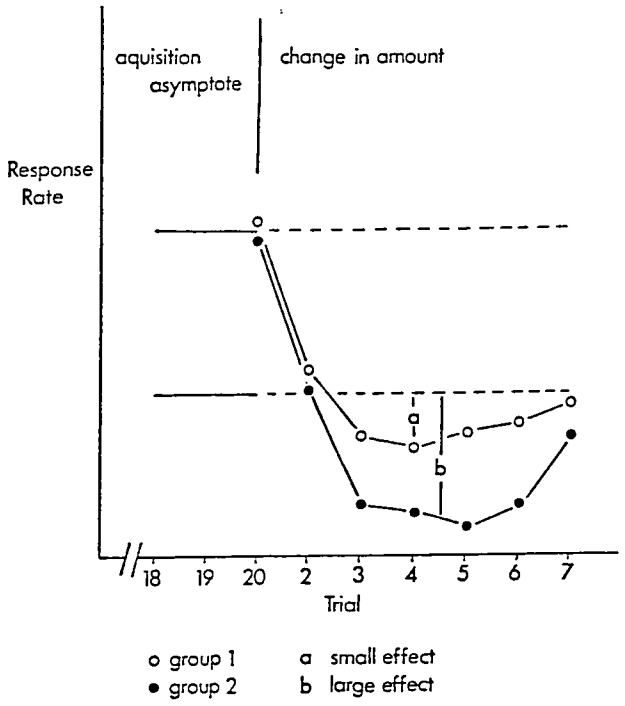
Although the results of early experiments (e.g. Crespi, 1942, 1944; Zeaman, 1949) appeared to demonstrate positive contrast, considerable doubt existed as to whether the 'overshoots' found were in fact positive contrast effects, or whether they were artifacts of experimental designs (see e.g. Black, 1968; Dunham, 1968; Spear, 1965; Spence, 1956). Some of this controversy may have been due to the fact that negative, but not positive, contrast was predicted by neo-Hullian learning theory (Black, 1968; Spence, 1956). It now appears that positive contrast is a valid phenomenon, albeit one which is harder to obtain than negative contrast (Flaherty, 1982).

The second use of contrast effects in the investigation of dopamine's rôle in reinforcement is the investigation of the effect of dopaminergic drugs on contrast effects induced by changes in the 'real' or 'explicit' magnitude of reinforcement (e.g. changes in the number of food pellets obtained as reinforcement, rather than the introduction or cessation of drug treatment assumed to effect the impact of reinforcers). A reduction in sensitivity to reinforcement, (perhaps produced by drug treatment throughout the whole of an experiment), may reduce the size of contrast effects. This is particularly likely if the effects of dopamine blockade are on motivational and emotional systems, given the 'emotional' nature of contrast (Benefeild, Ocós and Ehrenfreud, 1974; Baltzer, Huber and Weiskrantz, 1979; Crespi, 1942, 1944; Flaherty, 1982).

Weiskrantz and Baltzer (1975) showed that, within their specific experimental design (Baltzer and Weiskrantz, 1970), contrast effects were most reliable in animals which were only mildly food deprived. This effect appears to be due to increase in positive contrast at low drive levels, as a consequence of reduced baseline rates, and

hence reductions in ceiling effects (Panksepp and Trowill, 1971; Shanab, Sanders and Premack, 1969). Many studies have shown that low drive levels attenuate or eliminate negative contrast (Ehrenfreud, 1971; Ehrenfreud and Badia, 1962; Cleland, Williams and Di Lollo, 1969; Flaherty and Kelly, 1973), although negative contrast can be obtained at low drive levels (Capaldi and Singh, 1973; Panksepp and Trowill, 1971; Riley and Dunlap, 1979). Drug induced attenuations of contrast effects may therefore be indicative of effects on either motivational or emotional systems. Effects on positive contrast become difficult to interpret if significant reductions in baseline response rates are produced by the experimental manipulation. If the strength of the manipulation which reduces reinforcer sensitivity is chosen carefully, however, it may be possible to attenuate contrast effects (which are delicate phenomena) without significantly changing baseline response rates.

Figure 5.2 shows the idealised results from such an experiment in which two groups of animals are tested for negative contrast effects in a manner similar to the negative contrast group in figure 5.1. Group 1 have been treated with a manipulation which attenuates contrast, group 2 are the normal contrast control. Other animals have provided the low reward acquisition asymptote baseline. It can be seen from figure 5.2 that an attenuation of negative contrast implies that, during the contrast effect, the treated group are responding faster than the control group. This is clearly at odds with the predicted effects of a motor deficit which should reduce response rates whether or not that responding was part of a contrast effect. It is therefore apparent that a test of the effects of dopamine blockade on negative contrast effects may be able to dissociate potential reductions in sensitivity to reinforcers from motor impairments.



5.2 The effect of an imaginary contrast attenuating manipulation on response rates during contrast effects.

Both of the approaches described above are used in the experiments presented in this chapter. Experiments 5.1 and 5.2 examine the effects of flupenthixol on contrast effects induced by explicit changes in the quality of reinforcement. Experiment 5.3 investigates drug-induced contrast effects, flupenthixol's effects on contrast induced by shifts in the magnitude of reinforcement are also examined.

Experiment 5.1 – The Effect of α-Flupenthixol on Contrast Induced by Changes in the Concentration of a Glucose Solution Reinforcer.

5.1.1 Introduction.

This experiment investigates the effects of a low dose of flupenthixol on a negative contrast effect induced by a change in the value of reinforcement. Rather than using a more traditional runway or bar-pressing measures with food reinforcers, the present experiment assesses the effect of changes in the concentration of a glucose solution on rats licking rate when consuming the solution. Changes in the concentration of a liquid reinforcer have been shown to produce contrast effects when consummatory behaviour (licking) is the measured operant (Panksepp and Trowill, 1971; Riley and Dunlap, 1979). The effect of dopaminergic drugs on rats' lick rate of rewarding solutions has been studied by Gramling, Fowler and Collins (1984) and Gramling and Fowler (1986). As they note, the advantages of using this type of design when evaluating the effects of dopamine blockers on reinforcement systems are that the response makes very low motor demands and that associations between response, discriminative stimuli and reinforcement, which may potentially be disrupted by dopamine blockade, need not be learned. In addition, food or water deprivation is not needed in order to maintain responding for this type of reinforcer. Hedonic components of reinforcement are therefore likely to dominate any effects of central motivational state.

5.1.2 Method.

Subjects.

The subjects were 24 experimentally naive male DA strain rats (Bantin and Kingman, Hull) weighing approximately 200 gm at the start of the experiment.

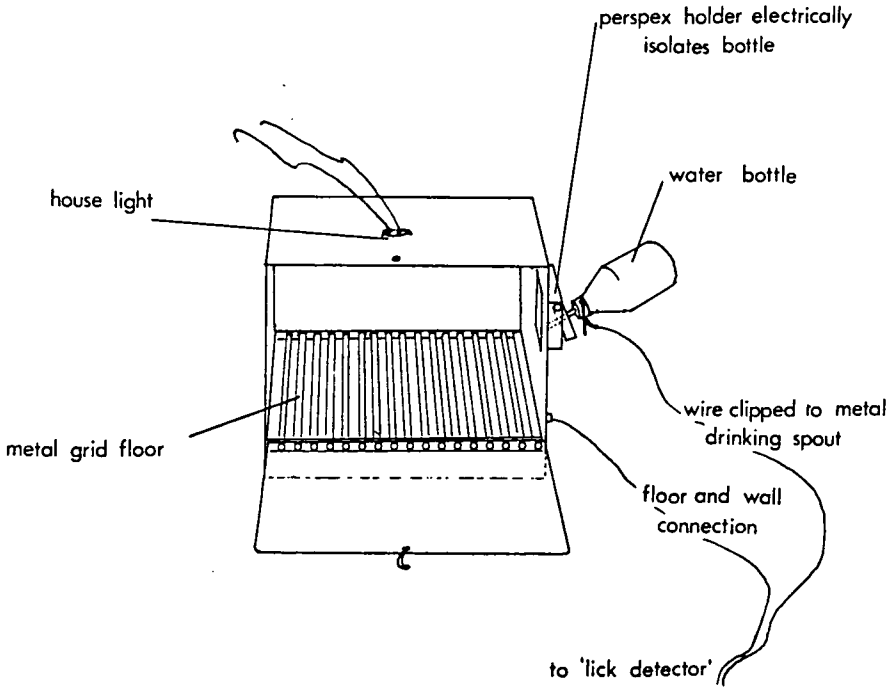
Drugs.

Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.06 mg/kg body weight and a injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

Four testing boxes were used, (see figure 5.3). Each was an aluminium box 22 cm wide × 16 cm deep × 17 cm high with a removable grid floor and a Perspex door. A rectangular hole 4 cm wide × 6 cm high was let into the centre of the end wall at a height of 8 cm. A single drinking bottle with a straight metal spout was

supported behind this hole at an angle of 30° from vertical. Each bottle contained either 5% or 25% (weight/volume) glucose solution. House lights were mounted in the centre of the ceiling of each box. Each testing box was housed inside a soundproof chamber.



5.3 The 'drinkometer' test box.

The grid floor and the spout of the drinking bottle were connected to an electrical circuit in such a way that the rat completed this circuit each time it licked the spout. The current passing through this circuit was negligible (less than 0.7 μa) and could not be detected by the experimenter when testing the circuit by licking. The changes in the resistance of the circuit brought about by the onset and offset of licking were converted into discrete TTL (transistor-transistor logic) voltages (0 volts corresponding to an open circuit, 5 volts corresponding to a completed circuit) and connected to a minicomputer (Plessey LSI 11/23) for data logging. The computer, together with an interface (Cambridge Electronic Design CED 502) were programmed in the 'C' language. The program collected data from all four boxes simultaneously, recording the onset or offset of each response from each box and the time (in milliseconds) between responses.

Procedure.

The experiment was conducted over 23 testing days. Animals were tested in blocks of four. Each animal was tested in the same box, at approximately the same time, each day. Half of the animals were injected with 0.06 mg/kg flupenthixol two and a half hours before their test session began, the remaining animals were injected with a similar volume of 0.09% saline. During a test session each animal was placed in its testing box, the doors were shut, and the animal was left undisturbed, with the opportunity to consume the glucose solution reinforcer, for 20 minutes. The computer logged any licking that occurred during the session. At the end of the session animals were removed from their boxes and returned to their home cages. There was no pre-training. Food and water were available ad lib at all times in the

animal's home cages. The liquid reinforcer (glucose solution) was available in the testing boxes throughout each session, but no food was provided.

The experiment was divided into three phases. During the first phase (days 1 to 6) the reinforcer was 25% glucose solution. During the second phase (days 7 to 17) the reinforcer was decreased to 5% glucose solution. During the final phase (days 18 to 23) there was a return to the high (25% glucose) reinforcer.

Analysis.

The raw data consisted of the total number of licking responses made by each animal on each day of the experiment. It is recognised that there are many ways of analysing this data. The first low reward day could be compared to some low reward baseline score such as the last low reward day or an average of the last few low reward days. A similar analysis could be carried out on data where individual differences between animals' scores were reduced by a taking ratios of the low rate scores being analysed with each animal's high baseline rate. As the decision of which scores constitute a baseline is somewhat arbitrary these types of analysis were rejected, instead two analyses which do not involve arbitrary selection of baselines were carried out.

If there is no increase in response rate over the experiment as a whole, that is, licking rates in phases 1 and 3 do not differ, then any change in lick rate during phase 2 can be attributed to a change in the rewarding impact of the low concentration reinforcer rather than any long term trend (e.g. an acquisition effect). Formal analyses were therefore carried out to test for differences between phases 1 and 3, and for changes in response rates over phase 2.

In the first analysis scores in phases 1 and 3 were compared in a 3-way split-plot analysis of variance of the factors phase and day (within phase) within subjects, and drug between subjects.

In order to avoid arbitrarily specifying some phase 2 days as 'baseline' and others as 'contrast' in the analysis of the phase 2 data, regression analyses were carried out on the individual scores of each animal over the entire phase. These regression slopes provided measures of the individual animals' changes in response rates over phase 2. Given that no long term change in response rates was shown over the experiment in the previous analysis, a change in response rates during phase 2 may be attributed to a change in animals' perception of the low value reward. That is, a change in response rates over phase 2 must reflect a contrast effect.

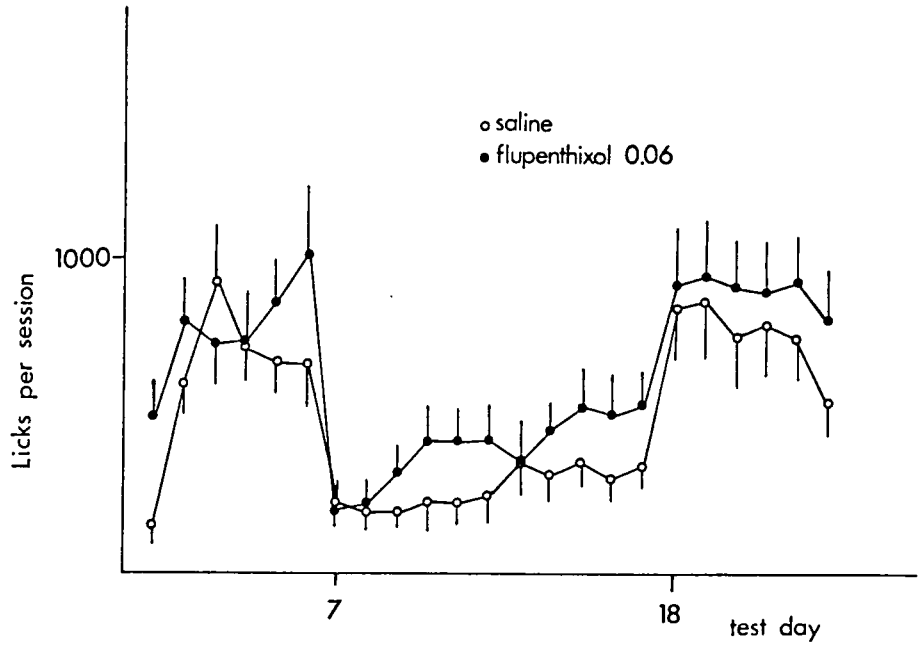
The measures of response rate change over phase 2 (the regression slopes) were then entered into a one way analysis of variance between drug groups using the procedure MANOVA in the SPSS-X package. This test is equivalent to an unpaired t-test, however, it not only performs a test of the group effect, but also provides a comparison of scores to a constant. This analysis provides two results. Comparison between the groups measures the effect of flupenthixol on the change in perception of the low reward over phase 2. Comparison of all regression scores with a constant of zero measures the significance of any general trend in the change of response rate over phase 2.

The second approach was to analyse scores from changeovers between phases 1 and 2 and phases 2 and 3. An 3-way split-plot analysis of variance was therefore performed on the factors changeover and day (within changeover) within subjects,

and drug between subjects. Changeover 1 consisted of scores from the last day of phase 1 followed by the first day of phase 2. Changeover 2 consisted of the scores of the first day of phase 3 followed by the last day of phase 2. The reversal of chronological order in changeover 2 means that both shifts are entered as being from high reward to low reward, but does not alter the analysis of variance itself.

5.1.3 Results.

The mean licking rates for each group obtained on each of the 23 test days are shown in figure 5.4.



5.4 Mean lick rates (and their standard errors) obtained over the full 23 days of experiment 5.1.

The analysis of data from phases 1 and 3 confirmed the evidence from figure 5.4 that there was no general increase in response rate over the experiment as a whole (phase, $F < 1$), indicating that there was no general learning or habituation effect over the experiment. In addition to this non-significant effect of phase, there was no drug main effect or phase by drug interaction.

The analysis did show significant variability of response rates across days, and a change in that variability between phases 1 and 3, (day, $F = 4.449$, df 5,110, $p < 0.01$; phase by day interaction, $F = 3.120$, df 5,110, $p < 0.05$). This day to day variability does not imply the existence of any long term trend which may confound the analysis of contrast effect. An examination of figure 5.4 shows that response counts of the saline group were very low on the first day of testing. This low score probably contributed considerably to both effects.

Once it has been demonstrated that there is no general increase in responding with time, a change in response rates during phase 2 may be explained in terms of changes in the subjects' perception of the low value reward over the 11 days of the phase. Regression analyses were carried out on each animals lick rates over the 11 days of phase 2. Analysis of variance of these regression slopes indicated that whilst there was no difference between the flupenthixol and saline groups, (drug, $F < 1$),

the pooled regression slopes differed significantly from zero (constant, $F = 9.162$, df 1,22, $p < 0.01$). In other words, responding increased significantly over phase 2. Such a change must reflect some form of contrast effect given no long term change between phases 1 and 3.

In the second analysis, the drop in responding between the last day of phase 1 (when animals were still being given the high value reinforcer) and the first day of phase 2 (when animals were switched to the low value reinforcer) were examined in relation to the increase in responding occurring between the last day of phase 2 and the first day of phase 3 (when animals were returned to high value reinforcement. The analysis did not reveal any drug effects. There was a highly significant day effect (day, $F = 39.208$, df 1,22, $p < 0.001$), this is not in the least surprising since data were entered into the analysis so that the first day within each changeover was a high reward day, and the second was a low reward day. Finally, there was a significant difference between the drop in response rates produced by reward downshift at the beginning of phase 2 and the increase in rates produced by upshift at the end of the phase (changeover by day interaction, $F = 9.698$, df 1,22, $p < 0.01$). This is indicative of a negative contrast effect, given certain assumptions about the 'control' changeover between phases 2 and 3.

If this analysis is to yield information about negative contrast and drug effects then it must be assumed that any positive contrast effect that does occur between phases 2 and 3 is negligible. This assumption, which is fully supported by the pattern of the data, is necessary as the change in response rate at upshift is being used as a control condition, (see figure 5.4, noting the flatness of phase 3). An analysis of regression scores from phase 3 carried out in a similar manner to that performed on the phase 2 data confirmed the lack of a positive contrast in phase 3 (drug, $F < 1$; constant, $F < 1$). There is a large amount of previous evidence that positive contrast effects are less likely to occur, and of smaller magnitude, than negative effects in the sequential paradigm (Flaherty, 1982), so this lack of positive contrast is not surprising. Moreover, if this assumption does not hold, its effect on the assessment of negative contrast and drug effects will be to make their tests more conservative. It therefore seems acceptable to regard the phase 2 and 3 changeover scores as a control condition for the assessment of the negative contrast effect which may be present at the phase 1 and 2 changeover.

5.1.4 Discussion.

The results of both types of analysis were in agreement that the change of reward between phases 1 and 2 produced a negative contrast effect, and that flupenthixol did not alter this contrast effect. Examination of figure 5.4 clearly shows the rise in response rates over phase 2 of both groups. Although it is tempting to discuss a possible group difference over phase 2, the significance of such a difference was not born out by any analysis.

If the increase in rates over phase 2 is to be interpreted in terms of contrast, it is important that there be no general increase in responding over the whole experiment. Examination of figure 5.4, and the results of the analysis, do not show any gross difference in rates during phases 1 and 3. Indeed, it would be surprising if a learning effect occurred since the subjects have had great experience of the measured response (licking) and there is no explicit learning requirement in the experiment. There does, however, appear to be an increase in response rates over the first few days of phase 1 in the saline group. One explanation for the

comparatively low rates of the saline group on the first few days is that animals in the saline group initially found exploring the test box rewarding and devoted part of their time to this, rather than to drinking, until they habituated to the test environment. Evidence from experiments presented in chapter 2 suggests that doses of flupenthixol lower than that used here can reduce exploratory behaviour, perhaps accounting for the higher initial drinking rates of the flupenthixol group.

Several factors may account for the failure of flupenthixol to alter contrast effects. It is possible that dopamine is involved in mediating the evaluation of secondary, and not primary, reinforcers, and thus did not effect the current experiment in which there is no explicit secondary reinforcer (although the experimental situation is likely to acquire secondary reinforcing properties). If flupenthixol affects perceived values of reinforcement but not motivational or emotional systems an attenuation of contrast is not necessarily predicted. Finally there may be problems with the sensitivity of the design. The contrast effect obtained was quite small, a weak attenuation of this effect may not have been detectable. The dose of flupenthixol may have been too low to produce behavioural effects in this design. The dose used was chosen in order to minimise confounding motor effects, a slightly larger dose might still produce anhedonic effects without seriously impairing motor performance.

It may also be predicted that the largest contrast dependent changes in response rates would occur at the beginning of each test session when an animal is expecting the baseline value of reinforcer. Analyses similar to those described were carried out on response rates taken from the first 4 minutes of each session, rather than over the full 20 minutes. The results of these analyses were, however, similar to those obtained from the full session data. Although a significant increase in response rates over phase 2 was found in the regression analysis, there was no drug effect. Phase 1 response rates were, however, significantly lower than those during phase 3. Although this difference can be attributed to very low response rates during the first 4 minutes of the first day, it does confound interpretation of the regression analysis results. If the increase in responding over phase 2 is interpreted as a decaying negative contrast effect, then it appears to be as small and long lived as the effect found when the full session data was analysed.

Finally, it has been demonstrated that dopamine is involved in the mediation of metabolic state controlled hunger mechanisms (e.g. Biggio, Porceddu, Fratta and Gessa, 1977; Liebowitz and Rossakis 1979a, 1979b). Dopamine's rôle in hunger control may confound the results of dopamine blockade experiments using rewards of high caloric value which modify animals' metabolic state quickly. Liebowitz has shown that dopaminergic inputs to the perifornical hypothalamus inhibit hunger. An implication of this is that dopamine blockade should interfere with this hunger inhibition, resulting in dopamine blocked animals remaining hungry when control animals are becoming sated. This effect might operate in the opposite direction to the hypothesised anhedonia effect of dopamine blockers and so may obscure anhedonic effects. The use of rewards with low or non-existent caloric value in undeprived animals could eliminate this potentially confounding effect.

Given the possible confounding problems outlined above, a second study was undertaken using a higher dose of flupenthixol and saccharine solution rewards (which have no caloric value) differing in concentration by a factor of ten.

Experiment 5.2 – The Effect of α-Flupenthixol on Contrast Induced by Changes in the Concentration of a Saccharin Solution Reinforcer.

5.2.1 Introduction.

Although the previous experiment showed evidence of contrast effects, no drug effects were found. It would not, however, be wise to conclude that dopamine blockers have no effect on contrast. It has been noted that manipulations of dopamine systems may effect central motivational states, in particular inhibition of hunger. In order to avoid such problems a non-nutritive reinforcer (saccharin solution), whose perceived value should be less effected by changes in hunger and satiety systems, was used in the current experiment. Since saccharin solutions become bitter and unrewarding at high concentrations (Kemble, Levine, Gregoire, Koepp and Thomas, 1972) selection of appropriate concentrations is very important. Concentrations were selected using data from Kemble's study, the high concentration chosen having produced maximum preference, and the low concentration, although much less rewarding, having still been significantly preferred to plain water.

The dose of flupenthixol used in the previous experiment was quite low. This low dose was chosen in order to avoid flupenthixol induced motor deficits. Although flupenthixol has been shown to effect behaviour in rats at even lower doses (Robbins and Watson, 1981, chapters 2 and 3 of this thesis), in general considerably higher doses are typically used (e.g. 0.1 to 0.4 mg/kg used in Ettenberg, Koob and Bloom (1981), Corbett, Stellar, Stinus, Kelly and Fouriezos (1983), Ettenberg and Carlisle, (1985)). Since the dose used in the previous experiment did not produce any deficit it was felt to be reasonable to use a higher dose in this experiment. A dose of 0.15 mg/kg was tested for one day with this group of animals using the experimental procedure described in this experiment. As response rates appeared to be greatly reduced at this dose, a lower dose of 0.1 mg/kg was used throughout the experiment proper.

This experiment retained the general design of the previous experiment, but was extended in order to allow a fuller investigation of both positive and negative contrast effects.

5.2.2 Method.

Subjects.

The same animals used in the previous experiment served as subjects. Animals were allocated to the drug and saline groups so that half of the animals in each group had been receiving flupenthixol in the previous experiment and half saline. Unfortunately one animal (saline group) died during the experiment, data are therefore only reported for 23 animals.

Drugs.

Cis-Z-Flupenthixol (α-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.10 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The apparatus used was the same as that used in the previous experiment. The liquid reward available in the drinking bottles was either 0.015% or 0.15% (weight/volume) sodium saccharin.

Procedure.

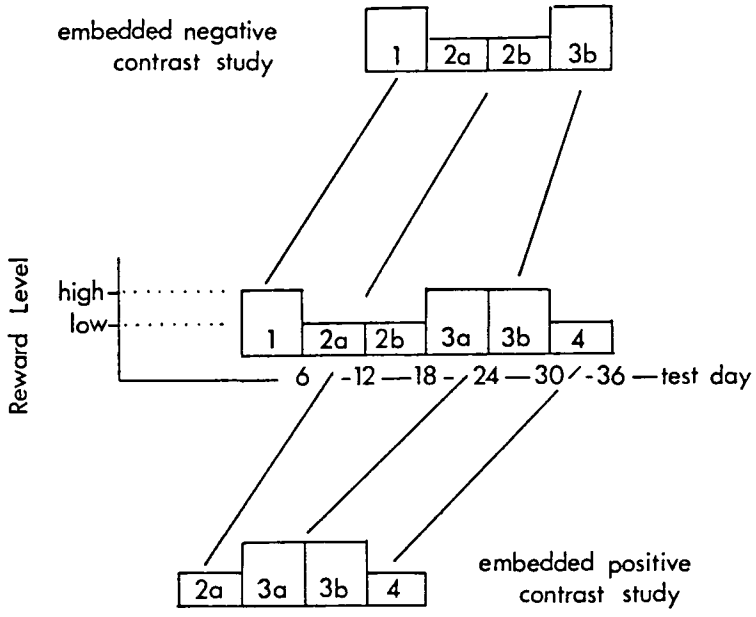
In general the procedure was the same as that used in the previous experiment, however, the following changes were made.

The experiment was conducted over 36 testing days, an extra phase having been added to aid the emergence and measurement of positive contrast effects. Conditions during the four phases were as follows. During the first phase (days 1 to 6) the reinforcer was 0.15% saccharin solution. During the second phase (days 7 to 18) the reinforcer was decreased to 0.015% saccharin solution. During the third phase (days 19 to 30) there was a return to the high value 0.15% saccharin reinforcer. In the final phase (days 31 to 36) the low value reinforcer 0.015% saccharin was used again.

Testing began three weeks after the end of the previous experiment, during this interval all animals were drug free.

Analysis.

The analyses used were similar to those used in the previous experiment, however, additional analyses were carried out to assess positive contrast effects. Two types of analyses were carried out for positive and negative contrasts, a regression slope based analysis and an analysis of response rate changes at phase boundaries. Both of these were described in the previous experiment.



5.5 A diagrammatic representation of the design of experiment 5.2.

The design of this experiment can be thought of as two embedded experiments, one testing negative contrast and the other positive contrast (see figure 5.5). These

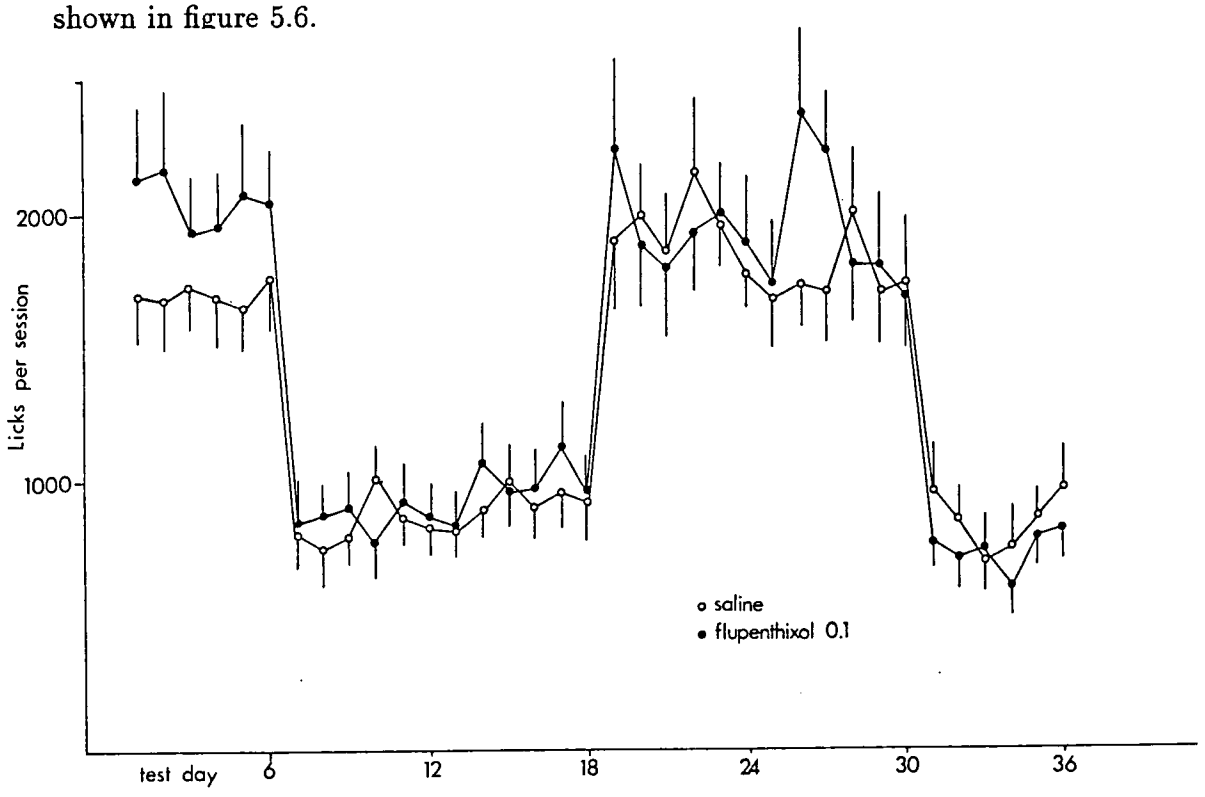
embedded experiments both consist of 3 blocks of trials, as in the previous experiment. The first block establishes an initial baseline response rate over 6 days. In the second block of 12 days a different value of reinforcer is used to examine a contrast effect. In the final 6 day block reinforcement is returned to the baseline value. Since the purpose of the initial and final blocks is to assess whether there has been any change in response rates over the contrast block which cannot be attributed to the contrast effect, test conditions in the initial and final blocks were matched as closely as possible.

In the embedded negative contrast experiment (and in the previous experiment) the reinforcement in the first block was high concentration solution, in the second block it was low concentration, and in the final block it returned to high concentration (phase 1, phase 2 and the last 6 days of phase 3 respectively). The last, as opposed to first, 6 days of phase 3 were used since any positive contrast effect occurring during phase 3 should have decayed somewhat by the end of the phase. As no positive contrast effect can be occurring during phase 1, the last 6 days of phase 3 provided the best match of testing conditions to phase 1.

In the embedded positive contrast experiment (see figure 5.5) the reinforcement in the first block was low concentration, changing to high concentration during the second block and returning to low concentration during the final block (the first 6 days of phase 2, phase 3 and phase 4 respectively). Since phase 4 was a period of low value reinforcement immediately following a period of high value reinforcement the first, rather than last, 6 days of phase 2 provided the best match of conditions as they too immediately followed a period of high value reinforcement.

5.2.3 Results.

The mean response rates obtained over the full 36 days of the experiment are shown in figure 5.6.



5.6 Mean lick rates and their standard errors obtained over the full 36 days of experiment 5.2.

The results of analyses of the embedded negative and positive contrast experiments are presented separately.

An examination of figure 5.6 indicates that the group receiving flupenthixol were responding at a higher rate than the saline group during phase 1. An additional two way analysis of variance of the factors drug and day was therefore carried out on data from that phase in order to establish whether the difference in response rates was statistically significant. The results of the analysis show that there was no significant difference between the groups during phase 1 (drug, $F = 1.499$, df 1,21, $p > 0.05$). Day and day by drug interactions were also non-significant (both F's < 1).

Negative Contrast Results.

The analysis of data from phases 1 and 3 showed no general increase in response rate over the negative contrast section of the experiment (block, $F < 1$). In addition to this non-significant effect of phase, no other effects or interactions were significant.

The analysis of regression slopes of each animals' scores over phase 2 did not show any significant difference from zero slope (constant, $F = 1.737$, df 1,21, $p > 0.05$), therefore no contrast effect appears to have occurred. In addition, no drug effect was shown (drug, $F < 1$).

The analysis of scores at the phase changeovers did not show any difference between the drop in response rate at the phase 1/2 changeover and the increase at the phase 2/3 changeover (changeover by day, $F < 1$). Such a difference would be indicative of a contrast effect. No drug effects were found (drug and interactions involving drug, F's < 1). A highly significant day effect was a consequence of the order in which scores were entered into the 'changeover' factor as explained in the previous experiment (day, $F = 131.109$, df 1,21, $p < 0.001$).

Positive Contrast Results.

The analysis of data from phases 2 (first 6 days) and 4 did not show any significant change in response rates between the initial and final blocks of the embedded positive contrast experiment (block, $F < 1$). Any reduction of response rate over phase 3 can therefore be attributed to a decaying positive contrast effect rather than any general reduction in response rates occurring over time. The analysis did show a significant difference in day to day variation in response rates between the initial and final phases (phase by day, $F = 2.630$, df 5,105, $p < 0.05$). There were no significant drug effects (drug, $F < 1$).

As in the previous experiment, given no difference in response rate between the initial and final phases, a change in response rates during phase 3 may be explained in terms of some change in the subjects' perception of the high value reward over the 12 days of the phase. The analysis of variance of the regression slopes of each animal's response counts over phase 3 indicated that whilst there was no difference between the flupenthixol and saline groups (drug, $F < 1$), the pooled regression slopes were significantly different from zero (constant, $F = 5.303$, df 1,21, $p < 0.05$). In other words, response rate decreased significantly over phase 3. This implies that animals' perception of the high reward was influenced by their prior experience of the low reward, that is, a positive contrast effect occurred. There were, however,

no significant drug effects.

The analysis of response rates at the changeovers between phases 2 and 3 and phases 3 and 4 supported the evidence from the analysis of variance of individual regression slopes for positive contrast. The increase in response rate between phases 2 and 3 was significantly larger than the decrease between phases 3 and 4 (changeover by day, $F = 34.597$, df 1,21, $p < 0.001$). Again, no drug effects were found (drug and all interactions involving drug, F's < 1). The highly significant day effect is an artifact of the method of analysis (day, $F = 44.879$, df 1,22, $p < 0.01$).

5.2.4 Discussion.

Although the experiment failed to produce a significant negative contrast effect, evidence for positive contrast was found. Both the analysis of phase changeovers and the regression based analysis were in agreement that response rates decreased significantly over phase 3. The comparison of response rates in phases 2 and 4 demonstrates that the drop in response rate found in phase 3 cannot simply be explained by a general decreasing trend. It can therefore be concluded that, as no other factors had changed, the high concentration saccharin solution became less reinforcing to the subjects as phase 3 proceeded. This reduction in the perceived value of the reinforcer can be interpreted as a positive contrast effect at the beginning of the phase, dependent on the subjects' prior experience of the low concentration solution, gradually diminishing over the phase. As in the previous experiment flupenthixol had no effect on this contrast.

One aspect of the data which must be considered is the apparent group difference in response rates during phase 1 which was not statistically significant. (Additional analyses showed that the difference was not significant on any individual day of the phase either.) It should be noted that overall the drug group was producing the faster response rate, so even this non-significant difference cannot be attributed to a drug induced motor deficit.

While it must be conceded that the contrast effects obtained in this experiment and in experiment 5.1 may appear weak compared to those obtained by Panksepp and Trowill (1971) and Riley and Dunlap (1979) the failure to demonstrate any effects of flupenthixol on contrast should not now simply be attributed to sensitivity problems. Roberts and Pixley (1965) and Rosen and Tessel (1970) both failed to demonstrate any effects of chlorpromazine, which has a dopamine blocking action (Guth and Spirtes, 1963), on successive negative contrast in more traditional food reinforced runway designs. Baltzer, Huber and Weiskrantz (1979) did, however, demonstrate a small attenuation of negative contrast, but no effect on positive contrast, with chlorpromazine. Their novel design, which differs markedly from other contrast designs, is discussed in more detail in the next experiment. They obtained far larger contrast attenuating effects with chlordiazepoxide, diazepam (both benzodiazepine anxiolytics) and amylbarbitone (a barbiturate). Furthermore, they found that chlorpromazine attenuated discrimination between high and low reward levels in the baseline conditions of their design, which may call its apparent contrast attenuating effect into question.

Gramling, Fowler and Collins (1984) and Gramling and Fowler (1986) assessed the effects of pimozide on rats licking sucrose solutions. Their designs did not test drug effects on contrast, however, they did find significant effect on lick rate when pimozide was introduced after a number of days of drug free training. Although

their detailed analyses of licking (measures not only of rate, but also of duration and inter-lick interval) showed differences between the introduction of pimozide and decreases in the concentration of the sucrose solution, their results suggest that an explicit investigation of drug induced contrast effects is called for.

The experiments described so far in this chapter have investigated contrast induced by changes in the concentration of liquid reinforcers. The measured response has been the subjects' consumption of the reinforcer, the subjects have not had to make intermediate responses to a secondary reinforcer (e.g. press a lever in a Skinner box in response to a discriminative stimulus) in order to obtain primary reinforcement. Although evidence for both positive and negative contrast effects has been found, there was no suggestion that these effects were sensitive to flupenthixol.

Although contrast effects for primary reinforcers in this type of design have been obtained by others (Riley and Dunlap, 1979; Panksepp and Trowill, 1971), the more usual paradigm is to study the effects of changes in reinforcement on second order responses e.g. rates of lever pressing in Skinner boxes or running speeds in runways. The next experiment investigates the effect of flupenthixol on contrast in a secondary reinforcement design.

Experiment 5.3 – A Combined Study of the Effect of α-Flupenthixol on Contrast for a Secondary Food Reinforcer and the Induction of Contrast Effects by the Introduction and Withdrawal of α-Flupenthixol Treatment.

5.3.1 Introduction.

If the hypothesis that dopamine blockers such as flupenthixol attenuate the rewarding impact of reinforcers holds, a number of predictions can be made about the effects of flupenthixol and contrast phenomena.

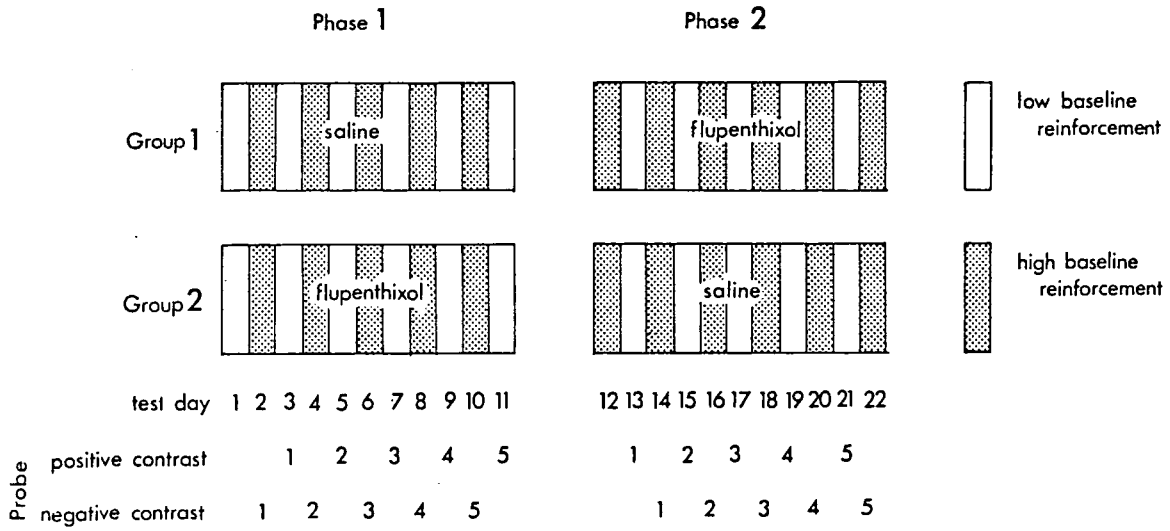
First, it is predicted that changes in the rewarding impact of reinforcers brought about by the introduction or termination of drug treatment should produce contrast effects. If an animal has been receiving a reinforcer under a particular drug condition for a number of days, a change in that drug condition should constitute a sudden change in the perceived value of the reinforcement from the previously expected value. If flupenthixol attenuates the rewarding impact of reinforcers, then administration of flupenthixol, after a number of days of drug free testing, should reduce the rewarding impact of the reinforcer, and hence produce a negative contrast effect. Correspondingly, if animals are repeatedly given flupenthixol, cessation of this administration should lead to relatively heightened reinforcement, and hence a positive contrast effect.

Royall and Klemm (1981) have reported apparent contrast effects following administration of a dopamine blocker (haloperidol) and an agonist (apomorphine) in

a saccharin solution reinforced runway task after a number of days of drug free testing. Their experiment did not include conditions in which termination of drug treatment was used to induce contrast effects. In addition to being unable to investigate contrast induced by release from drug treatment, the lack of conditions in which animals were running under drug treatment from the start of the experiment meant that there were no adequate control conditions with which contrast performance could be compared. The form of Royall and Klemm's results, does, however, indicate that contrast effects were produced. Transitory but marked changes in run rate followed the start of drug treatment.

One feature of Royall and Klemm's design which is worthy of note is the use of groups of animals receiving different reinforcer values in the runway task used. The use of high value reinforcers in the investigation of drug induced negative contrast and low value reinforcers in positive contrast reduces the likelihood of ceiling and floor effects which may prevent the detection of contrast (as suggested by Shanab, Sanders and Premack, 1969).

The present experiment will use high and low values of reinforcement to investigate the induction of contrast effect by the introduction of flupenthixol treatment (negative contrast) and the cessation of flupenthixol treatment (positive contrast) in an operant task. One group of animals will receive saline injections for the first half of the experiment and then be switched to flupenthixol, while the other group will receive flupenthixol initially and then switch to saline. Response rates on the final test day of the first half of the experiment from one group can be used as control scores with which to compare contrast effects in the other group that may occur on the first test day of the second half of the experiment. Separate groups of animals will not be used to assess contrast effects with high and low reinforcers, rather, animals will receive high and low reinforcement on alternate days, the value of reinforcement on a particular day being signalled by a discriminative stimulus. This design is shown schematically in figure 5.7.



5.7 Diagrammatic representation of the design of experiment 5.3.

The advantages of this type of design over one which uses separate groups of animals working for high and low reinforcement are that fewer animals are required, and that exposure to differing reinforcer values may enhance contrast effects (Benefield, Ocós and Ehrenfreud, 1974). The disadvantages are that the effects of the change in drug regime must be assessed over two days, rather than a single day in two groups, and that animals may not have as clear an expectation of reinforcement size at the start of any session as they would have if only exposed to a single value of reinforcement. Response rates were assessed both at the start of each daily session and for a period slightly later in the session when the animals were more likely to be fully aware of the reinforcement value being presented on that day.

The second potential effect of dopamine blockade is a reduction in the size of contrast effects brought about by changes in the (real) magnitude of reinforcement. If flupenthixol's effects on reinforcement perception are produced through an action on emotional or motivational systems, it may be predicted that flupenthixol will reduce the 'emotional' over-reaction to changes in expected reinforcement which underlie contrast effects. In other words, it may be expected that the contrast effect produced by a change from a four pellet reward to a one pellet reward will be smaller in flupenthixol treated animals than in controls. An embedded design based on that of Baltzer and Weiskrantz (1970) was used to assess the effects of flupenthixol on the size of contrast effects produced by sudden changes in (real) reinforcement magnitude. Two short periods are included in each test session where the reinforcement normally obtained on the other day, together with its associated discriminative stimulus, replace the baseline reinforcement value. Comparison of response rates during these probe periods with rates obtained during baseline periods of the session allow contrast effects induced by changes in the explicit magnitude of reinforcement to be assessed.

This embedded design allows both positive and negative contrast effects to be measured in individual animals repeatedly, as such, it is suited to examining potentially small drug effects on contrast which may be difficult to detect in a 'one-off' contrast test. Baltzer, Huber and Weiskrantz (1979) used this task to assess the effects of a range of drugs on contrast, they obtained a small but significant attenuation of contrast using the neuroleptic chlorpromazine. They also found that chlorpromazine attenuated within session discrimination between the probe and baseline conditions, this effect may confound interpretation of the contrast attenuation result. Unfortunately chlorpromazine blocks both noradrenaline and dopamine receptors (and a range of other sites, Guth and Spirtes, 1963) so their result is not a clear test of dopamine's rôle in reinforcement mediation. Flupenthixol, however, has been shown to have a purely dopaminergic action (Møller-Nielsen, Pedersen, Nymark, Franck, Boeck, Fjalland and Christensen 1973), and can therefore provide that test.

One of the reasons for using contrast designs in assessing dopamine's rôle in reinforcement is to eliminate motor control explanations of neuroleptic induced changes in response to reinforcers. Although the embedded contrast design can dissociate reinforcement and motor effects, it is still important to minimise motor deficits both for the main crossover experiment, and to ease comparison of contrast effects between groups which produce different baseline response rates. Two days of the experiment were run using a dose of 0.1 mg/kg flupenthixol, this dose appeared to impair responding. A lower (0.06 mg/kg) dose was therefore used in for the rest of the experiment.

In the previous two experiments animals were tested without being food deprived, in this experiment, however, a deprivation regime was used. Although responding can be obtained in a Skinner box from undeprived but well pre-trained animals, such an approach was thought to be potentially too unreliable. It has been shown that positive and negative contrast effects are obtained most reliably in animals which are only mildly food deprived (Panksepp and Trowill, 1971; Weiskrantz and Baltzer, 1975), a comparatively mild deprivation schedule which maintained animals at approximately 90% of their ad lib body weights was therefore used in this experiment.

5.3.2 Method.

Subjects.

The same animals used in the previous experiments served as subjects. Animals were allocated to the two groups so that each group consisted of animals from all four groups of the previous experiments.

Drugs.

Cis-Z-Flupenthixol (alpha-flupenthixol; Lundbeck, Copenhagen) dissolved in 0.9% saline at a dose of 0.06 mg/kg body weight and injected intra-peritoneally in a volume of 1.0 ml/kg body weight.

Apparatus.

The apparatus consisted of four Skinner boxes together with a controlling microcomputer and interface. Two of the boxes were Campden Instruments (London) Operant Test Chambers for rats, the other two boxes were replicas of these made in our workshops. All of the boxes were equipped with similar components. One Ralph Gebrands (Arlington, Massachusetts) solenoid driven food dispenser each. Two Campden Instruments retractable operant response levers, which required a response force of 9 gm for operation, each. One of the levers in each box was kept permanently retracted throughout the experiment. One Campden Instruments food hopper each. Each box was fitted with a house light in the centre of the ceiling, a white stimulus light above the extended lever, a red (less intense) stimulus light above the retracted lever and a light inside the food hopper. The boxes had metal grid floors. Each box was housed inside a soundproof chamber.

The operation of all four boxes was controlled by a BBC Model B microcomputer (Acorn Computers, Cambridge) connected to two Control Universal (Cambridge) 16 channel optically isolated laboratory interfaces. The routines which specified the components of the reinforcement schedule were written in BBC BASIC. An interrupt driven concurrent task scheduler and interface driver written in 6502 assembly language allowed these routines to control all four boxes simultaneously. Response data and timings of various events in the schedules were written to floppy discs for subsequent analysis.

Campden Instruments 45 mg food pellets were used as reinforcers.

Procedure.

The experiment consisted of ten days of pre-training and two consecutive eleven day test phases. Animals were maintained at 90% of their ad lib body weights throughout the experimental and pre-training periods.

Animals were magazine trained and gradually introduced to a pseudo-random variable interval 45 second (VI 45) reinforcement schedule over 10 days. Schedule operation was signalled by the stimulus light above the extended response lever. Once a successful response had been made this light was turned off for an 8 second inter-trial interval and two food pellets were dispensed into the food hopper. The pellets were dispensed at 4 second intervals, the food hopper light was turned on during the 8 seconds of the inter-trial interval. The solenoid operated food dispenser made a clearly audible click each time a pellet was dispensed.

The pseudo-random schedule generated intervals by selecting values between 0 seconds and twice the schedule value, so for a VI 45 schedule each interval is determined by a random number between 0 and 90. Chance extended sequences of very short or very long intervals were prevented by the following rule. The range of possible interval values was divided into three equal sections, for a VI 45, 0-30 seconds, 30-60 seconds and 60-90 seconds. If the random number generator produced more than three consecutive intervals within any section then it was re-run until an interval falling within another section was produced. This form of schedule has been found to ease VI training and reduce the likelihood that frustrated animals in negative contrast conditions completely cease responding when faced with chance sequences of very long intervals (James E. Wright, personal communication). VI schedules of this type were used throughout the experiment.

In the experimental period the low and high magnitudes of reinforcement used were one and four pellets respectively, both obtained through a VI 45 schedule. When a successful response was made pellets were dispensed at 4 second intervals, the inter-trial interval was therefore 16 seconds for the high reward condition and 4 seconds for the low reward condition. Differential stimuli were associated with each reward magnitude. For half of the animals the house light signalled the high reward and the red stimulus light above the second retracted lever signalled the low reward (with the house light extinguished), for the other animals these stimuli were reversed. In both conditions the signal light above the extended lever served as the discriminative stimulus signalling schedule operation. Each animals' daily test session lasted 45 minutes.

Animals were divided into two groups, one of which received flupenthixol for the first half of the experiment and was then switched to saline in the assessment of drug-induced positive contrast, while the other group received saline initially and were then switched to flupenthixol to assess negative contrast. Half of the animals in each group had high value reward signalled by the house light and half had it signalled by the red stimulus light. Both groups received the same value of reinforcer on any day. High and low reinforcement days alternated. For two days of testing, the first group were injected with 0.1 mg/kg flupenthixol and the second group with saline. This dose appeared to impair responding, so, after a drug-free day, the experiment proper was restarted. The experiment proper consisted of two eleven day phases. During phase 1 Group 1 received 0.06 mg/kg

flupenthixol each day and group 2 received saline. During phase 2 Group 1 received saline and Group 2 flupenthixol.

The design of the embedded experiment was based on that of Baltzer and Weiskrantz (1970). During any session, one level of reward was available during most of the session, while the contrasting level of reward and its associated stimulus was available during two relatively short (4 minute) 'probe' periods. These probe periods were timed to start at random times within specified 9 minute intervals. The first interval was centred one quarter of the way through the session (between 6 minutes 45 seconds and 15 minutes 45 seconds into the session) and the second three quarters of the way through (between 29 minutes 15 seconds and 38 minutes and 15 seconds). For each test session, random times were selected within these intervals. Once these times were reached, probes were programmed to start immediately after an animal had completed the interval it was currently working on and received its reinforcement. The magnitude of reinforcement available during baseline and probe periods alternated from day to day.

Animals were injected with either flupenthixol or saline two and a half hours before each daily session. They were weighed and fed at the end of the session.

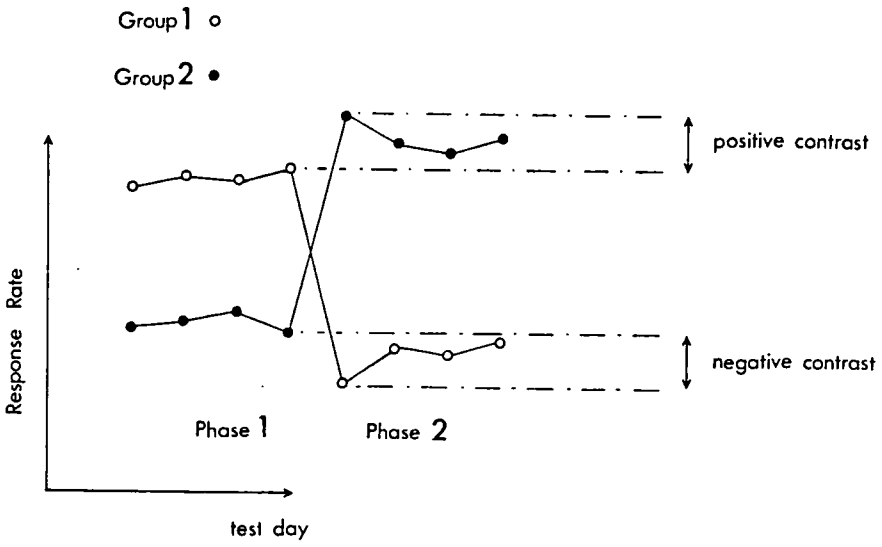
Analysis.

The time of every response, reinforcement, inter-trial interval and probe period was recorded for each animal daily. Response rates during the 4 minute period following the first reinforcement, the 4 minute probe periods and the 4 minutes preceding each probe period were extracted from this data. Inter-trial intervals during which reinforcement was being presented were not included when response rates were calculated. The 4 minutes following the first reinforcement and the 4 minutes preceding the first probe period are the earliest and latest periods in the session which can be used in the analysis of drug induced contrast effects.

Strong contrast effects can only be expected when animals clearly anticipate of the value of reinforcement they are to receive. If animals are correctly anticipating reward magnitude, a difference between response rates elicited by the high reinforcement stimulus and those elicited by the low reinforcement stimulus is predicted. Response rates in phase 1 were therefore compared in 2 way repeated measures analyses of variance with the factors day and magnitude for data from the earliest and latest periods in the session which could be used in the analysis of drug induced contrast effects. Further analyses were only conducted if response rates for high and low reinforcers differed.

Contrast effects must be attributable to the change in drug conditions between phases, not simply to the drug conditions themselves. The appropriate comparison is therefore between the response rates produced by animals which have been repeatedly exposed to a given drug condition and those which have just been switched to that condition. In this design that comparison is between the response rates produced by one group on the last day of phase 1 (the control group for the comparison) and those produced by the other group on the first day of phase 2 (the contrast group). Positive contrast was therefore assessed by comparing the response rates of group 1 on the last day of phase 1 with those of group 2 on the first day of phase 2. Both groups were receiving saline injections on these days, however, group 2 have previously been receiving flupenthixol while group 1 were on the last day of a series of saline injections. Negative contrast effect was assessed by comparing the

response rates of group 2 on the last day of phase 1 with those of group 1 on the first day of phase 2. On these days both groups were receiving flupenthixol, however, group 1 have previously been receiving saline while group 2 have not yet changed drug conditions and had been receiving flupenthixol injections for the previous ten days. These comparisons are schematically represented in figure 5.8. As there are clear predictions of the expected direction of effect, and the sensitivities of between groups comparisons of operant response rates are likely to be adversely effected by individual differences in rates, these analyses were performed as one-tailed t-tests. Separate analyses were carried out of the high and low reward magnitude data.



5.8 The response rate comparisons required for testing the occurence of contrast effects.

The session to session variability between response rates produced by each magnitude of reinforcer in each group was assessed with a series of repeated measures t-tests and magnitude of effects estimations. It is important to assess whether the difference in response rates between sessions at the phase changeover differs markedly from other session to session rate changes. In particular, in the positive contrast condition an increase in response rate between phases may simply be attributed to gradual task acquisition. The magnitudes of effects estimates allow comparisons to be made between the magnitudes of the response rate changes made between sessions. T-test results alone cannot do this. They can tell us whether response rates on two sessions differ, but not how much they differ. An increase in significance level indicates an increase in our confidence that the two rates differ, not an increase in the magnitude of that difference.

Magnitudes of effects were calculated using the method of Vaughan and Corballis (1968). One problem which occurs when magnitude of effects are estimated in repeated measures designs is that different underlying statistical structural models lead to different estimates. If it is assumed that the underlying statistical model is non-additive, (that is, it is assumed that interactions between treatments and subjects exist in the population independently of experimental errors – which is

the conservative position and most likely to be true in behavioural tasks (Keppel, 1973, p.408)), underestimates of magnitude of effects are unavoidable (Vaughan and Corballis, 1968, p.209). This underestimation is most extreme when the number of treatment levels is small. In the current case there are only two levels of treatment (session) in each test, so a very large underestimation of explained variance will occur.

Unbiased estimation of magnitude of effects is possible if it can be shown that an additive structural model is justified (i.e. population treatment by subject interactions are minimal). Tukey (1949) provides a test to determine whether an additive model is justified. This test was performed for each comparison, if data was found to conform to an additive model, magnitude of effects were recalculated using Vaughan and Corballis' procedure for an additive model. As the non-additive model produces the most conservative results, and Tukey's test leads to use of this model if it produces a significant F, Winer (1962) recommends using a numerically high significance level in order to avoid type 2 errors. Winer's suggested α level of 0.25 is used here. The use of analyses based on different models for different session to session comparisons would not normally be recommended, however, the likelihood of extreme underestimations of magnitude of effects when the non-additive model is used in a two treatment level case means that the unbiased additive model estimate is to be preferred whenever it can be justified. When the additive model cannot be used it must be stressed that magnitude of effects will be underestimated, although not to as great an extent as would be the case if the non-additive formula was used when an additive model was in fact justified.

Finally, split-plot analyses of variance with the factors day and group were carried out on response rates produced during phase 1 in order to assess whether flupenthixol significantly effected baseline response rates. Careful selection of drug dose should allow contrast effects to be produced by doses which do not significantly effect these rates. If the difference between groups is not significant a motor impairment explanation of the reduction in response rate associated with a putative negative contrast effect becomes less tenable.

The embedded experiment was analysed as follows. Contrast effects can be evaluated by comparing response rates during the probe period of one day with baseline rates of the previous day. Animals were receiving the same magnitude of reward during both of these periods, however, during the baseline period this was the expected level of reward, whilst during the next days's probe the same reward was an exception to that day's baseline. Separate analyses of the effect of flupenthixol on positive and negative contrast were carried out.

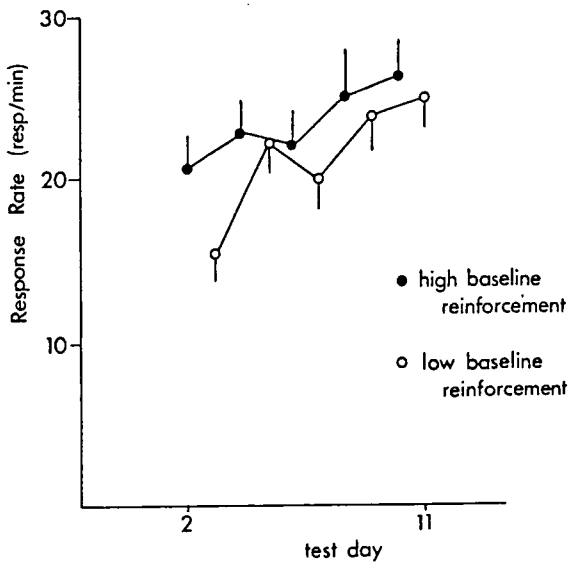
Contrast scores were calculated by dividing the difference between response rates during the probe periods and response rates during the previous day's baseline for the 4 minutes prior to the probe by their sum. That is, the contrast score on day n, c_n is given by:

$$c_n = \frac{p_n - q_{n-1}}{p_n + q_{n-1}}$$

where p_n is the response rate during the probe period on day n and q_{n-1} is the response rate during the 4 minutes pre-probe on day $n-1$.

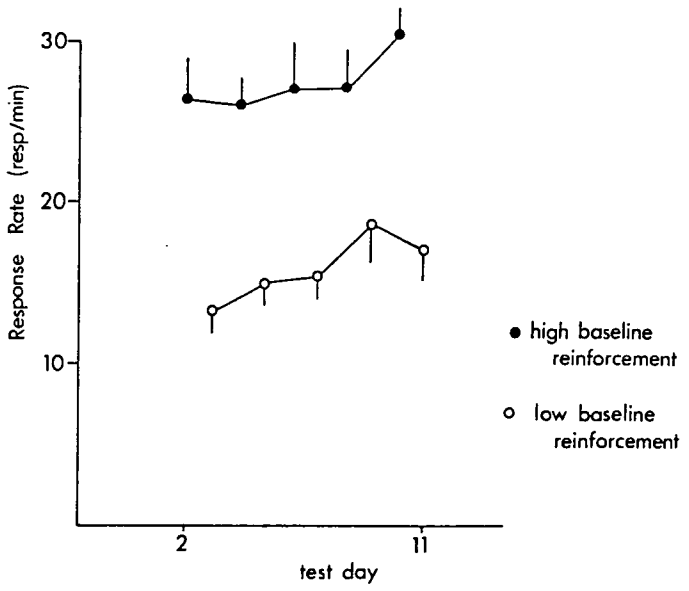
This produced five negative and five positive contrast scores for each animal during each phase of the experiment. Mean negative and positive contrast scores were calculated for each animal in each phase and these scores were entered into separate 2-way split plot analyses of variance for negative and positive contrast, each with the between subjects factor group and the within subjects factor phase. Since the drug treatment of the two groups is reversed between phases any effects of flupenthixol on contrast will be reflected in the group by phase interaction factor in the analysis.

5.3.3 Results.



5.9 Response rates produced for the high and low magnitudes of reinforcement during the first 4 minutes of each test session following the first reinforcement during phase 1. Means and standard errors of response rates are shown for all animals (groups 1 and 2 combined).

Initially analyses were conducted to assess differences in response rates between the high and low value reinforcers during phase 1. Phase 1 response rates produced in the 4 minute period following the first reinforcement are shown in figure 5.9, those produced during the 4 minutes preceding the first probe period are shown in figure 5.10.



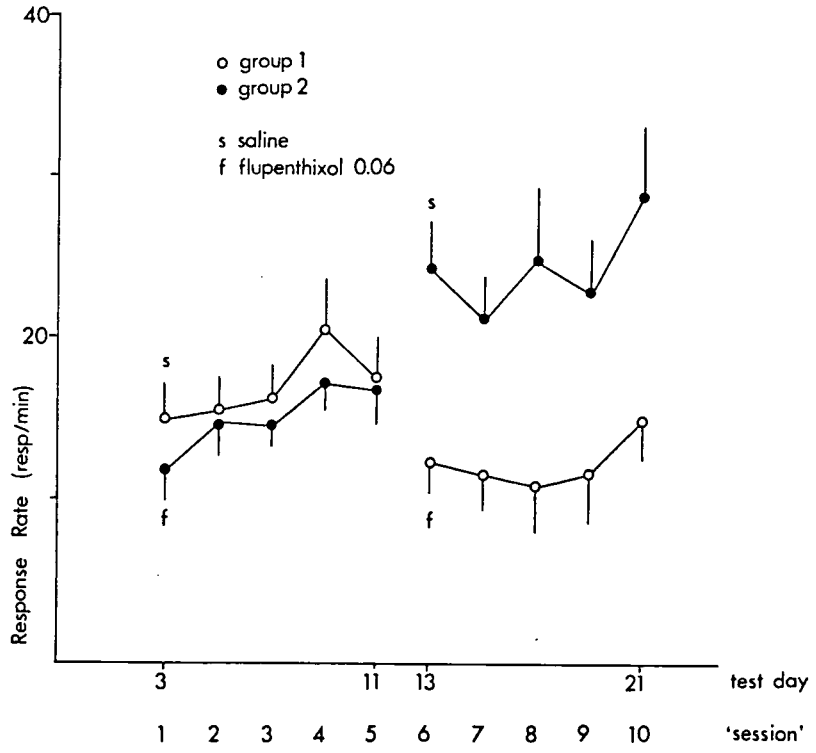
5.10 Response rates produced for the high and low magnitudes of reinforcement during the 4 minutes each test session preceding the first 'probe' period in phase 1. Means and standard errors of all animals' response rates are shown.

An analysis of response rates produced during the 4 minute period following the first reinforcement (see figure 5.9) did not show any difference between response rates produced on high and low magnitude days (magnitude, $F < 1$). The effect of flupenthixol on response rates approached, but did not reach, significance (drug, $F = 4.27$, df $1, 21$, $0.05 > p > 0.1$). The lack of difference between response rates produced in the high and low reward conditions makes the use of this first period inappropriate for contrast analyses.

A similar analysis of data from the 4 minute period preceding the first probe (see figure 5.10), which constitutes the latest part of the session which can be used in the drug induced contrast study, yielded the following results. A highly significant difference in the response rates produced by the high and low reinforcements was now found (magnitude, $F = 97.52$, df $1, 21$, $p < 0.0001$). A significant day to day variation in rates was also found. The factor day, however, showed significant sphericity (Huhyn and Feldt, 1970) hence a Greenhouse-Geisser epsilon correction was required (Howell, 1982). After this correction the effect was still found to be significant (day, $F = 3.21$, original df $4, 84$, corrected df $3, 55$, $p < 0.05$). No other significant effects were found, in particular the drug effect evident at the beginning of the session had disappeared (drug, $F < 1$).

Response rates of both groups during the 4 minute period preceding the first probe over the full experiment on low reinforcement sessions are shown in figure 5.11. The corresponding results for the high reinforcement sessions are shown in figure 5.12.

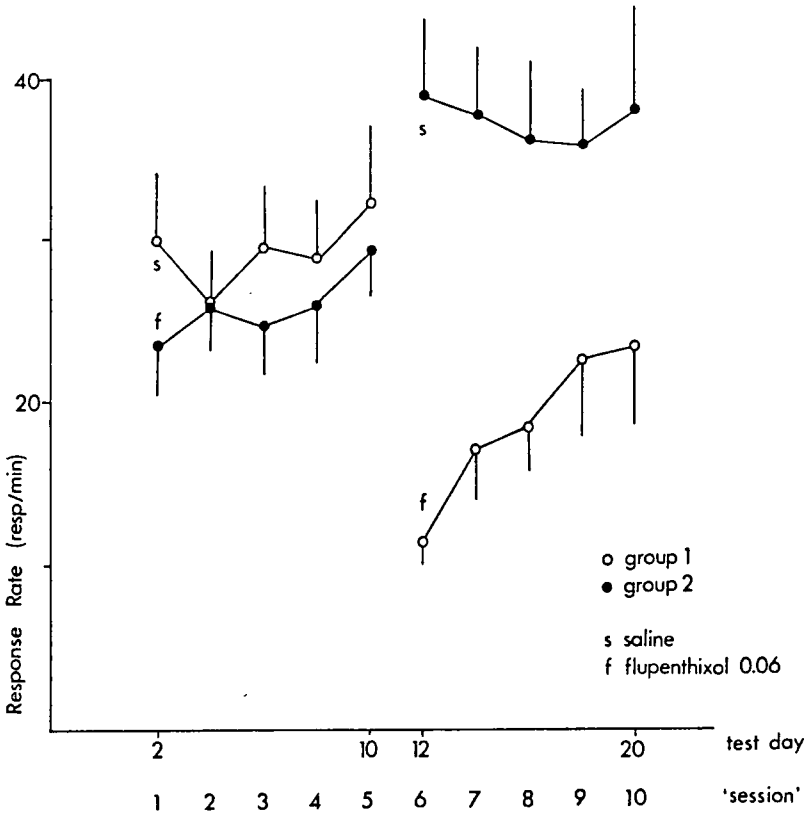
The results of the analyses of contrast effects for response rates during the 4 minute period preceding the first probe were as follows. Negative contrast effects were predicted when group 1 was shifted from saline to flupenthixol. In the high reward condition shown in figure 5.12 the response rates of group 1 on the first day



5.11 Low reinforcement response rates during the 4 minutes preceding the first 'probe' period. Mean rates and their standard errors are shown. Session numbers correspond to those used in table 5.2.

they were switched to flupenthixol were significantly lower than those produced by group 2 on the last day of phase 1 ($t = 6.31$, df 21, $p < 0.0001$, one-tailed). Although lower response rates were also shown after drug shift in the low reward condition, the difference between response rates from group 2 on the final day of phase 1 and those from group 1 on the first day of phase 2 were not significant. Positive contrast effects were predicted when group 2 was shifted from flupenthixol to saline. A comparison of response rates from the first day of phase 2 in group 2 with rates from the last day of phase 1 in group 1 showed a significant elevation of responding in the low reward condition (see figure 5.11) ($t = 1.91$, df 21, $p < 0.05$ one-tailed). A similar elevation of response rate was not found to be significant in the high reward condition.

The significant effects on response rates produced by the introduction of flupenthixol treatment in the high reward condition and the cessation of flupenthixol in the low reward condition are evidence for negative and positive contrast effects respectively. Analyses of session to session response rate changes were therefore carried out on group 1 response rates in the high reward condition and group 2 response rates in the low reward condition in order to assess possible alternative explanations of the contrast effects. The response rate in each session was compared with that produced in the next session (in each reward condition) by repeated measures t-tests. Estimates of the magnitude of effect (Vaughan and Corballis, 1968) accounted for by session to session changes were also calculated. The magnitude of effects estimator is only presented where t is greater than 1. Tukey's non-additivity test (Tukey, 1949) was performed on the data used in these comparisons. Magni-



5.12 High reinforcement response rates during the 4 minutes preceding the first 'probe' period. Means rates and their standard errors are shown. Session numbers correspond to those used in table 5.1.

tude of effects were calculated using the formula appropriate to the statistical model indicated by this test. The results of these tests are presented in tables 5.1 and 5.2. The warning that the magnitudes of effects calculated using the non-additive model are underestimates must be reiterated.

The results of the embedded experiment did not show any contrast effects in the probe periods. Although response rates during probe periods increased during high reinforcement probes and decreased during low reinforcement probes, these rates did not 'overshoot' the baseline rates for high and low reinforcement from the previous test days. Analyses of drug effects on contrast induced by changes of (real) reinforcement magnitude could not therefore be carried out.

Sessions	Rate Increase	t	Significance	Non-Additivity Test		Magnitude of Effects	
				F	Significance	Model	Effect Magnitude
S							
A 1 – 2	−3.84	1.74	n.s.	0.039	n.s.	*additive*	15.56%
L 2 – 3	3.47	1.06	n.s.	0.920	n.s.	*additive*	1.11%
I 3 – 4	−0.49	0.14	n.s.				
N 4 – 5	3.26	0.75	n.s.				
E							
5 – 6	−20.97	4.0	$p < 0.005$	20.203	$p < 0.01$	*non-additive*	39.45%
D 6 – 7	5.79	2.21	$p = 0.051$	3.187	$p < 0.25$	*non-additive*	7.38%
R 7 – 8	1.33	0.45	n.s.				
U 8 – 9	4.59	0.98	n.s.				
G 9 – 10	0.28	0.05	n.s.				

Table **5.1** – Session to session response rate changes for group 1 in the high reward condition. A negative contrast effect is expected between sessions 5 and 6 when the animals are switched from saline to flupenthixol.

Sessions	Rate Increase	t	Significance	Non-Additivity Test		Magnitude of Effects	
				F	Significance	Model	Effect Magnitude
D 1 – 2	2.94	0.52	n.s.				
R 2 – 3	−0.39	0.21	n.s.				
U 3 – 4	2.59	2.30	$p < 0.05$	2.115	$p < 0.25$	*non-additive*	5.05%
G 4 – 5	−0.31	0.24	n.s.				
5 – 6	7.57	5.12	$p < 0.001$	0.086	n.s.	*additive*	67.78%
S							
A 6 – 7	−3.06	2.62	$p < 0.05$	0.270	n.s.	*additive*	32.83%
L 7 – 8	3.71	1.05	n.s.	0.200	n.s.	*additive*	0.85%
I 8 – 9	−2.24	0.71	n.s.				
N 9 – 10	6.25	1.94	n.s.	0.601	n.s.	*additive*	18.72%
E							

Table **5.2** – Session to session response rate changes for group 2 in the low reward condition. A positive contrast effect is expected between sessions 5 and 6 when the animals are switched from flupenthixol to saline.

5.3.4 Discussion.

If dopamine is involved in the mediation of reinforcement, successive contrast effects can be predicted when drug treatment is suddenly initiated or withdrawn. The present experiment tested that prediction in a drug crossover design. In order to avoid floor and ceiling effects on rate changes two levels of reinforcement were used. The pattern of results obtained in the drug induced contrast design indicated that both negative and positive contrast effects were induced by switches to and from flupenthixol. Differences in response rates prior to the shift indicated that the dose of flupenthixol used only had a small effect on baseline response rates.

A state dependency mechanism could not account for both increases and decreases of response rates after changes in drug conditions. The negative contrast effect obtained may be explained in terms of a motor deficit which is subsequently adjusted to. The same explanation cannot, however, be applied to the positive contrast effect. Further evidence that the rate changes which occurred after drug switches were contrast effects is provided by the strong rate changes shown between the first two post shift sessions. In both the positive and negative contrast groups it can be seen that the rate 'overshoot' and 'undershoot' were transitory phenomena, with strong returns towards baseline rates being shown between the two immediately post-shift sessions.

The failure of the embedded within sessions contrast study to produce contrast effects can be attributed to the comparatively short duration of the experiment. Baltzer, Huber and Weiskrantz's (1979) drug studies using this type of design lasted from 88 to 110 days including at least 24 days of drug free training on the full contrast schedule (i.e. alternating high and low reward magnitude days with contrast probes) before drug testing began. It is not therefore surprising that no within sessions contrast effects were found in the limited period (22 days of the full schedule) of testing used in this experiment.

Before discussing the evidence for drug induced contrast effects and their implications for the rôle of dopamine systems in detail, the selection of the period from which response rates were analysed must be considered.

The first stage in the analysis of the data showed that animals were not producing significantly different response rates for the high and low reinforcements early in the test session. These intermediate response rates produced at the start of the session are not surprising. The animals have comparatively little experience of the complex changes in reinforcement they receive. At the start of a session we may expect little second order control of response rates by the stimuli signalling magnitude of reinforcement, and hence an inaccurate expectation of the number of food pellets to be obtained. As contrast effects rely on a sudden change in reinforcement from an expected value it was unlikely that effects would be demonstrated early in the session when animals did not appear to be certain whether they were working for the high or low magnitude of reinforcement. Once the rats have received a few reinforcements they are more likely to have correct expectations of the number of pellets they are to receive. A drug induced alteration of the reinforcing impact of this number of pellets may then produce contrast.

Once the first probe period of the embedded experiment has begun additional factors will confound the interpretation of drug induced contrast. The baseline period immediately preceding the first probe is therefore the latest part of the

test session which can be used in the assessment of drug induced contrast. When data from this period were analysed a significant difference had emerged between response rates for the high and low magnitudes of reinforcement. Detailed analyses of drug induced contrast effects were therefore carried out on data from this later period preceding the first probe.

Analyses showed that animals which had just switched drug conditions responded at significantly different rates from animals in the same drug conditions at the end of phase 1. The most striking of these effects was the dramatically low response rate shown by animals just switched to flupenthixol in the high reward condition ($p < 0.0001$, one-tailed). The response rate shown by animals just switched to saline was also significantly higher than the pre-switch rate of saline treated animals in the low reward condition ($p < 0.05$, one-tailed). These results are indicative of drug induced negative and positive contrast effects.

Similar decreases on switching to flupenthixol and increase when switching to saline were found in the low and high reward conditions respectively, however in these cases the between groups comparisons with the appropriate control scores were not statistically significant. The non-significance of these effects may be due to floor and ceiling effects.

Although the differences between the response rates of shifted and unshifted animals can be most parsimoniously explained in terms of drug induced contrast effects, alternative explanations must be considered.

It may be argued that the large decrease in response rates shown when group 1 were shifted to flupenthixol can be accounted for in terms of a motor deficit. This deficit must be transitory, however, otherwise a difference in response rates between animals receiving flupenthixol at the end of phase 1 and those just switched to flupenthixol at the beginning of phase 2 would not be expected. Analysis of the difference in response rates between animals receiving flupenthixol and saline during phase 1 did not show a significant drug effect, although mean response rates in the flupenthixol group were consistently lower than those in the saline group. It can be concluded that the steady-state response rate deficit produced by the comparatively low dose of flupenthixol used must be small. It should, however, be noted that flupenthixol produced a deficit approaching significance during an earlier period in each session. It appears that this deficit, which may be attributable to motor impairments, reinforcement devaluation, or a combination of effects, reduces response rates at the start of test sessions but is quickly recovered from or compensated for. It is possible, then, that the introduction of flupenthixol may produce a transitory response rate deficit, however, the size of the response rate difference produced earlier in sessions is not as large as that produced in the putative negative contrast effect.

While the rate decrease produced on switching to flupenthixol could possibly be explained in terms of a motor deficit, this is not the case for the higher response rates of animals just switched to saline. If this was the case, whilst an increase in the response rates of switched animals would be expected, the response rates of animals receiving saline at the end of phase 1 would match those just switched to saline. Instead a clear overshoot was observed. This overshoot is not only inconsistent with simple motor effects, but also rules out state-dependent explanations of the results.

It may be hypothesised that the 'overshoot' found when animals switched from

flupenthixol to saline was the result of an increase to the pre-shift level of response rates for saline treated animals plus a chance session to session increase in rate. When the magnitudes of session to session variations are analysed it becomes clear that random variations cannot account for the strong consistent rate increase that occurred when drug conditions were switched.

As the difference in response rates shown between groups 1 and 2 in phase 1 was very small, most of the increase in response rate when drug conditions were shifted must be explained in terms of a general session to session rate increase rather than release from a simple motor deficit. Figure 5.11 shows an increase comparable to that at shift between the last two days of phase 2. Estimations of magnitudes of effects allow the size and consistency within the group of animals of session to session changes in rates to be assessed. The results of such analyses (see table 5.2) show that the rate increase between phases (session 5 to 6) is a far stronger effect than any other session to session increase, indicating that an explanation based on random session to session rate increases is untenable.

The magnitude of effects results provide further convincing evidence that the rate change between sessions was the result of a contrast effect. Successive contrast is normally a transitory phenomenon (Crespi, 1942, 1944; Mackintosh, 1974), after the elevation of response rate produced by positive contrast a subsequent drop in rate is to be expected. Just such a drop is shown between sessions 6 and 7 in figure 5.11. Again the magnitude of effects results show this to be a strong phenomenon unlikely to be explained by random session to session variation. A similar pattern of results was found for the decrease in responding shown when group 1 were shifted from saline to flupenthixol in the high reward condition (see table 5.1). Figure 5.12 shows a strong decrease in response rate between session 5 and 6 was followed by a consistent increase between session 6 and 7 (both magnitudes were calculated on the basis of a non-additive structural model and hence are underestimates of the true strength of these effects). This pattern is indicative of a transitory negative contrast effect.

When the results of the positive contrast test, the negative contrast test and the patterns of results shown by magnitudes of effects estimation are considered together it must be concluded that drug induced positive and negative contrast effects have been demonstrated.

Although the most obvious explanation of drug induced contrast effects is that flupenthixol attenuates the reinforcing impact of the food rewards obtained by the animals, one final motor explanation must be considered.

The net reinforcing value of a reward may be conceptualised as the reward's intrinsic value less the cost of obtaining that reward. A motor deficit may increase response costs to an animal, and hence decrease the net value of any reward obtained. It is therefore conceivable that changes in response cost brought about by the introduction or cessation of flupenthixol may alter the net value of obtained reinforcers and hence produce contrast effects. Such an argument relies on two premises. First that changes in response cost can induce contrast effects, and second that dopamine blockade results in increased response cost.

Although not a perfect model of the general motor deficit which may be hypothesised to be a result of flupenthixol treatment, response cost and response deficits may be investigated by studying the forces of bar pressing in operant situations as

independent or dependent variables.

Many studies have been made on the effects of changes in reinforcement magnitude on the force of emitted responses (see Notterman and Mintz, 1965), however, very little literature exists on the effects of changes in required response force on operant behaviour (specifically response rates). Studies have been made of the effects of different levels of response force requirement on parameters of Herrnstein's matching law (e.g. McDowell and Wood, 1985), on fixed interval schedule response patterns (Azrin, 1958, although force was not the initially intended manipulation) and on simultaneous behavioural contrast (e.g. Hunter and Davison, 1982), however, studies of these types do not provide data on the effects of sudden changes in response cost of the type necessary to induce successive contrast effects. Skinner (1938) reports the results of a series of experiments on the differentiation of response according to force requirements in rats in which contrast effects are possible. No contrast effects were evident when force requirements were changed (see Skinner, 1938, p. 326, figure 110). However, the small changes in force requirements and short duration of training at each force level used by Skinner militate against the demonstration of contrast effects.

A review of the literature revealed one paper by Chung (1965) in which response force was used as an independent variable in a design where successive contrast effects were possible. Chung studied the effects of changing the force required for successful key pecks by pigeons on sixty second VI schedules. Auditory feedback was given for successful key pecks, which can be considered as secondary reinforcement of pecking force on a continuous reinforcement (CRF) schedule. Force requirements were changed after at least eight daily test sessions. These extended periods during which required force was unchanged increase the likelihood of contrast effects when force requirements changed.

The results of Chung's experiment show transitory enhancement and suppression of response rates following decreases and increases of force requirements respectively. These transient effects can only be compared with the subjects subsequent performance, no control groups were used. Some more serious methodological problems must be dealt with if these results are to interpreted as contrast effects.

First it should be noted that although Chung's data show impressive transitory enhancements of responding following a reduction of force requirement the corresponding suppression effects obtained with changes to stronger requirements appear weak (see Chung, 1965, p.3, figure 4; one apparently large suppression effect shown in figure 5 is not presented in figure 4, the time course of this particular effect cannot be determined from figure 5, it will be shown later that the time course of this effect is of particular interest). In other words, if these results are contrast effects, then Chung was obtaining strong positive but weak negative contrast, the opposite of the usual pattern of positive and negative contrast effects (e.g. Benefield, Ocós and Ehrenfreud, 1974; Black, House and Moss, 1973; Dunham, 1968). While this abnormal pattern does not provide evidence that the effects found were not contrast phenomena, it indicates that alternative mechanisms should be considered.

The second problem is that Chung's apparatus could only record responses made above the force requirement in operation on any day. It is therefore not clear whether the enhancement and suppression of rates found after changes in force requirements are the result of genuinely enhanced or suppressed rates of responding at the new force requirements relative to subsequent baseline rates, or whether they

are due to gradual response force differentiation at the new requirements.

As a new response force is differentiated the variability of response force about a mean approximating the new force requirement decreases to a level where approximately 65% of response fall above the required force (Notterman and Mintz, 1965). As sub-criterion responses are not reinforced, animals tend to respond on average at a force exceeding the force requirement. As a result, if responses are only recorded when they exceed the new requirement, the gradual decrease in emitted force variability and drop in the mean force of emitted responses will appear as a transitory enhancement of response rate when a reduction is made in required response force.

When the force requirement is decreased most of the responses made after the change will be reinforced, and, as noted above, differentiation to the new force requirement will appear as a transitory rate enhancement over the low force requirement baseline response rate. This is not the case when the force requirement is increased. Here, after the change in force requirement most responses fall below criterion. The differentiation of the new higher force is dependent on one of two mechanisms. A small percentage of the emitted responses may fall above the newer high force criterion and the selective reinforcement of these responses may lead to a gradual differentiation of higher force responding. Alternatively, if no responses are of sufficient force to obtain reinforcement, then the animal is essentially undergoing extinction. Notterman (1959) has shown that response force undergoes a transitory increase during extinction, this increase may be sufficient for some responses to fall above criterion and hence lead to differentiation of higher force responding. Whichever is the case, (a mixed mechanism is also possible), the gradual differentiation of the high force requirement will result in an apparent suppression of response rate below the high force baseline rate when only successful responses are recorded. The gradual acquisition of an increased force criterion is shown in Notterman and Mintz (1965, p.76).

As increasing force requirements differentially reinforces high force responses but decreasing force requirements reinforces both high and low force responses, differentiation of response force is likely to occur more rapidly when force requirements are increased to a level in which some responses are still reinforced (see Skinner, 1938). The small suppression and large enhancements of response rate found by Chung are consistent with this response differentiation explanation. Examination of the time course of the one large suppression effect obtained by Chung may have clarified the nature of possible differentiation processes underlying the suppression effects he obtained. Unfortunately, Chung (1965) does not present this data.

It may be concluded that Chung's results only provide very tentative evidence of force requirement-induced successive contrast. The alternative response force differentiation explanation outlined above is as consistent with the data as a contrast explanation. It also appears to explain the relative sizes of rate enhancements and suppressions which are not typical of contrast effects. In the results obtained in the present experiment, suppression of response rate following the introduction of flupenthixol is larger than enhancement following release from flupenthixol. Even if Chung's results can be interpreted as successive contrast effects, the pattern of results obtained in the current experiment bear more resemblance to the relationship between positive and negative contrast induced through reward magnitude shifts than to putative response cost induced effects. It should be noted that Chung

does not refer to the response suppression and enhancements he found as contrast effects. In further experiments on simultaneous behavioural contrast he found no effects of changes in force requirement on one key on response rates made to another concurrently available key, substantiating the contention that a contrast explanation of his earlier result is inappropriate. The failure of force requirement changes to induce simultaneous behavioural contrast has also been reported more recently by Hunter and Davison (1982).

Evidence of response cost induced contrast appears weak and inconsistent with the results of the present experiment. Studies of the effects of dopamine blockers on emitted response force add weight to the argument that a response cost based explanation of the results of the present experiment is untenable.

Recent experiments by Fowler, Gramling and Liao (1986) and Fowler, Lacerra and Ettenberg (1986) have shown that dopamine blockers do not cause decreases in the mean and peak force of responses made in operant situations even when the force requirement of response is very high (100 gm). In fact, when force requirements are of the order required in the current experiment (9 gm) dose dependent increases in emitted mean peak force and individual response durations were found (Fowler, Gramling and Liao, 1986, experiment 1, 4 gm condition; Fowler, Lacerra and Ettenberg, 1986, 10 gm condition). The doses of dopamine blockers used were sufficient to cause decreases in response rates produced to obtain reinforcement, however, reinforcers were obtained at similar rates in the 4 and 40 gm conditions of Fowler, Gramling and Liao (1986), regardless of drug condition (i.e. there was no interaction between drug and force requirement). These results are inconsistent with simple motor incapacitation models of the effects of dopamine blockade. The lengthened response durations may, however, be interpreted as evidence for a response initiation deficit.

The dose dependent increase in response force found in both of the Fowler studies (at least at low force requirements) is consistent with a reinforcement attenuating drug effect. Di Lollo, Ensminger and Notterman (1965) have shown that response force varies inversely with reinforcement magnitude, probably as a result of inaccurate response force discrimination at lower reinforcement levels. This effect is found even when there are no shifts in reinforcement magnitude (i.e. response forces are measured across groups of animals). The similarity of Fowler's seemingly paradoxical results with the equally paradoxical Di Lollo, Ensminger and Notterman result is striking, and points towards drug induced attenuation of the reinforcing impact of rewards. The result clearly rules out a simple motor deficit action which would predict the opposite pattern of results.

The relationship between response initiation deficits caused by neuroleptics (evidence for which has been found in many studies) and motivational systems will be discussed in the final chapter of this thesis. Response initiation deficits would not be predicted to add to simple response cost. Fowler's results provide evidence against the induction of simple response deficits by flupenthixol which could be used in a response cost argument of the type outlined earlier.

Finally, the lack of response rate differences between groups during phase 1 implies that any motor deficit, whether simple or initiation related, must have been minimal.

It may appear unusual that contrast effects can be obtained when there is no significant difference between baseline response rates for the more and less favoured alternatives (in this case the saline and flupenthixol conditions in phase 1). If flupenthixol is attenuating the perceived value of reinforcement a rate difference may be expected during the baseline phase. A small, non-significant, difference is indeed seen, yet highly significant differences follow drug changeover. Contrast effects are, however, often obtained when baseline responding differs little between the more and less preferred reinforcers (e.g. baseline lever pressing rates in Weiskrantz and Baltzer (1975), p.78 figure 3c; sucrose solution licking rates and times in Riley and Dunlap (1979), p.62 figure 1 ad lib condition; sucrose solution lick rates in Panksepp and Trowill (1971) p.52 figure 1 and Flaherty and Largren (1975), p.658 figure 3; runway speed for food reinforcers in Benefield, Ocós and Ehrenfreud (1974), p.650 figure 2). Moreover, although contrast effects are largest soon after reinforcement shifts, they may persist for considerable lengths of time (e.g. Benefield, Ocós and Ehrenfreud, 1974). The results of the present experiment are not, therefore, unusual.

It can be concluded that the results of the experiment can only be consistently explained by assuming that flupenthixol produced an attenuation of reinforcement. A rôle for dopamine in the mediation of reinforcement is therefore implied, confirming the conclusions drawn from earlier experiments in this thesis.

5.4 General Discussion.

The final experiment in this chapter provides further evidence that dopamine is involved in reinforcement processing, independently of its rôle in motor systems. The drug induced contrast method was quite different from the exploration choice procedures used in earlier experiments. The convergence of results from these different approaches adds further weight to the argument that dopamine's rôle in reinforcement systems plays an important part in the effects of dopaminergic drugs on behaviour.

The failure of flupenthixol to attenuate contrast effects induced by changes in reinforcer value in experiments 5.1 and 5.2 must be reconsidered in the light of the drug induced contrast effects demonstrated in experiment 5.3.

The possibility that the dose used was simply too low to show any behavioural effects in a task involving comparatively powerful reinforcers (in relation to exploration) must be rejected. While doses of 0.06 and 0.10 mg/kg failed to attenuate contrast, or indeed decrease licking rates, the 0.06 mg/kg dose induced contrast effects in experiment 5.3, (although again it did not significantly decrease baseline response rates). Gramling, Fowler and Collins (1984) found decreases in lick rates with 0.5 and 1.0 mg/kg pimozide, which are relatively high doses (for example, Wise and Schwartz (1981) found that a dose of 1.0 mg/kg pimozide abolished the acquisition of food reinforced lever pressing and that a dose as low as 0.125 mg/kg significantly retarded learning in the same situation). Similar results were found at the start of experiment 5.2 when a dose of 0.15 mg/kg flupenthixol was found to markedly decrease licking rates.

A number of factors may account for the difference in effects found between the two drinking experiments and the food reinforced bar pressing study; the nature of the response, animal's deprivation conditions, the nature of the reinforcer and the type of contrast manipulation.

Given dopamine's involvement in motor systems, differences in drug effects between response types may be expected. Dopamine'e motor and reinforcement effects may combine in their effects on motorically demanding responses, while motor impairments may contribute less to the effects on undemanding responses. It would, however, be predicted that tasks involving lever pressing would be more disrupted by dopamine blockade that those involving less demanding licking responses. Wauquier and Niemegeers (1979) showed just such an effect. Responding for intracranial self-stimulation was more sensitive to neuroleptic disruption when the operant was lever pressing than when it was licking.

Gramling, Fowler and Tizzano (1987) replicated the conditions of Gramling, Fowler and Collins (1984) in which the effects of pimozide on response to sucrose solutions was studied, but substituted a lever pressing operant requirement for the direct measure of lick rate used in the earlier study. Although two factors were changed, (the measured response and the change from direct consumption to an operant paradigm) it is worth noting that responses in both studies were sensitive to the same drug dose (0.5 mg/kg pimozide). A lower 0.25 mg/kg dose used in the later operant study failed to produce significant response rate reductions.

These results imply that the lack of drug effects found in experiments 5.1 and 5.2 cannot be attributed to the characteristics of the particular response measured.

During experiment 5.3 animals were maintained at 90% of their ad lib body weights, while in the two drinking experiments they were undeprived. If flupenthixol's effects on reinforcement are mediated by an attenuation of motivation it may be expected that effects would be weak in undeprived animals where there is little motivation to attenuate. On the other hand, if flupenthixol's effects on reinforcement perception act more directly on reinforcement itself, then it may be expected that drug effects will be strongest in situations where the reinforcer is weak, e.g. in undeprived animals.

Most studies of the effects of dopamine blockers on reinforcement have used deprived subjects (apart from studies where the reinforcer was intracranial self-stimulation (ICSS), but other issues complicate the inclusion of ICSS in the analysis of interactions between motivation and dopamine blockade), however, Gramling, Fowler and Collins (1984), and Gramling, Fowler and Tizzano (1987) studied the effects of dopamine blockade on undeprived animals licking, and lever pressing for, sucrose solutions. These studies may be compared with those of Xenakis and Sclafani (1981) who used only very mildly deprived animals (food deprived for 4 hours prior to testing), Geary and Smith (1985) also using mild deprivation (4 hours 45 minutes food deprivation) and Bailey, Hsiao and King (1986) using stronger deprivation (animals maintained at 85% ad lib weights). All of these studies also used sweet solutions as reinforcers (sucrose solutions, apart from Xenakis and Sclafani (1981) who used a saccharin and glucose mixture). Although procedures varied greatly across these studies, they all demonstrated effects of dopamine blockade regardless of deprivation conditions at doses ranging between 0.2 mg/kg pimozide (Bailey, Hsiao and King, 1986) and 0.5 mg/kg pimozide (Gramling, Fowler and Collins, 1984; Gramling, Fowler and Tizzano, 1986; Xenakis and Sclafani, 1981).

Lack of deprivation per se does not therefore seems a reasonable explanation of the failure of flupenthixol to effect responding found in experiments 5.1 and 5.2.

The studies cited above also show that the results of experiments 5.1 and 5.2 cannot be attributed to any peculiarity of sweet solutions as reinforcers.

The relationship between response and reinforcement differs between the lever pressing and licking experiments. Lever pressing must be maintained by learned cues (i.e. secondary reinforcers), while licking is a reflexive consummatory response. Gramling and Fowler (1985) studied the effects of a range of neuroleptics on water reinforcement in water deprived rats in two conditions; reflexive licks were simply licks of water reservoir, in an operant condition licks of a dry disc in the same place as the reservoir produced water reinforcement at another location in front of the test chamber (8.5 cm from the disc) on a continuous reinforcement schedule. Haloperidol had greater effect on operant than reflexive licking (no effects were obtained in the reflexive condition at doses of 0.06 and 0.12 mg/kg but lick rate reductions to 90 and 70% of control rates respectively were found in the operant condition). Ljungberg (1987) has also shown that, amongst a range of drugs, flupenthixol and haloperidol had greater effect on an operant (lever pressing) task reinforced by water than it had on direct water consumption. Doses of 0.05 mg/kg haloperidol or flupenthixol attenuated lever pressing, while doses of 0.10 mg/kg haloperidol or flupenthixol were required to attenuate water consumption.

Although there are differences between the studies noted above and the experiments presented in this chapter, there is a similarity between the drug doses found to be effective under corresponding conditions. Neither 0.06 nor 0.10 mg/kg doses of flupenthixol decreased licking rates or attenuated contrast effects in the reflexive drinking experiments. A decrease in licking rate was found on the two days when a higher 0.15 mg/kg dose was used. On the other hand, the 0.06 mg/kg dose was sufficient to induce contrast effects and reduce response rates, although not significantly, in the Skinner box experiment. These differences may be at least partially attributable to the differential sensitivity of reflexive and learned tasks to dopamine blockade.

Finally, the drug attenuation of contrast effects induced by changes in objective reinforcement value and the induction of contrast effects themselves by introduction or omission of dopamine blockers may be dependent on different mechanisms. Three possible actions of dopamine antagonists on reinforcement systems can be considered; attenuation of 'emotionality', attenuation of motivation and attenuation of reinforcement per se.

The definition of emotionality requires some discussion. As Fantino (1973, p.281) notes emotion "is a term so general as to defy definition". For the purposes of this discussion, however, an operational definition relating to contrast effects will suffice. In this context emotionality must be defined as a modulator of behaviour which is independent of motivation, (contrary to many definitions which hold the emotion is a special case of motivation, e.g. Leeper, 1948). Weiskrantz (1968), reporting results from his Ph.D. research, found that suppression ratios in a conditioned emotional response paradigm were independent of variations in baseline response rates produced by changes in deprivation levels. The definitional dissociation proposed is supported by these results. A dissociation between emotionality and reinforcement per se is also required. A definition which reduces essentially to 'reaction to extremely strong reinforcers' (e.g. Duffy, 1941) will not do. Two

definitions which capture the notion that emotion *alters* response maintained by reinforcers are those of Ferster and Perrott (1968, p.525), "Emotion is a state of the organism in which the form and frequency of several items of behavior in the ongoing operant repertoire are altered... The term 'emotional stimulus'... describes a stimulus which alters many ongoing performances of the organism's repertoire other than those directly affected by reinforcement or extinction." and Catania (1968, p.334) "... if a pre-aversive stimulus simultaneously alters heart rate, respiration, blood pressure, defecation, urination, and operant behavior maintained by reinforcement, the stimulus may be said to produce emotional behavior". Of course, the emotional stimuli referred to in these two definitions will be reinforcers themselves. Rolls (1986) notes that emotional states may be induced by the omission or termination of reinforcers in addition to their simple presentation (see Rolls, 1986, p.326, figure 1). An operational definition of emotion in the context of behavioural contrast may be constructed from a synthesis of Rolls' description of (some) eliciting conditions and Ferster and Perrott's (1968) and Catania's (1968) description of the consequences of emotional states that, amongst other things, alter ongoing operant responding. The working definition proposed is 'emotionality is a measure of the tendency of changes of reinforcement to elicit state changes in an organism which alter its ongoing performance of operant responses'. This definition is extremely specific to contrast effects, but, for the present purposes, that is what is required.

If, according to this working definition, dopamine blockade reduces emotionality, then an attenuation of contrast effects induced by changes in explicit reinforcement value would be expected, although baseline response rates may be unaffected. These are exactly the results obtained by Rosen and Tessel (1970) with chlordiazepoxide (a benzodiazepine, not a dopamine blocker) but not with chlorpromazine.

If only emotionality, and not motivation or reinforcement perception, is affected then drug induced contrast is not predicted. There is no reason to predict a change in reinforcement which may elicit an emotional state. If dopamine blockade has multiple effects then the contribution of emotional effects should attenuate drug induced negative contrast, but leave positive contrast on release from drug treatment unaffected. Although there is a change in reinforcement in both cases, emotionality is only attenuated in the case of negative contrast where drug treatment follows, rather than precedes, the change.

The results of a hypothesised attenuation of motivation (or drive) by dopamine blockade can be inferred from studies in which motivation was manipulated by varying deprivation conditions. As noted previously, contrast effects are obtainable in non-deprived rats when the reinforcers are sweet solutions and the measured response is licking rate (e.g. Riley and Dunlap, 1979). There is evidence that, within sweet solution consumption paradigms, contrast effects are more reliable in undeprived animals (Riley and Dunlap, 1979), and that positive contrast effects are larger in less deprived animals (Panksepp and Trowill, 1971, although this is probably the result of minimising ceiling effects). Baltzer and Weiskrantz (1975) obtained similar results in their operant paradigm. In most operant paradigms, however, negative contrast effects are attenuated at low drive levels (Flaherty, 1982). The situation is less clear for positive contrast because the occurrence of ceiling effects at high drive levels, however, it appears that the magnitude of successive contrast varies directly with deprivation level under appropriate conditions (Flaherty, 1982). Given these results an attenuation of negative contrast induced by explicit reinforcement changes would be expected if flupenthixol reduces drive, while the effects on positive

contrast are not clearly predicted.

The analogue of drug induced contrast, given a hypothesised motivational effect of flupenthixol, is the induction of contrast by changes in deprivation level. Zaretsky (1966) failed to obtain negative contrast effects when animals were switched from 22 hour food deprivation to 90 minutes food deprivation conditions. Capaldi (1973) has shown that contrast effects can be induced by shifts in deprivation, but only when reward magnitudes are large (e.g. twenty two 45 mg food pellets). Capaldi, Smith and White (1977) subsequently showed that changes in drive level occurring concurrently with changes in reward magnitude may reduce or eliminate contrast as a result of disruptions of reward expectancies. Given this result, and the low magnitude of reward used in the drug induced contrast study (one or four 45 mg food pellets) it does not seem likely that the drug induced contrast found can be attributed to a direct effect on motivational systems.

If flupenthixol attenuates perceived reinforcement magnitude more directly, then drug induced positive and negative contrast effects are predicted. The predictions for the explicit reinforcement shift experiments are less clear.

If flupenthixol attenuates the value of both the high and low concentration solutions, then any effect on contrast will be dependent on a differential (i.e. non-linear) drug effect on high and low value reinforcers and on the relationship between magnitude of contrast effects and difference between high and low value reinforcers. There is much evidence that the size of contrast effects increases with greater disparity between small and large rewards (e.g. Crespi, 1942, 1944). The question of non-linearity of drug effects is harder to answer. An examination of data from Geary and Smith's (1985) study indicates that 0.25 mg/kg pimozide may have proportionately greater effects on lower value reinforcers (e.g. the ratio of control to drug sucrose drinking rates over the first 3 minutes of testing was 1.9 for 40% sucrose solutions and 5.7 for 5% sucrose solutions, there did not, however, appear to be marked differences between effects on 40% , 20% and 10% concentrations). These data only give a very approximate indication that there may be differentially large effects of dopamine blockers on low value rewards, however, Bailey, Hsiao and King ((1986) also found greater effects of pimozide (0.2 mg/kg) on lever pressing rates lower concentration sucrose solution reinforcement. If these results are representative of the general case, it would be predicted that dopamine blockade would, if anything, increase perceived magnitude differences between high and low value reinforcers, and hence enhance contrast effects. However, examination of data from Rosen and Tessel (1970) shows that chlorpromazine has little effect on the ratio between running speeds obtained for high and low magnitudes of food reinforcers except at very high doses (5.0 mg/kg) where there was a reduction in the ratio between high and low reinforcement running speeds. Nevertheless, Rosen and Tessel (1970) found no evidence for effects of chlorpromazine on contrast magnitude, yet the drug markedly reduced running rates. Baltzer, Huber and Weiskrantz (1979) found a reduction in discrimination

between high and low value reinforcement with a lower dose of chlorpromazine (0.5 mg/kg). They also found a small attenuation of negative, but not positive, contrast.

Given these somewhat conflicting results it is not clear that attenuation of reinforcement perception predicts a change in contrast magnitude induced by shifts in explicit reinforcement magnitude. Any such effect is reliant upon differential effects on the perception of high and low reinforcement, as such, it is doubtful whether any effect on contrast magnitude will be large whether it is an enhancement or an attenuation.

Various mechanisms which may lie behind the effects of flupenthixol have been considered, they will now be assessed in relation to the experiment presented in this chapter.

A drug induced attenuation of emotionality (as defined above) is not supported by the evidence. Drug induced contrast effects would not be predicted as a consequence of an emotional deficit, while attenuation of explicit reinforcement shift induced contrast would be; the opposite of the results obtained.

The predictions derived from a drug-induced drive deficit also fail to match the results obtained, however they are less certain. The low magnitudes of reinforcement used in the drug induced contrast experiment militate against the induction of contrast effect through changes in effective drive level, nevertheless, such a result is possible. A motivational deficit would imply an attenuation of contrast effects induced by changes in the magnitude of food rewards, however, an attenuation is not necessarily predicted when sweet solutions are used as reinforcers. The results obtained do not seem likely to be the result of an attenuation of drive, however, such an attenuation cannot be clearly ruled out.

The predictions derived from a more direct effect on reinforcement are consistent with the results obtained. Drug induced positive and negative contrast is predicted and was obtained. An effect on contrast induced by changes in sweet solutions' concentration is dependent on differential effects on the perception of the value of the high and low concentration solutions. Evidence from studies of pimozide's effects on response to sweet solutions indicates that any differential effect is likely to lead to enhanced contrast if any effect at all is observed. No significant drug effects on contrast were found, however, the significant negative contrast found in experiment 5.1 and the positive contrast found in experiment 5.2 were both slightly larger in the drug condition (when contrast was assessed in terms of the difference in change of lick rate obtained between downshift and upshifts of reward concentration).

In summary, the results of experiment 5.3 clearly indicate that dopamine systems are involved in the perception of reinforcement. Differences between the results of that experiment and the two drinking experiments cannot be attributed to differences between the measured operant responses (bar-pressing versus licking), deprivation state of the animals or any peculiarities of sweet solutions as reinforcers. It is, however, possible that the reflexive nature of the drinking task made it less sensitive to dopaminergic manipulations.

When the potential mechanisms by which flupenthixol affected reinforcement perception were considered, it was clear that an effect on emotionality could not account for the results obtained. An effect on drive systems was also unlikely to

account for the results. It is concluded that the most likely action of flupenthixol was a more direct effect on reinforcement systems per se. The nature of such an action will be discussed in the following chapter.

Chapter 6

Discussion.

This thesis has addressed the question of whether dopamine systems play a rôle in reinforcement over and above any rôle they have in motor control. The results obtained in this thesis were not consistent with exclusively motoric effects of dopamine manipulations. It is clear that dopamine manipulations alter animals' response to reinforcers. This altered response was demonstrated for two disparate classes of reinforcement: food and exploration.

No direct attempts were made to assess the mechanisms by which alterations in animals' response to reinforcement occurred, however, the variety of experimental procedures used in this thesis, together with published studies, permit a model to be developed. A number of possible rôles for dopaminergic systems which could contribute to the effects found in this thesis will now be considered.

6.1 Emotion.

There is strong evidence against a drug effect simply on emotionality. The drug induced contrast effects shown in experiment 5.3 and by Royall and Klemm (1981) could not be produced by a simple change in emotionality. Furthermore, if dopamine blockade attenuates emotionality, reductions in the magnitude of contrast effects induced by objective changes in the value of reinforcement would be predicted. No such reductions were found in experiments 5.1 and 5.2, or in the food reinforced runway contrast studies of Roberts and Pixley (1965) and Rosen and Tessel (1970). The small contrast attenuation found by Baltzer, Huber and Weiskrantz (1979) with chlorpromazine is not clearly attributable to an emotional effect since baseline response rate differences between the high and low reinforcement value components were also attenuated. Phillips and LePiane (1986) have shown that pimozide attenuates the positive and negative contrast effects found when the current of ventral tegmental brain stimulation reward is gradually increased or decreased in steps over a test session. The contrast control condition was presentation of all current intensities in a random order. Rate-Intensity curves produced with random order presentation in vehicle and drug treated conditions were also compared. These comparisons indicated that the contrast attenuations obtained were due to drug induced attenuation of the reinforcing value of stimulation, and hence decreases in the relative values of the current intensities used in the contrast tests.

It has been noted that the attribution of the drug effects found by Baltzer, Huber and Weiskrantz (1979) to alterations in dopamine systems is complicated by the multiple action of chlorpromazine on dopaminergic and noradrenergic systems (Guth and Spirtes, 1963). It is less difficult to attribute the negative results of Roberts and Pixley (1965) and Rosen and Tessel (1970), who also used chlorpromazine, to a failure of dopamine blockade to reduce emotionality. As both dopamine and noradrenaline receptors are blocked by chlorpromazine, if either transmitter system is involved in emotionality an attenuation of contrast effects would be expected. As no such effect is found it can reasonably be concluded that dopamine blockade does not reduce emotionality. (There is a theoretical possibility that a putative reduction in emotionality produced by dopamine blockade is counterbalanced by an increase induced by noradrenergic blockade. There is, however, no evidence

which suggests that noradrenergic blockade increases emotionality (see e.g. Mason, 1981)).

A number of other phenomena observed with dopamine blockers are also inconsistent with an attenuation of emotionality (assuming a wider definition of emotion such as that of Rolls (1986) rather than the working definition used in chapter 5). For example, Ettenberg and Camp (1986) have shown that periodic administration of haloperidol during exposure to a food reinforced runway task (run with only one trial per day, in order to permit haloperidol treatment on selected individual trials) induces a partial reinforcement extinction effect in subsequent drug free extinction trials. In this design there is no change in the objective reinforcement value during drug trials which may induce an emotional state. Nor is the quality and magnitude of reinforcement (ten 45 mg unsweetened food pellets) or the deprivation state (85% ad lib body weight) so extreme that a characterisation of animals' response in terms of emotion, rather than reinforcement, seems justified.

The partial reward extinction effect is dependent on the frustrative after-effects of non-reward, which must represent an emotional state (see Amsel, 1962, 1967; Capaldi, 1967; Gray, 1975; whether Amsel's two process model, Capaldi's single process model, or Gray's acceptance of both, is the correct explanation of the partial reinforcement extinction effect, the following argument will still hold). Consider a hypothetical experiment in which a dopamine blocker is given on non-rewarded trials during partial reinforcement training. If dopamine systems are involved in emotionality then it would be expected that the partial reinforcement extinction effect would be attenuated by dopamine blockade as a result of reduced frustration on non-rewarded trials. This experiment has not been performed, however, Weiner, Feldon and Bercowitz (1987) have conducted an analogous experiment using d-amphetamine. As d-amphetamine is a dopamine stimulant it would be expected that if emotionality and dopaminergic activity are correlated then amphetamine would increase the partial reinforcement extinction effect if given on non-rewarded trials. In fact, Weiner, Feldon and Bercowitz (1987) found that amphetamine abolished the partial reinforcement extinction effect if given on non-rewarded trials or throughout training, but not if it was only given on reinforced trials. These results clearly indicate that effects of dopaminergic manipulations on animals' response to reinforcement cannot be attributed to emotional effects.

The emergence tests used in chapters 2 and 3 were intended to assess the contribution of emotional effects to changes in the distribution of exploratory behaviour found with flupenthixol, amphetamine and apomorphine. It was argued that in the context of the emergence test it was not possible to distinguish between heightened emotionality and increased sensitivity to the negatively reinforcing properties of exposure in the area outside the emergence tube. In terms of Rolls' (1986) model, an emotional state is being induced by a strong negative reinforcer. Emotionality will be altered if drug manipulations effect evaluation of the strength of negative reinforcement or if they effect emotional system directly. The two actions cannot be distinguished in this situation (unless various physiological measurement had been made concurrently). All emergence tests, apart from that using apomorphine (experiment 3.1.4), yielded null results. Apomorphine increased emergence time, indicating that the perceived negative consequences of exposure outside the emergence tube were heightened by apomorphine. It was concluded that this effect may have been due to increases in emotionality or potentiation of negative reinforcement perception, but not to apomorphine induced nausea. The contrast and partial re-

inforcement extinction effect studies discussed above indicate that apomorphine's effect on emergence should be attributed to changes in the impact of expected negative reinforcement, not emotionality.

6.2 Motivation.

Changes in motivation produced by dopaminergic drugs would lead to a change in the reinforcing impact of rewards analogous to the difference in reinforcing value of food between satiated and deprived animals. Two possible effects on motivation must be considered. First, an effect specifically on hunger via actions on hypothalamic systems. Second, a more general effect on drive which may underlie the attenuation of reinforcement with dopamine blockade.

6.2.1 Hunger.

Dopamine is involved in the central signalling of hunger. It has been shown by Leibowitz and Rossakis (1979a) that direct microinjection of dopamine into the perifornical hypothalamus inhibits feeding. This effect can be reversed by prior injection of neuroleptics into the perifornical hypothalamus (Leibowitz and Rossakis, 1979b). Dopamine systems appear therefore to be involved in at least one drive – hunger. Leibowitz and Rossakis' (1979b) results make clear, however, that the effect of centrally injected neuroleptics on this drive is to increase it (i.e. to block the inhibition of hunger by perifornical dopamine) and hence potentiate the perceived reinforcing value of food reward. This potentiation of the value of food reinforcers by dopamine blockade cannot account for the attenuation of reinforcement implied by the drug induced contrast and exploration choice experiments.

The rôle of dopaminergic neurons in the perifornical hypothalamus in hunger appears, however, to be independent of other more general rôles for dopamine in reinforcement and motor control (see e.g. Martin and Myers, 1976). It has been shown that amphetamine increases the rate of eating whilst decreasing the total amount of food consumed (Blundell and Latham, 1978, 1980). Moreover, low doses of amphetamine can increase food intake in undeprived (Dobrzanski and Dogget, 1976; Blundell and Latham, 1978; Winn, Williams and Herberg, 1982) and occasionally in food deprived animals (Holtzmann, 1974). These results indicate that, while amphetamine has an anorectic effect through its action on perifornical dopamine neurons, it also has actions on motor or reinforcement systems which may lead to increases in feeding (Winn, Williams and Herberg, 1982 demonstrate the dopaminergic nature of these low dose effects). The anorectic perifornical action dominates at high doses of amphetamine, at lower doses motor or reinforcement system stimulation outweighs hunger inhibition. (It should, however, be noted that amphetamine induced noradrenergic activity in the hypothalamus will also effect hunger and satiety; noradrenergic cells in the perifornical hypothalamus have been shown to inhibit hunger (Leibowitz and Brown, 1980b) while those in the paraventricular hypothalamus have been shown to stimulate feeding (Leibowitz and Brown, 1980a)).

Low doses of dopamine blockers and stimulants were used in the experiments presented in this thesis in order to minimise motor impairments and stereotypy. The results obtained do not show evidence for changes in hunger consistent with the hypothalamic system discussed above at these doses. The high licking rate shown by flupenthixol treated animals in the first phase of the saccharin drinking experiment (5.2) may appear to be an exception attributable to a drug induced

increase in hunger. The measured response was not motorically demanding and the dose of flupenthixol was the highest used in this thesis, both favouring the appearance of behavioural changes due to dopaminergic effects on hunger systems. Nevertheless, the drug effect was not statistically significant, and, although amphetamine induced anorexia is a common phenomenon, there is no evidence that non-centrally administered dopamine antagonists cause increases in food consumption (see e.g. Mason, 1984 pp.366-367). If any explanation of this non-significant effect is required, the one previously proposed (that the disruption of alternative exploratory behaviour may have increased licking time in the drug treated animals) is preferable to invoking an isolated instance of neuroleptic induced hunger.

It is concluded that although dopamine in the hypothalamus has a well documented rôle in hunger, the drugs used in the food and sweet liquid reinforced experiments in this thesis were not significantly effecting hunger through this system.

6.2.2 General Effects on Drive.

It must be made clear that 'drive' as discussed in this section refers to primary drives such as hunger, and not to conditioned drives (i.e. not to incentive motivation which will be discussed later). Care must therefore be taken in attributing the effects of manipulations of dopamine systems to changes in motivation, rather than effects on reinforcement or conditioned motivational stimuli (secondary reinforcers).

It has been noted that motivational changes are unlikely to be able to account for the drug induced contrast effects shown in experiment 5.3. A number of other studies also show effects inconsistent with motivational blunting as the mechanism underlying neuroleptic induced reinforcement attenuation.

In attempting to dissociate motor and hedonic drug effects, Wise has often pointed out that changes in responding due to neuroleptics can be shown to occur only after an animal has sampled reinforcement (e.g. deWit and Wise, 1977; Wise, 1982; Wise and Colle, 1984; Wise, Spindler, deWit and Gerber, 1978; Wise, Spindler and Legault, 1978; Yokel and Wise, 1975, 1976). For example, in Wise, Spindler, deWit and Gerber (1978) rats were trained while undrugged to lever press for food reinforcement on a continuous reinforcement schedule. When they were subsequently tested under pimozide they responded vigorously at the start of the test session, rates only decreasing after a number of reinforcements had been obtained. This effect was replicated in a food reinforced runway task. Again rats were trained undrugged, and when tested under pimozide runway latencies were unaffected on the first few trials but subsequently increased in drugged animals. Wise cites such effects as evidence against drug induced performance deficits, however, they provide stronger evidence against drive deficits (arguments can be made that neuroleptics may increase the rate of fatigue, hence providing a motor explanation for the initial sparing of performance (e.g. Anisman, 1982)). Although fatigue based explanations may be able to account for this effect, it is hard to see how drive attenuation could do so. If dopamine blockade reduced drive it would be expected that response for reinforcement would be effected before the reinforcer had been obtained. On the other hand, if the effect is more directly on reinforcement perception then reinforcement must be sampled before the drug induced attenuation of reinforcement can alter the animal's behaviour.

Fowler, Lacerra and Ettenberg (1986) compared the force and rate of responding

produced by satiated rats (presumably in a low drive state) with those of haloperidol treated animals. They found that while both satiation and haloperidol reduced response rates when compared to those of food deprived animals at the start of testing, haloperidol produced an increase in response force but satiation produced a decrease. Response characteristics produced by haloperidol do not appear to be the same as those resulting from an attenuated motivational state caused by satiation. As I noted previously, the effects of haloperidol on rate and force are similar to those found by Di Lollo, Ensminger and Notterman (1965) when comparing response characteristics for high and low value reinforcers. The difference in response characteristics found by Fowler Lacerra and Ettenberg (1986) cannot in itself, however, be treated as conclusive evidence for a non-motivational effect of haloperidol. The data for satiated animals were collected during the last three minutes of each fifteen minute test session. The animals in this group were food deprived, as were the haloperidol treated animals, however, they were assumed to be satiated during the final part of the session. The data for the haloperidol group were collected over the entire 15 minutes of each session. Although animals in the satiated group were indeed likely to be less motivated during the final three minutes when compared to the average state of the drug group over the full session, performance differences cannot be solely attributed to satiation. It would be quite reasonable to attribute the decreased response force of the satiated animals to fatigue rather than motivational effects. In conjunction with the contrast evidence and the initial response sparing evidence cited above, Fowler, Lacerra and Ettenberg's (1986) results are, nevertheless, intriguing. Their value as evidence against a drive attenuating model of neuroleptic action awaits more detailed studies currently underway in Fowler's laboratory.

6.3 Reinforcement.

Indirect effects on the impact of reinforcers through motivational or emotional systems have been ruled out as explanations of the effect of dopaminergic drugs on reinforced behaviour. The results obtained in this thesis also show that the effects of dopaminergic drugs cannot be interpreted simply in terms of motor deficits. This implies that dopaminergic drugs are directly effecting the impact of reinforcement.

The simplest model of dopamine's rôle in reinforcement is that dopaminergic neurons signal reinforcing events. As a consequence, dopamine blockade should result in attenuation of the perception of reinforcement value. This is essentially a restatement of Wise's anhedonia hypothesis (mark 2, Wise, 1982). Although this model is consistent with the results of the experiments in this thesis and with many of the other studies cited therein, there is evidence against a simple signalling rôle.

It has been shown that dopamine blockade does not impair the perception of reinforcers in stimulus-stimulus associative learning. Both appetitively and aversively significant stimuli (i.e. potential reinforcers of operant responses) have been shown to be correctly associated with neutral stimuli when presented to animals treated with dopamine blockers in the absence of operant response. Under subsequent drug free testing the neutral stimuli engaged behaviour appropriate to associated significant stimuli. Although behaviour in the drugged condition may be impaired, the development of associations shows that the qualities of the reinforcing stimuli were perceived correctly under drugged conditions even though they failed to engage appropriate behaviours. Examples of such transfers include demonstrations of tone-food associations made under high doses of pimozide to subsequent drug

free operant conditioning (Beninger and Phillips, 1981), conditioned emotional reactions to stimuli paired with shock (Hunt, 1956; Beninger, Mason, Phillips and Fibiger, 1980) and conditioned defensive burying of objects associated with shock (Beninger, MacLennan and Pinel, 1980).

These results lead Beninger (1983) to propose that "Normal dopamine functioning appears to be required for the establishment and maintenance of incentive learning in naive animals" (p.190). In other words, dopamine is not responsible for the signalling of reinforcement, but is required if that reinforcement is to elicit responses. Beninger's model specifically implicates dopamine in learned behaviour, implying that dopamine antagonists should not effect reflexive behaviours. The results of the exploration experiments presented in chapters 2 and 3, together with Wise and Colle's (1984) results, showing attenuations of free feeding not attributable to motor deficits with pimozide, indicate that reflexive behaviour for primary reinforcers can also be affected by dopamine blockade. Nevertheless, the position that dopamine is involved in the elicitation and maintenance of reinforcer directed responses is not fundamentally challenged by these results. Willner (1983) has indeed proposed that the meso-limbic dopamine system may act as an interface between reinforcement and motor systems regardless of the primary or secondary nature of the reinforcement.

Further evidence supporting this position has been provided by studies investigating the rôle of dopamine in intracranial self-stimulation (ICSS). The classic findings that dopaminergic drugs alter ICSS (Olds and Travis, 1960; Stein, 1962) were instrumental in the development of dopamine theories of reward. Subsequent studies have attempted to anatomically localise regions responsible for the rewarding consequences of stimulation. For example, Mora, Sanguinetti, Rolls and Shaw (1975) showed that injection of spiroperidol into the nucleus accumbens abolished medial forebrain bundle ICSS. Stephens and Herberg (1977) showed a similar effect, and, in addition, showed that dopamine blockade in the caudate-putamen also attenuated ICSS. These studies face the same problems as experiments using naturally reinforcers in that attenuation of ICSS may be due to effects on reinforcement or on performance. Fouriezos and Wise (1976) have shown that pimozide only attenuates ICSS after a few minutes of responding; they argue that this shows the deficit to be due to reinforcement rather than performance effects. An alternative fatigue based explanation of these results is, however, possible. The ICSS paradigm allows more sophisticated techniques to be employed in dissociating performance and reinforcement effects.

When a single stimulating electrode is implanted into a site in one hemisphere of an animal's brain the effects of intracranial drug injections into sites which are either ipsi-lateral or contra-lateral to the electrode can be used to dissociate reinforcement and performance effects of drugs on ICSS. If drug effects are due to performance deficits, then response rates for ICSS will be attenuated even if the stimulating electrode is in the contra-lateral hemisphere from an intracranial drug injection (i.e. deficits should be symmetrical between hemispheres). On the other hand, if the drug injection is disrupting a pathway involved in reinforcement then the effects of intracranial drug injections should be much stronger in the hemisphere in which the stimulating electrode is sited (i.e. deficits should be asymmetric).

Studies based on the logic described above, using injections of blockers or of the neurotoxin 6-hydroxydopamine (in conjunction with pretreatments protecting

noradrenergic cells), have produced mixed results. Broekkamp and van Rossum (1975) found symmetric response rate reductions when they injected haloperidol into the caudate-putamen and hence inferred a motoric origin for the deficit. This may well be the case, however, it is unclear whether the deficit was the result of a direct effect on the consequences of stimulation. The stimulating electrodes were sited in the ventral tegmentum which, in addition to some neostriatal projections, projects to many other areas (Moore and Bloom, 1978). It is therefore possible that responding was being maintained by activity in these other areas while neostriatal dopamine blockade was indeed simply causing a motor deficit. Neill, Paey and Gold (1978) injected haloperidol into the ventral anterior striatum and obtained a symmetric decrease in responding for lateral hypothalamic ICSS. They note, however, that unilateral hypothalamic stimulation may produce a bilaterally reinforcing effect (Hopkins and Kuypers, 1975; Wright, 1973), hence accounting for the symmetric effects on ipsi- and contra-lateral stimulation. Clavier and Fibiger (1977) found symmetric reductions in ICSS for electrodes in the substantia nigra pars compacta after 6-hydroxydopamine lesions of the nigro-striatal bundle, however, these deficits were only temporary. Christi, Ljungberg and Ungerstedt (1973) obtained asymmetric reductions in lateral hypothalamic ICSS after unilateral 6-hydroxydopamine injections in rats which showed strong rotational responses to systemic apomorphine (indicating extensive nigro-striatal lesions). Similar asymmetric deficits have been obtained after 6-hydroxydopamine lesions of the substantia nigra pars compacta by Phillips, Carter and Fibiger (1976) using electrodes in the caudate-putamen and by Koob, Fray and Iversen (1978) after combined nigral and tegmental lesions for hypothalamic ICSS. Carey (1985) has shown that nigro-striatal 6-hydroxydopamine lesions may appear to produce symmetric deficits due to recovery, however, apparently recovered lesions can be challenged by systemic dopamine blockers with resulting asymmetric effects on ICSS. It may be concluded that blockade of the nigro-striatal system can produce non-specific motor deficits, however, if lesions are extensive and stimulation is primarily of nigro-striatal cells then asymmetric effects indicative of reinforcement attenuation can be demonstrated.

The results of studies of meso-limbic dopamine systems are clearer. Asymmetric effects have been demonstrated after unilateral 6-hydroxydopamine lesions for ICSS in the sulcal pre-frontal cortex (Clavier and Gerfen, 1979) and after haloperidol injections into the nucleus accumbens for accumbens (Robertson and Mogenson, 1978) or tegmental ICSS (Mogenson, Takigawa, Robertson and Wu, 1980).

Overall it can be concluded that the effects of dopamine manipulations on ICSS are due to dopamine's involvement in reinforcement processing (with the possible exception of some motoric effects in nigro-striatal systems). It has, however, been shown that the reinforcing component of medial forebrain bundle of ICSS passes rostro-caudally, whereas dopamine neurons in the medial forebrain bundle send signals caudo-rostrally (Shizgal, Kiss and Bielajew, 1982). Furthermore, collision studies show that velocity of action potentials in these reinforcement signalling fibres is between 2 and 8 m/s, implying myelinated thick fibres (Bielajew and Shizgal, 1982). Dopaminergic fibres are thinner and non-myelinated, hence they transmit action potentials considerable more slowly (e.g. German, Dalsass and Kiser, 1980). The implication of these results, together with those from mapping studies which fail to show correlations between dopaminergic terminal fields and effective ICSS sites (Prado-Alcala and Wise, 1984; Prado-Alcala, Streather and Wise, 1984), is that although dopaminergic cells are involved in the processing of reinforcement

they are not involved in the actual reinforcement signalling.

In a series of elegant studies Mogenson has traced a neural circuit through the amygdala, the ventral tegmentum, the nucleus accumbens, the globus pallidus and subpallidal regions and on to the mesencephalic locomotor region (Mogenson, 1987; Mogenson and Yim, 1981). Activity in tegmental cells which project to the nucleus accumbens inhibits cells projecting to the globus pallidus which themselves inhibit the emission of behaviour; the net result of stimulating tegmental-accumbens connections is therefore to elicit behaviour. This has lead to the suggestion that the effects of medial forebrain bundle stimulation on dopaminergic cells is to disinhibit the elicitation of behaviour in response to the non-dopaminergic reinforcing substrate of ICSS. In conjunction with evidence of connections from limbic structures such as the amygdala to the ventral tegmentum Mogenson (1987) has proposed a 'dopamine "gating" mechanism', he suggests that "dopamine contributes to the modulation or gating of relevant or biologically significant stimuli that initiate behavioral acts" (Mogenson, 1987, p.151). This model, arrived at using very different evidence from that marshalled by Beninger (1983), is also consistent with the notion that dopamine blockade does not attenuate the perception of reinforcers per se, nor does it necessarily disrupt motor systems, its action on response to reinforcement is to reduce the extent to which reinforcement can elicit behaviour.

The two allied models I have described above (Beninger's and Mogenson's) account for a wide variety of dopaminergic effects. On the basis of Mogenson's evidence in particular, a rôle for dopamine in gating responsiveness to reinforcing stimuli can be accepted. Nevertheless, this rôle alone cannot account for some effects of dopamine system manipulations.

The drug induced contrast effects demonstrated in chapter 5 and by Royall and Klemm (1981) are not consistent with a dopamine gating mechanism, contrast effects must have been due to drug induced differences in the recalled impact of reinforcement. Modulation simply on the output side of the reinforcement system cannot account for the necessary differences in rewarding impact. A number of techniques attempt to measure the rewarding impact of ICSS through current titration or resetting procedures. For example Zarevics and Setler (1979) measured the point at which rats made a current resetting response on a lever when ICSS current obtained by pressing a second lever was gradually decreased. They found that low doses of pimozide did not decrease response rate on the ICSS lever, but did alter the threshold current at which animals responded on the reset lever. Pimozide increased the reset current threshold, indicating that it had decreased the rewarding impact of stimulation. Although this 'rate free' measure still involves the production of a response on the reset lever and even noting that at higher doses of pimozide response rate on the ICSS lever was attenuated, this result is nevertheless incompatible with simple dopamine response gating. In this experiment, the measured resetting response is actually produce more readily when animals are drugged, a result easily explained in terms of attenuated reinforcement impact but incompatible with reduced responsiveness to reinforcement. Finally, Sanghera, German and Kiser (1979) and Miller, Sanghera and German (1981) have recorded the activity of dopaminergic cells in the ventral tegmentum and substantia nigra during conditioned behaviour in awake behaving rats. Cells were identified as being dopaminergic by their electrophysiological characteristics and in some cases by their response to haloperidol. It is of particular interest that tegmental cells were identified which were active when discriminative stimuli indicating the onset of schedule

operation were presented and when conditioned stimuli indicating the availability of reward were presented, but which did not respond to the motor behaviour accompanying operant responses. These findings are not necessarily incompatible with a dopamine gating mechanism, but they do show that some dopaminergic cells respond specifically when stimuli signalling reinforcement are present but not during the emission of behaviour towards those stimuli occurs as the gating model may suggest.

The studies described above indicate that dopamine is involved in reinforcement processing to a greater extent than dopamine gating suggests. Although a rôle for dopamine in the modulation of behavioural response to reinforcers is likely, some rôle in computing or signalling the impact (but not presence) of those reinforcers is also suggested. The dopamine gating model circuit may account for the rôle of dopaminergic projection from the ventral tegmentum to the nucleus accumbens, however, this is only one of a number of dopaminergic projections originating in the ventral tegmentum.

Given that dopaminergic neurons are assumed not to signal reinforcement per se, but that dopaminergic activity is held to modulate both behavioural response to reinforcement (Mogenson's dopamine gating) and psychological response to reinforcement (as indicated by the results of contrast and ICSS titration experiments e.g. Zarevics and Setler, 1979), an extended form of gating model is required. An extended model which takes behavioural and anatomical findings into account is presented below. It must be stressed that this model is highly speculative, it forms the basis for suggestions for future research rather than being a distillation of the findings of a directed research program.

Consider the chain of events which may follow an encounter with a potentially reinforcing stimulus in the environment. Perceptual systems identify the stimulus, neural activity leading to stimulus classification is produced. The interaction between stimulus properties and memory may indicate that the stimulus has had reinforcing consequences in the past. In other words the stimulus may predict reinforcement. In addition activity reflecting the class of response which has lead to primary reinforcement being obtained may be produced. These activations (the reinforcement signal and action trace) may occur whether or not the drive state to which the reinforcer relates is present. Even if the drive is not currently present the reinforcing stimulus may warrant attention, although if the appropriate drive is present attention is more likely to be directed towards the stimulus. A comparison of current drive states with the predicted reinforcement indicates whether or not action in response to the reinforcer is justified. If a match between predicted reinforcement and drive is obtained, action is produced. Contingent on the results of that action, changes may occur in memory including modification of both sensory and action components of representation.

The learning of associations between reinforcing and neutral stimuli while under dopamine blockade indicates that perceptual processing, and allied mnemonic operations, are not severely disrupted, although there is evidence that disruptions of dopamine systems may produce sensory inattention (Marshall, Berrios and Sawyer, 1980). We may conclude that signals indicating that a stimulus is potentially reinforcing are not disrupted. It has been noted that the results of many studies show that dopamine blockade is not interfering with the signalling of drive states, however, the existence of drive state signals alone is not sufficient to elicit directed

action. It is possible that dopamine blockade disrupts the integration of reinforcement signals and drive signals. One consequence of this would be that reinforcers were less able to elicit behaviour (as in Mogenson's dopamine gating model). In addition, we may speculate that alterations occur in the associations which the reinforcing stimulus elicits and the ability of the reinforcing stimulus to maintain responding and possibly attention. An extended rôle for dopamine of this type would account for the findings discussed above which appear incompatible with Mogenson's dopamine gating model and Beninger's incentive response hypothesis. We may also speculate that the result of drive-reinforcer matches may be 'hedonia'.

Further speculation can directed to the mechanism by which dopaminergic systems are involved in drive-reinforcement integration. Given that dopamine is not involved in the signalling of either drive or reinforcement, its rôle must, in some way, be modulatory. In fact, given the slow conduction velocity of dopaminergic neurons (Bielajew and Shizgal, 1982) and the slow onset but long lasting action of dopamine receptors (see e.g. Stricker and Zigmond, 1984 p.261), modulatory, rather than signalling, rôles are to be preferred in models of dopamine's functions. In the system outlined above there is no need for signals other than drive state, reinforcement and perceptual classification on the input side of the system, we must therefore consider why any modulation would be required. One possibility may be that dopamine plays a part in an amplifying circuit. While drive state signals may long lasting, reinforcement signals, being dependent on external sensory input, may only be transitory. A positive feedback loop could amplify the effects of drive-reinforcement matches and hence prolong activation of motor and attentional systems allowing the reinforcer itself (rather than the signals indicating its availability) to be obtained and allowing additional environmental cues (perceptual classification signals) to be encoded into the associative complex predicting subsequent reinforcement.

An anatomical circuit consistent with this speculative model can be identified. The amygdala has been shown to be involved in the expression of emotional responses to normally significant stimuli, and in memory (Aggleton and Passingham, 1981, 1982), a rôle in reinforcement processing is therefore suggested. The amygdala receives inputs from a range of modality-specific association areas and from polysensory cortical regions (see e.g. Aggleton, Burton and Passingham, 1980), in addition, it receives inputs from regions implicated in motivational processes such as the hypothalamus (which may signal hunger, e.g. Leibowitz and Brown, 1980a,b, and thirst, e.g. Epstein, 1971; Huang and Mogenson, 1972) and the periaqueductal central grey (which may transmit pain related signals, e.g. Liebeskind and Paul, 1977). It has therefore been suggested that the amygdala may be involved in the integration of sensory information and emotion arousing signals (Aggleton and Mishkin, 1986). Projections from the amygdala to the ventral tegmentum form the first stage of Mogenson's dopamine gating circuit, however, the ventral tegmentum also makes reciprocal projections to the amygdala (Otterson, 1981) and has specifically been shown to be involved in reinforcement processing (e.g. Bozarth and Wise, 1986). These projections may, in turn, indirectly influence cells projecting from the nucleus of the diagonal band of Broca to the ventral tegmentum as the amygdala sends projections to the nucleus of the diagonal band (Aggleton, Friedman and Mishkin, 1987). This circuit is of particular interest since Gomita and Gallistel (1982) and Gallistel, Gomita, Yadin and Campbell (1985) have shown that the non-dopaminergic descending pathway signalling the reinforcing substrate of medial forebrain bundle ICSS arises in the vertical limb of the nucleus of the diag-

onal band and projects down the medial forebrain bundle to the ventral tegmentum. A loop can therefore be identified running from the amygdala to the nucleus of the diagonal band of broca (from which reinforcing signals may emanate) to the ventral tegmentum, from here dopaminergic cells project back to the amygdala. In addition there is a more direct loop consisting simply of reciprocal connections between the ventral tegmentum and the amygdala.

Cells in the ventral tegmentum project to the nucleus accumbens, the first stage of Mogenson's dopamine gating system. In addition, there are dopaminergic projection to cortical areas (Thierry, Tassin, Blanc, Stinus, Scatton and Glowinski, 1977; Loughlin and Fallon, 1984) and to the septum (Moore and Bloom, 1978). These connections may be involved in aspects of mnemonic and hedonic processing. Inhibitory feedback from cortical cells may also be involved in controlling and eventually suppressing the amplifying positive feedback circuit described above, Tassin (1987) has described studies which indicate that meso-limbic dopamine systems may be controlled by feedback from meso-cortical dopamine systems. Such negative feedback is highly necessary as the amplifying circuit described above would otherwise run out of control. Damping is required when actions have resulted in reinforcement being obtained, 'reinforcement' in this context may also have to include the frustrative effects of failing to obtain the expected consequences of the original reinforcement signal.

A highly speculative model has been proposed, it may have many faults, however, it can be used as a framework for studying the rôle of dopamine in reinforcement processing in more detail. One serious problem is the necessary assumption that all connections in the positive feedback loop are functionally excitatory. Dopamine has been shown to have inhibitory actions in the neostriatum (Siggins, 1978) and the nucleus accumbens (Mogenson, Swanson and Wu, 1983). Evidence does, however, exist for excitatory actions of dopamine (Fallon, 1987; Mintz, Hammond and Feger, 1986), and a functionally excitatory rôle could result from inhibition of inhibitory interneurons within the amygdala (see Chiodo and Berger, 1986 for evidence of such interactions in the striatum).

6.4 Implications of a Drive-Reinforcement Amplifier Model.

The key features of the speculative model described above are that dopamine is involved in the amplification of response to drive-reinforcement correlations and in the concomitant mnemonic, attentional and behavioural processes. The model does not involve dopamine in reinforcement or drive signalling but is consistent with the evidence marshalled by Beninger (1983) and Mogenson (1987) indicating a reinforcer-response elicitation rôle for dopamine. In addition, it is also consistent with the evidence presented previously which indicated that dopamine must also play a rôle in processing the final impact of reinforcement. A similar rôle for dopamine in the process by which reinforcement signals are converted into final rewarding effects has been proposed by Gallistel (1986).

6.4.1 Stimulant Effects.

The relationship between the model and effects of dopaminergic stimulants such as stereotypy and behavioural switching will now be considered. It has been noted previously that direct agonists such as apomorphine and indirect stimulants such as amphetamine may differ in their effects because indirect stimulants are dependent on ongoing neural activity for their functional effects, while direct agonists act

independently of ongoing activity. In terms of the model, indirect stimulants will enhance the response to drive-reinforcer matches whereas direct agonists will induce responses to stimuli, regardless of drive state.

The behavioural consequences of stimulation of the nigro-striatal dopamine system, which is not part of the model, must also be noted. The nigro-striatal system has also been associated with responsiveness to reinforcement, however, its rôle appears to be primarily concerned with the sensory-motor integration necessary for the control and sequencing of complex voluntary movements (see e.g. Evenden and Robbins, 1984; Jaspers, Schwartz, Sontag and Cools, 1984; Sabol, Neill, Wages, Church and Justice, 1985; Spirduso, Gilliam, Schallert, Upchurch, Vaughn and Wilcox, 1985). The particular involvement of reinforcement in nigro-striatally controlled movement appears to be due to the nigro-striatal systems rôle in specifically *voluntary* movements which will, in many cases, be prompted by reinforcement signals (Evarts, Kimura, Wurtz and Hikosaka,1984). Stimulation of the nigro-striatal system may underlie the repetitive, and sometimes incomplete, behavioural sequences characteristic of stereotypy.

Stimulation of the motor output section of the model (Mogenson's dopamine gating circuit) should lead to excessive emission of behaviour, as should stimulation of separate dopaminergic nigro-striatal neurons. Behaviour responses to minimally reinforcing stimuli should therefore be exaggerated. At high levels of stimulation this exaggerated behavioural responsiveness may contribute to the manifestation of stereotypy.

Stimulation of the proposed drive-reinforcement amplifier circuit should produce enhanced behavioural and psychological responses to reinforcers at low doses. When indirect stimulants are used the emission of responses will still be dependent on the existence of response-eliciting reinforcers. The enhanced efficacy of reinforcing stimuli may lead to competition between reinforcers of approximately equal values for control of behaviour. Given the necessary subsequent inhibitory feedback, increased rates of switching behaviour between those reinforcers is predicted, in line with the results of Robbins and Watson (1981).

In situations where one reinforcer dominates, one may expect amphetamine to increase responsivity to reinforcers which match current drive states well, at the expense of weaker drive-reinforcer matches. At high doses even minimal dopaminergic activity will result in stimulation and hence responding will be directed towards a single reinforcer, eventually stereotypy may be expected. Nieto, Makhlouf and Rodriguez (1979) have found just such a pattern of response with increasing doses of amphetamine. Stimulation by direct agonists such as apomorphine is not dependent on ongoing neuronal activity, the drive- reinforcement amplifying circuit will therefore be activated regardless of the presence of reinforcers of drives. If reinforcing stimuli are present then apomorphine is likely to elicit behaviour independently of drive-reinforcer matches. This difference between the effects of direct and indirect stimulants is reflected in the results of Robbins, Watson, Gaskin and Ennis (1983) who found that amphetamine selectively increased responding to a lever paired with reinforcement over a 'valueless' lever, while apomorphine increased responding on both levers.

The model is therefore consistent with a number of important stimulant effects. Its clinical implications can also be considered.

6.4.2 Parkinson's Disease and Schizophrenia.

The motor impairments characteristic of Parkinson's disease are classically associated with degeneration of the nigro-striatal dopamine system (Ehringer and Hornykiewicz, 1960); as such, cognitive deficits arising out of failure to respond appropriately to reinforcers may not be expected. Nevertheless, evidence exists for a range of psychological symptoms associated with the Parkinsonian syndrome which may reflect the involvement of reinforcement, motivation and emotional systems, although they may also be attributed to secondary responses to the primary disease symptoms (see e.g. Dakof and Mendlesohn, 1986). Although primary cognitive symptoms may on the most part be partially attributable to the degeneration of noradrenergic cells which has also been found in post-mortem Parkinson brains (Farley and Hornykiewicz, 1976), the possibility exists that, since there is some overlap between nigral cells innervating the caudate and putamen and tegmental cells innervating the nucleus accumbens (Moore and Bloom, 1978), there may be slight dysfunction of drive-reinforcement integration. The finding that extreme motivational states can temporarily overcome Parkinsonian motor initiation impairments (Mandell, Markham, Tallman and Mandell, 1962) offers evidence for such involvement.

The case for involvement of the proposed circuit in schizophrenia is stronger. It has been shown that the postmortem brains of schizophrenics show increased levels and asymmetries of dopamine in the amygdala (Reynolds, 1983). There are also reports of increased dopamine levels in other structures (e.g. the nucleus accumbens, Hornykiewicz, 1982). Atrophy of the amygdala and other limbic structures have been found in the brains of schizophrenics who died prior to the introduction of neuroleptic treatment in 1952 (Bogerts, Meertz and Schönfeld-Bausch, 1985), indicating that Reynold's findings are unlikely to be due to the effects of drug treatment. These studies indicate that dopamine is likely to be involved in the aetiology of schizophrenia and not just in recuperative processes as Alpert and Friedhoff (1980) have suggested. It has been pointed out by Katz (1982) that reinforcement models of dopamine function such as that of Wise (1982) lead to the peculiar prediction that, given there is a hyperdopaminergic state underlying schizophrenia, 'all schizophrenics should be fat and happy'. The drive-reinforcement integration model proposed predicts that, while schizophrenics would be over-reactive to reinforcing stimuli, they would also be reactive to reinforcers which were inappropriate to current drive states and may encode inappropriate associations in memory. One may also speculate that schizophrenic thought disorders may be cognitive equivalents of effects such as Nieto, Makhlouf and Rodriguez's (1979) finding that strong drive-reinforcer matches will lead to excessively focussed behaviour and that increases in the switching of behaviour will occur when animals are confronted with a number of approximately equal values reinforcers. The drive-reinforcer integration model does not seem as incompatible with hyperdopaminergic models of schizophrenia as a simple hedonia hypothesis, nevertheless, the gap between the highly complex nature of schizophrenia and the essentially simple models discussed here is so large that detailed positive predictions from such models must be viewed with great care.

6.5 Multiple Dopamine Receptor Types.

The multiple functions of dopamine systems present problems for experimental studies using pharmacological manipulations and for use of pharmacological intervention in the treatment of diseases associated with dopamine dysfunction. The

discovery of multiple classes of dopamine receptors (Cools, 1977; Kebabian and Calne, 1979; Sokoloff, Martres and Schwartz, 1980; Stoof and Kebabian, 1981) may provide a method of pharmacologically dissociating some of these functions.

There has been some controversy over the classification and terminology for receptor types (see Seeman, 1981), more importantly, however, the anatomical distribution of receptor types (see Seeman, 1981) and the pharmacological specificity of drugs selective for receptor types is not clear. The (in vitro) D-1 specific antagonist SCH 23390 (Hytell, 1983; Iorio, Barnett, Leitz, Houser and Korduba, 1983) has subsequently been shown also to act on D-2 receptors in vitro (Plantje, Daus, Hansen and Stoof, 1984), this result may be due to the use of a slightly different classification and model of D-1 vs. D-2 sites from that used by Hyttel (1983), see e.g. Quik, Emson and Joyce (1979)) and to show behavioural actions thought typical of D-2 receptor blockade (Mailman, Schultz, Lewis, Staples, Rollema and Dehaven, 1984).

Although there are problems in the pharmacological dissociation of receptor types in vivo (i.e. for behavioural experiments), results of studies with SCH 23390 may still give an indication of functional differences between D-1 and D-2 sites as its binding to D-2 sites is considerably weaker than that to D-1 sites (Plantje, Hansen, Daus and Stoof, 1984). The results of such studies are, however, equivocal. Molloy and Waddington (1984) have shown increases in grooming behaviour with the D-1 agonist R-SK&F 38393 which could be blocked by the D-1 antagonist SCH 23390 but not by the D-2 antagonist metoclopramide. No stereotypy was induced by R-SK& F38393, however, stereotypy induced by apomorphine could be blocked by both SCH 23390 and metoclopramide. Some dopaminergic behaviours appear to be mediated by D-1 receptors (i.e. grooming), however the implications of these results for the receptor origins of stereotypy are unclear. Christensen, Arnt, Hyttel and Svendsen (1984) have also shown blockade of stereotypy by SCH 23390, in addition they found that SCH 23390 produced catalepsy and antagonised amphetamine induced circling in unilaterally 6-hydroxydopamine lesioned rats. Iorio, Barnett, Leitz, Houser and Korduba (1983) failed to induce catalepsy with SCH 23390, but did show disruptions of conditioned avoidance response similar to those typically found with 'classical' neuroleptics. Hoffman and Beninger (1985) have shown that SCH 23390 reduces locomotor activity and rearing in rats. Taken together, these results do not indicate any clear dissociation between locomotor and reinforcement effects by D-1 and D-2 manipulations, however, such differences may be revealed by studies aimed more specifically at investigating reinforcement systems. A study by Lynch and Wise (1985) of the effects of various drugs (not including the type-specific drugs discussed above) on ICSS reward summation functions failed to find any correlation between drug effectiveness in shifting summation curves and in vitro D-1 or D-2 receptor affinities, however.

There do not appear to be clear dissociations between D-1 and D-2 changes in schizophrenia. Owen, Owen, Poulter and Crow (1984) have reported increases in the number of D-2 receptors in the caudate, putamen and nucleus accumbens of schizophrenics. On the other hand, using a slightly different classification (adenylate cyclase activity rather than labelled neuroleptic binding) Memo, Kleinman and Hanbauer (1983) found increases in D-1 sites in schizophrenics. O'Boyle and Waddington (1984) have shown selective losses of D-2 but not D-1 receptors in the neostriatum of aged rats, they speculate that this may reflect the basis of some pathological aspects of senescence.

Further investigations of functional differences between dopamine receptor types are clearly warranted, however, the evidence presented above does not allow any conclusions as to the differential involvement of any receptor type in the reinforcement processing which has been the subject of this thesis.

6.6 Conclusions.

The aim of this thesis has been to investigate whether the effects of dopaminergic drugs on reinforced behaviour can be dissociated from motor effects. The series of experiments investigating drug effects on exploration showed that reinforcement effects which could not be attributed to simple motor deficits were induced by dopaminergic drugs, even using this novel class of reinforcement. Although the operant analogue of his type of exploration choice experiment run in the Y-maze failed to show any drug effect, this does not necessarily indicate a lack of dopamine involvement in the reinforcing qualities of novelty. Choice, an indicator of drug effects on discrimination of reinforcers, was not significantly affected by flupenthixol, however, no measurement was made of latency to respond or any other indicator of the behaviour eliciting potential, or resulting impact of, reinforcement. In the light of studies which indicate that the discrimination of reinforcement per se is not affected by dopamine blockade the failure to detect any effect on discrimination is not surprising.

The final series of experiments showed that dopamine did not appear to be involved in emotional processes such as frustration. The drug induced contrast effects found in the final experiment clearly showed that dopamine is involved in the processing of reinforcement independently of any motor involvement, even reinforcement-related response-elicitation. A speculative model in which dopamine plays a rôle in the integration of drive and reinforcement signals was proposed to account for these results.

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