TITLE BEHAVIOURAL MICROANALYSIS OF DOPAMINE AUTORECEPTOR FUNCTION

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# BEHAVIOURAL MICROANALYSIS OF DOPAMINE AUTORECEPTOR FUNCTION.

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ABSTRACT

MUSCAT, R. BEHAVIOURAL MICROANALYSIS OF DOPAMINE AUTORECEPTOR FUNCTION.

Low doses of DA agonists are presumed to act by stimulating DA autoreceptors on the soma/dendrites and axon terminals of DA neurons. Low doses of apomorphine reduced food intake, in a microstructural analysis paradigm, by reducing both the time spent feeding and the rate of food ingestion. The reduction of eating time was shown to result from the stimulation of DA autoreceptors located on the cell bodies and dendrites of the mesolimbic DA system. The reduction of eating rate however, appeared to result from the activation of axon terminal DA autoreceptors. The significance of this dissociation is discussed in relation to the mechanisms through which presynaptic DA receptors on the same neuron may subserve different behavioural functions.

The observation that apomorphine administration resulted in a selective manipulation of the microstructural parameters of feeding, was then used to assess the action of antidepressant drugs on DA autoreceptor function. In both normal and chronically stressed rats, chronic antidepressant treatment failed to alter the sensitivity of DA autoreceptors. However, on withdrawal, the sensitivity of cell body DA autoreceptors appeared reduced, as apomorphine no longer in any way influenced the time spent feeding in the microstructural paradigm. The implications of these findings are discusssed in relation to the hypothesis that

antidepressant drugs increase DA function by reducing the sensitivity of presynaptic DA receptors.

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# BEHAVIOURAL MICROANALYSIS OF DOPAMINE AUTORECEPTOR FUNCTION

CONTENTS

CHAPTER 1

# DOPAMINE AUTORECEPTORS

## 1.1 DOPAMINE

Dopamine (DA) was first recorded in the CNS as early as the end of the 1950's (Montagu 1957) and soon localized intraneuronally (Carlsson et al, 1962). Shortly thereafter a deficit of DA within the CNS was suggested to be the underlying cause of Parkinson's disease (Hornykiewicz 1966). However, it was not until 1971 that DA was considered as a neurotransmitter in its own right within the CNS and not just a precursor of noradrenaline (Ungerstedt 1971a). Following the observation that DA systems are well represented in the brain, the electrophysiological, biochemical, pharmacological and behavioural properties of this system were soon forthcoming. Bunney's group demonstrated that DA neurons have firing rates of between 1 and 9 spikes per second, action potentials with an initial positive segment followed by a prominent negative phase and frequently a late positive component. In addition, a number of DA cells fire in what has come to be known as characteristic bursting fashion (Bunney et al, 1973). Hornykiewicz (1973), demonstrated that the caudate nucleus and the putamen contain the highest concentration of DA within the brain, followed by the nucleus accumbens, substantia nigra, globus pallidus and the amygdaloid complex. The main metabolite of DA in these brain regions is homovanillic acid (HVA) and some 80% of DA and HVA is found within the basal ganglia. In addition, it seemed that there were two receptor

subtypes for DA, named, D1 or D2 (Kebabian and Calne 1979); the stimulation of D1 receptors results in an increase in the activity of adenylate cyclase (cAMP) while stimulation of the D2 receptor does not activate cAMP but sometimes inhibits it (Onali et al, 1981).

Carlsson (1975), also noted that a particular characteristic of DA systems was that they possessed autoregulatory receptors. These autoreceptors are to be found presynaptically on axon terminals of DA neurones and also on the soma and dendrites of the cells of origin. Somatodendritic autoreceptors regulate the firing of DA neurones whereas the terminal autoreceptors seem to be involved in the control of DA synthesis and its release. Stimulation of these receptors by DA agonists results in a suppression of both the electrical conduction along DA neurones and impulse induced release of endogenous DA (Roth 1979). In behavioural terms this leads to reduction in, for example, locomotor behaviour, which is in direct contrast to an increase in activity which observed following the stimulation of postsynaptic DA receptors by DA agonists (Costall et al, 1980).

### 1.1.1 Anatomy of DA systems

The catecholamine neural systems emanate from a series of cell bodies in the brainstem that were designated A1 to A12 by Dahlstrom and Fuxe (1964). Of these cell groups A9, A10, and A12 are dopaminergic. They give rise to the nigrostriatal,

mesolimbic, and tuberoinfundibular pathways respectively, and these systems have been re-classified to include the well established cortical DA projections (Lindvall and Bjorklund 1974, 1978). There are, additionally, dopaminergic interneurons in the brainstem, superior cervical ganglion (S.I.F. cells), retina, olfactory bulb and carotid body. It is possible that additional dopaminergic structures will be identified in the future.

The nigrostriatal DA system is now one of the most widely studied pathways in the brain. The A9 cell group which gives rise to it, is located in the zona compacta of the substantia nigra. These are large multipolar cells which sit on the dorsal aspect of the substantia nigra. It can be seen that the large cells in question have multiple identations in the nuclei and a very prominent golgi apparatus. The dendrites extend into the zona reticulata where they are surrounded by nerve endings many of which are gabaergic. The axons of these proceed rostrally in a prominent pathway that ascends in the lateral hypothalamus just dorsalateral to the median forebrain bundle. They enter the crus cerebri at the midhypothalamic level, intermingle with the myelinated fibres in the internal capsule, and then fan out through the globus pallidus to enter the caudate and putamen (Moore and Bloom 1978). The fine structure of the pathway has been further consolidated by studies of axoplasmic flow. 3[H] dopamine injected into the substantia nigra is well transported to nerve endings in the caudate

putamen (Simon et al, 1979).

The next most prominent tract is the mesolimbic pathway. These A10 cell bodies extend medially from A9, forming a cap over the interpenduncular nucleus. It has been estimated that there are some 27-29,000 cells in the VTA of the rat (Halliday and Tork 1985, 1986) of these 18,000 (>70%) stained for tyrosine hydroxlase (Swanson 1982) which for the rat is the greatest midbrain concentration of DA (German et al, 1983). The axons ascend together with the axons of the nigrostriatal dopamine system following a slightly more medial course. They do not enter the crus cerebri but continue in a rostral direction just dorsal to the medial forebrain bundle to innervate limbic structures such as the nucleus accumbens, olfactory tubercle and possibly the amygdaloid complex and the hippocampus. Further analysis of the dopamine pathway to the nucleus accumbens has established that fibres sweeping along the medial aspect of the nucleus accumbens separate into a number of branches, the most abundant of which runs dorsally and rostrally into the deep layers of the frontal cortex (mPFC). A second branch turns dorsally above the corpus callosum and moves caudally to innervate the anterior limbic cortex. Another branch innervates the septum and remaining portion give terminals to the olfactory tubercle, (Lindvall and Bjorklund 1974). Additional evidence for mesolimbic projections has come from Simon et al, (1979), using HRP (retrograde) and 3H leucine (anterograde) procedures to map the DA projections from

the VTA. They have summarized their findings as follows;

1) DA projections to regions rich in DA terminals, eg, nucleus accumbens,

2) DA projections to regions suspected of containing DA terminal regions, eg, locus coerulus, and

3) DA projections to regions not known to contain DA terminal regions, eg, supraoptic nucleus and anterodorsal thalamic nucleus.

Recently, Oades and Halliday (1987), have suggested that the efferent projections of the VTA can be divided more systematically into five subsytems;

1. The mesorhombencephalic projection to the cerebellum and the inferior olive.

2. The mesodiencephalic pathway to the thalamic and hypothalamic nuclei.

3. A small but significant mesostriatal pathway to the anterior striatum.

4. The mesolimbic projection to the nucleus accumbens, tuberculum olfactorium, lateral septum and the interstiatal stria terminalis. Mesolimbic projections also serve the amygdaloid nuclei, olfactory nuclei, entorhinal cortex and the hippocampus.

5. The mesocortical pathway projects to the prefrontal, orbitofrontal and cingulate cortices.

Finally, the tuberoinfundibular dopamine system has cell bodies A12 located within the arcuate nucleus of the hypothalamus. These cells innervate the external layer of the

median eminence.

## 1.2 BEHAVIOURAL FUNCTIONS OF DOPAMINE SYSTEMS

Despite the vast amount of literature available on the behavioural functions of the nigrostriatal and mesolimbic dopamine pathways, they have yet to be fully understood. One of a number of controversies yet to be resolved is whether the mesolimbic pathway is specifically involved in locomotor activity and the nigrostriatal system in the complex stereotyped behaviour which involves sniffing, biting, licking and head movements which predominate after large doses of DA agonists, eg amphetamine (Randrup and Munkvad 1967; Schiorring 1979). A number of studies have demonstrated that local injection of amphetamine into either the nucleus accumbens or the caudate nucleus results in increased locomotor activity by the former and stereotyped behaviour by the latter (Creese and Iversen 1974; Costall et al, 1972; Jackson et al, 1975; Makanjuola et al, 1980). Some of the motor behaviours associated with the caudate-putamen nuclei are also observed following the stimulation of the D1 receptor found in this area and typically take the form of oral and facial dyskinesias (Rosengarten et al, 1983). In addition, the behaviours elicited by the stimulation of different dopaminergic brain loci may be antagonized by the specific DA receptor antagonists, eg haloperidol,(Kelly et al, 1975; Pijnenburg et al, 1975). However, others have suggested that the wide variety of behaviours recorded are only different

aspects of the same behavioural repetoire which thus implies that different behaviours are not necessarily mediated by different dopamine systems, (Kafetzopoulos 1986; Kokkinidis and Anisman 1980, 1981).

One model sees the primary function of DA systems as controlling the rate of behavioural output, independently of the motivational determinants of behaviour, which in turn supports the view that both the nigrostriatal and mesolimbic DA systems are involved in the behavioural stimulation resulting from the administration of amphetamine (Lyons and Robbins 1975). Consequently, dopamine sytems appear to be involved with a general behavioural activation, as administration of DA agonists, eg. amphetamine, results in an increase in open-field locomotion, activity and a number of unconditioned behaviours (Kelly et al, 1980). However, as suggested above, amphetamine-induced locomotion seems to be dependant on the integrity of the mesolimbic rather than the nigrostriatal DA sytem and thus behavioural activation may be independant of the type of motor arousal underlying stereotypy (Kelley and Stinus 1984).

In recent years considerable attention has been focussed on the question of whether the mesolimbic DA system constitutes a "reward pathway". Animals will self-administer DA receptor agonists but not agonists that have been shown to interact with other neurotransmitter receptor populations if trained to press a lever in the process (ICSS). In particular the D2 and the D1

receptor subtype seem to be involved as administration of sulpiride, a specific D2 receptor anatagonist and SCH23390, the specific D1 receptor antagonist, increased lever pressing in the ICSS paradigm (Nakajima and Mackenzie 1986; Woolverton 1986).

Wise and colleagues first noted that animals on neuroleptics show extinction like behaviour when placed in a self stimulation situation (Fouriezos and Wise 1976), or while responding for food reinforcement (Wise et al, 1978a,b). Gallistel et al, (1982), were also able to demonstrate that this effect of neuroleptics was not due to the motor debilitating effects of the drug as changing the environment resulted in a reinstatement of responding to baseline levels. Beninger and Freedman (1982), also using a two-task procedure observed extinction like behaviour with lever pressing following pimozide but no such effect in the motorically demanding wheel-runing paradigm. These findings argue against the notion that dopamine receptor blockade results in a severe motoric handicap and support the idea that DA systems are primarily involved in the rewarding aspects of behaviour supported by positive reinforcement (Wise 1982).

However, the assumption that neuroleptic pretreated animals behave as if they were undergoing extinction has not been validated experimentally. If anything the studies reported to date demonstrate quite the reverse of what is predicted from such a hypothesis. Animals on neuroleptics appear to mimic the behaviour of animals undergoing extinction as both show

reductions in response rates. However, transfering from neuroleptic pretreatment to extinction produces an increase in response rates (Beninger 1982; Mason et al, 1980; Tombaugh et al, 1980). These observations would suggest that DA sytems are not directly involved in the reward processes associated with positive reinforcement and thus do not support the anhedonia hypothesis. In addition, DA antagonist treatment and extinction do not seem to be functionally equivalent processes and therefore such comparisons should not be used as evidence to implicate DA systems in reward. As a result of such findings it has been suggested that the mesolimbic DA system is in fact responsible for the activating effects rather than the hedonic impact of positive reinforcement (Mogenson 1982; Salamone 1986).

DA systems however, are also implicated in feeding and drinking behaviour making studies using food and water reward difficult to interpret. However, the specificity of the DA systems in feeding behaviour have also been called into question. Leibowitz (1978), has argued that DA systems in the perifornical area of the hypothalamus are responsible for a satiety response, in that administration of DA to the PFH resulted in a dose dependent suppression of food intake. Indeed, a number of neuroleptics including pimozide totally attenuated the reduction in food intake caused by DA and amphetamine. However, systemic administration of DA antagonists also resulted in a reduction of food consumption. This effect has been interpreted as a non-specific action of DA antagonists, as

destruction of the nigrostriatal pathway following 6-OHDA administration also results in aphagia (Ungerstedt 1971b). Based on these findings it appears that the nigrostriatal DA pathway may mediate the arousal and motor component of feeding (Stricker and Zigmond 1976), whereas the hypothalamic DA mechanisms provide direct stimuli for terminating this behaviour. The DA receptor subtype within the perifornical hypothalamus appears to be of the D2 subtype which inhibits AC activity; the D1 agonist SKF 82526, did not in any way alter the effects of D2 agonists on AC activity (Carruba et al, 1985). This system of dopaminergic fibres also appears to be devoid of autoreceptors (Carruba et al, 1985). In contrast to the effects of DA agonists on feeding behaviour, intracerebral administration of dopamine increases water consumption (Poat et al, 1980; Setler 1973). It is also well established that dopamine receptor antagonists reduce the consumption of water (Block and Fischer 1975; Grupp 1976; Rowland and Engle 1977; Sumners et al, 1979). These data suggest some involvement of central DA mechanisms in the control of drinking responses but appear to be well less defined than the DA system involved in feeding behaviour.

The problem with DA mediated behaviours seems to be one of interpretation. Neuroleptic drugs suppress rewarded behaviour. However, the majority of paradigms used to study these effects require an active output of behaviour, and activating, hedonic, and rate effects become difficult to separate. However, a number of paradigms have now become available in which it is possible

to look at the contributions of the DA systems in reward. One paradigm described by Herrnstein (1961, 1971) exploits the matching law, in which the strength of the response is directly related to the reward received. In this case animals press a lever for food or water reinforcement, while in the paradigm used by Gallistel, animals press a lever for electrical stimulation. The number of responses can be plotted against the total number of reinforcements obtained in each paradigm and the curve obtained is sigmoid in shape. The motor parameter in the equation essentially sets the asymptote of the curve while the reinforcer efficacy is that value which maintains half maximal responding. The indirectly acting DA receptor agonist, amphetamine, increased the reinforcing value of the food reward and elelctrical stimulation. Pimozide, however, in addition to decreasing reinforcement efficacy also reduced motor capacity in a dose dependent manner (Gallistel et al, 1982; Gallistel and Karras 1984; Heyman 1983; Willner et al, 1987a). In the place location paradigm, which consisted of a box whose floor consisted of three rectangular wire mesh panels, haloperidol reduced motor activity compared to controls but not the time spent on the panel which contained the food dish (Salamone 1986). The minimal motor requirements demanded by such a task would first of all suggest that DA antagonists do not impair all reinforced behaviours and secondly, DA antagonists do not blunt the hedonic impact of food reward. However, in a two-bottle test in which animals had equal access to a bottle containing sucrose and one holding water, DA receptor anatgonists

reduced the consumption of sucrose but increased that of water (Towell et al, 1987a). It may be said that the motoric requirements of this paradigm are also minimal and reducing DA function resulted in a reduction in the rewarding properties of the sucrose solution. In that DA antagonists cause adipsia, the increase in water consumption cannot be said to result from a direct involvement of the physiological mechanisms involved in drinking (see above).

From a theoretical standpoint, it may be impossible to separate out the motoric and rewarding components of goal directed behaviour and the function of DA systems within this framework, as DA systems seem to be involved in incentive related motor activity. Thus treatments that reduce DA activity also interfere with performance in schedule induced or reinforcer induced behaviours (Keehn et al, 1976; Wallace et al, 1983). A general statement of the functional significance of DA pathways within the brain may simply be that they modulate motor output in relation to stimulus-related activation (Salamone 1986). Animals receiving neuroleptic pretreatment show a progressive deterioration of responding on a number of schedules (Salamone 1986; Willner et al, 1987a) but return to base-line responding when treatment has ceased (Fibiger et al, 1976).

## 1.3 PRESYNAPTIC DA RECEPTORS

In contrast to the locomotor stimulant effects of DA agonists at high doses, the most notable and most reported finding in the behavioural literature following the administration of low doses of DA agonists is the suppression of locomotion in rats and mice (Costall et al, 1981; Di Chiara et al, 1976; Strombom 1976). This effect is believed to arise from the stimulation of presynaptic DA receptors which results in the inhibition of DA synthesis and release (Farnebo and Hamberger 1971; Starke et al, 1978). However, a number of problems have arisen with regard to the role of DA autoreceptors in the reduction of locomotor behaviour and DA synthesis. Apomorphine and a number of other DA agonists also interact with noradrenergic receptor systems and it is recognised that sedation may also result from the stimulation of alpha 2 receptors. Indeed, one study reported that some DA antagonists were effective in reversing apomorphine-induced sedation while others were not (Sumners et al, 1981) and in another study, DA antagonists were totally ineffective (Costall et al, 1980). In addition to the problems associated with the findings that DA agonists may have effects on other neurochemical systems is the question of whether DA autoreceptors are actually present on the cell bodies and terminal regions of DA systems.

Opposition to the very presence of this receptor subtype has come from Laduron (1981), who has put forward the theory of "Partial Occupancy" to explain the biphasic action of apomorphine

and neuroleptic drugs. If a high dose of an exogenous substance is applied the occupation will be relatively homogenous so that all receptors will be reached by the drug. In contrast, at lower doses the disposition of the drug should occur randomly and this will result in only a few receptors occupied by the drug, ie. a partial occupation of the receptors. Low doses of a neuroleptic - would lead to a partial occupation of postsynaptic DA receptors which would result in an slight increase in DA synthesis through a positive feedback loop. As the great majority of the DA receptors are not blocked, the elevation of DA release will elicit hypermotility. Moreover, it is possible that such a concept could similarly explain the sedative effects observed with low doses of apomorphine. However, it is difficult to reconcile this hypothesis with the biochemical findings (see below). Also, kainic acid lesions of the neostriatum, which destroy the feedback pathways, fail to inhibit the effects of low doses of DA agonists on the synthesis of DA (Bannon et al, 1980).

A second alternative, the "reuptake" hypothesis has been put forward to explain the inhibition of DA synthesis by DA agonists and the biphasic effects of these drugs. It has been proposed that under normal conditions DA synthesis is partly inhibited by the transmitter newly taken up following the release into the synaptic cleft. Thus if the dopamine concentration in this area increases the amount of the transmitter uptake will also increase with the result that DA

synthesis will be more inhibited. On the contrary, if DA in the synapse decreases, the amine uptake will be proportionally reduced with the consequent disinhibition of DA sysnthesis (Cerrito et al, 1981). In addition, it has also been reported that DA agonists are highly hydrophobic and thus readily cross the presynaptic membrane to inhibit the enzyme machinery (tyrosine hydroxylase) responsible for the synthesis of dopamine. However, in synaptosomal preparations apomorphine inhibits DA synthesis at concentrations substantially lower than those needed to inhibit soluble tyrosine hydroxylase (Bitran and Bustos 1982). Furthermore, addition of the DA uptake inhibitors cocaine or benztropine to the preparation failed to inhibit apomorphine-induced reduction of DA synthesis. Demarest (1983), reported that amphetamine did reverse the reduction in dopamine synthesis by apomorphine in the striatum and the nucleus accumbens. This finding could be taken to support the reuptake hypothesis. However, it is also possible that amphetamine reduced the intracellular concentration of DA with the result that DA synthesis was increased by a reduction in end product inhibition.

Electrophysiological evidence unequivocally supports the findings that DA autoreceptors are present on the soma and dendrites of DA neurones. Microiontophoretic application of DA agonists to the cells of origin of both the nigrostriatal and mesolimbic pathways results in a cessation of impulse flow along these neurones (Groves et al, 1975; White and Wang 1984). In addition, amphetamine, applied systemically, inhibits cell

firing in the striatum as well as in the substantia nigra whereas haloperidol enhances cell firing in both these areas (Groves et al, 1975). Carlsson and Lindqvist (1963), put forward a neuronal feedback loop hypothesis to explain these systemic effects. However in the nigrostriatal system, while ablation of the feedback pathways with kainic acid did change the pattern of firing of nigral cells, this could not be explained simply by the removal of the inhibitory GABA input on to these cells (Doudet et al, 1984). In addition, the finding that nigral cells are sensitive to DA and are inhibited by DA antagonists (Bunney and Aghajanian 1973; Grace and Bunney 1983) demonstrates that these cells clearly possess dopamine receptors, which apparently are some 10 to 100 times more sensitive to DA agonists than are postsynaptic DA receptors (Skirboll et al, 1979).

The presence of DA autoreceptors has also been established on dendritic projections of nigral DA neurones. Geffen (1976), using slices of rat nigra from the zona reticulata, in which cell bodies are absent, demonstrated that DA was released into the preparation by raising the external potassium concentration. Cheramy (1981), reported that the release of DA from dendrites was involved in the self-regulation of DA cells as well as in the release of neurotransmitters in nigral afferents fibres and the regulation of the activity of non dopaminergic cells.

Autoreceptors on the cell bodies and dendrites of the mesolimbic DA system have similarly been reported to respond to DA agonists, which inhibit impulse flow in these neurones, whereas agonists of other classes exert weak or no effects at all (White and Wang 1984). The presynaptic DA receptors in the mesolimbic pathway also appear to be more sensitive to DA agonists than autoreceptors located in the substantia nigra, perhaps because the mesolimbic DA system seems to lack the well defined long loop feedback pathways found in the nigrostriatal DA system (Walker 1986). In addition, neurons projecting from the VTA to the prefrontal or anterior cingulate cortex have faster spontaneous firing rates than DA projections to the caudate and the accumbens. These neurons are insensitive to both rate and synthesis suppressant effects of DA agonists which would imply that these projections lack autoregulatory mechanisms on their soma or terminal regions (White and Wang 1984).

## 1.4 PHARMACOLOGY OF DA RECEPTORS

The classification of subpopulations of DA receptors has been the subject of a number of controversies. Two subtypes of DA receptors were initially defined by the observation that one population were coupled to adenylate cyclase while the second were not (Kebabian 1978; Kebabian et al, 1972). These two receptor subtypes were first refered to as alpha and beta (Kebabian 1978), but in order to avoid confusion between these receptor subtypes and those found on noradrenergic

terminals Kebabian and Calne (1979), suggested the D1 and D2 terminology. The occurrence of two receptor subtypes for DA has also been inferred from behavioural and the electrophysiological evidence, except that these receptors have been labeled excitatory or inhibitory ie DAe and DAi (Cools and van Rossum 1976; Cools et al, 1976; Cools and van Rossum 1980). It would appear that postsynaptic D1 receptors defined by Kebabian and Calne correspond to DAi, as application of D1 receptor agonists results in hyperpolarization, whereas stimulation of postsynaptic D2 receptors results in depolarization and consequently correspond to DAe (Grace and Bunney 1983; Herling 1981; Herling and Hull 1980). Costall and Naylor (1981), referred to these two subtypes of DA receptors as DA1 and DA2, but subsequently this terminology has been used to differentiate the peripheral DA receptor subtypes from those occuring centrally (Goldberg and Kohli 1983). Careful analysis of agonist/antagonist actions of these peripheral receptors has shown that R-sulpiride potently antagonizes the DA1 mediated renal vasodilation, whereas it is ineffective in blocking the D1 mediated DA stimulation of adenylate cyclase (Spano et al, 1979) while opposite effects have been reported for a number of ergot derivatives (Kebabian et al, 1978). However, with the emergence of very specific agonist and antagonist drugs of the two receptor subtypes it is possible that the peripheral and central receptor subtypes will eventually be shown to be identical (Goldberg et al, 1985).

This classification of DA receptors has been a subject of intense debate since Laduron (1981), proposed that there is only a single type of DA receptor while Seeman (1980), suggested four subtypes. At the moment the general viewpoint is that there are two subpopulations of DA receptors, D1 and D2; one possible reason for the proliferation of subtypes is the different conditions under which receptor binding studies are carried out in various laboratories (Iversen 1983). In addition, a number of models have been put forward that assume that receptors can exist in at least two conformations. The Monod model suggests that the transition from one state to the next depends on binding with the ligand in a concerted process whereas Kohland suggests the involvement of a sequential process (Kaiser and Jain 1985). The four subtypes according to these models could therefore represent high and low affinity states of D1 and D2 receptors (see Chapter 5).

Presynaptic DA receptors present on the soma and dendrites of the mesolimbic and nigrostiatal systems appear to resemble the D2 subtype in many respects. When applied iontophoretically to the zona compacta, DA and a number of DA agonists including apomorphine cause dose related suppression of the firing of these cells. The effects of these agonists is reversed by the selective D2 receptor antagonist sulpiride and zetidoline but not by SCH-23390, the specific D1 receptor antagonist (Freedman and Woodruff 1986). White and Wang (1984), reported that DA was the most effective exogenous agonist to inhibit the firing of these DA

cells and the specific D2 receptor antagonist, sulpiride, completely blocked the rate suppressant effects of DA and DA agonists (but not those of GABA). Similar observations have been made when exogenous DA agonists have been applied to dendrites in the zona reticulata. Again, the suppression of firing rates was reversed by antagonists of the D2 subtype but not by those reported to have D1 receptor affinity (Gessa and Mereu 1984). It has also been demonstrated that D1 receptor antagonists fail to block the responses mediated by eg. apomorphine, (emesis ,the inhibition of prolactin release and hypothermia) that are dependent on the integrity of postsynaptic D2 receptors (Iorio et al, 1983). The behavioural evidence though scant, also supports the suggestion that presynaptic DA receptors are of the D2 subtype (see Chapter 5) in that sulpiride but not SCH-23390 reversed apomorphine's inhibitory effect on locomotion (Cuomo et al, 1986). Finally, the pharamacological evidence would seem to support the hypothesis that both cell body and axon terminal DA autoreceptors are of the D2 receptor subtype as both have rather higher affinities for D2 agonists and antagonists. In terms of the behavioural function of these receptors there is no evidence (prior to the present study) to suggest a separate function for each receptor: stimulation of either cell body or axon terminal autoreceptors by DA agonists results in sedation (Costall et al, 1981).

### 1.4.1 Biochemical aspects of the stimulation of DA autoreceptors

The rate limiting step in the biosynthesis of DA is the conversion of tyrosine by tyrosine hydroxylase to L-DOPA (Blaschko 1957); following the stimulation of terminal autoreceptors it has been observed that tyrosine hyroxlase activity is reduced (Nowycky and Roth 1978). In contrast, electrical stimulation of the nigrostriatal pathway leads to an increase in the synthesis of DA following a change in the kinetic properties of tyrosine hydroxylase. Stimulation results in an increase in the enzyme affinity for the pteridine co-factor and substrate and a decrease in the affinity for DA (Murrin et al, 1976). In addition, Murrin and Roth (1987), have reported that maximal stimulation of the nigro-neostriatal fibres results in an increased synthesis of DA, which in turn could be reduced by DA agonists that interact with axon terminal autoreceptors. The abolition of neuronal firing by hemisection or treatment with Gamma-butyrolactone (GBL) also results in an increase in the synthesis of DA, as measured by the accumulation of its precursor DOPA (Carlsson 1975; Nowycky and Roth 1978; Roth et al, 1983). Again, in this model, DA agonists were found to depress synthesis whereas DA antagonists reversed this effect.

However, the role of DA autoreceptors in the release of DA has been questioned since it has been observed that changes in the release and synthesis of DA do not follow the same time course (Westfall et al, 1976). In addition, the initial

experiments (Farnebo and Hamberger 1971), in which it was demonstrated that neuroleptics increased the stimulation-induced release of DA while apomorphine had the opposite effects, have subsequently not been confirmed (Raiteri et al, 1978). The use of in vivo techniques could have caused this ambiguity as electrically invoked DA release may be reduced by a number of manipulations such as the stimulation of postsynaptic DA receptors and cell body autoreceptors in addition to the stimulation of axon terminal autoreceptors. In rat caudate slices, electrically evoked release of DA was inhibited by DA agonists while DA antagonists increased the release of DA (Arbilla et al, 1982a). It is of interest that spontaneous release of DA was not affected by apomorphine, haloperidol or calcium free Ringer; however, the electrically evoked release of DA was affected by these compounds (Starke et al, 1978). It would appear that the calcium dependent release of DA following electrical stimulation is modulated by presynaptic DA receptors but calcium independent release elicited by amphetamine is not (Arbilla et al, 1982b; Kamal et al, 1981). Similarly, in rat striatal slices it has been shown that apomorphine fails to inhibit spontaneous release of DA but it did block the formation of labelled DA (Bitran and Bustos 1982).

Roth et al, (1983), have put forward a tentative model in which it is envisaged that stimulation of DA autoreceptors by apomorphine reduces the influx of calcium leading to a reduction in DA release along with protein carboxymethylation of

calmodulin. The methylated calmodulin reduces the affinity of tyrosine hydroxylase for pteridine and tyrosine and increases its affinity for DA; as a consequence, DA synthesis is inhibited. Destruction of dopamine neurons by intraventricular 6-hydroxydopamine (6-OHDA) inhibits apomorphine effects on carboxymethlation (Roth et al, 1983) as well as haloperidol induced release of DA (Blaha and Lane 1984; Forni and Nieoullon 1984); however, in the lesioned rat amphetamine enhanced DA release. The evidence to date would suggest that axon terminal autoreceptors are indeed involved in the synthesis and calcium dependent release of DA though the precise mechanisms through which these molecular events are brought about still await resolution.

### 1.4.2 Behavioural consequences of DA autoreceptor stimulation

The consistent behavioural observation following the administration of low doses of DA agonists is hypokinesia (Costall et al, 1980; Di Chiara et al, 1976; Strombom 1976) which is also apparent following blockade of dopaminergic transmission with neuroleptic drugs (Beninger 1983). The former is assumed to reflect a selective stimulation of presynaptic DA receptors while the latter is assumed to occur following the blockade of postsynaptic DA receptors. However, with increasing doses of DA agonists stimulation of postsynaptic receptors also occurs resulting in locomotor and stereotyped behaviour (Kelly et al, 1975). The effects of autoreceptor stimulation by low doses of apomorphine are

blocked by D2 but not D1 receptor antagonists (Cuomo et al, 1986; Stahle and Ungerstedt 1986) but the stimulatory effects of this drug are reversed by non-specific DA receptor antagonists (Serra et al, 1983).

Apomorphine and 3-PPP have been used in the clinic as potential antipsychotic agents to some good effect (Tamminga et al, 1978; 1983). The predominant feature arising from the use of such compounds was the reduction in extrapyramidal side effects, relative to classical antipsychotic agents, and the lack of effect on serum prolactin levels (Brown and Campbell 1984). Neuroleptic administration in animals typically causes catalepsy as well as a suppression of conditioned avoidance responding (CAR). It has been pointed out that these effects of neuroleptic drugs correlate well with their clinical potency (Baldessarini 1980) and are dependent on the integrity of the nigrostriatal DA system (Carlsson 1978). The effects can be attenuated by pretreatment with anticholinergic agents (Baldessarini 1980; Carlsson 1978). The increase in striatal DA turnover following haloperidol administration is also blocked by pretreating with scopolamine (Anden 1972). However, very low doses of DA agonists, unlike neuroleptics, do not inhibit CAR, which could be related to the minimal effects of these agents on extrapyramidal motor functions (Ahlenius and Hillegaart 1986).

Interpretation of the effects of low doses of DA agonists on positively reinforced operant tasks is not entirely

straightforward. Apomorphine reduces responding in a CRF schedule by 30% at a dose of 60ug. However at 30ug no such effects are detected in the CRF paradigm but deficits are obtained in fixed interval schedules. In addition, apomorphine showed enhanced reductions in elevated fixed ratio schedules which in turn is concordant with the anhedonia hypothesis based on the law of effect (see above). That these effects can be explained by the simple notion that DA agonists reduce locomotor activity in rats is refuted by the observation that apomorphine spared renewal of responding caused by food delivery but abolished responding reinstated by secondary reinforcement (Carnoy et al, 1986). Low doses of DA agonists have also been reported to impair responses to novelty (Misslin et al, 1984). These findings would imply that low doses of DA agonists reduce or blunt the impact of incentive stimuli leaving primary reinforcement intact. Such a view would seem to be consistent with the position that DA systems are involved in the activating effects of rewarded behaviour ie, incentive motivation (Bindra 1974, 1978), rather than with reward per se.

Increases in yawning behaviour in rats has been observed following the administration of low doses of apomorphine (Gower et al, 1984; Stahle and Ungerstedt 1984). However, the location of the DA system involved in the yawning response has been questioned, as has the receptor subtype, as SCH-23390 attenuated the yawning response evoked by apomorphine in both control and reserpine pretreated rats (Morelli et al, 1986).

It has been suggested that DA systems may be implicated in the etiology and expression of anxiety (Taylor et al, 1982) since low doses of apomorphine seem to be beneficial in the management of certain states of anxiety associated with psychotic conditions seen in schizophrenics. However, low doses of neuroleptics have also been reported to relieve anxiety related symptoms in neurotic (Finnerty et al, 1976) and chronically anxious patients (Rogerson and Butler 1971). The animal literature also fails to distinguish between DA agonists and antagonists: both low doses of apomorphine and neuroleptics produce anxiolytic effects in the conflict paradigm (Hjorth et al, 1986; Merlo Pich and Samanin 1986). However, once again the exact identity of neuronanatomical locus through which these agents exert their anxiolytic like actions is lacking but the mechanism of action underlying neuroleptic-induced increases in punished responding does appear to involve central serotonin neurons (Leysen et al, 1978).

## 1.5 DA AND DEPRESSION

The catecholamine hypothesis of depression (Schildkraut 1965), suggested a reduction in the levels of the neurotransmitter noradrenaline rather than adrenaline or dopamine, in critical areas within the central nervous system (CNS). This hypothesis proved to be very attractive in that it could explain a number of pharmacological phenomena that result following the manipulation of noradrenergic systems. Reserpine which depletes noradrenergic terminals of NA, was used in the

treatment of hypertension also induced severe depression in a number of patients. In contrast, monoamine oxidase inhibitors used to treat tuberculosis, increase levels of NA and were found to produce an elevation in mood (Loomer et al, 1957). In addition, amphetamine which enhances the release of NA from presynaptic terminals, was also effective in elevating mood in normal volunteers (Lasagna et al, 1955). The finding that the levels of the major metabolite of NA, 3-methoxy-4-hydroxyphenyglycol (MHPG) were low in depressives (Mass 1968), further supported the hypothesis of a reduced NA function in depression.

However, a number of anomalies began to appear with respect to drugs that supposedly reduce NA function and thus in theory should exacerbate depression. Alpha-methyl-para-tyrosine (AMPT) inhibits the conversion of tyrosine to dihydroxyphenylalanine (DOPA) and thus reduces NA levels, did not cause depression (Mendels and Frazier 1974). In addition, Goodwin (1972), noted that in patients reported to be suffering from depression following reserpine pretreatment were actually not depressed but were sedate and lethargic. In contrast, amphetamine which increases NA has not been shown to be an effective antidepressant. However, little mention was made of the fact that;

1. the evidence for NA also inadvertantly supports a reduction in DA levels,
2. depression may be biochemically heterogenous.

The dopamine hypothesis of depression became prevalent following the observation by Randrup in 1975, that in some depressed patients HVA levels were abnormally low. HVA is the major metabolite of DA and its presence in cerebrospinal fluid (CSF) is thought to mainly originate from DA turnover in the CNS. The majority of studies to date have reported a reduction in the concentration of HVA in depressed patients (Banki 1977; Mendels et al, 1972; Papeschi and McClure 1971; Bowers et al, 1969) but a number of studies have found no difference in HVA levels in depressives and normal controls (Berger et al, 1980; Subramanyam 1975). The latter inconsistency may have resulted from the transport of HVA out of the CSF. This problem may be remedied by administering probenecid, which blocks the removal of acid metabolites out of the CSF; studies using this technique have all reported a decreased HVA accumulation in some or all depressed patients (Bowers 1972; van Praag et al, 1973; Sjostrom 1973). Low HVA levels in the CSF following probenecid pretreatment in patients with marked psychomotor retardation, is probably the most firmly established finding in the biochemistry of depression (Willner 1985).

### 1.6 DA AND ANTIDEPRESSANTS

For a number of years the possibility that antidepressants increase DA function was neglected, as it was believed that tricyclic antidepressants act by inhibiting the uptake of NA and 5-HT but not of DA (Carlsson 1970). This belief came to be questioned following the observation that antidepressants are

sometimes effective in the treatment of Parkinsons disease (Randrup et al, 1975), which results from a depletion of nigrostriatal DA (Hornykiewicz 1966). Subsequently it was demonstrated that antidepressants do in fact inhibit DA uptake albeit weakly and this effect was also demonstrated by some antidepressant drugs that do not block neuronal uptake of NA or 5-HT (Hytell 1978).

Antidepressant effects are usually seen following two to three weeks of treament so the acute effects of these drugs (which now appear to include a weak inhibition of DA uptake) may be ruled out as the mechanism of clinical action. A number of studies have reported that antidepressants may increase DA function by increasing the synthesis of DA (Leonard and Kafoe 1976; Sugrue 1980). However, only mianserin slightly increased DA content, while a number of antidepressants and ECS did not have any effect on DA levels (Evans et al, 1976; Sugrue 1980). In addition, turnover of DA was unaffected by chronic treatment with a number of antidepressant drugs (Sugrue 1980).

However, a considerable literature demonstrates an increase in the responsiveness of postsynaptic DA receptors following chronic antidepressant treatment. Chronic treatment with tricyclic antidepressants and the atypical antidepressants mianserin and iprindole produced an enhanced response to apomorphine and amphetamine-induced locomotor behaviour (Serra et al, 1979; Spyraki and Fibiger 1981; Maj et al, 1984a,b; Martin-Iverson et al, 1983; Willner and Montgomery

1981). These responses are believed to result from the stimulation of mesolimbic postsynaptic DA receptors (Moore and Kelly 1978), the results suggest that chronic antidepressant treatment either increased the affinity or the number of these receptors (see below). In addition, some studies have reported that repeated antidepressant treatment also caused an increase in the responsiveness of postsynaptic DA receptors in the nigrostriatal DA system, as amphetamine and apomorphine stereotypy were found to be increased following chronic treatment with a variety of antidepressant drugs (Willner and Montgomery 1981; Willner et al, 1984). However, these findings are less well supported as a number of studies have failed to demonstrate any enhancement of the stimulant-induced stereotyped behaviour with chronic antidepressant pretreatment (Delina-Stula and Vassout 1979; Maj et al, 1979; Spyraki and Fibiger 1981).

The biochemical evidence however, does not support an increased responsiveness of postsynaptic DA receptors. A number of studies have failed to show any increase in DA receptor binding in the striatum or for that matter, in the accumbens, with the specific ligand 3H haloperidol following chronic antidepressant treatment (Martin-Iverson et al, 1983; Tang and Seeman 1980; Tang et al, 1981; Willner 1983). Subsequently, it has been suggested that the sensitization of DA receptors may depend on the anticholinergic properites of antidepressant drugs in that antidepressants with negligible affinity for the cholinergic receptor subtype did not potentiate DA agonist

induced behaviours (Martin-Iverson et al, 1983). This promising hypothesis has now lost momentum as in three other studies the antidepressants used in the above study did potentiate DA-dependent behaviours (Arnt et al, 1984; Maj et al, 1984a,b). However, as results from receptor binding studies seem to vary from one laboratory to another it may be premature to rule out an increased sensitivity of postsynaptic DA receptors following chronic treatment with antidepressant drugs on this evidence alone. The behavioural evidence would also seem to support this view as sulpiride, an atypical neuroleptic, reversed the antidepressant effect on immobility in the Porsolt test in rats (Borsini et al, 1984; Pulvirenti and Samanin 1986). Central injection procedures would also suggest that the nucleus accumbens is involved in this effect as intracranial administration of sulpiride to this area resulted in similar findings (Cervo and Samanin 1987)..

### 1.6.1 Antidepressants and presynaptic DA receptors

While an increased response to DA agonists following chronic antidepressant treatment suggests a postsynaptic site of action, these data are also open to an alternative explanation: a reduction in the sensitivity of presynaptic DA receptors. The toning down of the inhibitory effects of autoreceptor stimulation by endogenous DA would in effect increase DA synthesis and release. Following acute administration of the antipsychotic drug molindone, increases in DOPAC have been reported while low doses of apomorphine were ineffective in reducing these levels (Conway

and Uretsky 1983). In addition, the doses of molindone used in this study correlate well with those that enhanced amphetamine-induced stereotypy.

Serra et al, (1977), reported that following chronic but not acute treatment with imipramine, amitriptyline and mianserin the sedative effects of a low dose of apomorphine were abolished. Subsequently, this effect was confirmed using a variety of antidepressant agents which included nomifensine ( a specific DA uptake inhibitor), doxepin (Zebrowska-Lupina and Kozyrska 1980), MAO inhibitors (Chiodo and Antleman 1982) and ECS (Serra et al, 1981). The electrophysiological evidence also seemed to support a reduction in the sensitivity of presynaptic DA receptors following chronic antidepressant treatment, as the reduction in DA cell firing following apomorphine was found to be attenuated (Chiodo and Antelman 1980). The biochemical evidence however, was equivocal. An increase in DA synthesis which should result from a reduction in the sensitivity of DA autoreceptors was observed by Serra et al, (1979), and Holcomb et al, (1982), following chronic treatment with antidepressant drugs. However, no increase in DA synthesis was observed following ECS, which did block the sedative effect of apomorphine (Farber et al, 1983).

In addition, Holcomb et al, (1982), using the GBL model to assess the inhibition of DA synthesis by apomorphine following the cessation of impulse flow, reported that chronic treatment with a typical or an atypical antidepressant did not change the response to apomorphine. A number of studies have also failed to

observe any changes in the sedative effect of apomorphine after chronic treatment with antidepressant drugs or ECS (Ehlers et al, 1983; Spyraki and Fibiger 1981). The finding that antidepressants increase DA cell firing has also been found wanting in that two studies found no change in the apomorphine-induced inhibition of DA cell firing following chronic antidepressant pretreatment (MacNeill and Gower 1982; Welch et al, 1982). It is difficult to draw any definite conclusions from these studies in relation to the effects of antidepressant drugs on DA autoreceptor sensitivity.

The problem of assessing the role of DA autoreceptor sensitivity in the apparent potentiation of dopaminergic function by antidepressants is compounded to some extent by methodological limitations. The major technique used to identify changes in DA function is that of a potentiation of DA agonist induced behaviours, which may result either from a reduction in the sensitivity of presynaptic DA receptors or from an increase in the sensitivity of postsynaptic DA receptors. This methodology therefore cannot determine with certainty the site of action through which antidepressants may exert their effects on DA systems. In earlier experiments in this laboratory (Towell 1984), presynaptic DA receptors were assayed by the effects of low doses of apomorphine on food intake in rats. As will be demonstrated below (Chapter 5), apomorphine anorexia provides a methodology which does differentiate between subsensitive presynaptic DA receptors and supersensitive postsynaptic DA receptors.

Like earlier studies of DA autoreceptors, the initial experiments on the effects of chronic antidepressant treatment on apomorphine anorexia also proved inconclusive. In the first experiment, the evidence for DA autoreceptor subsensitivity during chronic DMI treatment was at the best equivocal; in a second experiment, in which apomorphine was applied centrally, there was no evidence for this effect. Nevertheless, in both experiments, a reduction in DA autoreceptor sensitivity was apparent following the withdrawal from chronic treatment with the tricyclic antidepressant DMI (Towell 1984). In view of the potential clinical importance of antidepressant effects on DA systems and the advantage of this particular technique for studying these interactions (Chapter 2), the present experiments were carried out in order;

1. To establish the mechanisms of apomorphine anorexia.
2. To reevaluate the effect of antidepressants on DA autoreceptor function.

CHAPTER 2

## APOMORPHINE ANOREXIA

As noted in Chapter 1, low doses of DA agonists, such as apomorphine, stimulate presynaptic DA receptors (Carlsson 1975), and inhibit the synthesis and release of DA (Hjorth et al, 1982), which results in behavioural sedation. High doses of apomorphine, however, stimulate behaviour, by an interaction with postsynaptic DA receptors in the nucleus accumbens and striatum, which produces locomotor activation and stereotyped behaviour (Makanjoula et al, 1982; Kelly et al, 1975). The pharmacological evidence related to the behavioural effects of low doses of apomorphine is somewhat controversial. While some studies have found that apomorphine-induced sedation was blocked by neuroleptic drugs (Di Chiara et al, 1976; Montanaro et al, 1982), others have failed to antagonize apomorphine induced sedation with neuroleptics (Costall et al,1980) or have reported that some neuroleptics were effective but others were not (Sumner et al, 1981). These data in turn would suggest that the pharmacological and behavioural effects of a sedative dose of apomorphine may be heterogenous. However, all these studies used locomotor activity to monitor apomorphine induced sedation and in this paradigm it is very difficult to fractionate motor activity into meaningful and reliable behavioural components. Workers in this laboratory therefore decided to examine the sedative effects of apomorphine in a different paradigm that does lend itself to behavioural micronanalysis.

## 2.1 MICROSTRUCTURAL ANALYSIS OF FEEDING BEHAVIOUR

Both food deprived and non-deprived animals eat in discrete meals (Richter 1927), and it is possible to analyse feeding behaviour by measuring the number of meals taken, meal size, meal duration, inter-meal intervals and the average rate of eating during meals. Within meals, short bouts of eating are separated by episodes of other behaviours such as rearing and ambulation (Blundell 1981). So in addition, certain intra-meal parameters can be assessed, such as the number, size and duration of eating bouts. The relationship between bouts of eating and non-eating activities can also be assessed. Thus a drug may reduce food intake by either affecting the physiological mechanisms involved with feeding or by enhancing non-nutrional behaviours between eating bouts and disrupting feeding behaviour during a meal. The intrameal parameters are often called microstructural parameters of eating.

Using this methodology, Blundell and Latham (1980), reported that the anorectic agents, amphetamine and fenfluramine, had different affects on the microstructural characteristic of eating. Both drugs reduced consumption by reducing the time spent eating, but paradoxically, amphetamine increased the rate of consumption whereas fenfluramine reduced the rate of food intake. This finding demonstrates that the method employed is able to reveal different behavioural actions of anorectic drugs, and also implies that different modifications in the microstructure of eating may be related to the drugs varying actions on

particular neurochemical systems. In fact Blundell and Latham (1980), demonstrated that at equipotent doses, fenfluramine hypophagia was antagonized by the serotonergic antagonist methergoline but not by pimozide, a dopamine receptor antagonist, while the reverse was true of amphetamine anorexia.

The majority of microstructural studies of feeding behaviour have relied on observers to record whether the animal was eating or not (Blundell and Latham 1978; Cooper et al, 1979, 1980a, 1980b). Two problems are inherent in this procedure:

1. Subjective observation may result in drug effects that are distorted by artefacts of an inaccurate decision:
2. Observational analysis is both time consuming and labour intensive.

An alternative methodology, first used to analyze song patterns in zebra finchs (Slater 1974), and subsequently applied to feeding (Wiepkema 1971; Burton et al, 1981; Willner and Towell 1982) showed that changes between feeding and other behaviours may be deduced by examining the temporal distribution of feeding responses. The technique used to do this is log survivor analysis of the distribution of inter-response times (IRTs).

### 2.1.1 THE IRT METHOD

The theory behind log survivor analysis is that the frequency distribution of IRTs is made up of a number of underlying Poisson distributions, each associated with a behaviour having a constant probability of expression. The problem with IRT analysis is to resolve these discontinuities reliably. Such a method is

available in the technique of log survivor analysis;
45 mg pellets (Abels animal nutrient pellets), which are of uniform bite size are used to make it possible to record the time of all bites. From this information it is possible to calculate inter-response times (IRTs) and plot the frequency distribution of IRTs. The IRT distribution for values ranging between 1 and 36 sec is bell shaped with a long trailing tail (Fig 2.1A). IRT mode values are usually in the range of 4 to 8 sec and at IRT values of greater than 12 sec there are a significant reduction in responses which is the reason for the the long tail in the 36 sec distribution. The frequency distribution of IRTs can be transformed to a survivor plot, which shows the frequency of IRTs longer than any given time period. A log transform of this function results in a log survivor plot, from which reliable estimates of the time spent feeding may be deduced. A straight line on a log survivor represents a single Poisson function and so any discontinuity can be visualised as changes in the slope of the log survivor function. A "breakpoint" may then be identified (Fig 2.1B). The break point provides a "bout criterion" which is then applied to decide whether a particular inter-response interval is within or between eating bouts.

It is assumed that bouts of eating occured at IRTs shorter than the breakpoint whereas other behaviours such as ambulation were associated with IRTs longer than the breakpoint. Log survivor curves are therefore constructed for each subject and for every experimental session following which the breakpoints

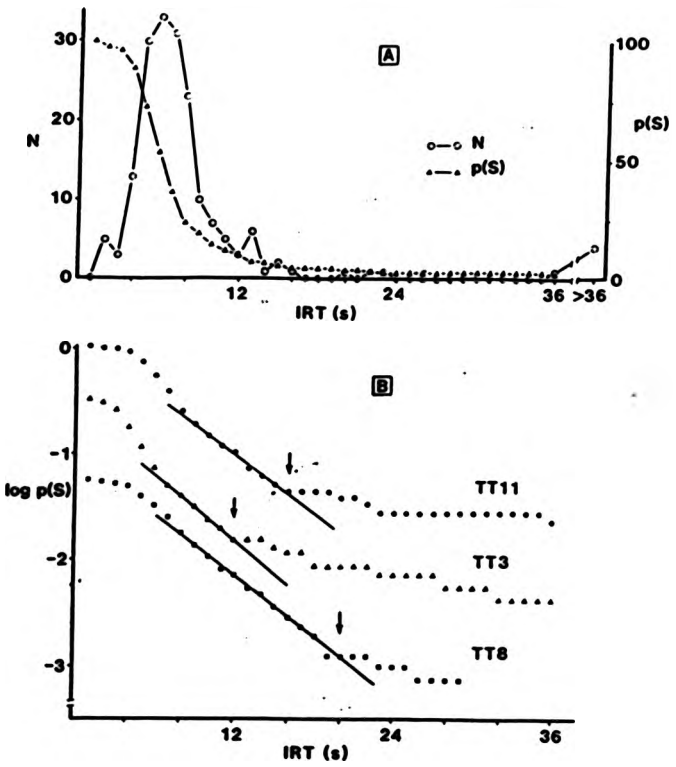



Fig. 2.1

A: The frequency distribution of inter-response times for a typical subject, and the the survivor transform, which shows the proportion of the frequency distribution lying to the right of each point in the frequency distribution. N is the number of responses in each 1s IRT bin and p(S) is the percentage of survivors.

B: Three typical log survivor functions. The uppermost curve is the log transform of the survivor function shown in A; for clarity, the other two examples are displaced down by one log unit. The breakpoint in each curve is marked by an arrow.

Willner and Towell 1982.

are determined and parameters of eating time and eating rate are then deduced.

In order to verify that changes apparent in eating rate in the microstructural paradigm are not artefacts of the method employed, the frequency distribution of IRTs can be plotted. A reduction in the rate of consumption should be readily visualized by a shift to the right in the distribution curve and an increase in the median IRT whereas an increase in eating rate should result in a shift to the left in the IRT frequency distribution and a decrease in the median IRT. Apomorphine reduces eating rate as calculated by the log survivor method (Willner at al, 1985), and also causes a rightward shift in the descending limb of the distribution curve which results in an increase in the median IRT. Amphetamine in contrast increases eating rate and causes a leftward shift in the IRT distribution. (In this case the 25th percentile was used to measure the shift in the ascending limb of the distribution as this parameter proved to be more sensitive to these changes than the median IRT.)(Towell et al, 1987b).

Prior to the work of Towell (1984), the log survivor method had only previously been used to analyse twenty-four hour feeding patterns (Booth 1972; Booth and Pain 1970; Burton et al, 1981). Apomorphine has a short duration of action (Burkman et al, 1974; Kaul et al, 1961), it was therefore necessary to determine whether log survivor analysis could also be used to analyse brief (thirty minute) feeding sessions. The assumption that IRTs shorter than the breakpoint represent eating within a bout,

whilst IRTs greater than the breakpoint represent eating between bouts, was validated by comparing results obtained with log survivor analysis and with those obtained through direct observation (Willner and Towell, 1982). Animals were filmed, and observed to spend long periods eating, directly facing the food tray and only moving to take a further food pellet; it was possible to identify from the film those inter-response intervals in which behaviours other than eating (rearing, grooming and walking) occured. Estimations of eating time by the log survivor method differed from those obtained from direct observation by only 2.1%. The values of eating rate were even closer (1.3%). The estimation of the number and length of bouts was less accurate with errors in excess of 40%. However, it is likely that a proportion of the gaps noted on the film were wrongly categorized, since at very short intervals these usually consisted of a single rear or turn by the rat, both of which are compatible with continued eating. When the very short gaps (<10 sec) were excluded from the calculation, the discrepancy in the number and length of bouts, though still marked, was considerably reduced (25% and 23% respectively). In conclusion, the method described produced very accurate estimates of eating rate and eating time and reasonably accurate estimations of the length of eating bouts and the intervals between these bouts of feeding.

## 2.2 MICROSTRUCTURAL ANALYSIS OF APOMORPHINE ANOREXIA

As noted above, DA agonists have biphasic effects on locomotor activity. Low doses produce sedation, but at higher doses, locomotor stimulation becomes apparent (Costall et al, 1980, 1981; Carlsson 1975). The sedative effects of apomorphine are believed to result from an interaction with presynaptic DA receptors on cell bodies and axon terminals (Skirboll et al, 1979; Sokoloff et al, 1980; Hjorth et al, 1982), while the activating effects result from the stimulation of postsynaptic DA receptors (Kelly et al, 1975; Kebabian and Calne 1979). However, Willner et al, (1985), reported that apomorphine reduced total food intake monotonically at all doses tested (Fig 2.2). Microstructural analysis showed that apomorphine reduced the rate of consumption and the time spent eating at all doses tested. However, at low doses (<0.1mg/kg) apomorphine reduced total eating time by reducing the length of eating bouts, whereas at high doses (>0.1 mg/kg) apomorphine reduced eating time by increasing the latency to initiate feeding and the length of gaps between feeding bouts. Nevertheless, despite these differences between the actions of presynaptic and postsynaptic doses of apomorphine, the monotonic dose response curve raises the question of whether the reduction in feeding by low doses of apomorphine really does result from the stimulation of presynaptic DA receptors.

The effect of apomorphine on eating time was assumed by Willner et al, (1985), to be mediated primarily by DA cell body autoreceptors in the mesolimbic system, since the effect could be

## APOMORPHINE DOSE-RESPONSE

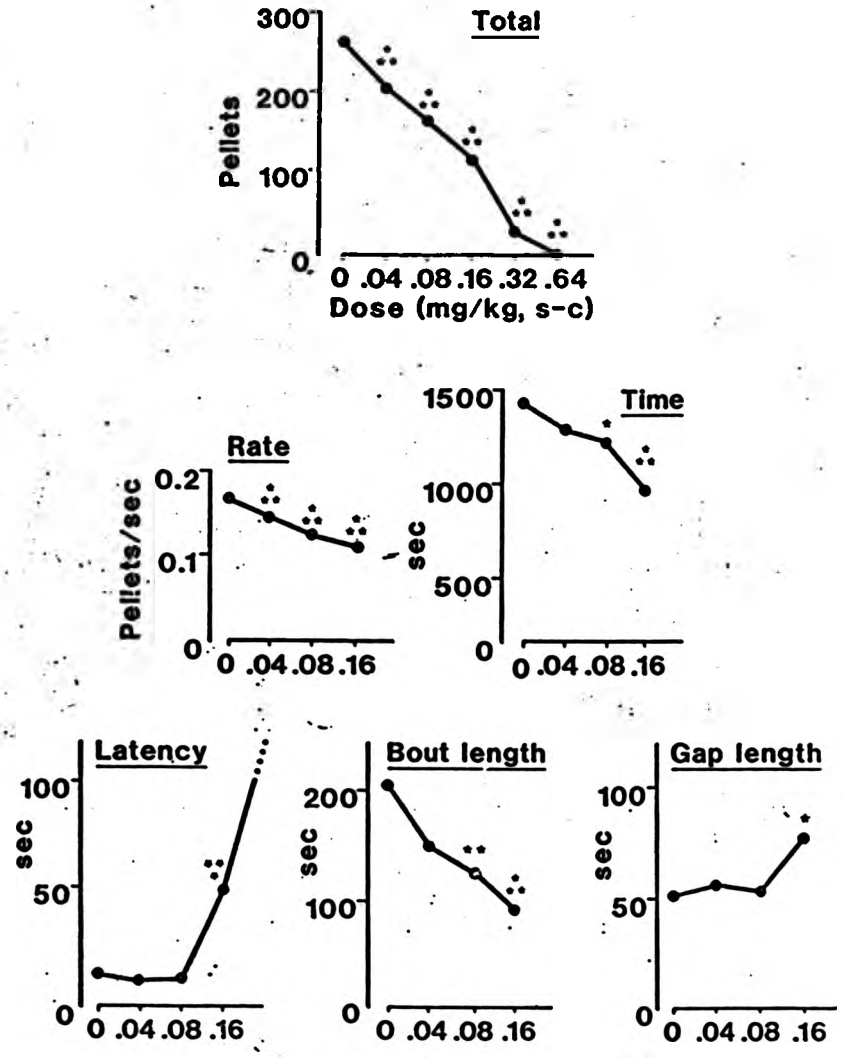



Fig. 2.2 Stars show differences between control and apomorphine. One star $p<.05$, two stars $p<.01$, three stars $p<.001$. Wellner et al, 1985.

reliably elicited by apomorphine infusions in the VTA. However, this finding is not conclusive evidence that apomorphine acts in the VTA after systemic administration. One of the main objectives of this thesis was resolve this problem unambiguously (see section 2.4).

A further problem with respect to the effects of apomorphine on the microstructural parameters was the finding that neuroleptics reversed the apomorphine-induced reduction of eating time but not of eating rate (Willner et al, 1985). A second objective was therefore to resolve the mechanism by which apomorphine reduces the rate of eating.

Taking the apomorphine-induced reduction in eating time as a measure of DA cell body autoreceptor function, Towell (1984), reported that in two out of five tests during chronic treatment with the tricyclic antidepressant DMI, apomorphine failed to reduce food intake. This finding would tend to support the observations of Serra et al, (1979), that chronic antidepressant treatment reduced the sedative response of apomorphine on locomotor activity. This, in turn would suggest that one of the mechanisms of by which antidepressants exert their effects is by reducing the sensitivity of presynaptic DA receptors. However, in a second experiment, no changes in the anorexic effect of apomorphine applied directly to the VTA were detected during the course of chronic treatment with DMI (Towell 1984). In both experiments (ie, using both peripheral and central administration of apomorphine) a reduction in the apomorphine response was

observed during withdrawal from chronic antidepressant treatment. This implies that DA autoreceptors are subsensitive only during withdrawal, and calls into question the assumption that reductions in the sensitivity of presynaptic DA receptors contribute to the effects of antidepressant drugs. A third objective of this thesis was therefore to reevaluate the effects of chronic antidepressant pretreatment on DA autoreceptor function following a conclusive resolution of the mechanisms by which low doses of apomorphine reduce eating time.

## 2.3 AN EVALUATION OF VARIABLE INTERVAL OPERANT RESPONDING AS A POTENTIAL EXPERIMENTAL TOOL

An initial experiment was carried out to examine whether variable interval (VI) responding could be used as an experimental tool in place of continous reinforcement (CRF). The schedule used was a VI of 30 secs. VI schedules have four potential advantages over CRF:

1. The schedule generates steady states of responding making it very amenable to study the effects of apomorphine on rate.
2. It is possible to introduce a number of different components within the schedule and thereby generate more complex experimental designs.
3. Satiation results much more slowly with these schedules so it is also possible to run longer sessions.
4. The schedules are cost effective, in that the number of pellets consumed in a session is considerably less than that in the CRF paradigm.

2.3.1 METHOD

Subjects

Adult male Lister hooded rats (Olac, Bicester, Oxon) weighing 250-350g were used. They were housed in pairs under conditions of controlled temperature and humidity, on a 12 h light-dark cycle (08.00-20.00 h light). Animals were maintained on 21-h food deprivation, with free access to food between 14.00 and 17.00 hours daily, and water available at all times. Behavioural testing was carried out between 10.00 and 14.00 hours.

Apparatus

Eight identical operant test chambers (Campden Instr. Ltd., London), enclosed in sound attenuating cubicles, were fitted with the right lever only. Recording of lever presses and delivery of food pellets were achieved by means of an on-line digital computer (Acorn System IV), which was programmed in Onlibasic, a language designed for the on-line control of behavioural experiments (Fray 1981). IRT's, were also recorded using a Cromemco Z2 microcomputer and thus the IRT frequency distribution for each animal receiving each treatment could be determined.

Procedure

Pharmacological studies were not initiated until all animals had achieved a steady state of responding, which required between 30 to 40 sessions. The duration of the test session was 30 min. Drug treatments were presented in a counterbalanced order, with a minimum of two drug-free days between tests. One group of animals (n=12) received four doses of apomorphine: 0, 0.04, 0.06 and 0.08

mg/kg; a further group (n=12) received four doses of pimozide: 0, 0.3, 0.45 and 0.6 mg/kg.

All injections were made in a volume of 1 ml/kg. Apomorphine HCl (Sigma) was dissolved in 0.02% ascorbate as an antioxidant, and injected subcutaneously in the scruff of the neck 10 min prior to test sessions. Pimozide (Janssen) was dissolved in minute quantities of glacial acetic acid and made up to the required volume with distilled water and injected intraperitoneally 2 h prior to test sessions. Appropriate vehicle solutions were used for control injections.

### 2.3.2 RESULTS

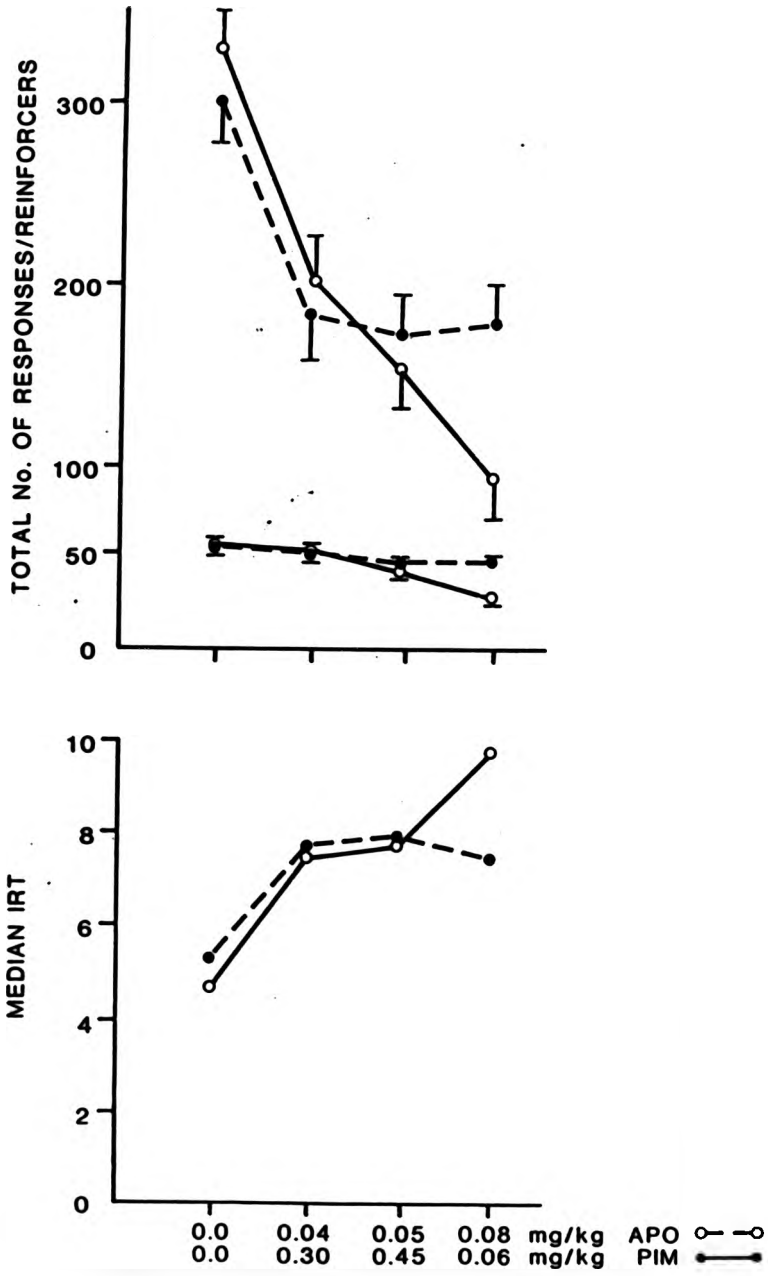
The effects of apomorphine and pimozide on the total number of responses and the total number of reinforcers recorded in the 30 minute session are shown in figure 2.3A. Apomorphine reduced the total number of responses at 0.04mg/kg, ($F(1,33) = 16.9$, $p<0.001$). However, with increasing doses of apomorphine there was no further reduction in response output. The reinforcement density was similarly reduced by apomorphine at all doses tested (min $F(1,33) = 6.2$, $p<0.05$). The reduction in response output by apomorphine was reflected in a reduction in the number of the shorter intervals in the IRT frequency distribution; the preponderance of the longer IRT's resulted in a significant increase in the median IRT (Fig 2.3B: min $F(1,33) = 5.2$, $p<0.05$). Again, however, there was no further increase the median IRT with increasing doses of apomorphine.

In contrast to apomorphine, pimozide reduced rate dose-dependently (Fig 2.3A: $F(1,33) = 26, 46.9, 89.4$, $P<0.001$). Except for the lowest dose tested, pimozide also reduced the total number of pellets obtained in the test session (Fig 2.3A: $F(1,33) = 16.7$ and $68.7$, $p<0.001$). Pimozide also caused a dose dependent increase in the median IRT (Fig 2.3B: $F(1,33) = 5.7, 6.5, 19.1$, $p<0.05, 0.05, 0.001$).

Apomorphine and pimozide produced very similar changes in responding, reinforcements and the median IRT at the lowest dose. However, the highest dose (double the lowest in both cases), the effects of pimozide were significantly greater on all three measures (Fig 2.3A,B: $F(1,33) = 89.4, 68.7, 19.1$, $p<0.001$).

Fig. 2.3

EFFECTS OF APOMORPHINE AND PIMOZIDE ON VARIABLE INTERVAL PERFORMANCE (VI 30)



### 2.3.3 DISCUSSION

In this experiment administration of apomorphine and pimozide in the variable interval schedule caused a concomittant reduction in responding and the number of reinforcements obtained in a session. In other words, apomorphine produced similar behavioural deficits to those seen following neuroleptic administration in positively reinforced tasks. However, the effects of pimozide increased dose-dependently while those of apomorphine did not. If it is assumed that the doses of apomorphine used in this study selectively stimulated presynaptic DA receptors, interpretation of the results is relatively straightforward. Stimulation of DA autoreceptors reduces DA availability in the synaptic terminal while pimozide reduces DA efficacy by blocking postsynaptic DA receptors. Therefore, following maximal stimulation of DA autoreceptors, increasing doses of DA agonists do not result in further reductions in responding (floor effect), whereas, increasing doses of DA antagonists further inhibit endogenous DA from activating postsynaptic DA receptors resulting in greater reductions in response maintainance. The results of the present experiment are fully consistent with this formulation. However, it is important to note, that the effects of apomorphine in the CRF paradigm were rather different: increasing doses of apomorphine, notably from 0.04 to 0.08 mg/kg, resulted in further reductions in food intake. The effect of apomorphine in the CRF paradigm appears to be more complex and therefore it may be unwise to use VI schedules to attempt to elucidate the effects observed in CRF

experiments.

A further problem becomes apparent on examination of the log survivor curves for VI and CRF schedules. The curves represented (Fig 2.4), are typical for animals performing in these two procedures. In the CRF paradigm a change in the slope of the curve is easily visualised at IRT values of less than 16 sec (approx), whereas in the VI curves a change is observable at IRT values of greater than 16 sec. Using the breakpoint then to deduce the times spent eating (CRF) or lever pressing (VI) it becomes apparent that the animals responding in the CRF paradigm did so for intervals ranging between 700-1600 sec, while in the VI paradigm animals responded for 1700 to 1800 sec. In the microstructural paradigm the animals were able to obtain as many food pellets as they desired and therefore spent more or less the first half of the session at the tray door, while in the rest of the session they carried out a variety of different behaviours (grooming, resting, ambulation), interspersed by occasional visits to the tray door. The animals performing under the VI:30 schedule spent most of the session lever pressing for food as the maximum number of pellets available was only 60. This explanation would account for the observation that the breakpoint in the VI procedure cannot be used, to distinguish between behaviours other than responding, to calculate an accurate estimate of the time spent lever pressing. The use of the breakpoint method however, demonstrated that responding in the VI schedule was continous throughout the session. The VI schedule would then be the

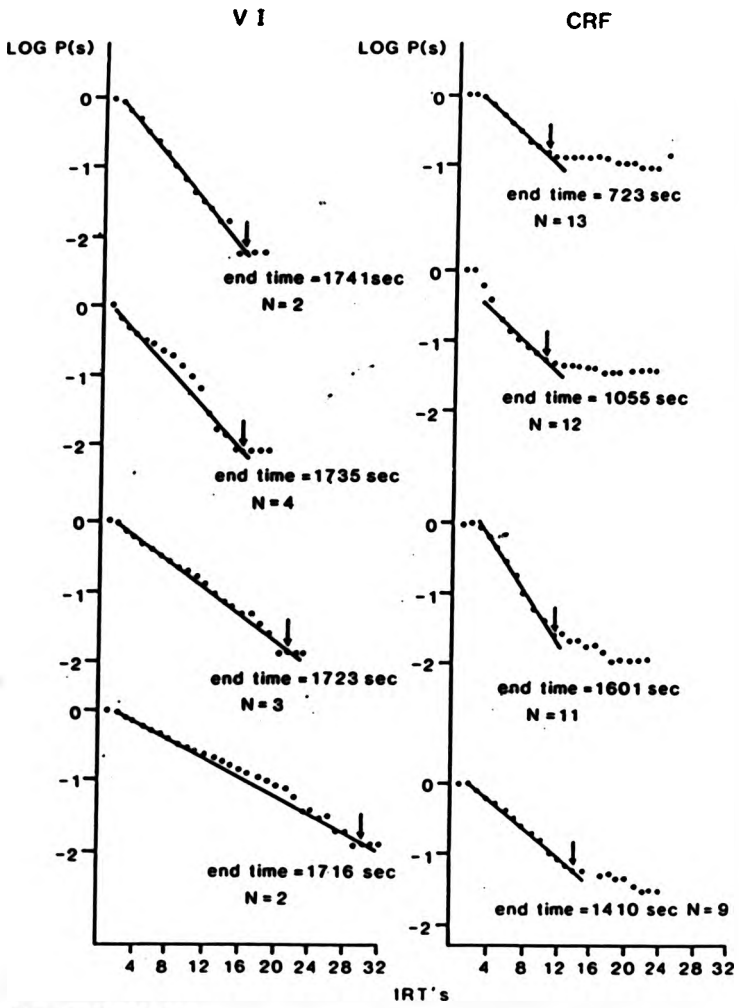



Fig. 2.4 Four typical log survivor functions for VI (variable interval) on the left and CRF (continuous reinforcement) on the right. The breakpoint is marked by an arrow. The end time under the arrow denotes the time spent lever pressing in the VI condition; in the CRF schedule this denotes the time spent feeding. N is the number of responses that occurred after the breakpoint in the 30 min session.

appropriate tool to use if one only wanted to investigate the effects of apomorphine on rate. We did, indeed, find that a variety of neuroleptics failed to attenuate the apomorphine response with the exception of sulpiride (results not shown) a specific D2 receptor antagonist (Jenner et al, 1978; Montanaro et al, 1982). However, for reasons outlined above, the VI procedure cannot demonstrate changes in the time spent lever pressing as lever pressing was continous throughout the session. Given that the effect observed during antidepressant withdrawal, in the CRF paradigm, was an attenuation of eating time, VI schedules cannot be used to investigate the mechanism by which antidepressants produce such an effect.

## 2.4 OBJECTIVES OF THE PRESENT WORK

For the reasons described above, the CRF paradigm was used in all subsequent experiments. Chapter 3 contains a detailed description of the methods used in these studies.

The first objective was to resolve the neurotransmitter systems involved in the apomorphine-induced reductions of eating time and eating rate (Chapter 4).

Having demonstrated that these reductions were in fact a consequence of the stimulation of central DA receptors, the second objective was to examine the relative contribution of pre and postsynaptic DA receptor systems to the apomorphine response (Chapter 5).

Given that apomorphine may interact with presysnaptic DA receptors, the third set of experiments made use of intracranial injection procedures to investigate the exact location and the relative contribution of these receptors to the apomorphine-induced reductions in eating time and eating rate (Chapter 6).

Finally, having established the receptors responsible for the apomorphine responses, two important points were addressed in Chapter 7 with respect to the mechanism of action of antidepressant drugs.

1. Does chronic antidepressant treatment unequivocally reduce DA autoreceptor sensitivity (and thus increase in DA output) ?
2. If so, is this effect relevant to the clinical actions of antidepressant drugs ?

CHAPTER 3

## GENERAL METHODS

This chapter gives an account of the general methods used in subsequent experiments; additional details are included where necessary in later chapters.

### 3.1 SUBJECTS

Male lister hooded rats (NIMR, Mill Hill or OLAC, Bicester) weighing approximately 300-400 g at the start were used in all experiments. They were individually housed under conditions of controlled temperature and humidity, on a 12 h light-dark cycle (09.00-21.00 light). Animals were maintained on 17-21 h food deprivation and fed with standard laboratory diet (Dixons, Ware, Herts). At the end of daily testing animals were given access to food pellets for 3 h. Water was freely available in the home cage at all times.

### 3.2 APPARATUS

Eight identical operant chambers (Campden Instr. Ltd., London), from which the levers had been removed, were programmed to deliver a 45 mg food pellet (Larkhall Labs. London), whenever the perspex food tray door was pressed, subject to the constraint that presses spaced less than one second apart were ineffective. The house and tray light were illumunated continously, and the chambers were housed in individual sound-attenuating boxes with smoked perspex viewing windows. Each response on the tray door was logged (to the nearest 0.1 sec) by a Cromemco Z2 microcomputer, which output the time of each response on a visual

display unit (VDU), and subsequently produced a listing of response times and inter-response times (IRTs), an IRT frequency distribution and a log survivor function (see below).

### 3.3 DRUGS

The following drugs were used in experiments described in this thesis; details of doses and times of administration can be found in the relevant chapters:

Amitriptyline hydrochloride (Merck, Sharp and Dohme, Hoddesdon, Herts): experiment 7.1.

(+)-Amphetamine sulphate (Smith Kline and French, Welwyn Garden City, Herts): experiment 5.3.

Apomorphine hydrochloride (Sigma, Poole, Dorset): all experiments.

Desipramine hydrochloride (Ciba, Horsham, Sussex): experiments 7.1 and 7.2.

Domperidone (Janssen, Wantage, Oxon): experiment 4.1.

Dopamine hydrochloride (Sigma, Poole, Dorset): experiment 4.1.

Metergoline (Farmitalia, Milan): experiment 4.2.

Mianserin hydrochloride (Beecham, Epsom, Surrey): experiment 7.1.

Naloxone hydrochloride (Sigma, Poole, Dorset): experiment 4.2.

Pargyline hydrochloride (Sigma, Poole, Dorset): experiment 4.1.

Phentolamine mesylate (Ciba, Horsham, Sussex): experiment 4.2.

Pimozide (Janssen, Wantage, Oxon): experiments 2.3 and 4.3.

(+/-)-Propranolol (Sigma, Poole, Dorset): experiment 4.2.

SCH-23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol) (Schering Corp. Kenilworth, New Jersey): experiment 5.2.

Scopolamine hydrobromide (Sigma, Poole, Dorset): experiment 4.2.

(+/-)-Sulpiride (Delagrange, Paris): experiments 4.3, 5.2, 6.1, 6.2A and 6.2B.

Tetrabenazine (Roche, Welwyn Garden City, Herts): experiment 5.1.

Yohimbine hydrochloride (Sigma, Poole, Dorset): experiment 4.2.

Apomorphine was dissolved in 0.02% ascorbate as an antioxidant; sulpiride, pimozide, domperidone and tetrabenazine were dissolved in minute quantities of glacial acetic acid and made up to the required volume with distilled water; SCH-23390 was dissolved in physiological saline; all other drugs were dissolved in distilled water. Apomorphine, SCH-23390 and yohimbine were administered subcutaneously in the scruff of the neck; all other drugs were administered intraperitoneally. All systemic injections were made in a volume of 1 ml/kg with the exception of domperidone which was injected at 2 ml/kg. Intracranial (i.c.) administration procedures were used in some experiments; full details are given in chapters 6 and 7.

### 3.4 HISTOLOGY

At the end of experiments 6.1, 6.2 and 7.1, animals were perfused under sodium pentobarbital (100 mg/kg i.p.) with buffered formalin and their brains were rapidly removed and stored in 10% formalin for approximately 5 weeks. The brains were then blocked in 20% gelatine and stored for a further 5 weeks. Frozen 100 micron sections were prepared using a microtome (MSE, London) and stained with cresyl fast violet in order to verify cannula placements microscopically using the atlas of Pellegrino

and Cushman (1967).

### 3.5 PROCEDURE

Animals were trained to press the perspex tray door in order to receive a 45 mg food pellet, under a schedule of continous reinforcement (CRF). Experiments were usually run on six days of each week (Mon-Sat). Sessions were of 30 min duration and following 3-4 weeks (18-24 sessions) of training all animals achieved asymptotic performance. In general, in each experiment all animals received drug treatments in a counterbalanced order with a minimum of two drug-free days between tests and (where animals were re-used) a minimum of seven days between successive studies. The exceptions were two studies in Experiment 4.2 and the experiments in Chapter 7, in which separate groups of animals were tested under each drug condition. Following the achievement of asymptotic performance 30 min sessions were run on test days; on intervening days, 10 min test sessions were usually run. Apomorphine (0.05mg/kg) was used in the majority of studies with the exception of those desribed in Chapter 7 in which 0.06 mg/kg was administered to ensure a consistent and reliable reduction in eating time as well as eating rate.

### 3.6 MICROSTRUCTURAL ANALYSIS

The frequency distribution of inter-response times (IRTs) between the taking of successive pellets, can be transformed to a survivor function, which shows the number or the proportion of IRTs greater than any given IRT. A logarithmic transformation of the proportions produces a log survivor function. The log

survivor function falls off steeply over IRT ranges that occur with high frequency, and the initial fall is in a straight line (indicating an underlying Poisson distribution). Usually the slope changes sharply to a shallower slope, enabling a breakpoint to be identified easily. A small proportion of log survivor curves do not have a clean breakpoint which is easily detectable. To aid identification of the breakpoint, grouped log survivor curves were constructed for each treatment condition, which defined a region in the log survivor curve where the breakpoint was likely to occur.

Following the identification of the breakpoint, the following parameters of feeding were calculated:

1. The number of bouts (B), which is equal to the number of gaps (i.e. intervals longer than the breakpoint) plus one.
2. Eating time (T), which is given by the total of all IRT's smaller than the breakpoint.
3. The length of eating bouts, which is given by T/B.
4. Eating rate: Since the time taken to eat the final pellet in each bout is neither known nor included in the calculation of eating time, the local eating rate is given by (N-B)/T (where N is the total number of responses), rather than by N/T. An eating rate of 0.1 pellets/s is equivalent to 0.27 g/min.

In order to confirm the authenticity of changes in eating rate derived by log survivor analysis, the raw data were also examined directly, by constructing IRT frequency distributions for each of the treatment conditions. A reduction in eating rate

means that each pellet takes a longer time to consume and this should be reflected by an increase in the median IRT. Apomorphine was found to reduce eating rate in the microstructural paradigm and accordingly increased the median IRT (Willner et al, 1985).

### 3.7 STATISTICAL ANALYSIS

Following the calculation of the microstructural parameters of feeding as described above, the data were subjected to analysis of variance, supplemented where appropriate by tests of simple main effects and planned comparisons. It was occasionally necessary to use non-parametric tests, when the variance in the data was inhomogenous.

CHAPTER 4

## APOMORPHINE ANOREXIA: A PHARMACOLOGICAL CHARACTERIZATION

Earlier experiments have suggested that apomorphine-induced anorexia may involve two distinct components. Low doses of apomorphine in the microstructural paradigm reduced food intake by decreasing both the time spent eating and the rate of food ingestion (Willner et al, 1985). The typical neuroleptic drug haloperidol and the atypical neuroleptic thioridazine both blocked the effect of apomorphine on eating time, and administration of apomorphine directly into the ventral tegmental area (VTA) selectively reduced eating time. By contrast, eating rate was unaffected by neuroleptic drugs, or by apomorphine infusions into the VTA. On the basis of this evidence, it was concluded that the reduction of eating time by apomorphine was mediated by DA autoreceptors in the VTA. However, the reduction of eating rate by apomorphine appeared to be mediated by a different mechanism.

The three experiments described in this chapter were designed to elucidate the pharmacological basis of this effect. The first experiment investigated the possibility that in part apomorphine anorexia might be mediated peripherally: the actions of the peripherally acting DA agonist and antagonist, DA and domperidone, were examined, as well as their interactions with apomorphine. The second experiment examined whether a range of antagonist drugs at receptors for neurotransmitters other than dopamine could attenuate the reduction in eating rate induced by

apomorphine; the drugs studied were antagonists at alpha 1, alpha 2 and beta adrenoreceptors, plus an antimuscarinic drug, a serotonergic antagonist and an opiate antagonist. In the final experiment the effect of neuroleptic drugs on apomorphine anorexia was re-examined, by challenging apomorphine with two additional neuroleptic drugs, pimozide and sulpiride.

## 4.1 METHODS

A total of 128 rats weighing 300-350 g were used in these experiments (48 NIMR, 80 Olac). Consumption of 45 mg food pellets was measured between 10.00 and 14.00 h in modified operant chambers as described in Chapter 2; food was freely available in the home cage from 14.00 to 17.00 h. Microstructural parameters were calculated for pellet feeding sessions as described in Chapter 3.

Drug doses and pretreatment times were as follows:
Apomorphine (0.05 mg/kg) and dopamine (1.0 mg/kg), 10 min; phentolamine (1.0 mg/kg), 20 min; yohimbine (1.0 mg/kg), 25 min: Pargyline (15 mg/kg), propranolol (5.0 mg/kg), naloxone (1.5 mg/kg), scopolamine (0.05 mg/kg) and domperidone (5.0 mg/kg), 30 min; methergoline (2.0 mg/kg) and sulpiride (5.0 and 7.5 mg/kg), 60 min; pimozide (0.3 and 0.4 mg/kg), 120 min.
Appropriate vehicle solutions were used for control injections.

In Experiment 4.1 the effects of apomorphine, DA, the monoamine oxidase inhibitor pargyline, and domperidone were tested in sixteen animals, using eight different drug

combinations; full details are shown in Fig. 4.1. In the first part of Experiment 4.2, separate groups of animals (n=12) were tested under four conditions: apomorphine, yohimbine, both drugs, control. One week later, the study was repeated using the same animals substituting metergoline for yohimbine. In the second part of Experiment 4.2, apomorphine was challenged with phentolamine, naloxone and scopolamine in one group of animals (n=16) and a further group (n=16) were used to test the effect of propranolol. In all four studies each animal was tested under all four drug conditions. In Experiment 4.3, the same design was used to test the effect of pimozide (n=16) and sulpiride (n=16). The experiment was repeated at two doses of each drug.

## 4.2 RESULTS

### Experiment 4.1

Apomorphine significantly reduced the total number of pellets consumed during the half-hour test session, (Fig 4.1A: ($F(1,105)= 14.4$, $p<0.001$). This effect was brought about primarily by a reduction in the rate of pellet consumption (Fig 4.1C: ($F(1,105)=16.3$, $p<0.001$); there was also a reduction in the time spent eating, though this change did not reach significance, (Fig 4.1B: ($F(1,105)= 1.71$, N.S.)). The peripheral DA antagonist domperidone did not in itself change performance on any of the three measures (max $F(1,105)=2.83$, N.S.), and also failed to attenuate the effects of apomorphine. In fact, the effect of apomorphine was greater following domperidone pre-treatment (Fig 4.1A-C): food consumption ($F(1,105)=11.3$, $p<0.01$)

and eating time ($F(1,105)=18.1$, $p<0.001$) were significantly lower after apomorphine and domperidone than after apomorphine alone.

Pargyline did not significantly alter any of the three measures (max $F(1,105)=1.0$, N.S.), but DA administered in the presence of pargyline produced a significant anorectic effect, ($F(1,105)=38.1$, $p<0.001$). However, unlike apomorphine anorexia, DA anorexia was caused predominantly by a reduction in eating time, ($F(1,105)=30.8$, $p<0.001$), in addition to a reduction in eating rate, ($F(1,105)=12.3$, $p<0.001$). Domperidone pretreatment completely reversed the anorexic effect of dopamine on all of the microstructural parameters (Fig 4.1A-C: total pellets, $F(1,105)=12.9$, $p<0.001$; time, $F(1,105)=5.4$, $P<0.05$; rate, $F(1,105)=6.9$, $P<0.05$). In contrast, apomorphine pretreatment resulted in a further reduction in the total number of pellets consumed ($F(1,105)=35.4$, $p<0.001$), consequent on a considerable reduction in both eating rate ($F(1,105)=16.6$, $p<0.001$), and eating time ($F(1,105)=20.7$, $p<0.001$).

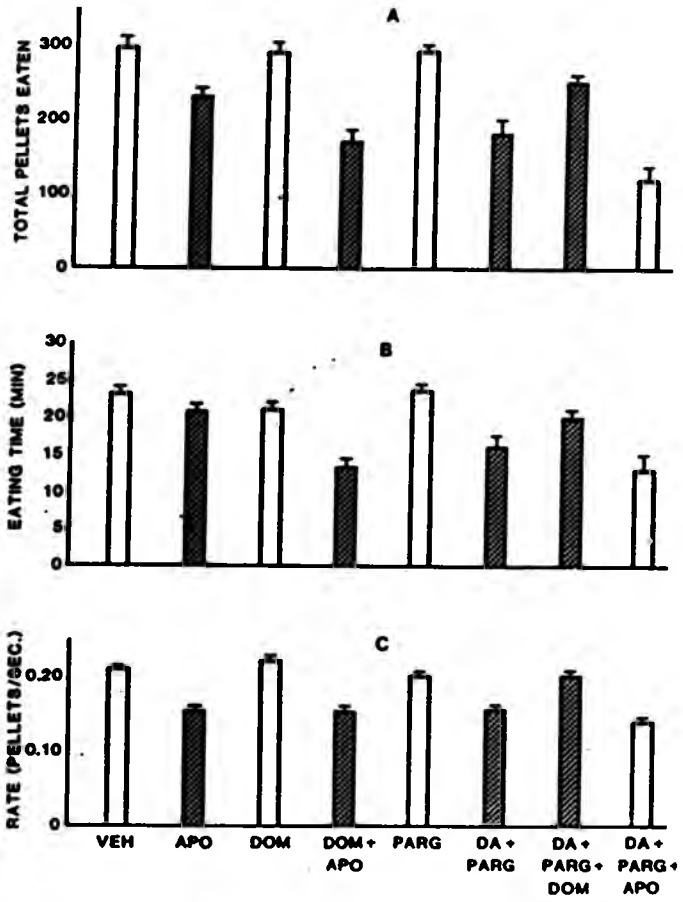



Fig. 4.1 A: Total food intake; B: eating time; C: eating rate. Values are means and standard error. VEH, vehicle; APO, apomorphine; DOM, domperidone; PARG, pargyline; DA, dopamine. APO, APO/DOM, DA and DA/DOM columns have been cross hatched to facilitate visual inspection of the figure.

Experiment 4.2

Total food intake was significantly reduced by pretreatment with five of the six antagonists: (Fig 4.2: phentolamine (24% reduction: $F(1,30)=27.5$, $p<0.001$), naloxone (30% reduction: $F(1,30)=45.6$, $p<0.001$), scopolamine (54% reduction: $F(1,30)=48.8$, $p<0.001$) and metergoline (17% reduction: $F(1,44)=8.4$, $p<0.01$)). The reduction in food intake resulted from a reduction in time spent eating (Fig 4.3), in the case of phentolamine ($F(1,30)=31.3$, $p<0.001$), naloxone ($F(1,30)=24.4$, $p<0.001$) and scopolamine ($F(1,30)=6.2$, $p<0.05$), the rate of eating (Fig 4.4) was also significantly reduced by scopolamine ($F(1,30)=25.8$, $p<0.001$) and metergoline ($F(1,44)=19.6$, $p<0.001$).

Despite these changes in baseline performance, none of the six antagonists significantly modified the actions of apomorphine. Yohimbine did partially attenuate the reduction of food intake, but this change did not reach statistical significance ($F(1,44)=2.9$, N.S.). As in experiment 4.1 apomorphine did not significantly reduce eating time in these studies (Fig 4.3). Effects of apomorphine on eating rate are shown in Fig 4.4. In all six studies, apomorphine substantially reduced eating rate (min $F(1,30)=14.6$, $p<0.001$); but in no case was this effect significantly attenuated by pretreatment with a potential antagonist (interaction: max $F(1,15)=3.3$, N.S.).

In order to guard against any unforeseen artefacts in the technique used to calculate eating rate, the effects of the six drugs were examined further by inspection of the raw data prior to log survivor analysis. The IRT frequency distribution curves for apomorphine/propranolol study are shown as an illustration (Fig 4.5). The reduction of eating rate by apomorphine is reflected in a reduction in the number of shorter IRTs and a shift to the right in the IRT frequency distribution. The preponderance of longer IRTs causes a significant increase in the median IRT ($F(1,30)=24.3$, $p<0.001$). The failure of propranolol to restore eating rate is confirmed by the fact that the propranolol/apomorphine curve is very similar to that of apomorphine alone, and by the lack of effect of propranolol on the median IRT (interaction: $F(1,15)=1.3$, N.S.). Similar data were obtained with the other five antagonists, (max $F(1,44)=3.4$, N.S.).

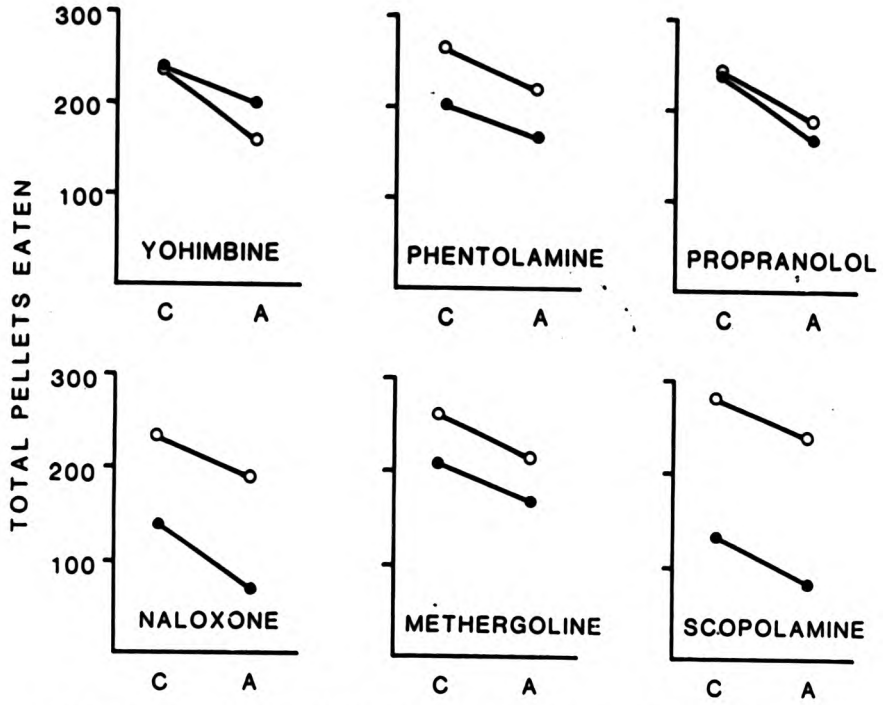



Fig. 4.2 Effects of apomorphine in combination with six potential antagonists on total pellets eaten. C, vehicle control; A, apomorphine. White circles, control; black circles, antagonist pretreatment.

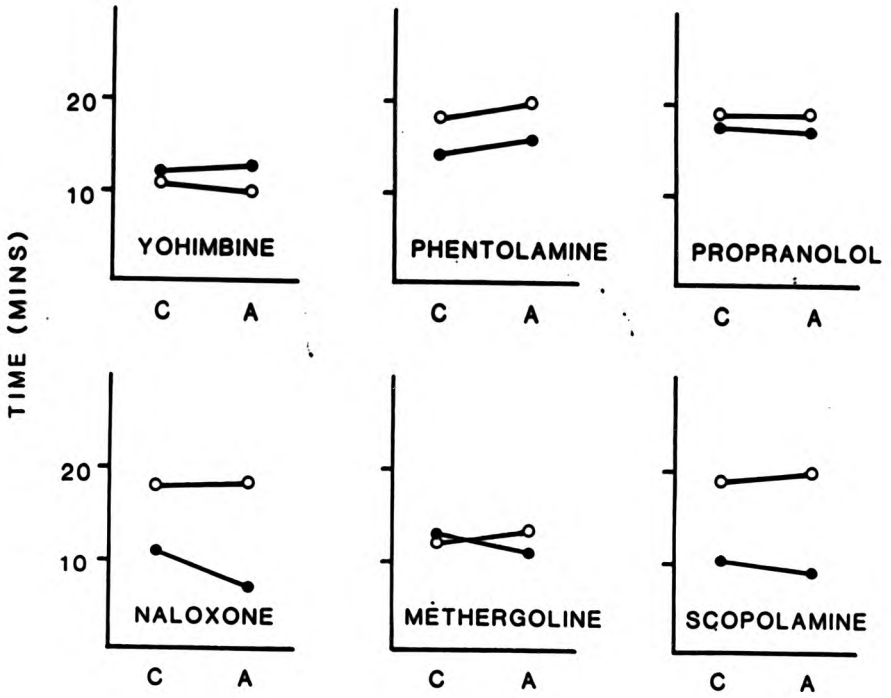



Fig. 4.3 Effects of apomorphine in combination with six potential antagonists on eating time. C, vehicle control; A, apomorphine. White circles, control; black circles, antagonist pretreatment.

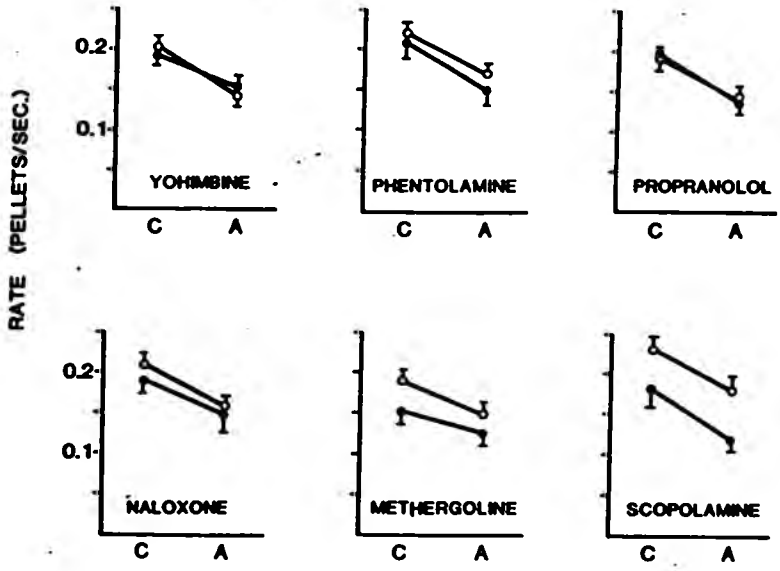



Fig. 4.4 Effects of apomorphine in combination with six potential antagonists on mean eating rate. C, vehicle control; A, apomorphine. White circles, control; black circles, antagonist pretreatment.

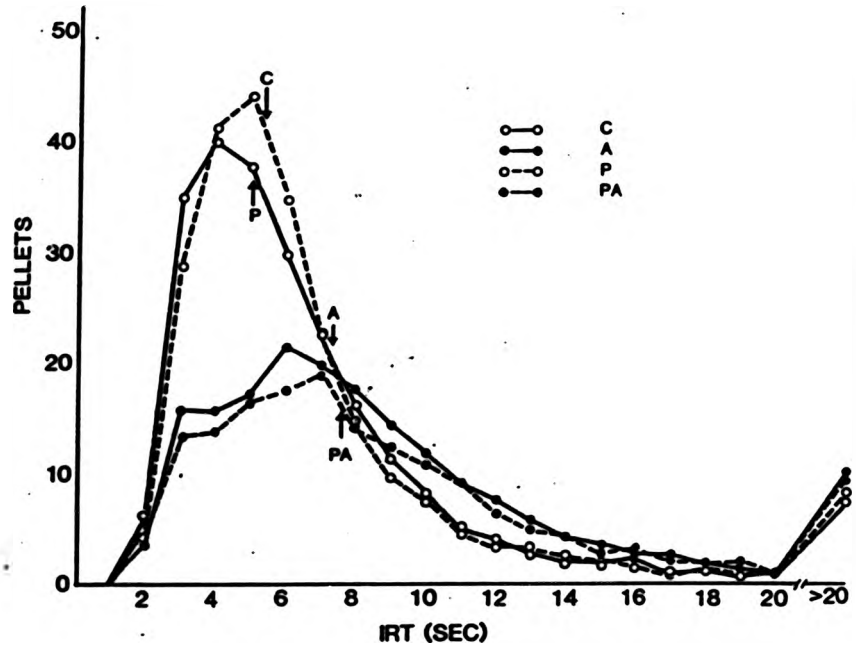



Fig. 4.5 Inter response time (IRT) frequency distribution for feeding responses in 1 sec time bins, following the administration of apomorphine and/ or propranolol. Arrows show the median IRT in each condition.

Experiment 4.3

Both doses of pimozide (0.4 & 0.3 mg/kg) substantially attenuated the anorexic response to apomorphine, (Fig 4.6A: interaction: $F(1,15)=8.1$, $p<0.01$), and ( Fig 4.6A: interaction: $F(1,15)=6.37$, $p<0.02$). At 0.3 mg/kg, pimozide alone had no effect on any measure, but pimozide reversed the effect of apomorphine on food intake (Fig 4.6A: interaction: $F(1,15)=8.1$, $p<0.01$) and eating rate, (Fig 4.6C: interaction: $F(1,15)=10.7$, $p<0.005$); in this experiment apomorphine did not significantly reduce the time spent eating. At 0.4 mg/kg, pimozide administered alone significantly reduced total food intake ($F(1,30)=16.7$, $p<0.01$), by reducing both eating time ($F(1,30)=5.9$, $p<0.05$), and eating rate ($F(1,30)=7.2$, $p<0.05$). In this experiment, apomorphine reduced both eating time and eating rate; pimozide reversed both of these effects (apomorphine/pimozide interactions: eating time $F(1,15)=8.1$, $p<0.01$; eating rate $F(1,15)=5.3$, $p<0.05$).

Both doses of sulpiride (5.0 & 7.5 mg/kg), when administered alone caused a small reduction in food intake which just reached significance at the lower dose used, (Fig. 4.7A: $F(1,30)=4.2$, $p<0.05$). Again, however, apomorphine failed to reduce food intake in sulpiride pretreated animals, (Fig 4.7A: interactions: $F(1,15)=9.5$, $p<0.01$ and $F(1,15)=9.3$, $p<0.01$). Apomorphine did not significantly reduce the time spent eating in these experiments. In both experiments, sulpiride reversed the apomorphine-induced reduction of eating rate, (Fig 4.7C: interactions: $F(1,15)=4.8$, $p<0.05$ and $F(1,15)=8.4$, $p<0.01$).

For both drugs, reversal of the effect of apomorphine on eating rate was confirmed by analysis of the IRT frequency distributions. As in Experiment 4.2, the median IRT was significantly increased by apomorphine; this effect was blocked by both doses of pimozide (interactions: Fig 4.6D: $F(1,15)=16.7$, $p<0.001$; $F(1,15)=4.8$, $p<0.5$) and by both doses of sulpiride (interactions: Fig 4.8D: $F(1,15)=8.2$, $p<0.01$; $F(1,15)=20.2$, $p<0.002$).

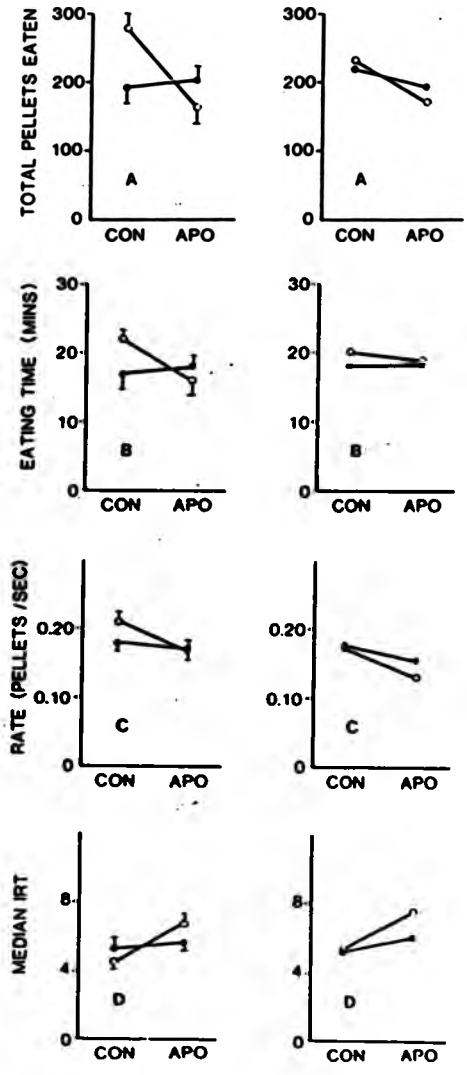



Fig. 4.6 Effects of apomorphine and pimozide (left 0.4, right 0.3 mg/kg) on A: total food intake; B: eating time; C: eating rate; D: median IRT. Values are means. CON, control; APO, apomorphine; white circles, control; black circles, pimozide pretreatment.

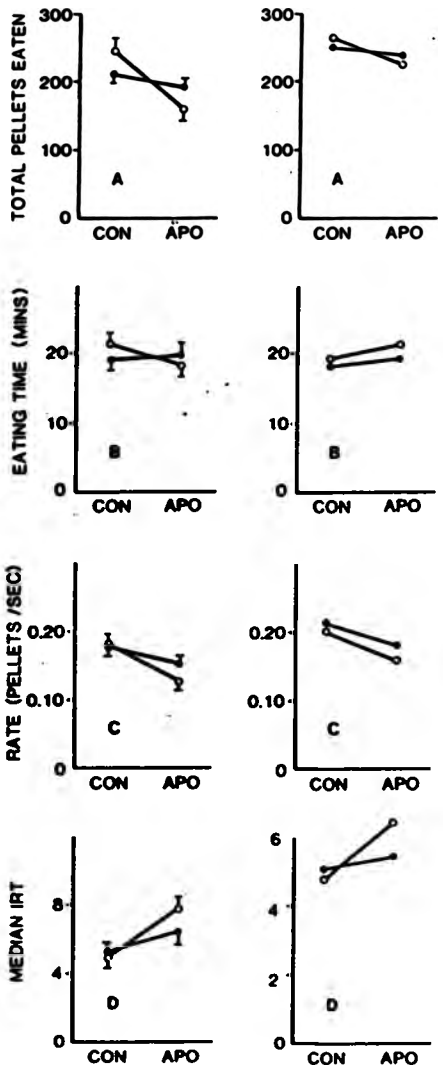



Fig. 4.7 Effects of apomorphine and sulpiride (left 5.0, right 7.5 mg/kg) on A: total food intake; B: eating time; C: eating rate; D: median IRT. Values are means. CON, control; APO, apomorphine; white circles, control; black circles, sulpiride pretreatment.

## 4.3 DISCUSSION

In experiment 4.1, administration of a low dose of apomorphine reduced food intake, mainly by reducing the rate of food consumption. DA also produced an anorexic response but this was mainly attributable to a reduction in the time spent eating. DA does not cross the blood brain barrier and the resulting anorexia may be a consequence of the stimulation of peripheral DA receptors present in the gastrointestinal system (GIT). Involvement of dopamine receptors in this system has been postulated for the inhibitory effects of dopamine on gastric acid secretion in dog and man, (Valenzuela et al, 1979). Dopamine receptors in the GIT may also be involved in the inhibition of gastric emptying (Brockaert , 1979). However, the evidence available for the localization of DA receptor systems within the GIT is not at all conclusive and furthermore there is evidence that the inhibitory effect on gastric motility following DA administration may be reversed by the alpha and beta adrenergic antagonists, phentolamine and propranolol (Brockaert et al, 1984). In addition, it is apparent that there are species differences as well as regional differences within the same animal, with respect to the localization of peripheral DA receptor populations, (Costall et al, 1982).

Many substances that affect the gastrointestinal system also act on the chemoreceptor trigger zone (CTZ) in the lower brain stem. Therefore, DA anorexia may alternatively be mediated by an action in the area postrema (Borrison 1974; Borrison and Wang

1951), within which lies the CTZ. Because the CTZ lies outside the blood brain barrier it is often difficult in in-vivo studies to exclude an effect on these structures, even for substances that do not cross the blood brain barrier.

Whatever the precise site of action of DA, it is clear that in addition to the difference in their microstructural characteristics apomorphine and DA anorexia are anatomically distinct. The peripherally acting DA antagonist domperidone, which, like DA, does not cross the blood brain barrier (Laduron and Leysen 1979) was effective in reversing DA anorexia but actually enhanced apomorphine anorexia. These data argue strongly that both components of apomorphine anorexia, the reduction in eating time and the reduction in eating rate, are mediated by central mechanisms.

Experiment 4.2 examined the possibility that the effects of apomorphine on eating rate might be mediated by one of six non-DA receptor systems. In the first instance the alpha 2 noradrenergic antagonist yohimbine was tested, as there are a number of studies in which the sedative effects of apomorphine were attenuated by alpha 2 antagonists, (Delbarre and Schmitt 1973; Sumners et al, 1981). Yohimbine did tend to antagonise the reduction in food intake produced by apomorphine, though in this study this effect was not significant. In an earlier experiment (not reported in detail) a larger dose of yohimbine (0.2 mg/kg), which reduced baseline performance, did appear to antagonise the effect of apomorphine on eating time. However, in neither of these

experiments was there any antagonism of the effect of apomorphine on eating rate. There is some evidence that yohimbine may also interact with DA autoreceptors (Scatton et al, 1980; Van Oene et al, 1984), though the apomorphine-induced reduction in mouse climbing behaviour which is believed to result from the stimulation of DA autoreceptors was not reversed by yohimbine (Costall et al, 1984).

At high doses, some of the behavioural effects of apomorphine are mediated by alpha 1 receptors (Maj et al, 1971, 1977); however the alpha 1 antagonist phentolamine was ineffective in reversing the reduction of eating rate, at a dose that reduced feeding when given alone. The beta antagonist propranolol also failed to attenuate the apomorphine reduction in food intake, at a dose which did partially antagonize amphetamine anorexia in the same paradigm, (Willner and Towell 1982).

The possible contribution to apomorphine-induced sedation of cholinergic, serotonergic and opiate systems, either directly or via modulation of dopamine activity is uncertain. In studies which investigated locomotor activity, cholinergic interneurons in the caudate nucleus appear to be postsynaptic to DA neurons, and administration of cholinergic antagonists have been shown to abolish haloperidol catalepsy, (Costa 1977). However, in the nucleus accumbens it would appear that the cholinergic input has a modulatory effect on the circuit initiating locomotor activity since carbachol, a cholinergic agonist, increased dopamine stimulated locomotion (Jones et al, 1981). In order for both

dopamine and carbachol to increase locomotion there would have to be at least one inhibitory interneurone in the circuit for locomotor activity between these synaptic inputs (Jones et al, 1981). In addition to a cholinergic input to the nucleus accumbens, a serotonergic input may also be involved, as serotonin abolished dopamine stimulated locomotion (Costall et al, 1976). There are no intrinsic 5-HT interneurons in the accumbens and thus this input is assumed to be from the midbrain raphe (Costall et al, 1976; Geyer et al, 1976). In addition, a preferential action of low doses of amphetamine on 5-HT systems within the nucleus accumbens may account for the inhibitory effect of amphetamine on locomotor activity (Hussey et al, 1983). Opiate receptor systems are also present in high concentrations in the mesolimbic brain regions (Snyder 1978); enkephalinergic neurons terminate presynaptically on nigrostriatal and mesolimbic dopamine nerve terminals, and apparently regulate the release of DA via activation of opiate receptors on the DA nerve terminal, (Pollard et al, 1977, 1978; Costall et al, 1978). The opiate antagonist, naloxone, has been found to potentiate methamphetamine stereotypy as well as abolish haloperidol catalepsy, (Balsara et al, 1984).

Despite this evidence of interactions between DA and ACh, 5-HT and enkephalin, in the present study, the muscarinic, serotonergic and opiate antagonists, scopolamine, methergoline and naloxone all failed to reverse apomorphine anorexia, at behaviourally active doses.

In an earlier report (Willner et al, 1985), it was demonstrated that haloperidol and thioridazine abolished the apomorphine-induced reduction of eating time but had little effect on the apomorphine-induced reduction of eating rate. The final experiment in this chapter however, showed that the effect of apomorphine on eating rate was in fact blocked by two additional neuroleptics, pimozide and sulpiride. Pimozide blocked all the effects of apomorphine, both at a dose (0.4 mg/kg) which itself substantially reduced feeding, and also at a dose (0.3 mg/kg) which had minimal effects on baseline performance. Similar considerations apply to sulpiride. Sulpiride slightly reduced food intake but subsequent experiments confirmed that sulpiride blocked all the anorectic effects of apomorphine, under conditions in which sulpiride itself had no significant effect on feeding (see Chapter 6).

Pimozide and sulpiride are relatively specific among DA antagonists. Pimozide has no other significant receptor interactions at moderate doses (Pinder et al, 1976). Sulpiride has some affinity for alpha 2 receptors (Montanaro et al, 1982), but the lack of effect of yohimbine on eating rate rules out an interaction with alpha 2 receptors as an explanation for this effect of sulpiride. It may therefore be concluded that, like the effect of apomorphine on eating time, the reduction of eating rate by apomorphine is also mediated by central DA receptors. However, it is clear from the evidence reviewed earlier that the receptors responsible are anatomically distinct from the cell

body autoreceptors in the VTA that appear to mediate the effect of apomorphine on eating time. The hypothesis now entertained and examined in the following chapters, is that the effects of apomorphine on eating time and eating rate may be mediated by different populations of central DA receptors.

CHAPTER 5

## PRELIMINARY ANATOMICAL LOCALIZATION OF APOMORPHINE ANOREXIA

It has been suggested that the reduction in eating time following systemic apomorphine may result from the stimulation of DA receptors in the VTA, as eating time was selectively reduced by direct application of apomorphine to this area (Willner et al, 1985). In the previous chapter it was shown that the reduction in eating rate following systemic apomorphine is also mediated by central DA receptors. The location of the DA receptors involved in both of these effects has yet to be firmly established but in view of the evidence reviewed in Chapter 1, the hypothesis put forward is that the site of action of low doses of apomorphine is a presynaptic one and the DA receptor is of the D2 subtype.

The three experiments carried out in this chapter were done in order to establish:

1. The relative contribution of pre and postsynaptic DA receptors to apomorphine anorexia.
2. The DA receptor subtype (D2/D1) involved in these effects.
3. The neuronal system mediating the anorectic response to low doses of apomorphine.

## EXPERIMENT 5.1: THE EFFECTS OF TETRABENAZINE PRETREATMENT ON APOMORPHINE ANOREXIA

In principle, it should be straightforward to distingiush pre-and postsynaptic effects of apomorphine, by destroying DA neurons, which should abolish any effects mediated presynaptically, while sparing postsynaptic mediated effects. Chemical lesions using 6-hydroxydopamine (6-OHDA) (in combination with desmethylimipramine (DMI) to protect noradrenergic neurons (Breese and Taylor 1971)), have been used to good effect to establish the anatomical bases of the effects of amphetamine: for example 6-OHDA lesions of the nucleus accumbens abolished the locomotor response to amphetamine, while lesions of the striatum reduced amphetamine-induced stereotypy (Kelly et al, 1975; Koob et al, 1978; Makanjoula and Ashcroft 1982). It has also been reported that 6-OHDA lesions of the mesolimbic neurons failed to attenuate the anorectic response to amphetamine (Koob et al, 1978) but lesions of the nigrostriatal system did attenaute amphetamine anorexia (Joyce and Iversen 1984).

In preliminary experiments (not reported in detail) it was found that the effects on apomorphine anorexia following 6-OHDA lesions of either the nucleus accumbens or the striatal complex were somewhat equivocal: the effects of apomorphine on eating time were attenuated, but the effects on eating rate appeared to be unaffected (Muscat et al, 1985). Although lesions of this type result in a marked reduction in transmitter availability and a concomittant disruption of behaviour, animals are still able to

perform though impoverished, on a number tasks, suggesting that following the lesion the system is still operative. Approximately 60% to 85% reductions in DA availability have been reported following local administration of 6-OHDA to the nucleus accumbens or the caudate nucleus (Kelly et al, 1975; Taylor and Robbins 1986): in Parkinsonism, motor disorders only sometimes become apparent following 90% destruction of DA terminals (Gessa and Corsini 1981). A major problem in interpreting results of this kind is the development of supersensitivty of postsynaptic DA receptors. DA agonists such as apomorphine, at doses which inhibited behaviour in the normal animal, have been found to increase activity in the 6-OHDA lesioned rat (Ungerstedt 1971b) as a result of a 40 fold increase in the sensitvity of postsynaptic DA receptors to low doses of apomorphine (Ungerstedt et al, 1978). This effectively means that 6-OHDA cannot be used to confirm presynaptic effects of low doses of apomorphine.

As an alternative to 6-OHDA there are several agents which are able to induce the disappearance of amines from their storage sites in the nerve terminal. Reserpine is probably the most familiar and widely used. This compound causes a dramatic fall in amine levels both in peripheral and central neurons within several hours (Glowinski and Baldessarini 1966). However, although recovery starts to take place within two days in the cell bodies, full recovery of amine levels in nerve terminals takes several days (Shore 1972). It appears that reserpine may

act irreversibly on vesicular membranes; the long time course of recovery may reflect the time of arrival of fresh-reserpine free vesicles provided by axoplasmic transport (Shore 1972).

Benzoquinolizines, on the other hand are a group of drugs, of which tetrabenazine is the best known, which exert similar amine depleting actions to reserpine (Pletscher et al, 1962). Tetrabenazine (TBZ) is less potent than reserpine, not as long acting and is more selective for amine stores in the CNS (Howard et al, 1981; Quinn et al, 1959). In addition, tetrabenazine causes a rapid depletion of amine stores probably by blocking uptake into storage granules. Of the three biogenic amines, DA appears to be most sensitive to TBZ. Reductions in DA levels are apparent within 15 min are still present 2 h later (Pettibone et al, 1984) and return to normal within 15 h (Pletscher 1968).

Sedative effects of low doses of apomorphine (<0.1 mg/kg) are believed to be mediated by presynaptic DA receptors, while high doses are believed to act postsynaptically (Di Chiara et al, 1976; Makanjoula et al, 1980; Kelly et al, 1975). It was therefore predicted that TBZ would abolish the anorexic effect of a low dose of apomorphine (0.05 mg/kg), but not those of a higher dose (0.15 mg/kg).

5.1.1 METHODS

16 rats (NIMR) weighing approximately 300g at the start of the experiment, were tested between 15.30 and 17.00 h daily. The animals were trained to feed by pressing the door of the pellet

dispenser in one of eight identical operant chambers; microstructural parameters of feeding were calculated as described in Chapter 2.

Drug treatments were presented in a counterbalanced order with a minimum of two drug free days between tests. The duration of test sessions was 30 min; on intervening days the session was 10 min. On experimental days the animals were administered apomorphine (0, 0.05 or 0.15 mg/kg) 10 min prior to testing, following pretreatment 2 h earlier with tetrabenazine (0, 1.0 or 2.0 mg/kg), in a 3 * 3 factorial design. The dose of TBZ was selected on the basis of a preliminary dose-response study: at higher doses than that used TBZ substantially reduced food consumption (75% reduction at 4.0 mg/kg).

5.1.2 RESULTS

Both low and high doses of apomorphine significantly reduced food intake (Fig 5.1A: $F(1,90) = 20.5, 54.1, p<0.001$). The reduction in consumption brought about by the low dose of apomorphine was predominantly attributable to a reduction in eating rate (Fig 5.1B: $F(1,90) = 15.5, p<0.001$). Apomorphine also reduced eating time: this effect was not statistically significant (Fig 5.1D: $F(1,90) = 1.3$, N.S.); there was, however, a significant reduction in the duration of eating bouts (Fig 5.1E: $F(1,90)=4.6, p<0.05$). The anorexic effect of the high dose of apomorphine was attributable to a reduction in the time spent eating (Fig 5.1D: $F(1,90) = 12.6, p<0.001$); and also to a further reduction in the rate of pellet consumption (Fig 5.1B:

F(1,90) = 14.3, p<0.001). At this dose, in addition to a decrease in the duration of eating bouts (Fig 5.1E: F(1,90)=11.2, p<0.01) there was also a non-significant increase in the duration of gaps between bouts (Fig 5.1F: F(1,90)=1.4, N.S.). At both doses of apomorphine the changes in eating rate were reflected in an increase in the median IRT (Fig 5.2C: F(1,90) = 12.0 and 20.7, p<0.001).

The lower dose of TBZ (1.0mg/kg) caused a slight but insignificant reduction in the total number of pellets consumed (Fig 5.1A: F(1,90) = 2.5, N.S.) which was attributable to a reduction in the rate of consumption (Fig 5.1B: F(1,90) = 6.8, P<0.05). At this dose, TBZ failed to attenuate significantly any of the effects of the low dose of apomorphine, but further accentuated the effects of the high dose (Fig 5.1A: F(1,90) = 60.8, p<0.001). This interaction was brought about by a further decrement in the time spent feeding (Fig 5.1D: F(1,90) = 30.7, p<0.001) and the length of eating bouts (Fig 5.1E: F(1,90)=18.0, p<0.001). TBZ did not however, alter the effects of apomorphine (0.15mg/kg) on eating rate or the median IRT (Fig 5.1B,C: F(1,90) = 0.1, 0.4, N.S.).

The higher dose of TBZ (2.0mg/kg) significantly reduced pellet consumption (Fig 5.1A: F(1,90) = 58.5, p<0.001), by reducing both eating rate (Fig 5.1B: F(1,90) = 51.9, p<0.001) and eating time (Fig 5.1D: F(1,90) = 13.8, p<0.001). At this dose of TBZ, the effects of the lower dose of apomorphine (0.05 mg/kg) on pellet consumption and eating rate were totally abolished

(Fig 5.1A,B: $F(1,90) = 0.1$, 0.1, N.S.). TBZ (2.0 mg/kg) also abolished the effects of the higher dose of apomorphine on eating rate and median IRT (Fig 5.1B and 5.2C: $F(1,90) = 2.4$, 0.1, N.S.) However, in the presence of tetrabenazine (2.0mg/kg) the high dose of apomorphine (0.15mg/kg) still reduced the total number of pellets consumed (Fig 5.1A: $F(1,90) = 16.4$, $p<0.001$), by reducing the time spent eating (Fig 5.1D: $F(1,90) = 19.1$, $p<0.001$).

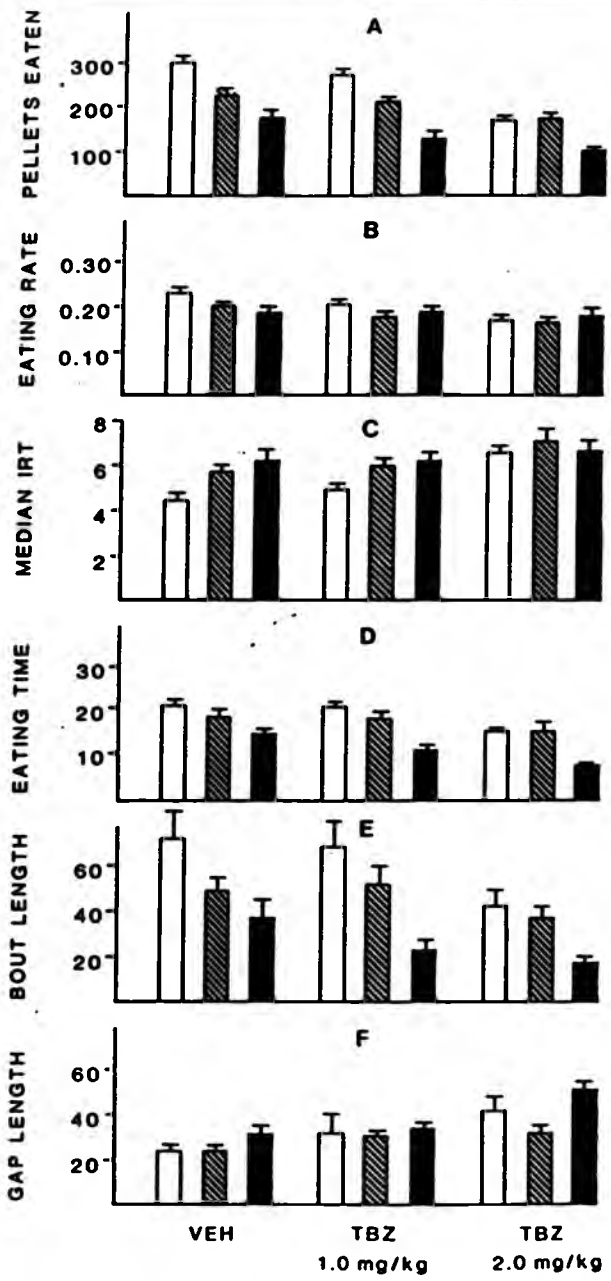



Fig. 5.1 Effects of apomorphine and tetrabenazine on (A) total food intake; (B) eating rate; (C) median IRT; (D) eating time; (E) bout length; (F) gap length. Values are means and standard error. VEH, vehicle; APO, apomorphine; TBZ, tetrabenazine. Open columns, vehicle pretreatment; cross hatched columns, apomorphine (0.05 mg/kg) pretreatment; filled columns, apomorphine (0.15 mg/kg) pretreatment.

### 5.1.3 DISCUSSION

The lower dose of tetrabenazine used in this study failed to attenuate any of the effects of a low dose of apomorphine. However, it may be assumed that at this dose only partial disruption of DA terminal stores may have occurred as tetrabenazine did not in itself reduce food intake in the 30 minute session. In contrast, the higher dose of tetrabenazine (2.0mg/kg), did reduce food intake, but abolished the effects of the lower dose of apomorphine. In addition, tetrabenazine also prevented the rate reducing effects of the higher dose of apomorphine. It is unlikely that these effects of tetrabenazine were floor effects: in experiment 4.2, both scopolamine and metergoline caused reductions in eating rate comparable to that brought about by TBZ, but nevertheless, in the presence of apomorphine further reductions in eating rate were observed.

The fact that tetrabenazine abolished the effects of both low and high doses of apomorphine on eating rate, supports the hypothesis that these effects are mediated by presynaptic DA receptors. TBZ did not, however, block the reduction in eating time that ensued following the administration of the higher dose of apomorphine. Indeed, both doses of TBZ enhanced this effect. These results suggest that the effects of the higher dose of apomorphine on eating time (though not on eating rate) may be mediated by postsynaptic DA receptors. It is generally assumed that the sedative effects of a low dose of apomorphine (<0.1 mg/kg s.c.) are mediated by presynaptic DA receptors while the

stimulant effects of higher doses (>0.1 mg/kg s.c.) are mediated by postsynaptic DA receptors (Carlsson 1975; Di Chiara et al, 1977; Stahle and Ungerstedt 1986a). The present data are fully consistent with this formulation.

## EXPERIMENT 5.2: ARE THE EFFECTS OF APOMORPHINE ON FEEDING MEDIATED BY D2 OR D1 RECEPTORS ?

Kebabian and Calne (1979), proposed the subdivision of dopamine receptors into two classes, D1 receptors which are functionally linked to cyclic AMP and D2 receptors that are not. In addition, it was also observed that in some cases D2 receptor stimulation results in the inhibition of cyclic AMP (Stoof and Kebabian 1984). Seeman (1980), suggested four classes of dopamine receptors, D1, D2, D3 and D4 but it now appears that the D3 and D4 sites may be conformational states of D1 (Leff and Creese 1983a, b) and D2 receptors (Caron et al, 1983; Grigoriadis and Seeman 1984, 1985; Sibley et al, 1982) respectively.

The pharmacological specificity of the rate reducing and time reducing effects of apomorphine may be further characterized by the administration of specific D2 and D1 receptor antagonists. As presynaptic DA receptors are presumed to be of the D2 subtype (Roth 1981), pretreatment with sulpiride, a specific D2 receptor antagonist (Jenner et al, 1978; Montanaro et al, 1982), should abolish the apomorphine-induced reduction in eating rate and this effect has already been demonstrated (experiment 4.3). However, this effect of sulpiride does not distingiush pre- and postsynaptic sites of action. The ventral tegmental area, the site of origin of the DA fibres to the nucleus accumbens has been found to possess only D2 receptor site as no DA sensitive adenylate cyclase activity has been identified on DA neurons in this region (Bockaert et al, 1977; Phillipson et

al, 1977). However, D2 receptor binding sites have also been demonstrated in the nucleus accumbens (Jastrow et al, 1984), and in this structure the D2 sites are located both pre and postsynaptically (Tassin et al, 1982). It is also apparent that in addition to D2 receptor sites, the nucleus accumbens also possess D1 receptor sites identified by DA-stimulated adenylate cyclase (Horn et al, 1974), or by the binding of D1 ligands (Schulz et al, 1985), in particular the specific D1 receptor antagonist, SCH-23390 (Hyttel 1983; Iorio et al, 1983). However, unlike D2 receptor sites, all D1 receptor sites are located postsynaptically: 6-OHDA lesions reduced the binding of D2 receptor ligands but did not affect D1 stimulated adenylate cyclase activity (Creese and Snyder 1979; Creese et al, 1977; Schwartz et al, 1978), whereas kainic acid lesions did not significantly alter binding of D2 receptor ligands but substantially reduced D1 increases in cyclic AMP activity (Sokoloff et al, 1980). Following however, the two kinds of selective lesion (6-OHDA and Kainic acid) of DA neurons, D2 receptor binding was significantly reduced (Sokoloff et al, 1980).

The specific D1 agonist SKF 38393 (Settler et al, 1978) has no marked stimulant effects (except for slight non-stereotyped sniffing, rearing and locomotion after high doses), but induces turning after unilateral lesions to the nigrostriatal pathway (Arnt and Hyttell 1984) and increases locomotor activity after 6-OHDA treatment (Breese and Mueller 1985). These effects of SKF

38393 are totally attenuated following the administration of the specific D1 receptor antagonist SCH-23390 (Hyttel 1983; Iorio et al, 1983). SCH-23390 also inhibited the actions of non-selective DA agonists such as amphetamine and apomorphine; SCH-23390 abolished apomorphine-induced stereotypy, amphetamine-induced stereotypy and amphetamine-induced hyperactivity (Christensen et al, 1984; Iorio et al, 1983; Schulz et al, 1985). However, in spite of its specificy for D1 receptors, it has also been observed that SCH-23390 prevented the behavioural effects of selectively acting D2 agonists, such as lisuride, pergolide and LY-17155, in normal animals (Mailman et al, 1984). In animals which have been pretreated with 6-OHDA, alpha-methyl-para-tyrosine (AMPT), or reserpine, SCH-23390 proved to be ineffective in reversing D2 receptor mediated behaviours. The explanation put forward for these effects of SCH-23390 was that D1 receptors in some way mediate the consequences of postsynaptic D2 receptor stimulation in normal animals (Carlson et al, 1986) but following DA depletion this mechanism becomes uncoupled (Arnt and Hyttel 1985).

In order to determine the DA receptor subtype involved in apomorphine anorexia, the present experiment examined the interactions of systemically administered apomorphine with the D1 receptor antagonist SCH-23390 (Hyttel 1983; Iorio et al, 1983) and the D2 receptor antagonist sulpiride (Montanaro et al, 1982). On the basis of the literature reviewed, it was assumed that

sulpiride would block responses mediated by pre- or postsynaptic D2 receptors, whereas SCH-23390 would block any postsynaptically mediated response.

5.2.1 METHODS

16 rats (NIMR) weighing 300-350g at the start of the experiment, were tested between 14.00 and 16.00 h, and allowed free access to food between 16.00 and 19.00 h. Following training to feed from pellet dispensers, as described in Chapter 3, drug treatments were presented in a counter-balanced order, with a minimum of two drug-free days between test. The duration of the test was 30 min and on intervening days the session length was usually 10 min. All sixteen animals were administered systemic apomorphine (0.05 mg/kg sc) or vehicle, following pretreatment with sulpiride (10 mg/kg i.p.), SCH-23390 (0.0125 mg/kg sc) or vehicle (saline sc) in a 2 * 3 factorial design. Following the termination of the experiment, measures of eating rate, eating time and other microstructural parameters were derived from log survivor analysis of the frequency distribution of IRTs, as decribed in Chapter 3.

Sulpiride was administered 1 hour before apomorphine injections and SCH-23390 30 min before apomorphine injections. Doses of apomorphine and sulpiride were based on those used in previous experiments. The dose of SCH-23390 was selected on the basis of a preliminary dose-response study: at doses higher than that used, SCH-23390 substantially reduced food consumption (45% reduction at 0.025 mg/kg; 70% reduction at 0.05 mg/kg).

Data were subjected to analysis of variance, supplemented where appropriate by tests of simple main effects and planned comparisons. In addition, in order to analyse separately the interaction of apomorphine with the two different drug treatments, after analysing raw scores, a second analysis was also carried out, on data obtained by subtracting each apomorphine score from the appropriate control score.

5.2.2 RESULTS

Systemic administration of apomorphine suppressed food intake (Fig 5.3: $F(1,45) = 65.4$, $p<0.001$) by reducing both eating time and eating rate (Fig 5.3: $F(1,45) = 11.3$, $p<0.01$; 59.6, $p<0.001$, respectively). Apomorphine also significantly increased the median IRT (Fig 5.3: $F(1,45) = 71.9$, $p<0.001$). The effect on eating time was further analysed by computing the mean duration of feeding bouts, the duration of gaps between bouts of feeding and the initial latency; apomorphine shortened the duration of feeding bouts (Wilcoxon $T(16) = 3.0$, $p<0.01$), but the duration of gaps and the latency to initiate feeding did not change significantly ($T(16) = 1.3$ and 0.4, N.S.).

Sulpiride, by itself had no noticeable effects, while SCH-23390, by itself, significantly impaired performance on all four measures (Fig 5.3: min $F(1,45) = 53.8$, $p<0.001$). Pretreatment with sulpiride substantially reduced the effect of apomorphine on food consumption, eating time, eating rate and median IRT (Fig 5.3: apomorphine/sulpiride interactions: ($F(1,30) = 13.6$, $p<0.001$; 5.6, $p<0.05$; 9.5, $p<0.01$; 19.8, $p<0.001$, respectively).

However, none of the effects were reversed by SCH-23390. Indeed despite suppressing feeding by itself, SCH-23390 actually enhanced the suppressant effect of apomorphine on food consumption and eating time, the effects of apomorphine on eating rate and median IRT were unaffected by SCH-23390 (Fig 5.3: apomorphine/SCH-23390 interactions, $F(1,30) = 8.7$, $p<0.01$; 8.0, $P<0.01$; 0.2, N.S.; 0.7, N.S., respectively). Sulpiride, but not SCH-23390, also reversed the effect of apomorphine on bout length ($F(1,30)= 6.7$, 0.1, $P<0.05$; N.S., not shown).

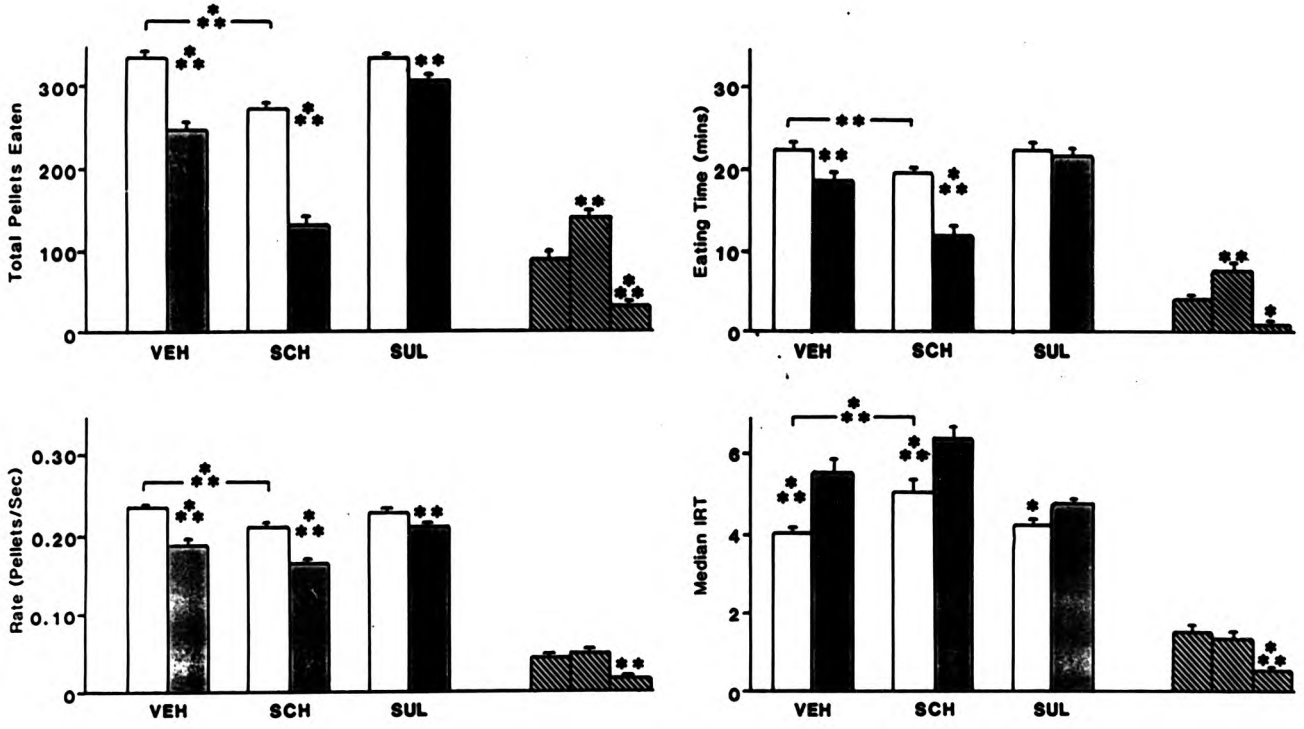



Fig. 5.2 Effects of systemic apomorphine on A: total food intake; B: eating time; C: eating rate; D: median IRT. Scores are means (+ standard error). White, control; black, apomorphine: pretreatments: VEH, vehicle; SCH, SCH-23390; SUL, sulpiride: the hatched bars to the right of each panel show the suppressant effect of apomorphine (i.e. white minus black). One star, $p < 0.05$; two stars $p < 0.01$; three stars $p < 0.001$.

### 5.2.3 DISCUSSION

The results of the present study provide further evidence for an involvement of the D2 receptor subtype in the effects of systemic apomorphine on both eating rate and eating time. The reversal by the selective D2 receptor antagonist sulpiride (Jenner et al, 1978; Montanaro et al, 1982), but not by the D1 receptor antagonist SCH-23390 (Hyttell 1983; Iorio et al, 1983) of the various effects of apomorphine on feeding confirm that these effects were mediated by D2 receptors. Although sulpiride has some affinity for alpha-2 adrenergic receptors (Montanaro et al, 1982), the more potent alpha-2 antagonist yohimbine did not antagonize the effects of systemic apomorphine on rate (Chapter 4). The fact that TBZ abolished all anorexic effects of a low dose of apomorphine (Expt. 5.1) strongly suggests that the relevant D2 receptors are presynaptic rather than postsynaptic. While SCH-23390 is a selective antagonist at D1 receptors (Hyttel 1983; Iorio et al, 1983), this compound also blocks the behavioural stimulant effects mediated by postsynaptic D2 receptors, suggesting that D1 and D2 receptors mediating behavioural stimulation may be functionally linked (Breese and Mueller 1985; Christensen et al, 1985). However, the sedative effects mediated by presynaptic D2 receptors are not antagonized by SCH-23390 (Iorio et al, 1983; Mereu et al, 1985). The fact that in the present study SCH-23390 failed to reverse any of the effects of apomorphine on feeding, supports the view that the receptors involved are located presynaptically.

## EXPERIMENT 5.3: DISSOCIATION OF THE DA PATHWAYS INVOLVED IN APOMORPHINE AND AMPHETAMINE-INDUCED ANOREXIA

A final experiment examined the interaction between low doses of apomorphine and amphetamine. The suppressant effects of amphetamine on feeding are well-documented and are known to be dependent on a stimulatory action at central catecholaminergic synapses (Blundell and Latham 1980; Cooper and Francis 1979; Samanin and Garattini 1981), which is brought about by the release of transmitter from presynaptic terminals (Stein 1964; Glowinski and Baldessarini 1966; Fuxe and Hanson 1967 ). Microstructural studies of amphetamine anorexia concur in showing that amphetamine suppresses food intake by decreasing the duration of eating, whilst concurrently increasing the rate of eating (Blundell and Latham 1978, 1980). Using the methods employed in the current study we have demonstrated that significant shifts to the shorter IRTs are detectable at doses of amphetamine as low as 0.125 mg/kg (Towell et al, 1987b).

A number of studies cited in the literature argue for both a noradrenegic and dopaminergic component in the mediation of the effects of a low dose of amphetamine (Leibowitz 1982; Willner and Towell 1982) while the effects of higher doses of amphetamine seem to be mediated by dopaminergic receptor systems (Lyon and Robbins 1975; Towell et al, 1987). However, it has also been reported that the dopamine receptor antagonist, pimozide, failed to reverse low dose amphetamine anorexia while abolishing the anorexia resulting from larger doses (Burridge and

Blundell 1979). In contrast to this observation, it has been reported that pimozide reversed both low and high dose amphetamine anorexia (Towell et al, 1987). In addition, the D1 receptor antagonist SCH-23390 (but not the D2 receptor antagonist sulpiride) (Gilbert and Cooper 1985) and 6-OHDA lesions of the nigrostriatal DA system (Joyce and Iversen 1984) have also been found to attenuate the anorectic response to a low dose of amphetamine, confirming an involvement of DA.

If amphetamine reduces feeding by increasing DA release from axon terminals, and apomorphine reduces feeding by decreasing DA release, it might be expected that the two drugs would tend to counteract one another. The present experiment therefore examined this interaction.

5.3.1 METHODS

Fifteen individually housed rats (OLAC), mean weight 380g, were used in this experiment. Testing for food reinforced door pressing, was carried out between 12.00 h and 14.00 h daily. Initially, test sessions were 30 min in duration. When animals were performing asymptotically, 30 min sessions were used on drug administration days, and 15 min sessions were run on intervening days. Pharmacological studies were initiated following the attainment of asymptotic performance.

Amphetamine (0.5 mg/kg), apomorphine (0.05 mg/kg), and vehicle treatments were administered in a 2 * 2 factorial design. Amphetamine was given 30 min and apomorphine 10 min before the

test session. Each animal received all four treatment combinations in a counter-balanced order, at two-day intervals. Microstructural analysis was carried out as described in Chapter 3. The 25th percentile rather than the median IRT was calculated as this measure was particularly sensitive to amphetamine (Towell et al, 1987).

5.3.2 RESULTS

Apomorphine caused a 19% reduction in food intake ($F(1,28) = 4.7$, $p<0.05$), an effect similar in size to that seen at this dose in earlier studies (Chapter 4; Willner et al, 1985). Unlike amphetamine, apomorphine caused a substantial decrease in eating rate ($F(1,28) = 16.6$, $p<0.001$), reflected in a rightward shift in the IRT frequency distribution and a significant increase in the 25th percentile ($F(1,28) = 25.9$, $p<0.001$).

Amphetamine caused a reduction in food intake (21%: $F(1,28) = 6.9$, $p<0.05$) similar to that seen with apomorphine. However the effects of apomorphine and amphetamine were additive (Fig 5.3: inset): amphetamine reduced food intake by 21% after vehicle treatment and by a further 22% in the presence of apomorphine ($F(1,28) = 5.0$, $p<0.05$), the interaction term being insignificant ($F(1,14) = 0.05$, N.S.). Effects of the two drugs on the shape of the IRT frequency distribution were also additive (Fig 5.4), as were their effects on eating time (apomorphine/amphetamine interaction: $F(1,14) = 0.5$, N.S.) and eating rate (apomorphine/amphetamine interaction: $F(1,14) = 1.6$, N.S.), (results not shown in detail).

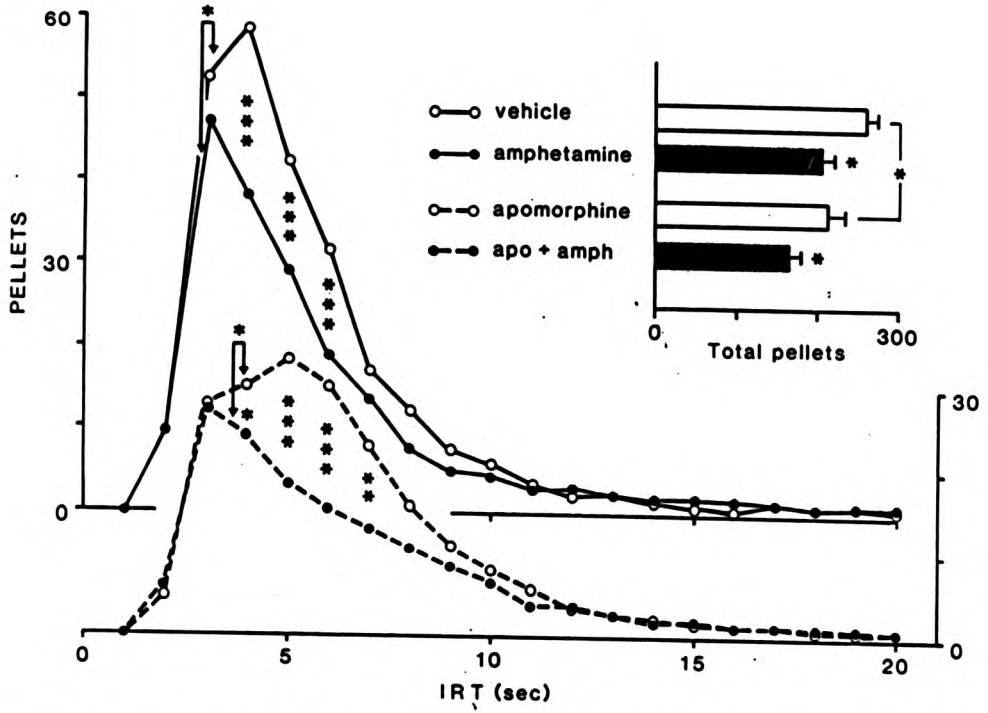



Fig. 5.3 Mean IRT frequency distributions for amphetamine (0.5 mg/kg) and apomorphine (0.05 mg/kg). For clarity, the two apomorphine curves have been displaced downwards; the vertical axis at the left refers to the upper curves while that at the right refers to the lower curves. The arrows mark the position of the 25th percentile of the frequency distribution. The inset shows total pellet intakes under the four conditions (mean and standard error). Stars show significant effects of amphetamine: One star, $p < 0.05$; two stars, $p < 0.01$; three stars $p < 0.001$.

5.3.3 DISCUSSION

The results of the present study rather surprisingly demonstrate that the effects of apomorphine and amphetamine were additive. It is difficult to see how this paradox might be resolved if the two drugs are acting at a common site. However, it seems likely that the effects of apomorphine and amphetamine are mediated by different population of DA receptors: since apomorphine anorexia is antagonized by the D2 receptor antagonist sulpiride but not by the D1 antagonist SCH-23390 (Expt. 5.2), while the reverse is true of amphetamine anorexia (Gilbert and Cooper 1985). A potential problem with this dichotomy as a possible explanation of the apomorphine and amphetamine-induced changes in eating rate is that pimozide reversed the effects of both these DA agonists. Pimozide has been reported to have a high specificty for D2 receptors in that it selectively blocked the inhibition of prolactin release by DA in the pituitary (Pinder et al, 1976) and has fewer side effects (acute dystonia and tardive dyskinesia) compared to haloperidol when administered in the clinic (Pinder et al, 1976; Runpiak et al, 1986). Nevertheless, pimozide is also among the most potent of drugs which inhibit dopamine-sensitive production of cyclic AMP in rat striatal homogenates (Miller et al, 1974). In addition, pimozide effectively reduced apomorphine and amphetamine-induced stereotypy (Janssen et al, 1968). These findings indicate that pimozide has also antagonistic actions at D1 receptor systems (Christensen et al, 1984), which may therefore provide an explanation for its antagonistic effects on both apomorphine and

amphetamine-induced anorexias.

Furthermore, it seems likely that apomorphine and amphetamine may reduce feeding by actions on different populations of DA neurons. The anorexic effects of apomorphine may be reliably elicited from the VTA but not from the substantia nigra (Willner et al, 1985). Amphetamine anorexia, by contrast, seems to be mediated by a system of fibres that originate in or around the substantia nigra (see Chapter 4, Introduction) and terminate in the lateral hypothalamus (McCabe et al, 1984). In addition, 6-OHDA lesions of the nigrostriatal sytem (Joyce and Iversen 1984) but not the nucleus accumbens (Koob et al, 1978) attenuated amphetamine-induced anorexia. The failure of amphetamine to antagonise apomorphine anorexia would suggest that the DA system originating in the substantia nigra is not involved in apomorphine anorexia thus supporting the hypothesis that it is the mesolimbic DA system that is responsible for the anorectic effects of apomorphine.

## 5.4 GENERAL DISCUSSION

The present series of experiments have demonstrated that following systemic apomorphine, the ensuing reduction in eating rate is mediated by:

1. Presynaptic DA receptors, since tetrabenazine prevented the effects of both pre- and postsynaptic doses of apomorphine on eating rate.

2. D2 receptors, as sulpiride (D2 receptor antagonist) but not SCH-23390 (D1 receptor antagonist) reversed the effects of a low dose of apomorphine.

3. The mesolimbic DA system, as amphetamine failed to attenuate the apomorphine-induced reduction in eating rate.

Observations 1 and 2, do not need any futher clarification, however, the last of these findings (3), may be open to different interpretations, in that presynaptic D2 receptors in the caudate-putamen could have contributed to the apomorphine response. This possibility remains open, because the DA system implicated in amphetamine anorexia originates in the substantia nigra but terminates in the lateral hypothalamus (McCabe et al, 1984). Following 6-OHDA lesions of the caudate, amphetamine failed to reduce food intake (Joyce and Iversen 1984). However the destruction by 6-OHDA of the DA fibres originating in the substantia nigra could have also lead to the destruction of cell bodies of the fiber system that projects to the lateral hypothalamus. The hypothalamus seems to have a high D1 receptor density (Dawson et al, 1986) whereas the caudate complex has a high density of both D1 (Dawson et al, 1986) and D2 (Gelhert and Wamsley 1985) receptor sites, the precise location of which still remains an enigma. Both the high D1 receptor density and the reversal by SCH-23390 of amphetamine anorexia (Gilbert and Cooper 1985) are consistent with an action in either the hypothalamus or the caudate nucleus. These sites would not, on the other hand, appear to be involved in the actions of

apomorphine.

While the apomorphine-induced reduction in eating rate is clearly mediated presynaptically, the position with respect to the apomorphine-induced reduction in eating time is less clearcut. Direct application of apomorphine to the VTA resulted in a reduction in eating time and bout length (Willner et al, 1985). However, systemic administration of high doses of apomorphine, in the presence or absence of tetrabenazine, also reduced eating time and bout length and also increased gap length (experiment 5.1; Willner et al, 1985). This leaves open the question of whether the reduction in eating time following a low dose of apomorphine is mediated pre or postsynaptically. This problem is resolved unambiguously by experiments described in the following chapter.

CHAPTER 6

## THE ROLE OF DOPAMINE AUTORECEPTORS IN APOMORPHINE ANOREXIA

Presynaptic inhibitory autoreceptors are selectively stimulated by low doses of DA agonists (Skirboll et al, 1979). Autoreceptor stimulation results in behavioural sedation (Di Chiara et al, 1976) and a concomittant reduction in food intake. The dopaminergic nature of this phenomenon is demonstrated by the attenuation of apomorphine anorexia following pretreatment with a variety of neuroleptic drugs (Chapter 4, 5; Willner et al, 1985).

The rate and time reducing effects of a low dose of apomorphine are abolished by the selective D2 antagonist sulpiride (Chapters 4 and 5) and the amine depleting drug, TBZ (Chapter 5). This would suggest that these effects of a low dose of apomorphine are mediated by presysnaptic DA autoreceptors. Administration of high doses of apomorphine also reduced eating rate and eating time. The reduction in rate following high doses of apomorphine was abolished by TBZ which would support the hypothesis that this effect of apomorphine was mediated presynaptically. However, TBZ did not abolish the reduction of eating time by a high dose of apomorphine, indicating a postsynaptic effect. On the other hand, eating time was selectively reduced by direct application of apomorphine to presynaptic receptors in the VTA suggesting a presynaptic site of action (Willner et al, 1985).

The precise site at which apomorphine reduces both eating time and eating rate, may be determined by central administration procedures. The hypotheses examined in these experiments were that the reduction of eating time by apomorphine is mediated by presynaptic receptors in the VTA, while injections of apomorphine to the nucleus accumbens would reduce eating rate via a separate population of DA autoreceptors located outside the VTA.

## EXPERIMENT 6.1: THE ROLE OF DA CELL BODY AUTORECEPTORS

The aims of the present study were, firstly to substantiate the findings that administration of apomorphine to the VTA selectively reduced eating time and secondly, to investigate whether systemic application of a low dose of apomorphine reduces eating time by an action at this site. The latter problem was investigated by administering sulpiride to the VTA. If apomorphine reduces eating time by an action on DA cell body autoreceptors in the VTA, but reduces eating rate by an action elsewhere, then the administration of sulpiride to the VTA should selectively antagonize the effect of systemic apomorphine on eating time while sparing the effect on eating rate.

### 6.1.1 METHODS

#### Surgery and Drugs

Eight rats (NIMR), weighing 360-400g, were implanted bilaterally under pentobarbital anaesthesia with cannulae aimed at the VTA. The co-ordinates, chosen according to the atlas of Pellegrino and Cushman 1967, were: anterior +2.9 mm, depth -3.4

mm and lateral + or -1.2 mm for VTA. The cannulae were of 26 gauge stainless steel (Arnold and Howell, London); injections through them were made using a microsyringe with a 33-gauge needle (V.A. Howe, London). At the end of the experiment, cannula placements were verified histologically (Fig 6.1).

Peripheral injections were made in a volume of 1 ml/kg: apomorphine was injected subcutaneously in the scruff of the neck 10 min prior to testing sessions; sulpiride was injected intraperitoneally 1h prior to testing sessions. All intracranial treatments were made up to a volume with sodium phosphate buffer (pH = 7.0) and injected at a volume of 0.44 ul, using a dispenser to deliver two 0.22 ul pulses, after which animals were immediately tested. Appropriate vehicle solutions were used for control injections.

Procedure

Behavioural testing was carried out between 14.00 and 16.00 h daily. Prior to surgery animals were given extensive training in feeding from a pellet dispenser; following a post-operative period of 14 days, pharmacological studies were not initiated until animals asymptotic performance had been re-established. In both parts of the experiment drug treatments were presented in a counter-balanced order with a minimum of two drug-free days between tests. The duration of test sessions was 30 min; on other days, the session length was usually 10 min.

In the first part of the experiment eight animals received apomorphine s.c.(0.05 mg/kg), sulpiride i.p. (10 mg/kg) or i.c. (4.4 ug bilaterally), and the appropriate vehicle injections, in a 2 * 3 factorial design. In the second part, which began after a 14 day interval, five of the animals received apomorphine i.c. (4.4 ug bilaterally), sulpiride i.p.(10 mg/kg), or vehicle injections, in a 2 * 2 factorial design. The other three animals from the first part could not be retested owing to movement of the implant.

Measures of eating rate and eating time were derived from log survivor analysis of the frequency distribution of inter-response times, as described in Chapter 3. Microstructural parameters were subjected to analysis of variance, supplemented where appropriate by tests of simple main effects; Wilcoxon tests were used to assess effects of apomorphine on median IRT.

### 6.1.2 RESULTS

In earlier studies, mean food consumption under control conditions in unoperated animals was of the order of 200-250 pellets, with substantially lower scores in operated animals, (Willner et al, 1985; see also earlier chapters). In the present experiment, performance in the first part was superior to that previously observed in VTA cannulated animals (mean pellet consumption + or - standard error = 157 + or - 11), and by the second part of the experiment, following a further recovery period, performance was in the lower end of the normal range (207 + or - 15). As, in each part of the experiment, the order of

presentation of conditions was counter-balanced, the changing baseline does not influence the interpretation of drug effects.

Systemic administration of apomorphine reduced food intake (Fig 6.2: $F(1,21) = 7.6$, $p<0.05$); by reducing both eating rate (Fig 6.2: $F(1,21) = 14.2$, $p<0.001$) and eating time, although in this experiment, the latter did not reach statistical significance (Fig 6.2: $F(1,21) = 0.5$, $p>0.05$). Sulpiride did not significantly affect feeding when administered alone, either peripherally or centrally, but sulpiride did substantially attenuate the anorectic effects of apomorphine (Fig 6.2: $F(1,28) = 16.5$, $p<0.001$; $F(1,28) = 13.2$, $p<0.001$) respectively. With peripheral sulpiride, attenuation of apomorphine anorexia was brought about by reversing the effects of apomorphine on both eating time and eating rate (Fig 6.2); after sulpiride pretreatment, apomorphine failed to alter either parameter ($F(1,21) = 0.3$, 0.3, respectively, $p>0.1$). By contrast, central sulpiride attenuated apomorphine anorexia by selectively increasing eating time (Fig 6.2: Apo vs Sul/Apo $F(1,21) = 4.8$, $p<0.05$), whilst sparing the effect of apomorphine on eating rate (Fig 6.2: Veh vs Apo $F(1,21) = 14.1$, $p< 0.001$).

Unlike peripheral apomorphine, central administration reduced food intake by a selective action on eating time (Fig 6.2: $F(1,8) = 8.2$, $p<0.05$); the rate of eating was unaffected (Fig 6.2: $F(1,8) = 0.1$, $p>0.05$). As in the first part of the experiment, sulpiride alone did not significantly affect feeding but sulpiride did attenuate the anorectic effect of central

apomorphine (Fig 6.2: $F(1,8) = 7.8$, $p<0.05$), by blocking the reduction of eating time (Fig 6.2: $F(1,8) = 7.5$, $p<0.05$).

As in earlier experiments, systemic apomorphine, which caused a decrease in eating rate as assessed by microstructural parameters, also caused a rightward shift in the IRT frequency distribution (Fig 6.3), with a significant increase in median IRT ($T(8) = 1$, $p<0.01$); central apomorphine, which did not decrease eating rate, did not change the shape of the curve (Fig 6.3), or significantly alter the median IRT ($T(5) = 5$, $p>0.05$). Systemic sulpiride blocked the effect of systemic apomorphine on eating rate, as assessed by microstructural analysis, and also blocked the rightward shift in the IRT frequency distribution (Fig 6.3: $T(8) = 13$, $p>0.05$). However, central sulpiride, which spared the effect of systemic apomorphine on eating rate, did not prevent the shift to longer IRTs (Fig 6.3: $T(8) = 1$, $p<0.01$).

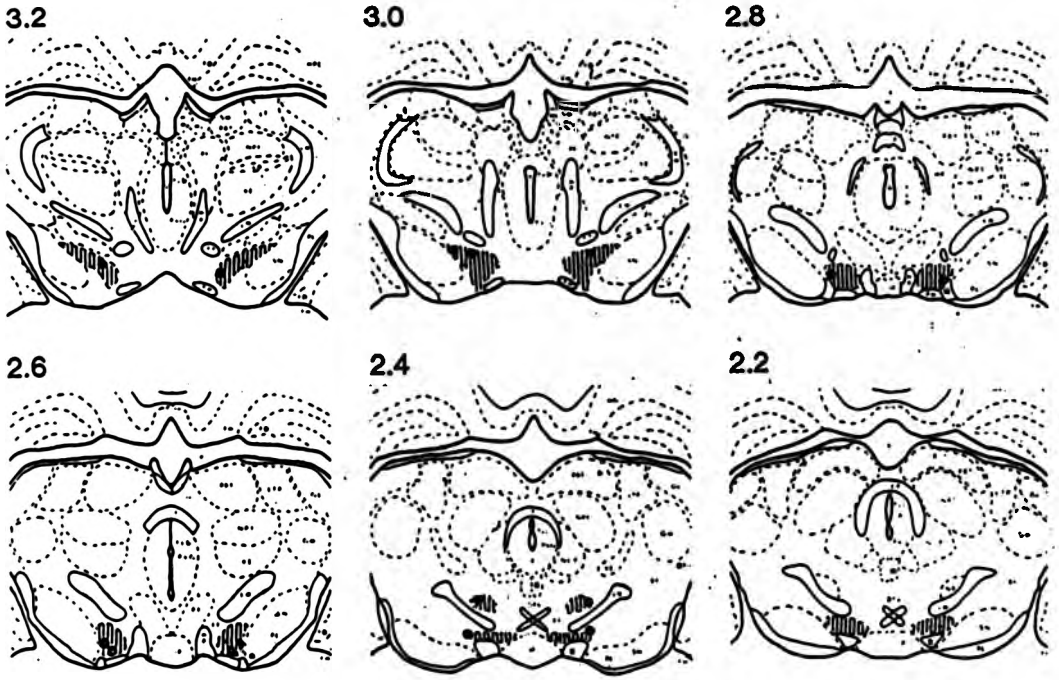



Fig. 6.1 Serial sections through the rat brain derived from the atlas of Pellegrino and Cushman (1967). The VTA has been indicated by shading. Cannula placements are shown by filled circles.

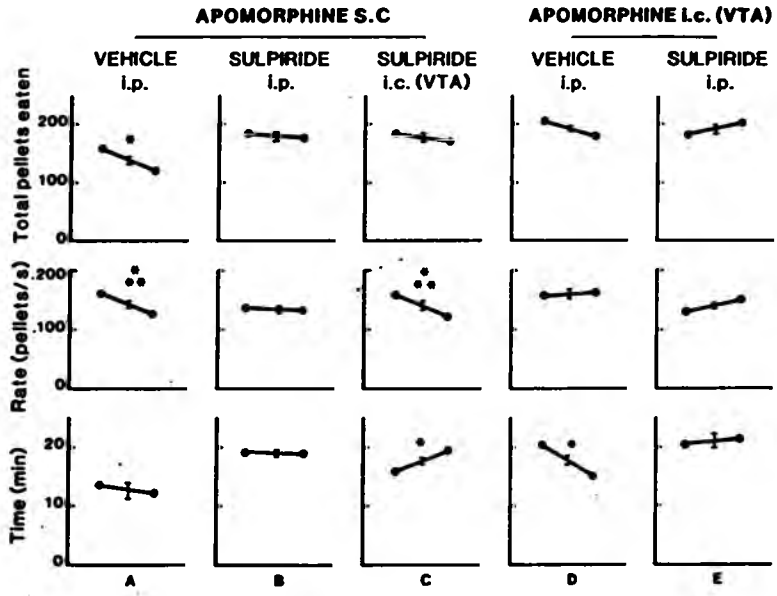



Fig. 6.2 The effects of apomorphine and sulpiride on mean food intake, eating rate and eating time. In every panel, control scores are shown on the left and scores after apomorphine on the right; error bars in the centre of the line joining control and apomorphine scores show the standard error of the difference between the two treatments. A, B and C show the effect of systemic apomorphine with no pretreatment (A), systemic sulpiride (B) and central sulpiride (C) pretreatment respectively. D and E show the effect of central apomorphine with systemic vehicle (D) and sulpiride (E) pretreatment respectively. Stars show the significant effects of apomorphine: One star, $p < 0.05$; two stars, $p < 0.01$; three stars, $p < 0.001$.

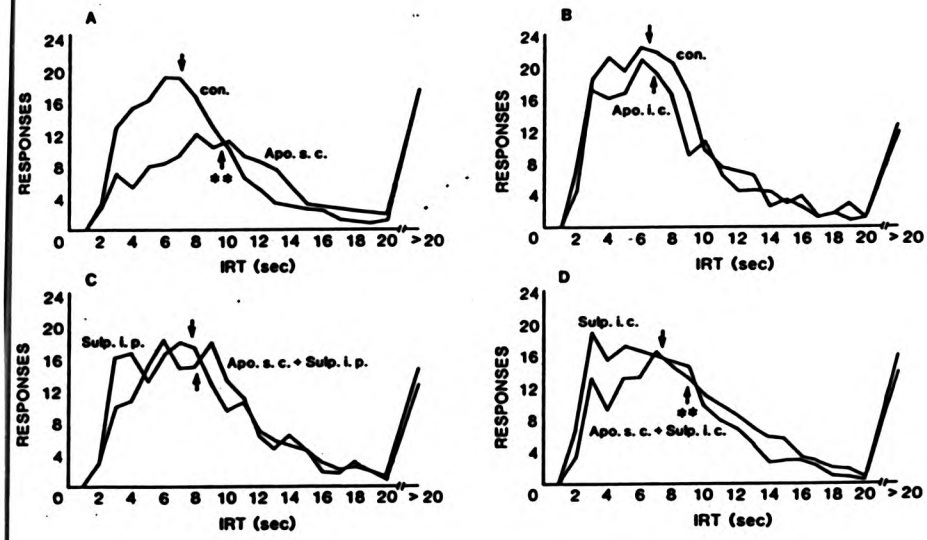



Fig. 6.3 IRT frequency distributions (mean of all five subjects). (A) systemic apomorphine; (B) central apomorphine; (C) systemic apomorphine and systemic sulpiride; (D) systemic apomorphine and central sulpiride.

### 6.1.3 DISCUSSION

These experiments confirm that the VTA is the site at which apomorphine reduces eating time, as suggested by the results of previous experiments (Willner et al, 1985): apomorphine infusions into the VTA selectively reduced eating time. In addition, sulpiride infusions into the VTA blocked the effect of systemic apomorphine on eating time only. This effect of central sulpiride provides strong evidence that systemic apomorphine reduces eating time through an action in the VTA.

In contrast to the clear effects on eating time, neither apomorphine nor sulpiride infusions into the VTA had any influence on eating rate, even though this parameter is more sensitive than eating time to the effects of systemic apomorphine (Chapters 4,5). The fact that systemic sulpiride did reverse the effect of apomorphine on eating rate, confirming the findings in Chapters 4 and 5, implies that the effect of apomorphine on eating rate is mediated by another population of DA receptors which is located outside the VTA.

## EXPERIMENT 6.2: THE ROLE OF DA AXON TERMINAL AUTORECEPTORS

The observation that TBZ abolished the rate reducing effects of apomorphine suggests that this effect is mediated by presynaptic DA receptors. The indirectly acting DA agonist amphetamine, which increased the rate of eating in the microstructural paradigm, failed to attenuate the rate reducing effect of apomorphine: 6-OHDA lesions of the caudate (Joyce and

Iversen 1984) but not the accumbens (Koob et al, 1978) attenuated the amphetamine-induced reduction in food intake. It would appear from these data that the rate reducing effects of apomorphine are mediated by presynaptic DA receptors in the mesolimbic projection from the VTA to the nucleus accumbens. A third and prominent DA pathway originates in the VTA and terminates in the frontal cortex; however, this system seems to be devoid of axon terminal autoreceptors (Bannon et al, 1982) and therefore is unlikely to be involved in this effect of apomorphine. That apomorphine infusions into the VTA did not have any effects on eating rate, effectively rules out the cell bodies of the mesocortical projection as the site of action.

The present experiment therefore investigated the effect on feeding of apomorphine applied directly to the axon terminals of the mesolimbic projection in the nucleus accumbens. The problem that arises with direct injections to this area is that unlike the VTA, the nucleus accumbens possesses both pre and postsynaptic receptor populations. However, stimulation of presynaptic DA receptors results in behavioural sedation (Di Chiara et al, 1976) while stimulation of postsynaptic DA receptors results in behavioural activation (Kelly et al, 1975). In order to evaluate the contribution of presynaptic and postsynaptic actions, apomorphine was administered at two doses and an attempt was made to relate the findings to effects on locomotor activity.

### 6.2.1 METHODS

**Surgery and Drugs**

Animals were implanted bilaterally under pentobarbital anaesthesia with cannulae aimed at the nucleus accumbens. For experiments 6.2A and 6.2B the co-ordinates, chosen according to the atlas of Pellegrino and Cushman 1967, were: anterior +8.0 mm, depth 0.0 - 1.0 mm and lateral + or - 1.0 - 3.5 mm. The cannulae were of 26 gauge stainless steel (Arnold and Howell, London); injections through them were made using a microsyringe with a 33-gauge needle (V.A. Howe, London). At the end of the experiment, cannula placements were verified histologically (Fig 6.4). All cannulae were located within the nucleus accumbens or ventral striatum, and adjacent to the anterior commisure. As a number of cannulae were somewhat posterior to the nucleus accumbens, a more anterior placement (+ 9.2 mm) was used for experiment 6.2C. These cannulae were all located within the nucleus accumbens (Fig. 6.4).

Sulpiride was injected intraperitoneally 1 hour prior to testing sessions. Intracranial injections of apomorphine were made bilaterally at a volume of 0.44 ul (two 0.22ul pulses) after which animals were immediately tested. Appropriate vehicle solutions were used for control injections.

**Procedure**

Behavioural testing was carried out between 14.00 and 16.00 h daily. Prior to surgery, the animals received extensive training in feeding from the pellet dispensers; following surgery,

pharmacological studies were not initiated until animals had re-attained asymptotic performance. In all experiments drug treatments were presented in a counter-balanced order with a minimum of two drug-free days between tests. Experiments 6.2A, 6.2B and 6.2C examined the effects of central apomorphine on feeding. The duration of feeding test sessions was 30 min; on drug-free days, the session length was usually 10 min. In experiment 6.2A eight animals received apomorphine (4.4 ug i.c., bilaterally), sulpiride (10 mg/kg i.p.) and the appropriate vehicle injections in a 2 * 2 factorial design. In experiment 6.2B twelve animals received apomorphine (2.2 ug i.c., bilaterally), sulpiride (10 mg/kg) or vehicle injections in a 2 * 2 factorial design. In experiment 6.2C, sixteen animals received three doses of apomorphine (0.0, 2.2 and 4.4ug i.c. * 2) through more anteriorly placed cannulae. For all three experiments, measures of eating rate, eating time and other microstructural parameters were derived from log survivor analysis of the frequency distribution of IRTs, as previously described.

Experiment 6.2D used ten of the animals previously tested in experiment 6.2B, following a drug-free period of eight days; experiment 6.2E used fourteen animals previously tested in experiment 6.2C, after a drug free period of ten days. The animals were tested in an open field: in the former experiment the apparatus consisted of a square wooden enclosure (75 * 75 * 23cm), ruled in 12.5 cm squares, with a smoked

perspex lid while in the latter experiment the enclosure was constructed from grey perspex (75 * 75 * 18 cm), and eight infrared beams at a height of 4.5 cm formed a 4 * 4 grid through which beam breaks were recorded automatically by a BBC microcomputer. Animals were habituated to the apparatus during a series of five daily six-minute test sessions. Following habituation animals received three central injections of apomorphine (0, 2.2 and 4.4 ug) in a counter-balanced order. Testing in the open-field commenced five minutes after the injection and lasted for six minutes; locomotor activity was assessed by the number of lines crossed (experiment 6.2D) or beams broken (experiment 6.2E); in experiment 6.2D, the incidence of rearing was also recorded.

### 6.2.2 RESULTS

**Experiment 6.2A**

The administration of apomorphine (4.4 ug bilaterally) to the nucleus accumbens significantly reduced food intake (Fig 6.5: $F(1,14) = 14.1$, $p<0.01$), by reducing both eating rate and eating time (Fig 6.5: $F(1,14) = 4.4$ and 3.6 respectively; $0.05<p<0.1$). Sulpiride (10 mg/kg) did not significantly affect feeding when administered alone, but did substantially attenuate the anorectic effect of apomorphine (Fig 6.5: interaction $F(1,7) = 7.4$, $p<0.05$). Effects of apomorphine on eating rate and eating time were both reversed by sulpiride pretreatment (Fig 6.5: interactions $F(1,14) = 6.1$, $p<0.05$ and 3.9, $0.05<p<0.1$ respectively).

The effect of apomorphine on eating time was further analysed by computing the mean duration of feeding bouts and of gaps between feeding bouts. Apomorphine did not change the duration of feeding bouts (means +/-standard error: control, 72 +/- 30; apomorphine, 67 +/- 28; T (8) = 0.1, p>0.1). However, apomorphine did significantly increase the duration of gaps (control, 32 +/- 4; apomorphine, 46 +/- 3; T (8) = 2.1, p<0.05). This effect is only seen with high ('postsynaptic') doses of apomorphine on systemic administration (Expt. 5.1; Willner et al, 1985).

Experiment 6.2B

Administration of apomorphine to the nucleus accumbens at a lower dose (2.2 ug) bilaterally produced a similar suppression of feeding (Fig 6.5: F(1,22) = 8.3, p<0.01). However, in this case, only eating rate was reduced (Fig 6.5: F(1,22) = 5.6 p<0.05), whilst eating time remained unchanged (Fig 6.5: F(1,22)= 0.4, p>0.1). As in experiment 6.2A, sulpiride by itself did not significantly change any parameter of feeding, but after sulpiride pretreatment apomorphine failed to reduce food intake or eating rate (Fig 6.5: F(1,22) = 0.8 and 0.2 respectively, p>0.1). Consistent with the effects on eating rate, apomorphine also increased the median IRT (Fig 6.6: Wilcoxon T (12) = 13, p<0.05), and sulpiride pretreatment blocked this effect (Fig 6.6: T (12) = 39, p>0.1).

Experiment 6.2C

Essentially similar effects of apomorphine were observed using the more anterior cannula placement (Fig. 6.7 A-C). Both doses of apomorphine significantly reduced food intake ($F(1,30) = 8.8, 16.3$; $p<0.01, 0.001$). The lower dose of apomorphine (2.2 ug) significantly reduced eating rate ($F(1,30) = 15.6$; $p<0.001$) but not eating time ($F(1,30) = 1.1$; N.S.); the higher dose (4.4 ug) reduced both eating rate ($F(1,30) = 5.9$; $p<0.05$) and eating time ($F(1,30) = 12.9$; $p<0.01$).

Experiment 6.2D,E

In the open field (Fig 6.8) the lower dose of apomorphine significantly reduced locomotor activity ($F(1,18) = 4.7$, $p<0.05$) and rearing ($F(1,18) = 8.5$, $p<0.01$). However, the higher dose did not significantly change either measure ($F(1,18) = 0.4$ and 0.0 respectively, N.S.). The same results were obtained in animals with more anterior cannula placements (experiment 6.2E: Fig 6.8): the lower dose of apomorphine (2.2 ug) significantly reduced locomotor activity ($F(1,26) = 8.5$; $p<0.01$), but the higher dose (4.4 ug) caused a non-significant increase ($F(1,26) = 1.6$; N.S.)

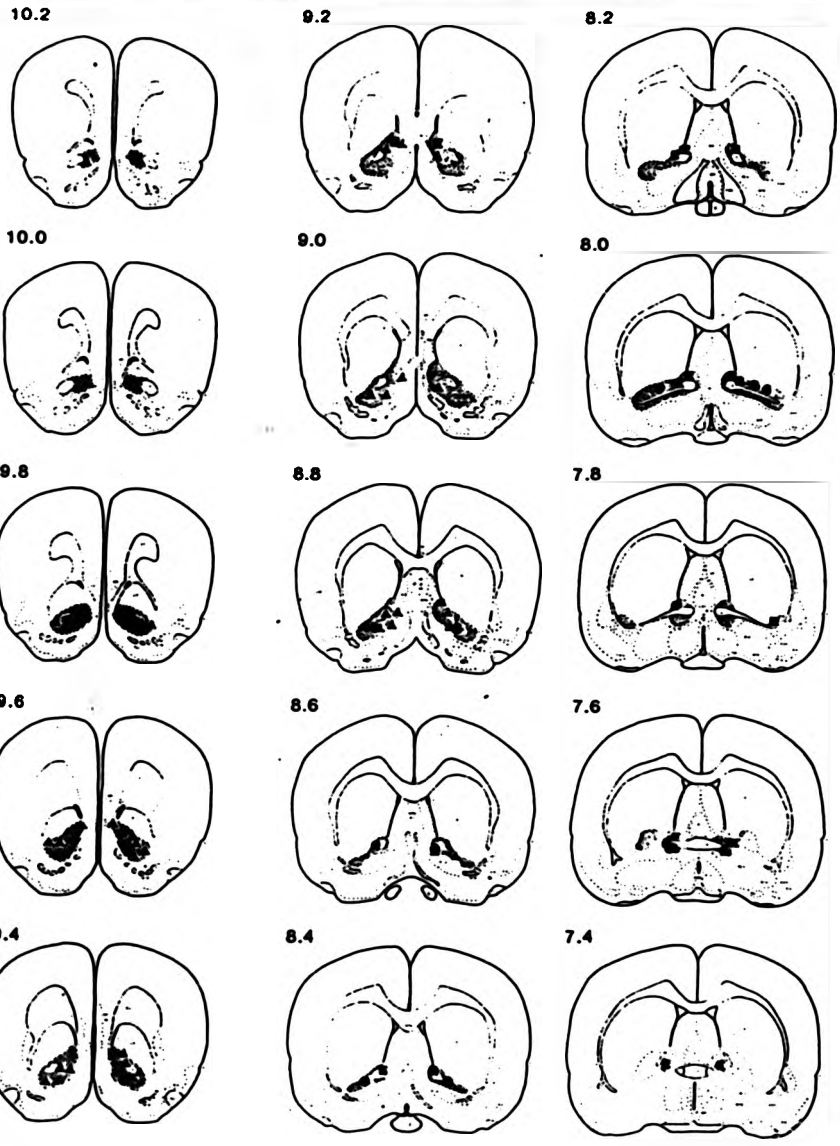



Fig. 6.4 Serial sections through the rat brain derived from the atlas of Pellegrino and Cushman (1967). The nucleus accumbens has been indicated by shading. Cannula placement is shown by filled circles (experiment 6.2A) filled squares (experiment 6.2B) and filled triangles (experiment 6.2C). According to the atlas of Pellegrino and Cushman (1967) the nucleus accumbens extends from anterior 10.2 to anterior 7.2; some authors consider sites posterior to 8.6 (approx.) to be in the ventral striatum (e.g. Beckstead et al, 1979).

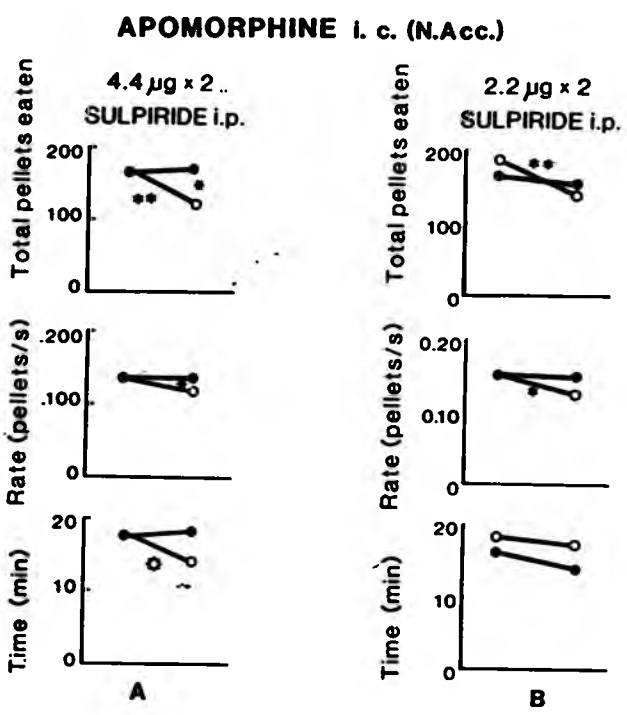



Fig. 6.5 The effects of central apomorphine and systemic sulpiride on mean food intake, eating rate and eating time. A and B show the effect of 4.4 and 2.2 ug apomorphine respectively. Scores are means (+ standard error). White, control; black, apomorphine. Pretreatments: VEH, vehicle; SUL, sulpiride. White star, $0.05 < P < 0.01$; one black star, $p < 0.05$; two black stars, $p < 0.01$.

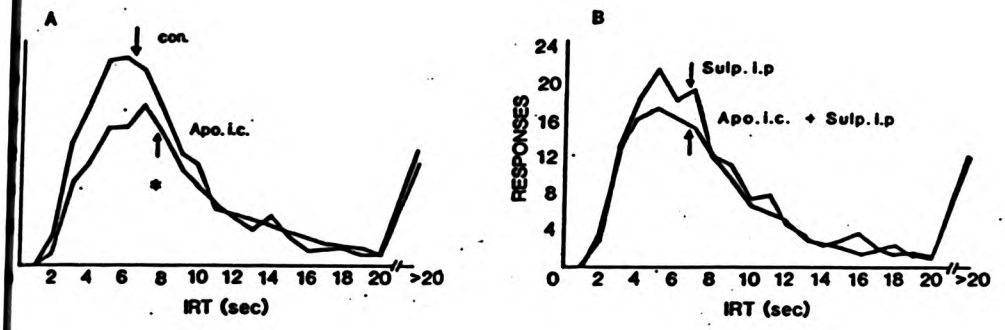



Fig. 6.6 IRT frequency distributions (mean of twelve subjects). (A) central apomorphine; (B) central apomorphine and systemic sulpiride. The arrows in each panel denote the median IRT.

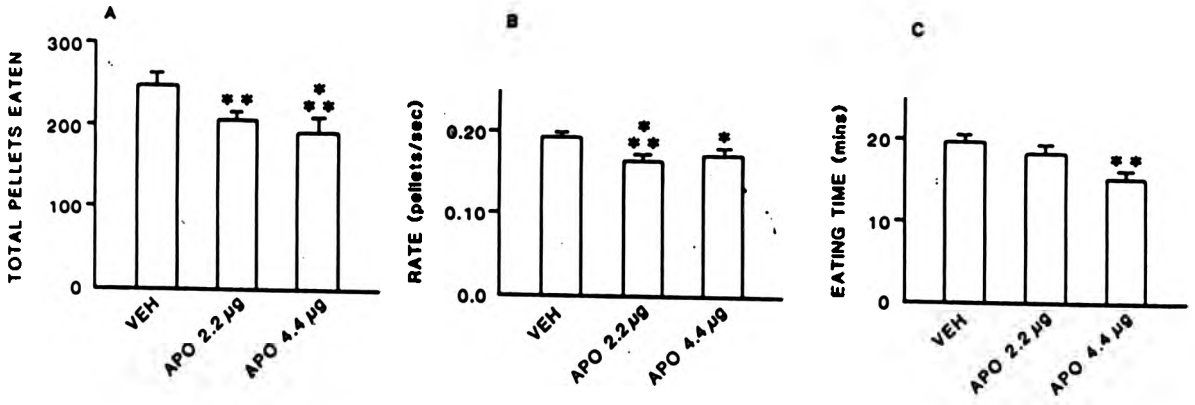



Fig. 6.7 Effects of central apomorphine on (A) total food intake (B) eating rate and (C) eating time, in animals with more anteriorly placed cannulae. Scores are means (+ standard error). One star, $p < 0.05$; two stars, $p < 0.01$; three stars, $p < 0.001$.

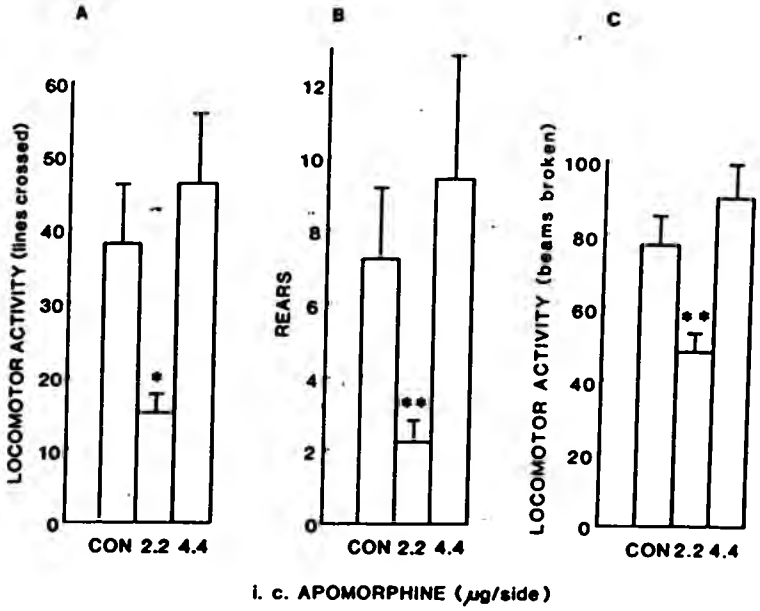



Fig. 6.8 The effects of two doses of central apomorphine on (A) locomotor activity and (B) rearing (experiment 6.2D) and (C) locomotor activity in animals with more anteriorly placed cannulae (experiment 6.2E). Stars show the significant effects of apomorphine. One star, $p < 0.05$; two stars, $p < 0.01$.

### 6.2.3 DISCUSSION

According to Pellegrino and Cushman (1967), the nucleus accumbens extends from anterior 10.2 to anterior 7.2; however, some authors consider sites posterior to 8.6 (approx) to be in the ventral striatum. Nevertheless, DA fibres from the VTA appear to terminate in this area (Beckstead et al, 1979; Simon et al, 1979). Examination of the data did not reveal any differences in behavioural effects systematically related to cannula placement. This was true for both feeding and locomotor behaviour.

The administration of the lower dose of apomorphine to the nucleus accumbens/ventral striatum selectively reduced eating rate. The reversal of this effect by sulpiride confirms the effect was mediated by DA receptors. The observation that sulpiride has some affinity for alpha 2 adrenergic receptors (Montanaro et al, 1982) does not undermine this conclusion, as the more potent alpha 2 antagonist yohimbine did not antagonize the effect of apomorphine on eating rate (Chapter 4). The fact that the reduction of eating rate by the lower dose of apomorphine was accompanied by behavioural sedation strongly suggests that at this dose apomorphine was acting presynaptically.

At the higher dose of apomorphine, the effect on feeding was less selective as both eating rate and eating time were reduced. Again, the reversal of these effects by sulpiride confirms the involvement of DA receptors in these phenomena. Earlier experiments have shown that systemic apomorphine alters

the duration of gaps between feeding bouts only at high doses thought to act postsynaptically (Expt. 5.1; Willner et al, 1985). The decrease in eating time caused by intracranial administration of the higher dose of apomorphine was brought by a selective increase in the duration of gaps. It is therefore suggested that stimulation of postsynaptic DA receptors might be responsible for this effect. An involvement of postsynaptic DA receptors at the higher dose of apomorphine is supported by the absence at this dose of sedative effects in the open field.

## 6.3 GENERAL DISCUSSION

The results of these experiments confirm the anatomical sites at which a low dose of apomorphine reduces eating time and eating rate. The low dose of apomorphine directly applied to the nucleus accumbens (or ventral striatum) selectively reduced eating rate without influencing eating time. Sulpiride the specific D2 receptor antagonist reversed this effect of apomorphine. The sedative effect of this dose of apomorphine was confirmed by the results of the open field study in which apomorphine reduced locomotor activity.

Although at the higher dose, apomorphine infusions into the nucleus accumbens reduced eating time, this effect is probably not involved in the reduction of eating time by a low systemic dose apomorphine, for two reasons:

1. The reduction in eating time following low doses of systemic apomorphine resulted from a reduction in bout length whereas the reduction in eating time following high doses of apomorphine

(i.c. or s.c.) was caused by a reduction in bout length and an increase in the gaps between bouts (gap length).

2. Sulpiride, the specific D2 antagonist reversed the effect of systemic apomorphine on eating time when administered directly to the VTA.

In that administration of a high dose of apomorphine to the nucleus accumbens brought about similar reductions in the microstructural parameters (reduction in eating time, bout length and an increase in gap length) to those seen following systemic administration of a high dose of apomorphine, it may be inferred that high doses of apomorphine reduce eating time by stimulating postsynaptic DA receptors in the nucleus accumbens.

As a result of these findings it would appear that eating time and eating rate are differentially controlled by two populations of autoreceptors, located, respectively on cell bodies and axon terminals of mesolimbic DA neurons. This suggests that autoreceptors on DA cell bodies and axon terminals may be differentially involved in behaviour even though pharmacologically both populations are of the D2 subtype. The implications of this dissociation are considered in Chapter 8.

CHAPTER 7

## CHANGES IN DA AUTORECEPTOR SENSITIVITY FOLLOWING CHRONIC ANTIDEPRESSANT TREATMENT

Antidepressant drugs are traditionally considered to act at noradrenergic or serotonergic synapses. However, a reduction of central dopamine turnover is a very robust finding in retarded depressions, and a number of DA receptor agonist drugs appear to be effective as antidepressants (Willner 1983). There is also more recent evidence that chronic treatment with antidepressant drugs or with electroconvulsive shock (ECS) causes a functional increase in dopaminergic transmission particularly in the mesolimbic system. One way in which chronic antidepressant treatment or ECS may bring about an overall increase in mesolimbic DA is by reducing the sensitivity of inhibitory presynaptic DA receptors. Serra et al (1979), have reported that small doses of apomorphine failed to elicit sedation in rats which had been chronically prtreated with imipramine, amitriptyline or mianserin. In addition, the administration of apomorphine failed to reduce DA synthesis in rats chronically treated with either antidepressant. The hypothesis that antidepressant treatment induces subsensitivity of DA autoreceptors is additionally supported by electrophysiological data. Chiodo and Antelman (1980, 1982), found that apomorphine administration selectively depressed the spontaneous activity of single DA cells in the pars compacta of the substantia nigra and the VTA, but following repeated administration of imipramine,

amitriptyline or iprindole, the inhibitory response to apomorphine was significantly attenuated.

However, these findings are controversial, in that a number of investigators have failed to observe DA autoreceptor subsensitivity following chronic antidepressant treatment. Holcomb et al (1982), failed to find any change in DA terminal autoreceptor sensitivity following chronic administration of imipramine or iprindole according to the same schedule as used by Serra et al, (1979). In a recent biochemical study it was reported that a number of antidepressants failed to alter DOPAC and HVA levels in the rat striatum following the administration of low doses of apomorphine (Diggory and Buckett 1984). Equally, Macneill and Gower (1982), were unable to find any change in apomorphine-induced inhibition of DA cell firing following chronic tricyclic antidepressant treatment. Indeed in one biochemical study it was reported that DA autoreceptor sensitivity was actually increased by chronic ECS (Reches et al, 1984).

In view of the potential theoretical and clinical significance of antidepressant effects on DA function, and the controversial nature of these observations, two experiments were carried out using the effects of apomorphine on the microstructural analysis of feeding behaviour to assess DA autoreceptor function. The first experiment examined the influence of DMI, amitriptyline and mianserin on the effect of apomorphine infusions to the VTA. The second experiment examined

the effects of systemic apomorphine in an animal model of depression.

EXPERIMENT 7.1: EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON DA CELL BODY AUTORECEPTORS

In previous chapters it has been demonstrated that microstructural analysis of the anorexic response to a low dose of apomorphine provides a sensitive behavioural assay of presynaptic dopamine receptor function and that the reduction of eating time by apomorphine appears to be mediated by DA cell body autoreceptors in the ventral tegmental area (VTA). Towell (1984), used these tools to assess the effects of chronic treatment with the antidepressant DMI on DA autoreceptor function. In the first of two experiments apomorphine was administered systemically while in the second study apomorphine was directly applied to the VTA. DMI did not change the effect of apomorphine on eating rate in either study, thus ruling out an effect of antidepressant treatment on DA terminal autoreceptor function. In addition, no consistent effects on eating time were seen during the course of chronic treatment with DMI. However, during withdrawal from DMI there was an attenuation of the effect of apomorphine on eating time, indicating a subsensitivity of DA cell body autoreceptors, which was clearly apparent in both experiments.

The implications of these findings are important in relation to the mechanism of clinical action of antidepressants. If DA autoreceptors were subsensitive during chronic DMI treatment,

then this effect might contribute to the clinical antidepressant action. However, if DA autoreceptors were subsensitive only following withdrawal of antidepressant treatment it would seem very unlikely that these effects would contribute to clinical improvements seen during treatment with antidepressant drugs.

The present experiment was a further attempt to clarify the status of DA cell body autoreceptors in the VTA during chronic DMI treatment. In addition to replicating these results of Towell (1984), the study also included two other antidepressants, the tricyclic antidepressant amitriptyline and the non-tricyclic antidepressant mianserin.

### 7.1.1 METHODS

#### Surgery and Drugs

Forty-eight rats (Olac) weighing 300g at the start of the experiment were divided into four groups (n=12). Each group received either vehicle injections (distilled water), or DMI, amitriptyline or mianserin at a dose of 2.5 mg/kg i.p.. Injections were given between 17.00 and 18.00h for forty-nine consecutive days. During the first forty days of treatment animals were run for thirty minutes daily in the operant chambers. The initiation of drug treatment was staggered over six consecutive days with two animals from each drug group commencing treatment on a given day. This treatment protocol allowed all animals to be implanted with cannulae on day 40 of drug treatment.

Cannulae aimed at the VTA were implanted bilaterally under pentobarbital anaesthesia. The co-ordinates, chosen according to the atlas of Pellegrino and Cushman (1967), were: anterior +2.9mm, depth -3.4mm and lateral + or - 1.2mm for VTA. The cannullae were of 26-gauge stainless steel (Arnold and Howell, London); injections through them were made using a microsyringe with a 33-gauge needle (V.A. Howe, London). At the end of the experiment, cannula placements were verified histologically (Fig 7.1). Following surgery, animals were retrained in the operant chambers until an asymptotic performance was re-established.

Procedure

On the day following the final antidepressant treatment, and on days three, six and ten during withdrawal animals received apomorphine (2 * 4.4ug i.c.) directly before the start of the session. Vehicle injections were given on the day before each apomorphine injection. All i.c. injections were made bilaterally at a volume of 0.44ul. Test sessions were thirty minutes in duration; on the intervening days, a ten minute session was run, with no drug treatments.

Measures of eating rate and eating time were derived from log survivor analysis of the frequency distribution of inter-response times, as described in chapter 3. To assess any differential effect of antidepressant drugs on control performance, an initial set of analyses of variance were carried out on data derived from the four vehicle injection sessions. Data were then transformed to represent the suppressive effect of

apomorphine on food intake, by subtracting the apomorphine score from its paired control score. These suppression scores were then subjected to analysis of variance. During the course of the experiment, the number of animals tested fell, as some of the implants were rejected (Fig 7.2). Rather than estimating scores for the missing subjects (which would be necessary for a 2-way analysis of variance), data for each of the four apomorphine tests were analysed separately by one-way analysis of variance. Where appropriate, analysis of variance was supplemented by tests of simple main effects.

### 7.1.2 RESULTS

None of the antidepressants tested significantly affected feeding scores on any of the four control days (max $F(1,37)$ = 3.5, 4.0, 2.8; $p > 0.05$ for totals, time and rate respectively). Over the four test days base line food intakes (mean +/- standard error) were: control: 170 +/- 15; amitriptyline: 156 +/- 16; DMI: 153 +/- 15; and mianserin: 162 +/- 14.

In control animals apomorphine reduced food intake on each of the four test days ($minF(1,8)=6.0$, $p<0.05$). Microstructural analysis revealed that the reduction of total food intake was mediated primarily by a decrease in eating time ($minF(1,8)=5.3$, $p<0.05$) with no significant changes in eating rate ($maxF(1,7)=3.3$, N.S.) These effects were of similar magnitude to those observed in experiment 6.1.

On the first test day (day forty-nine of treatment/ day one of withdrawal) none of the antidepressants significantly changed the reduction of total food intake by apomorphine. During withdrawal, however, all antidepressants attenuated apomorphine anorexia on all three test days (Fig 7.2). Taking each antidepressant separately, the effect of DMI was significant on all three withdrawal tests ($F(1,37) = 4.9$, $p<0.05$; $F(1,34) = 8.7$, $p<0.01$; $F(1,33) = 6.3$, $p<0.05$, respectively). Amitriptyline significantly attenuated apomorphine anorexia on day six of withdrawal ($F(1,34) = 8.3$, $p<0.01$) whilst the effect of mianserin was significant on day three of withdrawal only ($F(1,37) = 11.9$, $p<0.01$).

The microstructural analysis confirmed that these antidepressant-apomorphine interactions were mediated by effects on eating time rather than eating rate (Fig 7.2). The apomorphine-induced reduction in eating time was attenuated during the withdrawal phase of chronic drug treatment. This effect reached significance on day three of withdrawal from mianserin treatment ($F(1,37) = 14.9$, $p<0.001$), and on day six of withdrawal from amitriptyline and DMI treatment ($F(1,34) = 13.3$ and $5.6$, $p<0.001$ and $0.05$ respectively). On the final day of drug treatment, the suppression of eating rate was slightly increased by mianserin ($F(1,34) = 6.2$, $p<0.05$), but otherwise there were no significant effects of any antidepressant on eating rate.

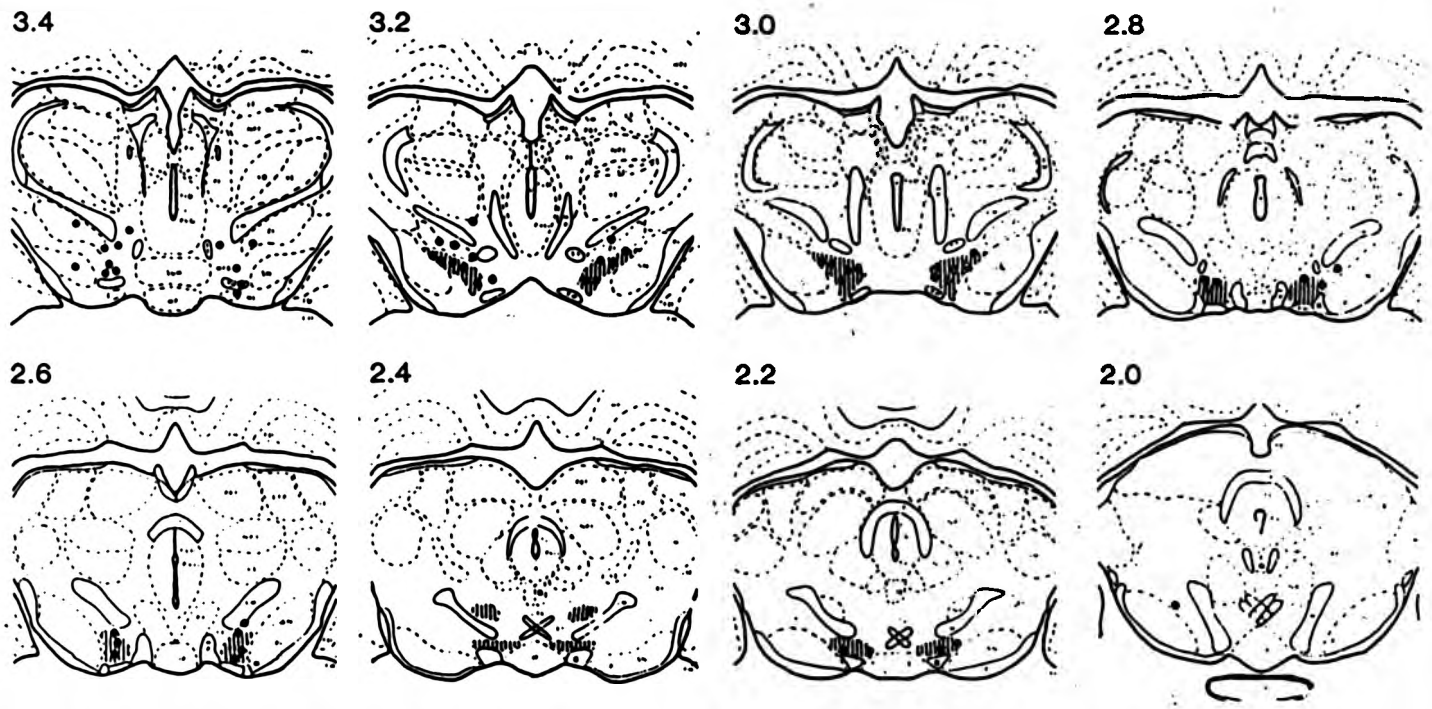



Fig. 7.1 Serial sections through the rat brain derived from the atlas of Pellegrino and Cushman (1967). The VTA has been indicated by shading. Cannula placement is shown by filled circles.

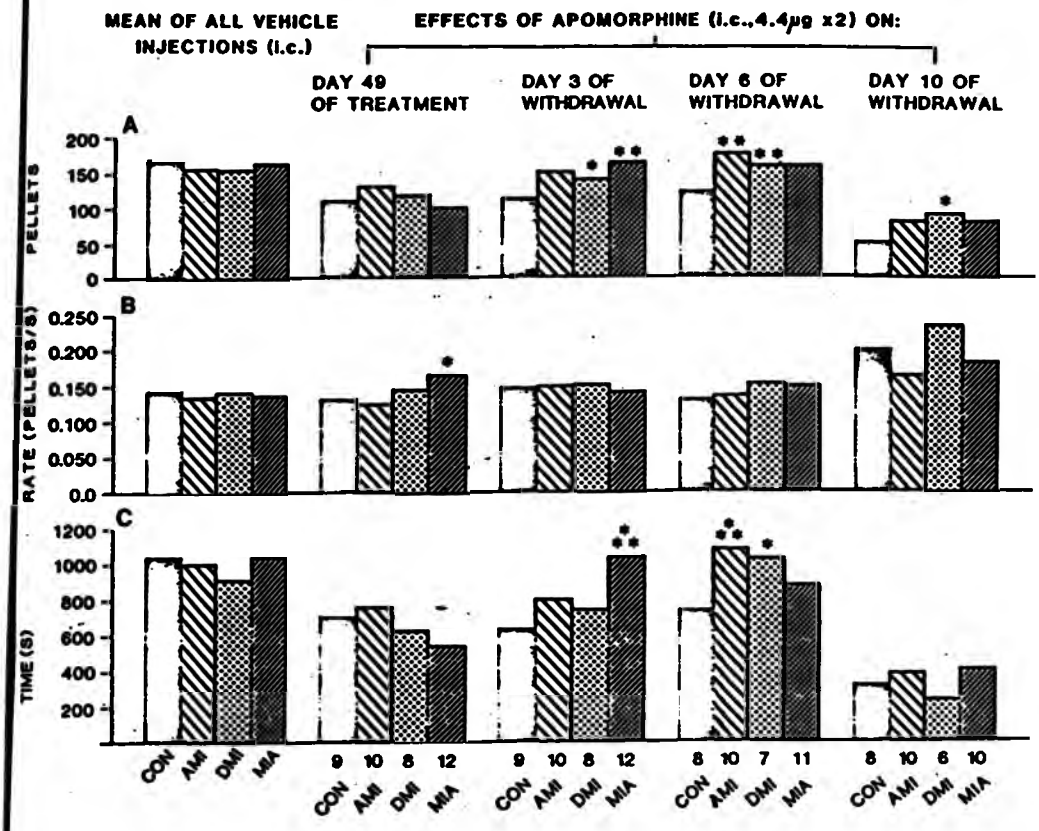



Fig. 7.2 Suppression of feeding by central apomorphine. A: Total food intake; B: Eating rate; C: Eating time. Scores are means. The set of columns at the left of each panel shows the means of data for the four control days; the other four sets of columns show the effects of apomorphine administered to the VTA. Figures below the columns show the number of animals tested on each occasion. Stars show significant differences between antidepressant and control treatments: one star, $p < 0.05$; two stars, $p < 0.01$.

### 7.1.3 DISCUSSION

The assumption underlying the present experiment was that anorexia caused by VTA infusions of apomorphine, and in particular, the apomorphine-induced suppression of eating time, may be used to asssay the responsiveness of DA cell body autoreceptors in the VTA (Chapter 6). Hence the attenuation of these effects represents a reduction in DA autoreceptor responsiveness. The present results confirm previous observations that this phenomenon is consistently seen during withdrawal from DMI not during chronic treatment, and extends this finding to amitriptyline and mianserin. In addition, DA autoreceptor subsensitivity was also absent on the first apomorphine test, which was 17-21h after the final antidepressant injection. In a recent study (Scavone et al, 1986) chronic imipramine (14 days) attenuated the apomorphine-induced reduction in locomotor activity and DA synthesis, but as in the present experiment, these effects were not observed until 48-72h following withdrawal.

The finding that DA autoreceptor subsensitivity only appears following withdrawal from chronic antidepressant treatment strongly suggests that this effect does not contribute significantly to the improvements seen in patients undergoing a course of antidepressant pretreatments. The question remains of whether the effect observed was an antidepressant effect which for some reason was masked by the presence of antidepressants or the opposite, a rebound "depressant" effect. However, it should

also be noted that these data may not be altogether relevant to the mechanism of clinical action of antidepressant drugs since this study was carried out in "normal" animals. It could therefore be argued that the antidepressant effects were observed "in vacuo". In line with this hypothesis is the observation that antidepressants failed to alter mood in normal subjects.

One way in which these issues could potentially be resolved is to ask whether DA autoreceptor sensitivity is abnormal in depression, and if so, whether the receptors are subsensitive or supersensitive. The following study examines this question in an animal model of depression.

## EXPERIMENT 7.2: EFFECTS OF CHRONIC DMI TREATMENT ON DA AUTORECEPTOR SENSITIVITY IN AN ANIMAL MODEL OF DEPRESSION

The majority of animal models of depression suffer from two major drawbacks; they are of questionable validity, and of brief duration (Willner 1984). An exception is a paradigm described by Katz. In this behavioural model, animals were subjected chronically and unpredictably to a variety of stressors, including electric shocks, immersion in cold water and others. Following a week of such treatment, deficits in open field performance were apparent, which could be prevented by a wide range of antidepressant drugs, but not by drugs of other classes. Indeed, as found in the clinical situation, chronic stress also

caused an increase in plasma corticosteroid levels, and this effect too was reversed by antidepressants (Katz 1981, 1984). This model has a reasonably high degree of validity (Willner 1984), and its prolonged duration makes it suitable to examine the effects of chronic treatment with antidepressant drugs.

Most of the studies using this model have examined open field behaviour and corticosteroid levels. However, in one study in which animals were exposed to the chronic stress regime, it was noted that the subjects failed to increase their fluid consumption when saccharin or sucrose were added to their drinking water (Katz 1982). This is a particularly relevant finding in that it implies a defective reward system, and models anhedonia which characterizes endogenous rather than reactive depression (Nelson and Charney 1981). The suggestion that chronic unpredictable stress produces a state analogous to endogenous rather than reactive depression is further substantiated by elevated corticosteroid levels observed by Katz and co-workers (Katz 1984; Katz and Hersch 1981; Roth and Katz 1981), since elevated adrenocortical activity is a specific diagnostic marker for the endogenous sub type of depression (Carroll 1982); while increased corticosterone levels are to be found in various other disorders such as Cushing's and Alzheimer's disease, within depression this effect seems to be specific to melancholia (Carrol 1982; Willner 1987).

We have developed a version of this model in which anhedonia is induced by exposure to ultra-mild stressors, such as periods of food or water deprivation and changes in temperature, lighting or housing conditions. Rats subjected to this regime showed substantial reductions in their preference for sweet solutions as measured in two-bottle tests; normal behaviour was restored by chronic administration of the tricyclic antidepressant DMI (Willner et al, 1987b). In the present study, these effects were replicated; in addition, DA autoreceptor sensitivity was examined at regular intervals during stress, DMI treatment, and withdrawal.

### 7.2.1 METHODS

#### Subjects

Forty eight male Lister hooded rats (NIMR, Mill Hill, London), weighing approximately 300 g at the start of the experiment were used. They were, with some exceptions described below, individually housed and maintained on a 12 h light/dark cycle, with food and water available ad libitum.

#### Fluid Consumption Tests

72 h before the start of the experiment, animals were given a continuous 48 h exposure to two bottles, one containing a 1% sucrose solution and the other containing tap water. The bottles were counterbalanced across the left or right side of the feeding compartment; this procedure was adopted throughout the experiment. Testing for fluid consumption was carried out in the animals home cage 6 h into the light cycle, between 14.00 and

15.00 h. Prior to testing, animals were food and water deprived for 23 h. Fluid consumption was recorded by reweighing preweighed bottles of test solution.

Feeding Tests

Consumption of 45mg food pellets was measured in operant chambers as described in Chapter 3. Testing was carried out between 14.00 and 17.30 h, after 21 h food deprivation; test sessions were thirty minutes in duration. Measures of eating rate and eating time were derived from log survivor analysis of the frequency distribution of inter-response times, as described in Chapter 3.

Stress Regime

The stress protocol consisted of 1. food and 2. water deprivation, 3. continuous lighting, 4. cage tilt (30 deg), 5. paired housing, 6. soiled cage (100 ml of water spilled into bedding), 7. exposure to reduced temperature (10 deg C), 8. intermittent white noise (85 db), 9. stroboscopic lighting (300 flashes/min), 10. exposure to empty water bottles following a period of water deprivation, 11. restricted access to food (scattering of a few 45 mg precision pellets in the animal's home cage), 12. novel odours (e.g. fresh air deodorant), 13. presence of foreign object in the home cage (e.g. piece of wood or plastic). The intensity of some of the stressors was gradually increased over the six weeks of testing (e.g. cage tilt increased from 30 to 50 deg; grouped housing increased to four per cage). These procedures were scheduled over a one week period in such a

way that at least one was in operation at any time, except during testing for fluid or food pellet consumption. Details of the scheduling of the stressors are shown Table 7.1.

Procedure

The animals were tested for their consumption of sucrose on Tuesday of each week (see Table 7.1). Following calculation of baseline intakes the animals were divided into two groups (n=24), matched for their sucrose and water consumption. One group was subjected to the stress regime, which was repeated for six consecutive weeks. Sucrose tests were readministered every Tuesday during the six weeks of stress, and for three further weeks. Every Thursday and Friday both groups of animals were tested in the pellet feeding procedure with vehicle injections on the Thursday and apomorphine injections (0.06 mg/kg s.c.) on the Friday. This dose of apomorphine was slightly higher than that used in experiments described in Chapters 4-6, in order to ensure a consistent and significant reduction of eating time throughout the three, three week phases (see below). At the end of the third week of stress, the groups were again divided (n=12) and matched for 1. sucrose and water consumption and 2. total pellet intakes. Each group received either DMI (5.0 mg/kg i.p.) or vehicle, for three weeks, according to a 2 * 2 factorial design. Testing was finally terminated three weeks into the withdrawal period so that the study was comprised of three, three week phases: phase 1. chronic stress, phase 2. chronic stress and DMI treatment, phase 3. withdrawal of both chronic stress and DMI.

Table 7.1

| | Food Depri-ation | Water Depri-vation | Continuous Lighting | Cage Tilt | Grouped Housing | Soiled Cage | Cold Room | Intermitt-ent White Noise | Strobo-scope Lighting | Empty Water Bottles | Restricted Access to Food | Odour | Foreign Object in Cage |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| am MON pm | 1500 | 1500 | | 1000 | | 1700 | 1500-1530 | | 1000-1200 | | | | |
| am TUES pm | TEST 1400-1500 | | 1700 | | | 1000 | | 1500-1800 | | | | | |
| am WED pm | 1700 | 1100 1700 | 1000 | | | | | | 1000 1700 | 1000-1100 | | | 1700 |
| am THURS pm | | 1000 | | 1000 1700 | 1700 | | 1000-1030 | | | | | | 1000 |
| am FRI pm | 1200 | | | | 1000 | | | 1200 1700 | | | 1000-1200 | 1700 | |
| am SAT pm | 1000 | 1000 | 1700 | | | | | | | | | 1000 | |
| am SUN pm | 1200 | 1200 | 1200 | 1700 | | | | | | | | | |

Analysis

All data were analyzed by analysis of variance, supplemented by tests of simple main effects. For the analysis of food consumption, eating time and eating rate, the nine sets of data were treated as 3 3-week phases (stress, stress + DMI, withdrawal). These analyses therefore had the following 5 factors: stress/control, DMI/vehicle, apomorphine/vehicle, phases (3), weeks (3).

For the analysis of fluid intakes it was not convenient to use the phases * weeks design as there were 4 test days in phase 1, but only 3 test days in each of phases 2 and 3. Sucrose preference scores (defined as [sucrose consumption / total consumption] * 100%) were therefore submitted to a 3-way analysis, using the factors stress/control, DMI/vehicle and weeks (10). The same design was used to analyze fluid consumption, with the addition of a fourth factor, sucrose/water.

7.2.2 RESULTS

Fluid Intakes

Application of the stress regime had no significant effects on water intakes ($F(1,88) = 0.02$, N.S.) but substantially reduced the consumption of sucrose ($F(1,88) = 52.5$, $p<0.001$) (Fig 7.3). The effect was already apparent after one week of exposure to stress ($F(1,440) = 13.9$, $p<0.001$). Sucrose consumption in vehicle-treated animals was even lower throughout the remainder of the stress period, and remained low for 2 weeks after termination of the stress regime (days 49, 56: $F(1,440) = 20.0$

and 12.5, $p<0.001$). In the final test session, 3 weeks after termination of the stress regime, sucrose consumption in these animals was still lower than in unstressed controls ($F(1,440) = 6.5$, $p<0.05$), though sucrose preference was normal on this final test (see below), owing to a small concomittant decrease in water consumption.

Unstressed animals administered DMI showed a small decrease in sucrose consumption after one week of treatment (day 28: $F(1,440) = 9.8$, $p<0.01$), but otherwise intakes of sucrose and water were unaffected by DMI in these animals. In stressed animals, DMI did not significantly alter fluid intakes during the first 2 weeks of treatment (days 28, 35: $F(1,440) = 0.03, 0.01$, N.S.), but an increase in sucrose consumption was seen after 3 weeks of DMI (day 42), compared to vehicle treated stressed animals. This difference was not quite significant ($F(1,440) = 2.9$, $p>0.05$), owing to an uncharacteristic rise in sucrose consumption in the vehicle treated group. However, large and significant differences in sucrose consumption between DMI and vehicle treated stressed animals were maintained throughout the withdrawal period (days 49, 56, 63: $F(1,440) = 22.8, 25.2, 13.4$, $p<0.001$).

The effects of stress and DMI were confirmed in analysis of sucrose preference scores (Fig 7.4). Sucrose preference was significantly reduced by 3 weeks of stress ($F(1,440) = 9.1$, $p<0.01$), remained low after 3 weeks of vehicle treatment ($F(1,440) = 7.2$, $p<0.01$), but was restored to a level not

significantly different from that of unstressed animals by 3 weeks of DMI treatment ($F(1,440) = 1.1$, N.S.). Sucrose preference in vehicle treated stressed animals had returned to normal by the final test session, 3 weeks after termination of the stress regime ($F(1,440) = 0.4$, N.S.), though as noted above, sucrose consumption was still abnormally low at this time.

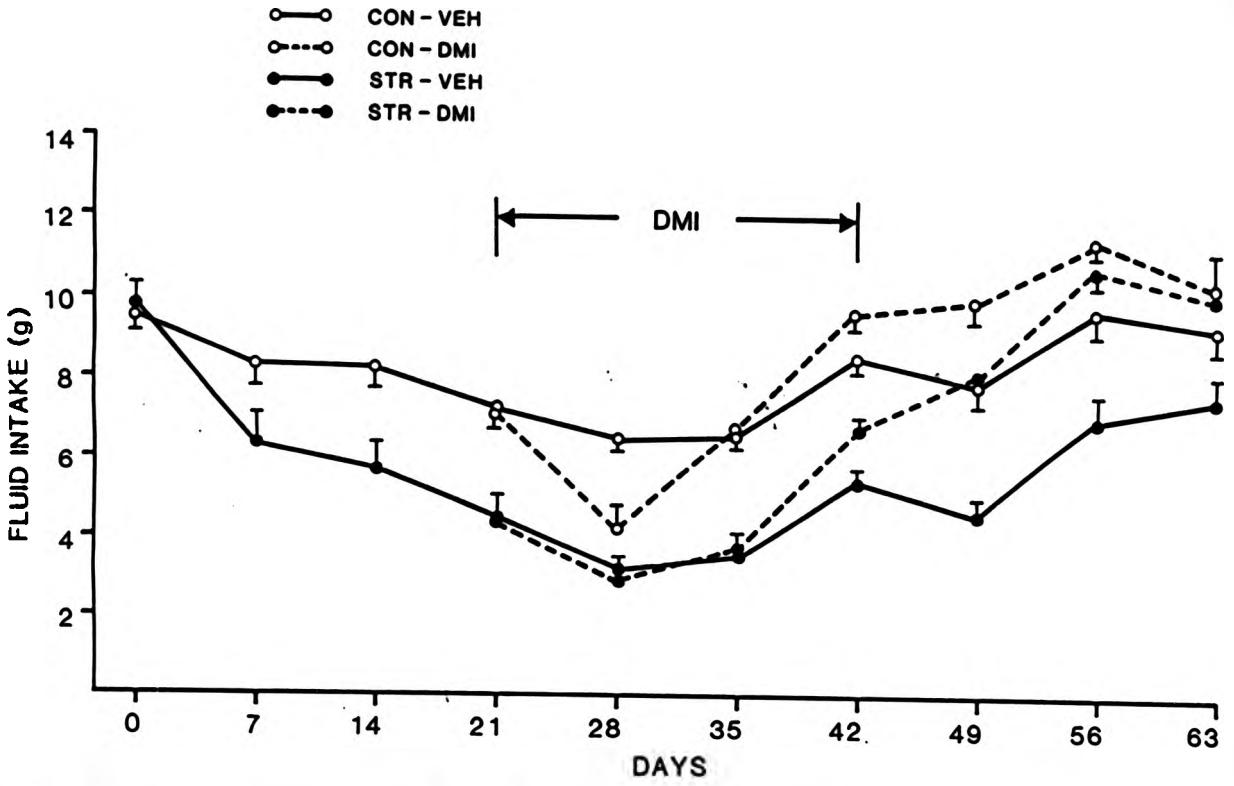



Fig. 7.3 The effects of stress and DMI on sucrose consumption. The results are expressed as means (+ standard error): CON - control, STR - stress, VEH - vehicle. DMI was administered from day 21 to day 42. For clarity, some standard error bars have been omitted.

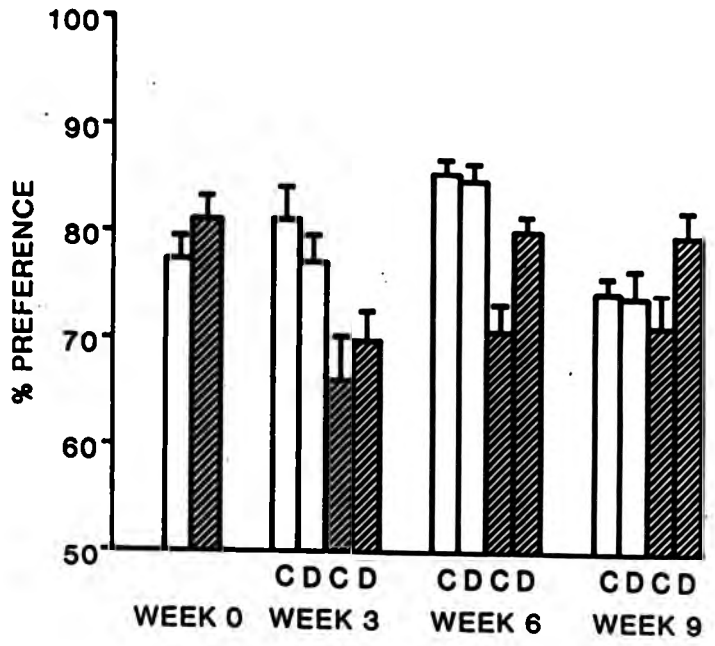



Fig. 7.4 The effects of stress and DMI on preference for sucrose (1.0%) over water. The results are expressed as means (+ standard error): open bars - control; closed bars - stress; C = vehicle pretreatment; D = DMI pretreatment.

Food Consumption

A preliminary analysis of food intake scores showed that on control days (i.e., those on which apomorphine was not administered), neither stress nor DMI significantly affected pellet consumption in any of the 3 phases of the experiment; the stress * DMI interactions were also non-significant. The 4 sets of data were therefore combined, for clarity of presentation (Fig 7.5A).

In vehicle treated, unstressed animals, apomorphine significantly reduced pellet consumption on every trial (min $F(1,220) = 13.4$, $p<0.001$). However, this effect was substantially attenuated in stressed animals. Higher pellet consumption in stressed animals (ie. a smaller suppression of feeding by apomorphine) was first seen in the third week of phase 1 (day 17: $F(1,220) = 12.0$, $p<0.001$), and continued, in vehicle treated animals, throughout phase 2 ($F(1,132) = 7.1$, $p<0.01$). Attenuation of the effect of apomorphine continued through the first 2 weeks of phase 3 (though not significant in week 7: $F(1,220) = 1.1$, N.S.; week 8: $F(1,220) = 4.2$, $p<0.05$), only returning to normal in the final week ($F(1,220) = 2.7$, N.S.).

DMI had a biphasic effect in unstressed animals, enhancing the effect of apomorphine in phase 2 ($F(1,132) = 10.7$, $p<0.01$), but attenuating the effect of apomorphine in phase 3 ($F(1,132) = 5.0$, $p<0.05$). The phase 2 enhancement was greatest in week 4 ($F(1,220) = 21.3$, $p<0.001$), substantially smaller in week 5 ($F(1,220) = 3.5$, $p<0.05$), and absent by week 6 ($F(1,220) = 0.1$,

N.S.). Similarly, the phase 3 attenuation was apparent in weeks 7 ($F(1,220) = 7.5$, $p<0.01$) and 8 ($F(1,220) = 5.6$, $p<0.05$), but absent in week 9 ($F(1,220) = 0.2$, N.S.).

In stressed animals DMI enhanced the effect of apomorphine throughout phase 2 ($F(1,132) = 12.1$, $p<0.001$): pellet intakes of DMI treated animals were lower than those of vehicle treated animals not only in weeks 4 and 5, but also in week 6 ($F(1,220) = 7.1$, 4.2, 6.4, $p<0.05$). In the final week of phase 2, therefore, stress attenuated the effect of apomorphine in vehicle treated animals ($F(1,220) = 5.5$, $p<0.05$), but not in DMI treated animals ($F(1,220) = 0.02$, N.S.).

Examination of microstructural parameters of feeding showed that the interactions of both stress and DMI with apomorphine were effected primarily by changes in eating time rather than in eating rate. Apomorphine significantly reduced eating rate (Fig 7.5B) in all 4 groups, in all 3 phases of the experiment (min $F(1,132) = 12.5$, $p<0.001$). However, with a single exception (week 6 in DMI-treated animals: $F(1,220) = 7.2$, $p<0.01$), all effects of stress and DMI on eating rate were non-significant.

By contrast, effects of stress and DMI on eating time (Fig 7.5C) were similar to effects on total food intake (Fig 7.5A), and if anything, more dramatic. While apomorphine consistently reduced eating time in vehicle treated, unstressed animals ($F(1,44) = 10.5$, $p<0.01$), this effect was totally abolished in vehicle treated stressed animals between weeks 3 and 8 inclusive:

on most of these tests apomorphine actually caused a non-significant increase in eating time (max $F(1,220) = 2.7$, N.S.). The normal reduction in eating time returned in the final test, three weeks after termination of the stress regime ($F(1,220) = 7.0$, $p<0.01$). Similarly, in DMI-treated unstressed animals, apomorphine failed to reduce eating time during the withdrawal phase (phase 3) of the experiment ($F(1,132) = 0.6$, N.S.).

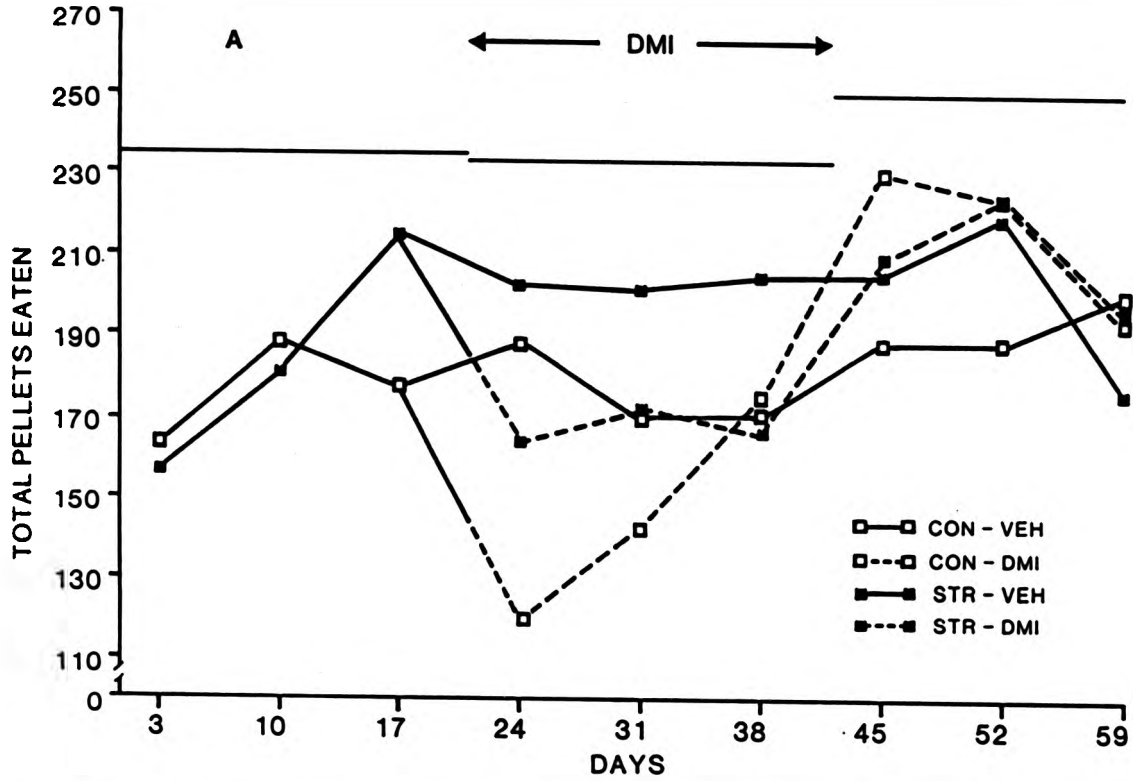
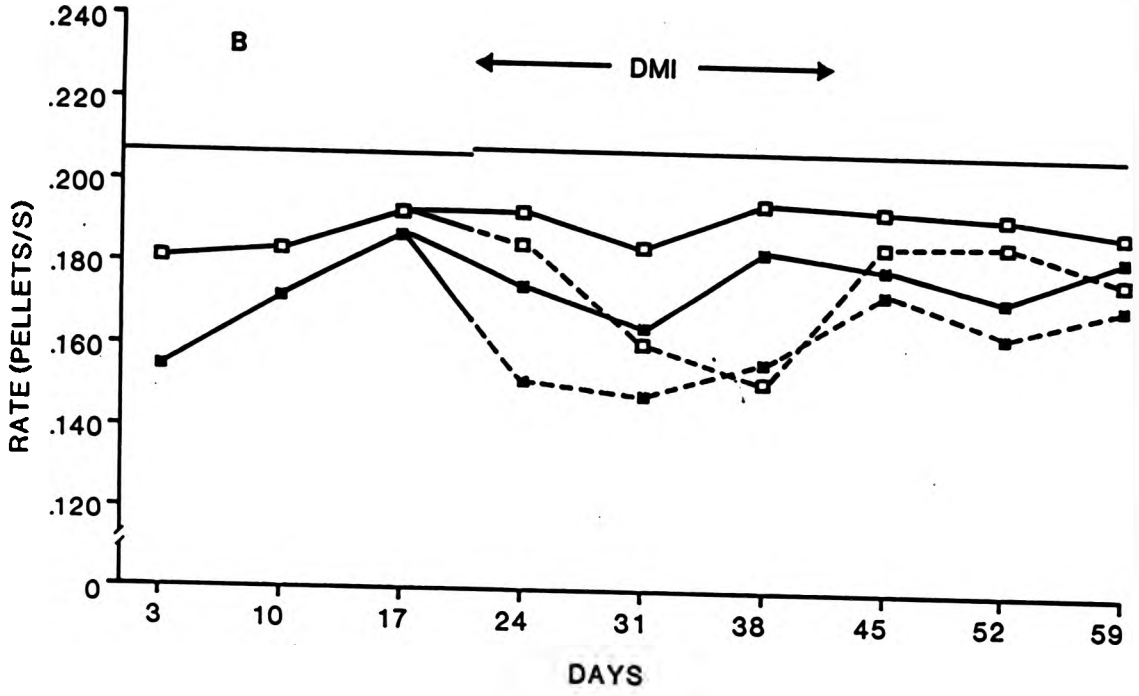
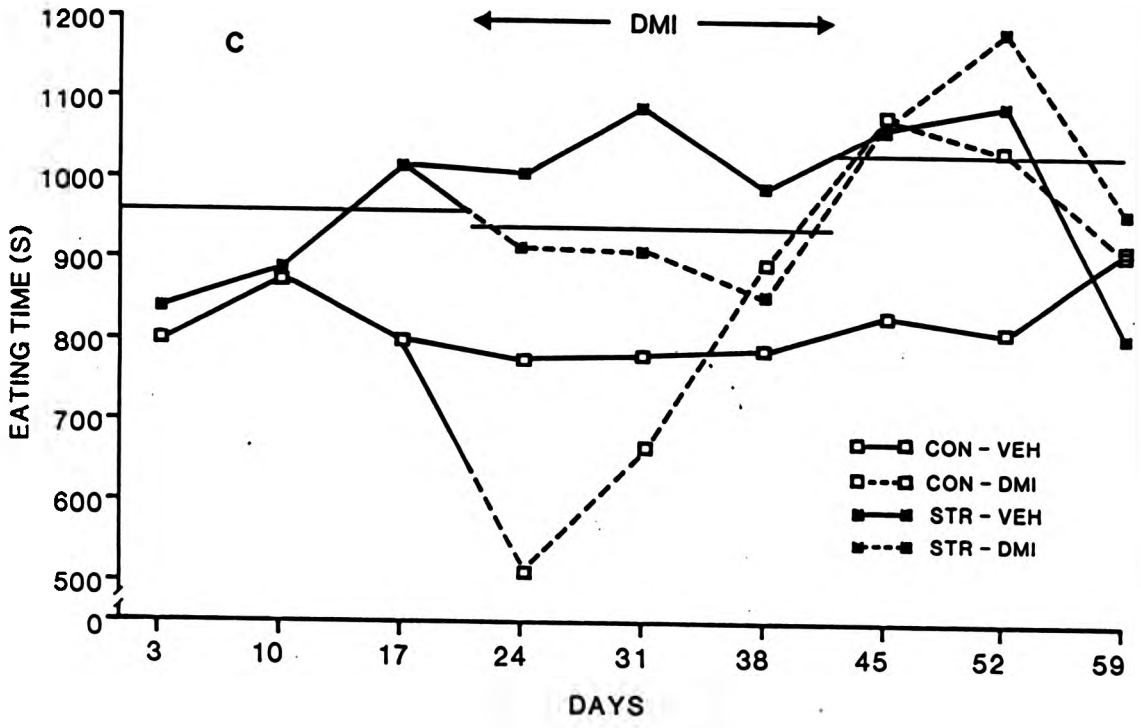



Fig. 7.5 The effects of stress and DMI on microstructural parameters of feeding following apomorphine administration (0.06mg/kg s.c.). A. total food intake B. eating rate C. eating time. The results are expressed as means: CON - control, STR - stress, VEH - vehicle. Horizontal lines show the mean of all vehicle treatments in each of the three three-week phases.


B
DMI
.240
.220
.200
.180
.160
.140
.120
0
RATE (PELLETS/S)
3
10
17
24
31
38
45
52
59
DAYS


C
DMI
EATING TIME (S)
1200
1100
1000
900
800
700
600
500
0
3
10
17
24
31
38
45
52
59
DAYS
CON - VEH
CON - DMI
STR - VEH
STR - DMI

### 7.2.3 DISCUSSION

In unstressed animals, DMI had a biphasic effect on apomorphine anorexia, initially enhancing the suppression of food intake and eating time, but attenuating the effect after withdrawal. These results replicate our earlier observations (Expt. 7.1, Towell 1984; Towell et al, 1986) and imply that during withdrawal from DMI, DA cell body autoreceptors in the VTA are subsensitive. However, an exactly comparable attenuation of apomorphine anorexia was also seen in stressed animals. In vehicle-treated stressed animals, this effect was present throughout the stress period; while in DMI-treated stressed animals, attenuation of the apomorphine effect disappeared at exactly the time their sucrose preference was begining to recover. These observations argue strongly against the hypothesis that the theraputic action of tricyclic antidepressant treatment results from the inhibition of DA autoreceptor function.

## 7.3 GENERAL DISCUSSION

Generalization for the results of experiment 7.2 to the clinical situation depends on the assumption that the animal model used to assess DA autoreceptor function is a valid one. The reduction in sucrose preference may reflect an underlying "anhedonia"; however, this behavioural deficit is open to other interpretations. The deficits in sucrose consumption may for example have occurred as a result of stress-induced decrements in caloric regulation. However, this explanation is ruled out by the observation that chronic stress produced deficits in both caloric

(sucrose) and non-caloric (saccharin) solutions (Willner et al, 1987b). It is also possible, that chronic stress might change the perceived stimulus properties of the sweet drinks to make them less palatable. There are however, no grounds, theoretical or empirical for believing this to be the case. A third possibility is that chronic stress somehow impairs the animals' ability to drink. This does not appear to be so, as saline consumption was unaffected by stress, even though total fluid intakes in unstressed saline-consuming animals were as high as in the sucrose-consuming control group (Willner et al, 1987). It seems more likely then, that the deficit in sucrose and saccharin preference is accounted for by a reduction in their rewarding properties than by sensory or motor changes. We have examined the possibility that this effect may be secondary to a stress-induced increase in blood glucose levels: if anything blood glucose levels were slightly lower in stressed animals (Willner et al, 1987b).

Acute pretreatment with DMI has been found to enhance the effect of apomorphine administered peripherally or centrally (Experiment 7.2; Towell 1984). The mechanism of these interactions is obscure. Tricyclics are known to potentiate the effects of high doses of apomorphine such as stereotyped behaviour (Menge and Brand 1971; Molander and Randrup 1976) and changes in blood pressure (Kadzielawa and Popielarski 1971). These effects of apomorphine seem to be mediated by DA systems as the potentiation of apomorphine stereotypy by tricyclics was

unaffected by pharmacological treatments which abolish NA transmission (Molander and Randrup 1976). There is also evidence that neither changes in the metabolism of apomorphine, nor the cholinergic effects of tricyclics, make a significant contribution (Molander and Randrup 1976; Rurak and Melzacka 1985). It is now accepted that tricyclics do in fact inhibit DA uptake (Halaris et al, 1975; Hytell 1978; Willner 1983), but it is unclear why this effect should enhance DA receptor stimulation by apomorphine. Consequently, there is as yet no satisfactory explanation for the synergistic effects of apomorphine and tricyclic antidepressants at postsynaptic or presynaptic DA receptors.

Following prolonged DMI treatment the enhancement of the apomorphine effects were greatly reduced. However, central administration of apomorphine provided no evidence that these effects of apomorphine were actually attenuated during chronic antidepressant treatment. Taken together, the present experiments (Expt 7.1 and 7.2) do not support the hypothesis that DA autoreceptors become subsensitive during the course of treatment with antidepressant drugs. The observation that DA autoreceptors on cell body/soma of the mesolimbic DA system are not subsensitive during chronic DMI does not in itself exclude the hypothesis of an increased DA output following chronic antidepressant treatment since such an effect could result from a desensitization of DA terminal autoreceptors. However, Holcomb et al, (1982) failed to observe any

changes in DA terminal autoreceptor responsiveness following chronic DMI. This in turn is consistent with the present finding (Experiment 7.2), that chronic DMI treatment failed to alter the reduction of eating rate by a low systemic dose of apomorphine. The evidence adduced in earlier chapters indicates that DA axon terminal autoreceptors mediate this effect.

In contrast to the acute and chronic effects of DMI the two experiments were consistent in confirming an attenuation of DA cell body autoreceptor responsiveness during withdrawal from chronic antidepressant treatment, which appears to be maximal after 6-7 days of withdrawal. While this conclusion may be drawn unambiguosly from the present model, this would not be the case, however in other behavioural models. In other models, therefore, it may be argued that the apparent absence of the sedative component may in fact have resulted from an increase in responsiveness of DA postsynaptic receptors (see eg. Serra et al, 1979). Chronic treatment with tricyclic and atypical antidepressants has been shown to enhance apomorphine and amphetamine-induced locomotor activity (Spyraki and Fibiger 1981), indicating that postsynaptic receptors in the mesolimbic DA system have become supersensitive. It should be noted however, that apomorphine in the present behavioural paradigm reduces food intake both at low doses which act at presynaptic DA receptors, and also at high doses which stimulate postsynaptic DA receptors (Chapter 5: Willner et al, 1985). Therefore any increase in postsynaptic DA receptor sensitivity brought about by chronic

antidepressant treatment would further reduce food intake. This effect was not observed in this study and hence rules out any contribution of an increased responsiveness of postsynaptic DA receptors following chronic antidepressant treatment to the attenuation of apomorphine anorexia. The effect may interpreted unambiguosly as a decrease in responsiveness of cell body autoreceptors.

It is at present unclear how to interpret the finding that DA autoreceptors are subsensitive in stressed animals displaying reduced sucrose preference. As DA autoreceptors are inhibitory (Skirboll et al, 1979), subsensitivity implies an increase in the activity of mesolimbic DA neurons. However, this conclusion is not easily reconciled with a decrease in the consumption of a highly rewarding solution since a considerable literature indicates that such an effect would involve a decrease in mesolimbic DA activity (Willner 1983; Wise 1982). It is possible, however that autoreceptor subsensitivity develops to compensate for chronically increased levels of dendritic DA release (Geffen et al, 1976). If this were the case, then autoreceptor subsensitivity might coexist with reduced DA turnover in terminal areas, since axonal and dendritic release of DA appear to be inversely related (Nieoullon et al, 1978, 1979).

Whatever the significance of a reduced DA autoreceptor sensitivity in stressed animals, the fact of its coexistence with a reduced sucrose preference strongly suggests that when the same effect is seen during withdrawal from antidepressant drugs, it is

not part of their spectrum of theraputic effects.

CHAPTER 8

## CONCLUDING DISCUSSION

### 8.1 SUMMARY CONCLUSIONS

The major findings of the present study may be summarised as follows:

1. Low doses (<0.1mg/kg) of apomorphine reduce food intake in the microstructural paradigm by reducing both the time spent eating and the rate of food consumption. These effects of apomorphine are mediated by central DA receptors of the D2 subtype.

2. Apomorphine reduced eating time by selectively stimulating cell body autoreceptors in the VTA. Apomorphine reduced eating rate by selectively stimulating axon terminal autoreceptors in the nucleus accumbens, one of the sites at which DA projections originating in the VTA terminate.

3. Using the apomorphine-induced reductions of eating time and eating rate to index DA autoreceptor function, chronic antidepressant treatment did not change the sensitivity of these receptors. However, following withdrawal, cell body autoreceptors were deemed to be subsensitive as there was an attenuation of the response to apomorphine on eating time.

4. This effect of antidepressant pretreatment does not appear to be relevant to the clinical action of these drugs.

The implications and detailed basis of these conclusions are now discussed.

## 8.2 APOMORPHINE ANOREXIA

In the microstructural paradigm, apomorphine reduced food intake by reducing eating time and eating rate. These effects of apomorphine were reversed by central DA anatagonists but not by the peripheral DA antagonist domperidone, or antagonists of other neurotransmitter systems (Chapter 4). This result, in effect, would suggest a homogenous interaction of apomorphine with central receptor systems. Indeed, the DA receptor involved in both of the responses to apomorphine is of the D2 subtype, as both effects were reversed by sulpiride but not SCH-23390 (Chapter 5). That these receptors are located presynapticaly was demonstrated following TBZ pretreatment. TBZ, which reduces DA availability by destroying terminal stores, inhibited the actions of low doses of apomorphine on eating time and eating rate. However, TBZ pretreatment failed to attenuate the reduction in eating time following a higher dose of apomorphine, which implies that at this dose apomorphine acts postsynaptically.

The observation that the reductions in food intake following apomorphine were prevented by TBZ, argues strongly against a postsynaptic site of action and in turn rules out the involvement of a special population of postsynaptic D2 receptors that are unusually sensitive to low doses of apomorphine. The existence of such a receptor population has been hypothesized by Gessa et al, (1985), on the basis that, in SCH-23390 pretreated animals, both low and high doses of apomorphine elicited a sedative response, which was antagonized by the D2 receptor antagonist, sulpiride. Presysnaptic DA receptors are several times more sensitive to DA

agonists (Skirboll et al, 1979), and following high doses of apomorphine stimulation of these receptors by endogenous DA is masked by the stimulation of postsynaptic receptors by the exogenous agonist (Wachtel et al, 1979). The fact that SCH-23390 unmasked the inhibitory response of a high dose of apomorphine would suggest that at this dose, SCH-23390 blocked the postsynaptic response but spared the presynaptic response (which is assumed to be mediated by D2 receptors). The findings reported by Gessa and colleagues are fully consistent with this formulation. There are no empirical data to support the notion of a hypersensitive population of sedative postsynaptic D2 receptors. Dawson et al, (1986), however, using autoradiogarphy techniques, reported that not only are there postsynaptic D2 and D1 receptors but also subpopulations of presynaptic D2 and D1 in various brain areas. The finding that SCH-23390 did not attenuate the effects of a presynaptic dose of apomorphine, shows that "presysnaptic" D1 receptors are not involved in this response.

That the responses to low doses of apomorphine on eating time and eating rate were mediated by presysnaptic DA receptors was confirmed by local administration procedures (Chapter 6). Apomorphine directly applied to the VTA resulted in a selective reduction of eating time; central administration of sulpiride to the VTA reversed the reduction in eating time but not eating rate following systemic apomorphine. In direct contrast to these findings, apomorphine infusions into the nucleus accumbens produced a reduction in eating rate but not eating time, and

behavioural sedation. Higher, non-sedative doses of apomorphine administered to the nucleus accumbens resulted in similar reductions in eating time and eating rate to those seen following administration of high systemic doses of apomorphine (Chapters 5 and 6). This in turn would suggest that both low and high doses of apomorphine reduce food intake by reducing eating time and eating rate. The pharmacological mechanisms by which these effects are brought about, are however, quite different; low dose effects are the result of the stimulation of presysnaptic DA receptors while the additional effects of high doses are brought about by an interaction of apomorphine with postsynaptic DA receptors.

Behaviourally, the observation that both low and high doses of apomorphine reduce eating time would suggest that DA agonists have a similar effects on feeding behaviour. On close examination of the data however, low doses of apomorphine appear to reduce eating time by reducing the length of feeding bouts whereas high doses also increase the latency to onset and the intervals between feeding bouts. The reduction in eating time following low doses of apomorphine results from a reduction in the impulse-induced release of DA into the synaptic terminal. In that reductions in DA availability result in a general decrease in behavioural activity (Beninger 1983; Heyman et al, 1986; Willner et al, 1987; Wise 1982), this would then explain why animals on apomorphine spend less time feeding. On the contrary, stimulation of postsynaptic DA receptors results in an increase in behavioural activity which in turn, from the above deductions,

ought to increase eating time. However, increasing doses of DA agonists are known to induce hyperactivity and stereotyped behaviours that are incompatible with feeding behaviour (Lyons and Robbins 1975). Following the administration of high doses of apomorphine the animals were not sedated and rearing behaviour was also in evidence, which would suggest that the animals spent less time feeding due to response competition. This effect of apomorphine was effectively picked up by the microstructural analysis of feeding in that an increase in the inter-bout intervals was observed. Similarly, Stahle and Ungerstedt (1986a), using an automated holeboard apparatus, noted that increasing doses of apomorphine reduced exploratory behaviour monotonically. The pre and postsynaptic effects of apomorphine could be reliably differentiated by the measurement of a number of behavioural characteristics including, activity, forward locomotion, rearing, hole exploration and corner-restricted behaviour. Low doses of apomorphine reduced most variables measured, however, they reported that the reduction of exploratory behaviour at higher doses involved qualitative change in the pattern of behaviour. The use of multivariate statistics demonstrated that a breakpoint may be determined for the transition from one typical pattern to the next; with increasing doses of apomorphine the change occurred at doses between 0.1 and 0.2 mg/kg.

The reduction of eating rate by apomorphine, appears to be caused by the stimulation of axon terminal autoreceptors. These receptors reduce DA availability in the synaptic terminal by

inhibiting its release by an interaction with calcium gating mechanisms (Geffen et al, 1976; Korf et al, 1976; Roth et al, 1983). Behaviourally, this effect of apomorphine on DA axon terminal autoreceptors, may be interpreted as a reduction in the effort the animals are prepared to sustain while feeding. The mesolimbic DA system has for some time been implicated in the activating aspects of positively reinforced behaviours (Beninger 1983). In addition, it has been proposed that the VTA is a site through which biologically significant stimuli converge and influence the firing rate of the mesolimbic DA neurons (Mogenson and Yim 1981). The information arriving at the nucleus accumbens is then influenced by inputs from other brain areas (eg amygdala), believed to be involved with motivational behaviour, before being relayed to the motor system. Typically neuroleptic pretreated animals fail to maintain responding for food or saccharin reinforcements beyond the first few trials (Geary and Smith 1985; Towell et al, 1987a; Wise et al, 1978). The performance decrement that results following administration of DA antagonists in positively reinforced procedures, has been interpreted as an inability to sustain effort (Neill and Justice 1981). In a similar way, animals on apomorphine were capable of responding but did so less vigorously while responding for food reinforcement.

It is therefore suggested that the reduction of eating time by apomorphine following the stimulation of cell body autoreceptors, resulted from a decrease in the firing rate of the mesolimbic DA system which in turn caused a reduction in response

maintainance. On the other hand, the stimulation of axon terminal autoreceptors by apomorphine reduced the effort expended by animals while responding for food, which was reflected by a reduction in the rate of consumption.

The hypothesis that DA autoreceptors subserve different behavioural functions is difficult to reconcile physiologically. One potentially relevant difference between axon terminal and cell body autoreceptor action is that the stimulation of cell body autoreceptors may alter the pattern of firing. A characteristic of DA neurons is that they have two active modes, a single spiking and a burst firing mode (Bunney et al, 1984). In addition, it seems that the activity of these neurons is interchangeable in the freely moving, undrugged animal (Freeman et al, 1985). Since bursting activity is highly correlated with an augmented DA release, it is tempting to speculate that as a result of this activity the effects of other inputs into the nucleus accumbens are diminished in order to maintain the animals attention to the currently ongoing behaviour. Amphetamine administration which results in a augmentation of DA release, produces response perserveration in the attentional switching model (Evenden and Robbins 1983, 1985), first described by Kristofferson (1967), to measure attentional switching in humans. In addition, 6-OHDA lesions of the nucleus accumbens antagonized the amphetamine induced performance deficits in this paradigm (Robbins et al, 1986). Apomorphine in the microstructural paradigm reduced eating time by selectively stimulating DA cell

body autoreceptors. This action of apomorphine may have resulted in an inhibition of bursting activity of these neurons and the concomitant augmented DA release, which was observed in the paradigm by a reduction in response maintainance.

As well as reducing eating time apomorphine also reduced eating rate, in this case by a selective stimulation of DA axon terminal autoreceptors. This effect of apomorphine is potentially more difficult to resolve. The reduction in the release of endogenous DA that results from the stimulation of these autoregulatory receptors may be more significant with respect to the single spike mode than the bursting mode. It is possible that the rate of ongoing behaviour is determined by a minimal level of DA in the synaptic terminal that is maintained by single spiking activity. This is supported by the observation that axon terminal autoreceptors do not become functional until DA levels in the synaptic cleft are such that reuptake mechanisms are saturated (Cubeddu et al, 1983).

This account of the function of mesolimbic DA neurons has little or no empirical data to support it. However, it does provide for a number of testable predictions. Firstly, using extracellular recording techniques it should be possible to show that the stimulation of cell body autoreceptors selectively reduces the number or frequency of spike trains. Secondly, if the fluctuation in DA release is monitored from moment to moment, stimulation of axon terminal autoreceptors should decrease the release of DA by single spikes, but not maximal

concentrations that result following a burst of spikes. It must be stressed again, that these assumptions are highly speculative. The second of the two hypotheses is not currently testable in that the preparations used to monitor DA in vivo have an insufficiently fine temporal resolution; with in vivo dialysis the time required to collect measurable samples is in the order of 20 min while electrical events occur in milliseconds (Zetterstrom and Ungerstedt 1984; Zetterstrom et al, 1985). However, the first hypothesis is currently testable and we are hoping to carry out the appropriate electrophysiological studies in the near future.

## 8.3 DOPAMINE AND ANTIDEPRESSANT FUNCTION

Since the recognition by Randrup et al, (1975), that DA systems might be involved in the pathogenisis of depression, a plethora of literature has evolved around the mechanisms by which antidepressant drugs may increase DA efficacy. The hypothesis examined in this thesis was that antidepressants exert their effects by reducing the sensitivity of DA autoreceptors and thus increasing DA output. No evidence for this hypothesis was forthcoming from the experiments described in Chapter 7.

Acute antidepressant pretreatment, consistently resulted (in all studies), in an amplification of the apomorphine response (Experiment 7.1; Towell 1984). The mechanism by which this effect is brought about is not known, but it may result from the increase in DA levels following the inhibition of DA uptake by antidepressants (Hytell 1979). This in turn would lead to an

increase in the stimulation of DA autoreceptors, and an enhancement of the apomorphine response on acute administration. Similarly, changes in the sensitivity of postsyanptic DA receptors following chronic antidepressant treatment, were only apparent following amphetamine administration. Antidepressant pretreatment resulted in the potentiation of amphetamine-induced hyperactivity but had no effects on basal actvity levels. (Spyraki and Fibiger 1981).

However, chronic antidepressant treatment did not cause any changes in the sensitivity of DA autoreceptors as determined by the response to low doses of apomorphine. This result is a further addition to the body of growing evidence that if antidepressants effectively increase DA efficacy in the clinical situation, the mechanism of action is not a reduction in the sensitivity of DA autoreceptors (Spyraki and Fibiger 1981; Spyraki et al, 1987).

Finally, both typical and atypical antidepressants reduced the sensitivity of DA cell body autoreceptors following withdrawal from treatment. Scavone et al, (1987), also reported that 72 h following the last antidepressant injection, animals failed to respond to a sedative dose of apomorphine in the locomotor activity paradigm. This result, in effect, should remove any remaining uncertainties as to the clinical relevance of a reduction in the sensitivity of presynaptic DA receptors since improvements in depressive symptomology are manifested during chronic antidepressant treatment rather than withdrawal.

A consistent finding in the literature is the observation that postsynaptic receptors in the mesolimbic DA system become supersensitive during chronic treatment with antidepressant drugs (Spyraki and Fibiger 1981; Spyraki et al, 1987; Willner 1987). In studies using the locomotor activity paradigm, this effect could be misinterpreted as a decrease in autoreceptor sensitivity (see eg Serra et al, 1979). However, supersensitivity of postsynaptic DA receptors would be demonstrated in the microstructural paradigm by a further reduction in food intake. In effect, the findings reported showed the contrary; following withdrawal of chronic antidepressant treatment there was an attenuation of the effect of apomorphine on food intake. Using this particular paradigm, therefore, unambiguous conclusions may be drawn in relation to the effects of antidepressant pretreatments on DA autoreceptor sensitivity.

However, the problem inherent in all these types of studies is that the effects of antidepressant pretreatments were monitored in normal animals. Indeed, the findings that receptor sensitivity may change over time, has resulted in the general acceptance that receptor systems are dynamic rather than static and therefore function homeostatically. Thus a reduction in transmitter output may be remedied by an increase in the sensitivity of postsynaptic receptors and an increase in the release of transmitter may be overcome by a down regulation of these receptors. Effects observed with antidepressant pretreatments in normal animals might therefore reflect this system of operation in action. Bearing in mind these receptor

modulations, the results of studies in normal animals provide only limited insight into the mechanisms by which antidepressant drugs may alleviate depressive episodes in the clinical situation.

## 8.4 DOPAMINE AND STRESS

It has been postulated that antidepressants may work by mimicking the process of adaptation to stress. Stone (1979), observed that the induction of stress or the inception of chronic treatment with antidepressants, both resulted in a reduction in the sensitivity of beta adrenergic receptors. The mechanism by which these treatments cause the subsensitivity of beta adrenergic receptors is somewhat different. Antidepressants increase NA in the synaptic terminal by inhibiting its uptake (Carlsson et al, 1969; Maitre et al, 1982) whereas stress reduced the activity of monoamine oxidase, the enzyme that degrades monoamines intraneuronally (Welch and Welch 1970); the overall result however, is an increase in intrasynaptic NA concentrations.

Acute stress is found to decrease both NA and DA levels by increasing the utilization and synthesis of these catechoalmines. It would appear that these adaptive changes occur in order to keep pace with immediate enviromental demands and therefore, assure adequate supplies of transmitter. Exposure to a number of stressful stimuli may result in a excessive stimulation of neuronal systems which may then result in a down regulation of postsynaptic receptors (eg beta adrenergic receptors) in order to

minimize the potential hazards of over stimulation. However, following repeated exposure to uncontrollable stress there comes a point when demand outstrips supply (Anisman and Sklar 1979; Weiss et al, 1980, 1981; Willner 1985) and then reductions in transmitter levels become apparent. The reduction in amine levels may then be held responsible for mediating the debilitating effects of stress on performance. The analogy that is consequential to these observations is that following antidepressant pretreatment, the reduction in sensitivity of DA autoreceptors may result from acute increases in DA levels. DA autoreceptor subsensitivity was not observed in normal animals except during withdrawal from treatment but in stressed animals a reduction in sensitivity of DA autoreceptors has been demonstrated (Antelman and Chiodo 1984). These observations would seem to suggest that it is not a good idea to compare neurochemical adaptations to stress with ones resulting from antidepressant treatments in normal animals. On reflection, even though the initial changes seem to involve an increase in transmitter availability, subsequently, the similarities cease to exist. It may therefore be advisable to look at the mechanism by which antidepressants influence DA receptor function in a framework that models the affective disorder in question.

Evidence is accumulating that chronic low grade stress may be important factor in the development of depression (Aneshensel and Stone 1982; Jahoda 1979); in particular a specific relationship to endogenous depression has been postulated (Willner 1985, 1987). One of the characteristic features of this subtype of

depression is a decrease in the performance of rewarded behaviours. In that a reduction in DA decreases responding for ICSS (Koob et al, 1978; Mogenson et al, 1979) as well as in other positively reinforced tasks, it follows that a decrease in DA function may be a good candidate to mediate some of the major dysfunctions seen in depression. We have developed a potential animal model of depression in which a reduction in sucrose preference has been brought about by exposure to ultra-mild, relatively naturalistic stressors. This effect was interpreted as a gradual development of anhedonia through the course of several weeks of exposure to the stress regime. Animals in this model when "depressed", displayed a reduction in DA autoreceptor sensitivity determined by the apomorphine response. This result would seem surprising in light of the evidence that a reduction in the sensitivity of these receptors would mean an increase in DA output.

However, an increase in DA transmission is not easily reconciled with the low preference for a highly rewarding solution. In other studies, we have demonstrated that drugs that block the action of endogenous DA, decreased sucrose preference in two bottle preference tests (Towell et al, 1987a). As described in Chapter 7, it is possible that the down regulation of cell body autoreceptors represent a homeostatic compensation for chronically increased levels of dendritic DA release (Geffen et al, 1976). Alternatively, DA autoreceptor subsensitivity may be secondary to the increased cholinergic activity that results from exposure to inescapable stressors (Aprison et al, 1975;

Finkelstein et al, 1984; Romano and Shih 1983). The fibre system that seems to sustain ICSS, originates in the lateral hypothalamus and the substantia innominata and terminates on DA cells in the VTA (Gallistel et al, 1981). The substantia innominata also receives a cholinergic input from the midbrain (Lewis and Schute 1978) and the inhibition of these neurons may result in a reduction in the stimulation of mesolimbic DA neurons, and might perhaps be responsible for a reduced sensitivity of cell body autoreceptors.

## 8.5 DOPAMINE, DEPRESSION AND ANTIDEPRESSANT FUNCTION

We have suggested that with the use of a valid animal model of depression (Willner et al, 1987) it may be possible to study the physiological effects of antidepressant agents on a clinically relevant time scale. In the chronic stress model, the typical antidepressant DMI, did attenuate the anhedonic deficits on chronic treatment. However, this effect did not result from a down regulation of DA autoreceptors. This observation casts serious doubts over the assumption that chronic antidepressant pretreatments increase DA efficacy by reducing the sensitivity of presysnaptic DA receptors. It may be that the reversal of the sucrose deficits in this model by DMI, result from a change in the sensitivity of postsynaptic DA receptors. This line of inquiry is currently under investigation.

It must be said however, that in our attempts to understand complex disease states such as depression, animals models have proved to be valuable on the understanding that they too

are limited in the amount of information they can provide. No model can seriously pretend to come up with all the necessary answers. However, unlike biochemical assays, behavioural models provide data that provide information about the overall functioning of neurotransmitter systems.

Finally, theories of depression should now incorporate a role for the mesolimbic DA system in the endogenous subtype of depression. The animal literature also reflects this point as there is a significant amount of evidence to suggest that there is more than a casual relationship between a reduction in DA function and endogenous depression. The DA autoreceptor hypothesis of antidepressant action should be discarded and the focal point of future research switched to the mechanisms by which antidepressant drugs are able to sensitize postsynaptic DA receptors. However, as far as presynaptic DA receptors are concerned, an effort should be made to understand how different behaviours may arise following the stimulation of cell body or axon terminal DA autoreceptors on the same population of DA neurons.

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TITLE BEHAVIOURAL MICROANALYSIS OF DOPAMINE AUTORECEPTOR FUNCTION

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