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BEHAVIOURAL ASSAYS OF THE EFFECTS OF ANTIDEPRESSANT DRUG TREATMENT ON THE FUNCTIONING OF CATECHOLAMINE SYSTEMS.

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ABSTRACT

TOWELL,A. BEHAVIOURAL ASSAYS OF THE EFFECTS OF ANTIDEPRESSANT DRUG TREATMENT ON THE FUNCTIONING OF CATECHOLAMINE SYSTEMS.

The effects of antidepressant drugs on central beta-adrenergic and dopaminergic receptor function were investigated using the anorexic effects of low doses of amphetamine and apomorphine as assays of beta-adrenergic function and presynaptic dopamine function respectively. Anorexia was typically examined using a microstructural analysis of feeding, which was validated observationally.

Amphetamine anorexia was characterized by a decrease in eating time and an increase in eating rate. At 0.5 mg/kg both beta-adrenergic and dopaminergic antagonists reversed anorexic effects, whilst anorexia at 1.0 mg/kg was reversed by dopaminergic antagonists only. An enhancement of amphetamine anorexia was seen following acute desmethylimipramine treatment; this effect was exactly compensated for over chronic treatment, implying no net change at beta-adrenergic synapses. However, applying amphetamine intracranially showed that approximately 75% of the acute enhancement of amphetamine anorexia was mediated peripherally, suggesting an attenuation of beta-receptor function during chronic antidepressant drug treatment. Some further data suggested that an alpha-adrenergic change could also have contributed to the antidepressant drug-induced attenuation of anorexia.

Low doses of apomorphine, specific for presynaptic dopamine receptors, induced an anorexia characterized by decreases in both eating time and eating rate. The dopamine receptor antagonists haloperidol and thioridazine reversed apomorphine anorexia by reversing eating time but not eating rate. Administration of apomorphine into nuclei A9 and A10 reduced total food intake and eating time but not eating rate. These findings imply that presynaptic dopamine receptors mediate the effects of apomorphine on eating time. Acute treatment with desmethylimipramine, enhanced apomorphine anorexia. During chronic treatment the apomorphine-induced reduction in eating time was sometimes attenuated, suggesting a presynaptic dopamine receptor subsensitivity. Anorexia was also enhanced following acute desmethylimipramine treatment with intracranial administration of apomorphine. Again, there was no clear evidence of subsensitivity following chronic treatment, but some evidence for subsensitivity in nucleus A10 during withdrawal.

The significance of a reduced beta-receptor function and an increased dopamine function following chronic antidepressant drug treatment are discussed in relation to the biological basis of depression.

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CHAPTER ONE

MONOAMINES AND DEPRESSION

1.1. INTRODUCTION

In the 1950s, a biological orientation to depression was adopted by many, which was based on the observations that drugs used to treat other medical conditions such as tuberculosis and hypertension could either elevate or depress mood. The first clinical trials of the tricyclic antidepressant drug (TAD), imipramine, were reported in 1957 (Crane 1957; Loomer et al 1957) after which many new TADs were synthesized and made available for prescription. TADs are now amongst the most prescribed treatments for depression. However, despite the immense research initiative into the psychobiology of depression, there is still uncertainty over the exact mechanism by which TADs and other antidepressant drug (AD) treatments work.

The incidence of depressive disorders is known to have increased in western society over the past few decades (World Health Organisation figures). This increase has been attributed partially to socio-economic factors, such as increased industrialization and the subsequent stresses it makes on our lives, and has also been partially attributed to the acceptance and acknowledgement of a role for medicine in psychological problems and mood disorders in our society. However, it is also clear that reports of an increase in the depressive disorders are at least in part dependent on the definition of depressive illness and the subsequent criteria used to diagnose such states.

1.2. DEFINITIONS AND CLASSIFICATIONS

Clinically, the term depression can refer not just to a state of depressed or lowered mood, but to a syndrome which comprises mood disorder, psychomotor changes, and a variety of somatic and vegetative distirbances. These changes may all be present, but none, including depressed mood, is essential. In fact, according to DSM III, the third edition of the American Psychiatric Associations Diagnostic and Statistical Manual (1980), the only essential criterion to diagnose a major depression is that the syndrome be present for at least two weeks. This system therefore encompasses 'masked depressions' where the patient often satisfies certain criteria but will not admit to being unhappy. It has been claimed that the DSM III system is a classification system which describes the clinical features of affective disorders (and other psychiatric disorders) comprehensively and at "the lowest order of inference necessary to identify the disorder in a reliable way" (U. Malt 1983) thereby avoiding the criticism of diagnostic invalidity due to overcomplexity. Even at this level though, depression can be seen to take a variety of forms.

For the purposes of both treatment and research, it is necessary to decide when the emotional state of feeling depressed (which we all feel from time to time) changes into an abnormal clinical symptom. It is clear that internationally acceptable criteria are needed to diagnose depression and in so doing, to further classify the depressions into specific sub-groups,

categories or dimensions- reflecting its heterogenous nature. Some clinical taxonomies relevent to the scope of this thesis, i.e. to the psychobiology of depression, will be discussed briefly. Such forms or subgroups of depression are based on symptomatic, familial and genetic characteristics, on biological measures or the therapeutic response or on outcome. It is worth noting that genetic, biological and outcome data are mainly used for research purposes and are not used routinely in clinical diagnosis.

1.2.1. BIPOLAR-UNIPOLAR

Two such subgroups characterized by these criteria are bipolar and unipolar depressions. These data are summarized in table 1. More recently, further subdivisions have been defined in bipolar illness (bipolar I, II and III) based on family history and the neccesity for hospitalization (Depue and Monroe 1979). The usefulness of this latter system has yet to be assessed clinically. It must be mentioned though that the unipolar-bipolar distinction does not necessarily reflect a dichotomy. The problem is that, unlike bipolar depressions, unipolar depressions are extremely heterogenous, and it may be possible that there are some unipolar diseases (endogenous, retarded; see 1.2.3) which are not phenomenologically distinguishable from bipolar disease. However, the diagnostic criteria used by the bipolar-unipolar taxonomy yield a high degree of concordance amongst clinicians; these criteria, unfortunately, do not encompass all the syndromes of affective disease, rendering the unipolar-bipolar distinction ineffective as a global taxonomy.

TABLE 1

SOME MAJOR DIFFERENCES REPORTED BETWEEN BIPOLAR AND UNIPOLAR DEPRESSIONS

	BIPOLAR	UNIPOLAR	AUTHOR
History of mania	Positive	Negative	Perris (1966)
Family history	Positive	Negative	Taylor and Abrams (1980)
Age of onset	Younger	Older	Taylor and Abrams (1980)
Sex distribution	Male=Female	Male Female	Taylor and Abrams (1980)
Hypomanic response to tricyclic antidepressant drug and l-dopa	Positive	Negative	Bunney (1978) Murphy et al (1971)
Antidepressant drug response to lithium carbonate	Good	Poor	Taylor and Abrams (1980)
Platelet monoamine oxidase activity	Decreased	Normal	Gerson et al (1979)
Psychomotor activity during depressive episode	Negative	Positive	Kupfer et al (1974)

1.2.2. PRIMARY-SECONDARY

This classification is based on the existence of a prior psychiatric history and was developed by Robins and Guze (1972) to define depression for research purposes. A primary depressive is a patient with an affective disorder who has had no pre-existing diagnosable psychiatric illness other than depression or mania. In contrast, a secondary depressive is a patient with an affective disorder who has previously had or currently has another diagnosable psychiatric illness. As this system is based on chronology and not on clinical features of the depression, it does not necessarily follow that primary and secondary depressions differ. In fact they do not; patterns of symptoms do not differ between the two groups (Wood et al 1977). This system does not appear to be consistent with other systems (e.g. Spitzer et al 1980), the exception being that secondary depression is rarely seen in bipolar patients (Akiskal et al 1979).

1.2.3. ENDOGENOUS-REACTIVE

The endogenous-reactive distinction is based primarily on aetiology. Reactive depressions are associated with attributable environmental and psychological precipitants whilst endogenous depressions have no clear psychological precipitant.

Multivariate statistical procedures have consistently provided evidence in support of endogenous depression as a distinct diagnostic category (Charney and Nelson 1981). Endogenous depression corresponds to the DSM III diagnosis of major

depression with melancholia, which is defined as the inability to experience pleasure, together with three of the following: distinct quality of mood, excessive or inappropriate guilt, marked psychomotor change, anorexia, early morning awakening and having more severe symptoms in the morning.

In addition to producing evidence to isolate endogenous depression as a diagnostic category, a number of different multivariate techniques have also shown that the symptom most strongly associated with endogenous depression is psychomotor change, in particular retardation, rather than severity of depressed mood or lack of reactivity (Nelson and Charney 1981).

Biological markers also provide some support for the endogenous/non-endogenous distinction. The most widely used of these is the dexamethasone suppression test. Depressed people have elevated cortisol levels in plasma and cerebral spinal fluid (CSF), due to the failure of the brain to inhibit adrenocorticotrophic hormone release. Dexamethasone is a synthetic corticosteroid, which suppresses adrenocorticotrophic hormone release. When dexamethasone is given to endogenously depressed patients, they usually show normal suppression of blood cortisol level. However, in depressed people, dexamethosone suppression of cortisol does not last as long as it does in normal controls. Depressed people therefore escape from suppression significantly earlier than normal. Escape from dexamethosone supprssion was seen in 43% of endogenous depressions, but only in 4% of non-endogenous depressions

(Carroll 1982).

A second biological marker of importance is a decreased rapid eye movement sleep latency, defined as the latency from the onset of sleep to the first period of rapid eye movement sleep. Like the dexamethasone suppression test, this change also appears to be specific for endogenous depression (Akiskal 1980).

It is noteworthy that the term 'reactive' was originally used in the context of depressions being 'reactive to treatment', as opposed to depressions being 'reactive to stress'; in this thesis the latter and more recent definition has been used. The endogenous-reactive distinction has also been used to encompass the psychotic/neurotic distinction which refers to clinical symptomatology, and the autonomous/reactive distinction which refers to the course of the depression (see Willner 1984, for a discussion of the current status of these distinctions). Multivariate analysis of symptomology supports the concept of a psychotic depression, but not a diagnostically homogenous neurotic depression. This is consistent with the view of some authors that neurotic depression is what endogenous depressions are not (e.g. Foulds 1975).

The distinction between endogenous and reactive depressions has been questioned as both types of depression are equally likely to be prec eded by pschosocial stressors (e.g. Brown and Harris 1978). The apparent presence of precipitants in one group and

their absence in the other, has been explained by two artefacts of reporting: endogenous depressives tend to underreport serious events as precipitants, because to some extent they see themselves as having been responsible, whilst reactive depressives tend to over-report events as precipitants, which may appear to an observer as being rather trivial inconveniences which are not related to their depression (Willner 1984c).

The previous discussion, which has reviewed some of the various taxonomies currently in use, emphasizes the heterogenous nature of affective illness. What is required, then, is a global taxonomy that can encompass the various classifications without losing diagnostic validity. DSM III, through its multiple axes or dimensions, provides the most comprehensive and up to date taxonomy available.

1.3. THE CASE FOR A BIOLOGICAL APPROACH TO THE TREATMENT AND STUDY OF DEPRESSION

With the recent onset of cognitive psychotherapies (Beck et al 1980; Kovacs 1980) that in some cases have proved as effective as some antidepressant drugs in the treatment of depression, it has become increasingly necessary to justify a biological approach to the research and treatment of depression. The starting point for such a justification is that although psychological and environmental factors are known to precipitate depressions to a great extent, the course of endogenous depression is subsequently largely independent of such factors. The exception being the claimed therapeutic treatment success of the cognitive

psychotherapies mentioned above. Furthermore, therapies which selectively change monoamine transmission are known to be clinically effective in the treatment of some depressions and therefore support a biological orientation towards depression. This finding is classically exemplified by the study of Klein and Davis (1969) who reviewed 65 reports comparing a number of tricyclic antidepressant drug (TAD) treatments and concluded that in approximately 80% of these reports TADs were more effective than placebos. Efficacy of the monoamine oxidase inhibitors (MAOIs) has generally been found to be less than that of the TADs, but these drugs may have specific indications in groups of atypical or hysterical depressions with prominent apathy and discouragement (mixed anxiety depression), (Paykel 1972). However, these drugs are not favoured over the TADs because of the risk of hypertensive crisis and convulsions induced by reactions to diets, such as the 'cheese reaction'. Another antidepressant treatment, electro-convulsive tratment (ECT), has been shown to be at least as effective as TAD treatment in the treatment of affective disease. The main disadvantages of ECT over TADs are the side effects it produces (e.g. retrograde amnesia); these iatrogenic symptoms which were thought to be transient are now thought to be more permanent (Kendall 1981).

Although a small literature implicates acetylcholine in depression, and an even smaller literature implicates other non-monoaminergic transmitters, by far the most work has concentrated on the monoamines- noradrenaline (NA), dopamine (DA) and serotonin (5-HT).

1.4. MONOAMINE HYPOTHESES OF DEPRESSION

1.4.1. THE CATECHOLAMINE HYPOTHESIS

The CA hypothesis of depression can be summarized by the proposition that depression resulted from a deficiency of NA at certain functionally important synapses in the CNS and that the action of AD treatments was to increase NA activity at these synapses (Bunney and Davis 1965; Schildkraut 1965). The hypothesis was attractive in that it provided an explanation of the pathogenesis of depression together with a proposed mechanism of action of the clinically effective TADs. It incorporated several lines of evidence, amongst them:-

(i) Reserpine (0.5 mg/day), used in the treatment of hypertension, induced a severe depression in 15-20% of patients receiving this drug, that was thought at the time to be clinically indistinguishable from 'true' clinical depression (Bunney and Davis 1965). This drug was postulated to cause depression via a depletion of functionally active CA systems (Carlsson 1961).

(ii) Around the same time, iproniazed and isoniazid, drugs used to treat tuberculosis, were found to produce an elevation in mood, contrasting with the lowering of mood often accompanying such a chronic illness (Loomer et al 1957). These drugs were postulated to act via the inhibition of monoamine oxidase, and were assumed to increase monoamine transmission in the brain.

(iii) Amphetamine, a drug acting primarily by increasing synaptic CA levels, was effective in elevating mood in normal subjects

(Lasagna et al 1955) and was thought to be an effective AD.

(iv) TADs were demonstrated to inhibit NA re-uptake into the presynaptic terminal, thereby enhancing NA transmission (Glowinski and Axelrod 1964).

(v) A few years later, 3-methoxy-4-hydroxyphenyglycol (MHPG), was identified as the main metabolite of noradrenaline in rat brain and subsequently in human CSF (Schanberg et al 1968). On the basis of metabolite levels, taken from a heterogenous sample of depressives, Schildkraut later proposed a system of diagnosis that encompassed a variety of postulated depressions, some of them being linked with schizophreniform symptoms (Schildkraut et al 1978). Maas et al 1968;

The CA hypothesis was valuable in two ways. First, it afforded a parsinomous explanation of the treatment and pathogenesis of depression in terms of the role of NA; and, secondly, it proved to have immense heuristic value in that it could be readily tested, permitting focused biochemical, pharmacological and clinical research.

1.4.2. THE DOPAMINE HYPOTHESIS

The CA hypothesis of depression was centred primarily on NA. Adrenalin, which was known to be poorly represented in the CNS (although more recent evidence would suggest established adrenalin pathways, Hokfelt et al 1978) gained little attention. DA, the precursor of NA, was not at that time (1965) widely accepted as a transmitter in its own right, and also gained little attention until the identification of its cell bodies and

pathways by Ungerstedt in 1971 (see chapter 2, section 2.3.1). Emphasis was therefore directed away from DA in the formulation of the CA hypothesis, although its neurotransmitter potential was well recognised in other clinical and laboratory contexts (Hornkiewicz 1966).

In fact a substantial amount of evidence cited in support of the CA hypothesis can be used in favour of a DA hypothesis - reserpine depletes DA by interfering with its storage (Carlsson et al 1957), monoamine oxidase inhibitors also protect DA from metabolism (Molinoff and Axelrod 1971), and in animals, amphetamine stimulation is blocked by DA antagonists such as pimozide but not by NA antagonists. Additionally, some depressed patients showed evidence of reduced central DA function as determined by homovanillic acid (HVA) concentration in the CSF (Goodwin et al 1973), though this was proposed to mediate the motor retardation often seen in depressed people, and was thought to be a secondary characteristic of depression (Praag et al 1971). However, TADs appeared not to block synaptic re-uptake of DA, except at relatively high doses. In consequence, DA did not gain much attention until Randrup et al (1975) published a review promoting a DA hypothesis of depression. This review cited evidence from a number of sources that DA depletion in people was associated with depression.

1.4.3. THE SEROTONIN HYPOTHESIS

The possibility that serotonin (5-HT) is involved in depression was raised following the observation that reserpine-induced sedation in rats was found to be associated not only with decreased levels of NA and DA but also 5-HT (Carlsson 1961). However, weighing against an involvement of 5-HT, was the finding that the precursor of NA (L-DOPA) reversed reserpine-induced sedation whilst the precursor of 5-HT (5-HTP) did not (Carlsson et al 1967). However, the additional observations that MAOIs also elevate 5-HT (Spector et al 1963) and that TADs block the uptake of both NA and 5-HT (Carlsson et al 1969) supported a 5-HT hypothesis.

Other lines of evidence have also suggested a role for 5-HT in the affective disorders. The earliest of these was probably the observation by Woolley and Shaw (1954), that 5-HT and LSD were structurally similar; they therefore speculated a role for 5-HT in the regulation of mood and associated disorders. In addition, the 5-HT precursor, tryptophan, was also thought to possess AD properties (Coppen et al 1963) and a reduction in 5-HIAA (5-hydroxyindoleacetic acid) in CSF was also observed (Ashcroft et al 1966) in a proportion of depressed patients and has subsequently been shown to be a consistent finding (Willner 1984c).

1.5. AN EVALUATION OF THE MONOAMINE HYPOTHESES

1.5.1. BIOCHEMISTRY

Shortly after the formulation of the monoamine hypotheses of depression, evidence began to emerge that was contrary to the predictions made by the hypotheses. A substantial amount of such evidence arose from biochemical studies attempting to measure levels of biogenic amines and their metabolites in blood, urine and spinal fluid of depressed patients. It has become increasingly apparent, that the measure of a single metabolite in a sample is dependent upon drug regime, age, sex, length of illness, time of testing, diet and many other variables. Worse, these metabolite levels may not reflect whole brain monoamine levels or activity.

1.5.1.1. NORADRENALINE

The original NA hypothesis of depression implies lower rates of generation and output of NA metabolites in depressed patients. MHPG is thought to be the major central metabolite of NA (Maas and Landis 1968, Schanberg et al 1968). Studies of CSF MHPG in depression have given largely negative results. A reduction of CSF MHPG has been reported in depressed patients (e.g. Subrahmanyam 1975), but others have failed to replicate this result (e.g. Berger et al 1980). However, it is possible that CSF measures of NA turnover may give a poor estimate of NA turnover in the brain (Bertillson et al 1982), whilst some peripheral measures of NA turnover might reflect central NA function better than lumbar CSF measures (Peyrin and Pequignot

1983).

The actual proportion of urinary MHPG originating in the human CNS is controversial; estimates vary between 20% (Blomberg et al 1980) and 80% (Ebert et al 1975). The usual finding in undifferentiated groups of depressed patients, or patients diagnosed as suffering from endogenous depression or primary affective disorder is a reduced excretion of MHPG (e.g. Puzynski et al 1980). A return to normal MHPG levels in patients who recovered from depression, but not in those who did not, has also been reported (Pickar et al 1978). MHPG excretion appears to be reduced during depressive episodes in some, but not all, bipolar patients (e.g. Beckman and Goodwin 1980) and in some unipolar patients (e.g. Pickar et al 1978). There appears also to be a group of unipolars in whom MHPG is abnormally high (Schildkraut et al 1981).

There is also some evidence that MHPG levels can predict responsiveness to different TADs. It has also been suggested that pretreatment urinary MHPG excretion may be predictive of TAD response (Maas et al 1972, Schildkraut 1971). Low levels of MHPG were found predictive of response to imipramine or desipramine in one study (Mass et al 1972), while higher levels of MHPG correlated with an antidepressant response to amitryptiline in another study (Schildkraut et al 1971). Furthermore, patients with low pretreatment MHPG responded to a challenge with amphetamine with a brightening of mood, and either no change or a slight increases in MHPG. Patients with normal or high

pretreatment levels of MHPG, when challenged with amphetamine, show reduced MHPG and no change in mood. On the basis of these findings, Maas has proposed two subtypes of depression, type A correlating with low MHPG and type B correlating with high MHPG. The type B findings have not been consistently replicated (e.g. Maas et al 1982; Mendewicz et al 1982), whilst the type A findings have proven to be more robust (e.g. Maas et al 1982; Schatzberg et al 1980).

1.5.1.2. DOPAMINE

Biochemical studies of DA turnover seem to be more consistent in that decreased concentrations of HVA are usually found in the CSF of depressed patients (e.g. Banki 1977), although this has not always been reported (e.g. Berger 1980). Probenecid blocks the active transport of organic acid metabolites such as HVA out of the CSF and thereby raises relative concentrations. Use of this drug has yielded the more consistent finding of decreased CSF HVA accumulation in some or all depressed patients (e.g. Berger 1980). Decreased post-probenecid CSF HVA accummulation is a particularly consistent finding in depressed patients with psychomotor retardation as a symptom (Banki 1977, Willner 1983).

1.5.1.3. SEROTONIN

There have been several studies on the concentration of the 5-HT metabolite 5-HIAA in the cerebro-spinal fluid. Although there is some argument as to the significance of cerebro-spinal fluid 5-HIAA levels (Curzon et al 1971, Ashcroft et al 1973), it is

generally thought that decreased brain 5-HT turnover is indicated by low lumbar CSF concentrations of its metabolite, 5-HIAA, which have been reported in many studies (e.g. Curzon et al 1980) although a few disagree (e.g. Berger et al 1980). Low lumbar cerebro-spinal fluid values probably indicate that 5-HT turnover is low in part of the nervous system only, as ventricular CSF 5-HIAA appears normal (Curzon 1980). The disagreements on lumbar 5-HIAA values may derive from differences in patient selection as Asberg et al (1976) found that values were distributed bimodally. Low values correlate highly with suicidal and violent behaviour. This suggests that the abnormality of 5-HT metabolism could be causally related to symptomatology. These data therefore support a classical indoleamine hypothesis of depression in some patients.

1.5.2. PHARMACOLOGY - NORADRENALINE, DOPAMINE AND SEROTONIN

Body fluid assessment and measuring of monoamine function are rather inconsistent, and in any case hard to interpret in terms of brain biochemistry. This is mainly due to methodological problems and to the fact that depression may be biochemically heterogenous. Therefore, it is difficult to evaluate the original monoamine hypotheses from these studies, with the possible exception of the DA hypothesis. This hypothesis would tend to be supported from the biochemical studies reviewed, at least in the case of depressions with psychomotor retardation as a symptom.

Extensive pharmacological studies have been carried out in both

animals and man involving manipulations of biogenic amine levels in an effort to evaluate the original hypothesis. Drugs which increase functional monoamine levels in the brain should alleviate depression, whilst treatments which reduce monoamine levels should exacerbate depression.

A major challenge to the monoamine hypothesis came from a re-appraisal of the original reserpine results which concluded that the majority of cases of depression reported were actually a 'pseudo-depression' syndrome of sedation and lethargy. The actual incidence of true depressions during reserpine administration was thought to be around only 5% to 9%, with the majority of these cases having a prior history to the disease (Goodwin et al 1972). Furthermore, reserpine given to animals induces a psychomotor retardation that can be reversed by l-dopa; however, l-dopa given to depressed patients induces psychomotor activation but seems to have no AD properties (Goodwin 1972).

Like reserpine, alpha-methyl-para-tyrosine (AMPT), which inhibits the rate-limiting step in the synthesis of DA and NA, should also exacerbate depression if the CA hypothesis is correct, but no consistent findings have emerged from these studies (Mendels and Frazer 1974). Clearly, therefore, treatments which reduce CA levels do not necessarily cause depression in humans.

As stated earlier, it would be predicted that drugs which increase CA levels, such as amphetamine, should alleviate

depression. Amphetamine has not been shown to be an effective AD; rather, it increases motor activity and by doing so alleviates some of the symptoms associated with depression. Furthermore, cocaine, another psychostimulant drug which acts in part via CA re-uptake blockade (the same supposed mechanism as TADs), also appears not to be an effective AD. Similar findings have also emerged from studies using l-dopa as a drug therapy (Goodwin et al 1970). These studies therefore do not support the original hypothesis. More recent studies, though, using the directly acting DA receptor agonists bromocryptine and piribedil, have indicated that such drugs have AD properties (e.g. Waehrens et al 1981, Reus et al 1980).

If brain 5-HT deficiency has a causal role in depression, then treatments which increase 5-HT synthesis or increase responses dependent on 5-HT should have a therapeutic effect. When tryptophan is given alone, varied results are obtained; though it is generally found that tryptophan potentiates other ADs, such as MAOIs (Coppen et al 1963) and the 5-HT re-uptake blocker clorimipramine (Walinder et al 1976). Tryptophan availability can be measured in at least two different ways, and it is generally thought that free rather than total tryptophan (free and albumin-bound) in plasma influences the availability of tryptophan to the brain. This in turn influences synthesis and, in some circumstances, functional activity of 5-HT (Kennett and Joseph 1981). Low levels of free tryptophan in plasma have been found in some groups of patients (e.g. Burns and Mendells 1979) though not in others (e.g. Moller et al 1979).

Studies wih the other 5-HT precursor, 5-hydroxy-L-trytophan (5-HTP), at first showed it to be ineffective as an AD treatment (e.g. Glassman 1969). However, more recent studies, using larger doses of 5-HTP have given more positive results (e.g. Van Praag 1983) and would tend to support a classic indoleamine hypothesis of depression.

The conventionally assumed pharmacological mechanism of TADs (reuptake blockade) has also been questioned. This problem was highlighted with the introduction of two 'atypical' ADs, mianserin and iprindole, which were shown to possess therapeutic properties, but were relatively ineffective at uptake blockade at monoaminergic synapses (Gluckman and Baum 1969; Randrup and Braestrup 1977; Zis and Goodwin 1979). Moreover, as the clinical effects of TADs usually take between two to four weeks to become apparent, beginning in the mid-1970s, many workers suggested that amine uptake blockade, which occurs immediately, may not be the mechanism of clinical action of TADs. This viewpoint may be rather premature, as it has been claimed that intravenous (i.v.) administration of TADs can show a therapeutic response as early as the second day of treatment (e.g. Becker 1971). Nevertheless, the long latency in the therapeutic response during TAD therapy had a profound influence on research. Attention was shifted from acute to chronic studies.

1.6. THE REVISED NA HYPOTHESIS

When the shift away from acute towards chronic studies began, it became evident that chronic TAD treatment actually reduced some aspects of NA function, shown for example by the development of beta receptor subsensitivity at NA synapses. A reduction in NA function following chronic TAD treatmentis a finding inconsistent with the original NA hypothesis (Schildkraut et al 1970, Segal et al 1974). On the basis of these observations, Segal et al (1974) proposed an NA hypothesis of depression, which was diametrically opposed to the original hypothesis; i.e. that depression resulted from an overstimulation of NA receptors which was corrected by chronic TAD treatment. The fact that certain subpopulations of patients showed a reduced MHPG excretion, which was originally reported in support of the 'too little' hypothesis, was now explained in terms of postsynaptic supersensitivity at NA synapses. This resulting overstimulation brought into play a negative feedback mechanism, which reduced the activity of NA neurones, causing a decrease in NA synthesis, and the observed reduction in MHPG excretion. It was further postulated that this mechanism could only partially compensate for the supersensitive receptors, and the system still remained in a state of overstimulation. The effect of tricyclics would be to increase receptor stimulation once more, which would further activate the negative feedback system, and, finally, reduce the activity of NA neurons sufficiently to compensate fully for abnormal receptor activity (Segal et al 1974, Mandel et al 1975). This proposed mechanism of TAD action accords with the adaptive processes of

the brain and the role of receptors in mediating these processes. It is therefore the effects of AD treatments on monoamine receptors which will now be reviewed with a view to gaining further insight into the psychobiology of depression.

1.7. ANTIDEPRESSANT DRUGS AND NORADRENALINE

1.7.1. ACUTE EFFECTS

One of the best documented effects of acute TAD treatment is to block NA re-uptake into the pre-synaptic cleft. Although all the tricyclic antidepressant drugs are very similar in structure, they can be usefully classified by the type of amine on their side chain (tertiary or secondary). Furthermore, secondary TADs (e.g. desethylimipramine) are more potent uptake blockers than the tertiary TADs (e.g. amitriptyline), (Maitre et al 1982). One consequence of NA uptake blockade is the stimulation of inhibitory presynaptic alpha 2 autoreceptors, which results in a decrease in firing rate (McMillan et al 1980) and NA turnover (Rosloff and Davis 1978). Therefore, TADs have two opposing synaptic effects - inhibition of uptake and decreased turnover. The net result as revealed by electrophysiological recording is an increase in adrenergic transmission at post-synaptic beta receptor sites (e.g. Huang 1979). At alpha receptors, however, the picture is less clear, since in addition to the effects already described, TADs also act as post-synaptic alpha-receptor antagonists (Brown et al 1980). Secondary TADs tend to enhance alpha adrenergic transmission, as do with tertiary TADs at low doses; on the other hand, tertiary TADs at high doses tend to

block transmission (Menkes and Aghajanian 1981).

'Atypical' TADs, which do not block re-uptake, have nevertheless been shown to potentiate adrenergic transmission through a variety of mechanisms. For instance, mianserin is a potent antagonist at presynaptic alpha 2 receptors (Maggi et al 1980) and consequently increases NA turnover (Sugrue 1980). Iprindole, which is comparable to DMI and imipramine in clinical potency (Bradshøw et al 1974), seems to be devoid of effects on NA uptake or turnover (Rosloff and Davis 1974), but it has been shown to potentiate the electrophysiological effects of iontophoretically applied NA (Bevan et al 1975).

1.7.2. CHRONIC EFFECTS

The effect of chronic TAD treatment on presynaptic receptors has been assessed by observing concurrent changes in clonidine-induced sedation, a supposed index of presynaptic receptor function (Drew et al 1979). Desensitization of alpha-2 autoreceptors following chronic TAD treatment is an effect observed with a number of AD treatments (Kostowski et al 1983) including ECT and REM sleep deprivation, although there are some exceptions (e.g. Bhavsar et al 1981, 1983). Presynaptic alpha-2 receptor desensitization has also been demonstrated in depressed patients receiving amitriptyline (Charney et al 1983). However, receptor binding studies do not confirm these findings. Binding measures of presynaptic adrenergic receptors are usually increased or unchanged by chronic AD treatment (see Willner 1984c). The development of autoreceptor subsensitivity during

chronic AD treatment results in an enhancement of NA turnover, as measured by the production of MHPG (e.g. Roffman et al 1977). The increase in release, together with the acute adrenergic facilitation effects of TADs result in an increased stimulation of post-synaptic receptors.

Chronic TADs have variable effects on post-synaptic alpha-2 receptors, making it difficult to draw any conclusions (Willner 1984c). In contrast, chronic TAD treatment increases the functional efficacy of the more numerous post-synaptic alpha-1 receptors as indexed by clonidine stimulation of locomotor activity (Modigh 1975, Maj et al 1979). This effect has also been reported following repeated electroconvulsive shock (Ehlers et al 1983) and has been confirmed electrophysiologically (e.g. Menkes and Aghajanian 1981). As before, receptor binding studies do not confirm an enhancement of alpha-1 transmission, probably due to difficulties inherent in binding methodology (Menkes et al 1983).

The most widely reported effect of chronic TAD treatment and most other AD treatments, is a decrease in beta-receptor sensitivity, as assessed by the ability of NA to stimulate cAMP production, a beta-receptor mediated response (Vetulani and Sulser 1975). This decrease in functional sensitivity of beta-receptors is accompanied, in most cases, by a reduction in cortical beta-receptor binding (Banerjee et al 1977).

In order for a 'too much' hypothesis of depression to work, it is insufficient that the changes at beta-receptors merely compensate for acute AD-induced increases in NA transmission, they must actually over-compensate. If they do not, the net result will still be an increase in NA transmission rather than a decrease. So a simple reduction in binding, for instance, cannot be used as evidence to support the 'too much' hypothesis. On the other hand, although, electrophysiological evidence was used to support a reduced beta-receptor function (Schultz et al 1981), due to a shift in baseline firing rate this evidence is inconclusive. Without a steady baseline firing rate, it is impossible to interpret the effects of iontophoretically applied NA. Evidence showing an increase in baseline firing rate following chronic DMI treatment, in support of a decrease in beta-adrenergic function is therefore controversial. In fact, only one study shows an increase (Huang 1979) and most others show no change, with a variety of AD treatments (e.g. De Montigny et al 1981; see section 5.1.). A major focus of this thesis will be to assess beta-receptor subsensitivity using behavioural techniques.

1.8. ANTIDEPRESSANT DRUGS AND DOPAMINE

1.8.1. ACUTE EFFECTS

Although originally believed not to affect DA, it now appears that ADs do block DA uptake, although less potently than that of NA and 5-HT (Hytell 1978). However, many TADs are also DA receptor antagonists. Therefore, depending on which of these two effects predominate, some TADs enhance DA function, others decrease DA function, whilst a third group show no consistent

change (Carlsson and Lindquist 1978).

1.8.2. CHRONIC EFFECTS

Behavioural studies point to a functional increase in DA activity following chronic AD treatment. In particular, the mesolimbic DA system has been found to be sensitive to TAD induced facilitation, as indexed by an enhancement of the locomotor stimulant effects of apomorphine (e.g. Green et al 1981), amphetamine (Wielosz 1981), and a number of other psychostimulants - all known to reflect mesolimbic activity at the moderate doses used (e.g. Kelly et al 1975; Pijnenberg et al 1973). The addition of the striato-nigral feedback loop in the nigrostriatal DA system, which provides a homeostatic regulation to this system, may well be reflected in the relative lack of enhancement of psychostimulant-induced stereotypy following chronic AD treatment (Willner 1983c).

Biochemical studies do not provide evidence for an increased responsiveness to post-synaptic DA receptors following chronic AD treatments. Binding of spiroperidol, assumed to label post-synaptic DA receptors, shows no change following chronic AD treatments (e.g. Bergstrom et al 1979, Rosenblatt et al 1979). However, as mentioned earlier, the relevance of binding techniques to measure physiological properties of receptors has been questioned (Menkes et al 1983). Electrophysiological studies have not yet been carried out which directly address this question. So if one were to reach a conclusion solely from biochemical evidence, it would have to be that chronic AD

treatment does not increase the sensitivity of post-synaptic DA receptors. However, DA receptor agonists, including apomorphine, when injected into the nucleus accumbens following chronic TAD treatment, do enhance DA activity (indexed by locomotor activity). Heal et al 1978 This would suggest that behavioural stimulation by the action of apomorphine following TAD treatment is mediated postsynaptically.

It is, however, also possible that the TAD-induced increase in DA responsiveness has a presynaptic contribution. Chronic treatment with TADs does not appear to affect DA synthesis or turnover, measured by accummulation of the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Sugrue 1980). However, evidence is accumulating that chronic AD treatments may desensitize DA autoreceptors. The sedative effects of low doses of apomorphine are thought to be dependent on DA autoreceptor stimulation. Serra et al (1979) first demonstrated that apomorphine-induced sedation was reversed by chronic amitriptyline, imipramine and mianserin (10 days) treatment but not by acute treatment with these drugs. This autoreceptor desensitization effect has been demonstrated with a range of other TAD treatments (Zebrowska-Lupina et al 1980) as well as treatment with MAOIs, ECS (Serra et al 1981) and lithium (Harrison-Read 1980).

The effect has also been demonstrated electrophysiologically as shown by a reduction of the inhibitory effect of a low dose of

apomorphine on DA cell firing (Chiodo and Antelman 1980a,b). It has also been shown biochemically by blocking the apomorphine-induced inhibition of DA release as measured by the accumulation of the DA metabolite DOPAC (Serra et al 1979). In fact Chiodo and Antelman (1980b) observed more than a reduction in apomorphine-induced inhibition of DA cell firing: following chronic imipramine or amitriptyline treatment, they observed excitation by apomorphine in approximately 25-50% of the cells they studied. It was suggested that this progressive switch in cell firing effect might potentially explain the shift from hypomania to mania, sometimes seen in bipolar depressed patients receiving TAD treatment. In support of DA autoreceptor subsensitivity are the findings that the actual number of active DA cells in A9 and A10 areas increased following chronic DMI treatment (Chiodo and Bunney 1982). In addition, autoreceptor subsensitivity appears to depend simply on the passage of time, as opposed to repeated drug treatment: a similar degree of autoreceptor subsensitivity was seen 10 days after 2 days of imipramine treatment as compared to 2 days after 10 days of treatment (Chiodo and Antelman 1980c). Similar results were also obtained with ECS (Antelman et al 1982).

However, subsequent studies have shown that autoreceptor subsensitivity is not as robust an effect as it first seemed. Spyraki and Fibiger (1981) and Chiodo et al (1983) were unable to demonstrate attenuation of apomorphine-induced sedation after chronic TAD treatment. No change in apomorphine-induced inhibition of DA synthesis has also been reported following

chronic TAD treatment (Holcombe et al 1982). These findings do not support the hypothesis of a DA autoreceptor mediation of TAD therapy. This topic, which is the second major issue addressed in this thesis, will be examined in chapters 6, 7 and 8.

1.9. ANTIDEPRESSANT DRUGS AND SEROTONIN

1.9.1. ACUTE

Acute tricyclics have generally been found to block 5-HT uptake, with the exception of the atypical AD and some typical tricyclics such as DMI, which are relatively weak at uptake blockade (Maitre et al 1982). In addition, firing rate of 5-HT containing cells in the raphe nuclei, and rate of synthesis and turnover are reduced following acute AD treatment. These effects are proportional to the blockade of 5-HT uptake and are therefore probably mediated by autoreceptors (Carlsson and Lindquist 1978, De Montigny et al 1981). Post-synaptic receptor blockade is also a property of many TADs. Post-synaptic function appears to be enhanced by zimelidine (a specific 5-HT uptake inhibitor with AD properties) on a variety of measures including potentiation of 5-HTP stimulation of locomotor activity (Buus Lassen 1978). Mianserin, on the other hand, appears to have the reverse effect - showing an attenuation of post-synaptic function, as measured by a reduction of 5-HTP induced behavioural arousal (Gold et al 1980) for example.

Suppression of 5-HTP-induced arousal following mianserin appears to be mediated by blockade of post-synaptic 5-HT receptors, in particular the 5-HT2 receptors, which bind mianserin with high affinity (Barbacchia et al 1983). Therefore, in considering the net effect of acute AD on 5-HT transmission, blockade of post-synaptic receptors must be set against the increase in receptor bombardment brought about by uptake blockade. The fact that many ADs show some degree of blockade of 5-HT receptors, which seem to predominate over their 5-HT enhancing effects, has been used as evidence to support the hypothesis that some depressions may be a reflection of hyperactive 5-HT mechanisms - a 'too little' hypothesis (Aprison et al 1982). However, the affinity of AD for 5-HT2 receptors is less than that of traditional 5-HT blockers (Peroutka and Snyder 1982); furthermore, spiroperidol, which has the highest affinity for 5-HT2 receptors, seems to have no AD properties, Therefore, blockade of post-synaptic 5-HT2 receptors cannot be used as evidence for the 'too little' hypothesis, as this mechanism does not seem to mediate the therapeutic response of AD.

1.9.2. CHRONIC EFFECTS

Chronic AD treatment seems to have no effect on autoreceptor desensitization as measured electrophysiologically (e.g. Svensson 1980). A single unit recording study did however find a recovery on the number of normally active raphe cells following chronic zimelidine treatment; this change was associated with a decrease in autoreceptor sensitivity to LSD (which is a selective 5-HT

autoreceptor agonist at low doses) (Blier and Demontigny 1982).

A decrease in 5-HT uptake in rat brain has been reported following chronic ECT (Minchin et al 1983), though no change was seen with a variety of other AD (Segawa et al 1982). Chronic TADs and ECT (e.g. Langer et al 1981) decrease imipramine binding. Imipramine is thought to bind to a site closely associated with 5-HT specific uptake sites. Mianserin and iprindole do not produce these changes in imipramine binding. The effects of chronic TAD treatment on turnover seem to be broadly similar to their acute effects, i.e. DMI has no effect on turnover, whilst zimelidine decreases turnover (e.g. Sugrue 1980, see section 1.9.1). To summarize, most treatments enhance presynaptic 5-HT function by reducing 5-HT uptake, the exception being mianserin and iprindole.

The post-synaptic response to iontophoretically applied 5-HT following chronic TADs, iprindole and ECT is enhanced in subcortical structures only (Willner 1984c). Behavioural evidence also indicates an increased responsiveness of 5-HT receptors following chronic AD treatment. In particular ECT, as indicated, for example, by an increase in 5-HTP induced head twitches (Lebrecht and Nowak 1980). Evidence from studies employing other AD is less clear, mainly due to methodological inconsistencies such as the dose of drug used, the duration of treatment and the latency to testing following treatment. Powerful inhibitors of uptake such as zimelidine have been found in different studies to reduce and to enhance 5-HTP-induced behaviours over chronic

treatment. TADs and atypical AD are usually reported to enhance 5-HT receptor function (e.g. De Montigny and Aghajamian 1978). The enhancement of 5-HT transmission observed with the uptake inhibitors and TADs is time dependent, becoming apparent several hours after treatment (withdrawal). This finding would seem to have important implications for the clinical setting and would merit further investigation.

Binding studies show that chronic AD treatments do not, in general, change 5-HT1 receptor binding (Lucki and Frazer 1982). In contrast, chronic AD treatments reduce the binding of 3H-spiroperidol to 5-HT2 receptors, although some regional differences have been reported (Fuxe et al 1982). This effect of ADs on 5-HT2 binding seems quite specific (e.g. Peroutka and Snyder 1980) although amphetamine shares this effect, a treatment which has no AD potential when administered chronically (Nielsen et al 1980). However, ECS has the opposite effect, i.e. it increases the number of 5-HT2 receptors (Vetulani et al 1981). This finding makes it extremely unlikely that changes in 5-HT2 receptor binding mediate the clinical effects of AD - a conclusion reached earlier in this review.

In summary, the evidence reviewed in this section would tend to support the original indoleamine hypothesis of depression, that depression arises from 'too little' 5-HT and that AD treatments increase 5-HT function.

1.10. DISCUSSION

From the previous sections on AD and monoamines, it will be apparent that the results of receptor binding studies are generally inconsistent with those obtained by electrophysiological and behavioural methods. Given that binding studies represent a 'static' assessment of any one aspect of synaptic function, these generally discrepant data will be disregarded and emphasis will be directed towards functional measures of synaptic function i.e. electrophysiological, neuroendocrine and behavioural measures.

The problem that arises, is to decide in which direction the overall functioning of a system changes following chronic AD treatment, and to relate this increase or decrease in potentially multi-synaptic functioning to a particular monoamine hypothesis of depression. This thesis addresses these problems in relation to NA- and DA- mediated mechanisms.

1.11. AN EXCESS NORADRENALINE HYPOTHESIS

For a 'too much' hypothesis of depression to be plausible, beta-receptor subsensitivity must not merely compensate for acute AD-induced increases in NA transmission, but it must actually over-compensate. The net result will then be a decrease in NA transmission. Evidence for a reduction of beta-receptor function to overcompensate for the acute AD-induced enhancement of beta-receptor function is controversial. Receptor binding studies cannot provide evidence to answer this question, whilst only one electrophysiological study supports an overcompensation of beta-

receptor function following chronic AD treatment (see section 5.1. for a more detailed discussion of this evidence).

Attempts to assess beta-adrenergic function via behavioural methods have been hampered by the fact that there are no models which precisely reflect beta-adrenergic receptor stimulation. One such attempt to model beta-receptor function utilized the dorsal bundle extinction effect (DBEE), which is thought to reflect a decrease in beta-adrenergic function during resistance to extinction. During chronic DMI treatment, in three different behavioural paradigms, no increase in resistance to extinction was seen except when the animals were tested during withdrawal (Montgomery and Willner 1980, Willner et al 1981). These results would suggest that beta-receptor subsensitivity is unmasked by withdrawing DMI, and simply compensates for the acute NA enhancing effects of the drug. However, resistance to extinction only appears when dorsal bundle lesions are made prior to acquisition (Mason and Fibiger 1979). It follows that if chronic DMI treatment is mimicking a functional lesion of the dorsal NA bundle, resistance to extinction should be observed only when animals are treated during acquisition.

We carried out an experiment to test this hypothesis (Willner and Towell 1982a). Rats were trained to press a lever for food rewards, then given a five-week break. Animals which received 14 daily DMI injections, ending 4 days before the extinction session, showed resistance to extinction; but no effect was seen

in animals which received DMI during acquisition. These results show that resistance to extinction induced by DMI is probably not mediated by changes in the efficacy of the dorsal NA bundle, and this behaviour cannot therefore be used with confidence to assay NA function.

A second behavioural assay approach relies on the anorexic effects of small doses of amphetamine on food intake; an effect known to have a beta-adrenergic component (e.g. Leibowitz 1975, see chapter 2, section 2.7.). As in the DBEE experiments, attenuation of amphetamine anorexia was only observed during withdrawal from DMI or iprindole, but not during the course of chronic treatment (Willner and Montgomery 1980, 1981, Willner, Towell and Montgomery 1984). Taken together, the simplest conclusion to be drawn from the biochemical, electrophysiological and behavioural studies of beta-adrenergic function during chronic antidepressant treatment is that changes in receptor function simply act to restore NA systems to pre-drug levels rather than to decrease them.

Interpretation of these behavioural results is complicated by the fact that some ADs inhibit the metabolism of amphetamine (see chapter 5, experiment 5). However, mianserin, which does not have this effect did attenuate amphetamine anorexia during the course of chronic treatment (Willner, Towell and Montgomery 1984), suggesting a decrease in

beta-adrenergic function. However, since an alpha-adrenergic system stimulates feeding (see chapter 2, section 2.5.2.1), changes in amphetamine anorexia might equally well represent an increase in alpha-adrenergic function. This problem forms the subject of chapter 5, experiment 6.

1.12. A DOPAMINE DEFICIT HYPOTHESIS

The hypothesis that chronic AD treatments increase DA transmission has been reviewed in section 1.8.2. and would appear to be supported by the behavioural evidence. However, the mechanism of this enhancement of transmission is uncertain. Furthermore, although primarily the mesolimbic pathway has been implicated in depression, controversy has developed over which DA pathways are affected by AD, and whether these changes are mediated pre- or post-synaptically.

Low doses of apomorphine have been shown to be selective for presynaptic DA receptors and to produce behavioural sedation. We have observed that low doses of apomorphine (0.05 mg/kg) produce a marked and reliable anorexia (chapter 6). Apomorphine anorexia was therefore used as a model to index autoreceptor function (chapter 6), and the effects of chronic DMI treatment on this model were also tested (chapter 7). Furthermore, the contribution of nigrostriatal and mesolimbic pathways towards mediating autoreceptor function were assessed in studies using central administration of apomorphine (chapter 7).

The behavioural assay techniques, briefly described here, depend on changes induced by TADs on pharmacological probes that inhibit feeding behaviour. It is therefore timely to review the role of CAs in feeding behaviour as a background to the experimental chapters.

CHAPTER TWO

CATECHOLAMINES AND FEEDING

2.1. INTRODUCTION

To understand the mechanism of action of drugs which cause loss of appetite, such as amphetamine, it is necessary to understand how the brain produces hunger and satiety, and how the experiential and psychological states that follow such changes relate to feeding behaviour. This analysis of anorexic drug action spans across several domains of explanation such as the biochemical, physiological, cognitive and experiential. As the main thrust of this thesis is to develop behavioural assays of synaptic function in rodents through the use of pharmacological probes, such as amphetamine or apomorphine, it is possible to ignore the cognitive and experiential levels of explanation and concentrate on the biochemical and physiological levels.

2.2. THE EARLY WORK

The earlier studies concerned with elucidating the mechanisms involved in feeding behaviour correlated gross electrolytic lesions with changes in food intake. Hetherington and Ranson (1940) demonstrated that electrolytically induced ventromedial hypothalamic (VMH) lesions produced hyperphagia and obesity; Anand and Brobeck (1951) showed that lesions to the lateral hypothalamus (LH) produced aphagia and adipsia. These studies have been successfully replicated and have received further support from electrical brain stimulation investigations: electrical stimulation in the LH elicits feeding or drinking (Miller 1960), whereas electrical stimulation of the VMH blocks

ongoing ingestive behaviour in deprived animals (e.g. Sclafani and Maul 1974).

At the onset of the neurochemical era, Grossman and colleagues amongst others (in the 60's) set about to examine how the LH and VMH syndromes were mediated. This early work on the neurochemistry of feeding demonstrated that stimulation of adrenergic receptors within the hyothalamus resulted in feeding (e.g. Booth 1967). Briefly, this work eventually led to the hypothesis that alpha adrenergic stimulation in the paraventricular nucleus (PVN) led to feeding and beta-adrenergic stimulation in the perifornical hypothalamus (PFH) led to satiety. This literature is reviewed later in the chapter. However, the simplicity of this 'neurochemical' hypothesis has been questioned on anatomical as well as behavioural grounds. Therefore, in order to evaluate the 'neurochemical' hypothesis a brief review of CA neuroanatomy will follow. It should be noted that the neurochemical hypothesis of alpha adrenergic feeding in the PVN and beta adrenergic satiety in the PFH is distinct from the clasical two centre hypothesis of LH feeding and VMH satiety.

2.3. ANATOMY OF CA SYSTEMS

The mapping of CA pathways in the CNS was made possible by the development of histofluorescence techniques such as the formaldehyde (Falck et al 1962) and glyoxylic acid (Lindvall and Bjorklund 1974) methods, which have allowed cell bodies and terminal areas to be readily visualized in detail. These methods

have been used in conjunction with potentially 'transmitter-specific' neurochemical lesion techniques (such as 6-OHDA) to further classify CA pathways.

2.3.1. DOPAMINE

The CA axons arise from a series of cell bodies that were designated A1 to A12 by Dahlstrom and Fuxe (1964). Of these cell groups, A8, A9, A10, and A12 are dopaminergic. They give rise to three major pathways as defined by Ungerstedt (1971): (1) the nigro-striatal system (A8, A9) which terminates in the caudate nucleus, (2) the mesolimbic system (A10) which terminates in the nucleus accumbens and (3) the tuberoinfundibular system (A12). Unlike the nigrostriatal and mesolimbic systems the tuberoinfundibular system has not been directly implicated in behaviour. These systems have been re-classified by some to include the well established cortical projections of the DA systems - particularly the mesocortical system which emanates from A10, passes through the nucleus accumbens and terminates in the frontal cortex (Lindvall and Bjorklund 1978).

2.3.2. NORADRENALINE

The anatomy of NA systems as identified by Ungerstedt (1971), using the histofluorescence method comprises two major pathways.

(i) Ventral noradrenergic bundle(VNAB). This arises from cell groups A1, A2, A5 and A7 in the medulla oblongata and pons and

innervates the whole hypothalamus and several major terminal region of the mesencephalon.

(ii) Dorsal noradrenergic bundle (DNAB). This originates in the locus coeruleus (A6). The fibres ascend in the medial forebrain bundle to innervate the geniculate body, thalamic nuclei, the hippocampus and cortex. The cortical projections of the locus coeruleus have been found to have the highest fibre density in the medial cortex (Lindvall and Bjorklund 1978).

Additionally, a projection from the locus coeruleus to the hypothalamus has been identified (Lindvall and Bjorklund 1978). An adrenaline innervation of parts of the hypothalamus from cell bodies thought to lie closely in the region of A1 and A2 has also been shown (Hokfelt 1974). The hypothalamus is therefore heavily innervated by DA, NA and adrenalin pathways, together also with 5-HT pathways, originating in the medial raphe nucleus (which have not been reviewed).

2.4. LESION STUDIES

Brobeck (1946) first speculated that the effects of VMH lesions (to increase food intake and body weight) might by caused by an interuption of pathways in the vicinity of the VMH rather than by destruction of the cell bodies of the VMH. Similar objections have also been raised about the notion that the aphagia from LH lesions arises from destruction of LH cell bodies, most notably by Morgane (1961). This is to cast doubt on the evidence in support for a 'centre' concept, where a discrete region of the brain is supposed to carry out the integration necessary and

sufficent to organise behaviour such as appetite or satiation.

2.4.1. LH APHAGIA - A RE-EXAMINATION

Speculation that LH aphagia is not in fact entirely mediated by destruction of LH cell bodies, arose from the general anatomical observations that the LH is relatively cell poor and is traversed by numerous diffuse fibre systems, some of which originate or terminate in the A9 and A10 cell bodies. Ungerstedt (1970, 1971) proposed that LH aphagia was mediated by destruction of nigrostriatal DA connections, following his finding that bilateral intra-ventricular injection of 6-OHDA (which was shown to destroy the nigrostriatal DA neurons, amongst others) produced marked aphagia and adipsia. Destruction of NA systems or the mesolimbic DA system produced no such effects. However, this nigrostriatal mediation of LH aphagia has been questioned on several grounds.

First, 6-OHDA has considerable non-specific neurotoxic effects when used in the concentrations employed in these studies- these observations do not provide any evidence for a crucial involvement of the nigrostriatal DA.

Secondly, although the behavioural profiles consequent on nigrostriatal and LH destruction are similar, they differ in a number of details: nigrostriatal (NS) rats are not especially adverse to quinine adultered food as LH rats are; and less importantly as regards aphagia, NS rats are not somnolent (though

they are akinetic) as LH rats are, and NS rats do not show certain gross autonomic changes as seen after LH lesions (Blundell et al 1979).

Thirdly, the reduction in striatal DA level is not proportional to the degree of aphagia shown, e.g. a 50% reduction in striatal DA following a LH lesion produces a complete disruption of ingestive behaviour (Zigmond and Stricker 1973), whereas a 90% reduction following other neurochemical treatments e.g. lesions with copper sulphate results in only mild aphagia (Grossman 1976). It is therefore possible to conclude that striatal DA plays a role in LH aphagia but cannot wholly account for the behavioural effects of LH lesions. It is also probable that the reductions in striatal DA following both 6-OHDA and LH lesions are instrumental in producing aphagia as a consequence to some extent of gross sensory-motor impairment and lowered arousal (effects which are well documented e.g. Marshall et al 1974); behavioural states which are incompatible with spontaneous ingestive behaviour but not specific to it.

Lesions in the dorsal midbrain tegmentum are known to produce a marked aphagia and adipsia, together with more persistent deficits (unlike aphagia which lasts up to a few weeks only). These deficits are in the rats' feeding response to glucoprivation, or to hydrational challenges, such as a decrease in the consumption of sucrose and saccharin solutions (Leibowitz et al 1980c, Grossman et al 1978). However, an interesting point in relation to LH aphagia, to emerge from the Leibowitz study is

the report of a relatively 'normal' neurological profile following midbrain electrolytic lesions. Furthermore, 6-OHDA injected into the same area failed to produce a marked aphagia or adipsia (Leibowitz et al 1980c) suggesting that the aphagia and adipsia observed after dorsal midbrain electrolytic lesions cannot be attributed to damage of the various ascending CA fibres.

Evidence against DA mediation of LH aphagia has also been obtained by Grossman et al (1978) who found that kainic acid lesions of the LH produced a small but significant aphagia and adipsia in the absence of neurological impairment. There was also no significant depletion of DA in the hypothalamus, striatum and telecephalon. These data therefore suggest that the LH syndrome may encompass a number of different syndromes mediated by different regional anatomy: dorsal midbrain tegmentum damage (specifically related to the regulation of food and water intake), 6-OHDA damage (related to sensory-motor disabilities), and kainic acid damage, involving specific destruction of neurons inherent to the LH, but not known to be related to any specific component of aphagia.

It should be mentioned however, that although a wealth of evidence fails to implicate nigrostriatal DA in aphagia, more recent evidence does suggest a role of A10 neurons in the feeding elicited by stimulation of the MFB at the level of the LH. Injections of spiroperidol into the nucleus accumbens,

ipsilateral to the stimulating electrode, significantly attenuated the elicited feeding response; no change was seen on contralateral administration. These changes were also correlated with changes in the electrophysiological characteristics of cell bodies in the VTA (Mogenson 1982).

Another explanation of the aphagia seen following DA depletion is based on the idea that DA is implicated in the rewarding properties of food. DA depletion therefore renders food unrewarding (Wise et al 1982). However, this view is not adopted by Beninger (1983), who sees DA as being implicated in the incentive value of food stimuli. In this model, DA neurons are seen in part to mediate reinforcing stimuli on learning. This may work by reinforcing stimuli increasing the incentive motivational properties of neutral stimuli that are associated with them. Normal DA functioning appears to be required for the establishment and maintenance of incentive learning in naive animals.

2.4.2. VMH HYPERPHAGIA - A RE-EXAMINATION

The classic VMH syndrome has also been criticised along similar lines. Firstly, the anatomical specificity of the lesion has been questioned. Discrete lesions of the VMH region e.g. within the bounds of the VMN (ventromedial nucleus) produce no hyperphagia, but larger lesions (e.g. those produced at conventional current levels by the electrolytic method) which damage tissue surrounding the VMH produce hyperphagia (Gold

1973). More specifically, damage to the tissue directly adjacent to the VMH skirting the fornix produces hyperphagia. Also knife cuts in that region are sufficient to produce the classic VMH obesity syndrome (Gold 1973, Sclafani 1977). As mentioned earlier, this area is known to be heavily innervated by both NA and 5-HT pathways. An attempt to identify the specific pathway involved in VMH hyperphagia was made through studies using 6-OHDA which indicated that destruction of the VNAB gave rise to a hyperphagic syndrome (Ahlskog 1974). Thus, it was possible that the original VMH lesion was identical to the VNAB lesion. This hypothesis received little support for a number of reasons. Firstly, hyperphagia seen in VMH rats is more severe than in VNAB rats. Secondly, VNAB rats overeat only at night whilst VMH rats overeat both day and night. Thirdly, hypophysectomy blocks hyperphagia in VNAB rats but not in VMH rats. Fourthly, VNAB rats show around a 50% attenuation of the anorexic effect of amphetamine whilst no such attenuation is seen in VMH rats. Fifthly, VMH rats are finicky, especially towards quinine adulterated diets, VNAB rats are not. Sixthly, the hyperphagic effects of the two lesions are additive, arguing against them having the same mechanism. Finally, VNAB rats showed a 94% depletion of brain NA whereas VMH rats show no significant depletion of NA (Ahlskog et al 1975).

The anatomical specificity of the 6-OHDA-induced VNAB lesion has also been questioned. Selective lowering of NA by bilateral 6-OHDA injection produced a maximal effect on lowering brain NA at 4 ug with no significant change in food intake. Only at 12 ug

did increases in food intake become apparent, suggesting that non-specific damage around the injection site is responsible for increases in food intake (Oltmans et al 1977 ; Lorden et al 1976). However, this notion was contradicted by other studies which demonstrated normal food intake with the same low and high doses (4 ug and 12 ug) of 6-OHDA following DMI pretreatment (which was used to protect NA cells by its reuptake blockade action). These results support an interpretation in favour of NA mediation of 6-OHDA-induced hyperphagia (Hernandez and Hoebel 1982). It is feasible that the 'non-specific' damage which some claim to produce hyperphagia can be attributed to damage incurred by the adrenalin fibres of the central tegmental tract (CTT) and DA fibres of the A8 and A9 cells groups (see section 2.7.).

In summary, the LH and VMH lesions appear both to have a CA involvement. However, as 6-OHDA spares adrenalin fibres (Goldstein et al 1978), which innervate parts of the hypothalamus, it is possible that a component of both LH aphagia and VMH hyperphagia is mediated in part, by adrenergic activity of the hypothalamus: in particular the PVN and PFH. This hypothesis is addressed within the context of a review on neurochemical studies of feeding behaviour which follows.

2.5. PHARMACOLOGICAL STUDIES

2.5.1. FEEDING

Grossman (1962) first demonstrated that the l-isomer of NA (l-NA) injected into the hypothalamus of a fully satiated rat produced a feeding response. Adrenalin also produced this feeding response, with greater potency than NA (Booth 1968), whereas DA, 5-HT and the d-isomer of NA (d-NA) were ineffective (Booth 1968). Dose dependency of NA- and adrenalin-induced feeding were also shown (Miller et al 1964 and Booth 1968 respectively), together with facilitation of feeding by adrenalin and NA in hungry rats (Leibowitz 1970). The original site at which Grossman implanted and obtained the LH feeding has not been fully confirmed. Subsequent studies by Booth (1967) have shown sites medial to the fornix at the more rostral hypothalamic level to be more sensitive to NA elicited feeding. The sites identified by Booth were subsequently confirmed by Grossman (1968) in a re-examination of his own histology. This particular area has been extensively studied by Leibowitz (1975, 1978a), amongst others, who has identified the PVN within the anterior hypothalamus to be the site most sensitive to adrenergic feeding effects. This sensitivity is exemplified by the latency of NA to stimulate feeding at the PVN: in the region of 0.5 - 2.0 minutes at exceptionally low threshold doses (1.0 - 4.2 ng)in satiated rats. But Mathews et al (1978) found that NA applied to the border of the PVN was the most sensitive site in eliciting NA-induced feeding.

Pharmacological classification of the NA-induced adrenergic

effect was established in the earlier studies of Grossman (1960, 1962). Booth (1968) and Slangen et al (1969) reaffirmed Grossmans finding that NA-induced feeding could be reduced by prior hypothalamic administration of phentolamine, an alpha-adrenergic blocker. This finding has been successfully replicated by Leibowitz (1978a), amongst others, who found that administration of phentoleamine by i.c. and peripheral routes was also effective in blocking NA feeding. Complementary to an alpha-adrenolytic disruption of NA feeding is the finding that alpha-agonists, such as clonidine and metaraminol, injected into the PVN, elicit a reliable sustained feeding response (Leibowitz 1975). The beta-agonist, isoproteronol, is ineffective in eliciting feeding at the PVN and similarly the beta-antagonist propranolol at relatively large doses is without effect in blocking NA feeding (Leibowitz et al 1980a).

Thus, evidence so far clearly supports the notion of an alpha-adrenergic mechanism mediating the feeding facilitatory effects of exogenously applied NA. However, this is far from establishing whether endogenous NA transmitter systems have a physiological role in the control of naturally motivated feeding. Attempts to answer this question utilize drug-induced release of endogenous transmitter to induce behavioural change. Slangen and Miller (1969) applied both tetrabenzine to deplete local CA stores and nialamide (a MAOI) to arrest breakdown of NA into the PFH, this drug conbimaton produced an eating response in satiated rats. Booth (1968) and Slangen et al (1969) showed that the TAD,

DMI (NA re-uptake blocker) when injected into the LH showed some tendency to stimulate feeding. This result was more positively re-affirmed by Montgomery et al (1971), who showed that DMI potentiated feeding in hungry rats at a LH site but not in satiated rats. Amphetamine, a drug that increases synaptic concentrations of CAs, has also been shown to elicit a feeding response when injected into the VMH (Leibowitz 1970b).

A more recent study by Leibowitz (1978a) reported that the TAD DMI, protriptyline and amitriptyline injected into the PVN elicited feeding in satiated rats. This AD-induced feeding response was blocked in a dose-dependent fashion by phentolamine. AD-induced feeding was also blocked by PVN pretreatment with CA synthesis inhibitors, at doses which were shown to be without effect when administered together with exogenous NA. In conclusion, the AD facilitation of NA feeding at the PVN seems to depend on the integrity of the presynaptic uptake site and postsynaptic alpha-adrenergic receptors.

Biochemical support for the role of endogenous NA in feeding elicited from the PVN comes from a study by Martin and Myers (1975), who demonstrated that the PVN, particularly its dorsomedial and ventromedial areas, exhibited the most dramatic changes in indices of NA turnover or release as a function of changes in feeding behaviour. More solid evidence from van der Gugten et al (1977) demonstrated increased NA concentrations in the PVN and certain other brain nuclei following feeding. These studies taken in isolation simply point to a correlation between

changes in NA and feeding, but taken in the context of the pharmacology, they support the hypothesis of endogenous NA-induced feeding within the PVN.

2.5.2. SATIETY

2.5.2.1. ADRENERGIC MECHANISMS

LH sites have been implicated in the suppression of feeding behaviour. Adrenalin produces a marked anorexia when injected into the LH together with an alpha antagonist (phentolamine), (Leibowitz 1970a). The purpose of pretreatment with the alpha antagonist is to confirm that adrenalin acts at beta-adrenergic receptors, as stimulation of alpha-adrenergic receptors by adrenalin might mask any anorexia by inducing an increase in food intake. That the anorexic effect is beta-adrenergic is shown by an attenuation of food intake in food-deprived rats following a LH injection of the beta-adrenergic agonist isoproterenol. Isoproterenol-induced suppression of food intake was reversed by prior administration with propranolol (beta blocker), but not by the alpha blocker phentolamine (Leibowitz 1973, Goldman et al 1971). The assumption that this beta-adrenergic hypophagic effect has to do with natural satiety processes is speculative. It may be that the hypophagia is a consequence of (un)palatability or a sickness artefact.

A controversy exists as to the beta-adrenergic specificity of feeding suppression, as it has also been reported that NA injected into a perifornical LH site elicits satiety instead of

feeding, whilst phentolamine injected into the same site elicits feeding (Margules 1970). In addition, Margules obtained evidence that NA suppressed feeding in the dark and facilitated feeding in the light (Margules et al 1972). However, Margules tested with milk rather than with solid food and his NA injections may have been affecting a system concerned with thirst rather than hunger. It seems unlikey that the water content of milk influenced the effects of NA on feeding, as it has been reported that NA injected into the dorsal perifornical region and the lateral aspect of the PVN elicited feeding irrespective of whether the rats' food was solid or liquid (Matthews et al 1978). As Margules maintained his rats on solid food and presented milk only during test periods, this factor could be responsible for the observed suppression of intake (Margules 1972). Matthews et al also reported a facilitation of feeding by NA in the light with no effect on the amount of food eaten in the dark. This result also differs from Margules and may possibly be explained in terms of dose of drug used, injection site and baseline food intake values.

It is apparent from the Margules work that the circadian cycle is a variable in the expression of neurochemically-induced behavioural effects, and that the type of diet is also important when measuring a behaviour as complex as feeding. For example, Leibowitz has proposed a carbohydrate specific preference to NA induced feeding, although a preliminary investigation failed to confirm this effect (Towell and Booth, unpublished data 1980).

NA appears to be the endogenous neurotransmitter mediating alpha-adrenergic feeding effects (see section 2.5.1.). It is unclear which transmitter might mediate the beta-adrenergic 'satiety' effect. It is possible that NA could play a dual role in instigating feeding and satiety, but with the more recent finding that adrenalin innervates parts of the hypothalamus (see section 2.3.2.), it is plausible that adrenalin innervation might mediate beta-adrenergic satiety.

2.5.2.2. DOPAMINERGIC MECHANISMS

As mentioned earlier in section 2.4.1., Ungerstedt (1971) proposed that DA depletion was important in mediating aphagia. More specifically, aphagia was presumed to be mediated by the depletions of DA in the nigro-striatal system (Ungerstedt 1974). The resultant sensori-motor incapacitation following nigro-striatal DA depletion was thought to be incompatible with normal ingestive behaviour. Evidence in support of this hypothesis comes from studies showing that neuroleptics (especially the butyrophenones) injected peripherally cause aphagia, that is also presumed to result from lowering of striatal DA transmission (e.g. Blundell and Burridge 1979).

However, not all neuroleptics cause aphagia - the phenothiazine neuroleptics have been shown to increase feeding when injected peripherally, or centrally at an LH site, (Leibowitz 1976). This increase in feeding may possibly result from the stimulation of alpha-adrenergic receptors in the nearby PVN, or perhaps from a

direct blockade of a group of DA receptors in the PFH. This hypothalamic DA system, which is inhibitory to feeding, is thought to be located specifically in the lateral perifornical region of the hypothalamus (Leibowitz and Rossakis 1979b). Both DA and adrenalin in the PFH suppress feeding in a dose-dependent way. The effects of DA and adrenalin are selectively enhanced by the uptake inhibitors benzotropine and DMI respectively which indicates that there may be distinct presynaptic nerve endings for the release of DA and adrenalin.

DA-induced suppression of feeding at the PFH is readily antagonized by intracranial (i.c.) pretreatment with a variety of neuroleptics but not with antagonists of alpha-adrenergic, beta-adrenergic, cholinergic and serotonergic receptors (Leibowitz 1980). Adrenalin is also effective at the PFH in suppressing feeding, with a latency of between 1 and 2 minutes (Leibowitz and Rossakis 1979a). Leibowitz has used this short latency time for adrenalin as evidence to implicate the existence of a population of receptors close to the cannulae tip which mediate adrenalin's suppressive effects on feeding. Other evidence would suggest however, that after a latency of 1 minute or so, adrenalin is likely to be very widely diffused through the brain.

Adrenalin-induced feeding was blocked by pretreatment with beta-antagonists, more specifically those of the B2 subtype, but not by alpha-adrenergic, cholinergic and serotoninergic receptor antagonists. The receptor antagonism produced by propranolol was stereospecific and reversible by adrenalin. Neuroleptics were

also found to be effective in reversing adrenalin's suppressive effect (Leibowitz and Rossakis 1979a). Taken together with the finding that beta-adrenergic blockers were ineffective at preventing DA-elicited suppression of feeding, these observations lead to the hypotheses that adrenalin and DA are acting on two independent populations of hypothalamic receptors, and that the integrity of the DA receptors is necessary for even the beta-adrenergic suppression of feeding to occur.

2.6. DRUG-INDUCED ANOREXIA

2.6.1. PHARMACOLOGY

That suppression of feeding can be mediated by endogenous DA is indicated by the effects of amphetamine, a psychomotor stimulant which is known to amplify the release of endogenous NA and DA from presynaptic endings (Carlsson 1970). When amphetamine is injected into the PFH, as when injected i.p., it produces an anorexia which can be antagonized by CA synthesis inhibitors (Leibowitz 1976), DA blockers (see experiment 4), and beta blockers (see experiment 3). The evidence for beta-adrenergic blockade is controversial. However amphetamine anorexia is not mediated solely by DA release. Injection of beta-adrenergic receptor blocking drugs into the PFH attenuated the anorexic effect of centrally or peripherally administered amphetamine (e.g. Leibowitz et al 1980b). Furthermore, it has recently been reported that neuroleptics were ineffective in blocking low-dose amphetamine anorexia (0.5 mg/kg; Burridge and Blundell 1979) but

that lesions to the VNAB did attenuate the hypophagic response to this low dose of amphetamine (Ahlskog 1974). Most studies however, fail to show attenuation of amphetamine anorexia with beta-blockers when the drugs are administered peripherally (e.g. Lehr et al 1973). This problem is addressed in experiment 3.

Other indirectly acting CA agonists (e.g. mazindol) have also been shown to produce an anorexia, which is selectively antagonized by DA blockade (see Leibowitz and Rossakis 1978 for details). Further evidence suggesting that endogenous DA mediates suppression of food intake, arises from studies using l-dopa, a CA precursor. When injected peripherally (e.g. Sanghvi et al 1975) or centrally (Leibowitz and Rossakis 1979c), l-dopa has been shown to suppress food intake. The central effect at the PFH was totally antagonized by local administration of dopa decarboxylase inhibitors and by a combination of haloperidol and propranolol, but only partially attenuated when haloperidol and propranolol were used in isolation. This suggests that both DA and NA at the PFH play some role in suppressing food intake in the rodent.

2.6.2. ANATOMY

DA-, adrenalin- and NA-containing varicosities occur in the region of the PFH (Leibowitz 1979). Increased whole brain DA has been reported following satiety (Samanin and Garrantini 1981). The relationship between increased whole brain DA and hypothalamic CA containing varicosities is obscure. However, Dunnett (1983) and Rolls (1983) have proposed that

physiologically identified DA cell bodies in the A9 region terminate in the hypothalamus. Although, a more thorough mapping of this proposed pathway is needed before any firm conclusions can be drawn, it might be that hypothalamic DA has a direct control over the physiological mechanisms controlling food intake and connections to substantia nigra provide the overriding and essential fine tuning and co-ordination of behaviour for this hypothalamic mediation to occur.

In support of this argument are the findings of Leibowitz et al (1980) who suggest that the ventral midbrain may be a source of cell bodies, both DA (A8, A9) and NA (A1, C1, A5) which project to the PFH and mediate satiety, (see section 2.7. for a more detailed discussion). However, a relevant question in all these types of studies is whether behavioural and neurochemical change resulting from lesions reflects a direct neuronal link between the midbrain and hypothalamus, as opposed to an indirect link due to damage of midbrain cells that have an impact upon the activity of hypothalamic CA neurons (Brownstein et al 1976).

These studies are consistent with the hypothesis that DA neurons contained within the PFH are not specific to the initiation of satiety, but rather through contact with other midbrain DA systems, serve as 'regulators' for the behavioural conditions whereby eating and satiety occur. For example, a low level of hypothalamic DA activity might facilitate eating, by stimulating motor responses compatible with feeding; a high level of

hypothalamic DA activity might hinder eating, by producing motor responses incompatible with eating, thereby indirectly initiating satiety or maybe an incompacity to eat.

2.7. AMPHETAMINE, CATECHOLAMINES, FEEDING AND SATIETY

The behavioural effects of amphetamine can be used as evidence to support the dual-role DA hypothesis of feeding. Amphetamine potentiates central CA transmission (e.g. Fuxe and Ungerstedt 1969); the predominant CA thought to mediate the behavioural effects of amphetamine is DA although there is some evidence for an NA mediation at lower doses (e.g. Ahlskog 1974). Low systemic doses of amphetamine (0.125-0.25 mg/kg) can produce feeding in hungry animals (Blundell and Latham 1979; Dobrzanski and Doggett 1979), whereas higher doses (0.4 mg/kg upwards) produce a marked anorexia (Burridge and Blundell 1979). This enhancement of feeding could represent a presynaptic action of amphetamine on DA receptors but this is unlikely as, when amphetamine is injected directly into the striatum at comparatively low doses, it significantly increases food intake. This suggests that amphetamine exerts its facilitatory and inhibitory effects on food intake purely via a DA mechanism (as NA is not found in the corpus striatum). The concept of DA having a dual role in feeding behaviour is consistent with the hypothesis of Lyons and Robbins (1976), who attempt to explain amphetamine action in terms of an increased tendency to respond to stimuli within a decreasing number of response categories.

That there is a beta-adrenergic component to the suppression of

feeding is well established (see section 2.5.2.1.). It is difficult to determine whether amphetamine anorexia includes a beta-adrenergic component because of concommitant DA receptor stimulation. However, lesions to the VNAB attenuate the anorexic effect of a fairly low dose of amphetamine (0.5 mg/kg) by approximately 50%. This suggests that amphetamine-induced anorexia has an NA component.

A more detailed analysis of the behavioural consequences of the VNAB lesion (see section 2.4.2.) suggests that its facilitatory effects on feeding could either be attributed to other damage in the vicinity of the VNAB (eg Oltmans et al 1976) or conversely, to specific NA damage as reported by Ahlskog (1974) and more recently by Hernandez and Hoebel (1982). Around the VNAB lesion area, axons are highly collateralized, therefore any damage aimed at a specific pathway is likely to be broadened by transsynaptic changes to other pathways. It is possible that Ahlskog's VNAB lesion also damaged ventral adrenergic fibres in the vicinity of the VNAB and DA fibres from the A8 and A9 cell groups. Lesions specifically aimed at the CTT (of which the VNAB is a component) in fact cause around a 40% reduction in the potency of amphetamine to induce anorexia, presumably arising from loss of the (beta) adrenergic component. That amphetamine anorexia is not totally abolished may be explained in terms of the DA receptor component to this behaviour (Leibowitz and Rossakis 1979b).

Alternative explanations have been put forward to account for the effects of amphetamine on feeding behaviour. Tordoff et al (1982) present evidence suggesting that low-dose amphetamine anorexia is mediated by hepatic glycogenolysis: removal of the sympathetic nerves to the viscera attenuates the effects of amphetamine. This mechanism depends on stores of glycogen which are sustained during ad lib feeding, and so it is likely that free feeding animals do have a peripheral component which contributes to amphetamine anorexia. Food-deprived animals, on the other hand, have depleted stores of glycogen and therefore this peripheral component cannot account for amphetamine anorexia in most experiments. This is reflected by studies which show the anorexic effects of central amphetamine at doses ineffective peripherally (e.g. Hoebel 1977).

A recent binding study gave indication that amphetamine bound to two distinct sites in synaptosomal membrane preparations from the rat hypothalamus (Paul et al 1983). However, the fact that phenylethylamine derivatives show affinity for specific binding sites, which was used as evidence to identify the 'amphetamine receptor', is probably merely a reflection of their similar chemical structure. The most likely explanation is that these two hypothalamic receptor binding sites represent NA and DA uptake sites respectively, or possibly pre- and post-synaptic NA sites, since amphetamine has been shown to have a direct action on post-synaptic sites at very high doses (Carlsson 1970).

2.8. SUMMARY

In summary, therefore, the effects of amphetamine on food intake vary with site of action and dosage. However, evidence does support the hypothesis that low dose amphetamine anorexia (0.5 mg/kg) can be used to index central beta-receptor activity (e.g. Ahlskog 1974). This position is confirmed in chapter 4, and this rationale underlies the use of low dose amphetamine anorexia to assess TAD function, as described in chapter 5. Futhermore, treatments which reduce midbrain DA levels also reduce feeding, though the exact behavioural mechanisms by which these different treatments reduce food intake differs markedly (see chapters 6 and 7).

If drugs which suppress feeding are to be used to index TAD function, it is clear that a detailed understanding of their behavioural action as regards feeding is required. To this end, the next chapter will review some of the different behavioural mechanisms by which a drug can reduce food intake.

CHAPTER THREE

MICROSTRUCTURAL ANALYSIS OF FEEDING BEHAVIOUR

3.1. INTRODUCTION

Studies of food intake, have typically been concerned with the amount of food consumed within a fixed time period. As illustrated in the previous chapter, amount eaten has proved useful as a dependent variable in analysing drug effects. However, if one is interested in the behavioural mechanisms that produce drug effects, then this measurement procedure is clearly inadequate. The point is exemplified by the ambiguities that arise when a drug is shown to suppress food intake. A reduction of weight of food consumed in a fixed time fails to indicate whether a drug is acting to inhibit the onset of eating, to slow the process of eating, or to terminate an eating episode prematurely. For this reason, a more detailed behavioural analysis of feeding has been used by several psychopharmacologists.

3.2. MICROSTRUCTURAL PARAMETERS AND DRUG EFFECTS

Freely feeding and food deprived rats eat in discrete 'meals' (Richter 1927). Accordingly, it is possible to analyse feeding behaviour by measuring various parameters of meal patterns, such as the number of meals taken over a given period, meal sizes, meal durations, inter-meal intervals, and the average rate of eatin during meals. Within meals, short bouts of eating are separated by short episodes of other behaviours such as ambulation and rearing (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. Such data could demonstrate that a drug can suppress food intake independently of any process related to the control of food intake itself, either by enhancing non-nutritional behaviours between eating bouts or by disrupting feeding behaviour during a meal. The intrameal parameters are often called 'microstructural' characteristics of eating.

A number of experiments have in fact yielded demonstrations of the subtle differences that the actions of drugs can exert on feeding. For instance, Blundell and Latham (1978) selected doses of amphetamine (1.0 mg/kg), fenfluramine (3.0 mg/kg) and mazindol (5.0 mg/kg) which each produced an approximate 50% reduction in food intake measured over one hour and examined these drug effects on the microstructure of feeding. Trained and independently concordant observers recorded whether the animal was eating or not. Unlike the other drugs amphetamine increased the latency to begin eating. Mazindol reduced the frequency of bouts whilst not affecting the mean weight of food consumed per bout. Fenfluramine had the reverse effect of reducing the mean weight of food consumed per bout without affecting the frequency of bouts. All three drugs reduced the duration of bouts and therefore the time spent eating. The rate of eating per bout was lowered by fenfluramine, but paradoxically, despite their hypophagic effeccts the other drugs increased eating rate.

Given that microstructural analysis of feeding behaviour can reveal variation among the actions of these anorectic drugs, it might be that the modifications in the microstructure of eating, may be related to the drugs' varying actions on particular neurochemical systems. Evidence for this hypothesis is that pimozide (a DA antagonist) reversed amphetamine's suppressant effect on food intake by increasing eating time, decreasing the latency to initiate feeding and decreasing the rate of eating. Fenfluramine, on the other hand, was antagonised by the serotoninergic receptor antagonist methergoline: the fenfluramine-induced increase in the latency to initiate feeding, decrease in the number of eating bouts, and decrease in rate of eating, were all reversed by methergoline (Blundell and Latham 1980).

One of the most interesting results to emerge from the work of Blundell and Latham (1980), is the increase in eating rate brought about by amphetamine. This effect could explain why low doses of amphetamine are sometimes seen to increase food intake (see chapter 2, section 2.7.). However, Cooper et al (1979, 1980a, 1980b) were unable to show an amphetamine-induced increase in eating rate. Spiroperidol and chlorodiazepoxide were found to antagonise amphetamine anorexia, mainly by reversing the amphetamine-induced reduction in eating time, and in the case of chlorodiazepoxide, also by reducing the increase in latency brought about by amphetamine. However, the procedures used in these studies differ from those used by Blundell and Latham

(1980), in that feeding tests were of 10 minute duration instead of 1 hour. Given that both the Blundell and Latham (1980) and Cooper et al (1979, 1980a,b) studies employed similar feeding regimes (approximately 18 hours food-deprivation before the test session), and used the same dose of amphetamine (1.0 mg/kg), it is possible that the discrepancy arises from differences in the duration of the feeding test. In a 10 minute feeding test, following 18 hours food deprivation, it is unlikely that an animal would eat in discrete bouts; it is likely that an animal would eat virtually continuously throughout the 10 minute feeding test. However, this is impossible to calculate without relevant data taken from longer feeding sessions, which would enable one to calculate the mean length of the first bout of eating.

3.3. CRITICISMS OF MICROSTRUCTURE METHODOLOGY

Studies such as the above have been criticised on several grounds. Feeding was observed over a period of only one hour, or indeed 10 minutes, excluding useful data that can be obtained from longer periods of access to food, which mimic the natural environment more closely. Animals are usually maintained on deprivation schedules and as such, given that increases in behavioural arousal modify brain neurotransmitter systems, data are obtained from 'abnormal' rats. In addition, animals are relatively insensitive to suppressant effects of drugs as eating is so vigorous, especially when the animals have been adapted to a food dprivation cycle. Also, on a deprivation schedule, peripheral metabolism is 'abnormal', affecting both eating and

drug catabolism.

In an effort to overcome criticisms of this kind, there has been a move to monitor food intake during much longer periods of access to food (e.g. 24 hours; Blundell and McArthur 1981). It is clearly impractical to expect observers to monitor such sessions, although time-lapse video recording can provide some useful information. Instead, arbitary criteria are used to define whether an animal is eating or not. For instance, a rodent meal has been defined as consuming 6 or more 45 mg food pellets, with a termination criterion of 10 minutes without taking a pellet (Hsiao et al 1979), or 5 or more pellets each separated from the last by less than 5 minutes (DeCastro 1981). It is apparent that these criteria do not take into account individual differences in eating patterns. Clearly, the criterion of what constitutes a meal is critical to every parameter calculated on the basis of such a decision. Therefore, drug effects may be distorted by artefacts of that decision. Furthermore, the differences in the definition of a meal make it difficult to compare data across studies from different laboratories.

Probably the most important criticism of microstructural analysis of feeding behaviour - what exactly constitutes a 'meal' - can be countered by establishing meal-start and meal-end criterion for each animal. This can be (and has been) achieved by direct observation of one animal at a time, but this is both labour intensive and time consuming. An alternative is to use the method

of log survivor analysis of the frequency distributions of inter-response times (IRTs) to derive a meal criterion. The short half-life of many drugs and the need to increase the probability of eating within a short time, (so that sufficient data can be obtained for IRT analysis), make it necessary to use short test sessions with food deprived rats when carrying out experiments involving acute drug injections. However, the criticisms of brief feeding sessions outlined above do not apply when microstructural analysis of feeding behaviour is used as a tool for neuropharmacological analysis, as the purpose here is to study brain mechanisms in their own right and not brain mechanisms in relation to naturalistic feeding behaviour.

3.4. MICROSTRUCTURAL ANALYSIS OF FEEDING BEHAVIOUR BY THE IRT METHOD

A record of the time taken between successive responses to take food, (the inter-response time or IRT), during a feeding session can be plotted as an IRT frequency distribution. The theory behind IRT analysis is that this global distribution in fact constitutes a number of underlying Poisson distributions, each associated with a behaviour having a constant probability per opportunity during a feeding session, e.g. meal taking, exploratory activity and sleep. The problem in IRT analysis is to resolve these natural discontinuities reliably. This task is simplified and made more objective by transforming the IRT frequency distribution into a log survivor function, i.e. the log frequency of IRTs longer than any given time period. A straight

line on a log survivor function represents a single Poisson function and so any discontinuity can be visualised as changes in the slope of the log survivor function.

This methodology has previously been applied to study drug effects on consumatory behaviour in rats over a 24 hour period (Booth 1972, Booth and Pain 1970). These authors identified breaks in survivor slope at less than 1 minute and at 7-10 minutes, using intervals between photoelectrically detected approaches to food in rats. Booth claimed that these findings established 'performance based' criteria for defining a meal, instead of the investigator using arbitary preconceptions. He interprets a pause of under 1 minute as being characteristic of consumatory control during a meal, 1-10 minutes as development of satiety during a meal, and pauses over 10 minute as ranging over intermeal intervals (Booth 1972). This criterion of what constitutes a meal was not computed for each individual animal however, but was observed from data aggregated over a sample of rats (n=6), thus obscuring possible differences between animals. So in the 24 hour IRT analysis of Booth, presumably pauses equal to or under 10 minutes represent the taking of a meal, and pauses of under 1 minute represent bouts during a meal.

In a 24 hour IRT analysis, Burton et al (1981) found no objective evidence to support a meal criterion from their data, but they were able to identify a bout-criterion from their log-survivor plot of each animal (n=8), which typically consisted of a cluster of feeding responses separated by less than 25 seconds.

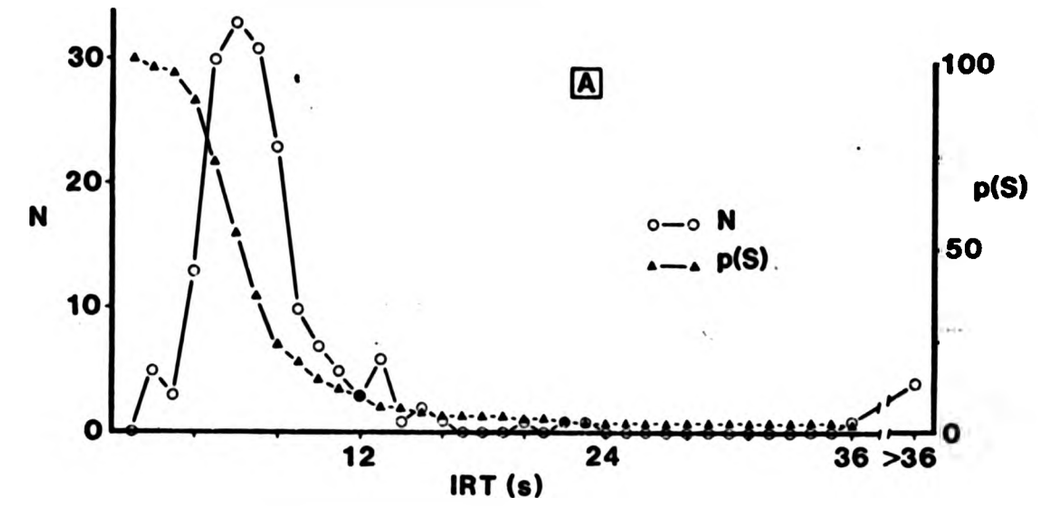
They adopted an arbitary meal criterion of 10 minutes as being representative of the literature (Kissileff 1970). Meals were typically composed of between 2 to 5 bouts.

3.5. MICROSTRUCTURAL ANALYSIS OF BRIEF FEEDING SESSIONS

The aim of the present work was to examine effects on feeding of drugs which at the doses used only suppress feeding for brief periods. An experiment was therefore carried out to determine whether log survivor analysis could be used to analyse feeding sessions brief enough to test such drugs i.e. 30 minutes. Before describing this validation exercise, some general features of the microstructural method, as used in several of the experiments in this thesis, will first be outlined.

3.5.1. APPARATUS AND PROCEDURE

Operant chambers (Campden Instruments Ltd., London), from which the levers had been removed, were programmed to deliver a 45 mg food pellet (Campden Instruments Ltd., 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 light and tray light were illuminated continuously, 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 figure 1A).



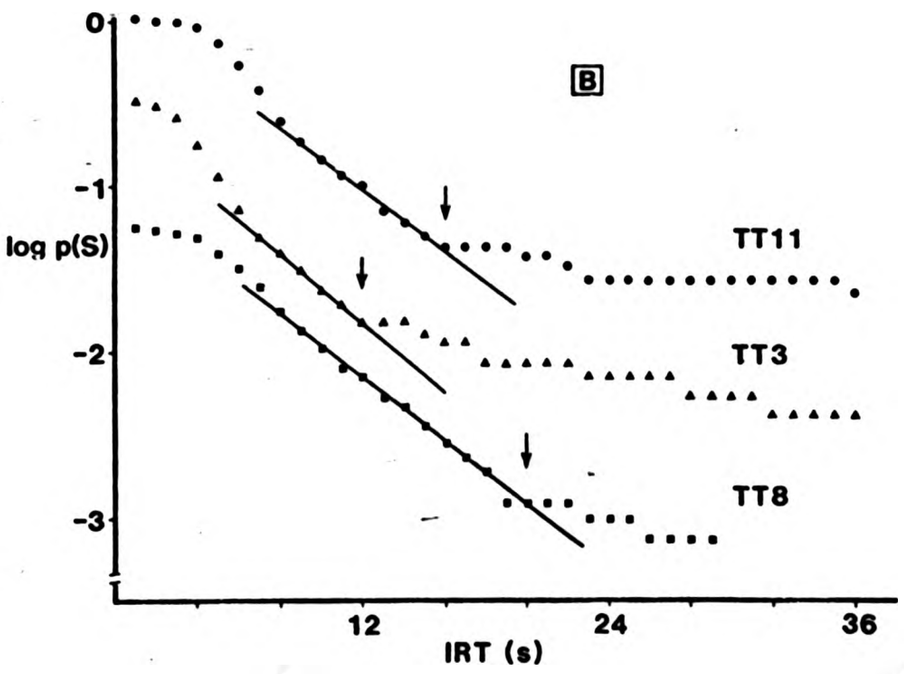


Fig. 1

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.

Animals were food deprived for between 17-21 hours, with water available ad libertum. Before the start of each experiment, animals were trained to press for food reward to asymptotic performance. On experimental days, when drug treatments were given, the animals were tested for 30-minute sessions in the boxes. On days between drug treatments, animals were given ten-minute sessions. All testing took place between 10.00 h and 14.00 h. The animals were fed with standard laboratory diet (Dixons, Ware, Herts.) at 14.00 h and deprived at 17.00 h.

3.5.2. ANALYSIS

The IRT frequency distribution, a distribution of the time taken between successive responses to take food, can be transformed to a survivor function, which shows the number or the proportion of IRT's greater than any given IRT (figure 1A). A logarithmic transformation of the proportions produces a log survivor function (figure 1B). 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 fairly easily. A small proportion of log survivor curves do not have a clean breakpoint which is easily detectable (figure 2A; data taken from experiment 2). To aid identification of the breakpoint, grouped log survivor curves are constructed for each treatment condition (figure 2B), which define a region in the log survivor curve where the breakpoint is likely to occur.

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FIGURE 2A

LOG SURVIVOR CURVES FOR VARIOUS DOSES OF AMPHETAMINE

In these examples, which were seldom, the breakpoint was difficult to detect.

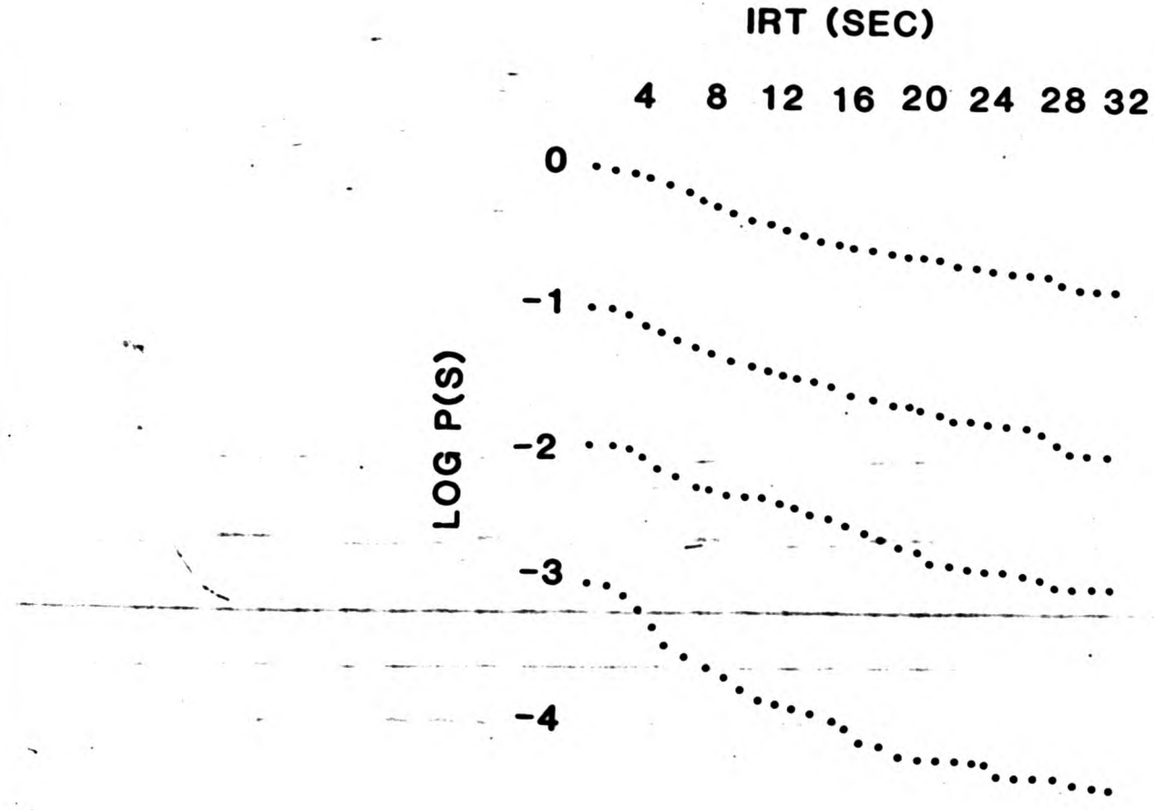
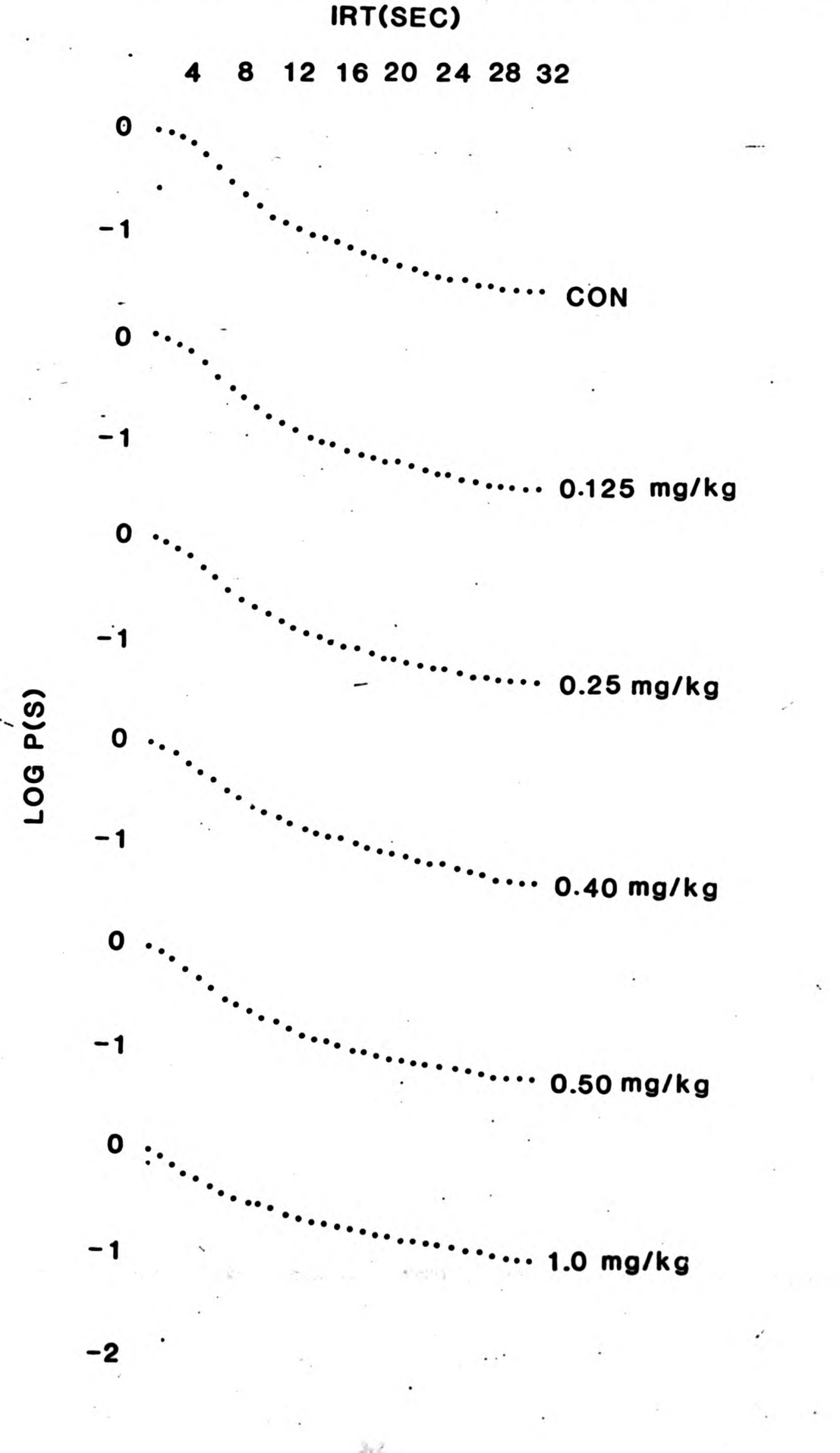


FIGURE 2B
GROUPED LOG SURVIVOR CURVES FOR VARIOUS DOSES OF AMPHETAMINE



The reliability of identifying the breakpoint has been assessed. Five independent judges estimated the position of the breakpoint for every log survivor function in 21 sets of 12-20 curves. The lowest concordance coefficient between the 5 judges was 0.75, and the majority of concordances were above 0.85.

Following identification of the breakpoint, the following parameters of feeding are 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 IRTs 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.

3.6. EXPERIMENT 1: VALIDATION OF MICROSTRUCTURAL ANALYSIS OF FEEDING BEHAVIOUR BY LOG SURVIVOR ANALYSIS

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 with those obtained through direct observation.

3.6.1. METHOD

Subjects

Twelve male Lister hooded rats (weight 330-400 g) were housed in pairs and maintained on 21-hour food deprivation, with water available ad lib. The animals had had prior experience of continuously reinforced lever pressing for food rewards.

Behaviour in the apparatus was recorded on videotape, using a video camera adapted for low intensity light. By the use of a second camera filming the VDU, and a video-mixer, the occurrence and time of each response on the tray door was also recorded on the film.

Procedure

Following a pretraining period in which 10-min daily sessions were run until all animals achieved asymptotic performance of continuous reinforcement, the animals were given a single 30-min session, which was recorded and filmed as described. The animals were 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-reponse intervals in which behaviours other than eating (rearing, grooming and walking) occurred. Microstructural parameters were calculated following identification of the breakpoint, as outlined above.

3.6.2. RESULTS

The rats consumed a mean of 218 pellets (9.8 g) in the 30-minute session (range: 148-268). Inspection of the log survivor curve for each animal (e.g. figure 1B) showed breakpoints varying from 12 to 25 sec (mean +/- SE = 16.8 +/- 0.9 sec). If the IRT frequency distributions are simply summed across animals, without regard to the differences in breakpoint, the occurrence of behaviours other than eating appears to increase almost linearly for IRTs between 10 and 30 sec (figure 3). However, a very different picture is shown by the distribution of IRTs around the breakpoint (figure 4). The incidence of behaviours other than eating now shows a marked discontinuity: other behaviours were relatively rare (5.8 +/- 0.8% of inter-response intervals) at IRTs shorter than the breakpoint, and highly likely (88.4 +/- 2.6% of intervals) at IRTs longer than the breakpoint. It is clear that using the breakpoint for each individual to provide an eating criterion (figure 4) affords a far clearer discrimination between eating and not eating, than would any arbitrarily chosen or aggregate criterion (figure 3).

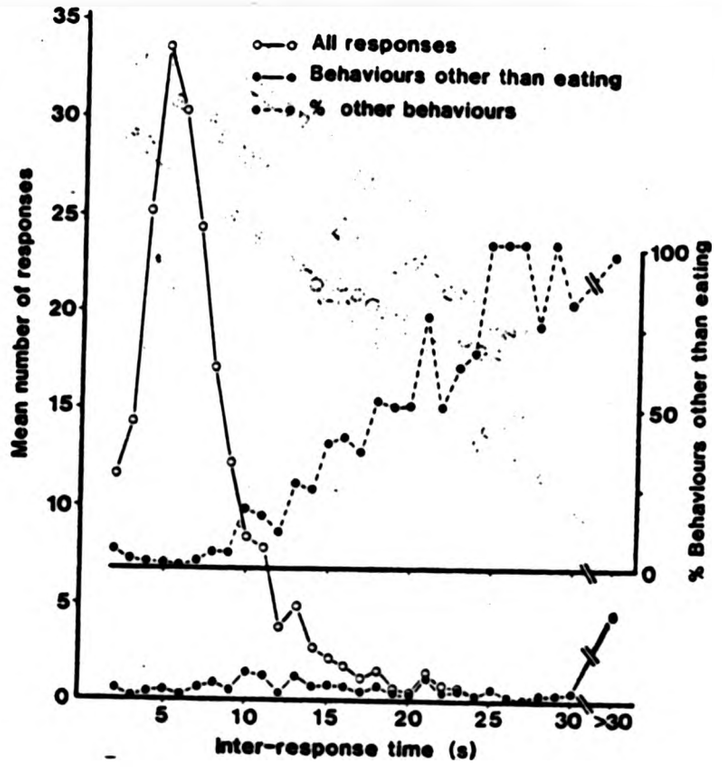


Fig.3

The frequency distribution of IRTs (mean of all subjects), the distribution of those inter-response intervals in which behaviours other than eating were observed, and the latter as a proportion of the total. For clarity, the percentage scale has been displaced upwards.

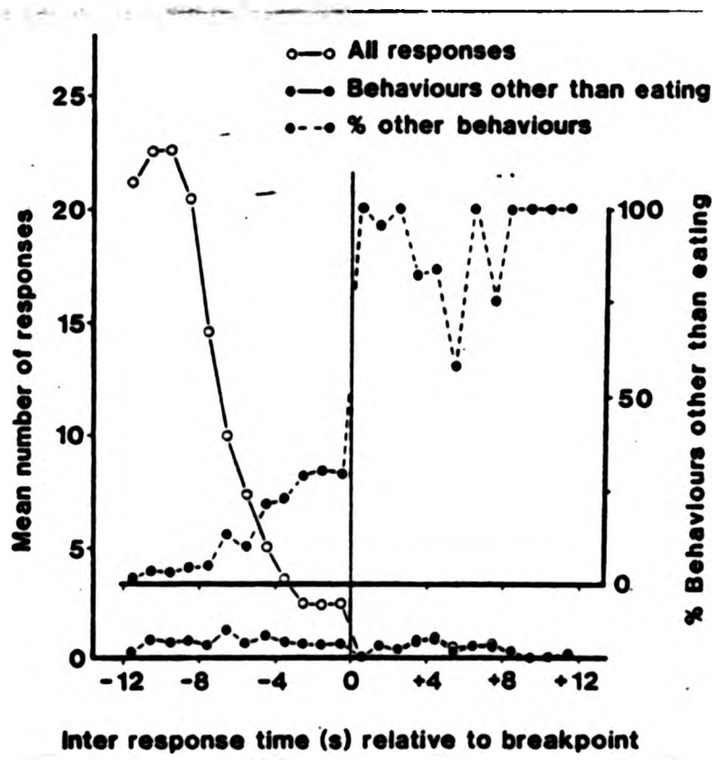


Fig.4

For each subject, the breakpoint was identified by log survivor analysis (see text), and the frequency distribution of inter-response times plotted for 12 seconds either side of the breakpoint. The figure shows the IRT frequency distribution (mean of all animals), the distribution of those inter-response intervals in which behaviours other than eating were observed, and the latter as a proportion of the total. For clarity, the percentage scale has been displaced upwards.

TABLE 2

COMPARISON OF MICROSTRUCTURAL PARAMETERS DERIVED FROM DIRECT OBSERVATION AND FROM LOG SURVIVOR ANALYSIS

	Eating rate (pellets/s)	Eating time (s)	Number of bouts	Bout length (s)
Calculated	.162(±.007)	1289(±70)	12.3(±1.3)	128(±21)
True	.164(±.007)	1205(±55)	23.1(±1.8)	62(±6)
Error %	-1.3(±1.0)	6.5(±1.7)	-43.7(±6.3)	41.0(±7.4)
Adjusted		1308(±55)	16.9(±1.3)	83(±7)
Error %		-2.1(±1.4)	-25.0(±6.6)	22.9(±7.7)

Microstructural parameters were calculated using the bout criterion derived from log survivor analysis (see text). "True" values were obtained by direct observation. The "adjusted" values add 4.5 s per bout to "true" eating time, and exclude gaps of less than 10 s when counting the number of bouts. The percentage error terms refer to calculated values in relation to true/adjusted values. All values are means (± standard error).

Estimates of eating time and local eating rate were calculated using the breakpoint as described above. Also, the values of these parameters were corrected, by excluding from eating bouts the 5.8% of short inter-response intervals which the film showed to be false positives, and including the 11.6% of long intervals which were false negatives (Table 2). Compared with these corrected values, the crude values under-estimated eating rate by 1.3% (+/- 1.0%) and over-estimated eating time by 6.5% (+/- 1.7%). Eating rate appears to be a very robust measure, which is not significantly affected ($t = 1.3$, $p>0.1$) by the small proportion of errors. Although eating time was less robust, the figure of 6.5% overestimates and exaggerates the error, since the corrected eating time makes no allowance for the final pellet of each bout. If it is assumed that these pellets were consumed in the modal time of 4.5 sec (figure 3), then a further corrected estimate of eating time may be made (table 2). This figure is higher than the calculated value by an insignificant 2.1% (+/- 1.4%) ($t = 1.5$, $p>0.1$). Thus, as the effects of the two types of error to some extent cancel one another out, the original crude values for both eating time and eating rate are very close to their true values.

Estimates of the number and length of bouts were 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; indeed, it was sometimes

possible to see that an animal did continue to eat whilst moving away from the food dispenser. If very short gaps (<10 sec) are excluded from the calculation (table 2), then the discrepancy in the number and length of bouts, though still marked, is considerably reduced (25 and 23% respectively).

3.6.3. DISCUSSION

During a microstructural analysis, the emphasis is directed not to 'what constitutes a meal', but rather to whether at any moment an animal is eating or not. In order that there is a high probability that they will eat during the half hour test session, animals were deprived for 21 hours. This is necessary for log survivor analysis, because the method depends on a large number of data points. If there were evidence of a meal ending, instead of the animals eating in discrete bouts within a meal, then the calculated microstructural parameters of feeding could be misleading. As the intermeal gap length would now have to be calculated instead of the interbout gap length, there would be an apparent increase in gap length. However, in the experiments presented in this thesis gaps of more than 10 minutes (the criterion of a meal generally accepted in the literature) are almost never seen in control animals.

In conclusion, the method here described is clearly more successful than the use of arbitrary criteria for discriminating between eating and not eating. Compared with continuous observation, the method produces very accurate estimates of

eating rate and eating time. The method underestimates the number and over-estimates the length of eating bouts, but it does have the advantage that the bout criterion is unambiguous, rather than relying on the often difficult subjective judgement of whether an animal is eating or not. Error arises from the fact that the frequency of continuously reinforced feeding reponses decreases as IRT increases, which means that there are more responses at IRT values below the breakpoint than above (figure 4); the error is therefore relatively constant between subjects. As will be shown in the following chapter, results obtained using the present method are consistent with those obtained by previous authors using conventional observational methods.

This microstructural analysis technique has been successfully used to analyse feeding behaviour in a number of experiments, including those in the next chapter, which assess the relative contributions of NA and DA mediated-mechanisms to amphetamine-induced suppression of feeding.

CHAPTER FOUR

MICROSTRUCTURAL ANALYSIS OF AMPHETAMINE ANOREXIA

4.1. INTRODUCTION

The experiments in this chapter investigate the proposed beta-adrenergic and dopaminergic mediation of the food-intake-suppressant effects of amphetamine using microstructural analysis of feeding. It is known that amphetamine anorexia has a dopaminergic component as well as a beta-adrenergic one. Basic to the present approach, therefore, are methods to distinguish the contributions of NA (or adrenalin) and DA-mediated mechanisms to amphetamine anorexia. This distinction is not simple because the relative contribution of NA and DA seem to depend on amphetamine dose. Leibowitz (1975) has provided evidence suggesting that amphetamine anorexia was attenuated by beta-adrenergic antagonists. This NA (or adrenalin) mediation is thought by some to be specific to lower doses of the drug (Burridge and Blundell 1979). The first experiment in this chapter was therefore designed to establish dose-response relationships for the effects of amphetamine on microstrutural parameters of feeding, in order to assess the relative contributions of NA and DA-mediated mechanisms to this anorexia in the second and third experiments of this chapter respectively.

4.2. EXPERIMENT 2

4.2.1. INTRODUCTION

Although a number of dose-response studies have been carried out on amphetamine anorexia, they are controversial in that their

findings vary at low doses of the drug: two studies show a stimulation of feeding at around 0.1 and 0.25 mg/kg (Blundell and Latham 1978, Dobrzanski and Doggett 1976), whilst the more common finding is a slight reduction of feeding (Cooper et al 1979). Intracranial administration of amphetamine also has variable effects on feeding, depending on the site of injection. Low doses of amphetamine (0.125 mg/kg and 0.25 mg/kg) injected into the striatum produce a stimulation of feeding (Winn et al 1982); the same is true of administration into the PVN (Leibowitz 1980) and parts of the LH (see experiment 5). However, the more common finding following amphetamine injection in the LH is a marked reduction in feeding (Leibowitz and Rossakis 1978). Findings at higher doses from both peripheral and central studies all concur in showing a marked anorexia.

This experiment examined the dose-response relationships of amphetamine anorexia using the microstructural analysis of feeding outlined in the previous chapter. The experiment was also used as a further validation study of the log survivor analysis of eating, by comparing these results with those obtained by Blundell and Latham (1980) through direct observation (see chapter 3, section 3.2.).

4.2.2. METHOD

Subjects

Twenty-four male Lister hooded rats (OLAC), weight 280-350g, were individually housed and maintained throughout the experiment on a

21-hour food deprivation schedule, in which food was available between 14.00h and 17.00h daily, with water available ad lib. The animals had been previously trained to press the tray door of the operant chamber for food reward.

Apparatus

The animals were trained to feed by pressing the door of the pellet dispenser in one of six identical operant chambers, as described in chapter 3. Ten-minute daily sessions were run until all animals reached asymptotic performance.

Drugs and Procedure

Six treatment conditions were used: control (distilled water) and five doses of d-amphetamine sulphate (Smith, Kline and French) 0.125, 0.25, 0.40, 0.50 and 1.0 mg/kg. Doses were calculated as salts and all injections were made i.p. at a volume of 1 ml/kg. All animals received each treatment once, over six experimental days, according to an individually randomized design. Animals were injected between the hours of 10.00 and 13.00, 30 minutes before their test session which lasted for 30 minutes.

Log survivor curves were constructed for each session for each animal; in addition, a set of curves was constructed for each treatment condition using the grouped data from all animals. In this and all subsequent microstructural analysis experiments reported in this thesis, breakpoints were determined for each of the individual curves by two independent judges, who were blind

as to the treatments administered. When there was a disagreement, which was seldom, the value was chosen which was closer to the centre of the breakpoint region in the grouped curves. The grouped curves for the present experiment were presented in chapter 3, section 3.5.2. Following identification of the breakpoint, the microstructural parameters of feeding were calculated, and subjected to analysis of variance, supplemented by tests of simple main effects.

4.2.3. RESULTS

Amphetamine caused a small and reliable reduction in total food intake at 0.40 (14%) and 0.50 (13%) mg/kg ($F(1,115) = 9.11$ and 7.88 respectively, $p<.01$). This reduction was enhanced at 1.0 mg/kg (37%) and was highly significant ($F(1,115) = 64.95$, $p<.001$; see figure 5A). Lower doses of amphetamine either had no effect (0.125 mg/kg) or caused a small (5%) but insignificant reduction (0.25 mg/kg). The anorexia observed at 0.40, 0.50 and 1.0 mg/kg is entirely attributable to significant reductions in eating time ($F(1,115) = 20.09$, 38.08, 125.84 respectively, $p<.001$; see figure 5C). Amphetamine also caused an increase in eating rate, which was significant at 0.50 and 1.0 mg/kg ($F(1,115) = 8.14$ and 8.99 respectively, $p<.01$, figure 5B).

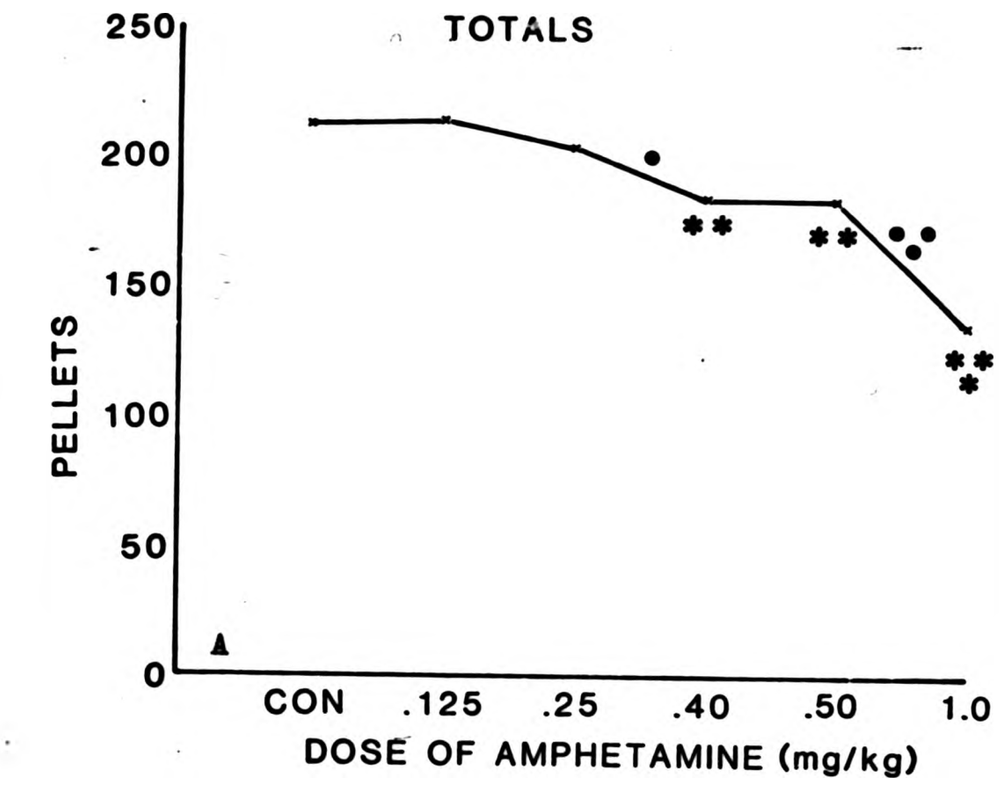
The reductions in eating time appeared to be brought about primarily by reductions in bout length, which - like the effects on eating time - were highly significant ($F(1,115) = 18.45$, 27.27, 63.45, $p<.001$) at 0.40, 0.50 and 1.0 mg/kg respectively

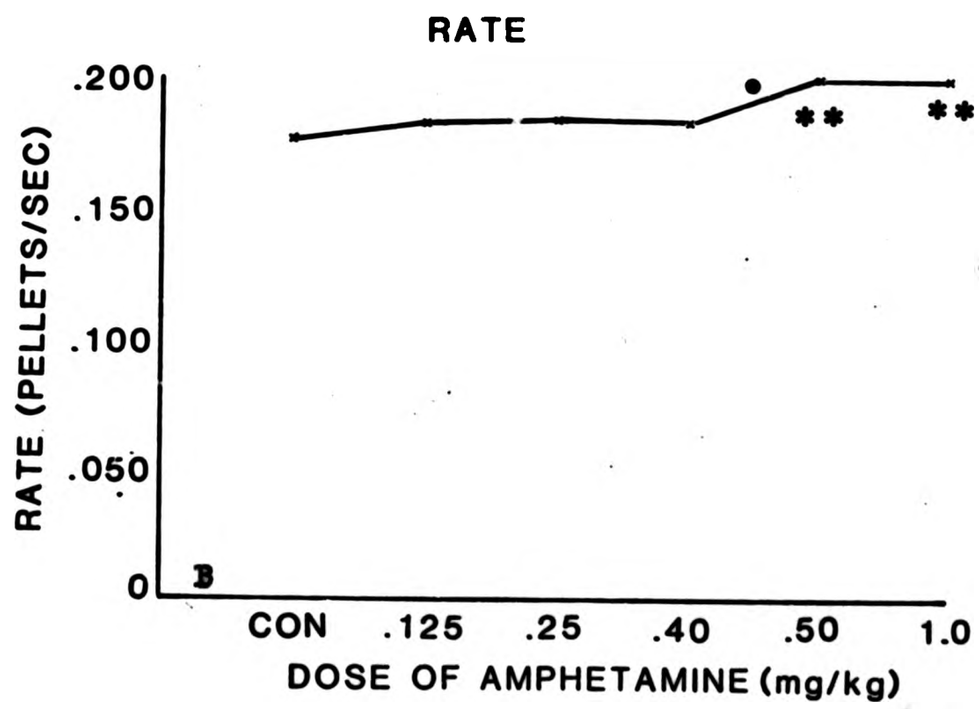
(figure 5D). At these doses, amphetamine caused significant increases in the number of bouts (0.40 mg/kg, $F(1,115) = 8.18$, $p<.01$, 0.50 and 1.0 mg/kg, $F(1,115) = 19.68$ and 37.51 respectively, $p<.001$, figure 5F). Gap length was very slightly increased, at 0.25 mg/kg and above, though these changes did not reach statistical significance (figure 5E). Amphetamine significantly increased the latency to initiate feeding at a dose of 1.0 mg/kg ($F(1,115) = 8.26$, $p<.01$ figure 5G). Means and S.Es. together with overall F-ratios for the microstructural parameters are given in table 3.

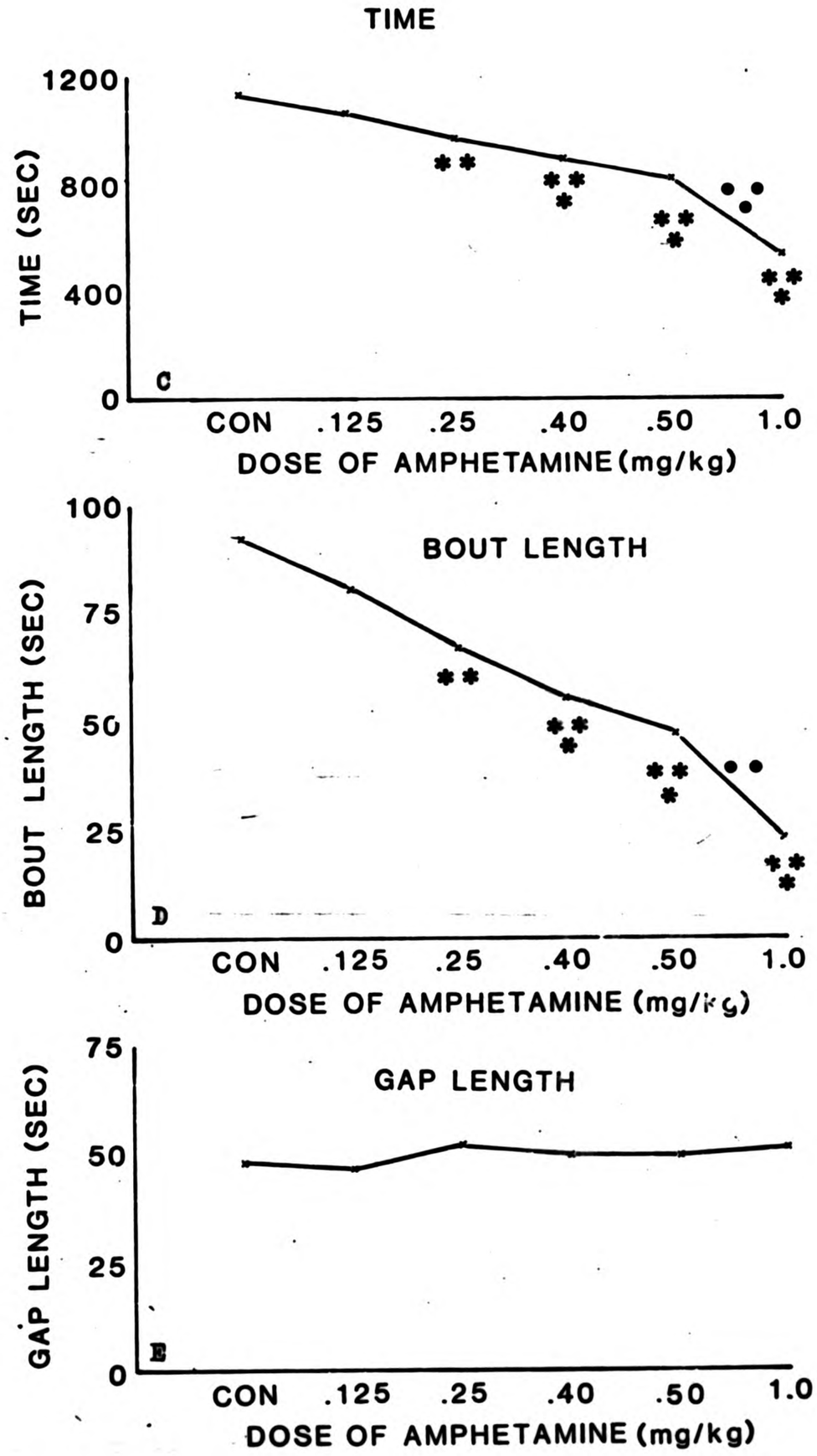
FIGURE 5

THE EFFECTS OF DIFFERENT DOSES OF AMPHETAMINE ON FEEDING.

Stars show difference from control. Dots show difference between doses. One symbol $p<.05$, two symbols $p<.01$, three symbols $p<.001$.



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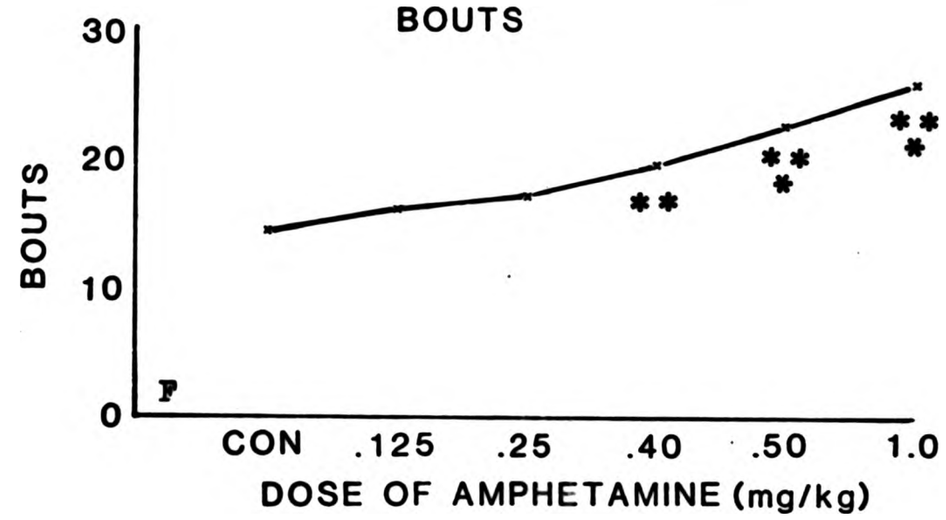
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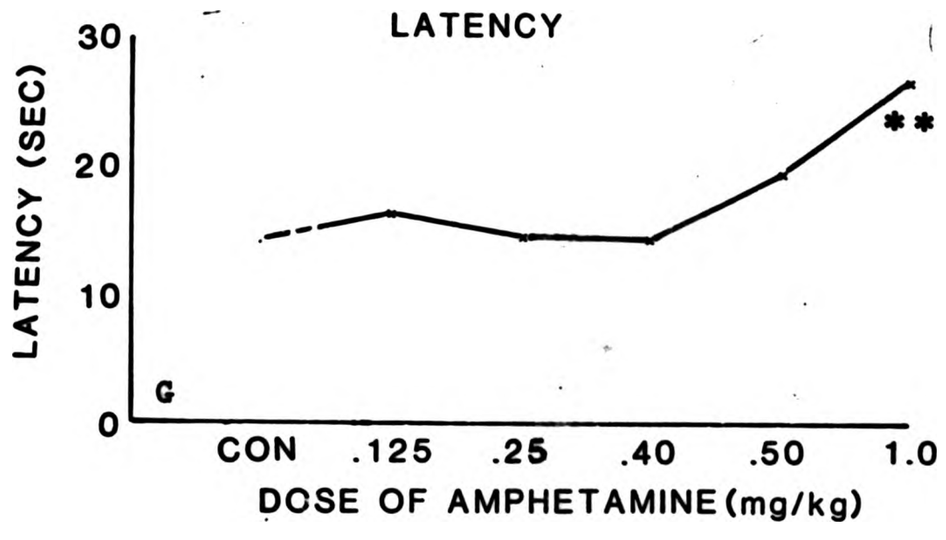
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TABLE 3

CHANGES IN MICROSTRUCTURAL PARAMETERS FOLLOWING VARIOUS DOSES OF AMPHETAMINE

Microstructural Parameter	Dose of amphetamine (mg/kg) 0	0.125	0.25	0.40	0.50	1.00	F Ratio d.f. 5,115	p
Total	213	214	204	184	186	136	18.59	.001
Rate	0.176	0.184	0.187	0.184	0.202	0.203	2.98	.05
Time	1133.77	1081.21	988.88	903.24	816.4	558.9	33.13	.001
Bout length	92.9	81.9	67.5	56.1	48.2	24.6	16.36	.001
Gap length	49.5	47.4	52.0	50.5	50.0	51.5	0.20	n.s.
Bouts	14.6	16.2	17.3	19.9	22.8	26.0	10.82	.001
Latency	14.59	16.70	14.92	14.53	19.23	26.85	2.52	.05
Change in food intake %	-	+0.5	-5.0	-13.6	-12.7	-36.6	-	-

Differences between control and 1.0 mg/kg amphetamine are shown by p values.

4.2.4. DISCUSSION

The most striking result to emerge from this study is the dose-related decrease in eating time, which become apparent at a dose as low as 0.25 mg/kg. Reductions in eating time therefore appear to be responsible for the anorexia observed following amphetamine treatment (Blundell and Latham 1980, Cooper et al 1979, 1981). Accompanying a reduction in eating time is an increase in eating rate at the two highest doses of amphetamine (0.50 and 1.0 mg/kg). This result is paradoxical in that, without a concomitant reduction in time, an increase in eating rate would actually increase food intake. This experiment showed no increase in food intake following low doses of amphetamine, as the small increase in rate and small decrease in time exactly cancelled each other out at 0.125 mg/kg. The increase in food intake sometimes observed at this dose can possibly be explained by a proportionally greater increase in eating rate.

The dose-related reduction in eating time could be caused by an increase in gap length, a decrease in bout length or an increased latency, or any combination of these parameters. In fact the main component underlying the decrease in eating time is a strong dose-related reduction in bout length. Changes in gap length were not significant. However, doses of amphetamine that reduced food intake did cause non-significant increases in gap length compared to control values, whilst a dose of 0.125 mg/kg, which did not change food intake, slightly reduced gap length - a result consistent with stimulation of feeding. An increased

latency observed at 1.0 mg/kg contributed to the dramatic reduction in eating time seen between 0.5 mg/kg and 1.0 mg/kg. However, latency measures at other doses showed no significant changes, probably due to the high motivational state of the animal to eat, imposed by 21 hour food deprivation. In addition to these parameters supporting a decrease in eating time, the number of bouts taken by the animals increased in a dose-related fashion with significance being reached at 0.4 mg/kg ($p<.01$), 0.5 and 1.0 mg/kg ($p<.001$).

The results obtained in this study are consistent with those of Blundell and Latham (1980), but not of Cooper et al (1979), who observed a significant reduction of food intake at 0.25 mg/kg. Furthermore, at the higher dose of 1.0 mg/kg, Cooper et al were unable to show any increase in rate brought about by amphetamine treatment. As discussed in the previous chapter, this result is probably an artefact of their methodology.

Estimation of the microstructural parameters of feeding depends on the identification of the breakpoint. The mean breakpoint values for the 6 amphetamine doses are presented in table 4 (which also includes comparable data for experiments 3 and 4). The breakpoint values differed significantly between the control and 1.0 mg/kg treatments ($F(1,115) = 4.90$, $p<.05$). If there were a problem in the methodology of microstructural analysis, the fairly consistent increase in rate following amphetamine treatment could be an artefact. For this reason IRT frequency curves were constructed to represent the raw data graphically

(figure 6). A clear shift in peak towards the left can be seen with increasing doses of amphetamine. This is further evidence for an increase in eating rate with amphetamine which is independent of the log survivor analysis.

(figure 6). A clear shift in peak towards the left can be seen with increasing doses of amphetamine. This is further evidence for an increase in eating rate with amphetamine which is independent of the log survivor analysis.

TABLE 4

MEAN BREAKPOINTS FOR EXPERIMENTS 2, 3 and 4

EXPERIMENT 2

Dose of amphetamine (mg/kg)	Mean breakpoint
0	16.42
0.125	16.16
0.25	16.87
0.40	16.5
0.50	15.0
1.00	14.45

EXPERIMENT 3

Treatment	Mean breakpoint
Control	17.5
Propranolol	18.3
Amphetamine	17.8
Amphetamine/Propanolol	16.8

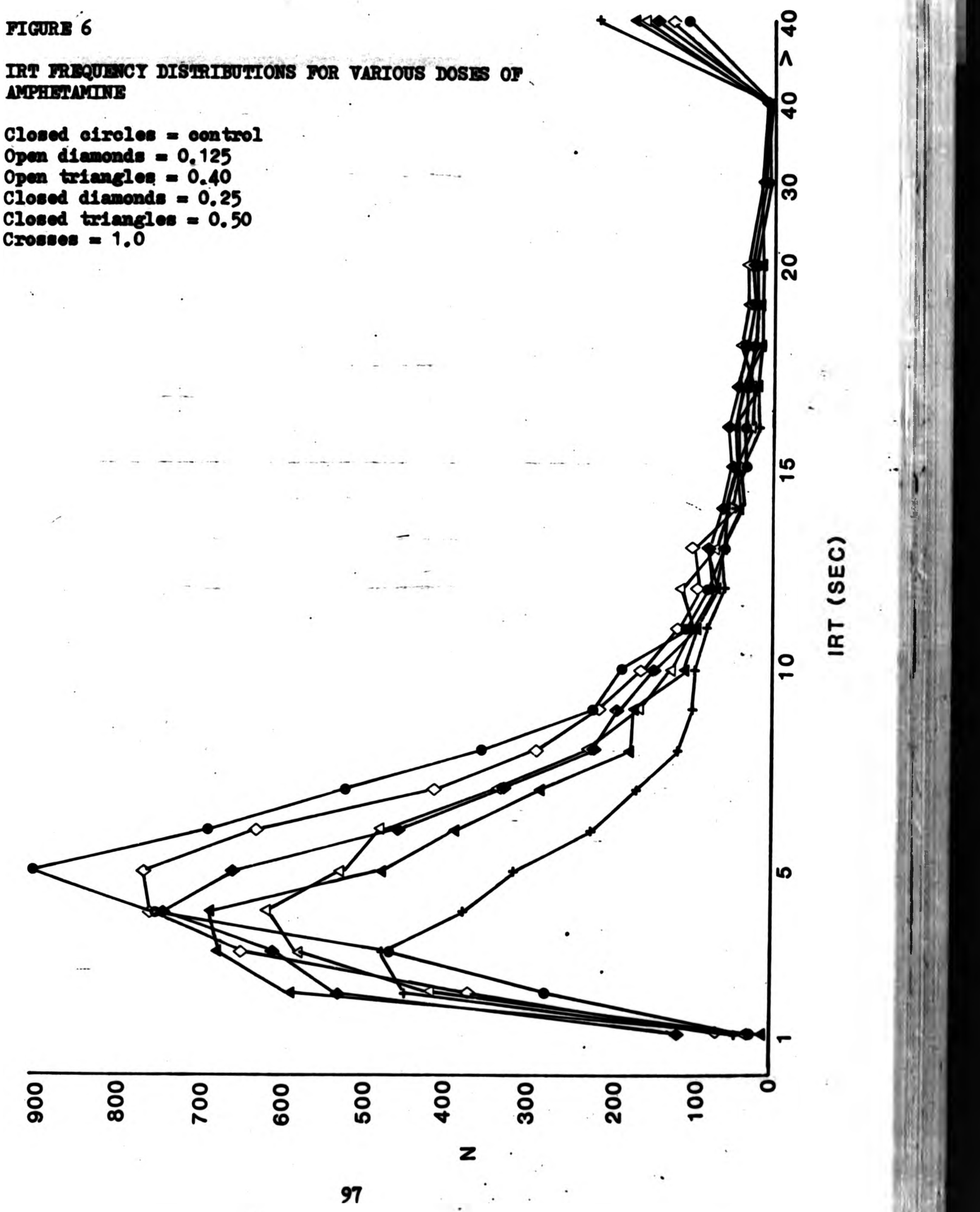
EXPERIMENT 4

Treatment (mg/kg)	Mean breakpoint	Treatment (mg/kg)	Mean breakpoint
Control	17	Control	18
Amphetamine 0.5	12	Amphetamine 0.5	16
Amphetamine 1.0	13	Amphetamine 1.0	16
Pimozide 0.45	20	Thioridazine 5.0	19
Pimozide 0.45 + amphetamine 0.5	17	Thioridazine 5.0 + amphetamine 0.5	19
Pimozide 0.45 + amphetamine 1.0	16	Thioridazine 5.0 amphetamine 1.0	18

FIGURE 6

IRT FREQUENCY DISTRIBUTIONS FOR VARIOUS DOSES OF AMPHETAMINE

Closed circles = control
Open diamonds = 0.125
Open triangles = 0.40
Closed diamonds = 0.25
Closed triangles = 0.50
Crosses = 1.0



In summary then, the main mechanism by which amphetamine produces anorexia is a decrease in eating time, and at the highest dose, an increased latency to eat. A concomitant increase in rate is also observable. A theory of amphetamine action has been proposed by Lyons and Robbins (1975), who explain the behavioural effects of amphetamine in terms of increased response rates within a decreasing number of response categories. Winn et al (1982) present evidence in support of this hypothesis: amphetamine injected into the striatum at very low doses was found to stimulate feeding and, in the absence of food, other behaviours such as locomotion. As the dose of amphetamine was increased, feeding was suppressed as well as other complex behavioural sequences, until finally simpler acts such as rearing and repetitive motor stereotypies were observed. These findings suggest that the decreased food intake after amphetamine does not represent a true anorexia but simply reflects the facilitation of behaviours that are incompatible with feeding. However, Dobrzanski and Doggett (1979) and Blundell and Latham (1978) have reported that the increased feeding with low doses of amphetamine was unaccompanied by evidence of any increase in general activity. This raises the possiblity that low doses might be selective for brain mechanisms specifically involved in the control of food intake, such as the hypothalamic mechanism sensitive to NA, which is known to enhance feeding (see chapter 2, section 2.5.1.).

Studies using DA receptor antagonists to reverse amphetamine

anoreia have nearly all reached similar conclusions. In most studies, pretreatment with a DA-receptor blocking drug can reverse a large component of amphetamine anorexia, (e.g. Kruk 1973). Burridge and Blundell (1979), on the other hand, have reported that whilst the DA receptor blocker pimozide (amongst other typical neuroleptics) was effective in reversing the anorexic effect of higher doses of amphetamine (1.0 mg/kg and 2.0 mg/kg), thioridazine and clozapine, which are atypical neuroleptics, did not attenuate amphetamine anorexia at any dose. Typical neuroleptics are known to antagonise DA-mediated hyperactivity or amphetamine stereotypy (Costall and Naylor 1975), whereas atypical neuroleptics are not. On the basis of such findings and their own observations, Burridge and Blundell (1979) postulated that the anorexia observed following 1.0 and 2.0 mg/kg of amphetamine treatment is due to the inability of animals to eat on account of competing behavioural responses such as stereotypy, whilst low dose anorexia is a 'true' anorexia mediated in the LH.

Another finding to emerge from thr Burridge and Blundell study was evidence of non-DA mediation of the low dose anorexia. All neuroleptics, including pimozide, failed to reverse the anorexic effect of 0.5 mg/kg amphetamine, and when reversal was apparent at the 1.0 and 2.0 mg/kg dose, it was not total, thereby implicating the involvement of another mechanisms, most probably NA. In addition to the lesion studies of Ahlskog and Leibowitz cited in chapter 2, which support this view, is the finding that

low doses of amphetamine exert a more potent blockade of the re-uptake of NA than DA (Samanin et al 1978).

4.3. EXPERIMENT 3: MICROSTRUCTURAL ANALYSIS OF THE INVOLVEMENT OF BETA-RECEPTORS IN AMPHETAMINE ANOREXIA

4.3.1. INTRODUCTION

Studies employing central drug administration also suggest that amphetamine anorexia is not mediated solely by DA. Injection of beta-adrenergic receptor blocking drugs in the region of the perifornical hypothalamus attenuate the anorexic effect of centrally or peripherally administered amphetamine; this and several other lines of evidence strongly support the concept of a beta-adrenergic satiety system in the perifornical hypothalamus (see chapter 2, section 2.5.2.2.). On the basis of these results, it would be expected that amphetamine anorexia should also be attenuated by peripherally administered beta-blockers. Paradoxically, however, this does not appear to be the case. Preliminary studies in this laboratory failed to demonstrate attentuation of amphetamine anorexia by the beta-blocker propranolol, and with one exception, (Sanghvi et al 1975), previous investigations have had similar results (e.g. Dobrzanski and Doggett 1979).

The resolution of this paradox may lie in the observation that propranolol impairs the metabolism of amphetamine (Shoeman et al 1974). This effectively increases the dose of amphetamine reaching the brain, which would tend to mask a partial blockade of the anorexic effect. In the present experiment, this

possibility was investigated using the technique of microstructural analysis. It was reasoned that if the dopaminergic and beta-adrenergic systems control different parameters of feeding, then these might be differentially affected by propranolol. Specifically, if any amphetamine-induced microstructural changes are mediated by beta-receptors, then such changes might be blocked by propranolol, whilst at the same time microstructural changes, which are mediated by DA receptors, would be enchanced by propranolol because of a higher amphetamine dose, arising from impairment of amphetamine metabolism.

The dose of propranolol used in this experiment was chosen on the basis of the consideration that the dose should be as high as possible, but should not itself produce an anorexic effect, since that would unduly complicate interpretation of the results. In preliminary studies, we found that a small (20%) but significant anorectic effect was produced by 10 mg/kg propranolol; a dose of 5 mg/kg was therefore chosen for the present study.

4.3.2. METHOD

Subjects

Twenty-four male Lister hooded rats (weight 360-430 g) were trained to feed by pressing the door of the pellet dispenser in one of three identical operant chambers, as described in chapter 3, experiment 1. Ten-minute daily sessions were run until all animals attained asymptotic performance.

Drugs and Procedure

On experimental days, the animals received two intraperitoneal injections: propranolol HC1 (5 mg/kg) (Sigma) was administered 60 minutes before the start of the session and d-amphetamine sulphate (0.5 mg/kg) (Smith, Kline and French) 30 minutes before. Control injections in both cases were distilled water (1 ml/kg). Each animal received all four treatment combinations in a counterbalanced order, at two-day intervals. On the intervening days, a ten-minute session was run, with no drug treatments. During experimental sessions, which were 30 minutes long, a computer recorded each response on the tray door, as described in chapter 3, experiment 1. Analysis of microstructural parameters of feeding was carried out as described previously (see chapter 3, experiment 1). Results were analysed by analysis of variance, supplemented by tests of simple main effects. The mean breakpoint in the four conditions varied between 16.8 and 18.3 seconds; the differences were not significant (all F-ratios<1, see table 4).

4.3.3. RESULTS

Amphetamine caused a small (13%) but highly significant ($F(1,46) = 12.83$, $p<0.001$) decrease in food intake (figure 7A), which was apparently blocked by propranolol pretreatment (interaction: $F(1,23) = 3.2$, $0.05<p<0.1$). However, this conclusion would be seriously misleading. Total food intake may be broken down into eating rate and eating time (figures 7B and 7C), and propranolol actually increased the amphetamine-induced changes in both these

parameters: eating rate was only very slightly increased by amphetamine alone, but a substantial increase was seen following propranolol pretreatment ($F(1,46) = 9.22$, $p<.01$); eating time was decreased by amphetamine, and this effect was also somewhat greater following propranolol pretreatment ($F(1,46) = 17.42$, $p<.001$). It is the combination of decreased eating time and increased eating rate, which results in no significant net change in total intake, following propranolol pretreatment.

A description of the distribution of behaviour within the session is given by the mean length of feeding bouts, the mean length of gaps between bouts, and the initial latency; these three parameters determine the total feeding time. As in experiment 2, amphetamine reduced eating time and therefore reduced bout length and increased gap length. In the present experiment the amphetamine-induced increase in gap length was statistically significant ($F(1,46)= 11.21$, $p<.01$, figure 7E) but the decrease in bout length was not (figure 7D). Propranolol treatment blocked the effect of amphetamine on gap length (figure 7E). There were smaller, but insignificant effects on latency (figure 7F).

Propranolol significantly increased bout length ($F(1,46) = 5.35$, $p<.01$, figure 7D); this effect led to an increase in eating time ($F(1,46) = 6.15$, $p<.01$, figure 7C), and is reflected in an increase in bout size ($F(1,46) = 6.44$, $p<.05$, figure 7G), and a decrease in the number of bouts ($F(1,46) = 5.21$, $p<.05$, figure

7H). As in the case of eating rate, propranolol increased the effect of amphetamine on bout length ($F(1,46) = 7.45$, $p<.01$, figure 7D), bout size ($F(1,46 = 7.88$, $p<.01$, figure 7G) and the number of bouts ($F(1,46) = 7.22$, $p<.01$, figure 7H): on each of these measures, significant effects of amphetamine were seen following propranolol pretreatment, but amphetamine alone produced small and insignificant effects.

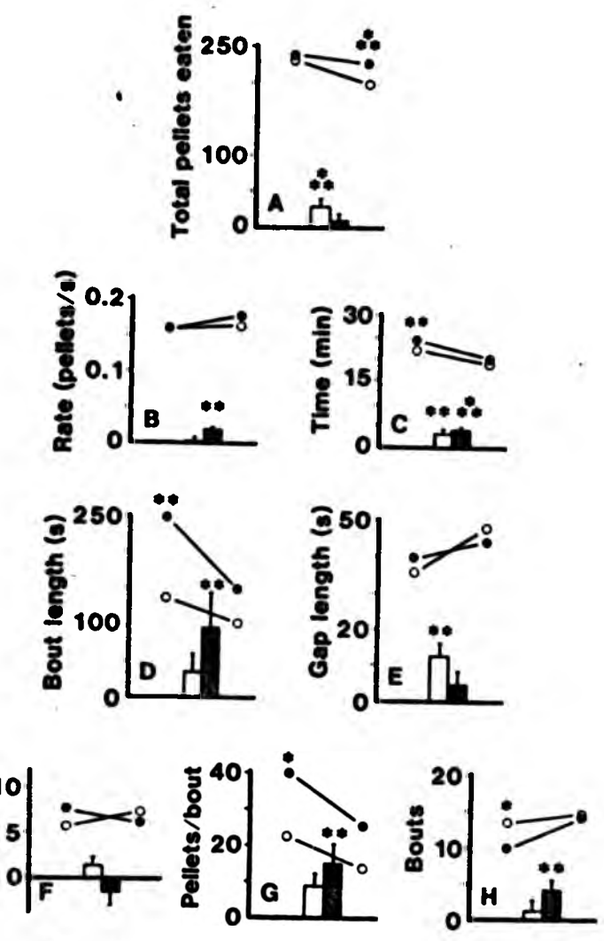


Fig.7

Effect of amphetamine and propranolol on microstructural parameters. A: Total food intake; B: Local eating rate; C: Eating time; D: Bout length; E: Gap length; F: Latency; G: Bout size; H: Number of bouts. Circles show the scores in each condition: Left - control, right - amphetamine; white - control, black - propranolol. Bars show the difference brought about by amphetamine (mean + standard error): white - control, black - propranolol pretreatment. One star - $p < 0.05$; two stars - $p < 0.01$; three stars - $p < 0.001$.

In order to explore these interactions further, the correlations between effects of amphetamine on the different microstructural parameters were examined (table 5). In both pretreatment conditions, the effect of amphetamine on eating time was significantly correlated with the change in total food intake; after propranolol pretreatment, there was also a significant negative correlation between the decrease in food intake and the increase in eating rate. In both conditions, a significant correlation was seen between the increase in eating rate and the decrease in bout length (even though in the control condition there was no significant net change in either). However, changes in these parameters were uncorrelated (in one case, there was a significant negative correlation) with increases in gap length. In the control condition only, changes in gap length were significantly correlated with changes in total food intake.

TABLE 5

INTERCORRELATIONS BETWEEN AMPHETAMINE-INDUCED CHANGES IN MICROSTRUCTURAL PARAMETERS

	Total ↓	Rate ↑	Time ↓	Bout Length ↓	Gap Length ↑
Total ↓		- .28	.46*	.24	.47*
Rate ↑	- .45*		.63**	.45*	- .08
Time ↓	.50**	.46*		.80**	.12
Bout length ↓	.35*	.40*	.81**		
Gap length ↑	.09	- .18	- .12	- .44*	

The table shows correlations (Spearman rank-order correlation coefficients) between the changes induced by amphetamine in different microstructural parameters. One star, $p < 0.05$; two stars, $p < 0.01$. The upper part of the table shows values obtained in control conditions; the lower part shows values obtained following propranolol pretreatment. Arrows show the direction of change; underlined parameters were those in which significant net changes were seen.

4.3.4. DISCUSSION

The apparent outcome of this experiment was an attenuation of amphetamine anorexia by propranolol. However, it is clear from the microstructural analysis that this result is largely fortuitous, since propranolol, amphetamine and the propranolol-amphetamine combination each produced a different pattern of behavioural changes.

Propranolol did not affect eating rate, but increased bout length, and consequently, bout size and eating time. Whilst these effects did not cause a significant increase in food intake, it is clear that appropriate testing circumstances might reveal hyperphagia, and this has, in fact, been observed (Dobrzanski and Doggett 1979). This result is consistent with the finding that hyperphagia was caused by lesions to adrenergic systems innervating the perifornical hypothalamus (Leibowitz and Brown 1980b), and with the concept of a beta-adrenergic satiety system.

In this experiment amphetamine anorexia was caused by a decrease in eating time which led to an increased gap length. In contrast to the effect of amphetamine alone, after propranolol pretreatment, gap length was the only parameter (other than latency) which was not significantly altered by amphetamine. The animals showed, on the one hand, a different hypophagic effect (decreased bout length), and on the other, a hyperphagic effect (increased eating rate). As a result, there was no significant

net change in food intake. Since the interaction of propranolol with amphetamine produces such contradictory effects, it is clear that, depending on the dose and specific experimental conditions, the outcome might be a decrease in the efficacy of amphetamine (the present study and Sanghvi et al 1975), no change (Dobrzanski and Doggett 1979, Frey and Schulz 1973),or even an increase (Schmitt 1973). It is important, however, not to lose sight of the fact that in the present study, propranolol did block the effect underlying amphetamine anorexia, and also blocked the correlation between changes in gap length and changes in total food intake.

The starting point for the interpretation of these results is the observation that propranolol interferes with the metabolism of amphetamine. To what extent may the effects of propranolol be understood as simply an increase in the dose of amphetamine? In this study, only a single dose of amphetamine, 0.5 mg/kg was tested. However, it is well established that amphetamine at 1 mg/kg significantly increases eating rate and decreases eating time, a result supported by experiment 2. It has also been reported (or it is possible to calculate from published figures) that bout length and bout size were decreased by amphetamine (Blundell and Latham 1978, Blundell and Latham 1980; see also experiment 4). Data on the length of gaps has not previously been reported in the literature, but from published figures it is possible to calculate that amphetamine caused a substantial increase in gap length. Furthermore, the results from experiment 2 (figure 5E)

show a slight increase in gap length at doses of 0.125 mg/kg or above, although these increases did not reach statistical significance. The effects of propranolol are therefore consistent with a functional increase in the dose of amphetamine, with one exception: gap length. Propranolol blocked the effect of amphetamine on gap length, where an increase would be predicted from an increase in dose.

Not only was gap length the only parameter which was significantly altered by amphetamine alone in this experiment, but also, this was the one parameter which was not significantly intercorrelated with all the others. The results therefore suggest the involvement of two separate mediating systems. At low doses, amphetamine induces anorexia by increasing gap length (i.e. reducing the tendency to begin eating), and at higher doses (assumed to result from propranolol pretreatment), a number of other mechanisms come into play. The anorexic effect of the low dose appears to be mediated by beta-receptors, since the increase in gap length was blocked by propranolol. The other effects appear to be dopaminergically mediated, since it has been reported that the changes in eating rate, bout length and bout size were antagonized by DA receptor blocking drugs (see chapter 3). It is of great relevance to the present argument that in the study of Blundell and Latham (1980), gap length (calculated from published figures) was the one feeding parameter which was unaffected by the DA receptor blocker pimozide.

4.4. EXPERIMENT 4: MICROSTRUCTURAL ANALYSIS OF THE INVOLVEMENT OF DOPAMINE RECEPTORS IN AMPHETAMINE ANOREXIA

4.4.1. INTRODUCTION

The amphetamine-induced decrease in eating time, increase in eating rate and increase in latency to initiate feeding have been shown to be reversed by the DA-receptor antagonist, pimozide (Blundell and Latham 1980). In order to confirm an involvement of DA in amphetamine anorexia under the present experimental conditions, this experiment examined pimozide challenge to two doses of amphetamine (0.5 mg/kg and 1.0 mg/kg). The effects of the atypical neuroleptic, thioridazine, found to be ineffective at reversing amphetamine stereotypy and locomotor activity (Bentall and Herberg 1980) were also examined.

4.4.2. METHOD

Subjects

Twelve male Lister hooded rats (weight 280-320g), were individually housed and maintained on 21-hour food deprivation, with water available ad lib. Animals had had previous experience of amphetamine administration (subjects were used in experiment 2) but had been drug free for approximately one month.

Drugs and Procedure

The animals were reintroduced to the six identical operant chambers, in which they had previously been trained to feed by pressing the door of the pellet dispenser (experiment 2), and ten

minute daily sessions were run until all animals returned to asymptotic performance. On experimental days, all animals received two intraperitoneal injections. As in experiment 3, doses of pimozide and thioridazine used in this present experiment were chosen on the basis of the consideration that the doses should be as high as possible, but should not in themselves produces an anorexic effect. The doses chosen, 0.45 mg/kg of pimozide and 5.0 mg/kg of thioridazine, were taken from a dose-response study of these drugs carried out in this laboratory by R. Muscat.

Pimozide (N=6, (0.45 mg/kg)), or thioridazine (N=6, (5.00 mg/kg)), were administered two hours, and d-amphetamine sulphate (0.5 mg/kg or 1.0 mg/kg), 30 minutes before the start of the session. Control injections in all cases were vehicle, (either glacial acetic acid in the case of pimozide, distilled water in the case of amphetamine and physiological saline in the case of thioridazine), at a volume of 1 ml/kg. Each animal received all four treatment combinations in a counterbalanced order, at two day intervals; on intervening days a ten minute session was run with no drug treatments. During experimental sessions, which were thirty minutes long, a computer recorded each response on the tray door, as described previously.

Analysis of microstructural parameters of feeding was carried out as described in experiment 1. Results were analysed by analysis of variance, supplemented by tests of simple main effects.

4.4.3. RESULTS

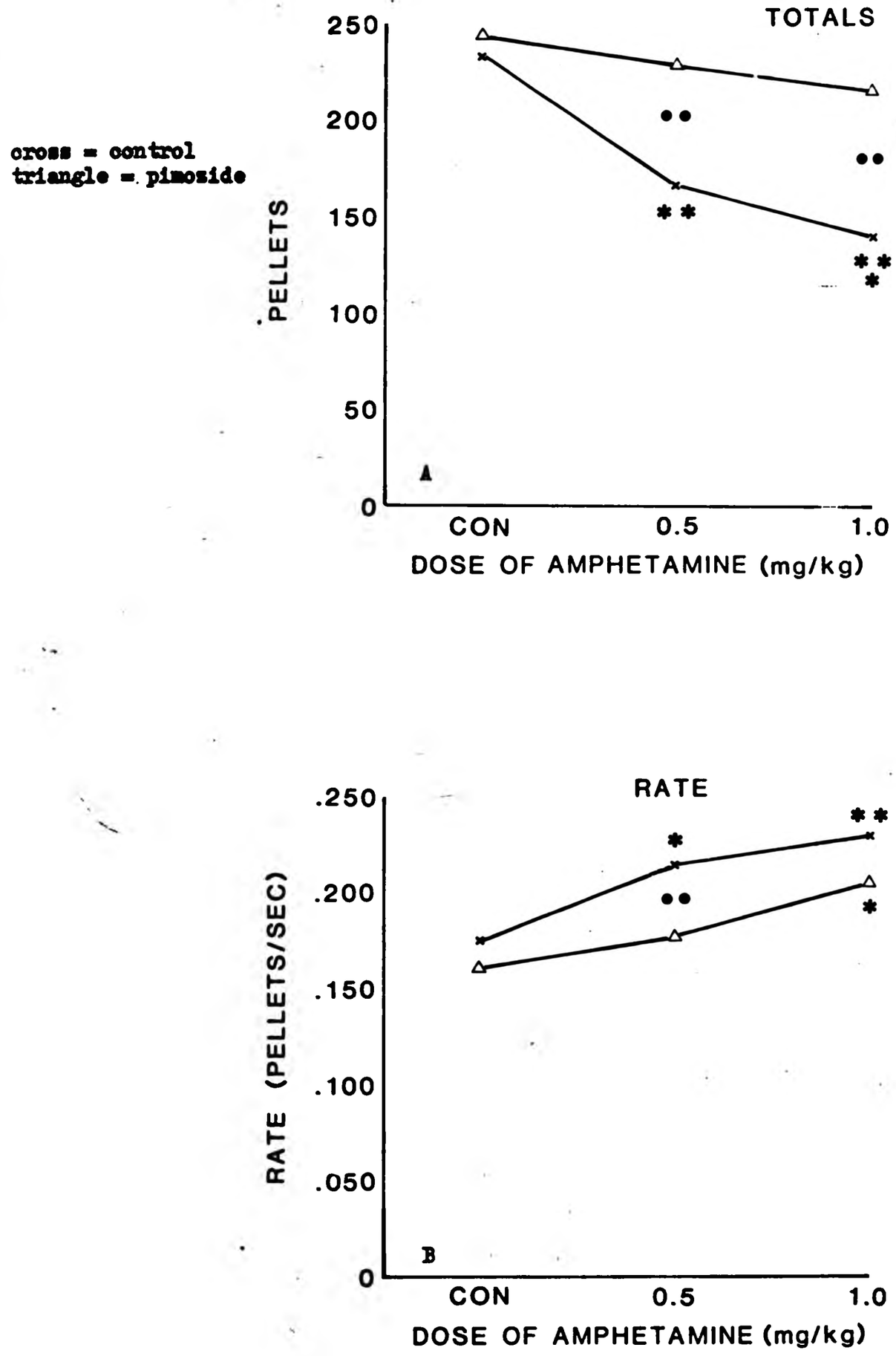
4.4.3.1. Pimozide

Amphetamine caused a dose related reduction in food intake (0.5 mg/kg, $F(1,20) = 11.88$, $p<.01$; 1.0 mg/kg, $F(1,20) = 22.63$, $p<.001$, figure 8A). As in experiments 2 and 3 amphetamine caused anorexia by decreasing eating time ($F(1,20) = 24.04$ and 36.41, $p<.001$ at 0.5 mg/kg and 1.0 mg/kg respectively, figure 8C); this was caused by slight increases in gap length and latency with decreases in bout length, although none of these changes reached significance owing to the small number of subjects (figures 8E, 8G and 8D respectivly). Amphetamine also increased the rate of eating in a dose-related fashion (0.5 mg/kg, $F(1,20) = 5.70$, $p<.05$; 1.0 mg/kg, $F(1,20) = 10.57$, $p<.01$, figure 8C), and also increased the number of eating bouts (0.5 mg/kg, $F(1,20) = 13.58$, $p<.01$; 1.0 mg/kg, $F(1,20) = 17.46$, $p<.001$, figure 8F).

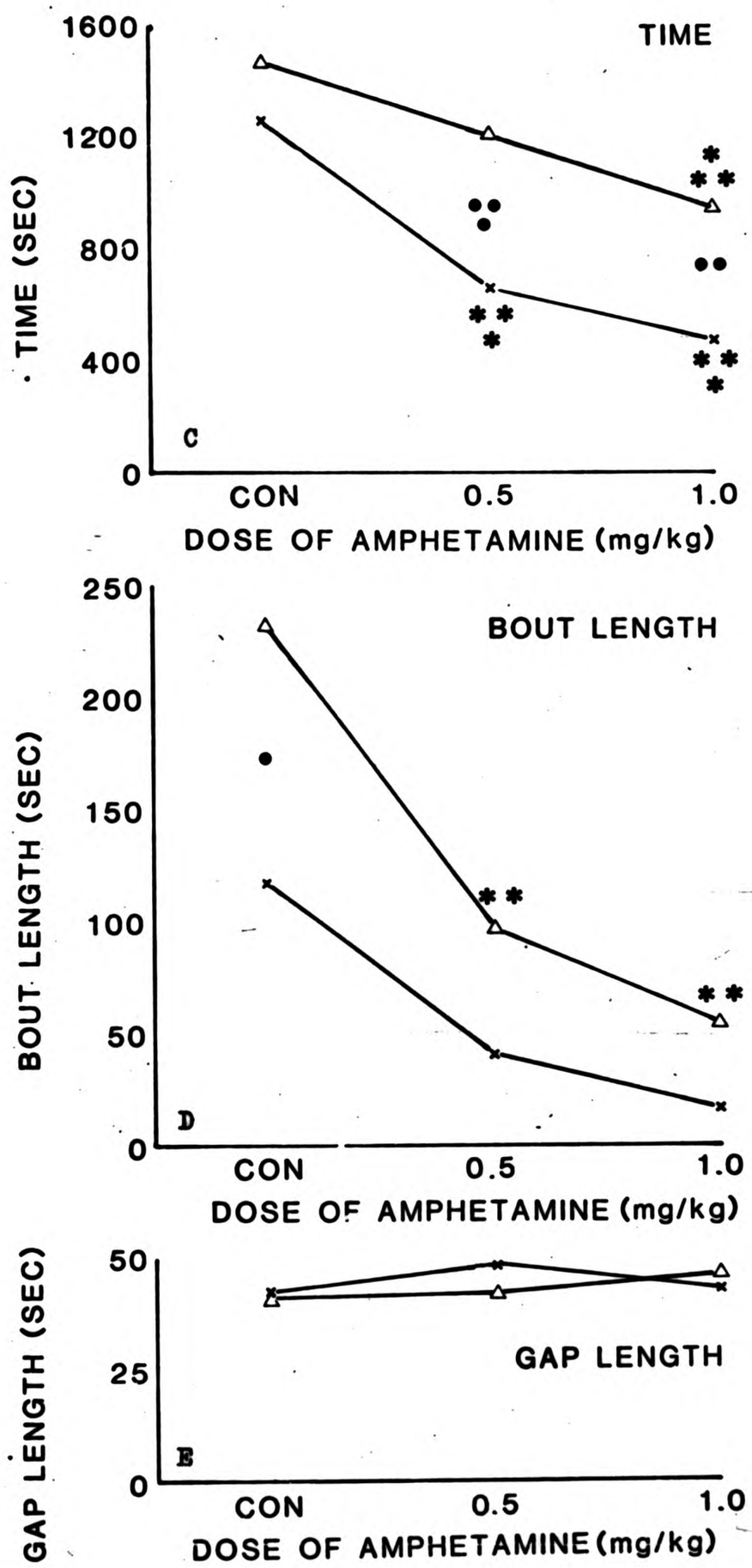
Following pimozide pretreatment, the reduction in food intake brought about by amphetamine was greatly attenuated ($F(1,15) = 10.29$ and 14.64, $p<.01$, for 0.5 and 1.0 mg/kg respectively, figure 8A). Pimozide pretreatment also blocked the amphetamine-induced reduction in eating time (0.5 mg/kg, $F(1,15) = 18.32$, $p<.001$; 1.0 mg/kg, $F(1,15) = 11.95$, $p<.01$, figure 8C), and reversed the slight changes in gap length, latency and bout length (figures 8E, 8G and 8D respectively), though none of these latter effects reached significance. Pimozide pretreatment attenuated the amphetamine-induced increase in rate at 0.5 mg/kg ($F(1,15) = 6.06$, $p<.05$), but this effect did not reach

significance at 1.0 mg/kg (figure 8B). The pretreatment also attenuated the amphetamine-induced increase in number of bouts (0.5 mg/kg and 1.0 mg/kg, $F(1,15) = 8.42$ and 5.82 respectively, $p<.05$, figure 8F). Pimozide significantly increased bout length ($F(1,15) = 4.76$, $p<.05$, figure 8D); this effect led to an increase in eating time (figure 8C) and a slight increase in the total number of pellets (figure 8A). Changes in these parameters were not significant.

FIGURE 8 THE EFFECTS OF PIMOZIDE PRETREATMENT ON AMPHETAMINE ANOREXIA



Stars show differences from control. Dots show the effects of pimozide pretreatment. One symbol $p<.05$, two symbols $p<.01$, three symbols $p<.001$.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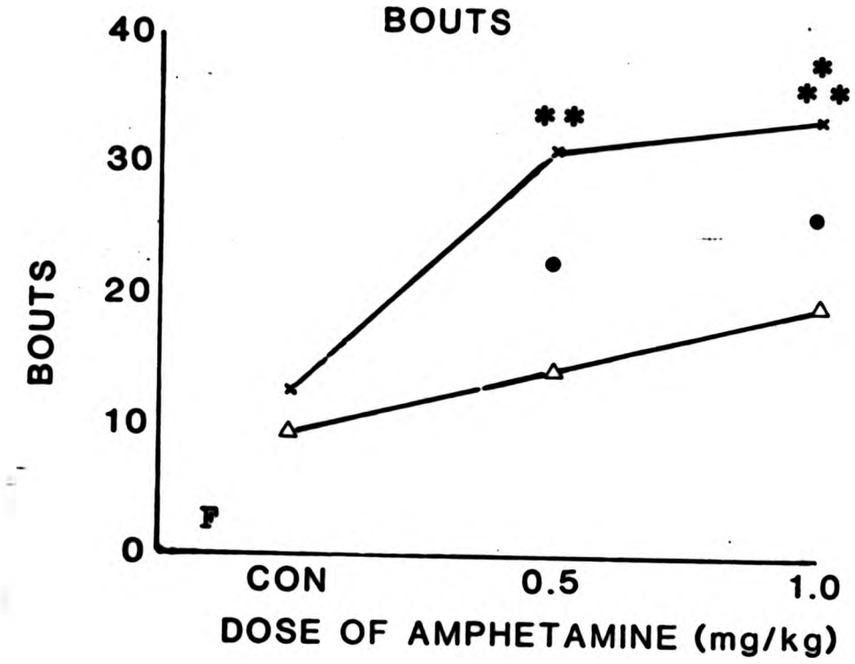
DOSE OF AMPHETAMINE (mg/kg)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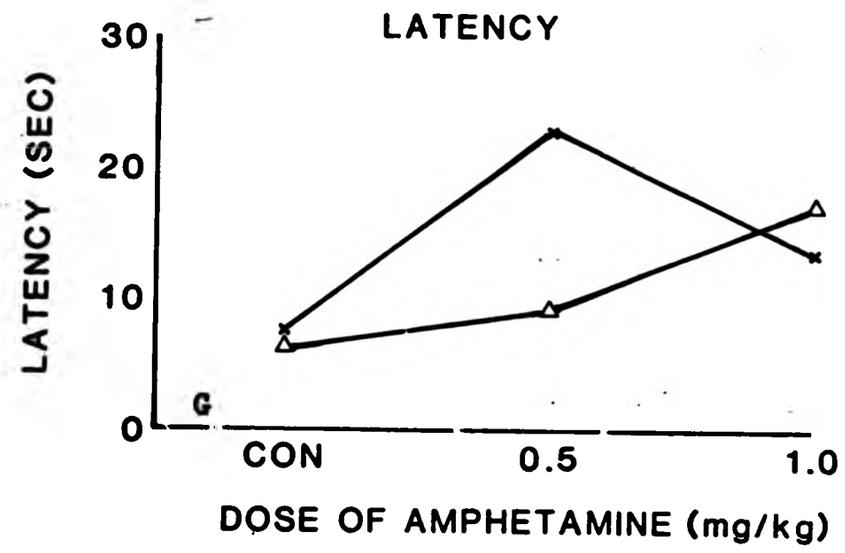
DOSE OF AMPHETAMINE (mg/kg)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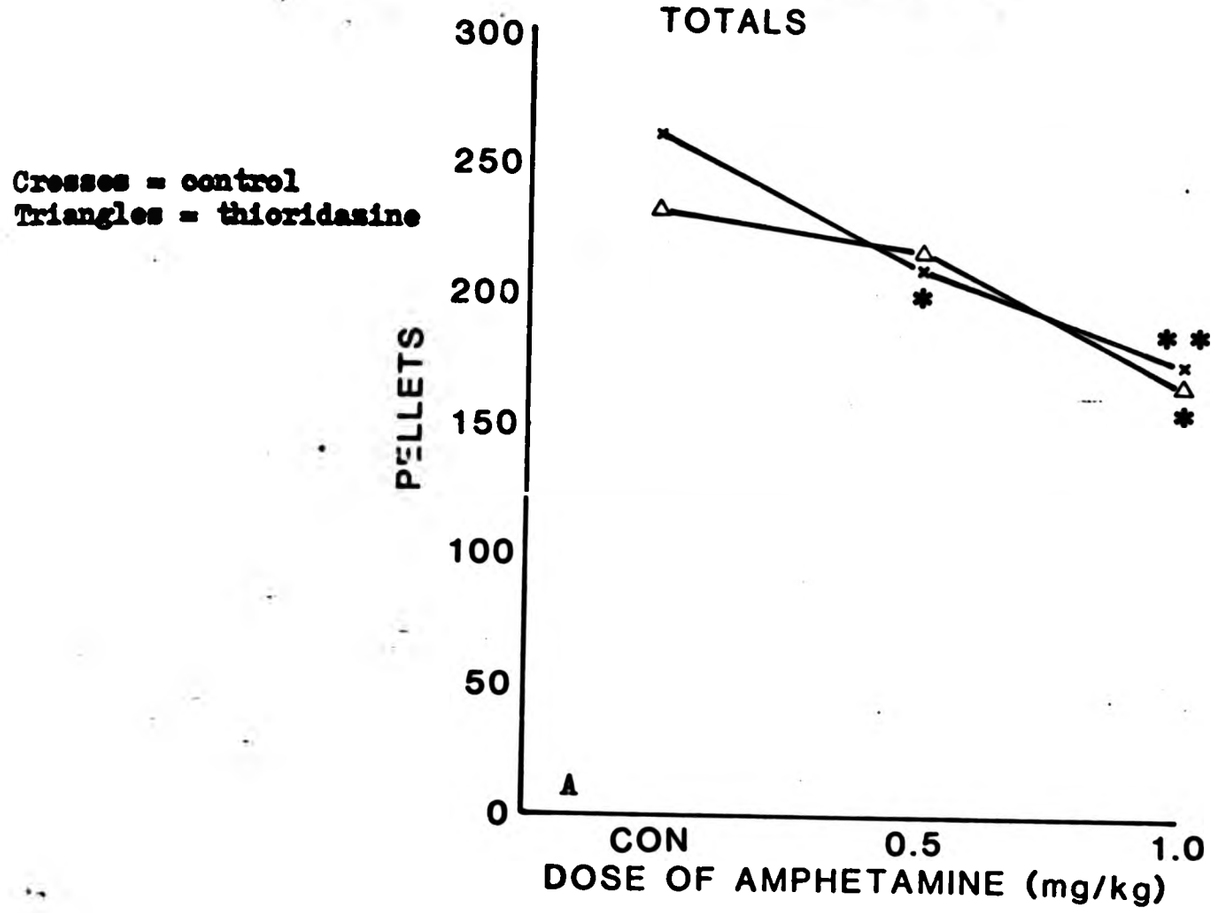
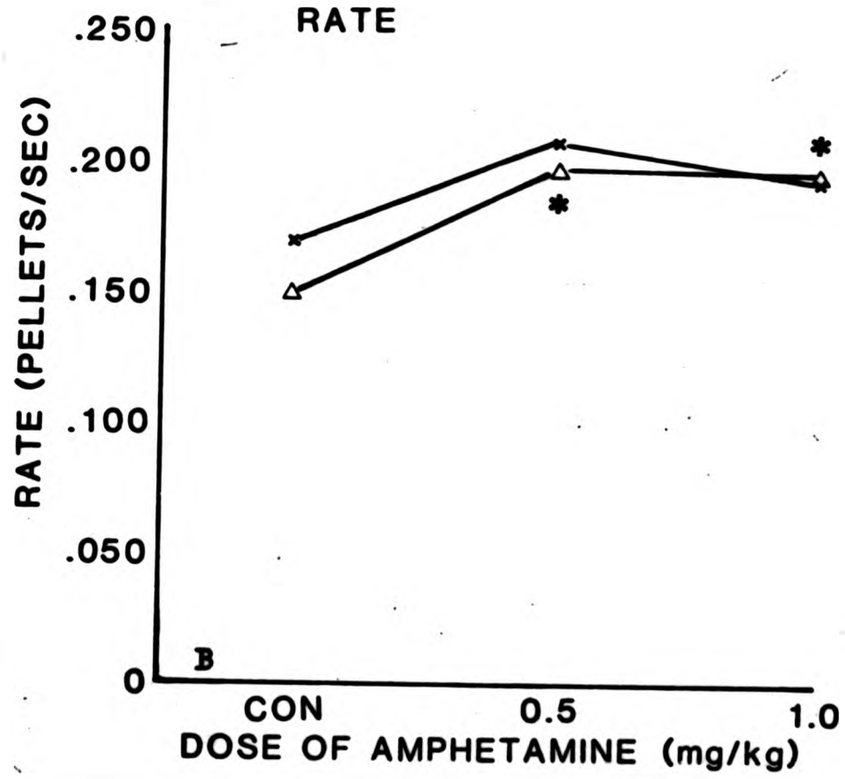
4.4.3.2. Thioridazine

The effects of amphetamine on food intake were similar to those seen in the pimozide experiment. Amphetamine significantly reduced food intake (0.5 mg/kg, $F(1,20) = 4.95$, $p<.05$; 1.0 mg/kg, $F(1,20) = 14.02$, $p<.01$, figure 9A). Amphetamine reduced eating time (0.5 mg/kg and 1.0 mg/kg, $F(1,20) = 17.78$ and 29.76 respectively, $p<.001$, figure 9C) and bout length (0.5 mg/kg, $F(1,20) = 3.78$, $p>.05$; 1.0 mg/kg, $F(1,20) = 4.70$, $p<.05$, figure 9D) and increased gap length (figure 9E) and latency (figure 9G), although these latter effects did not reach significance. Unlike the significant increases in rate seen following amphetamine treatment in experiments 2 and 3, the increases in rate observed in this experiment did not reach significance (figure 9B). On the other hand, a significant increase was observed in the number of bouts taken (0.5 mg/kg, $F(1,20) = 7.53$, $p<.01$; 1.0 mg/kg, $F(1,20) = 20.78$, $p<.001$, figure 9F) following amphetamine.

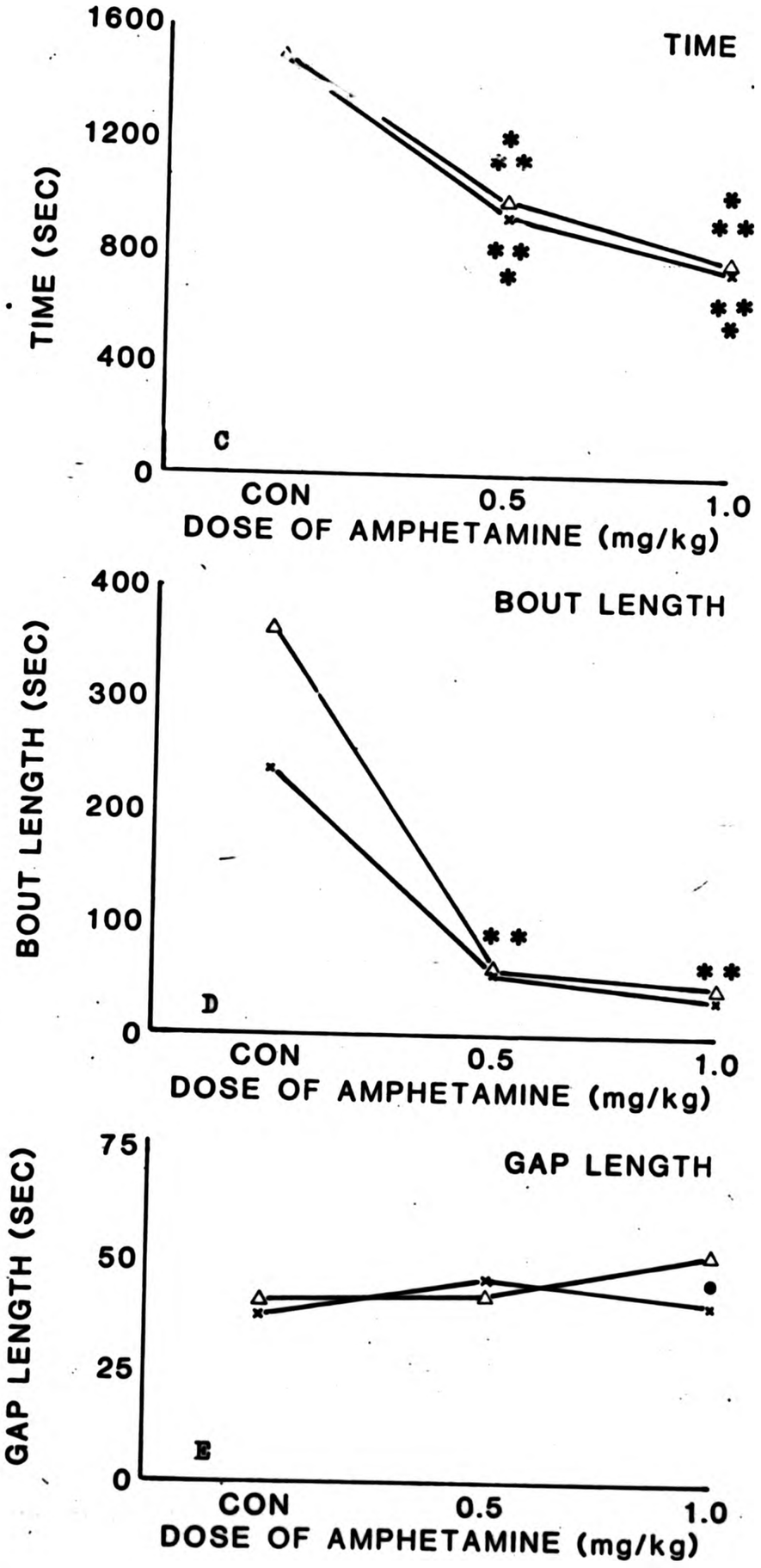
Thioridazine pretreatment had minimal effects on the amphetamine-induced changes in total food intake, time and rate (figures 9A, C and B respectively). Thioridazine pretreatment attenuated the increase in the number of bouts shown by amphetamine at 1.0 mg/kg ($F(1,15) = 5.95$, $p<.05$, figure 9F), but actually enhanced the increase in latency at this dose ($F(1,15) = 7.97$, $p<.05$, figure 9G). Thioridazine alone had minimal effects on microstructural parameters; like pimozide, thioridazine significantly increased bout length (figure 9D), but not eating time (figure 9C) or total food intake (figure 9A); in fact thioridazine slightly reduced total intake.

FIGURE 9 THE EFFECTS OF THIORIDAZINE PRETREATMENT ON AMPHETAMINE ANOREXIA





Stars show differences from control. Dots show the effects of thioridazine pretreatment. One symbol $p<.05$, two symbols $p<.01$, three symbols $p<.001$.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DOSE OF AMPHETAMINE (mg/kg)
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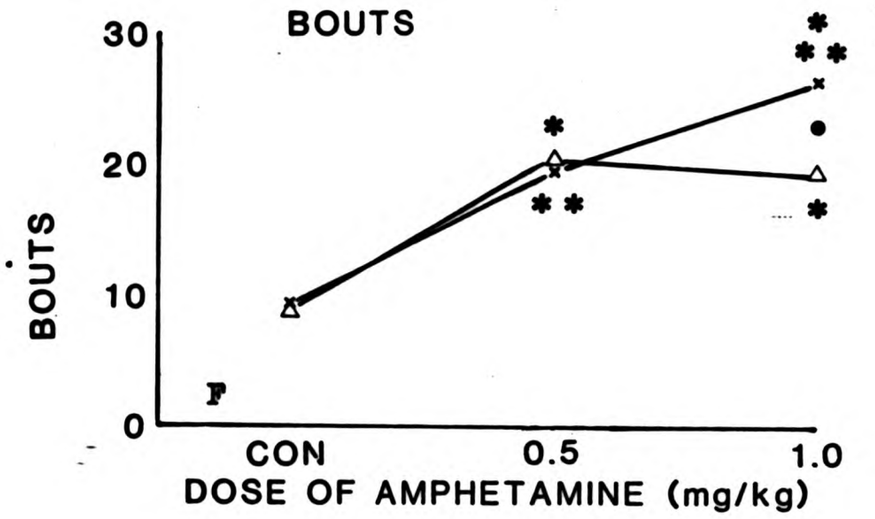
DOSE OF AMPHETAMINE (mg/kg)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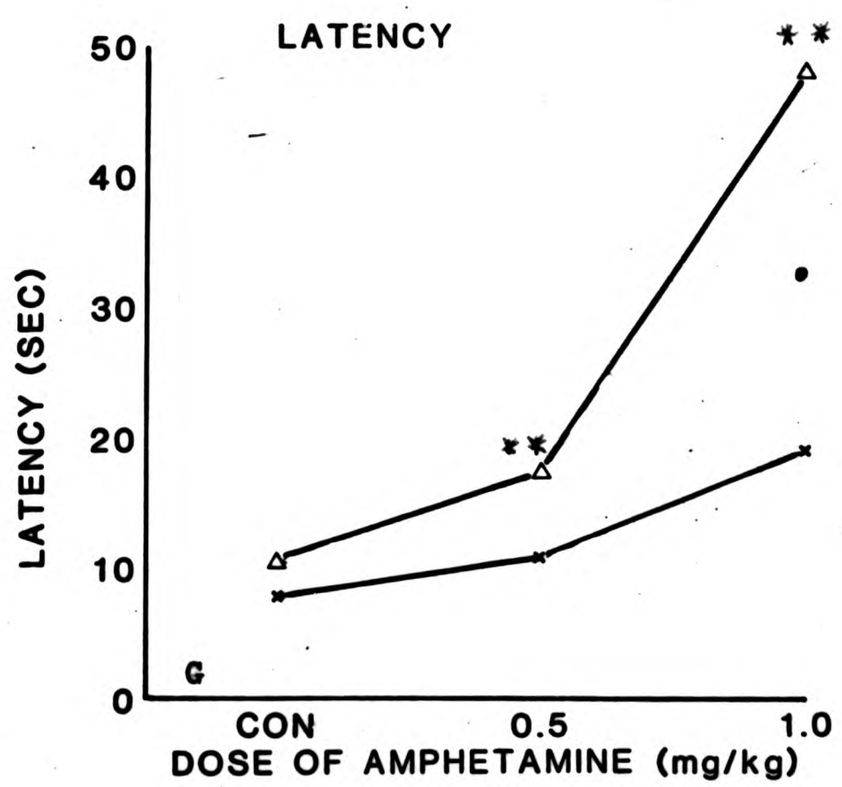
DOSE OF AMPHETAMINE (mg/kg)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DOSE OF AMPHETAMINE (mg/kg)

4.4.4. DISCUSSION

The anorexic effects of both doses of amphetamine were blocked by the typical neuroleptic pimozide, but not by the atypical neuroleptic thioridazine. Pimozide blocked amphetamine anorexia by attenuating the amphetamine-induced reduction in eating time, allowing animals to spend more time eating. The increased rate of eating following amphetamine was also reversed, though only at the 0.5 mg/kg dose. Blundell and Latham (1980) also showed significant reversal of the effects of amphetamine on eating time, rate and latency.

However, these effects of pimozide pretreatment are not entirely consistent with the literature. The main discrepancy between these results and those of Burridge and Blundell (1979) is the reversal of low dose amphetamine anorexia. This difference is difficult to resolve as the methodologies and baseline amphetamine results of the two studies are similar. In both studies, 0.5 mg/kg amphetamine produced around a 25% reduction in feeding. The dose of pimozide used in the Burridge and Blundell study (0.5 mg/kg) was slightly higher than the dose used in the present study (0.45 mg/kg). A higher dose could in principle induce motor incapacitation, which would disrupt eating. However, these authors conducted their own dose-response studies of pimozide, which showed no impairment of total food intake at 0.5 mg/kg. This conclusion is supported by other studies carried out in this laboratory, that showed no significant change in microstructural parameters following 0.5 mg/kg pimozide. Another

difference between the present study and that of Burridge and Blundell (1979) is the time between neuroleptic drug treatment and the amphetamine injection; Burridge and Blundell allowed two hours whilst this experiment used one and a half hours. However, a brief survey of the literature shows corresponding time periods of between one and a half hours to four hours. It therefore seems unlikely that this difference in procedure alone could explain the different result. Whatever the interpretation of the discrepancy, the present experiment has produced clear evidence that low-dose amphetamine anorexia does involve a DA component. This result is consistent with studies using central drug administration which show blockade of amphetamine anorexia with DA receptor antagonists (see chapter 2, section 2.7.). More specifically, this experiment has suggested that eating rate and eating time may be under the control of DA systems which are antagonised by neuroleptic drugs.

This experiment also confirmed the inability of the atypical neuroleptic, thioridazine, to reverse amphetamine anorexia, even at a dose ten fold greater than that used by Burridge and Blundell (1979). Bentall and Herberg (1980) showed that thioridazine at doses of 5, 10 and 20 mg/kg were ineffective at reversing amphetamine-induced locomotion and stereotypy, whilst a much smaller dose of spiroperidol was effective. The implications of these findings seem to be that the established antipsychotic action of thioridazine (Creese et al 1976) depends on some property of the drug other than its ability to block the

DA receptors that are stimulated by amphetamine.

Classical neuroleptics given acutely, such as haloperidol, are known to increase DA cell firing . The atypical neuroleptics, such as clozapine and thioridazine, have been shown on acute treatment to increase DA cell firing in A10 neurones only (White and Wong 1983, Chiodo and Bunney 1983). This selective dissociation of drug action between A9 and A10 neurons is further seen following chronic thioridizine treatment, which causes a reduction in transmission at A10 neurons only. It may be the selectivity of atypical neuroleptics for A10 neurons which gives them their antipsychotic properties and perhaps also, for that matter, their relatively low incidence of side effects.

However, if thioridazine is specific for A10 systems, it should reverse the amphetamine-induced locomotion which is known to result from stmulation of this system (see chapter 6, section 6.1.). As mentioned above, this is not the case. It may be that thioridazine exerts its neuroleptic properties largely through a presynaptic DA action which is not entirely reflected in amphetamine-induced locomotion.

4.5. GENERAL DISCUSSION

The microstructural analysis of the action of amphetamine on feeding behaviour, which was carried out in this chapter, has confirmed previous findings of Blundell and Latham (1978, 1980), that the feeding parameters mediating amphetamine anorexia are a

reduction in eating time together with an increase in eating rate. In addition, experiment 3 showed that a low dose of amphetamine also increased gap length, a finding which would support the decrease in eating time. A slight increase in gap length following amphetamine treatment was also seen in experiments 2 and 4 (figures 5E,8E and 9E), but failed to reach significance. This may be due to the lability of gap and bout length results in this microstructural procedure (see chapter 3, experiment 1, discussion).

Given the evidence in experiment 2 of changes in feeding at various doses of amphetamine, experiments 3 and 4 set about to characterize the neurochemical substrates underlying these changes. As pimozide was effective in antagonizing amphetamine-induced changes in eating rate, eating time, bout length and the number of bouts taken during the feeding session, it is possible that DA mechanisms might mediate both low and high dose amphetamine anorexia.

Nevertheless, a beta-adrenergic contribution was also evident in reversal by propranolol pretreatment of the increase in gap length following a low dose of amphetamine. That DA antagonists reversed low-dose amphetamine anorexia might suggested an overall cotrol of DA systems over beta-adrenergic systems in the modulation of feeding behaviour. This is indicated by the results of studies using intracranial drug administration which show that DA-induced suppression of feeding cannot be reversed by beta-blockers, but adrenalin-induced suppression of feeding

can be reversed by DA receptor antagonists (see chapter 2, section 2.5.2.).

The amphetamine-induced increase in gap length represents a decrease in the likelihood of starting eating, an effect thought to be mediated by beta-receptors. This effect of amphetamine could be an effect on the normal control of eating. However, the putative DA-mediated effect of amphetamine appears less likely to be a specific motivational effect. Lyon and Robbin's (1976) theory, that amphetamine increases the intensity of ongoing behaviour within a decreasing number of response categories, is supported by the correlations (experiment 3) between the amphetamine-induced increase in eating rate and shortening of bouts: increases in eating rate were significantly correlated with decreases in bout length, both in the control condition and also following propranolol pretreatment (table 5). Thus, it may be that amphetamine has two anorexic effects, one genuine and the other an artefact of the (psycho)motor stimulant effect of amphetamine not specific to eating. The specific effect, which is only apparent at low doses, appears to be mediated by both beta-adrenergic and dopaminergic systems, whilst the stimulant effect seems to be mediated by dopaminergic systems only.

CHAPTER FIVE

THE EFFECTS OF ANTIDEPRESSANT DRUGS ON AMPHETAMINE ANOREXIA

5.1. INTRODUCTION

The previous chapter has suggested that amphetamine anorexia can be used as a tool to index beta-receptor function. This chapter will examine interactions between AD and amphetamine anorexia.

The classic action of TADS is to increase adrenergic transmission through blockade of re-uptake at NA synapses, which only takes a few hours or so (e.g. Carlsson et al 1966). This action of TADs was used as evidence to support the CA hypothesis of depression (see section 1.4.1.). However, as a result of elevated NA transmission (Wolfe et al 1978), subsensitivity of postsynaptic beta-receptors develops during chronic treatment as measured by the c-AMP technique (Vetulani et al 1976). This chronic effect of TADs and virtually all other antidepressant treatments is now frequently assumed to reflect the mechanism of clinical action of these drugs and has been used as evidence to support the revised CA hypothesis of depression, that depression arises from an elevation of NA transmission which is decreased by AD (Sulser 1978). However, subsensitivity of beta-receptors could be nothing more than a compensation for the acute adrenergic enhancement induced by ADs. In this case the beta-receptor subsensitivity following chronic AD treatment would return NA systems to their pre-drug state. If, depression did result from an over-stimulation of NA systems, then for AD treatments to be

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effective, they would have to functionally reduce NA transmission to a level lower than the pre-drug state. Only then would the net result be an overall decrease in NA transmission.

There have been several attempts to address the issue of the net effect of ADs on beta-adrenergic function. Biochemical studies have typically examined changes in basal cAMP levels as an index of beta-adrenergic function. In general, chronic AD treatment has not been found to alter basal cAMP levels, suggesting that there is no net change in beta-adrenergic function (e.g. Frazer and Mendels 1977). As some cortical cAMP is produced at sites other than beta-receptors, the value of this approach is limited. However, one study examined the cAMP response in the cortex following electrical stimulation of the locus coeruleus and found the cAMP response to be clearly reduced by DMI but not by other ADs, except mianserin and chlorimipramine at high doses (Korf et al 1979).

Other biochemical models used to assess beta-adrenergic function include pineal cAMP and pineal melatonin (see Willner 1984 for a full discussion). All these biochemical models have largely shown that there is little evidence to suggest that beta-adrenergic function is significantly altered following chronic AD treatment.

Electrophysiological studies reach a similar conclusion. Although decreased sensitivity to iontophoretically applied NA has been reported following chronic AD treatment in cingulate

cortex (Olpe and Schellenberg 1980; Delina-Stula et al 1982) and in cerebellar Purkinje cells (Schultz et al 1981), the baseline firing rate of Purkinje cells was reduced by approximately 50-70% (Schultz et al 1981). As NA is inhibitory on Purkinje cells, the reduction in baseline firing represents an increase in adrenergic function and makes it impossible to interpret the reduced effect of NA. No baseline data were reported in the cingulate cortex studies.

NA has an inhibitory effect on hippocampal cell function. Chronic DMI has been reported to increase the baseline firing rate of hippocampal cells (Huang 1979), thereby indicating a decrease in beta-adrenergic function. In contrast, four other studies have found that the response of hippocampal cells to iontophoretically applied NA was unchanged by chronic DMI and a variety of other AD treatments including ECT (De Montigny 1980; De Montigny and Aghajanian 1978, De Montigny et al 1981, Gallager and Bunney 1979). However, it was shown that changes in firing rate of hippocampal cells following DMI treatment were an exact reflection of changes in the firing of locus coeruleus cells (Huang et al 1980). Therefore, the proportional reduction of firing in response to stimulation of the locus coeruleus was unaltered by chronic DMI treatment, despite changes in basal firing rate (Huang 1979).

5.2. BEHAVIOURAL ASSAYS OF BETA-ADRENERGIC FUNCTION DURING CHRONIC DMI TREATMENT

Attempts to assess beta-adrenergic function via behavioural methods have been hampered because there are no models which which are thought to unequivocally reflect beta-adrenergic receptor stimulation. An approach to assess beta-adrenergic receptor function has recently been developed by Mason and Angel (1983). This model is based on the duration of thiopentone anaesthesia in the rat and is assumed to be sensitive to the net functional activity of the locus coeruleus NA system. Drug manipulations, which increase activity in the locus coeruleus, have been shown to shorten thiopentone anaethesia (Mason and Angel 1983). Chronic DMI treatment (10 mg/kg) once daily for 10 or 20 days was found to increase the duraton of thiopentone anaesthesia, suggesting a net decrease in beta-adrenergic function. The problem with this study is that testing was carried out 48h into withdrawal and therefore does not assess the net effect of chronic DMI treatment at beta-adrenergic synapses.

A second approach to assess beta-receptor function utilized the DBEE (dorsal bundle extinction effect), which is thought to reflect a decrease in beta-adrenergic function during resistance to extinction. During chronic DMI treatment, no increase in resistance to extinction was seen in three different behavioural paradigms, except when the animals were tested during withdrawal (Montgomery and Willner 1980; Willner et al 1981b). These results would suggest that beta-receptor subsensitivity is unmasked by

withdrawing DMI, and simply compensates for the acute NA enhancing effects of the drug. However, there is some uncertainty as to whether the resistance to extinction induced by DMI is in fact mediated by the DNAB (Willner and Towell 1982b; see chapter 1, section 1.11).

A third behavioural approach to index beta-receptor function uses the anorexic effects of small doses of amphetamine on food intake; as discussed in the previous chapter this effect appears to have a beta-adrenergic component. Acute pretreatment with DMI or iprindole potentiated the anorexic effect of low doses of amphetamine (Willner and Montgomery 1980; Willner, Towell and Montgomery 1984). However, after two weeks' treatment, amphetamine anorexia in drugged animals did not differ significantly from that in controls. The anorexic response to amphetamine remained tolerant, showing neither enhancement nor attenuation, after more than two months' treatment (Willner and Montgomery 1981a). This tolerance to the acute potentiating effect of ADs is probably mediated by change in NA function, since tolerance was not seen to the enhancement of amphetamine-induced stereotyped behaviour (Willner and Montgomery 1981a; Willner, Towell and Montgomery 1984) - a presumed DA mediated effect (Kelly 1977).

In these studies amphetamine anorexia was attenuated during withdrawal from DMI or iprindole. Again, an underlying NA mediation is implicated, since amphetamine-induced locomotor

activity and stereotyped behaviour were not attenuated - in fact both behaviours were slightly enhanced (Willner and Montgomery 1980a, 1981, Willner, Towell and Montgomery 1984). These results suggest that during withdrawal from DMI or iprindole, NA transmission is decreased. However, during drug treatment, this effect is exactly offset by the acute, potentiating effects of the drugs, resulting in no net increase or decrease in NA transmission.

This conclusion relies on the assumption that the initial potentiation of amphetamine anorexia is not dependent on changes on amphetamine metabolism. However, it is known that DMI and iprindole impair amphetamine metabolism effectively making more amphetamine available to produce an enhancement of its effects (Consolo et al 1967, Lewander 1968, Lemberger et al 1970). If the potentiation of amphetamine anorexia following acute AD treatment is artefactual, then the tolerance which develops during chronic treatment does not give a true representation of beta-adrenergic function. If the potentiation of amphetamine anorexa by DMI is an artefact of metabolism then the changes seen in amphetamine anorexia during chronic treatment may actually reflect a reduction in beta-adrenergic function. It is clearly necessary therefore to assess the contribution of the metabolic artefact to the potentiation of ampheamine anorexia by ADs.

Two recent observations suggest that the enhancement of amphetamine anorexia by acute AD pretreatment may in fact be an artefact of impaired amphetamine metabolism. First, as noted

above, high doses of DMI and iprindole enhanced amphetamine anorexia, and when these drugs were given acutely, tolerance was seen to the acute potentiating effects over chronic treatment, and attenuation of anorexia was seen during withdrawal. However, at lower doses, these drugs produced a similar enhancement following acute AD treatment, but no tolerance to this effect developed during chronic treatment (Willner, Towell and Montgomery 1984). Secondly, mianserin, an atypical AD known to enhance NA by blocking inhibitory alpha-two receptors, (Sugrue 1980) did not enhance amphetamine anorexia with acute pretreatment, but did attenuate anorexia during chronic treatment and also during withdrawal (Willner, Towell and Montgomery 1984). These results suggest that results obtained with DMI and iprindole may have been artificially elevated during drug treatment; unlike these drugs, mianserin is without effect on amphetamine metabolism.

5.3. EXPERIMENT 5: CENTRAL AND PERIPHERAL CONTRIBUTIONS TO THE ENHANCEMENT OF AMPHETAMINE ANOREXIA BY DMI

5.3.1. INTRODUCTION

In view of the descrepant results following acute and chronic drug treatment with a variety of AD, it is clearly necessary to assess the extent to which the enhancement of amphetamine anorexia by acute DMI pretreatment depends on a peripheral metabolic artefact. The following experiment was based upon the assumption that DMI should not potentiate the anorexic effect of

amphetamine administered directly to the perifornical hypothalamus, if the enhancement of amphetamine anorexia by DMI depends upon peripheral factors, such as a metabolic interaction in the liver. If, however, the enhancement of anorexia is mediated by an interaction at central synapses, the effect should be present with both peripheral and central adminisration of amphetamine.

As reviewed in chapter 2, the anorexic action of amphetamine appears to be mediated by DA and beta-adrenergic synapses in the PFH: administration of beta-blockers or DA receptor antagonists to this region, or destruction of the ventral noradrenergic bundle or DA fibre systems, attenuates or abolishes the anorexic effect of peripherally administered amphetamine; conversely, administration of amphetamine to the lateral hypothalamus reduces food intake, through an action at DA and beta-adrenergic receptors in the perifornical area. Administration of amphetamine to other hypothalamic areas, most notably the paraventricular nucleus, increases food intake by an action at alpha-adrenergic receptors. Consequently, the concurrent administration of an alpha-receptor blocker such as phentolamine was used, in order to observe the beta-adrenergic anorexic effect of centrally administered amphetamine.

5.3.2. METHOD

Subjects

A group of 12 Lister hooded rats (Olac, Bicester, Oxon), weighing approximately 300g at the time of surgery, were housed individually under conditions of controlled temperature and humidity, on a 12-hour light-dark cycle (09.00h to 21.00h light). Animals were maintained on 21-hour food deprivation, and fed with standard laboratory diet (Dixon, Ware, Herts) from 14.00h to 17.00h daily. Water was freely available at all times.

Procedure

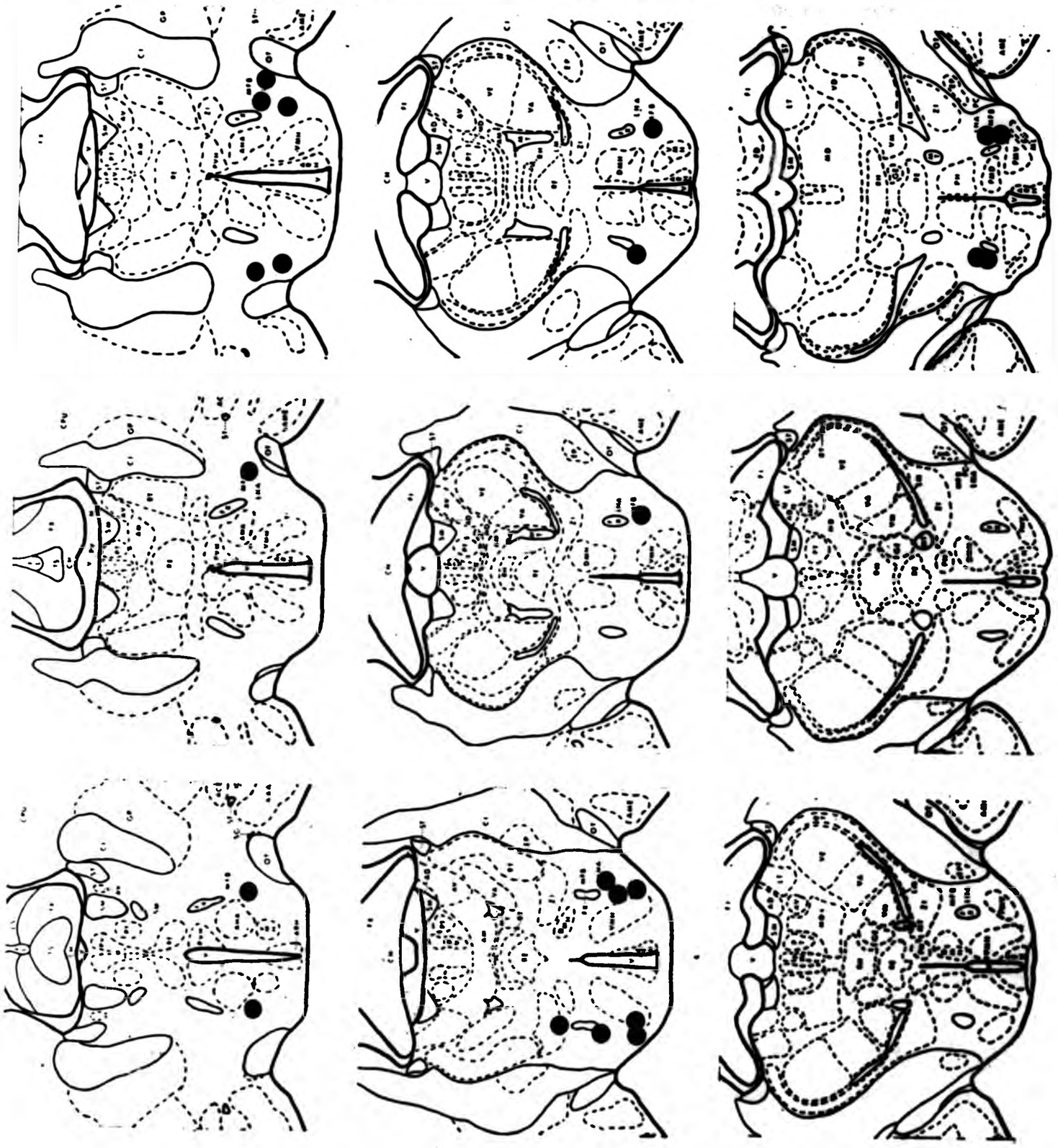
At 14.00h each day, a weighed amount of food was placed in each animal's cage. Uneaten food was removed briefly at 14.30h, weighed, and then returned to the cage. Food uneaten at 17.00h was removed and weighed; all results reported refer to the first 30 minutes of the feeding session. Food intake scores were subjected to one-way analysis of variance, supplemented by Scheffe contrasts.

Following stabilization of food intake, cannulae aimed at the PFH were implanted bilaterally under pentobarbital anaesthesia, as described below. The coordinates, chosen according to the atlas of Pellegrino and Cushman (1967), were anterior 5.6mm, medial 1.6mm and ventral 2.4mm; at the end of the experiment, cannulae placements were verified histologically. The cannulae were of 26-gauge stainless steel (Arnold and Horwell, London); injections through them were made using a microsyringe with a 33-

gauge needle (V.A. Howe, London). Details of the micro-cannula system and surgery are given below.

Following their last injection, animals were perfused with buffered formalin under sodium pentobarbital (100 mg/kg i.p.) and their brains were rapidly removed and stored in 10% formalin for approximately 10 weeks. Frozen 100um sections were stained with fast cresyl violet and cannula placement was verified histologically. Placements were identified as being ventral or lateral to the fornix, at the level of, or slightly posterior to, the ventromedial hypothalamus (see figure 10).

FIGURE 10
Consecutive sections of the rat brain derived from the atlas of Pellegrino and Cushman (1967). Circles show cannulae placement to be in the vicinity of the fornix. Abbreviations of structure close to the circles are given below: ARH nucleus arcuatus hypothalami, AHA hypothalamic area anterior, DMH nucleus dorsomedialis hypothalami, FX fornix, LHA hypothalamic area lateral, MFB medial forebrain bundle, PVH paraventricular nucleus of hypothalamus, PMV premamillary nucleus ventral, OT optic tract, V ventricle, VMH ventomedial nucleus of hypothalamus, ZI zona incerta.



Micro-cannula System (see figures 11A and 11B)

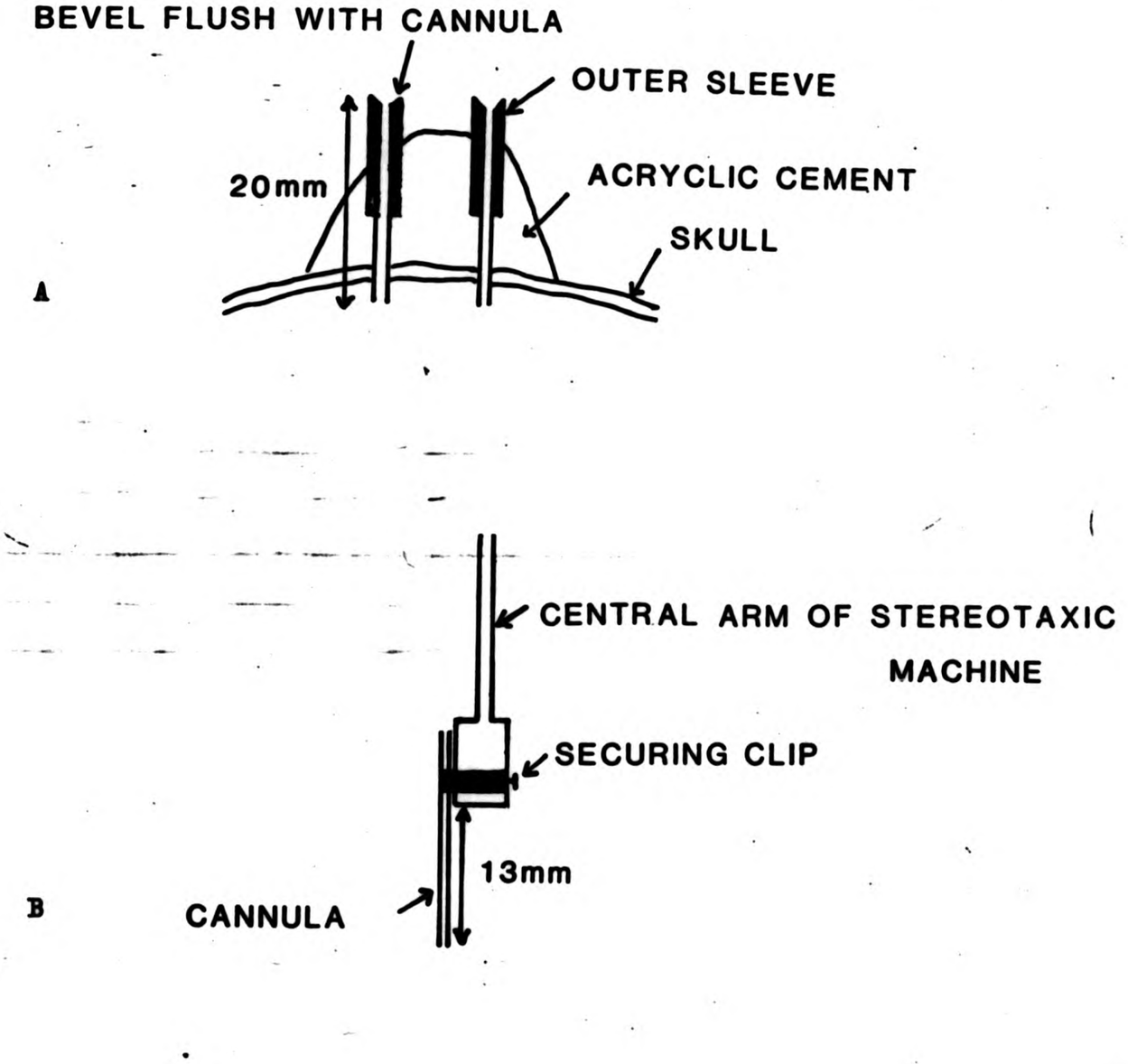
The dimensions of the cannulae used in these experiments were determined by the smallest possible needle size currently available (33 gauge: outer diameter = 0.2mm inner diameter = 0.1mm). Therefore 26 gauge (outer diameter = 0.46-0.47mm; inner diameter = 0.24-0.28mm) stainless steel cannulae (Arnold and Horwell, London) were cut to 20mm lengths using a grinding machine. Where necessary, the ends of the cannulae were de-burred by inserting a small length of injection needle into one end of the cannula and gently rubbing this needle up and down the length of the cannula.

Outer sleeves were constructed from 2.5mm diameter screws. These served two purposes: first, they strengthened the cannulae and prevented the rat from damaging the implant by scraping its head against the metal bars at the top of the cage; and, secondly, the sleeves served as guides to facilitate the entry of the needle into the cannula.

Using a size 0.5mm drill, a hole was drilled centrally along the length of the screw, using the centre of the posidrive head as a guide. The head was removed by a grinding machine leaving a 0.5cm length of threaded screw. The remaining length was mounted in a vice machined side up and a contersunk drill was lowered to enlarge the existing aperture. The sleeve was cleared of shavings by inserting a piece of 26 gauge tubing through it.

FIGURE 11

MICROCANNULA SYSTEM



Injections were made through the guide cannula with Hamilton 33 gauge stainless steel needles (V.A.Howe, London) reduced to a 20mm length. The needle was filled and attached to a filled Hamilton 10ul syringe. A visual check was made that no bubbles were present in either. The syringe was centred in a Hamilton repeating dispenser which enabled the delivery of 0.22ul of solution or multiples of that amount.

Surgery

Animals were anaesthetized under sodium pentobarbital (40-50mg/kg i.p.). Cannulae were implanted bilaterally using a student stereotaxic machine (David Kopf Instruments). As the machine did not permit implantation from both right and left sides, it was necessary to devise a system which would enable bilateral implantation during a single operation. On the central arm (or electrode carrier) of the machine, the cannula was inserted in a pre-machined groove and adjusted such that 13mm of cannula portruded from the base plate (see figure 11B). Following normal calibration of the machine co-ordinates, and insertion of the first cannula, it was possible to insert the second cannula by placing it in the groove in exactly the same way as before. This procedure ensured a reasonable degree of accuracy in placement.

Five small holes were made in the skull using a 10 BA self-tapping drill operated by a Radio Spares 12-volt hand drill. Two of the holes were used for cannula insertion, whilst the other three were used as anchorage points to secure the implant. Into

the three anchorage holes were inserted cheese head 10 BA stainless steel screws (Clerkenwell Screws, London) which were tightened by approximately four complete revolutions. After cannulae were inserted in place, dental cement was liberally applied to the implant area such that all three screws and the base of the cannulae were covered. When the cement had dried, the sleeves were carefully positioned onto the cannulae, bevel side up. Care was taken to ensure that the top of the cannula sat at the bottom of the bevel (see figure 11A). More dental cement was applied to the implant to ensure that the sleeves were securely placed. Animals were removed to their home cages and a minimum of one week was allowed before their first injection. During this time animals were handled and mock-injected every other day.

Drugs and Procedure

Drug trials began 14 days after surgery, when food intakes were stable. All drugs were dissolved in distilled water and made up to volume with phosphate buffer, pH 7.0, which was also used for control injections. All intraperitoneal (i.p.) injections were made at a volume of 1 ml/kg. Intracranial (i.c.) injections were made in a volume of 0.44ul. A minimum of two drug-free days were allowed between successive treatments.

DMI (Geigy, 7.5 mg/kg) was administered i.p. at 17.00h on the day before feeding tests. D-Amphetamine sulphate (Smith, Kline and French) was administered either at 0.5 mg/kg i.p. 30 min. before feeding, or at 200 nM i.c. 5 mins before feeding. Phentolamine

hydrochloride (Ciba) was administered at 50nM i.c., 10 mins before feeding. Doses were calculated as salts. The treatment combinations and the sequence of treatments are shown in table 6.

5.3.3. RESULTS

Phentolamine and DMI, alone or in combination, did not significantly change food intake from that seen after a control injection (1 vs 4, 8, 9: maximum $F(1,81) = 0.90$, $p>0.1$). Intracranial administration of amphetamine significantly increased feeding (1 vs 2: $F(1,81) = 9.88$, $p<0.01$), but a significant reduction of feeding was apparent when i.c. amphetamine was preceded by phentolamine (1 vs 3: $F(1,81) = 24.45$, $p<0.001$). In fact, the anorexic effect of i.c. amphetamine, with phentolamine pretreatment, did not differ significantly from that seen after i.p. administration of amphetamine, at approximately 5 times the dose (3 vs 6: $F(1,81) = 1.80$, $p>0.1$). Phentolamine did not interact significantly with amphetamine administered i.p. (6 vs 10: $F(1,81) = 2.47$, $p>0.1$).

The effects of DMI pretreatment on amphetamine anorexia are shown in figure 12. DMI greatly enhanced the anorexic effect of i.p. amphetamine (5 vs 6: difference = 2.51g; $F(1,81) = 67.57$, $p<0.001$), but only slightly enhanced the anorexic effect of i.c. amphetamine (3 vs 7: difference = 0.65g; $F(1,81) = 4.53$, $p<0.05$). The potentiation of peripherally induced anorexia by DMI was approximately 4 times the size of the potentiation of centrally induced anorexia (5 vs 7: $F(1,81) = 22.55$, $p<0.001$).

TABLE 6

THE EFFECT OF VARIOUS DRUG TREATMENTS ON FOOD INTAKE

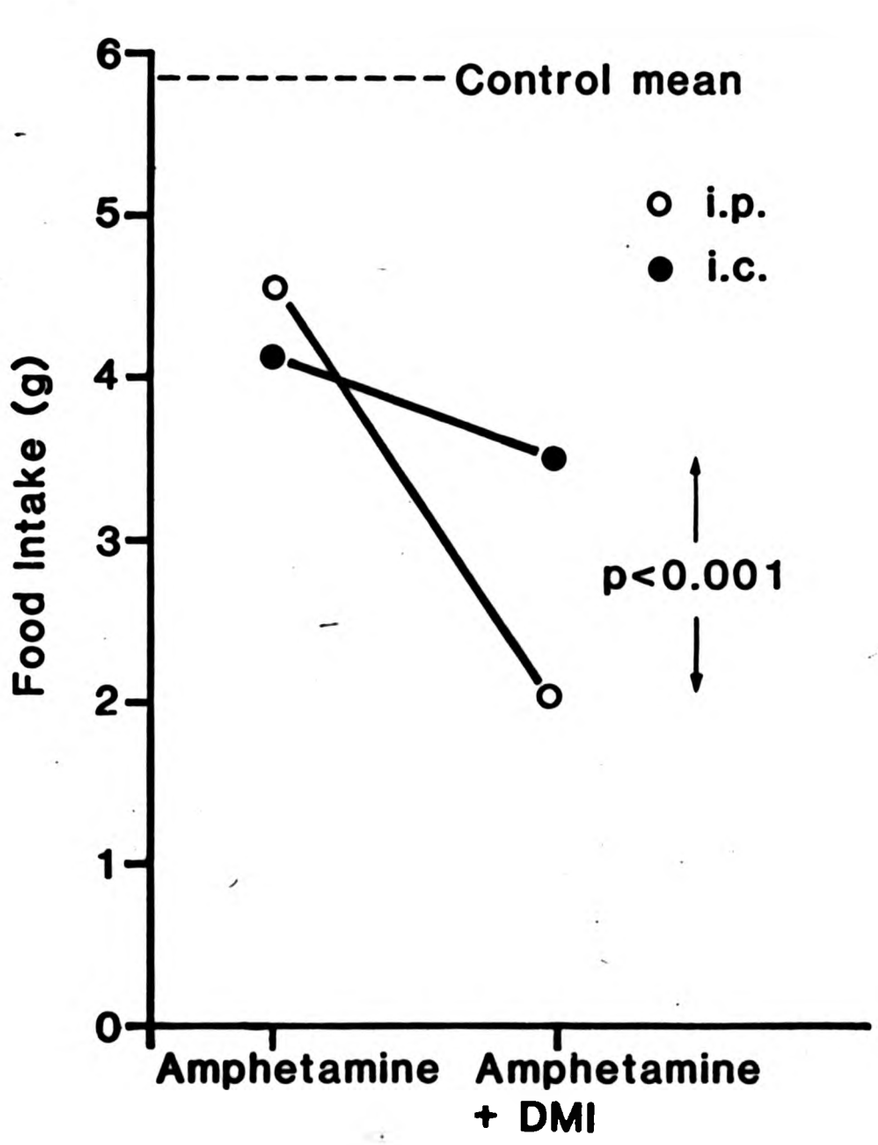
	PRETREATMENT TIME				FOOD INTAKE (g)*
	21h	30 min	10 min	5 min	Mean ± Standard Error
1.				SAL i.c	5.66 ± 0.30 a
2.				AMP i.c.	6.62 ± 0.48 b
3.			PHEN i.c.	AMP i.c.	4.15 ± 0.27 c
4.	DMI i.p.	SAL i.p.			5.92 ± 0.32 a
5.	DMI i.p.	AMP i.p.			2.05 ± 0.29 d
6.		AMP i.p.			4.56 ± 0.35 c
7.	DMI i.p.		PHEN i.c.	AMP i.c.	3.50 ± 0.31e
8.	DMI i.p.		PHEN i.c.		5.87 ± 0.29 a
9.			PHEN i.c.		5.95 ± 0.26 a
10.		AMP i.p.	PHEN i.c.		4.08 ± 0.36 c

SAL: Saline; DMI: desmethylimipramine; PHEN: phentolamine; AMP: amphetamine

* Treatments with the same suffix do not differ significantly; differences between treatments with different suffixes are highly significant ($p<0.01$ or better), with the exception of c vs e, for which $p<0.05$.

FIGURE 12

EFFECT OF DMI PRETREATMENT ON THE ANOREXIC EFFECTS OF AMPHETAMINE, i.c. (OPEN CIRCLES) AND i.p. (CLOSED CIRCLES). PHENTOLAMINE PRETREATMENT WAS USED WITH i.c. INJECTIONS. THE DOTTED LINE REPRESENTS THE MEAN OF THE FOUR CONTROL PROCEDURES (1, 4, 8 and 9 in table 6).



5.3.4. DISCUSSION

The sequence of treatments was not randomized in this study, owing to practical constraints. However, it seems unlikely that this factor compromised the results: control procedures were administered at various points throughout the series (1,4,8,9), with no change in baseline food intake. Furthermore, there was no discernible tendency towards either sensitization or tolerance to amphetamine as a function of repeated administration. It is also noted that phentolamine was not administered in combination wih DMI and peripheral amphetamine. Again, however, it is unlikely that this is a significant omission, as phentolamine did not interact with either DMI or peripheral amphetamine administered separately.

With this reservation, the results are straightforward. Blockade of alpha-receptors by phentolamine unmasked an anorexic effect of i.c. amphetamine, as has been previously reported (see introduction). This anorexic effect was slightly enhanced by DMI; beta-adrenergic synapses in the PFH are the most likely site for this interaction. DMI also enhanced the anorexic effect of peripherally administered amphetamine, but much more substantially. The discrepancy between the sizes of the two enhancements suggests that under these conditions approximately three quarters of the potentiation by DMI of peripherally induced anorexia is mediated peripherally. This potentiation is artefactual in origin: DMI inhibits the inactivation of amphetamine by the liver, resulting in higher circulating levels

of amphetamine.

The anorexic effect of amphetamine, at low doses, is mediated in part by beta-adrenergic mechanisms (see Introduction), whereas most other behavioural effects of amphetamine, including the anorexic effect of higher doses, are mediated primarily by DA. Consequently, it is uncertain whether any potentiation of the effect of i.c. amphetamine by systemic DMI would be observed if behavioural measures other than the anorexic effect of a low dose of amphetamine were used.

As stated above, chronic TAD treatment (at high doses) attenuates amphetamine anorexia, but only in withdrawal. This finding has previously been interpreted as chronic AD treatment having no net effect at beta-adrenergic synapses. In light of the present results, this conclusion is no longer valid. It now appears that chronic AD treatment may cause a net reduction in the output of the relevent beta-adrenergic synapses.

However, since chronic TAD treatment also enhances central alpha1 receptor function (Maj et al 1980, 1981) and this system has been implicated in the stimulation of feeding (Leibowitz 1980), an attenuation in anorexia over chronic treatment might well represent an increase in alpha-adrenergic function instead of a decrease in beta-adrenergic function. This intepretation is examined in the following experiment.

In conclusion, acute DMI pretreatment has been shown to significantly potentiate amphetamine anorexia via a peripheral mechanism. A direct consequence of this finding is to unmask an attenuation of anorexia during chronic treatment. However, the implications of this finding with regard to beta and alpha-adrenergic mechanisms are unclear.

5.4. EXPERIMENT 6: THE EFFECTS OF CHRONIC MIANSERIN TREATMENT ON AMPHETAMINE ANOREXIA

5.4.1. INTRODUCTION

Although the attenuation of amphetamine anorexia following chronic AD treatment has been used as evidence of beta-receptor subsensitivity, the results are open to more than one interpretation. When amphetamine is used to elevate NA transmission, both the alpha-adrenergic feeding system and the beta-receptor satiety system could theoretically be stimulated. In fact the results of the previous experiment showed evidence of alpha-adrenergic stimulation by amphetamine, which caused an increase in food intake. Consequently, an attenuation in amphetamine anorexia following chronic DMI treatment, which has been taken to reflect a decrease in beta-receptor activity, could reflect an increase in alpha-receptor activity; chronic AD treatment has been shown to have both effects.

It is difficult to address this issue experimentally, because of the peripheral interaction between DMI and amphetamine which was demonstrated in the previous experiment. Mianserin, however, does

not appear to impair aphetamine metabolism. Acute pretreatment with mianserin had no significant effects on amphetamine anorexia, or on other effects of amphetamine, such as stereotyped behaviour (Willner, Towell and Montgomery 1984). Mianserin does, however, attenuate amphetamine anorexia with chronic administration.

One way of assessing the contribution of alpha-adrenergic mechanisms to this attenuation of amphetamine anorexia by mianserin is to administer the alpha-adrenergic blocker phentolamine concurrently. If attenuation of anorexia by chronic mianserin is still seen, then this would suggest a beta-adrenergic involvement. However, if mianserin-induced attenuation of amphetamine anorexia is blocked by phentolamine, then this would suggest an alpha-adrenergic involvement.

A problem in drug interaction studies of this kind is that changes in amphetamine anorexia following phentolamine pretreatment could mask any concomittant change in beta-receptor function. This problem was highlighted in chapter 4, experiment 3, in which a microstructural analysis of feeding behaviour was used to unmask propranolol-amphetamine interactions. It is for these reasons that the microstructural technique was used in this experiment to examine mianserin-amphetamine-phentolamine interactions.

5.4.2. METHOD

Subjects

Twenty-four male Lister hooded rats (weight 330-365g), were individually housed and maintained on 21-hour food deprivation with water available ad lib.

Drugs and Procedure

The animals were trained to feed by pressing the door of the pellet dispenser in one of six identical operant chambers, as described in experiment 1, chapter 3. Daily ten-minute sessions were run until all animals reached asymptotic performance. Animals were divided into two groups of 12. Mianserin hydrochloride (10 mg/kg, Beecham, Epsom, England) was administered at approximately 17.00h daily, for a period of 21 days to one group whilst the other group received the same number of control injections (distilled water). Amphetamine (0.5 mg/kg, Smith, Kline and French) was administered 30 minutes prior to feeding (i.e. 19-20 hours following mianserin pretreatment), whilst phentolamine (Ciba, 1.0 mg/kg) was administered 10 minutes prior to feeding. All injections were made at a volume of 1 ml/kg and the drugs were administered i.p. Doses were calculated as salts. Control injections consisted of the vehicle (distilled water).

Treatments (amphetamine, phentolamine and a combination of amphetamine and phentolamine) were administered in a counterbalanced order between days 14 and 21 of mianserin

treatment. An amphetamine-phentolamine challange was also carried out two days into withdrawal (see table 7). At least one day intervened between successive amphetamine-phentolamine treatments, on which control injections were given.

Anorexia was measured as suppression scores. Suppression was calculated as the mean food intake (total number of pellets taken during the half hour session) of the day directly before and the day directly after the amphetamine test day, minus food intake on the amphetamine test day. Microstructural parameters of feeding were calculated as suppression scores in the same manner. Suppression scores for each microstructural parameter were subjected to an analysis of variance, and where appropriate were supplemented by tests of simple main effects. An additional analysis was carried out on control data taken at the beginning of the experiment (day 14) and at the end of mianserin treatment (day 20; see table 7). The purpose of this analysis was to establish if chronic mianserin treatment changed baseline scores, as such an effect would complicate interpretation of the main analysis. Only data on the total food intake, eating rate and eating time are presented, as consideration of the other microstructural parameters adds no further information.

5.4.3. RESULTS

Mianserin did not significantly change total food intake, eating time or eating rate during the course of chronic treatment ($F(1,22) = 3.10$, 0.21 and 2.62, respectively, $p>.05$). As observed

in previous experiments (2, 3 and 4) amphetamine caused a reduction in food intake (figure 13A) which was characterized by a decrease in eating time (figure 13C) and an increase in eating rate (figure 13B).

Mianserin pretreatment attenuated both amphetamine anorexia and the amphetamine-induced decrease in eating time and enhanced the amphetamine-induced increase in eating rate, although none of these effects reached statistical significance (figures 13A-C).

The effect of phentolamine treatment on the amphetamine-mianserin interaction was to slightly block the mianserin-induced attenuation of amphetamine anorexia, and also to slightly block the mianserin-induced attenuation of the amphetamine change in eating time. The mianserin-induced enhancement of the amphetamine-induced increase in eating rate was totally blocked following phentolamine treatment. A more descriptive analysis of the effect of phentolamine on the mianserin-amphetamine interaction is given in table 7. The suppressant effect of amphetamine on total food intake and eating time was attenuated by 31% and 18% respectively following mianserin pretreatment. When phentolamine was conurrently administered, the mianserin-induced attenuation of amphetamine's suppressant effects on totals and time were slightly blocked (15% and 10% respectively). However, the mianserin-induced enhancement of the amphetamine-induced increase in eating rate was totally abolished by phentolamine.

FIGURE 13A

SUPPRESSION OF FEEDING BY AMPHETAMINE

White = control, black = mianserin,
P = phentolamine, A = amphetamine,
A/P = amphetamine/phentolamine, W/D = amphetamine/phentolamine during withdrawal.
See text for details

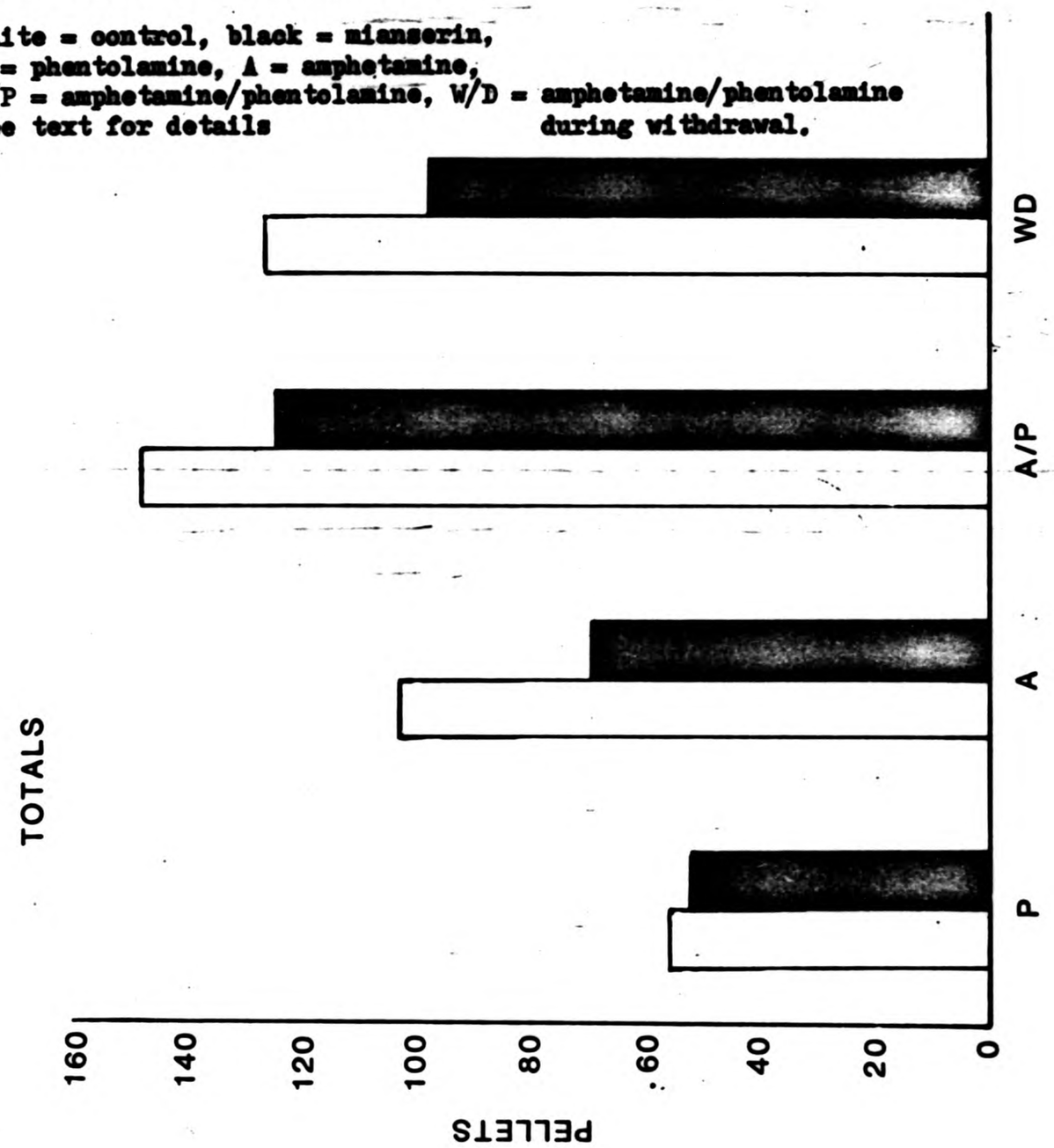


FIGURE 13B

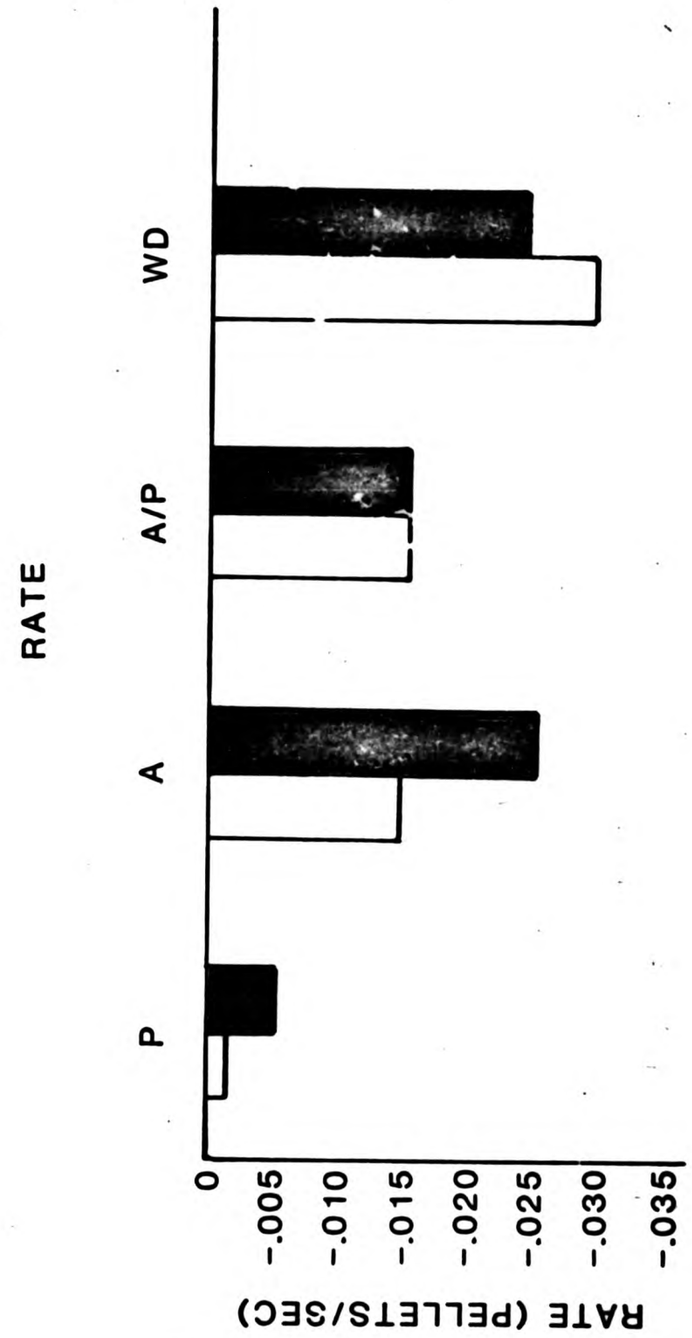


FIGURE 13C

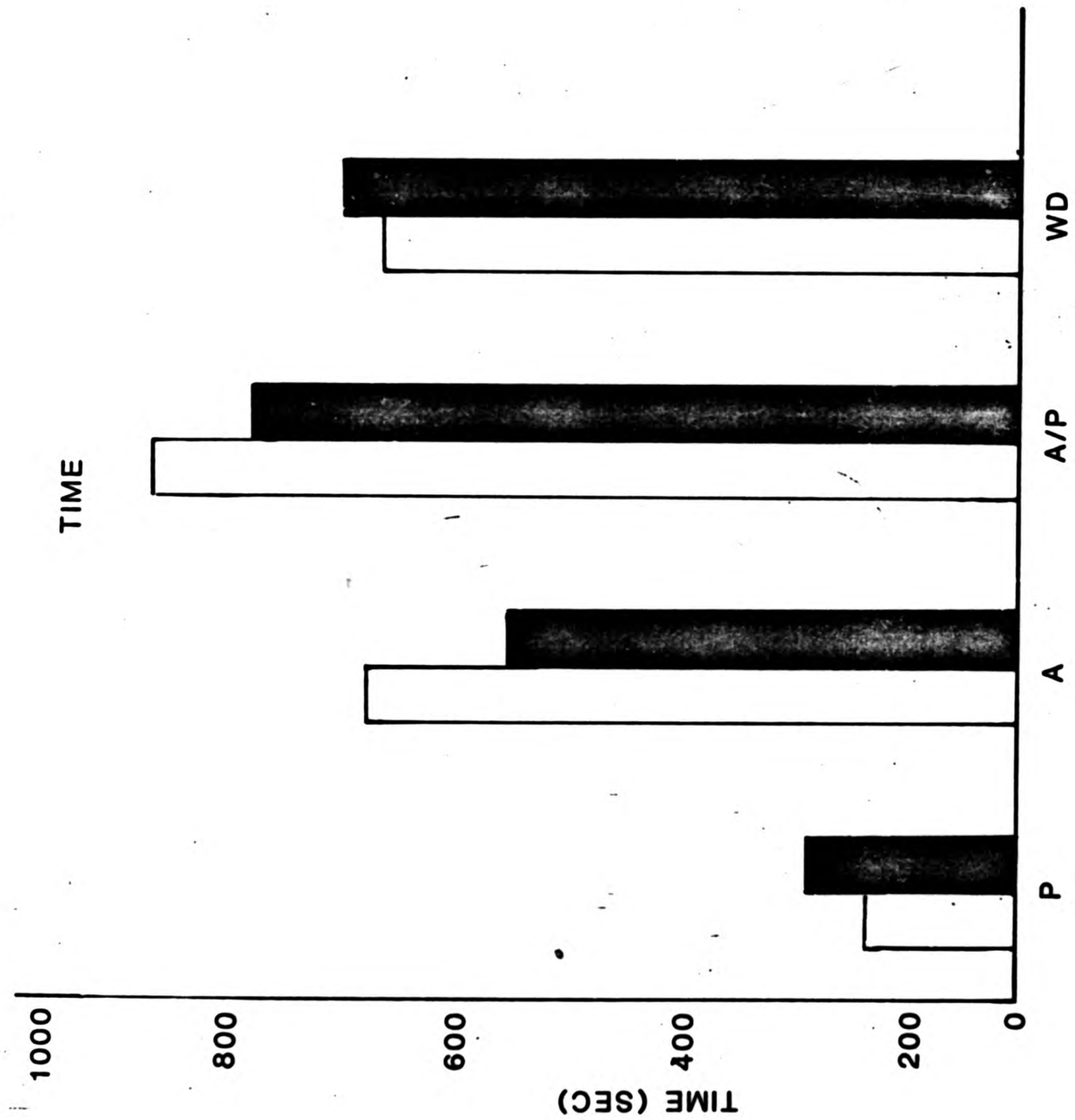


TABLE 7

EFFECTS OF CHRONIC MIANSERIN TREATMENT ON AMPHETAMINE ANOREXIA

Sequence of drug treatments in four groups of animals (n=6).
See text for details.

	Days of drug treatment						↓	Withdrawal		
Drug pretreatment	14	15	16	17	18	19	20	21	22	23
Gp 1 Control	C	A/P	C	P	C	A	C	C	A/P	C
Gp 2 Mianserin										
Gp 3 Control	C	A	C	P	C	A/P	C	C	A/P	C
Gp 4 Mianserin										

Mean suppression of food intake following amphetamine or phentolamine treatment

	Mianserin pretreatment				Control pretreatment			
	P	A	A/P	A/P	P	A	A/P	A/P
Pellets	53	70	124	97	56	102	146	127
Time	295	558	782	702	237	680	870	666
Rate	-.005	-.025	-.015	-.024	-.001	-.014	-.015	-.029

	Suppressant effect of amphetamine (in absolute and %) following mianserin pretreatment		Suppressant effect of amphetamine (in absolute and %) following phentolamine and mianserin pretreatment	
Pellets	32	(31%)	22	(15%)
Time	122	(18%)	88	(10%)
Rate	.011	(79%)	0	(0%)

A = amphetamine
P = phentolamine
C = control

5.4.4. DISCUSSION

Phentolamine pretreatment enhanced the suppression of total food intake and eating time in control and mianserin treated animals. Chronic mianserin treatment attenuated amphetamine anorexia, an effect that has recently been reported (Willner, Towell and Montgomery 1984), although this effect was not statistically significant in this experiment. This lack of significance in the mianserin-induced attenuation of amphetamine anorexia precludes a proper analysis of the effects of phentolamine on the mianserin-amphetamine interaction. In fact, phentolamine pretreatment decreased the magnitude of the mianserin-induced attenuation of amphetamine anorexia, although this effect was not significant. The decrease of the mianserin-induced attenuation of amphetamine anorexia and the reduction in eating time following phentolamine pretreatment could be evidence for an enhancement of alpha-adrenergic function during chronic mianserin treatment. However, the mianserin-induced enhancement of the amphetamine-induced increase in eating rate was totally blocked by phentolamine. This might suggest that some component of eating rate is also mediated by alpha-adrenergic mechanisms. This conclusion is examined in the next chapter.

5.5. GENERAL DISCUSSION

Willner and Montgomery (1980) proposed that the effects of DMI on amphetamine anorexia could be understood as compensatory adaptive changes, which occur during chronic treatment (a presumed beta-adrenergic subsensitivity effect), and which carry through into

withdrawal. However, if the acute enhancement of amphetamine anorexia is an artefact of the metabolic effects outlined in experiment 5, then the model would be incorrect. Instead of there being no effect on amphetamine anorexia during chronic treatment, there would be an attenuation of amphetamine anorexia. This revised model would postulate a more plausible explanation of the clinical action of AD than the earlier model, which concluded that there was no net change in the output of NA systems.

If attenuation of amphetamine anorexia following mianserin pretreatment does indeed have an alpha-receptor component, it is likely that the other and larger component of the mianserin-induced attenuation of amphetamine anorexia is beta-adrenergic. The most likely mechanism for attenuation of amphetamine anorexia is a beta-receptor subsensitivity which becomes apparent during chronic mianserin treatment. This interpretation is consistent with that of the previous experiment, in its conclusion that chronic DMI, iprindole and mianserin treatment reduce NA transmission through the mechanism of beta-receptor subsensitivity. It must be noted, however, that even if ADs do lower NA transmission by inducing beta-receptor subsensitivity which over-compensates for the NA stimulatory effect of ADs; it does not necessarily follow that beta-receptor subsensitivity is responsible for the clinical effects of ADs. In fact, recent evidence suggests that it is not (see chapter 8, section 8.3.).

CHAPTER SIX

ASSAYING PRESYNAPTIC DOPAMINE RECEPTORS: VALIDATION STUDIES

6.1. INTRODUCTION

The preceding chapter considered the contribution of beta-receptor subsensitivity as a possible mediator of AD treatment. It is also evident that AD treatments interact with DA systems. This evidence has been presented in chapter one, section 1.8., and can be briefly summarized by the hypothesis that chronic AD treatment increases DA transmission. In addition to clear effects on postsynaptic measures of DA transmission, it is also possible that chronic AD treatment induces subsensitivity of presynaptic DA receptors. This chapter examines a behavioural assay for presynaptic DA receptor activation.

The dose response curve for the effects of apomorphine on locomotor activity is biphasic ; low doses cause sedation whilst higher doses cause stimulation of behavioural activity (Costall et al 1980, 1981; Carlsson 1975). This biphasic profile of apomorphine is explained by the proposal that low doses of the drug act as an agonist specifically at presynaptic DA autoreceptors, whilst higher doses are agonistic to both pre and postsynaptic sites (Skirboll et al 1979). Autoreceptor stimulation results in inhibition of DA release, producing sedation, whilst postsynaptic stimulation enhances DA transmission to increase behavioural activity (DiChiara et al 1978; Carlsson 1975). The anatomical substrates for the

postsynaptic effects of apomorphine have been identified from experiments in which DA was injected directly into the terminal areas. Enhancement of locomotor activity has been attributed to the stimulation of the mesolimbic DA system (Makanjuola et al 1980), whilst the mesocortical DA pathway has been implicated in attenuation of locomotor activity (Tassin et al 1978); the nigrostriatal DA pathway mediates the stereotyped behaviour seen at very high doses of apomorphine (Fog et al 1971).

In addition to the nigrostriatal and mesolimbic systems being regulated by autoreceptors the nigrostriatal system is also regulated by a striato-nigra feedback circuit (Bunney and Aghajanian 1976). The nigrostriatal system is therfore capable of buffering receptor changes to a greater extent than the mesolimbic system and in consequence will show less change to treatments which selectively interact with autoreceptors. In contrast, the mesocortical system, an extension of the mesolimbic system, appears to be devoid of autoregulation (Chiodo and Bunney 1983).

This simplistic account of the anatomical localisation of apomorphines behavioural effects is complicated by evidence implicating a multiplicity of DA receptor sites. Up until the late 1970s DA receptors were classified by their ability to stimulate cAMP formation (Creese 1982). DA1 (D1) receptors stimulated cAMP formation whilst D2 receptors did not (Kebabian and Calne 1979). Recently, a classification of DA receptors has emerged based on the relative affinities for various DA agonists

and antagonists to bind with specific receptor types. D3 receptors are thought to be located on DA terminals and might correspond to autoreceptors whilst D4 receptors are thought to be associated with endings of cortico-striatal neurons (Sokoloff et al 1980).

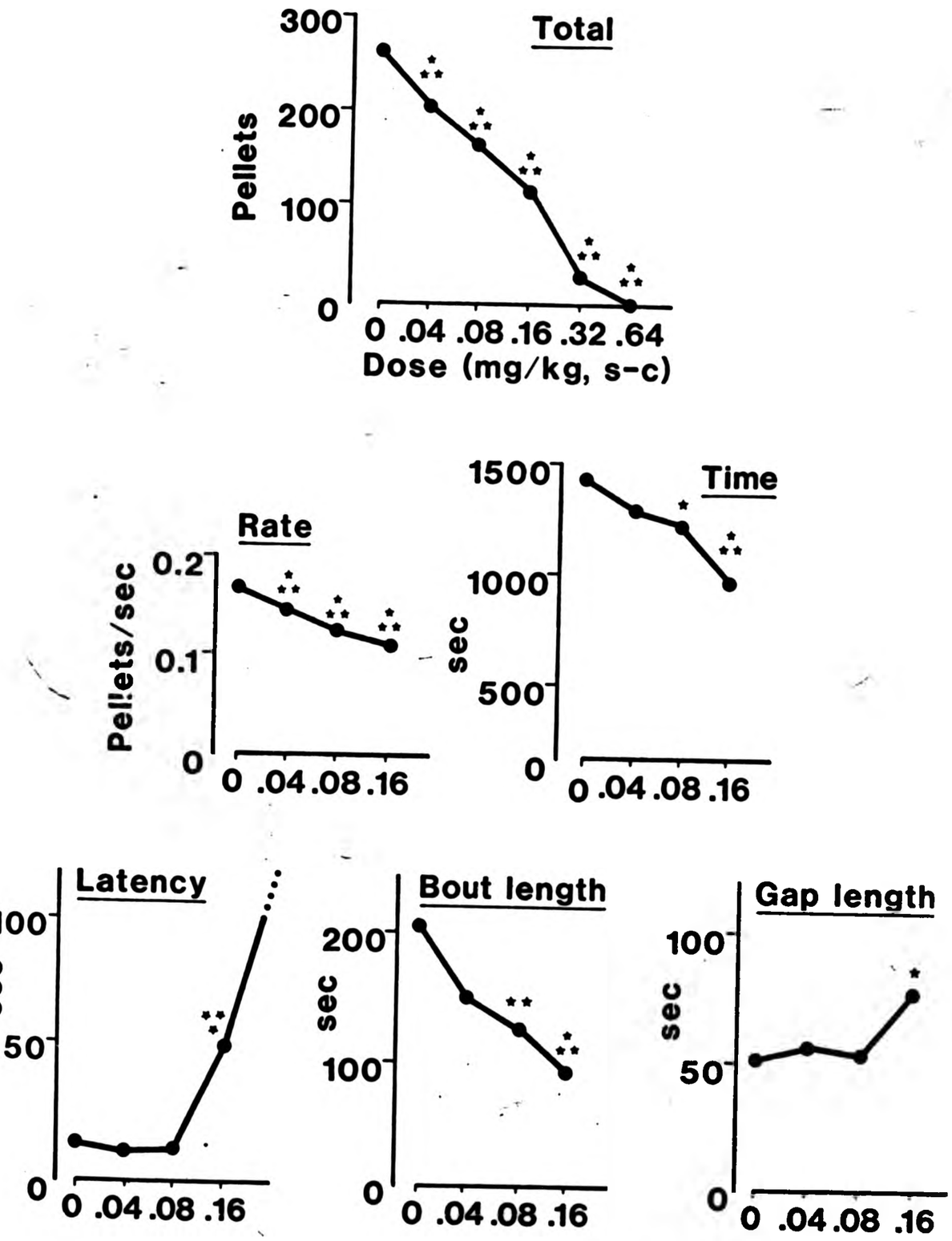
As outlined in chapter one, the behavioural effects of low doses of apomorphine have been used to index presynaptic DA activity during AD treatment and some studies have reported autoreceptor subsensitivity following chronic AD treatment (e.g. Serra et al 1979). These studies typically examined changes in locomotor activity, a behaviour that is quantified either by complex and often unreliable rating scales or by the use of photocell matrixes. Interpretation of these data are often complicated by the limitations of these procedures. It is for these reasons, together with the practical consideration that a microstructural feeding technique had already been set up in the laboratory, that we decided to examine the possibility that autoreceptor activity could be assayed by the effects of apomorphine on food intake.

The results of a microstructural study of feeding under various doses of apomorphine carried out in this laboratory (Willner and Jackson unpublished) are shown in figure 14. Apomorphine at all doses tested reduced total food intake ($p<.001$), eating rate ($p<.001$) and eating time (0.08 mg/kg, $p<.05$; 0.16 mg/kg, $p<.001$). Doses of apomorphine specific for presynaptic receptors (0.04, 0.08 mg/kg) reduced bout length (0.08 mg/kg, $p<.01$), but did not affect latency or gap length. Postsynaptic doses (>0.16 mg/kg)

further reduced bout length ($p<.001$) and also increased latency ($p<.001$) and gap length ($p<.05$). This dissociation between pre- and postsynaptic doses of apomorphine on latency and gap length, together with the dose-related effect on total, time, rate and bout length indicate that apomorphine anorexia appears to be a sensitive tool for studying presynaptic DA receptor stimulation. This conclusion is examined in this chapter, by attempting to reverse apomorphine anorexia with treatments known to be DA antagonists, at doses selective for presynaptic blockade.

FIGURE 14

APOMORPHINE DOSE-RESPONSE



Stars show differences between control and apomorphine.
One star $p<.05$, two stars $p<.01$, three stars $p<.001$.

6.2. EXPERIMENT 7: THE EFFECTS OF HALOPERIDOL PRETREATMENT ON APOMORPHINE ANOREXIA

6.2.1. INTRODUCTION

Although biochemical evidence suggests that presynaptic DA receptors do mediate apomorphine-induced behavioural sedation (Sokoloff et al 1980; Hjorth et al 1982), the behavioural evidence for this hypothesis is controversial. It has been reported that apomorphine-induced hypomotility together with the concurrent decrease in DOPAC concentrations was reversed by pimozide (0.3 mg/kg), haloperidol (0.05 mg/kg), sulpiride (10.0 mg/kg) and a variety of other neuroleptics at doses which in themselves did not influence motor activity or brain DOPAC concentrations (DiChiara et al 1976). However, another study in the same species (mice) confirmed reversal by pimozide (0.3mg/kg) and sulpiride (20 mg/kg) but failed to replicate reversal of apomorphine-hypomotility by haloperidol (0.05 and 0.1 mg/kg). This study also demonstrated reversal with two alpha blockers, yohimbine and prazosin (Summners et al 1981).

A third study, by Costall et al (1980), failed to antagonize apomorphine inhibition of locomotor activity in mice with a large range of neuroleptics, including those mentioned so far. However, no precise details of the doses of apomorphine were given by Costall et al (1980), apart from the fact that doses were in the ug/kg range! These authors attributed problems in replication to the strain of mice used and differences in methodologies encountered in other studies. More recently, using rats as subjects, Montanaro et al (1982) showed reversal of

apomorphine-induced hypomotility using low doses of -sulpiride but not +sulpiride, and also showed reversal of both apomorphine hypomotility and apomorphine hypermotility with the same doses of haloperidol (.03, .05 and 1.0 mg/kg). However, no data were presented on haloperidol induced changes in motility, making the results of this study difficult to evaluate.

Protais et al 1983, have further examined the dose-response relationship of apomorphine on locomotor activity in mice and found it to be not byphasic but polyphasic. In this model, attenuation of locomotor activity occured at 0.025mg/kg and 0.015mg/kg; restoration of this hypokinesia occured at 0.075mg/kg and 0.0375mg/kg respectively. The hypokinesia produced at 0.025mg/kg was effectively antagonised by haloperidol only (0.020 and 0.040 mg/kg); other typical and atypical neuroleptics were without effect. Antagonism of the hypokinesia produced at 0.015mg/kg of apomorphine was apparent across a range of neuroleptics at relativey small doses (e.g. 0.050mg/kg haloperidol). From the relative potency of neuroleptics to antagonise hypokinesia and its respective restoration, Protais et al chracterize their 0.025mg/kg and 0.0150mg/kg hypokinesia as being mediated by D3 and D4 receptors respectively. However, to the authors knowledge this is the first demonstration of a polyphasic dose-response curve for apomorphine and the shift in phase occurs within a very narrow dose range. The result therefore requires confirmation before the conclusions can be taken seriously.

On the basis of these different results, sometimes using animals from the same species, it is uncertain whether apomorphine dose-response studies on mice, and their subsequent pharmacological characterizaton can be meaningfully extrapolated to apomorphine dose-response studies using rats or other species. This problem will at least be partially resolved when attempts at replication are carried out using similar procedures to those used in previous studies.

Given the lack of consistent replicability of neuroleptic reversal of apomorphine-induced hypomotility, it is important for the present purpose to establish whether the anorexia induced by a low dose of apomorphine in rats could be antagonised by a non-sedative doses of neuroleptic drugs.

6.2.2. METHOD

Subjects

12 male Lister hooded rats (weight 330-410g), were individually housed and maintained on 21 hour food deprivation with water available ad libitum. Animals had had prior experience of continous reinforcement lever pressing and acute neuroleptic treatment, but had been drug free for a period of approximately 2 months.

Drugs and Procedure

Animals were trained to feed by pressing the door of the pellet dispenser in one of six identical operant chambers, as described

in chapter 3, experiment 1. Ten-minute daily sessions were run until all animals attained asymptotic performance. On experimental days, the animals received two injections: apomorphine hydrochloride (0.05mg/kg; Sigma) was administered s.c. in the scruff of the neck 10 mins. before the start of the session and haloperidol (0.02mg/kg; Jansen) was administered i.p. 30 minutes before the start of the session. Apomorphine was dissolved in 0.02% ascobate solution, whilst haloperidol was dissolved in distilled water. Drug solutions were prepared fresh daily. Control injections consisted of vehicle solutions, administered s.c for ascorbate and i.p for distilled water. All injections were at a volume of 1 ml/kg. Each animal received all four treatment combinations in a counterbalanced order, at two day intervals. On the intervening days, a ten minute session was run, with no drug treatments. Microstructural analysis of feeding was carried out as described in chapter 3, experiment 1. Results were analysed by analysis of variance, supplemented by tests of simple main effects.

6.2.3. RESULTS

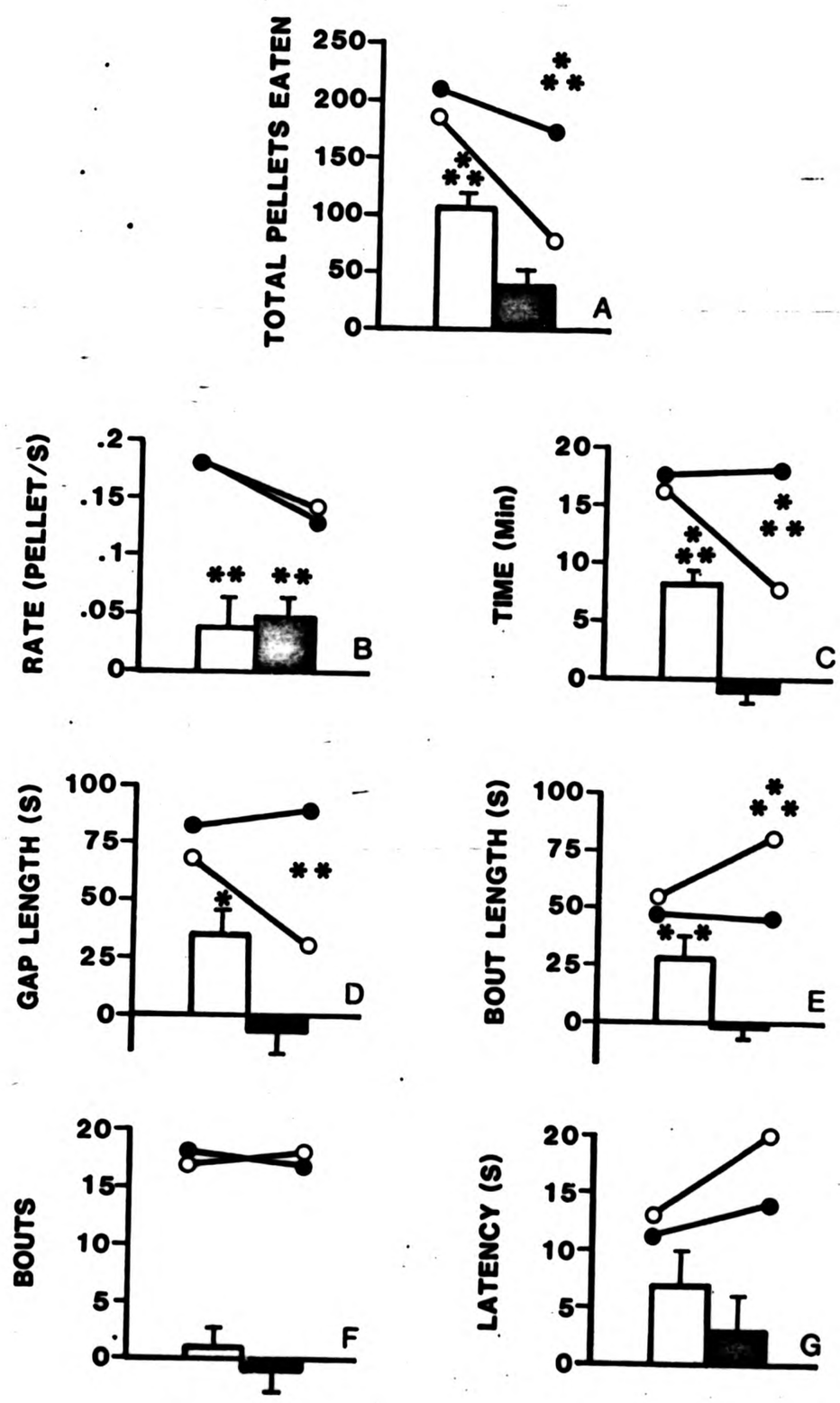
Apomorphine caused a significant (46%) reduction in food intake ($p<.001$, figure 15A) which was blocked by haloperidol pretreatment (interaction: $F(1,11)=12.56$, $p<.01$). Microstructural analysis showed that the reduction of food intake by apomorphine was produced by reductions in both eating rate and eating time. Haloperidol pretreatment blocked the effect on eating time. However, haloperidol did not block the effect on eating rate (figures 15B and C). It is therefore the increase in eating time

following haloperidol pretreatment that is responsible for the restoration of eating following apomorphine.

Examination of other microstructural parameters adds little further information. Apomorphine increased gap length and reduced bout length; both effects were reversed by haloperidol pretreatment (figures 15D and 15E). No significant changes were seen in the number of bouts taken during the session or the latency to start eating (figures 15F and 15G). Haloperidol alone slightly increased the total number of pellets eaten, eating time, bout time and the number of bouts taken during the session, and decreased gap time and latency- none of these effects were significant.

FIGURE 15

HALOPERIDOL-APOMORPHINE



Circles show scores in each condition. Left - control, right - apomorphine; white - control, black - haloperidol. Bars show difference brought about by apomorphine, (mean and standard error): white - control, black - haloperidol pretreatment. One star $p<.05$, two stars $p<.01$, three stars $p<.001$.

These effects were further investigated by examining intercorrelations between the various microstructural effects of apomorphine, with and without haloperidol pretreatment. The apomorphine-induced decreases in eating time and bout length, were significantly correlated with each other and with the concomitant reduction in total food intake (table 8). After haloperidol pretreatment, the apomorphine-induced reduction in eating rate was slightly enhanced and the reduction in eating time was reversed to control values; these two parameters were now both significantly correlated with the reduction in food intake. No other significant correlations between apomorphine-induced changes in parameters were seen. It is noteworthy that the reduction in eating rate was not correlated with the reduction in total food intake following apomorphine alone, but was correlated with the reduction in total food intake following haloperidol pretreatment.

TABLE 8

INTERCORRELATIONS BETWEEN APOMORPHINE-INDUCED CHANGES IN MICROSTRUCTURAL PARAMETERS

	Total	Rate	Time	Bout length	Gap length
Total		-0.3	0.63*	0.64*	-0.13
Rate	0.65*		-0.2	-0.21	0.18
Time	0.80**	0.2		0.85**	-0.26
Bout length	0.49	-0.15	0.48		-0.1
Gap length	-0.03	0.05	-0.34	0.35	

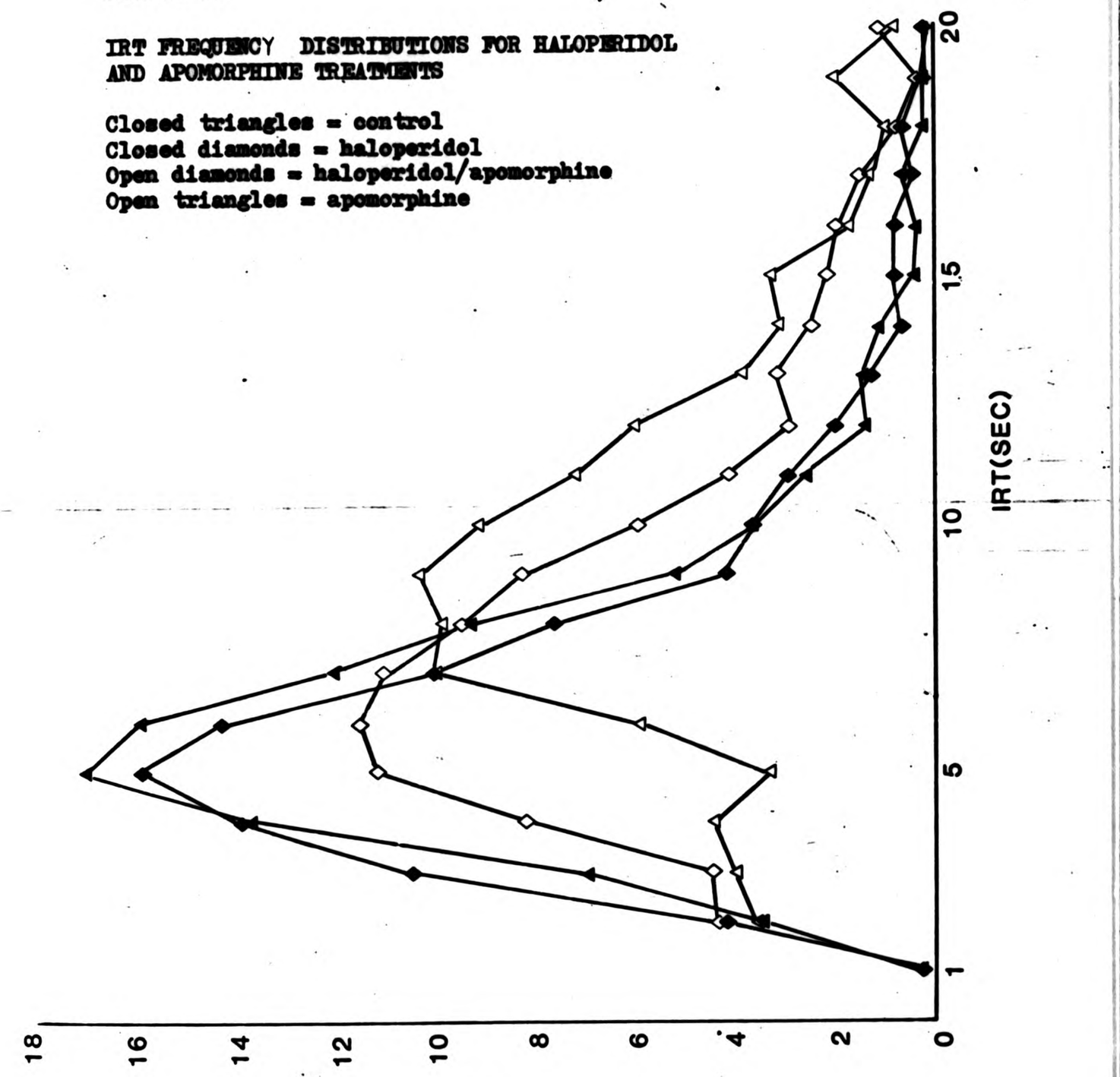
The table shows correlations (Spearman rank-order correlation coefficients) between the changes induced by apomorphine in different microstructural parameters. One star $p<.05$, two stars $p<.01$. The upper part of the table shows values obtained in control conditions; the lower part shows values obtained following haloperidol pretreatment.

As no reversal was seen in the apomorphine-induced decrease in rate following haloperidol pretreatment a closer, descriptive analysis was carried out on these data (figure 16). The purpose of this analysis was to unmask any potential artefactual distortions arising from the microstructural analysis method. Frequency distributions of IRTs were constructed for the four treatment conditions, control, haloperidol, apomorphine and haloperidol-apomorphine. Any decreases in eating rate will be readily visualised by a shift in the peak of small IRTs to the right of the distribution. When the four treatment distributions are superimposed they appear as two pairs: One pair represents haloperidol and control treatments whilst the other, shifted to the right, represents apomorphine and haloperidol-apomorphine treatments. These observations are consistent with the conclusion derived from the microstructural analysis that haloperidol did not reverse the effect of apomorphine on eating rate.

FIGURE 16

IRT FREQUENCY DISTRIBUTIONS FOR HALOPERIDOL AND APOMORPHINE TREATMENTS

Closed triangles = control
Closed diamonds = haloperidol
Open diamonds = haloperidol/apomorphine
Open triangles = apomorphine



6.2.4. DISCUSSION

Haloperidol pretreatment antagonised the reduction in food intake brought about by low doses of apomorphine. This antagonism is presumed to be mediated by presynaptic DA receptors, as no sedative effects were seen following haloperidol treatment. Microstructural analysis revealed that the antagonistic effect of haloperidol on apomorphine anorexia was consequent on an increase in eating time and bout length together with a decrease in gap length. It is the reduction in gap length and increase in bout length that results in the animals spending more time eating and therefore increasing their total food intake.

The apomorphine-induced reduction in rate was not reversed, suggesting that this effect of apomorphine is not in fact mediated by classical presynaptic DA receptors. This conclusion is supported by the findings of experiment 10, which show that apomorphine injected directly into nucleus A9 and A10, reduces eating time, but has no significant effect on eating rate. The intercorrelations presented in table 8 also support this conclusion, in that in haloperidol pretreated animals there was a significant correlation between changes in total food intake and changes in eating rate.

In conclusion, it appears that apomorphine-induced decreases in eating time can be reversed by haloperidol pretreatment at such a dose as to implicate a presynaptic DA involvement. The next experiment examines this conclusion, using the atypical

neuroleptic thioridazine to reverse apomorphine anorexia.

6.3. EXPERIMENT 8: THE EFFECTS OF THIORIDAZINE PRETREATMENT ON APOMORPHINE ANOREXIA

6.3.1. INTRODUCTION

The DA hypothesis of depression (see chapter 1 section 1.4.2.), which states that certain types of depression are due to a lowering of DA transmission in the brain, has received support from studies which show that treatments that functionally increase brain DA transmission are effective as AD in certain sub-populations of patients (Robertson and Trimble 1982, Jenner and Marsden 1982). Paradoxically, amongst these DA enhancing treatments are the atypical neuroleptic drugs that are known to decrease DA transmission and be without serious side effects in the treatment of schizophrenia. However these drugs (in paticular thioridazine and sulpiride) have been shown to possess AD properties; and some studies have shown thioridazine and sulpiride to be of similar potency or indeed more potent than amitriptyline in the treatment of depression (Smith et al 1973; Aylward et al 1980). It is possible that thioridazine exerts its AD effects through antagonism of pre-synaptic DA receptors, dis-inhibiting DA release and increasing DA transmission. Typically, it is claimed that patients show response to thioridazine or sulpiride therapy after 4 or 5 days treatment or so, as compared with two weeks or so for TAD therapy. It was therfore of interest to investigate the effect of acute thioridizine pretreatment on apomorphine anorexia, which is mediated in part by presynaptic DA receptors (see experiment 7).

The effect of subchronic thioridazine treatment was also studied in this experiment. If, as hypothesized, thioridazine exerts its clinical antidepressant effects by disinhibiting DA neurons and increasing DA transmission, apomorphine anorexia should be antagonised in thioridizine treated animals by an enhancement of DA transmission, an effect which should persist and might become more apparent over treatment.

6.3.2. METHOD

Subjects

Twenty-four male hooded rats (390-450g) (Olac, Bicester) were individually housed and maintained on 21 hour food deprivation, with water available ad libitum. The animals had had prior experience of continously reinforced lever pressing for food rewards and had received acute neuroleptic drug treatment four months prior to the experiment.

Drugs and Procedure

Animals were trained to feed by pressing the door of the pellet dispener in one of six identical operant chambers. Ten-minute daily sessions were run until all animals attained asymptotic performance. Animals were assigned to one of two groups (n = 12) which were matched for prior lever pressing performance. Animals were tested daily in the operant chambers for half-hour sessions between the of 10.00 and 14.00 hours.

On each of eight consecutive days, animals received two

injections two hours before a test session. One group received thioridazine (6 mg/kg; Sandoz), whilst the other received physiological saline. Ten minutes before the commencement of the session, all animals received either apomorphine s.c. (0.05 mg/kg; Sigma) or its vehicle s.c. (0.05% ascorbate) on alternate days. Thioridazine was dissolved in physiological saline whilst apomorphine was dissolved in 0.05% ascorbate to prevent rapid oxidisation of the drug. All injections were made at a volume of 1 ml/kg. Apomorphine or its vehicle were administered s.c. in the scruff of the neck whilst thioridazine was administered i.p. Analysis of microstructural parameters of feeding were carried out as described in chapter 3, experiment 1. Results were analysed by analysis of variance, supplemented by tests of simple main effects.

6.3.3. RESULTS

In both the control and thioridazine treated groups, food intake rose steadily through the course of the experiment. This increase in food intake can probably be accounted for by the fact that animals had not attained asymptotic performance during half-hour experimental sessions, as they were only trained on 10 minutes sessions (due to financial constraints).

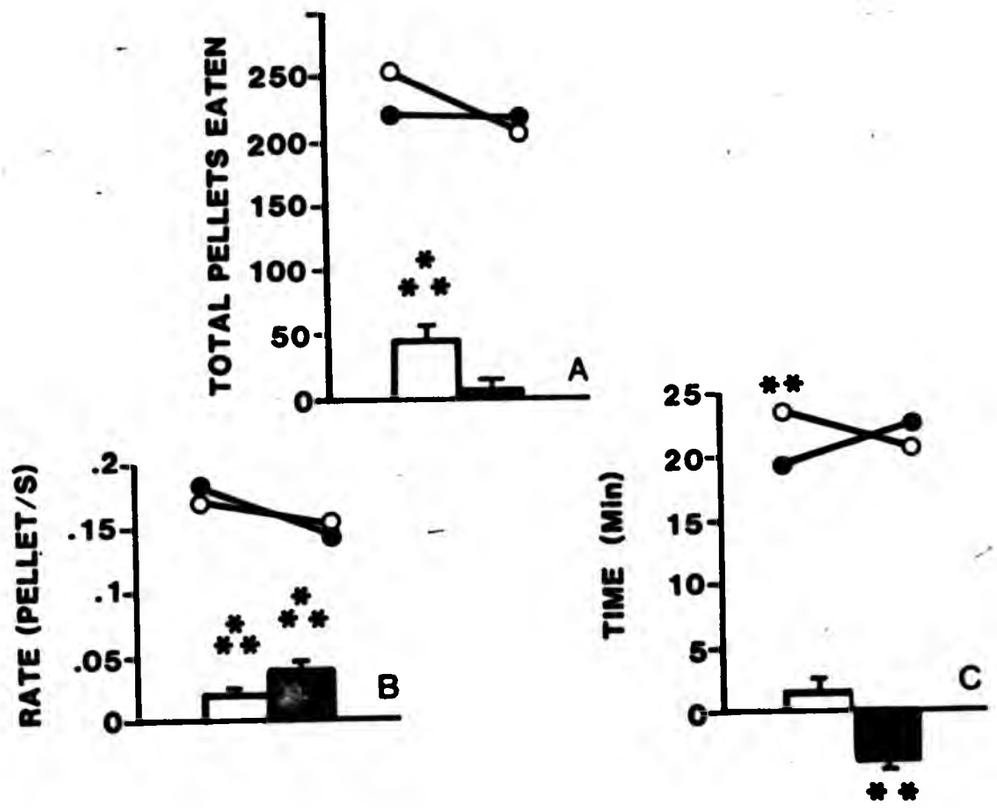
On the first control day, (figure 17A) thioridazine pretreatment decreased food intake; however on subsequent control days thioridazine had no significant effect on food intake (not shown)

Apomorphine caused a significant reduction in food intake over each of the four days when it was administered($F(3,66)=3.39$, $p<.05$). This effect was blocked by thioridazine pretreatment on day 1 (figure 17A) but on subsequent tests, thioridazine pretreatment had no effect on apomorphine anorexia (not shown).

Results of the microstructural analysis are only presented in detail for the first apomorphine trial. Apomorphine significantly reduced eating rate ($F(1,88)=12.06$, $p<.001$, figure 17B) and increased latency ($F(1,88)=32.18$, $p<.001$, results not shown). Eating time was slightly decreased by apomorphine ($F(1,88)=1.18$, $p>.05$, figure 17C). Thioridazine alone significantly decreased eating time ($F(1,88)=10.87$, $p<.01$, figure 17C). However, thioridazine pretreatment reversed the reduction of food intake by apomorphine ($F(1,88)=5.56$, $p<.01$, figure 17A), by reversing the effects of apomorphine on latency ($F(1,88)=1.79$, $p>.05$, results not shown) and eating time ($F(1,88)=7.76$, $p<.01$, figure 17C). The apomorphine-induced reduction in eating rate was unaffected by thioridazine pretreatment ($F(1,88)=.07$, $p>.05$, figure 17B).

On trials 2,3 and 4 apomorphine reduced eating rate in both control and thioridazine pretreated groups ($F(1,132)=7.48$, $p<.001$, results not shown), but otherwise analysis of the microstructural parameters on trials 2,3 and 4 was generally unremarkable: No significant differences were apparent with the single exception of a significant difference in gap length on the third control day ($F(1,88)=5.25$, $p<.05$, results not shown).

FIGURE .17



Circles show scores in each condition. Left - control, right - apomorphine; white - control, black - thioridazine. Bars show the difference brought about by apomorphine (mean and standard error): white - control, black - thioridazine pretreatment. One star $p<.05$, two stars $p<.01$, three stars $p<.001$.

6.3.4. DISCUSSION

On the first test day, thioridazine pretreatment reversed the reduction in food intake brought about by apomorphine (figure 17A) by reversing eating time (figure 17C) and latency . However, eating rate was not reversed by thioridazine, suggesting again that this parameter is not mediated by DA (figure 17B). The reversal of total food intake and eating time was masked by differences in baseline scores between thioridazine and control treatments. This difference in baseline scores cannot argue against the reversal of apomorphine-induced efects on total food intake and eating time, as the mean value of both these parameters was actually increased above that of apomorphine following thioridazine pretreatment. The reversal of the apomorphine-induced reduction in latency by thioridazine is consistent with a restoration of normal food intake. These results agree with the findings of the previous experiment, where haloperidol was shown to reverse the number of pellets eaten and the time spent eating but not the rate of eating.

It is likely that the (15%) increase in food intake following haloperidol could have been caused by specific presynaptic blockade, thereby increasing DA transmission and facilitating feeding. The reduction in feeding following thioridazine (12%), on the other hand, could have been caused by both presynaptic and postsynaptic DA receptor blockade, the consequence of which was to functionally reduce DA transmission, and cause a reduction in feeding.

Thioridazine pretreatment is thought to have non-specific effects on DA transmission at relatively low doses. Thioridazine (5, 10 and 15 mg/kg) has been shown to be without effect at blocking amphetamine induced locomotor activity and stereotypy, unlike typical neuroleptics which produce a dose related blockade (Bentall and Herberg 1980). Iversen and Koob (1977) showed blockade of amphetamine induced locomotion with 4.0 mg/kg thioridazine, but were subsequently unable to replicate this result. Thioridazine is also known to be a potent blocker of central Ach receptors (Herberg et al 1980), and also of alpha-receptors (Jenner and Marsden 1982) at relatively low doses. This failure of thioridazine to reverse amphetamine-induced behaviours may well be accounted for by its interaction with preynaptic DA receptors.

Factors that were likely to contribute to the general lack of signficant effects of thioridazine on microstructural parameters on trials 2,3 and 4 are the general increase in feeding over the four test days, the lack of selectivity of thioridazine at pre- and post-synaptic sites, together with concurrent adaptive changes in receptor sensitivity that might take place in response to these factors. In conclusion, taking these factors into account, it is possible that thioridazine exerts its potential AD activity by facilitating DA transmission through presynaptic DA receptor blockade. However, as thioridazine only blocked apomorphine anorexia on day 1, it appears that the animals rapidly became tolerant to repeated thioridazine treatment. It is unlikely then that the therapeutic potential of thioridazine

as an AD is mediated via presynaptic DA blockade.

6.4. GENERAL DISCUSSION

Microstructural characterization of apomorphine anorexia showed that it was caused primarily by reductions in both eating rate and eating time. In experiment 7, a non-sedative dose of haloperidol reversed apomorphine anorexia, by reversing the apomorphine-induced reduction in eating time but not eating rate. In experiment 8, the atypical neuroleptic thioridazine also reversed the effect of apomorphine on eating time, but again had no effect on eating rate. These results suggest that the reduction in eating rate following apomorphine is not mediated by DA receptors.

Some results from experiment 10, described more fully in the following chapter will be mentioned briefly here in support of this conclusion. Aministration of apomorphine into either of the dopamine cell body areas, nuclei A9 and A10, reduced total food intake (figure 18). The reduction in food intake was more apparent in A10 ($F(1,144)=11.31$, $p<.01$) than in A9 animals ($F(1,144)=6.41$, $p<.05$) but in both cases the effect was brought about by reductions in eating time which were significant in A9 animals ($F(1,144)=8.40$, $p<.01$, figure 18C) but not in A10 animals. However, in contrast to the previous experiments where systemic apomorphine decreased eating rate, eating rate was sligthly increased with central administration and reached significance in A9 animals ($F(1,144)=5.83$, $p<.05$, figure 18B). The differences between the effects of central and peripheral

apomorphine administration are shown clearly in figure 19. Eating rate is slightly increased with central administration in marked contrast to systemic administration (figure 19B). Both eating time and total food intake show similar effects, independent of the route of apomorphine administration (figures 19C and A respectively).

FIGURE 18

THE EFFECTS OF INTRACRANIAL APOMORPHINE ADMINISTRATION ON FEEDING

Stars show difference from control. One star $p<.05$, two stars $p<.01$, three stars $p<.001$.

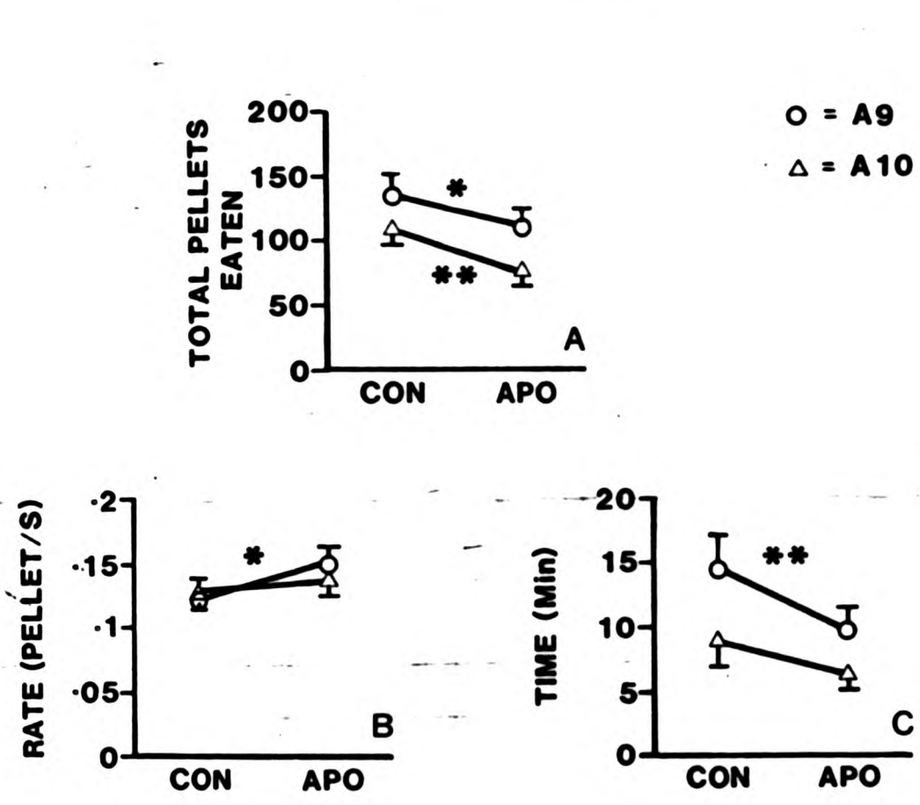
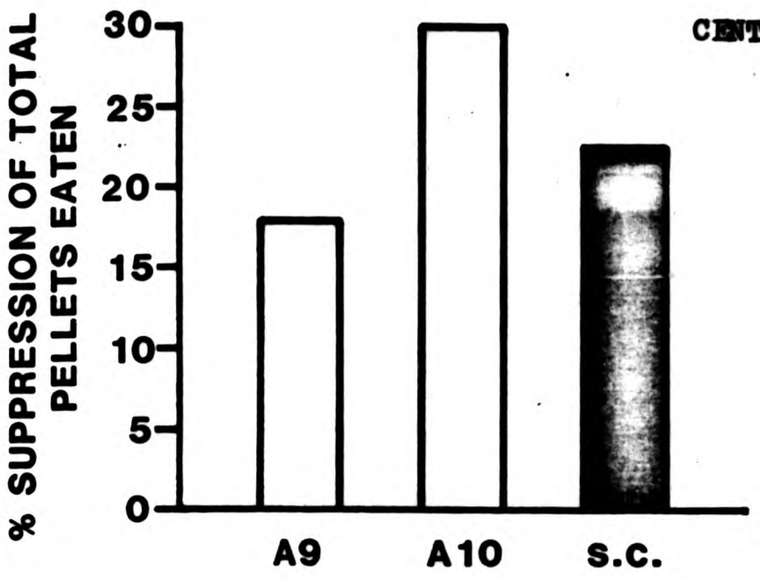
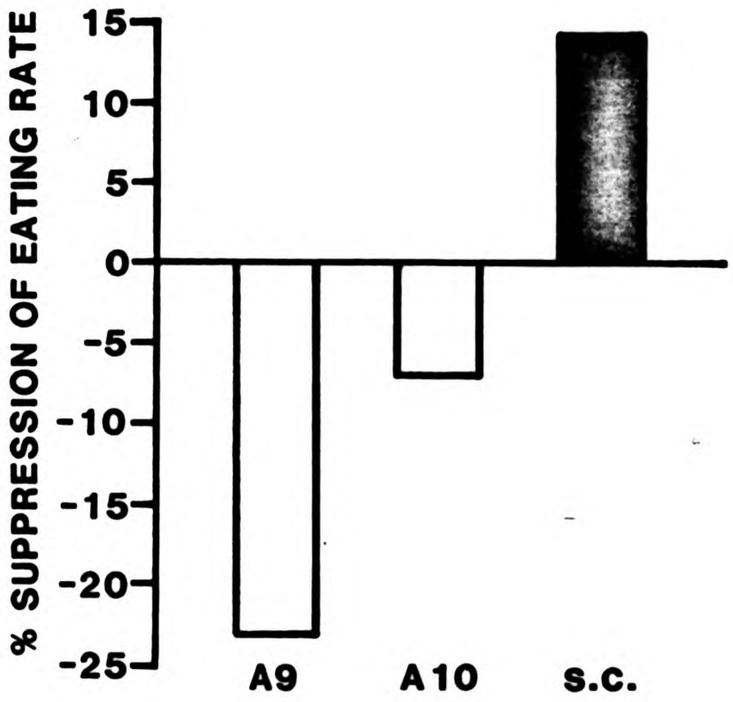


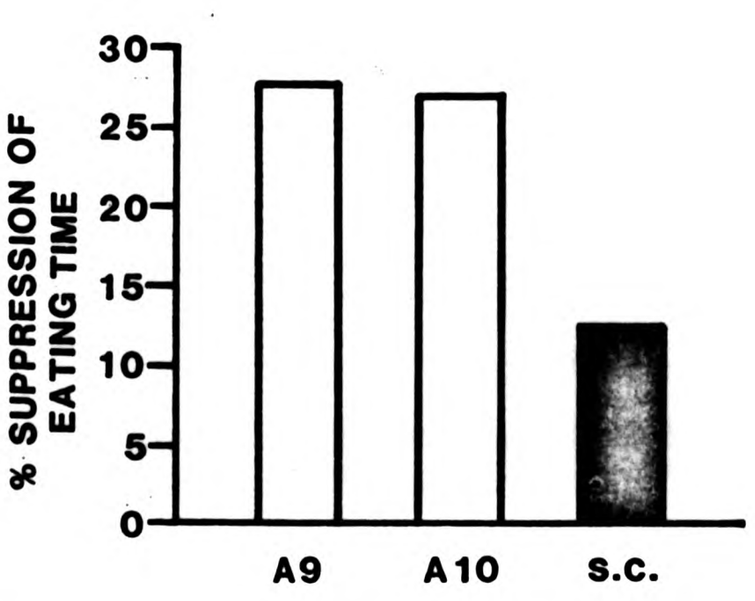
FIGURE 19 (see text for details)

DAY 1 A9-A10

CENTRAL VERSUS SYSTEMIC EFFECTS OF APOMORPHINE







As alpha blockers have been shown to reverse apomorphine-induced hypomotility in mice, it would be of interest to see if the reduction in rate, or indeed any other microstructural parameters could be reversed by alpha-receptor blockade. In fact, in a recent study carried out in this laboratory, yohimbine was found to be similar to haloperidol, in reversing anorexia by reversing eating time but not eating rate. We are currently attempting to establish the neurochemical basis of the reduction of eating rate by apomorphine so far without success.

In conclusion, the apomorphine-induced reduction in eating time appears to be a robust and usable assay for pre-synaptic DA receptors. The next chapter presents the results of two experiments in which low doses of apomorphine were used to index presynaptic DA receptor function during chronic DMI treatment.

CHAPTER SEVEN

ASSAYING PRESYNAPTIC DOPAMINE RECEPTORS DURING CHRONIC DMI TREATMENT

7.1. INTRODUCTION

In support of a DA hypothesis of depression, that depression arises from a lowering of DA transmission at certain functionally important synapses in the brain, are the findings that chronic TAD treatments increase DA transmission (e.g. Modigh 1975). A mechanism by which ADs could increase DA transmission is by inducing autoreceptor subsensitivity, to disinhibit DA neurons. The evidence in support of this hypothesis is controversial (see chapter 1, section 1.8.2.).

Serra et al (1979) first reported evidence for autoreceptor subsensitivity in that chronic treatment with imipramine, amitriptyline and mianserin counteracted or reversed the effect of small doses of apomorphine on motor activity and potentiated the central stimulant response to larger doses of apomorphine. Changes in apomorphine responses were seen after 10 but not 2 days of imipramine treatment and persisted up to 4 days of imipramine withdrawal. This effect was subsequently confirmed using other AD treatments, including MAOIs and ECS (Serra et al 1982) and also lithium (Harison-Read 1980).

Spyraki and Fibiger (1981), however, failed to confirm autoreceptor subsensitivity following chronic DMI treatment- a conclusion which is supported by at least three unpublished studies (Willner 1983c). It is noteworthy that Spyraki and

Fibiger report that following chronic DMI treatment there was a tendency towards a reduced effectiveness of low doses of apomorphine in producing hypmotility, although this did not reach statistical significance in their study. It may be that although autoreceptor subsensitivity was apparent it was not of sufficient magnitude to be detected by their apparatus using apomorphine-induced hypomotility as an assay.

The previous chapter proposed apomorphine aorexia as an assay for presynaptic DA receptors. More specifically it was shown that the aomorphine reduction in eating time was mediated by presynaptic DA receptors. The present experiments investigated the effects of chronic DMI treatment on apomorphine anorexia.

7.2. EXPERIMENT 9: ASSAYING PRESYNAPTIC DOPAMINE RECEPTORS DURING CHRONIC DMI TREATMENT USING SYSTEMIC APOMORPHINE

This experiment attempted to assay the sensitivity of presynaptic DA receptors by means of the anorexic response to a low dose of apomorphine (0.06 mg/kg).

7.2.1. METHOD

Subjects

Twenty-four male hooded rats (350-420g)(Olec, Bicester) were individually housed and maintained on 21 hour food deprivation, with water available ad libitum.

Drugs and Procedure

Animals were trained to feed by pressing the door of the pellet dispenser in one of six identical operant chambers as outlined in chapter 3, experiment 1. Ten-minute daily sessions were run until all animals attained asymptotic performance. Animals were then assigned to one of two matched groups (n=12), either receiving DMI (7.5 mg/kg i.p.) or saline treatment for 32 consecutive days. Testing was carried out between 10.00 and 14.00 h. The animals had free access to food from 14.00 to 17.00 h and DMI was administered between 17.00 and 18.00 h.

On each test day, all animals received apomorphine s.c. (0.05 mg/kg; Sigma) ten minutes before the commencement of the test session. Vehicle injections (0.05% ascorbate) were given on the day before and the day after each apomorphine treatment. Thus the experiment consisted of successive groups of 3 test days. On the intervening days, animals were run in the apparatus for ten minutes. Animals were tested with apomorphine at six day intervals during drug treatment and at 3 day intervals during 10 days of withdrawal. Apomorphine was dissolved in 0.05% ascorbate to prevent rapid oxidisation of the drug, and DMI was dissolved in distilled water. All injections were made at a volume of 1 ml/kg. Analysis of microstructural parameters of feeding were carried out as described in chapter 3, experiment 1.

An initial analysis of variance was carried out on all data derived from vehicle injections on days before and after the

apomorphine probe days. These analyses showed that there was no significant difference between DMI and control pretreatment either on total food intake (F (1,22) = 0.20, p>.05) or on any other microstructural parameter. Having established there was no differential effect of DMI on control treatments data were transformed into suppression scores according to the formula:

$$\frac{\text{day before + day after}}{2} - \text{apomorphine day}$$

These suppression scores were then subjected to analysis of variance, supplemented by tests of simple main effects.

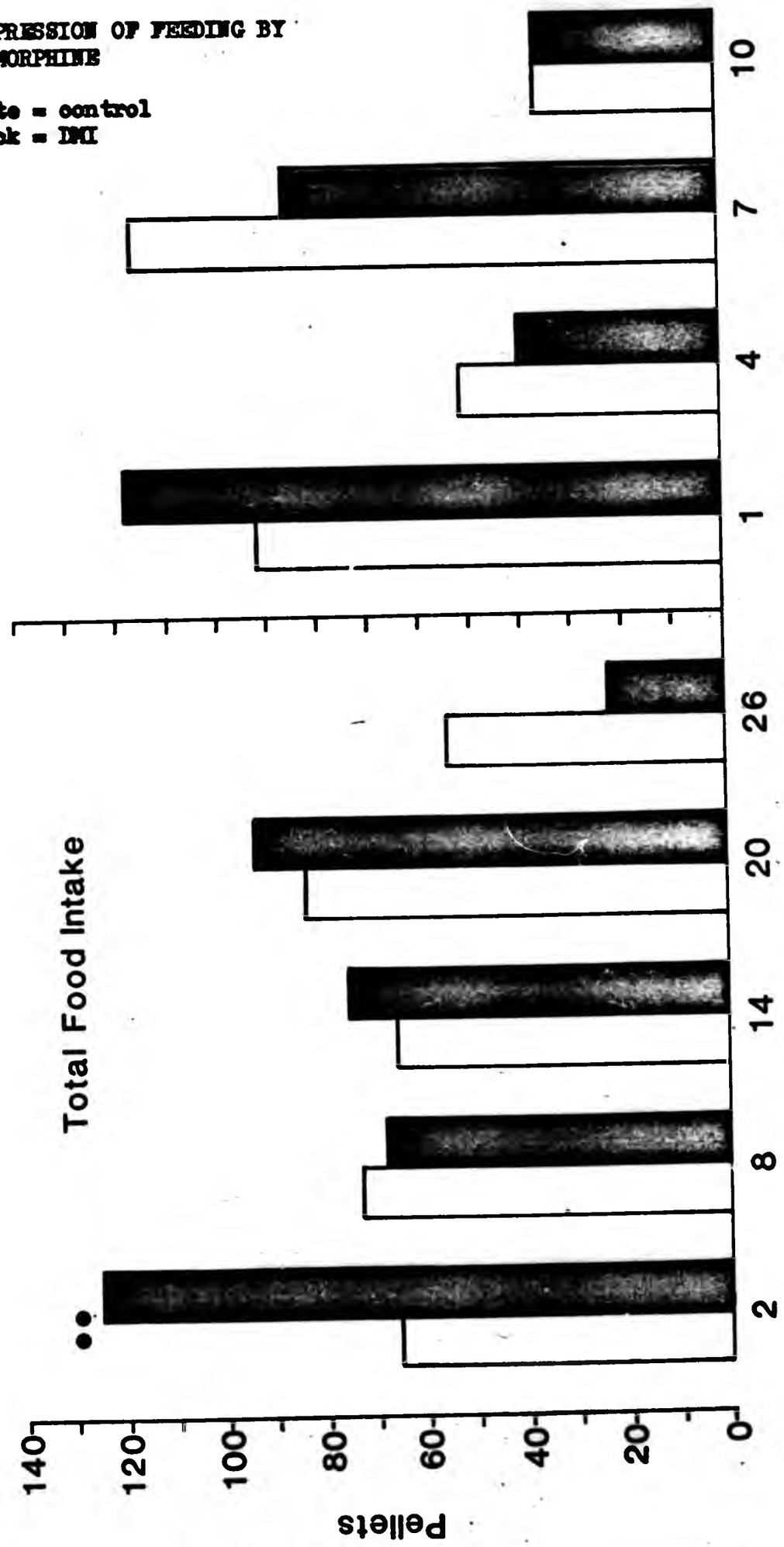
7.2.2. RESULTS

As observed in previous experiments (e.g. experiment 7), apomorphine anorexia (figure 20A) was characterized by a decrease in both eating rate (figure 20B) and eating time (figure 20C). DMI pretreatment had no significant effects on the apomorphine-induced decreases in eating rate (figure 20B). However the change in eating time was significantly enhanced (F (1,198) = 8.09, p<.01) by acute (2 days) DMI pretreatment (figure 20C), causing an enhanced anorexic effect (F (1,198) = 9.26, p<.01, figure 20A). Subsequently, during chronic DMI treatment, the effect was reversed: significant attenuation of the decrease in eating time was observed on days 8 and 26 of DMI and day 7 of withdrawal (F (1,198) = 4.58, 5.55 and 5.94 respectively, p<.05, figure 20C), though not on the other test days.

FIGURE 20A

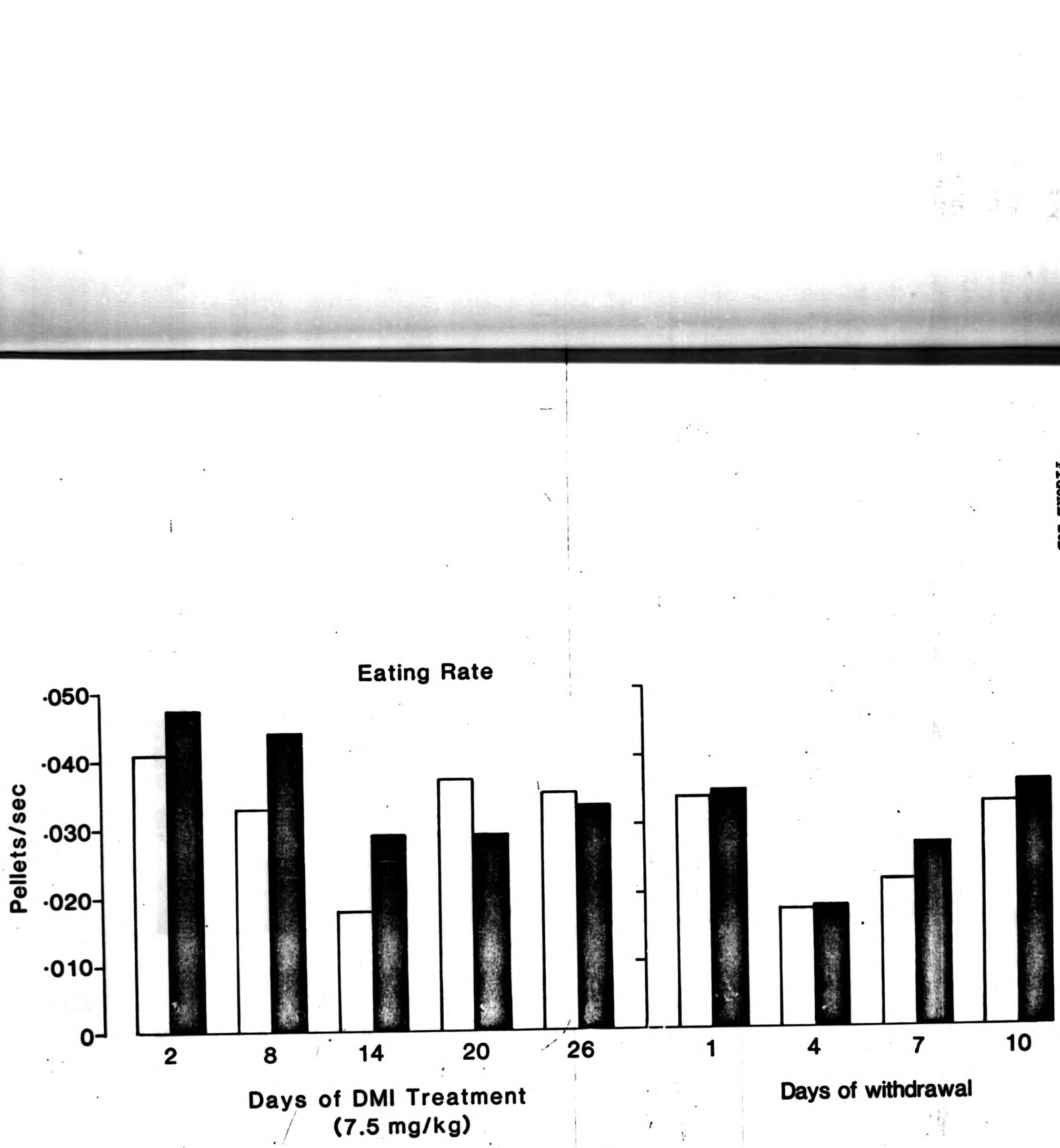
SUPPRESSION OF FEEDING BY APOMORPHINE

White = control
Black = DMI



Dots show the effect of DMI pretreatment. Stars show the suppressant effect of apomorphine. One symbol $p<.05$, two symbols $p<.01$, three symbols $p<.001$.

FIGURE 20B


Eating Rate
Pellets/sec
·050
·040
·030
·020
·010
0
2
8
14
20
26
1
4
7
10
Days of DMI Treatment
(7.5 mg/kg)
Days of withdrawal

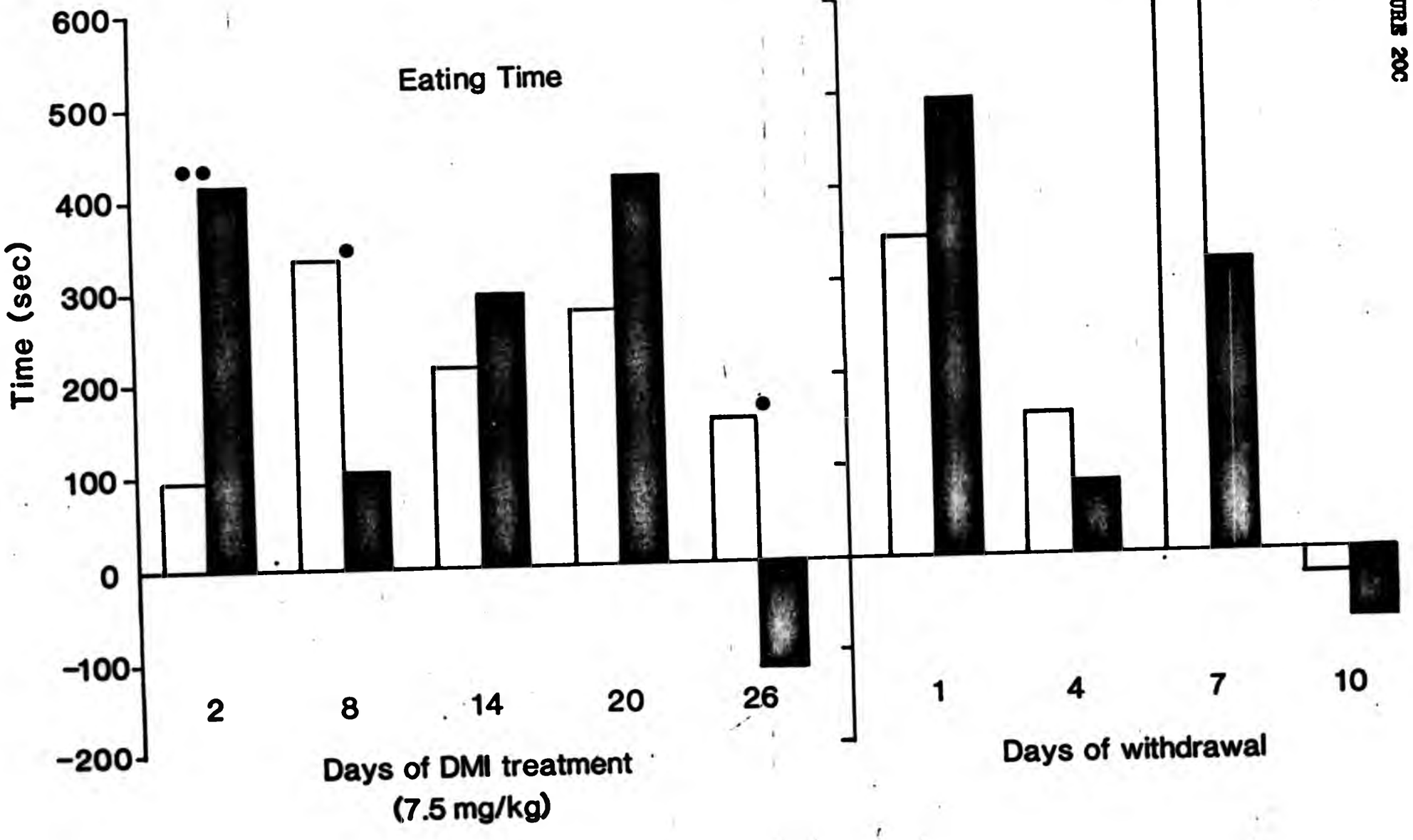

Eating Time
Time (sec)
600
500
400
300
200
100
0
−100
−200
2
8
14
20
26
Days of DMI treatment
(7.5 mg/kg)
1
4
7
10
Days of withdrawal

FIGURE 20

7.2.2. DISCUSSION

Acute pretreatment with DMI significantly enhanced apomorphine anorexia, probably by stimulating presynaptic DA receptors to reduce DA transmission. This initial increase in stimulation of autoreceptors was associated with an enhancement of the apomorphine-induced decrease in eating time. It is the reversal of this acute effect of DMI on eating time, which becomes apparent during chronic treatment, that provides evidence for autoreceptor desensitization. This result extends previous accounts of autoreceptor desensitization (Serra et al 1979, Chiodo and Antelman 1980), in that the effect was demonstrated during maintained DMI treatment, not simply during withdrawal, and furthermore that the desensitization may be related to an initial increase in stimulation of presynaptic DA receptors.

It is difficult to explain why the attenuation of the decrease in eating time was observed only on days 8 and 26 of DMI and on day 7 of withdrawal. On the remaining test days, (with the exception of day 2 which showed an enhancement of the effects of apomorphine on eating time), there were no significant results. However, there were some differences between control and DMI pretreated animals both within and between apomorphine probe days. The configuration of results between apomorphine probe days conformed approximately to a sinusoidal function. This configuration of results is consistent with control theory (Bayliss 1966). Control theory would account for these results in

terms of homeostatic compensatory deviations. Such deviations are claimed to allow a system to obtain an optimal state or set point, by allowing the system to search around the set point. In other words, DA systems might be searching through extremes of synaptic function to obtain autoreceptor subsensitivity. It should be noted, however, that these fluctuations in results between apomorphine probe days were not apparent when apomorphine was administered intracranially (see figure 22C).

In summary, this experiment has produced results which provide some evidence for the hypothesis that chronic DMI treatment induces autoreceptor subsensitivity at DA synapses, and by so doing, enhances dopaminergic transmission.

It is possible that there could be clearer results with central administration of apomorphine as each DA system can be manipulated independently from the other. The next experiment examined the relative contributions of the nigrostriatal and mesolimbic pathways to DMI-induced autoreceptor subsensitivity.

7.3. EXPERIMENT 10: ASSAYING PRESYNAPTIC DOPAMINE RECEPTORS DURING CHRONIC DMI TREATMENT USING CENTRAL APOMORPHINE ADMINISTRATION

7.3.1. INTRODUCTION

The dose-dependent pattern of behavioural effects produced by DA agonists such as apomorphine is well documented: moderate doses produce locomotor hyperactivity, whilst higher doses produce stereoyped behaviour (e.g. Kelly et al 1975). It is now generally accepted that the locomotor stimulant effect of moderate doses is mediated by the mesolimbic DA system, whilst the stereotyped behaviour seen at higher doses is mediated by the nigrostriatal system (e.g. Makanjuola et al 1982). Changes seen in locomotor activity and stereotypy following AD treatment have therefore been used to index DA transmission in the mesolimbic and nigrostriatal pathways.

Following chronic, but not acute AD treatment, behaviours elicited by moderate doses of DA receptor agonists are enhanced, indicating increased responsiveness in the mesolimbic system (e.g. Spyraki and Fibiger 1981; Willner 1983c). These results are also open to the interpretation that increases in postsynaptic responsiveness reflect the absence of the sedative component of stimulant drug action, as a result of AD-induced autoreceptor subsensitivity, rather than an increase in postsynaptic responsiveness. This possibility has been ruled out in the case of ECS , in that enhancement by repeated ECS of the stimulant effect of DA was seen when DA was injected directly into the

nucleus accumbens (Heal et al 1978). In addition, when reserpine is given at a dose to selectively abolish presynaptic DA activity, the locomotor stimulant effect of apomorphine was still enhanced by repeated ECS treatment (Modigh 1979). However, it is still possible that ADs might increase DA function primarily by the mechanism of autoreceptor subsensitivity.

The effects of repeated AD treatments on the nigrostriatal system are less clear. Some studies have shown increases in apomorphine and amphetamine-induced stereotyped behaviour following chronic treatment with a variety of AD (e.g. Willner, Towell and Montgomery 1984), but others have failed to show this effect (e.g. Delina-Stula et al 1979). However, difference in methodologies employed by these studies, such as different rating scales and drug doses, may account for some of these discrepancies (see Willner 1983b for a fuller discussion). There is at least some evidence which points to an increase in nigrostriatal responsiveness similar to that observed in the mesolimbic system following chronic AD treatment.

The purpose of this experiment was to evaluate the contribution of autoreceptor subsensitivity to changes in the responsiveness of nigrostriatal and mesolimbic DA systems during chronic DMI treatment.

7.3.2. METHOD

Subjects

A group of 28 Lister hooded rats (Olac, Bicester, Oxon), weighing

approximately 300g at the time of surgery, were housed inividually under conditions of controlled temperature and humidity, on a 12 hour light-dark cycle (09.00h to 21.00h light). Animals were maintained on 17-21 h food deprivation, and fed with standard laboratory diet (Dixon, Ware, Herts) from 14.00h to 17.00h daily. Water was freely available at all times.

Drugs and Procedure

The animals were trained to feed by pressing the door of the pellet dispenser in one of six identical operant chambers, as described in experiment 1. Ten minute daily sessions were run until all animals reached asymptotic performance. Animals were divided in to 2 matched groups. Cannulae aimed at either nucleus A9 or A10 were implanted bilaterally under pentobarbital anaesthesia. The coordinates, chosen according to the atlas of Pellegrino and Cushman (1967), were anterior +2.7mm, depth -3.3mm and lateral + or -2.5 for nucleus A9, and anterior +2.9mm, depth -3.4mm and lateral + or -1.2mm for nucleus A10. At the end of the experiment, animals were perfused with buffered formalin under sodium pentobarbital (100 mg/kg i.p.) and their brains were rapidly removed and stored in 10% formalin for approximately 10 weeks. Frozen 100um sections were stained with fast cresyl violet and cannula placement was verified histologically. Cannulae were confirmed to be in the vicinity of either nucleus A9 or A10 (see figure 21).The cannulae were of 26-gauge stainless steel (Arnold and Horwell, London); injections through them were made using a microsyringe with a 33-gauge needle (V.A. Howe, London). Details

of the micro-cannula system and surgery are given in chapter 5, experiment 5. Following surgery, animals returned to training sessions in the operant chambers until asymptotic performance was once again reached. The A9 and A10 groups were further subdivided into 2 matched groups .

DMI (7.5 mg/kg i.p., Geigy) was administered at 17.00h each day for 21 consequetive days to one of the A9 groups, whilst its vehicle (distilled water) was administered to the other. The same treatments were administered to the A10 animals. On test days, animals received apomorphine hydochloride (7.24nM i.c., Sigma) or its vehicle (0.05% ascorbate) directly before the start of the session. Apomorphine was dissolved in 0.05% ascobate solution and made up to volume with phosphate buffer, whilst DMI was dissolved in distilled water. Drug solutions were prepared fresh daily. Control injections consisted of vehicle solutions, administered i.c. for ascorbate and i.p. for distilled water. All i.p. injections were at a volume of 1 ml/kg, whilst i.c. injections were at a volume of 0.44 ul.

Apomorphine was administered on days 4, 7, 13 and 20 of DMI treatment, and on days 3 and 6 after withdrawal; control injections were given on the day before and the day after apomorphine probes. On test days, sessions lasted 30 minutes. On the intervening days (see table 9), a ten minute session was run, with no central drug treatments. Microstructural analysis of feeding was carried out as described in chapter 3. Results were analysed by a 4-way analysis of variance, supplemented by

tests of simple main effects.

7.3.3. RESULTS

Like systemic apomorphine, administration of central apomorphine significantly reduced food intake. On the first apomorphine probe day (day 4) apomorphine anorexia was more apparent in A10 than A9 animals ($F(1,144)=11.31$ and 6.41, $p<.01$, $p<.05$ respectively, figure 22A). As noted in section 6.4., these effects of apomorphine were brought about by reductions in eating time, but not in eating rate. The apomorphine-induced reduction in eating time, was significant in A9 animals ($F(1,144)=8.40$, $p<.01$, figure 22C), but failed to reach significance in A10 animals. In contrast to the results with systemic administration of apomorphine, eating rate was actually increased by the administration of apomorphine into nucleus A9 ($F(1,144)=5.83$, $p<.05$, figure 22B) and unchanged in nucleus A10 ($F(1,144)=0.59$, $p>.05$, figue 22C).

Taking the results over all six test days, apomorphine anorexia was greater with A10 infusions ($F(1,24) = 9.96$, $p<.01$, figure 22A) and again was characterized by decreases in eating time ($F(1,124)=36.93$, $p<.001$, figure 22C).

DMI pretreatment enhanced apomorphine anorexia on day 4 (the first probe day) in both A9 and A10 animals ($F(1,144)=7.52$ and 10.09 respectively, $p<.01$, figure 22A). This effect was maintained until day 7 in A10 animals ($F(1,144)=6.66$, $p<.05$, figure 22A). Sub-acute DMI pretreatment in A10 animals, enhanced the apomorphine-induced reduction in eating time

(F(1,144)=8.40, p<.01, figure 22C). No other significant changes in microstructural parameters were seen following sub-acute DMI pretreatment. The effects of chronic DMI treatment were minimal: a reduction in eating time was seen on day 20 of treatment in A9 animals (F(1,144)=7.49, p<.01, figure 22C). In A10 animals however, an enhancement in the apomorphine increase in gap length was seen on day 7 of DMI treatment (F(1,144)=17.06, p<.001, results not shown), an effect which was reversed on day 20 of DMI treatment (F(1,144)=4.42, p<.05, results not shown).

During withdrawal from DMI in A10 animals only, apomorphine anorexia, and the apomorphine-induced suppression of eating time were attenuated in contrast to the (non-significant) enhancement of these parameters during the course of chronic DMI treatment (figures 22A and 22C respectively). A separate analysis of variance was carried out comparing the last apomorphine probe day during DMI treatment with the first probe of withdrawal from DMI in A10 animals. The apomorphine-induced reduction in food intake was now shown to be significantly attenuated following withdrawal from DMI (F(1,24)=7.80, p<.05, figure22A); this effect did not quite reach statistical significance in eating time (F(1,24)=4.05, p>.05, figure22C). In addition, in the second withdrawal probe, the apomorphine-induced suppression of eating time was almost totally abolished. This effect did not reach statistical significance in the main analysis of variance but was clearly significant on a Mann-Whitney U-test (n=7, U=4, p<.01).

FIGURE 21

Consecutive sections of the rat brain derived from the atlas of Pellegrino and Cushman (1967). Symbols show typical cannulae placement to be in the vicinity of either nucleus A9 or A10. Abbreviations of structures close to symbols are given below: LM Lemniscus medial, PC Pedunculus cerebri, SN substantia nigra, TT tractus mamillotegmentalis, VTN nucleus ventralis tegmenti, ZI zona incerta.

Circles = A9, triangles = A10

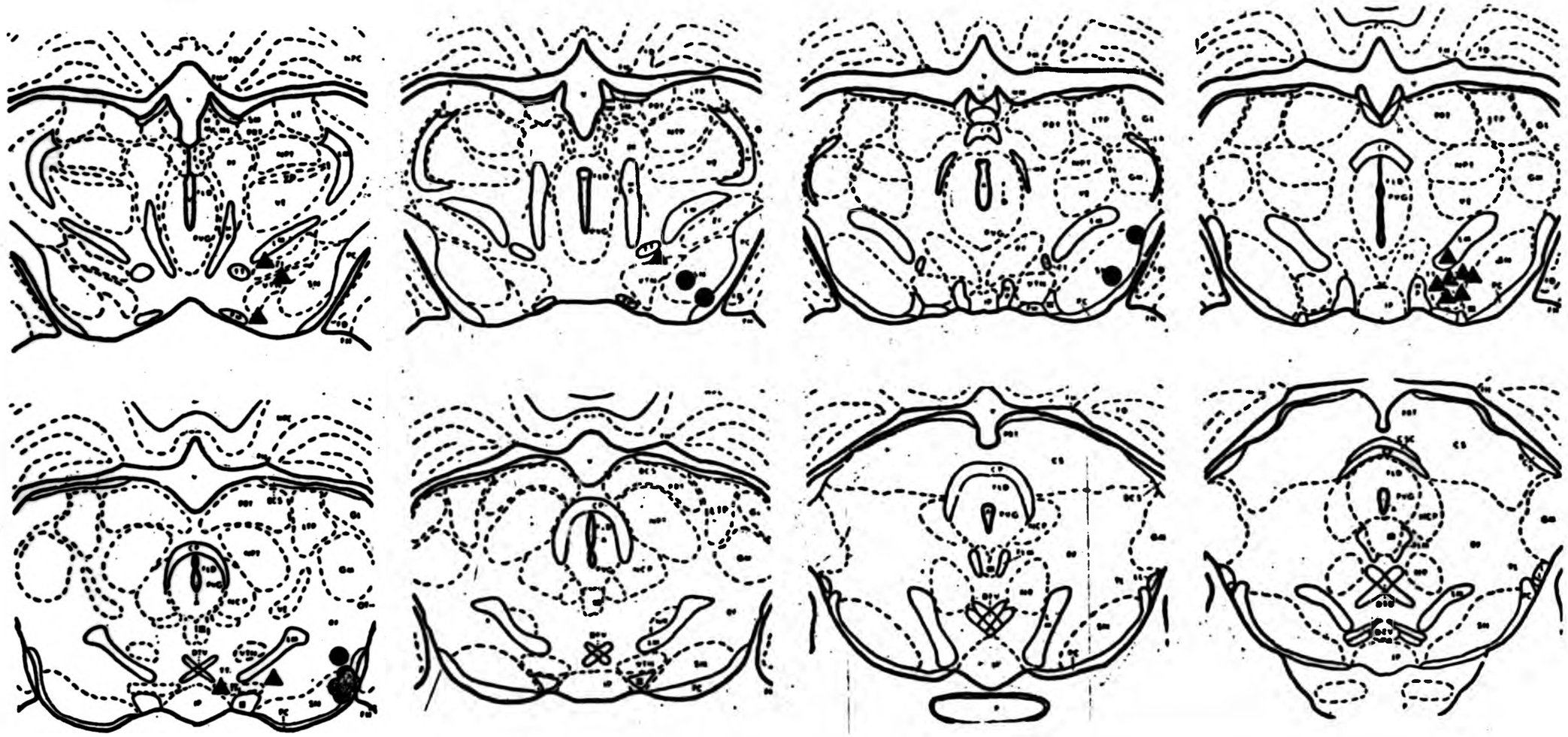


TABLE 9

MEAN SUPPRESSION OF FOOD INTAKE FOLLOWING CENTRAL APOMORPHINE ADMINISTRATION

Scores refer to the first 30 minutes of feeding

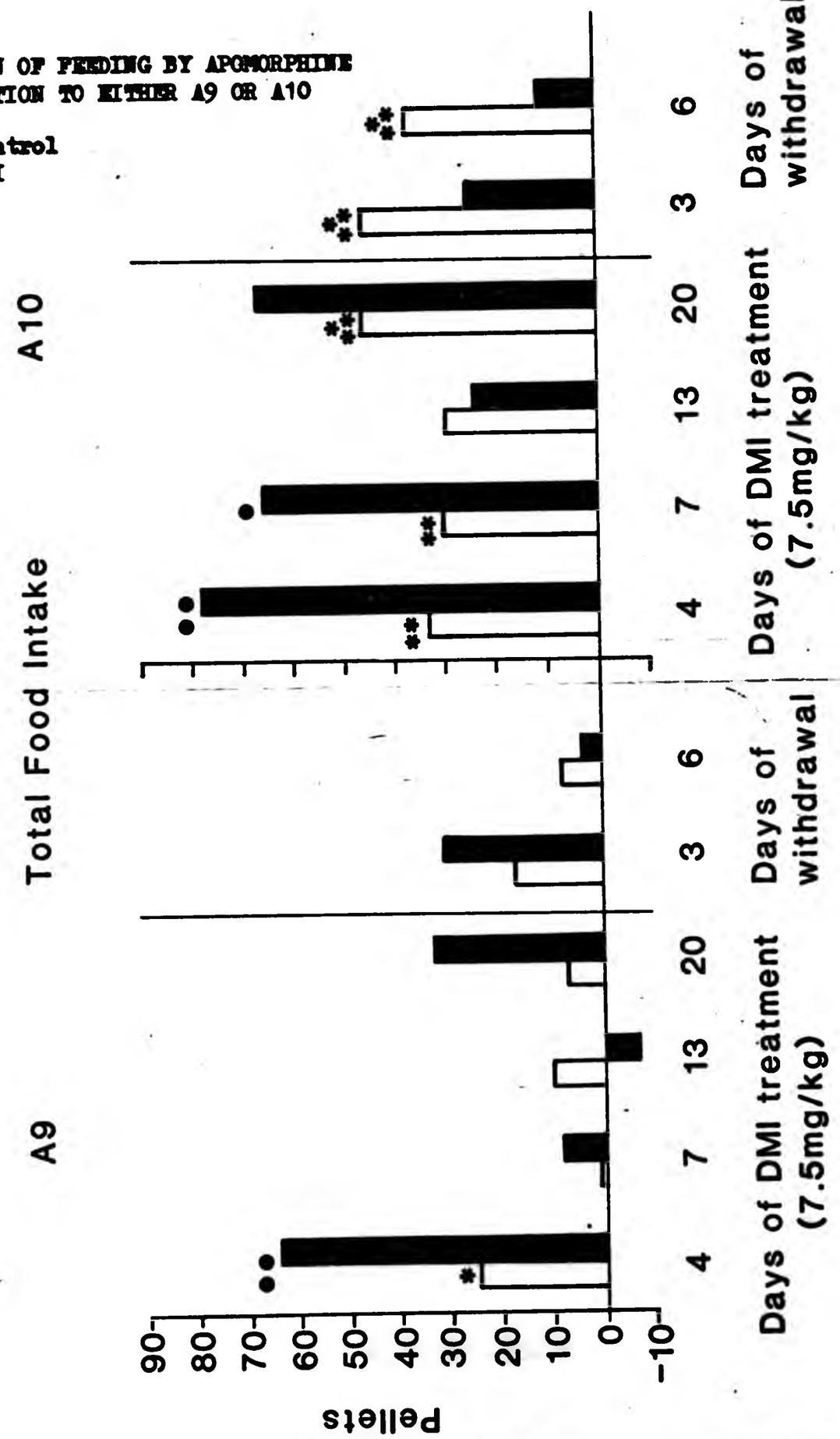
Treatment	C	A	C	C	A	C		C	A	C		C	A	C↓ Withdrawal		C	A	C	C	A	C
Day of DMI treatment	3	4	5	6	7	8		12	13	14		19	20	21		23	24	25	26	27	28
A9 CON n=7		25		1				10				7				17			8		
A9 DMI n=7		64		8				-7				33				31			4		
A10 CON n=7		33		30				29				46				46			37		
A10 DMI n=7		78		66				24				67				25			11		

C = ascorbate control injection
A = apomorphine injection

FIGURE 22A

SUPPRESSION OF FEEDING BY APOMORPHINE ADMINISTRATION TO EITHER A9 OR A10

White = control
Black = DMI



Dots show the effect of DMI pretreatment. Stars show the suppressant effect of apomorphine. One symbol $p<.05$, two symbols $p<.01$, three symbols $p<.001$.

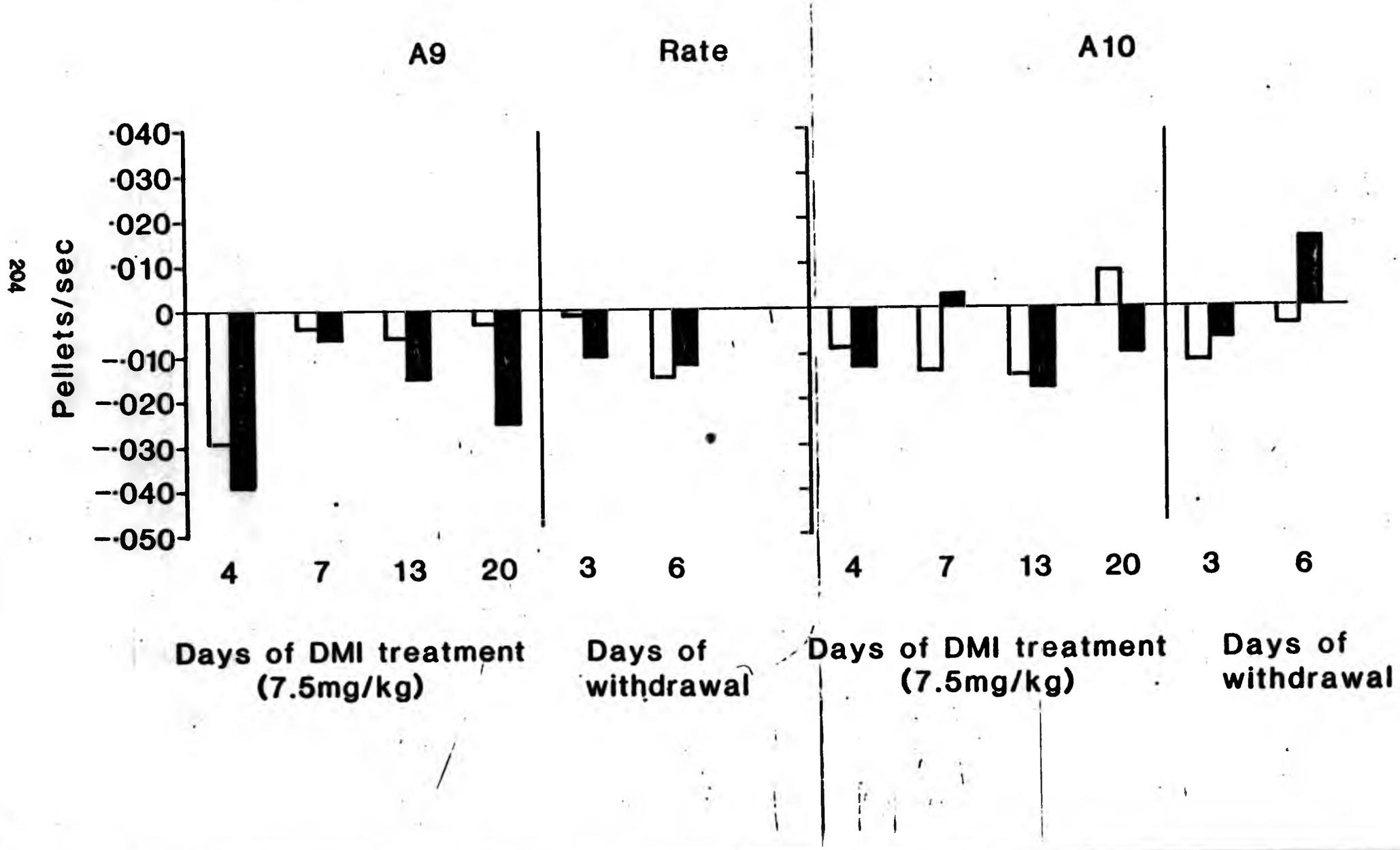

A9
Rate
A10
·040
·030
·020
·010
0
-·010
-·020
-·030
-·040
-·050
Pellets/sec
204
4
7
13
20
3
6
Days of DMI treatment (7.5mg/kg)
Days of withdrawal

FIGURE 22 b

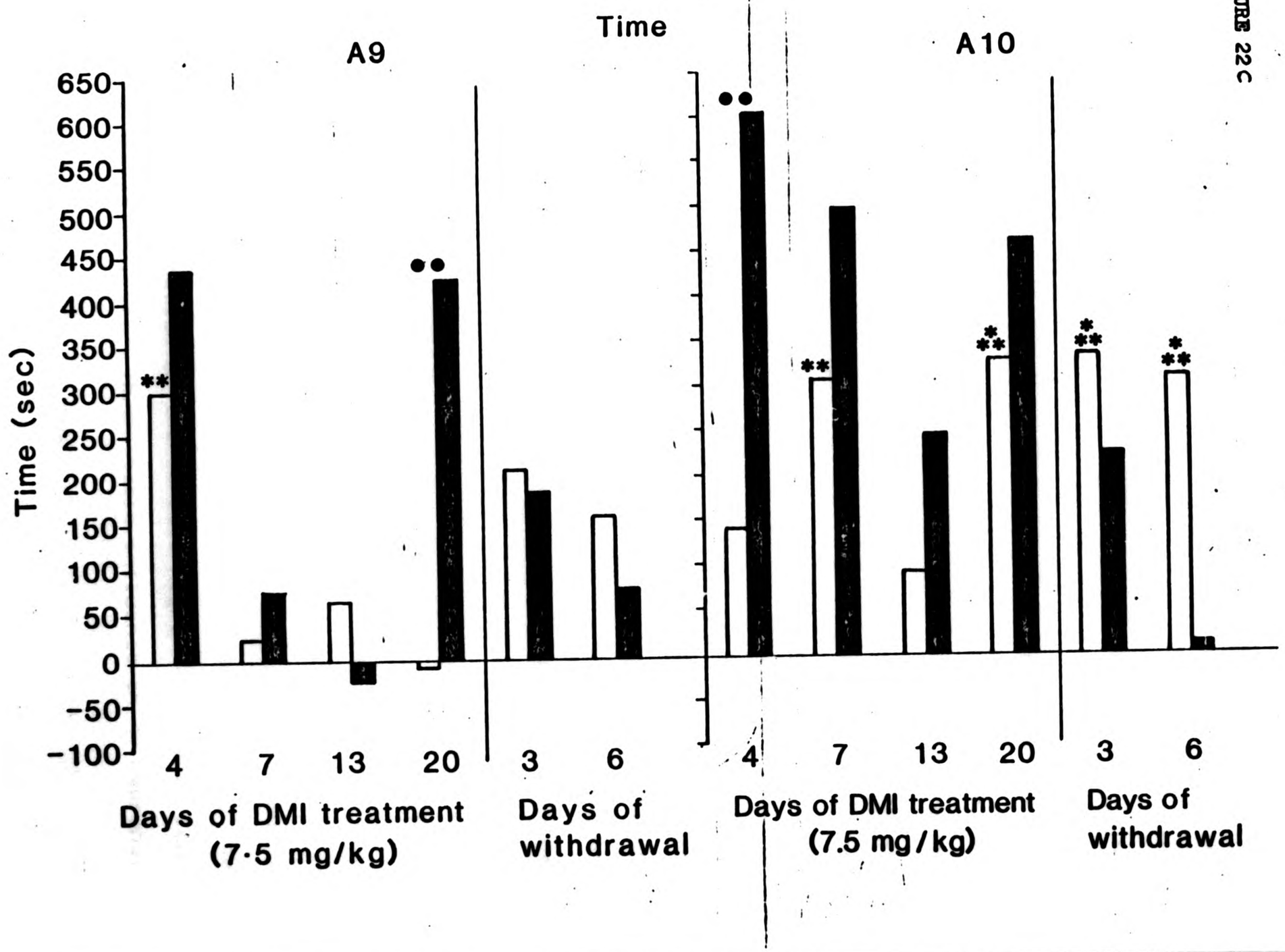

A9
Time
A10
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
Time (sec)
**
••
4
7
13
20
3
6
Days of DMI treatment (7·5 mg/kg)
Days of withdrawal
Days of DMI treatment (7.5 mg/kg)
Days of withdrawal

FIGURE 22C

7.3.4. DISCUSSION

Like the previous experiment, acute pretreatment with DMI significantly enhanced apomorphine anorexia, in both A9 and A10 animals, probably by stimulating presynaptic DA receptors. In the case of A10 animals, this initial increase in stimulation of autoreceptors was associated with an enhancement of the apomorphine-induced decrease in eating time. The apomorphine-induced decrease in eating time has been shown to be reversed by DA antagonists (see chapter 6) and can therefore be used as an index of presynaptic DA receptor function. The initial increase in stimulation in A9 animals is more difficult to characterize as no significant effects were seen on day 4 of treatment in any of the microstructural parameters.

As no reversal of the acute enhancing effects of DMI were seen over chronic treatment, it would appear that there is no evidence for autorecetor desensitization. However, a closer examination of the results from A10 animals showed that during withdrawal from DMI there was an attenuation of apomorphine anorexia and the aomorphine-induced decrease in eating time. These effects during withdrawal from DMI did not reach significance in the main analysis but were confirmed statistically, in a more selective analysis of the chronic component of the experiment.

The result that A10 animals seem to be preferentially affected by apomorphine in comparison to A9 animals, can possibly be explained by the function of the striato-nigral feedback loop which provides an inhibitory homeostatic influence on A9 neurons.

It is quite possible that the behavioural consequence of autoreceptor stimulation could be masked in the nigrostriatal system, but not in the mesolimbic system.

In summary, no clear evidence for autoreceptor subsensitivity during DMI treatment was found in either the A9 or A10 DA systems. However, during withdrawal from DMI, apomorphine anorexia and the apomorphine-induced decrease in eating time were both attenuated in A10 animals. This provides some indication for autoreceptor subsensitivity in the mesolimbic system during withdrawal from DMI.

7.4. GENERAL DISCUSSION

As in the previous experiments of chapter 6, confirmation of a non-dopaminergic mediation of the effects of apomorphine on eating rate was given by both the peripheral and central experiments. In the case of the peripheral experiment, the apomorphine-induced reduction in eating rate was unaffected by DMI. In the case of the central experiment, not only did DMI fail to interact with apomorphine on this measure, but apomorphine failed to lower eating rate. This result is strong evidence for a non-dopaminergic mediation of the apomorphine-induced reduction in eating rate.

Apomorphine administered both peripherally and centrally caused an anorexia that was enhanced by acute DMI pretreatment. As apomorphine anorexia has been shown to be mediated by DA autoreceptors, the mechanism underlying this initial enhancement

of apomorphine anorexia was probably a stimulation of DA autoreceptors. In both experiments, the microstructural parameter which primarily mediated autoreceptor subsensitivity appeared to be eating time, and was shown by the attenuation of the apomorphine-induced decrease in this measure. Evidence that chronic DMI treatment caused a subsensitivity of DA autoreceptors was sometimes seen in the peripheral experiment, and seen even less in the central experiment.

A possible explanation of the inconsistent results in the peripheral experiment is the development of postsynaptic supersensitivity, as 0.06 mg/kg apomorphine might have a postsynaptic action in addition to its presynaptic action. As, in this model, pre and postsynaptic effects work in the same direction, (i.e. they decrease feeding), a postsynaptic supersensitivity could mask presynaptic subsensitivity. To investigate this possibility, a replication of the peripheral experiment was carried out using a lower dose of apomorphine (0.03 mg/kg). The results of this experiment are not presented in full, as they were essentially the same as the experiment using 0.06 mg/kg apomorphine- there was an enhancement of the effects of apomorphine on food intake and eating time by acute DMI, however, there were no clear interactions thereafter. These results suggest that 0.06mg/kg apomorphine has a primarily presynaptic action. The conclusions of the peripheral experiment using 0.06mg/kg apomorphine therefore appear to be valid.

The central experiment did not produce results consistent with the hypothesis of DMI induced autoreceptor subsensitivity. However, on withdrawal from DMI, there was a tendency for DMI to attenuate the apomorphine-induced reductions of both totals and time in A10 animals only. The experiments presented in this chapter extend all previous accounts of autoreceptor subsensitivity following chronic AD treatment in that testing was carried out throughout drug treatment and not just during withdrawal from DMI. In fact, according to Antelman et al (1982), the development of autoreceptor subsensitivity depends simply on the passage of time (approximately 10 days) following acute TAD treatment. In light of the Antelman et al result it is therefore possible that autoreceptor subsensitivity could appear during maintained TAD treatment.

It is of interest to these experiments that Antelman and Chiodo (1984) have reported that immobilization stress can induce the same time dependent subsensitivity of A9 autoreceptors that they had previously reported following ECS (Chiodo and Antelman 1980, see section 1.8.). Given that both s.c. apomorphine injections and i.c. injections both involve immobilisation to some extent and can both be stressful to the animal, these injection procedures could theoretically contribute to autoreceptor subsensitivity.

These two experiments have therefore shown that the phenomenon of AD-induced autoreceptor subsensitivity, as indexed behaviourally, is not a robust effect. This conclusion is consistent with both

the biochemical, electrophysiological and behavioural literature: where some studies support AD-induced autoreceptor subsensitivity (e.g. Serra et al 1979), whilst others do not (Willner 1983c). If AD-induced autoreceptor subsensitivity is to be seriously considered as a possible mechanism of clinical action, it is clear that the exact conditions and extent to which autoreceptor subsensitivity occurs should be more fully elucidated.

CHAPTER EIGHT

CONCLUDING DISCUSSION

8.1. SUMMARY CONCLUSIONS

The major conclusions of the present investigation may be summarised as follows:

(1) At low doses (0.5 mg/kg) amphetamine anorexia appears to have both a beta-adrenergic and dopaminergic component, whilst at higher doses (1.0 mg/kg) amphetamine anorexia appears only to have a DA mediation.

(2) The enhancement of amphetamine anorexia following acute DMI treatment is an artefact of impaired amphetamine metabolism. In consequence, chronic DMI treatment does not just merely compensate for acute beta-receptor stimulation, but will actually over-compensates to reduce beta-receptor function. The implication of this result is that central beta-receptors could be over-stimulated in depression.

(3) The decrease in eating time following a low dose of apomorphine can be used to index presynaptic DA receptor function.

(4) There is some evidence that chronic DMI treatment induces a subsensitivity of preynaptic DA receptors although this effect was not consistently seen during treatment or withdrawal.

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

8.2. AMPHETAMINE ANOREXIA

A catecholaminergic mediation of amphetamine anorexia was shown by the results of the experiments in chapter 4. Microstructural

analysis of amphetamine anorexia showed that suppression of food intake could be accounted for by a dose-dependent decrease in eating time (figure 5C). Reductions in eating time were brought about by dose-dependent decreases in bout length (figure 5D) and an increase in gap length (figure 5C). Amphetamine anorexia was also characterized by an increased eating rate - which could potentially increase food intake.

The amphetamine-induced reductions in total food intake, eating time and bout length and the increase in eating rate were reversed by pimozide pretreatment (figures 8A, C, D and B respectivly). However, the amphetamine-induced increase in gap length, which was seen at a dose of 0.5 mg/kg, was reversed by propranolol pretreatment (figure 7E). These results suggest that both beta-adrenergic and DA mechanisms mediate amphetamine anorexia at low doses (0.5 mg/kg), whilst amphetamine anorexia at high doses (1.0 mg/kg) is mediated primarily by DA .

The above evidence, for a dose-dependent CA mediation of amphetamine anorexia, is consistent with some of the literature which shows that lesions of the VNAB attenuated amphetamine anorexia at low doses but not at higher doses of the drug. Studies employing central drug administration also confirm that amphetamine anotrexia is not mediated solely by DA. Injection of beta-adrenergic receptor blocking drugs in the region of the PFH attenuate the anorexic effect of peripherally and centrally administered amphetamine (see chapter2, section 2.5.2.).

These dose-dependent results of amphetamine are also consistent with Lyon and Robbin's theory of stimulant action, which accounts for amphetamine anorexia in terms of an increased intensity of ongoing behaviour (increased eating rate), within a decreasing number of response cateories (shortening of eating bouts).

8.3. NA AND DEPRESSION

From the evidence cited in chapter 2 and the results of experiment 3, it is likely that low dose (0.5 mg/kg) amphetamine anorexia can be used to index central beta-adrenergic receptor function. When low dose amphetamine anorexia was used as a behavioural assay to study the effects of chronic TAD treatment, attenuation of amphetamine anorexia was observed during withdrawal from DMI or iprindole, but not during the course of chronic treatment (Willner and Montgomery 1980, Willner, Towell and Montgomery 1984, see section 5.2.). These results suggested that the effects of chronic TAD treatment merely compensate for the acute NA-enhancing effects of the drug by inducing a beta-receptor subsensitivity, and by implication, does not cause a functional decrease in beta-receptor function. However, the results obtained using central administration of amphetamine suggest that the enhancement of amphetamine anorexia by acute pretreatment with DMI (and possibly with iprindole) results from inhibition of the peripheral metabolism of amphetamine.. If, as experiment 5 suggests, approximately 75% of the enhancement of amphetamine anorexia following acute DMI treatment is an artefact of impaired amphetamine metabolism, then it follows that

chronic DMI treatment would attenuate amphetamine anorexia during the course of chronic treatment, as well as during withdrawal. An attenuation of amphetamine anorexia following chronic DMI treatment is supported from the finding that mianserin, which does not inhibit amphetamine metabolism, did attenuate amphetamine anorexia during the course of chronic treatment (Willner, Towell and Montgomery 1984).

As amphetamine anorexia has been used and validated as an assay of beta-adrenergic receptor function, the attenuation of anorexia seen following chronic AD treatment is evidence of a reduction in beta-receptor function. However, since an alpha-adrenergic system stimulates feeding, and chronic AD treatment enhances alpha-adrenergic function, changes in amphetamine anorexia could represent an increase in alpha adrenergic function. The results of experiment 6 indeed showed indications of alpha-adrenergic augmentation: the attenuation in amphetamine anorexia following chronic mianserin treatment was partly blocked by the alpha-antagonist phentolamine. It is therefore possible that chronic AD treatment produces changes in both beta- and alpha-adrenergic function, a conclusion consistent with the literature (see section 1.7.).

The evidence cited so far suggests that chronic AD drug treatment reduces central beta-adrenergic function. The inference has been made that central beta-receptors are over-stimulated in depression (e.g. Segal et al 1974). It might therefore be expected that AD which most reduce the sensitivity of beta-

receptors would be the most potent AD clinically. However, this is not the case - there is a highly significant negative correlation between clinical potency and beta-receptor binding (Willner 1984a), i.e. the more potently a drug desensitizes beta-receptors, the less potent it is clinically.

Some hormonal effects on beta-receptors also support the conclusion that beta-receptors desensitization is not responsible for the clinical effects of ADs (Willner 1984a). NA-stimulated c-AMP generation is known to be decreased by cortisol (Mobley and Sulser 1980) and by adrenocorticotrophic hormone (Kendall et al 1982). If antidepressants work by normalizing supersensitive beta-receptors, then depression should be associated with reduced circulating levels of these steriod hormones. However, depressive episodes are accompanied by abnormally elevated pituitary-adrenal activity (Carroll 1982) and cortisol levels (Carroll et al 1976), and there is a high incidence of depression in Cushing's disease, which is known to result from elevated cortisol (Kelly et al 1980). Similarly, beta-receptor responsiveness is lower in females than in males (Sulser and Mishra 1982) and is decreased by estradiol (Biegon et al 1982). However, depression is two to three times more frequent in women than in men (Weissman and Paykel 1974), and symptoms of depression are claimed to have been experienced by approximately 50% of women taking oral contraceptives (Parry and Rush 1979).

In summary, it is suggested that although the desensitization of cortical beta-receptors by AD supports the revised NA hypothesis of depression, this phenomenon is probably not responsible for the therapeutic effects of these drugs.

8.4. APOMORPHINE-ANOREXIA

Low doses of apomorphine thought to be selective for presynaptic dopamine receptors produce a dose-dependent anorexia (see section 6.1.). Microstructural characterization of apomorphine anorexia showed that it was caused by reductions in both eating rate and eating time (figure 4B and C respectively). Non-sedative doses of haloperidol and thioridazine reversed apomorphine anorexia by reversing the apomorphine-induced reductions in eating time (figures 15C and 17C respectively). However, eating rate was unaffected by haloperidol or thioridazine (figure 15B and 17B respectively). These results suggest that the reduction in eating rate following apomorphine is not mediated by dopamine receptors. This conclusion is supported by the results of experiment 10 in which administration of apomorphine into dopamine cell body areas did not reduce eating rate but did reduce eating time.

As the changes in eating time brought about by apomorphine were reversed by DA-receptor antagonists at doses which did not appear to interact with postsynaptic receptor sites, it is concluded that these changes may be mediated by presynaptic dopamine receptors. However, the changes in eating rate brought about by

apomorphine are not reversed by DA-receptor antagonists, and therefore remain to be characterized pharmacologially. Recent work in our laboratory (Towell, Willner and Muscat, unpublished) has shown that the alpha-2 receptor blocker yohimbine mimicked haloperidol in reversing eating time but not eating rate, whilst the serotonin blocker methergoline and the opiate antagonist maloxone did not interact with apomorphine on either measure.

The intercorrelations of the microstructural parameters observed in experiment 7 showed that the apomorphine-induced reduction in eating time was correlated with the reduction in total food intake, whereas the apomorphine-induced reduction in rate was not. Therefore, apomorphine anorexia and, more specifically, the apomorphine-induced reduction in eating time can be used to index presynaptic DA receptor function.

8.5. CHRONIC DMI TREATMENT AND PRESYNAPTIC DOPAMINE RECEPTORS

Apomorphine anorexia was used as an assay of presynaptic DA receptor function during chronic DMI treatment. Enhancement of apomorphine anorexia is likely to represent a stimulation of autoreceptors, whilst an attenuation of apomorphine anorexia is likely to represent a reduction in autoreceptor stimulation, probably through the desensitization of presynaptic DA receptors. With systemic administration of apomorphine acute DMI treatment enhanced apomorphine anorexia and the apomorphine-induced decrease in eating time. It is assumed that these changes in apomorphine anorexia result from the stimulation of autoreceptors, the consequence of which is a lowering DA

transmission (see section 8.4.). Some evidence was seen of pre-synaptic DA receptor subsensitivity following chronic DMI treatment. Autoreceptor subsensitivity was shown by an attenuation of the apomorphine-induced reduction in eating time. However, autoreceptor subsensitivity was not seen consistently throughout chronic treatment and withdrawal, but was seen only on days 8 and 26 of DMI treatment and on day 6 of withdrawal. No effects were seen on the apomorphine-induced reduction in eating rate throughout DMI treatment, a result consistent with this measure not having a presynaptic DA mediation (see section 8.4.).

When apomorphine was applied centrally to DA cell body areas, it produced a similar anorexia to that seen with systemic administration. Acute pretreatment with DMI significantly enhanced apomorphine anorexia. In the case of A10 animals, DMI-induced stimulation of autoreceptors was associated with an enhancement of the apomorphine-induced reduction in eating time. In A9 animals, however, no significant changes were seen in any of the microstructural parameters, making it difficult to account for the enhancement of apomorphine anorexia following acute DMI treatment. Following chronic DMI treatment, there was no significant reversal of the acute enhancing effects of DMI in either A9 or A10 animals. However, there appeared to be some tolerance towards repeated DMI treatment, and in A10 animals there was a tendency towards an attenuation of the apomorphine-induced reduction in totals and eating time following withdrawal from DMI.

In summary, using systemic apomorphine to index presynaptic DA function, there is some evidence for autoreceptor subsensitivity following chronic DMI treatment. This result extends previous accounts which report autoreceptor subsensitivity during withdrawal only. Central administration of apomorphine yielded no clear evidence of autoreceptor subsensitivity during chronic DMI treatment. It would appear therefore that TAD-induced autoreceptor subsensitivity, as indexed behaviourally by apomorphine, is not a robust effect. Therefore, before autoreceptor subsensitivity can be considered as a potential mediator of AD therapy, the exact conditions under which autoreceptor subsensitivity occurs will have to be established.

8.6. THE USE OF BEHAVIOUR TO STUDY BRAIN SYSTEMS

Using biochemical techniques to assess the integrated functioning of synaptic systems is problematic as only a static component of the system is being measured, more often than not in vitro. Biochemical studies do not therefore, represent the functional state of a system. In contrast, electrophysiological recording techniques can be used to measure the functional state of a system. Typically, the method involves recording from an anatomical location within a particular system. These are, however, limitations in the use of electrophysiological recording techniques such as sampling biases and recording artefact. These limitations can be overcome to an extent, by observing changes in behaviour known to be mediated by receptors that input a particular system. It should then be possible to assess the

integrated and dynamic functioning of the system using behaviour.

The experiments in this thesis have made use of behaviour elicited by CA agonist probes to study neural mechanisms of action of antidepressant drugs. For this approach to be effective, it is essential to select agonist-induced behaviours that represent specifically and solely the synaptic mechanisms under investigation. For example, it is a problem if the behaviour under investigation is consequent on post-synaptic receptor stimulation (as amphetamine-induced suppression of food intake seems to be), because any changes in behaviour resulting from presynaptic receptor stimulation are liable to be masked. Therefore, amphetamine-induced-suppression of food intake cannot be used to index presynaptic receptor activity. Similarly, apomorphine-induced suppression of food intake at higher doses of the drug is a resultant of two opposing effects- an inhibitory presynaptic effect and an excitatory postsynaptic effect. Therefore, apomorphine-induced suppression of food intake at these higher doses cannot be used to index presynaptic function as, again, any presynaptic effects will be masked by postsynaptic effects. However, the behaviour resulting from low doses of apomorphine, can be used to index presynaptic activity at doses which are specific for presynaptic receptors and do not interact with postsynaptic receptors sites.

Often, however, drugs act at a multiplicity of receptor types. This can and does make interpretation of behavioural experiments

difficult at times. These data do, however, have the advantage that they address the major issue - that of integrated synaptic functioning. Finally, in the context of psychopathology, behaviour is what we want to understand.

ABBREVIATIONS

ACh	Acetylcholine
AD	Antidepressant drug
cAMP	cyclic-Adenosine monophosphate
AMPT	Alpha-methyl-paratyrosine
CA	Catecholamine(s)
CNS	Central nervous system
COMT	Catechol-3-O-methyl-transferase
CRF	Continuous reinforcement
CSF	Cerebrospinal fluid
CTT	Central tegmental tract
DBEE	Dorsal bundle extinction effect
DBH	Dopamine-beta-hydroxylase
DMI	Desmethylimipramine
DNAB	Dorsal noradrenergic bundle
DOPAC	Dihydroxyphenylacetic acid
ECS	Electroconvulsive shock
ECT	Electroconvulsive treatment
EEG	Electroencephalogram
5-HIAA	5-Hydroxyindole acetic acid
5-HT	5-Hydroxytryptamine
5-HTP	5-Hydroxy-L-tryptophan
HVA	Homovanillic acid
IRT	Inter-response time
LH	Lateral hypothalamus
LSA	Log survivor analysis
LSD	Lysergic acid diethylamide
MAO	Monoamine oxidase inhibitors
MHPG	3-Methoxy-4-hydroxyphenylglycol
5-MEODMT	5-Methoxy-N,N-dimethyltryptamine
mg/kg	milligrams per kilogram
MSA	Microstructural analysis
NA	Noradrenaline
6OHDA	6-hydroxydopamine
PCPA	Para-chlorophenylalanine
PFH	Perifornical hypothalamus
PVN	Paraventricular nucleus
REM	Rapid eye movement
TAD	Tricyclic antidepressant
VMA	3-Methoxy-4-hydroxymandelic acid
VMH	Ventromedial hypothalamus
VNAB	Ventral noradrenergic bundle

REFERENCES

Ahlskog, J.E. (1974). Food intake and amphetamine anorexia after selective forebrain norepinephrine loss. Brain Res. 82, 211-240.

Ahlskog, J.E.,P.K.Randell and B.G.Hoebel. (1975). Hypothalamic hyperphagia: dissociation from hyperphagia following destruction of noradrenergic neurons. Science, 399-401.

Akiskal, H.S. (1980). External validating criteria for psychiatric diagnosis: Their application in affective disorders. J. Clin. Psychiat. 41:12, Sec. 2, 6-15.

Akiskal, H.S., Rosenthal, R.H., Rosenthal, T.L., Kashgarian, M., Khani, M.K., and Puzantian, V.H. (1979). Differentiation of primary affective illness from situational, symptomatic and secondary depressions. Arch. Gen. Psychiat. 36, 635-643.

American Psychiatric and Association (1980). DSM III - Diagnostic and Statistical Manual of Psychiatric Disorders, 3rd Edition A.P.A., Washington.

Anand, B.K., and Brobeck, J.R. (1951). Hypothalamic control of food intake in rats and cats. Yale J. Biol. Med. 24, 123-140.

Antelman, S.M., and Chiodo, L.A. (1984). Stress-induced sensitization: a framework for viewing the effects of ECS and other antidepressants. In ECT: Basic Mechanisms, B. Lerer, R.D. Weiner and R.H. Belmaker (Eds.), pp. 28-32. John Libbey, London.

Antelman, S.M., Chiodo, L.A., and Degiovanni, L.A. (1982). Antidepressants and dopamine autoreceptors: implications for both a novel means of treating depression and understanding bipolar illness. In Typical and Atypical Antidepressants: Molecular Mechanisms, E. Costa and G. Racagni (Eds.), pp. 121-132.

Aprison, M.H., Hintgen, J.N., and Nagayama, H. (1982). Testing a new theory of depression with an animal model: Neurochemical-behavioural evidence for postsynaptic serotonergic receptor involvement. In New Vistas in Depression, S.Z. Langer, R. Takahashi, T. Segawa and M. Briley (Eds.). Pergamon Press, New York, pp. 171-178.

Asberg, M., Thoren, P., Traskman, L., Bertilsson, L., and Ringleerger, N. (1976). 'Serotonin depression' - a biochemical subgroup within the afective disorders. Science 191, 478.

Ashcroft, G.W., Crawford, T.B.B., Eccleston, D., Sharman, D.F., McDougall, E.J., Stanton, J.S., and Binns J.K. (1966). 5-Hydroxyindole compounds in the cerebrospinal fluid of patients with psychiatric or neurological diseases. Lancet ii, 1049.

Ashcroft, G.W., Blackburn, I.M., Eccleston, D., Glen, A.I.M., Hartley, W., Kinlock, N.E., Lonergan, N., Murray, L.G., and Pullar, I.A. (1973). Changes on recovery in the concentration of tryptophan and the biogenic amine metabolites in the cerebrospinal fluid of patients with affective illness. Psychol. Med. 3, 319.

Aylward, M., Maddock, J., Dewland, P.M., and Lewis, P.A. (1980). Sulpiride in depressive illness. A controlled clinical comparison of sulpiride and amitriptyline in patients with primary depressive illness. The Amsterdam Symp. on Depressive Illness, September.

Balsara, J.J., Bapat, T.R., Gada, V.P., and Chandorkar, A.G. (1982). Small Doses of Apomorphine Induce Catalepsy and Antagonize Methampetamine Stereotypy in Rats. Psychopharmacology 78, 192-194.

Banerjee, S.P., Kung, L.S., Riggi, S.J., and Chanda, S.K. (1977). Development of beta-adrenergic subsensitivity by antidepressants. Nature 268, 455-456.

Banki, C.M. (1977). Correlation between CSF metabolites and psychomotor activity in affective disorders. J. Neurochem. 28, 255-257.

Barbaccia, M.L., Brunello, N., Chuang, D.M., and Costa, E. (1983). On the mode of action of imipramine: Relationship between serotonergic axon terminal function and down-regulation of beta-adrenergic receptors. Neuropharmacol. 22, 373-383.

Bayliss, L.E. (1966). Living Control Systems. English Universities Press, London.

Beck, A.T. (1983). Cognitive therapy of depression: New perspectives. In Treatment of Depression: Old Controversies and New Approaches, P.J. Clayton and J.E. Barrett (Eds.), pp. 265-290. Raven Press, New York.

Becker, J. (1979). Vulnerable self-esteem as a predisposing factor in depressive disorders. In The Psychobiology of the Depressive Disorders: Implications for the Effects of Stress, R.A. Depue (Ed.), pp. 317-334. Academic Press, New York.

Beckman, H., and Goodwin, F.K. (1975). Antidepressant response to tricyclics and urinary MHPG in unipolar patients: Clinical response to imipramine or amitryptiline. Arch. Gen. Psychiat. 32, 17-21.

Beckman, H., and Goodwin, F.K. (1980). Urinary MHPG in subgroups of depressed patients and normal controls. Neuropsychobiol. 6, 91-100.

Beninger, R.J. (1983). The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6, 173-196.

Bentall, A.C., and Herberg, L.J. (1980). Blockade of amphetamine-induced locomotor activity and stereotypy in rats by spiroperidol but not by an atypical neuroleptic, thioridazine. Neuropharm. 19, 699-703.

Berger, P.A., Faull, K.F., Kilkowski, J., Anderson, P.J., Kraemer, H., Davis, K.L., and Barchas, J.D. (1980). CSF monoamine metabolites in depression and schizophrenia. Amer. J. Psychiat. 137, 174-180.

Bergstrom, D.A., and Kellar, K.J. (1979). Effect of electroconvulsive shock on monoaminergic receptor receptor binding sites in rat brain. Nature 278, 464-466.

Bertilsson, L., Asberg, M., Lantto, O., Scalia-Tomba, G.P., Traskman-Bendz, L., and Tybring, G. (1982). Gradients of monoamine metabolites and cortisol in cerebrospinal fluid of psychiatric patients and healthy controls. Psychiat. Res. 6, 77-83.

Bevan, P., Bradshaw, C.M., and Szabadi, E. (1975). Effects of iprindole on responses of single cortical and caudata neurones to monoamines and acetylcholine. Brit. J. Pharmacol. 55, 17-25.

Bhavsar, V.H., Dhumal, V.R., and Kelkar, V.V. (1981). The effect of some anti-epilepsy drugs on enhancement of the monoamine-mediated behavioural responses following the administration of electroconvulsive shocks to rats. Europ. J. Pharmacol. 74, 243-247.

Bhavsar, V.H., Dhumal, V.R., and Kelkar, V.V. (1983). The effect of estradiol on the alterations in monoamine-mediated behavioural responses induced by administration of electroconvulsive shocks of imipramine to female rats. Neuropharmacol. 22, 751-756.

Biegon, A., Snyder, L., and McEwen, B.S. (1982). Effect of chronic treatment with ovarian steroids on neurotransmitter receptors in the rat brain. Soc. Neurosci. Abstr. 8, 648.

Blier, P., and De Montigny, C. (1982). Effects of repeated zimelidine administration on serotonergic neurotransmission: Single-cell studies in the rat. Soc. Neurosci. Abstr. 8, 486.

Blombery, P.A., Kopin, I.J., Gordon, E.K., Markey, S.P., and Ebert, M.H. (1980). Conversion of MHPG to vanillylmandelic acid: Implications for the importance of urinary MHPG. Arch. Gen. Psychiat. 37, 1095-1098.

Blundell, J.E. (1981). Bio-grammar of feeding: pharmacological manipulations and their interpretations. In Theory in Psychopharmacology, Vol. 1, S.J. Cooper (Ed.). Academic Press, London, pp 233-275.

Blundell, J.E., and Burridge, S.L. (1979). Control of feeding and the psychopharmacology of anorexic drugs. In Obesity and its treatment, J. Munro (Ed.). MTP Press, Lancaster.

Blundell, J.E., and Latham, C.J. (1978). Pharmacological manipulation of feeding behaviour: possible influences of serotonin and dopamine on food intake. In Central mechanisms of anorectic drugs, S. Garattini and R. Samanin (Eds.). New York: Raven Press, pp. 83-109.

Blundell, J.E., and Latham, C.J. (1980). Characterisation of adjustments to the structure of feeding behaviour following pharmacological treatment: Effects of amphetamine and fenfluramine and the antagonism produced by pimozide and methergoline. Pharmacol. Biochem. Behav. 12, 717-722.

Blundell, J.E., and Latham, C.J. (1979). Pharmacology of food and water intake. In Chemical Influences on Behaviour, K. Brown and S.J. Cooper (Eds.). Academic Press, pp. 201-249.

Blundell, J.E., and McArthur, R.A. (1981). Behavioural flux and feeding: Continuous monitoring of food intake and food selection, and the video-recording of appetitive and satiety sequences for the analysis of drug action. In Anorectic Agents: Mechanisms of Action and Tolerance, S. Garattini and R. Samanin (Eds.). Raven Press, New York, pp 19-43.

Booth, D.A. (1967). Localization of the adrenergic feeding system in the rat diencephalon. Science 158, 515-517.

Booth, D.A. (1968). Mechanism of action of norepinephrine in eliciting an eating response on injection into the hypothalamus. J. Pharmacol. Exp. Ther. 160, 336-348.

Booth, D.A. (1972). Some characteristics of feeding during streptozotocin-induced diabetes in the rat. J. comp. physiol. Psychol. 80, 238-249.

Booth, D.A., and Pain, J.F. (1970). Effects of a single insulin injection on approaches to food and on the temporal pattern of feeding. Psychon. Sci. 21, 17-19.

Bradshaw, C.M., Roberts, M.H.T., and Szabadi, E. (1974). Effects of imipramine and desipramine on responses of single cortical neurones to noradrenaline and 5-hydroxytryptamine. Brit. J. Pharacol. 52, 349-358.

Brobeck, J.R. (1946). Mechanisms of the development of obesity in animals with hypothalamic lesions. Psychol. Rev. 26, 541-559.

Brown, G.W., and Harris, T. (1978). Social Origins of Depression. Tavistock Publications, London.

Brown, J., Doxey, J.C., and Handley, S. (1980). Effects of alpha-

adrenoceptor agonists and antagonists and of antidepressant drugs on pre- and postsynaptic alpha-adrenoceptors. Europ. J. Pharmacol. 67, 33-40.

Brownstein, J.M., Palkovits, M., Tappaz, M.L., Saavedra, J.M. and Kizer, J.S. (1976). Effect of surgical isolation of the hypothalamus on its neurotransmitter content. Brain Res. 117, 287-295.

Bunney,B.S. and Aghajanian.G.K. (1976). D-amphetamine-induced inhibition of central dopaminergic neurons: mediation by a striato-nigral feedback pathway, Science, 192, 391-393.

Bunney, W.E. (1978). Psychopharmacology of the switch process in affective illness. In Psychopharmacology: A Generation of Progress, M.A. Lipton, A. DiMaschio and K.F. Killam (Eds.). Raven Press, New York, pp. 1249-1259.

Bunney, W.E., and Davis, J.M. (1965). Norepinephrine in depressive reactions. Arch. Gen. Psychiat. 13, 483-494.

Burns, D.B., and Mendells, J. (1979). Serotonin and affective disorders. In W.B.Essman and L.Valzelli (Eds.). Current Developments in Psychopharmacology, vol. 5, pp 293-359. Spectrum Press, New York.

Burridge, S.B., and Blundell, J.E. (1979). Amphetamine anorexia: antagonism by typical but not atypical neuroleptics. Neuropharm. 18, 453-457.

Burton, M.J., Cooper, S.J. and Popplewell, D.A. (1981). The effect of fenfluramine on the microstructure of feeding and drinking in the rat. Br. J. Pharmac.72, 621-633.

Buus Lassen, J. (1978). Potent and long-lasting potentiation of two 5-hydroxytryptophan-induced effects in mice by three selective 5-HT uptake inhibitors. Europ. J. Pharmacol. 47, 351-358.

Carlsson, A. (1961). Brain monoamines and psychotropic drugs. Neuropsychopharmacology 2, 417-421.

Carlsson, A. (1970). Amphetamine and brain catecholamines. In International Symposium on Amphetamines and Related Compounds, E. Costa and S. Garattini (Eds.). Raven, New York, pp. 289-300.

Carlsson, A. (1975). Dopaminergic autoreceptors. In Chemical tools in catecholamine research, Vol. II, O. Almgren, A. Carlsson and L. Engel (Eds.). Marcel Dekker, New York, pp. 49-65.

Carlsson, A., and Lindqvist, M. (1967). Metatyrosine as a tool for selective protection of catecholamine stores against reserpine. Eur. J. Pharmacol. 2, 187-192.

Carlsson, A., and Lindqvist, M. (1978). Effects of antidepressant agents on the synthesis of brain monoamines. J. Neur. Trans. 43, 73-91.

Carlsson, A., Fuxe, K., Hamburger, B., and Lindqvist, M. (1966). Biochemical and histochemical studies on the effect of imipramine-like drugs and (+) amphetamine on central and peripheral catecholamine neurons. Acta Physiol. Scand. 67, 481-497.

Carlsson, A., Corrodi, H., Fuxe, K., and Hokfelt, T. (1969). Effects of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-alpha-ethyl-m-tyramine. Europ. J. Pharmacol. 5, 357-366.

Carroll, B.J. (1982). The dexamethasone suppression test for melancholia. Brit. J. Psychiat. 140, 292-304.

Charney, D.S., Heninger, G.R. and Sternberg, D.E. (1983). Alpha-2 adrenergic receptor sensitivity and the mechanism of action of antidepressant therapy: The effect of long-term amitryptiline treatment. Brit. J. Psychiat. 142, 265-275.

Charney, D.S., and Nelson, J.C. (1981). Delusional and nondelusional unipolar depression: Further evidence for distinct subtypes. Am. J. Psychiat. 138, 328-333.

Chiodo, L.A., and Antelman, S.M. (1980a). Electroconvulsive shock: progressive dopamine autoreceptor subsensitivity independent of daily drug treatment. Science 210, 799-801.

Chiodo, L.A., and Antelman, S.M. (1980b). Repeated tricyclic antidepressants induce a progressive 'switch' in the electrophysiological response of dopamine neurons to autoreceptor stimulation. Europ. J. Pharmacol. 66, 255-256.

Chiodo, L.A., and Antelman, S.M. (1980c). Repeated tricyclic induce a progressive dopamine autoreceptor subsensitivity independent of daily drug treatment. Nature 287, 451-454.

Chiodo, L.A., and Bunney, B.S. (1982). Effects of chronic neuroleptic treatment on nigral dopamine cell activity. Soc. Neurosci. Abstr. 8, 482.

Chiodo, L.A., and Bunney, B.S. (1983). Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. J. Neurosci. 3, 1607-1619.

Consolo, S., Dolfina, E., Garattini, S., and Valzelli, L. (1967). Desipramine and amphetamine metabolism. J. Pharm. Pharmacol. 19, 253-256.

Cooper, S.J., and Francis, R.L. (1979). Feeding parameters with two food textures after chlordiazepoxide administration, alone

or in combination with d-amphetamine or fenfluramine. Psychopharamcology 62, 253-259.

Cooper, S.J., and Francis, R.L. (1980). Interactions of chlordiazepoxide and anorectic agents on rate and duration parameters of feeding in the rat. Psychopharmacology 69, 261-265.

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

Coppen, A., Shaw, D.M. and Farrell, J.P. (1963). Poentiation of the antidepressant effect of a monoamine-oxidase inhibitor by tryptophan. Lancet 1, 90-90.

Costall, B., and Naylor, R.J. (1975). Detection of the neuroleptic properties of clozapine, sulpiride and thioridazine. Psychopharmacology 43, 69-74.

Costall, B., Naylor, R.J., and Nohria, V. (1980). On the ability of apomorphine derivatives to reduce spontaneous locomotor activity in mice. Neurosci. Lett. suppl. 5:S, 462.

Costall, B., Lim, S.K., and Naylor, R.J. (1981). Characterisation of the mechanisms by which purported dopamine agonists reduce spontaneous locomotor activity of mice. Eur. J. Pharmacol. 73, 175-188.

Crane, G.E. (1957). Iproniazid (Marsilid) phosphate, a therapeutic agent for mental disorders and debilitating diseases. Psychiat. Res. Rep. Amer. Psychiat. Ass. 8, 142-152.

Creese, I. (1982). Dopamine receptors explained. Trends in Neuroscience 5, 40-43.

Creese, I.,Burt, D.R. and Snyder, S.H. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192: 481-483.

Curzon, G., Gumpert, E.J.W., and Sharpe, D.M. (1971). Amine metabolites in the lumbar cerebrospinal fluid of humans with restricted flow of cerebrospinal fluid. Nature (New Biol.) 231, 189.

Curzon, G., Kantamaneni, B.D., Van Boxel, P., Gillman, P.K., Bartlett, J.F., and Bridges, P.K. (1980). Substances related to 5-hydroxytryptamine in plasma and in lumbar and ventricular fluids of psychiatric patients. Acta Psychiat. Scand. 61, Suppl. 280, 3-20.

Dahlstrom, A., and Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system - I. Demonstration of monoamines in the cell bodies of brain stem neurones. Acta Physiol. Scand. 62, Suppl. 232, 1-55.

Davitz, J. (1969). The Language of Emotion. Academic Press, New York.

De Castro, J.M. (1981). The stomach energy content governs meal patterning in the rat. Physiol. Behav. 26, 795-798.

De Montigny, C. (1980). Electroconvulsive shock treatment increases responsiveness of forebrain neurons to serotonin: A microiontophoretic study in the rat. Soc. Neurosci. Abstr. 6, 453.

De Montigny, C. (1981). Enhancement of the 5-HT neurotransmission by antidepressant treatments. J. Physiol. (Paris) 77, 455-461.

De Montigny, C., and Aghajanian, G.K. (1978). Tricyclic antidepressants: Long-term treatment increases responsivity of rat forebrain neurons to serotonin. Science 202, 1303-1306.

De Montigny, C., Blier, P., Caille, G., and Kouassi, E. (1981). Pre- and postsynaptic effects of zimelidine and norzimelidine on the serotonergic system: Single cell studies in the rat. Acta Psychiat. Scand. 63, Supple. 290, 79-90.

Delina-Stula, A., and Vassout, A. (1979). Modulation of dopamine-mediated behavioural responses by antidepressants: effects of single and repeated treatment. Europ. J. Pharmacol. 58, 443-451.

Delina-Stula, A., Hauser, K., Baumann, P., Olpe, H.R., Waldmeier, P., and Storni, A. (1982). Stereospecificity of behavioural and biochemical responses to oxaprotiline - a new antidepressant. In Typical and Atypical Antidepressants: Molecular Mechanisms, E. Costa and G. Racagni (Eds.), pp. 265-275. Raven Press, New York.

Depue, R.A., and Monroe, S.M. (1979). The unipolar-bipolar distinction in the depressive disorders: implications for stress-onset interactions. In The Psychobiology of the Depressive Disorders: Implications for the Effects of Stress, R.A. Depue (Ed.), pp. 23-53. Academic Press, New York.

DiChiara, G., Porceddu, M.L., Vargiu, L., Argiolas, A., and Gessa, G.L. (1976). Evidence for dopamine receptors mediating sedation in the mouse brain. Nature 264, 564-566.

Dobrzanski, S., and Doggett, N.S. (1979). The effect of propranolol, phentolamine and pimozide on drug-induced anorexia in the mouse. Psychopharmacology 66, 297-300.

Drew, G.M., Gower, A.J., and Marriott, A.S. (1979). Alpha-2-Adrenoceptors mediate clonidine-induced sedation in the rat. Brit. J. Pharmacol. 67, 133-141.

Dunnett, S.B., Tarbuck, A., Bevan, R., and Winn, P. (1983).

Regulatory impairments following ibotenic acid lesions of the lateral hypothalamus: a comparison with the classic electrolytic lesion syndrome. British Feeding Group Conference, Oxford University, 15 April 1983.

Ebert, M., and Kopin, I.J. (1975). Origins of urinary catecholamine metabolites. Differential labeling by dopamine C14. Trans. Ass. Am. Physns. 28, 256-262.

Falck, B., Hillarp, N.A., Thieme, G., and Torp, A. (1962). Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytochem. 10, 348-354.

Foulds, G.A. (1975). The relationship between the depressive illnesses. Brit. J. Psychiat. 123, 531-533.

Fog, R., and Pakkenburg, H. (1971). Behavioural effect of dopamine and p-hydroxyamphetamine injected into corpus striatum of rats. Exp. Neurol. 31, 75-86.

Frazer, A., and Mendels, J. (1977). Do tricyclic antidepressants enhance adrenergic transmission? Am. J. Psychiat. 134, 1040-1042.

Frey, H., and Schulz, S. (1973). On the central mediation of anorexic drug effects. Biochem. Pharmac. 22, 3041-3049.

Fuxe, K., Ogren, S.O., Agnati, L.F., Andersson, K., and Eneroth, P. (1982a). Effects of subchronic antidepressant drug treatment on central serotonergic mechanisms in the male rat. In Typical and Atypical Antidepressants: Molecular Mechanisms, E. Costa and G. Racagni (Eds.). Raven Press, New York, pp. 91-107.

Fuxe, K. Ogren, S.O., Agnati, L.F., Andersson, K., and Eneroth, P. (1982b). On the mechanism of action of antidepressant drugs: Indications of reductions in 5-HT neurotransmission in some brain regions upon subchronic treatment. In New Vistas in Depression, S.Z. Langer, R. Takahashi, T. Segawa and M. Briley (Eds.). Pergamon Press, New York, pp. 49-63.

Gallager, D.W., and Bunney, W.E. (1979). Failure of chronic lithium treatment to block tricyclic antidepressant-induced 5-HT supersensitivity. Naunyn-Schmiedebergs Arch. Pharmacol. 307, 129-133.

Gershon, E.S., Targum, S.D., and Leckman, J.F. (1979). Platelet monoamine oxidase (MAO) activity and genetic vulnerability to bipolar (BP) affective illness. Psychopharmacol. Bull. 15, 27-30.

Glassman, A.H. (1969). Indoleamines and affective disorders. Psychosom. Med. 2, 107-114.

Glowinski, J., and Axelrod, J. (1964). Inhibition of the uptake of tritiated-noradrenaline in the intact rat brain by

imipramine and structurally related compounds. Nature 204, 1318-1319.

Gluckman, M.I., and Baum, T. (1969). The pharmacology of iprindole, a new antidepressant. Psychopharmacol. 15, 169-185.

Gold, R.M. (1973). Hypothalamic obesity: the myth of the ventromedial nucleus. Science 182, 488-490.

Gold, R., Morgenstern, R., and Fink, H. (1980). Effects of atypical antidepressants on LSD potentiated apomorphine hypermotility in rats. Acta Biol. Med. Ger. 39, 917-921.

Goldstein, M., Lew, J.Y., Matsumoto, Y., Hokfelt, T. and Fuxe, K. (1978). Localization of function of PNMT in the CNS. In Psychopharmacology: A Generation of Progress, M.A. Lipton, A. DiMascio and K.F. Killman (Eds.). Raven Press, New York, p.261-269.

Goldman, H.W., Lehr, D., and Friedman, E. (1971). Antagonistic effects of alpha- and beta-adrenergically coded hypothalamic neurons on consummatory behaviour in the rat. Nature (London) 231, 453-455.

Goodwin, F.K. (1972). Behavioural effects of L-dopa in man. In: R.I. Shader (Ed.). Psychiatric Complications of Medical Drugs, pp 149-174.Raven Press, New York.

Goodwin, F.K., Ebert, M.H., and Bunney, W.E. (1972). Mental effects of reserpine in man: a review. In: R.I.Shader (Ed.). Psychiatric Complications of Medical Drugs, pp 73-101.

Goodwin, F.K., and Post, R.M. (1973). The use of probenecid in high doses for the estimation of central serotonin turnover in affective illness and addicts on methadone. In Serotonin and behaviour, J. Barchas and E. Usdin (Eds.). Academic Press, New York, p. 469.

Goodwin, F.K., Murphy, D.L., Brodie, H.K.H., and Bunney, W.E., Jr. (1970). L-Dopa, catecholamines and behaviour: a clinical and biochemical study in depressed patients. Biol. Psychiat. 2, 341-366.

Goodwin, F.K., Post, R.M., Dunner, D.L., and Gordon, E.K. (1973). Cerebrospinal fluid amine metabolites in affective illness: The probenecid technique. Am. J. Psychiat. 130, 73-79.

Green, A.R., Costain, D.W., and Deakin, J.F.W. (1980). Enhanced 5-hydroxytryptamine and dopamine-mediated behavioural responses following convulsions. III. The effects of monoamine antagonists and synthesis inhibitors on the ability of electroconvulsive shock to enhance responses. Neuropharmacol. 19, 907-914.

Grossman, S.P. (1960). Eating and drinking elicited by direct

adrenergic or cholinergic stimulation of hypothalamus. Science 132, 301-302.

Grossman, S.P. (1962a). Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. Am. J. Physiol. 202, 872-882.

Grossman, S.P. (1962b). Effects of adrenergic and cholinergic blocking agents on hypothalamic mechanisms. Am. J. Physiol. 202, 1230-1236.

Grossman, S.P., Dacey, D., Halaris, A.E., Collier, T., and Routtenberg, A. (1978). Aphagia and adipsia after preferential destruction of nerve cell bodies in hypothalamus. Science 202, 537-539.

Gudeman, J.E., Schatzberg, A.F., Samson, J.A., Orsulak, P.J., Cole, J.O., and Schildkraut, J.J. (1982). Towards a biochemical classification of depressive disorders, VI: Platelet MAO activity and clinical symptoms in depressed patients. Am. J. Psychiat. 139, 630-633.

Harrison-Read, P.E. (1980. Behavioural evidence for increased dopaminergic activity after long-term lithium pretreatment in rats. IRCS Med. Sci. 8, 313.

Herberg, L.J., and Wishart, T.B. (1980). Self-stimulation and locomotor changes indicating "Latent" anticholinergic activity by an atypical neuroleptic, (thioridazine). Neuropharmacology 20, 55-60.

Hernandez, L., and Hoebel, B. (1982). Overeating after midbrain 6-OHDA: Prevention by central injection of selective CA re-uptake blockers. Brain Res. 245, 333-343.

Hetherington, A.N., and Ranson, S.W. (1942). The spontaneous activity and food intake of rats with hypothalamic lesions. Am. J. Physiol. 136, 609-617.

Hjorth, S., Carlsson, A., Clark, D., Svensson, D., Wikstrom, H., Sanchez, D., Lindberg, P., Hacksell, U., Arvidsson, L-E., Johansson, A., and Nilsson, J.L.G. (1982). Pharmacological manipulation of central dopamine (DA) autoreceptors - biochemical and behavioural consequences. In Dopamine receptor agonists, A. Carlsson and J.L.G. Nilsson (Eds.). Acta Pharmaceutica Suecica Suppl. 1983:1. Swedish Pharmaceutical Press, Stockholm, 130-137.

Hoebel, B.G. (1977). The psychopharmacology of feeding. In Handbook of Psychopharmacology, Vol. 8, Drugs, Neurotransmitters, and Behavior, L.L. Iversen, S.D. Iversen, and S.H. Snyder (Eds.). Plenum, New York, pp. 55-129.

Hokfelt, T., Fuxe, K., Goldstein, M., and Johansson, O. (1974). Immunohistochemical evidence for the existence of adrenaline

neurons in the rat brain. Brain Res. 66, 235-251.

Hokfelt, T., Elde, R., Fuxe, K., Johansson, O., Ljungdahl, A., Goldstein, M., Luft, R., Efendic, S., Nilsson, G., Terenius, L., Ganten, D., Jeffcoate, S.L., Rehfeld, J., Said, S., Perez de la Mora, M., Possani,L., Tapia, R., Teran, L., and Palacios, R. (1978). Aminergic and peptidergic pathways in the nervous system with special reference to the hypothalamus. In The Hypothalamus, S. Reichlin, R.J. Balderssarini, and J.B. Martin (Eds.). Raven, New York, pp. 69-135.

Holcomb, H.H., Bannon, M.J., and Roth, R.H. (1982). Striatal dopamine autoreceptors uninfluenced by chronic administration of antidepressants. Europ. J. Pharmacol. 82, 173-178.

Hornykiewicz, O. (1966). Dopamine (3-hydroxytyramine) and brain function. Pharmacol. Rev. 18, 925-964.

Hsiao, S., Wang, C.H., and Schallert, T. (1979). Cholecystokinin, meal pattern, and the intermeal interval: Can eating be stopped before it starts? Physiology and Behaviour 23, 909-914.

Huang, Y.H. (1979). Chronic desipramine treatment increases activity of noradrenergic postsynaptic cells. Life Sci. 25, 709-716.

Huang, Y.H., Maas, J.W., and Hu, G. (1980). The time course of noradrenergic pre- and post-synaptic activity during chronic desipramine treatment. Europ. J. Pharmacol. 68, 41-47.

Hytell, J. (1978). Inhibition of [3H] dopamine accumulation in rat striatal synaptosomes by psychotropic drugs. Biochem. Pharmacol. 27, 1063-1068.

Iversen, S.D., and Koob, G.F. (1977). Behavioural implications of dopaminergic neurons in the mesolimbic system. In Advances in Biochemistry and Psychopharmacology, Vol. 16, E. Costa and G.L. Gessa (Eds.). Raven Press, New York, pp. 209-214.

Jenner, P., and Marsden, C.D. (1982). The mode of action of sulpiride as an atypical antidepressant agent. In Typical and Atypical Antidepressants: Clinical Practice, E. Costa and G. Racagni (Eds.). Raven Press, New York.

Jones, D.L., Berg, S.L., Dorris, R.L., and Dill, R.E. (1981). Biphasic locomotor response to intra-accumbens dopamine in a nonhuman primate. Pharm. Biochem. & Beh. 15, 243-246.

Kebabian, J.W., and Calne, D.B. (1979). Multiple receptors for dopamine. Nature 277, 93-96.

Kelly, P.H. (1977). In Handbook of Psychopharmacology, Vol. 8, L.L. Iversen, S.D. Iversen and S.H. Snyder (Eds.). Plenum Press New York and London, pp. 295-331.

Kelly, P.H., Seviour, P.W., and Iversen, S.D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 94, 507-522.

Kendell, R.E. (1976). The classification of depressions: A review of contemporary confusion. Brit. J. Psychiat. 129, 15-28.

Kendell, R.E. (1981). The present status of electroconvulsive therapy. Brit. J. Psychiat. 139, 265-283.

Kennett, G.A., and Joseph, M.H. (1981). The functional importance of increased brain tryptophan in the serotonergic response to restraint stress. Neuropharmacol. 20, 39-43.

Kissileff, H. (1970). Free feeding in normal and "recovered lateral" rats monitored by a pellet detecting eatometer. Physiol. Behav. 5, 163-173.

Klein, D.F., and Davis, J.M. (1969). "Diagnosis and Drug Treatment of Psychiatric Disorders." Williams and Williams, Baltimore, Maryland, p. 480.

Korf, J., Sebens, J.B., and Postrema, F. (1979). Cyclic AMP in the rat cerebral cortex after stimulation of the locus coeruleus: Decrease by antidepressant drugs. Eur. J. Pharmacol. 59, 23-30.

Kostowski, W., and Malatynska, E. (1983). Antagonism of behavioural depression produced by clonidine in the mongolian gerbil: A potential screening test for antidepressant drugs. Psychopharmacol. 79, 203-208.

Kovacs, M. (1980). Efficacy of cognitive and behaviour therapies for depression. Am. J. Psychiat. 137, 1495-1501.

Kruk, Z.L. (1973). Dopamine and 5-hydroxytryptamine inhibit feeding in rats. Nature (London) 246, 52-53.

Kupfer, D.J., Weiss, B.L., Foster, F.G., Detre, T.P., Delgado, J., and McPartland, R. (1974). Psychomotor activity in affective states. Arch. Gen. Psychiat. 30, 765-768.

Langer, Z.A., Zarifian, E. Briley, M., Raisman, R. and Sechter, D. (1981). High affinity binding of 3H-imipramine in brain and platelets and its relevance to the biochemistry of affective disorders. Life Sci. 29, 211-220.

Lasagna, L., Felsinger, J.M., and Beecher, H.K. (1955). Drug induced mood changes in man: (1) Observations on healthy subjects, chronically ill patients and post-addicts. J. Am. Med. Ass. 157, 1006-1020.

Lebrecht, V., and Nowak, J.Z. (1980). Effect of single and repeated electroconvulsive shock on serotonergic system in rat

brain. II. Behavioural studies. Neuropharmacol. 19, 1066-1071.

Lehr, D., and Goldman, W. (1973). Continued pharmacological analysis of consummatory behaviour in the albino rat. Eur. J. Pharmac. 23, 197-210.

Leibowitz, S.F. (1970a). Hypothalamic beta-adrenergic "satiety" system antagonizes an alpha-adrenergic "hunger" system in the rat. Nature (London) 226, 963-964.

Leibowitz, S.F. (1970b). Reciprocal hunger-regulating circuits involving alpha- and beta-adrenergic receptors located, respectively, in the ventromedial and lateral hypothalamus. Proc. Natl. Aca. Sci. U.S.A. 67, 1063-1070.

Leibowitz, S.F. (1973). Alpha-adrenergic receptors mediate suppression of drinking induced by hypothalamic amphetamine injection. Fed. Proc. 32, 754.

Leibowitz, S.F. (1975). Ingestion in the satiated rat: role of alpha and beta receptors in mediating effects of hypothalamic adenergic stimulation. Physiol. Behav. 14, 743-754.

Leibowitz, S.F. (1976). Brain catecholaminergic mechanisms for control of hunger. In Hunger: Basic Mechanisms and Clinical Implications, D. Novin, W. Wyrwicka and G. Bray (Eds.). Raven, New York, pp 1-18.

Leibowitz, S.F. (1978a). Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol. Biochem. Behav. 8, 163-175.

Leibowitz, S.F. (1978b). Identification of catecholamine receptor mechanisms in the perifornical lateral hypothalamus and their role in mediating amphetamine and L-DOPA anorexia. In Central Mechanisms of Anorectic Drugs, S. Garattini and R. Samanin (Eds.). Raven, New York, pp. 39-82.

Leibowitz, S.F. (1979). Midbrain-hypothalamic catecholamine projection systems mediating feeding stimulation and inhibition in the rat. In Catecholamines: Basic and Clinical Frontiers, Vol. 2, E. Udsin, I.J. Kopin, and J.D. Barchas (Eds.). Pergamon, New York, pp. 1675-1677.

Leibowitz, S.F., and Brown, L.L. (1980a). Histochemical and pharmacologial analysis of noradrenergic projections to the paraventricular hypothalamus in relation to feeding stimulation. Brain Res., in press.

Leibowitz, S.F., and Brown, L.L. (1980b). Histochemical and pharmacological analysis of catecholaminergic projections to the perifornical hypothalamus in relation to feeding inhibition. Brain Res., in press.

Leibowitz, S.F., and Brown, L.L. (1980c). Analysis of behavioral

deficits produced by lesions in the dorsal and ventral midbrain tegmentum. Physiol. Behav., in press.

Leibowitz, S.F., and Rossakis, C. (1978). Analysis of feeding suppression produced by perifornical hypothalamic injection of catecholamines, amphetamines, and mazindol. Eur. J. Pharmacol. 53, 69-81.

Leibowitz, S.F., and Rossakis, C. (1979a). Mapping study of brain dopamine- and epinephrine-sensitive sites which cause feeding suppression in the rat. Brain Res. 172, 101-113.

Leibowitz, S.F., and Rossakis, C. (1979b). Pharmacological characterization of perifornical hypothalamic dopamine receptors mediating feeding inhibition in the rat. Brain Res. 172, 115-130.

Leibowitz, S.F., and Rossakis, C. (1979c). L-DOPA feeding suppression: effect on catecholamine neurons of the perifornical lateral hypothalamus. Psychopharmacology 61, 273-280.

Leibowitz, S.F., Hammer, N.J., and Brown, L.L. (1980). Analysis of behavioural deficits produced by lesions in the dorsal and ventral midbrain tegmentum. Physiology & Behaviour 25, 829-843.

Lemberger, L.,E. Sernatinger and Kuntzman,R. (1970). Effect of desmethylimipramine, iprindole and DL-erytho-alpha(3,4-dichlorphenyl)-beta-(t-butyl amino) propanol HCl on the metabolism of amphetamine, Biochem. Phamacol. 19, 3021.

Lewander, T. (1968). Effects of amphetamine on urinary and tissue catecholamines in rats after inhibition of its metabolism with desmethylimipramine. Eur. J. Pharmacol. 5, 1-9.

Lindvall, O., and Bjorklund, A. (1974). The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiol. Scand. Suppl. 412, 1-48.

Lindvall, O., and Bjorklund, A. (1978). Organization of catecholamine neurons in the rat central nervous system. In Handbook of Psychopharmacology, Vol. 9, Chemical Pathways in the Brain, L.L. Iversen, S.D. Iversen, and S.H. Snyder (Eds.). Plenum, New York, pp. 139-231.

Loomer, H.P., Saunders, J.C., and Kline, N.S. (1957). A clinical and pharmacodynamic evaluation of iproniazid as a psychic energizer. Psychiat. Res. Rep. Amer. Psychiat. Ass. 8, 129-141.

Lorden, J., Oltmans, G.A., and Margules, D.L. (1976). Central noradrenergic neurons: Differential effects on body weight of electrolytic and 6-hydroxydopamine lesions in rats. J. Comp. Physiol. Psychol. 90, 144-155.

Lucki, I., and Frazer, A. (1982). Prevention of the serotonin syndrome in rats by repeated administration of monoamine oxidase inhibitors but not tricyclic antidepressants. Psychopharmacol. 77, 205-211.

Lyon, M., and Robbins, T.W. (1975). The action of central nervous system stimulant drugs: A general theory concerning amphetamine effects. In Current Developments in Psychopharmacology 2, M.V. Essman and L. Valzelli (Eds.). Spectrum Publications Inc.

Maas, J.W., and Landis, D.H. (1968). In vivo studies of the metabolism of norepinephrine in the central nervous system. J. Pharmacol. Exp. Ther. 163, 147-162.

Maas, J.W., Fawcett, J.A., and Dekirmenjian, H. (1972). Catecholamine metabolism, depressive illness, and drug response. Arch. Gen. Psychiatry 26, 252-261.

Maas, J.W., Kocsis, J.H>, Bowden, C.L., Davis, J.M., Redmond, D.E., Hanin, I., andRobins, E. (1982). Pretreatment neurotransmitter metabolites and response to imipramine or amitriptyline treatment. Psychol. Med> 12,37-43.

Maggi, A., U'Prichard, D.C., and Enna, S.J. (1980). Differential effects of antidepressant treatment on brain monaminergic receptors. Europ. J. Pharmacol. 61, 91-98.

Maitre, L., Baumann, P.A., Jaeckel, J., and Waldmeier, P.C. (1982). 5-HT uptake inhibitors: Psychopharmacological and neurobiological criteria of selectivity. In Serotonin in Biological Psychiatry, B.T. Ho, J.C. Schoolar and E. Usdin (Eds.). Raven Press, New York, pp. 229-246.

Maj, J., Mogilnicka, E., and Klimek, V. (1979). The effect of repeated administration of antidepressant drugs on the responsiveness of rats to catecholamine agonists. J. Neur. Trans. 44, 221-235.

Maj, J., Mogilnicka, E., and Kordecka-Magiera, A. (1980). Effects of chronic administration of antidepressant drugs on aggressive behaviour induced by clonidine in mice. Pharmacol. Biochem. Behav. 13, 153-154.

Maj. J., Mogilnicka, E., Klimek, V., and Kordecka-Magiera, A. (1981). Chronic treatment with antidepressants: Potentiation of clonidine-induced aggression in mice via noradrenergic mechanism. J. Neur. Trans. 52, 189-197.

Makanjuola, R.O.A., and Ashcroft, G.W. (1982). Behavioural effects of electrolytic and 6-hydroxydopamine lesions of the accumbens and caudate-putamen nuclei. Psychopharmacol. 76, 333-340.

Makanjuola, R.O.A., Dow, R.C., and Ashcroft, G.W. (1980). Behavioural responses to stereotactically controlled injections

of monoamine neurotransmitters into the accumbens and caudate-putamen nuclei. Psychopharmacol. 71, 227-235.

Malt,U. (1983). Classification and diagnosis of depression. Acta. psychiat. scand., suppl. 302,7-35.

Mandell, A.J., Segal, D.S., and Kuczenski, R. (1975). Metabolic adaptation to antidepressant drugs: Implications for pathophysiology and treatment in psychiatry. In Catecholamines and Behaviour, Vol. 2, A.J. Friedhoff (Ed.). Plenum Press, New York, pp. 197-208.

Mason, S.T., and Fibiger, H.C. (1979). Noradrenaline, fear and extinction. Brain Res. 165, 47-56.

Margules, D.L. (1970). Alpha-adrenergic receptors in hypothalamus for the suppression of feeding behavior by satiety. J. Comp. Physiol. Psychol. 73, 1-12.

Margules, D.L., Lewis, M.J., Dragonica, J.A., and Margules, A.S. (1972). Hypothalamic norepinephrine: Circadian rhythms and the control of feeding behavior. Science 178, 640-643.

Marshall, J.F., and Teitelbaum, P. (1974). Further analysis of sensory inattention following lateral hypothalamic damage in rats. J. Comp. Physiol. Psychol. 86, 375-395.

Martin, G.E., and Myers, R.D. (1975). Evoked release of [14C] norepinephrine from the rat hypothalamus during feeding. Am. J. Physiol. 229, 1547-1555.

Mason, S.T., and Angel, A. (1983). Behavioural evidence that chronic treatment with the antidepressant desipramine causes reduced functioning of brain noradrenaline systems. Psychopharmacology 81, 73-77.

Mathew, R.Y., Ho, B.T., Kralik, P., Taylor, D., and Kralik, J.L. (1980). MAO, DBH and COMT: The effect of anxiety. J. Clin. Psychiat. 41:12, Sec 2, 25-28.

Matthews, J.W., Booth, D.A., and Stolerman, I.P. (1978). Factors influencing feeding elicited by intracranial noradrenaline in rats. Brain Res. 141, 119-128.

McMillen, B.A., Warnack, W., German, D.C., and Shore, P.A. (1980). Effects of chronic desipramine treatment on rat brain noradrenergic responses to alpha-adrenergic drugs. Europ. J. Pharmacol. 61, 239-246.

Mendels, J., and Frazer, A. (1974). Brain biogenic amine depletion and mood. Arch. Gen. Psychiat. 30, 447-451.

Mendlewicz, J., Pinder, R.M., Stutemeijerm, S.M. and van Dorth,R. (1982). Monoamine metabolites in cerebrospinal fluid of depressed patients during treatment with mianserin or

iprindole. J. Affect. Dis. 4,219-226.

Menkes, D.B., and Aghajanian, G.K. (1981). Alpha1-adrenoceptor-mediated responses in the lateral geniculate nucleus are enhanced by chronic antidepressant treatment. Europ. J. Pharmacol. 75, 27-35.

Menkes, D.B., Aghajanian, G.K., and Gallager, D.W. (1983). Chronic antidepressant treatment enhances agonist affinity of brain alpha-adrenoceptors. Europ. J. Pharmacol. 87, 35-41.

Miller, N.E. (1960). Motivational effects of brain stimulation and drugs. Fed. Proc. 19, 846-853.

Miller, N.E., Gottesman, K.S., and Emery, N. (1964). Dose response to carbachol and norepinephrine in rate hypothalamus. Am. J. Physiol. 206, 1384-1388.

Minchin, M.C.W., Williams, J., Bowdler, J.M., and Green, A.R. (1983). The effect of electroconvulsive shock on the uptake and release of noradrenaline and 5-hydroxytryptamine in rat brain slices. J. Neurochem. 40, 765-768.

Mobley, P.L., and Sulser, F. (1980). Adrenal corticoids regulate sensitivity of noradrenaline receptor coupled adenylate cyclase in brain. Nature 286, 608-609.

Modigh, K. (1975). Electroconvulsive shock and postsynaptic catecholamine effects: Increased psychomotor stimulant effect of apomorphine and clonidine in reserpine pretreated mice by repeated ECS. J. Neur. Trans. 36, 19-32.

Modigh, K. (1979). Long lasting effects of ECT on monoaminergic mechanisms. In Neuropsychopharmacology, B. Saletu, P. Berner and L. Hollister (Eds.). Pergamon, Oxford, 11-20.

Mogenson, G.J., and Wu, M. (1982). Neuropharmacological and electro physiological evidence implicating the mesolimbic dopamine system in feeding responses elicited by electrical stimulation of the medial forebrain bundle. Brain Res. 253, 243-252.

Molinoff, P.B. and Axelrod, J. (1971). Biochemistry of catecholamines. Ann. Rev. Biochem. 40, 465-500.

Moller, S.E., Kirk, L., and Honore, P. (1980). Relationship between plasma ratio of tryptophan to competing amino acids and the response to L-tryptophan treatment in endogenously depressed patients. J. Affect. Disord. 2, 47-59.

Montanaro, N., Dall'Olio, R., Gandolfi, O., and Vaccheri, A. (1982). Neuroleptic versus antidepressant activity of sulpiride isomers in the rat. In Typical and Atypical Antidepressants: Molecular Mechanisms, E. Costa and G. Racagni (Eds.). Raven Press, New York.

Montgomery, A., and Willner, P. (1981). Effects of subchronic desmethylimipramine (DMI) treatment on the classically conditioned eyeblink response in rabbits. Commun. Psychopharmacol. 4, 519-525.

Montgomery, R.B., Singer, G., and Purcell, A.T. (1971). The effects of intrahypothalamic injections of desmethylimipramine on food and water intake of the rat. Psychopharmacologia 19, 81-86.

Morgane, P.J. (1961). Alterations in feeding and drinking behavior of rats with lesions in globi pallidi. Am. J. Physiol. 201, 420-428.

Murphy, D.L., Brodie, H.K.H., and Goodwin, F.K. (1971). Regular induction of hypomania by L-dopa in "bipolar" manic-depressive patients. Nature 229, 135-136.

Nelson, J.C., and Charney, D.S. (1981). The symptoms of major depression. Am. J. Psychiat. 139, 1-13.

Nielsen, E.B., Nielsen, M., Ellison, G., and Braestrup, C. (1980). Decreased spiroperidol and LSD binding in rat brain after continuous amphetamine. Europ. J. Pharmacol. 66, 149-154.

Olpe, H.R., and Schellenberg, A. (1980). Reduced sensitivity of neurons to noradrenaline after chronic treatment with antidepressant drugs. Europ. J. Pharmacl. 63, 7-13.

Oltmans, G.A., Lorden, J.F., and Margules, D.L. (1977). Food intake and body weight: effects of specific and nonspecific lesions in the midbrain path of the ascending noradrenergic neurons of the rat. Brain Res. 128, 293-308.

Paul, S.M., Hulihan-Giblin, B., and Skolnick, P. (1982). (+)-Amphetamine binding to rat hypothalamus: Relation to anorexic potency of phenylethylamines. Science 218, 487-489.

Paykel, E.S. (1972). Depressive typologies and response to amitriptyline. Brit. J. Psychiat. 120, 147-156.

Paykel, E.S., Parker, R.R., Penrose, R.J., and Rassaby, E.R. (1979). Depressive classification and prediction of response to phenelzine. Br. J. Psychiat. 134, 572-581.

Pellegrino, L.J., and Cushman, A.J. (1967). A Stereotaxic Atlas of the Rat Brain. Appleton-Century-Crofts, New York.

Perris, C. (1966). A study of bipolar (manic-depressive) and unipolar recurrent depressive psychoses. Acta Psychiat. Scand. 42, Suppl. 194, 1-188.

Peroutka, S.J., and Snyder, S.H. (1980). Long-term antidepressant treatment decreases spiroperidol-labelled serotonin receptor

binding. Science 210, 88-90.

Peyrin, L., and Pequignot, J.M. (1983). Free and conjugated 3-methoxy-4-hydroxyphenylglycol in human urine: Peripheral origin on glucuronide. Psychopharmacol. 79, 6-20.

Pickar, D., Sweeney, D.R., Maas, J.W., and Heninger, G.R. (1978). Primary affective disorder, clinical state change, and MHPG excretion: a longitudinal study. Arch. Gen. Psychiat. 35, 1378-1383.

Pijnenburg, A.J.J., and van Rossum, J.M. (1973). Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. J. Pharm. Pharmacol. 25, 1003-1004.

Post, R.M., Goodwin, F.K., Gordon, E., and Watkin, D.M. (1973a). Amine metabolites in human cerebrospinal fluid: effects on cord transection and spinal fluid block. Science 179, 897.

Post, R.M., Gordon, E., Goodwin, F.K., and Bunney, W.E. (1973b). Central norepinephrine in affective illness: MHPG in the cerebrospinal fluid. Science 179, 1002.

Praag, H.M. van, and Korf, J. (1971). Retarded depression and the dopamine metabolism. Psychopharmacol. 19, 199-203.

Protais, P., Bonnet, J.J., and Costentin, J. (1983). Pharamcological characterization of the receptors involved in the apomorphine-induced polyphasic modifications of locomotor activity in mice. Psychopharmacology 81, 126-134.

Puzynski, S., Bidzinski, A., and Hauptmann, M. (1980). Correlations between the urinary levels of biogenous amines and their metabolites in patients with endogenous depression before and after treatment. Psychiatr. Pol. 14, 457-463.

Randrup, A. and Braestrup, C. (1977). Uptake inhibition of biogenic amines by newer antidepressant drugs: relevance to the dopamine hypothesis of depression. Psychopharmacology, 53, 309-314.

Randrup, A., Munkvad, I., Fog, R., Gerlach, J., Molander, L., Kjellberg, B., and Scheel-Kruger, J. (1975). Mania, depression and brain dopamine. In Current Developments in Psychopharmacology, Vol. 2, W.B. Essman and L. Valzelli (Eds.). Spectrum Publications, New York, pp. 206-248.

Reus, V.I., Lake, C.R., and Post, R.M. (1980). Effect of piribedil (ET-495) on plasma norepinephrine: Relationship to antidepressant response. Commun. Psychopharmacol. 4, 207-213.

Richter, C.P. (1927). Animal behaviour and internal drives. Q. Rev. Biol. 2, 397-443.

Robertson, M.M., and Trimble, M.R. (1982). Major tranquillizers

used as antidepressants: a review. J. affect. Disord. 4, 173-193.

Robins, E., and Guze, S.B. (1972). Classification of affective disorders: The primary-secondary, the endogenous-reactive and the neurotic-psychotic concepts. In Recent Advances in the Psychobiology of the Depressive Illness, T.A. Williams, M.M. Katz and J.A. Shields (Eds.). U.S. Government Printing Office, Washington, pp. 283-293.

Roffman, M., Kling, M.S., Cassens, G., Orsulak, P.J., Reigle, T.G., and Schildkraut, J.J. (1977). The effects of acute and chronic administration of tricyclic antidepressants on MHPG-SO in rat brain. Psychopharmacol. Commun. 1, 195-206.

Rolls, E.T., Griffiths, C., Yaxley, S., and Simpson, S. (1983). Analysis of functions of hypothalamic and related neurones in feeding. British Feeding Group Conference, Oxford University, April.

Rosenblatt, J.E., Pert, C.B., Tallman, J.F., Pert, A., and Bunney, W.E. (1979). The effect of imipramine and lithium on alpha- and beta-receptors in rat brain. Brain Res. 160, 186-191.

Rosloff, B.N., and Davis, J.M. (1974). Effect of iprindole on norepinephrine turnover and transport. Psychopharmacol. 40, 53-64.

Rosloff, B.N., and Davis, J.M. (1978). Decrease in brain NE turnover after chronic DMI treatment: No effect with iprindole. Psychopharmacol. 56, 335-341.

Samanin, R., Bendotti, C., Bernasconi, S. and Potaccini, R. (1978). Differential role of brain monoamines in the activity of anorectic drugs. In: Central Mechanisms of Anorectic Drugs, S, Garrantini and R., Samanin (Eds.), Raven Press, New York, pp.233-242.

Samanin, R. and Garanttini, S. (1981). Neuropharmacology of Feeding. In: Drugs and Appeite. T.silverstone (Ed.). Life Sciences Research Report.

Sanghvi, I.S., Singer, G., Friedman, E., and Gershon, S. (1975). Anorexigenic effects of d-amphetamine and L-DOPA in the rat. Pharmacol. Biochem. Behav. 3, 81-86.

Schanberg, S.M., Breese, G.R., Schildkraut, J.J., Gordon, E.K., and Kopin, I.J. (1968). 3-methoxy-4-hydroxyphenylglycol sulfate in brain and cerebrospinal fluid. Biochem. Pharmacol. 17, 2006-2008.

Schatzberd, A.F., Orsulak, P.J., Rosenbaum, A.H., Maruta, T., Kruger, E.R., Cole, J.O. and Schildkraut, J.J. (1980). Towards a biochemical classification of depressive disorders. IV.

Pretreatment urinary MHPG levels as predictors of antidepressant drug response to imipramine. Commun. Psychopharm. 4,441-445.

Schildkraut, J.J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiat. 130, 509-522.

Schildkraut, J.J., and Kety, S.S. (1967). Biogenic amines and emotion. Science 156, 21-30.

Schildkraut, J.J., Orsulak, P.J., Gudeman, J.E., Schatzberg, A.F., Rohde, W.A., Labrie, R.A., Cahill, J.F., Cole, J.O., and Frazier, S.F. (1978). Norepinephrine metabolism in depressive disorders: implications for a biochemical classification of depressions. In Depression: Biology, Psychodynamics and Treatment, J.O. Cole, A.F. Schatzberg and S.H. Frazier (Eds.). Plenum Press, New York, pp. 75-101.

Schildkraut, J.J., Orsulak, P.J., Schatzberg, A.F., Cole, J.O., and Rosenbaum, A.H. (1981). Possible pathophysiological mechanisms in subtypes of unipolar depressive disorders based on differences in urinary MHPG levels. Psychopharmacol. Bull. 17, 90-91.

Schildkraut, J.J., Winokur, A., and Applegate, C.W. (1970). Norepinephrine turnover and metabolism in rat brain after long term administration of imipramine. Science 168, 867-869.

Schildkraut, J.J., Winokur, A., Dreskoczy, P.R., and Hensle, J.H. (1971). Changes in norepinephrine turnover in rat brain during chronic administration of imipramine and protriptyline: a possible explanation for the delay in onset of clinical antidepressant effects. Am. J. Psychiat. 27, 72-79.

Schmitt, H. (1973). Influence d'agents interferant avec les catecholamines et la 5-hydroxytryptamine sur les effets anorexigenes de l'amphetamine et de la fenfluramine. J. Pharmac. 4, 285-294.

Schultz, J.E., Siggins, G.R., Schocker, F.W., Turck, M., and Bloom, F.E. (1981). Effects of prolonged treatment with lithium and tricyclic antidepressants on discharge frequency, norepinephrine responses and beta receptor binding in rat cerebellum: Electrophysiological and biochemical comparison. J. Pharmacol. Exp. Ther. 216, 28-38.

Sclafani, A., and Maul, G. (1974). Does the ventromedial hypothalamus inhibit the lateral hypothalamus? Physiol. Behav. 12, 157-162.

Sclafani, A., and Berner, C.N. (1977). Hyperphagia and obesity produced by parasagittal and coronal hypothalamic knife cuts: further evidence for a longitudinal feeding inhibitory pathway. J. Comp. Physiol. Psychol. 91, 1000-1018.

Segal, D.S., Kuczenski, R., and Mandell, A.J. (1974). Theoretical implications of drug-induced adaptive regulations for a biogenic amine hypothesis of affective disorder. Biol. Psychiatry 9, 147-159.

Segawa, T., Mizuta, T., and Uehara, M. (1982). Role of the central serotonergic system as related to the pathogenesis of depression: Effect of antidepressants on rat central serotonergic activity. In New Vistas in Depression, S.Z. Langer, R. Takahashi, T. Segawa and M. Briley (Eds.). Pergamon Press, New York, pp. 3-10.

Serra, G., Argiolas, A., and Gessa, G.L. (1981). Opposite Changes in DA Autoreceptors Sensitivity Induced by Chronic Antidepressants and Neuroleptics. In Apomorphine and Other Dopaminomimetics, Vol. 1: Basic Pharmacology, G.L. Gessa and G.U. Corsini (Eds.), pp. 133-142. Raven Press, New York.

Serra, G., Argiolas, A., Fadda, F., Melis, M.R., and Gessa, G.L. (1981). Repeated electroconvulsive shock prevents the sedative effect of small doses of apomorphine. Psychopharmacol. 73, 194-196.

Serra, G. Argiolas, A., Klimek, V., Fadda, F., and Gessa, G.L. (1979). Chronic treatment with antidepressants prevents the inhibitory effect of small doses of apomorphine on dopamine synthesis and motor activity. Life Sci. 25, 415-424.

Serra, G., Melis, M.R., Argiolas, A., Fadda, F., and Gessa, G.L. (1981). REM sleep deprivation induces subsensitivity of dopamine receptors mediating sedation in rats. Europ. J. Pharmacol. 72, 131-135.

Shoeman, D.W., Sirtori, C.R., and Azarnoff, D.L. (1974). Inhibition of amphetamine tolerance and metabolism by propranolol. J. Pharmac. Exp. Ther. 191, 68-71.

Skirboll, L.R., Grace, A.A., and Bunney, B.S. (1979). Dopamine auto- and postsynaptic receptors: Electrophysiological evidence for differential sensitivity to dopamine agonists. Science 206, 80-82.

Slangen, J.L., and Miller, N.E. (1969). Pharmacological tests for the function of hypothalamic norepinephrine in eating behavior. Physiol. Behav. 4, 543-552.

Smith, R.C. (1973). The psychopharmacologic management of patients manifesting symptoms of neurotic depressive reaction. Scientific exhibit, 126th Ann. Meeting, Amer. Psychiat. Ass., Honolulu, Hawaii, 7-15.

Sokoloff, P., Martres, M.P., and Schwartz, J.C. (1980). 3H-Apomorphine labels both dopamine postsynaptic receptors and autoreceptors. Nature 288, 283-286.

Spector, S., Hirsch, C.W., and Brodie, B.B. (1963). Association of behavioural effects of pargyline, a non-hydrazide MAO inhibitor with increase in brain norepinephrine. Int. J. Neuropharmacol. 2, 81-93.

Spitzer, R.L., and Williams, J.W.B. (1980). Classification of mental disorders and DSM-III. In Comprehensive Textbook of Psychiatry, 3rd. Ed., Vol. 1, H.I. Kaplan, A.M. Freedman and B.J. Sadock (Eds.). Williams and Wilkins, Baltimore, pp. 1035-1072.

Spyraki, C., and Fibiger, H.C. (1981). Behavioural evidence for supersensitivity of postsynaptic dopamine receptors in the mesolimbic system after chronic administration of desipramine. Europ. J. Pharmacol. 74, 195-206.

Subramanyam, S. (1975). Role of biogenic amines in certain pathological conditions. Brain Res. 87, 355-362.

Sugrue, M.F. (1980). Changes in rat brain monoamine turnover following chronic antidepressant administration. Life Sci. 25, 423-429.

Sulser, F. (1978). New perspectives on the mode of action of antidepressant drugs. Trends Pharmacol. Sci. 1, 92-94.

Sulser, F., and Mishra, R. (1982). Regulation of central noradrenergic receptor function and its relevance to the therapy of depression. In New Vistas in Depression, S.Z. Langer, R. Takahashi, T. Segawa and M. Briley (Eds.). Pergamon Press, New York, pp. 37-47.

Sumners, C., DeVries, J.B., and Horn, A.S. (1981). Behavioural and neurochemical studies on apomorphine-induced hypomotility in mice. Neuropharmacology 20, 1203-1208.

Svensson, T.H. (1978). Attenuated feedback inhibition of brain serotonin synthesis following chronic administration of imipramine. Naunyn-Schmiedebergs Arch. Pharmacol. 302, 115-118.

Taylor, M.A., and Abrams, R. (1980). Reassessing the bipolar-unipolar dichotomy. J. Affect. Disord. 2, 195-217.

Tordoff, M.G., Hopfenbeck, J., Butcher, L.L., and Novin, D. (1982). A peripheral locus for amphetamine anorexia. Nature 297, 148-150.

Ungerstedt, U. (1971a). Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Suppl. 367, 1-48.

Ungerstedt, U. (1971b). Adipsia and aphagia after 6-hydroxy-dopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiol. Scand. Suppl. 367, 95-122.

Ungerstedt, U. (1974). Neuropharmacology of the control of food intake. Vth International Conference on Physiology of Food and Fluid Intake, Jerusalem, pp. 33-34.

van der Gugten, J., and Slangen, J.L. (1977). Release of endogenous catecholamines from rat hypothalamus in vivo related to feeding and other behaviors. Pharmacol. Biochem. Behav. 7, 211-219.

Van Praag, H.M. (1983). In search of the mode of action of antidepressants: 5-HTP/tyrosine mixtures in depressions. Neuropharmacol. 22, 433-440.

Vetulani, J., and Sulser, F. (1975). Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP generating system in limbic forebrain. Nature 257, 495-496.

Vetulani, J., Stawarz, R.J., and Sulser, F. (1976). Adaptive mechanisms of the noradrenergic cyclic AMP generating system in the limbic forebrain of the rat: adaptation to persistent changes in the availability of norepinephrine. J. Neurochem. 27, 661-666.

Vetulani, J., Lebrecht, U., and Nowak, J.Z. (1981). Enhancement of responsiveness of the central serotonergic system and serotonin-2 receptor density in rat frontal cortex by electroconvulsive shock treatment. Eur. J. Pharmacol. 81, 85.

Waehrens, J., and Gerlach, J. (1981). Bromocryptine and imipramine in endogenous depression: A double-blind controlled trial in out-patients. J. Affect. Dis. 3, 193-202.

Wallinder, J., Skott, A., Carlsson, A. and Ross, B. (1976). Potentiation of antidepressant action of chlormipramine by tryptophan. Arch. Gen. Psychiat.33,1384-1389.

Weissman, M.S., and Paykel, E.S. (1974). The Depressed Woman: A Study of Social Relationships. University of Chicago Press, Chicago.

White, F.J., and Wang, R.Y. (1983). Comparison of the effects of chronic haloperidol treatment on A9 and A10 dopamine neurons in the rat. Life Sci. 32, 983-993.

Wiberg, A., Gottfries, C.G., and Oreland, L. (1977a). Low platelet monoamine oxidase activity in human alcoholics. Med. Biol. 55, 181-186.

Wiberg, A., Wahlstrom, S., and Oreland, L. (1977b). Brain monoamine oxidase activity after chronic ethanol treatment of rats. Psychopharmacol. 52, 111-113.

Wielosz, M. (1981). Increased sensitivity to dopaminergic

agonists after repeated electroconvulsive shock (ECS) in rats. Neuropharmacol. 20, 941-945.

Willner, P. (1984a). The ability of antidepressant drugs to desensitize beta-receptors is inversrly correlated with their clinical potency. J. of Affect. Dis. 7,53-58.

Willner, P. (1984b). The validity of animal models of depression. Psychopharm. 83,1-16.

Willner, P. (1984c). Depression: Towards a psychobiological synthesis. John Wiley, New York, in press.

Willner, P. (1983a). Dopamine and depression: A review of recent evidence. I. Empirical studies. Brain Res. Rev. 6, 211-224.

Willner, P. (1983b). Dopamine and depression: A review of recent evidence. II. Theoretical approaches. Brain Res. Rev. 6, 225-236.

Willner, P. (1983c). Dopamine and depression: A review of recent evidence. III. The effects of antidepressant treatments. Brain Res. Rev. 6, 237-246.

Willner, P., Towell, A. and Montgomery, T. (1984). Changes in amphetamine-induced anorexia and stereotypy during chronic treatment with antidepressant drugs. European J. Pharmacol. 98, 397-406.

Willner, P., and Montgomery, A. (1980). Attenuation of amphetamine anorexia in rats following subchronic treatment with a tricyclic antidepressant. Commun. Psychopharmacol. 4, 101-106.

Willner, P., and Montgomery, A. (1981). Behavioural changes during withdrawal from the tricyclic antidepressant desmethylimipramine (DMI). I. Interactions with amphetamine. Psycopharmacology 75, 54-59.

Willner, P., Montgomery, A. and Bird, D. (1981). Behavioural changes during withdrawal from desmethylimipramine (DMI).II. Increased resistance to extinction. Psychopharmacology 75, 60.

Willner, P., and Towell, A. (1982a). Evidence suggesting that DMI-induced resistance to extinction is not mediated by the dorsal noradrenergic bundle. Brain Res. 238, 251-253.

Willner, P., and Towell, A. (1982b). Microstructural analysis of the involvement of beta-receptors in amphetamine anorexia. Pharmacol. Biochem. Behav. 17, 255-262.

Winn, P., Williams, S.F., and Herberg, L.J. (1982). Feeding stimulated by very low doses of d-amphetamine administered systemically or by microinjection into the striatum. Psychopharmacology 78, 336-341.

Wolfe, B.B., Harden, R.K., Sporn, J.R., and Mounoff, P.B. (1978). Presynaptic modulation of beta-adrenergic receptors in rat cerebral cortex after treatment with antidepressants. J. Pharmac. Exp. Ther. 207, 446-457.

Wood, D., Othmer, S., and Reich, T. (1977). Primary and secondary affective disorder: I. Past social history and current episodes on 92 depressed inpatients. Compr. Psychiat. 18, 201-210.

Woolley, D.W., and Shaw, E. (1954). Some neurophysiological aspects of serotonin. Br. med. J. ii, 122.

Zebrowska-Lupina, I., and Kozyrska, C. (1980). The studies on the role of brain dopamine in the action of antidepressant drugs. Naunyn-Schmiedebergs Arch. Pharmacol. 313, Suppl. 80, R20.

Zigmond, M.J., and Stricker, E.M. (1973). Recovery of feeding and drinking by rats after intraventricular 6-hydroxydopamine or lateral hypothalamic lesions. Science 182, 717-719.

Zis, A.P. and F.K. Goodwin. (1979). Novel antidepressants and the biogenic amine hypothesis of depression. The case for iprindole and mianserin. Arch. Gen. Psychiat. 36,1097.

APPENDIX 1

PUBLISHED MATERIAL

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Evidence suggesting that DMI-induced resistance to extinction is not mediated by the dorsal noradrenergic bundle

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Key words: extinction — desmethylimipramine — dorsal noradrenergic bundle — rat

Rats were trained to press a lever for food rewards, then given a 5 week break, followed by a single extinction session. Animals which received 14 daily desmethylimipramine (DMI) injections, ending 4 days before the extinction session, showed resistance to extinction; no effect was seen in animals which received DMI during acquisition. The opposite pattern of results would be predicted if the effect were mediated by changes in the efficacy of the dorsal noradrenergic bundle.

We have recently reported that subchronic treatment with the tricyclic antidepressant desmethylimipramine (DMI) induces resistance to the extinction of learned behaviours. This effect has been observed in rats lever pressing or running for food rewards[11], and in rabbits performing a classically conditioned eyeblink response[7]. The effect was only seen during withdrawal from the drug, although other antidepressants may also cause resistance to extinction during maintained drug treatment[2].

Resistance to extinction is also induced by lesions to the dorsal noradrenaline (NA) bundle[5,6]. Subchronic treatment with tricyclic antidepressants reduces a number of parameters of noradrenergic function, notably NA synthesis[8] and β-adrenergic receptor sensitivity[1,9]. Whilst there is some doubt as to the significance of these changes during continued drug treatment[3], it appears likely that during withdrawal from tricyclics, noradrenergic transmission is functionally depressed[10]. We have therefore suggested that DMI-induced resistance to extinction might be mediated by a decrease in the functional efficacy of the dorsal bundle[11].

A test of this hypothesis is afforded by the observation[4] that the dorsal bundle extinction effect depends upon changes induced during acquisition: it was reported that the effect was only seen in animals lesioned prior to acquisition, but not in animals lesioned following acquisition and before extinction. Hence, if DMI-induced resistance to extinction is mediated by the dorsal bundle, then the effect should be seen in animals treated with DMI during acquisition, but not in animals receiving DMI between acquisition and extinction.

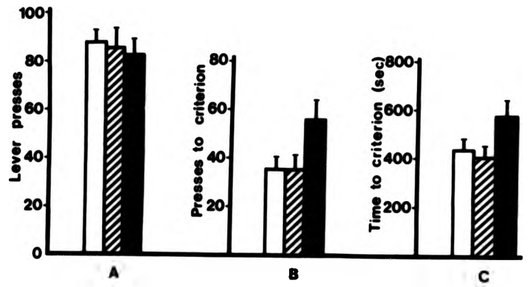

Fig. 1. White bars, controls; hatched bars, DMI treatment during acquisition; black bars, DMI treatment between acquisition and extinction. Values are means (+ standard error); n = 12. A: lever presses in the reacquisition session. B: presses to criterion in extinction. C: time to criterion in extinction.

Thirty-six male Lister hooded rats (weight 250–300 g), housed in pairs and maintained on 21-h food deprivation, were trained to lever press for food reward in an operant chamber (Campden Instruments Ltd., London). Following the attainment of asymptotic performance, they were maintained on continuous reinforcement (CRF) for 14 daily 10-min sessions. During this time, one group of animals (n = 12) received injections of DMI each evening (7.5 mg/kg i.p.; Geigy Pharmaceuticals Ltd., Macclesfield, U.K.). Following an interval of 19 days without training or injection, a second group (n = 12) received 14 daily DMI injections. Control injections in both cases were distilled water (1 ml/kg). On the third day after the final injection, all animals received a single 10-min CRF reacquisition session. The following day they were allowed to make 10 reinforced presses, then extinguished to a criterion of 2 min with no lever presses.

The 3 groups did not differ significantly in their performance on the reacquisition session (Fig. 1A: $F(2,33) = 0.2$, $P > 0.25$). In extinction, the group which received DMI during acquisition were indistinguishable from controls. However, resistance to extinction was shown by the group treated with DMI several weeks after acquisition (and extinguished during withdrawal): they made more presses and took a longer time to reach the extinction criterion than the other groups (Fig. 1B: $F(1,33) = 5.5$, $P < 0.025$ and Fig. 1C: $F(1,33) = 4.4$, $P < 0.05$).

If, as claimed[4], the dorsal bundle extinction effect is only seen in animals lesioned prior to acquisition, then, contrary to our earlier hypothesis, the present results are not consistent with dorsal bundle mediation of DMI-induced resistance to extinction. The possibility of a dopaminergic involvement is currently under investigation.

1 Banerjee, S. P., Kung, L. S., Riggi, S. J. and Chanda, S. K., Development of beta-adrenergic receptor subsensitivity by antidepressants, *Nature (Lond.)*, 268 (1977) 455–456.
2 Egan, J., Earley, C. J. and Leonard, B. E., The effect of amitryptiline and mianserine (Org. GB94) on food motivated behaviour of rats trained in a runway: possible correlation with biogenic amine concentration in the limbic system, *Psychopharmacology*, 61 (1979) 143–147.

3 Maas, J. W., Neurotransmitters and depression: too much, too little or too unstable? *Trends Neurosci.*, 2 (1979) 306–308.
4 Mason, S. T. and Fibiger, H. C., The dorsal bundle extinction effect: dependence on subtle changes in acquisition, *Brain Research*, 166 (1979) 341–348.
5 Mason, S. T. and Iversen, S. D., Effects of selective forebrain noradrenaline loss on behavioural inhibition in the rat, *J. comp. physiol. Psychol.*, 91 (1977) 165–173.
6 Mason, S. T. and Iversen, S. D., Theories of the dorsal bundle extinction effect, *Brain Res. Rev.*, 1 (1979) 107–137.
7 Montgomery, T. and Willner, P., Effects of subchronic desmethylimipramine (DMI) treatment on the classically conditioned eyeblink response in rabbits, *Commun. Psychopharmacol.*, 4 (1981) 519–525.
8 Segal, D. S., Kuczenski, R. and Mandell, A. J., Theoretical implications of drug-induced adaptive regulation for a biogenic amine hypothesis of affective disorder, *Biol. Psychiat.*, 9 (1974) 147–159.
9 Sulser, F., New perspectives on the mode of action of antidepressant drugs, *Trends Pharmacol. Sci.*, 2 (1979) 92–94.
10 Willner, P. and Montgomery, T., Neurotransmitters and depression: too much, too little, too stable — or not unstable enough? *Trends Neurosci.*, 3 (1980) 201.
11 Willner, P., Montgomery, T. and Bird, D., Behavioural changes during withdrawal from desmethylimipramine (DMI) II. Increased resistance to extinction, *Psychopharmacology*, 75 (1981) 60–64.

Pharmacology Biochemistry & Behavior, Vol. 17, pp. 255–262, 1982. Printed in the U.S.A.

Microstructural Analysis of the Involvement of Beta-Receptors in Amphetamine Anorexia

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WILLNER, P. AND A. TOWELL. *Microstructural analysis of the involvement of beta-receptors in amphetamine anorexia*. PHARMAC, BIOCHEM. BEHAV. 17(2) 255–262, 1982.—Rats were trained to take food by pushing the door of the pellet dispenser in an operant chamber. Log survivor analysis of the inter-response time frequency distribution was used to determine whether or not an animal was eating, at any time during a thirty minute session. This information was used to compute eating time, eating rate, and the mean length of bouts of eating and gaps between eating bouts. Video-recordings confirmed that the method discriminated eating from not eating with an accuracy of approximately ninety percent. Amphetamine (0.5 mg/kg) significantly reduced total food intake and eating time, and increased gap length; propranolol (5 mg/kg) significantly increased eating time and bout length. Following propranolol pretreatment, amphetamine significantly reduced eating time and bout length but also significantly increased eating rate; as a result there was no significant decrease in total food intake. The possible mediation of these effects by beta-adrenergic and dopaminergic systems is discussed.

Feeding Microstructural analysis Log survivor analysis Amphetamine Propranolol Catecholamines Rats

AMPHETAMINE has for many years been treated as a reference drug in pharmacological studies of anorexia. However, uncertainty still exists as to the mechanisms which mediate amphetamine anorexia. In common with many other actions of amphetamine the anorexic effect is attenuated by neuroleptic drugs, which are known to be dopamine (DA) receptor blocking agents [1, 6, 13, 15, 16, 19, 20, 33] and by lesions to dopaminergic pathways in the brain [9, 14, 21, 26]. However, it was recently reported that neuroleptics were ineffective in blocking the anorexic effect of a low dose of amphetamine (0.5 mg/kg) [6], suggesting that transmitters other than DA might be involved, particularly at low doses.

Studies employing central drug administration confirm that amphetamine anorexia is not mediated solely by DA. Injection of beta-adrenergic receptor blocking drugs in the region of the perifornical hypothalamus also attenuated the anorexic effect of centrally or peripherally administered amphetamine [18, 19, 20, 22]; this and several other lines of evidence strongly support the concept of a beta-adrenergic satiety system in the perifornical hypothalamus [2, 8, 18, 19, 20, 22, 23, 24, 26]. On the basis of these results, it would be expected that amphetamine anorexia should also be attenuated by peripherally administered beta-blockers. Paradoxically, however, this does not appear to be the case. Preliminary studies in this laboratory failed to demonstrate attenuation of amphetamine anorexia by the beta-blocker propranolol, and with one exception [27] previous investigations have had similar results [13, 15, 17, 28].

The resolution of this paradox may lie in the observation that propranolol impairs the metabolism of amphetamine [29]. This effectively increases the dose of amphetamine, which would tend to mask a partial blockade of the anorexic effect. In the present study, this possibility was investigated using the technique of microstructural analysis [31]. Previous workers have demonstrated that anorexic drugs do not simply reduce total food intake, but also produce characteristic changes in the fine structure of behaviour—for example, amphetamine reduces food inintake primarily by reducing eating time, whilst fenfluramine acts primarily by slowing down the rate of eating [4, 5, 10, 11]. It was reasoned that if the dopaminergic and beta-adrenergic systems control different parameters of feeding, then these might be differentially affected by propranolol. Specifically, if any amphetamine-induced microstructural changes are mediated by beta-receptors, then such changes might be blocked by propranolol whilst at the same time, owing to the increase in amphetamine dose, microstructural changes which are mediated by DA receptors would be enhanced by propranolol.

The sine qua non of microstructural analysis of feeding is knowing at any time whether a subject is eating or not. This is usually achieved by direct observation [4, 5, 10, 11, 12]. However, direct observation is extremely time consuming and labour intensive. We were therefore interested in developing an automated method. Such a method is available in the technique of log survivor analysis [30]: by inspection of

the frequency distribution of inter-response times, it is possible to establish a bout criterion for each subject; this criterion is then applied to decide whether a particular inter-response interval is within or between eating bouts. This method has only previously been used to analyse twenty-four hour feeding patterns, involving thousands of responses [7,30]. Experiment 1 was carried out to determine whether log survivor analysis could also be used to analyse brief (thirty minute) feeding sessions. Experiment 2 describes the application of the technique to the interaction between propranolol and amphetamine.

EXPERIMENT 1

METHOD

Subjects

Twelve male Lister hooded rats (weight 330–400 g) were housed in pairs and maintained on 21-hour food deprivation, with water available ad lib. The animals had had prior experience of continuously reinforced lever pressing for food rewards.

Apparatus

An operant chamber (Campden Instruments Ltd., London), from which the levers had been removed, was programmed to deliver a 45 mg food pellet whenever the perspex food tray door was pressed, subject to the constraint that presses spaced less than one second apart were ineffective. The house light and tray light were illuminated continuously, and the chamber was housed in a sound attenuating box with a smoked perspex viewing window. Each response on the tray door was logged (to the nearest 0.1 sec) by a Cromemco Z2 microcomputer, which displayed the time 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). Behaviour in the apparatus was also recorded on videotape, using a video camera adapted for low intensity light. By the use of a second camera filming the VDU, and a video-mixer, the occurrence and time of each response on the tray door was also recorded on the film.

Procedure

Following a pretraining period in which 10-min daily sessions were run until all animals achieved asymptotic performance, the animals were given a single 30-min session, which was recorded and filmed as described. The animals were observed to spend long periods eating, directly facing the food tray and only moving to take a further food pellet. From the film, it was possible to identify those inter-response intervals in which behaviours other than eating (rearing, grooming and walking) occured.

Microstructure Analysis

The IRT frequency distribution can be transformed to a survivor function, which shows the number, or the proportion, of IRTs greater than any given IRT (Fig. 1A). A further log transform produces a log survivor function (Fig. 1B). The log survivor function typically falls off steeply, usually in a straight line (indicating an underlying normal distribution), which at the breakpoint changes sharply to a much shallower slope. The assumption underlying log survivor analysis, and tested in the present experiment, is that IRTs shorter than the breakpoint represent responses within a continuous bout of feeding, whilst IRTs longer than the breakpoint represent gaps between feeding bouts.

Following identification of the breakpoint the following parameters of feeding may be calculated: (1) The number of bouts (B) is equal to the number of gaps (i.e. intervals longer than the breakpoint) plus one. (2) Eating time (T) is given by the total of all IRTs smaller than the breakpoint. (3) The length of eating bouts is given by T/B. (4) 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.

RESULTS AND DISCUSSION

Subjects consumed a mean of 218 pellets (9.8 g) in the 30-minute session (range: 148–268). Inspection of the log survivor curve for each animal (Fig. 1B) showed breakpoints varying from 12 to 25 sec (mean±standard error=16.8±0.9 sec). If the IRT frequency distributions are simply summed across animals, without regard to the differences in breakpoint, the occurrence of behaviours other than eating appears to increase almost linearly for IRTs between 10 and 30 sec (Fig. 2). However, a very different picture is shown by the distribution of IRTs around the breakpoint (Fig. 3). The incidence of behaviours other than eating now shows a marked discontinuity: other behaviours were relatively rare (5.8±0.8% of inter-response intervals) at IRTs shorter than the breakpoint, and highly likely (88.4±2.6% of intervals) at IRTs longer than the breakpoint. It is clear that using the breakpoint to provide an eating criterion for each individual (Fig. 3) affords a far clearer discrimination between eating and not eating than would any arbitrarily chosen criterion (Fig. 2).

Estimates of eating time and local eating rate were calculated by use of the breakpoint, as described above (Table 1). The true values of these parameters were also calculated, by excluding from eating bouts the 5.8% of short inter-response intervals which the film showed to be false positives, and including the 11.6% of long intervals which were false negatives. Compared with these true values, the calculated values under-estimated eating rate by 1.3 (±1.0)%, and over-estimated eating time by 6.5 (±1.7)%. Eating rate appears to be a very robust measure, which is not significantly affected ($t=1.3$, $p>0.1$) by the small proportion of errors. Although eating time is accurate to 6.5%, this figure is actually an over-estimate of the error, since the true eating time makes no allowance for the final pellet of each bout. If it is assumed that these pellets were consumed in the modal time of 4.5 sec (Fig. 2), then a further estimate of true eating time may be made (Table 1). This figure is higher than the calculated value by an insignificant 2.1 (±1.4)% ($t=1.5$, $p>0.1$). Thus as the effects of the two types of error to some extent cancel one another out, the values calculated for both eating time and eating rate are very close to their true values.

Estimates of the number and length of bouts were 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, both of which are compatible with continuous eating; indeed, it was sometimes possible to see that an animal did continue to eat whilst moving away from the food dispenser. If very short gaps (<10 sec) are excluded from the calculation (Table 1), then the discrepancy in the number and length of bouts, though still marked, is considerably reduced (25 and 23% respectively).

In conclusion, the method here described is clearly more successful than the use of arbitrary criteria, for discriminating between eating and not eating. Compared with continuous observation, the method produces very accurate estimates of eating rate and eating time. The method underestimates the number and over-estimates the length of eating bouts, but it does have the advantage that the bout criterion is unambiguous, rather than relying on the often difficult subjective judgement of whether an animal is eating or not. The error arises from the fact that the frequency of responses decreases as IRT increases, which means that there are more responses to the left of the breakpoint than to the right (Fig. 3); the error is therefore relatively constant between subjects. As will be shown below, results obtained using the present method were consistent with those obtained by previous authors using conventional observational methods (Note 1 and Refs. [4, 5, 10, 11, 12]).

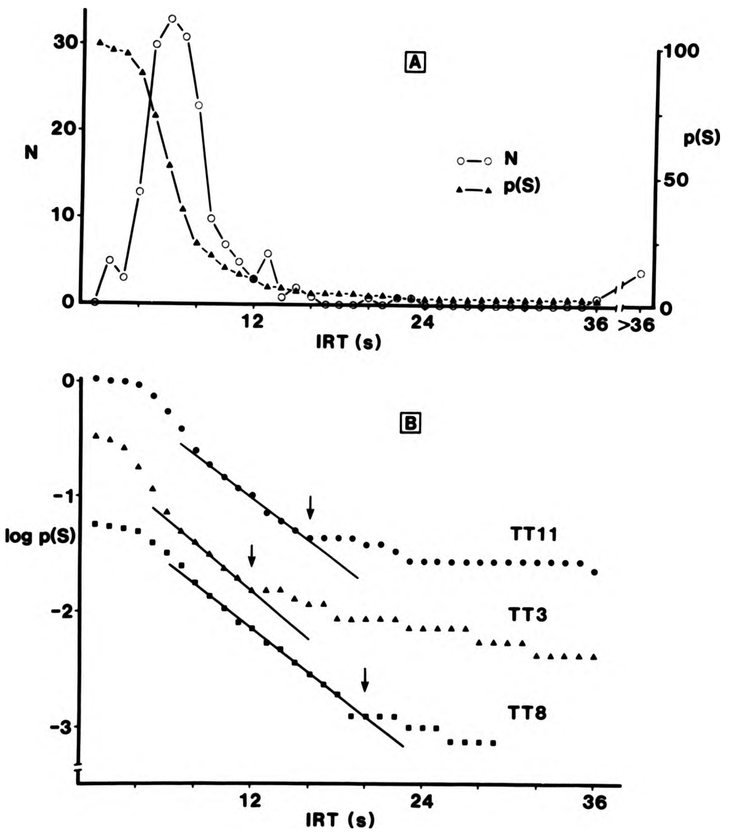

FIG. 1. A: The frequency distribution of inter-response times for a typical subject, and the survivor transform, which shows the proportion of the frequency distribution lying to the right of each point in the frequency distribution. N is the number of responses in each 1s IRT bin and p(S) is the percentage 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 half a log unit. The breakpoint in each curve is marked by an arrow.

EXPERIMENT 2

The doses of amphetamine and propranolol used in the present experiment were chosen on the basis of the following considerations:

(1) We have found that whilst log survivor analysis produces reliable estimates of microstructural parameters

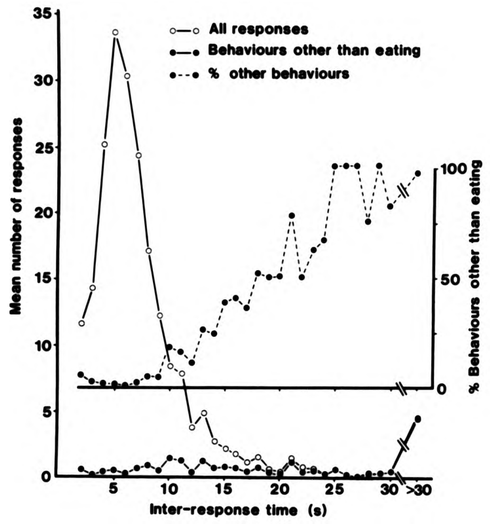

FIG. 2. The frequency distribution of IRTs (mean of all subjects), the distribution of those inter-response intervals in which behaviours other than eating were observed, and the latter as a proportion of the total. For clarity, the percentage scale has been displaced upwards.

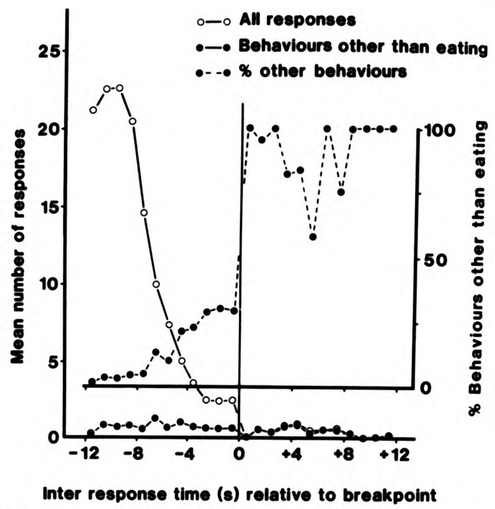

FIG. 3. For each subject, the breakpoint was identified by log survivor analysis (see text), and the frequency distribution of inter-response times plotted for 12 seconds either side of the breakpoint. The figure shows the IRT frequency distribution (mean of all animals), the distribution of those inter-response intervals in which behaviours other than eating were observed, and the latter as a proportion of the total. For clarity, the percentage scale has been displaced upwards.

TABLE 1

COMPARISON OF MICROSTRUCTURAL PARAMETERS DERIVED FROM DIRECT OBSERVATION AND FROM LOG SURVIVOR ANALYSIS

	Eating Rate (pellets/sec)	Eating Time (sec)	Number of Bouts	Bout Length (sec)
Calculated	0.162 (±0.007)	1289 (±70)	12.3 (±1.3)	128 (±21)
True	0.164 (±0.007)	1205 (±55)	23.1 (±1.8)	62 (±6)
Error %	−1.3 (±1.0)	6.5 (±1.7)	−43.7 (±6.3)	41.0 (±7.4)
Adjusted		1308 (±55)	16.9 (±1.3)	83 (±7)
Error %		−2.1 (±1.4)	−25.0 (±6.6)	22.9 (±7.7)

Microstructural parameters were calculated using the bout criterion derived from log survivor analysis (see text). True values were obtained by direct observation. The adjusted values add 4.5 sec per bout to true eating time, and exclude gaps of less than 10 sec when counting the number of bouts. The percentage error terms refer to calculated values in relation to true/adjusted values. All values are means (±SE).

when animals are making hundreds of responses, it becomes difficult to identify the breakpoint when the number of responses is small. It is therefore necessary to use a low dose of amphetamine which produces a relatively small anorectic effect; we chose a dose of 0.5 mg/kg, which in a previous study [32] produced an anorectic effect of roughly 30%.

(2) The dose of propranolol should be as high as possible, but should not itself produce an anorectic effect, since that would unduly complicate interpretation of the results. In preliminary studies, we found that a small (20%) but significant anorectic effect was produced by 10 mg/kg propranolol; a dose of 5 mg/kg was therefore chosen for the present study.

METHOD

Twenty-four male Lister hooded rats (weight 360–430 g) were trained to feed by pressing the door of the pellet dispenser in one of three identical operant chambers, as described above. Ten-minute daily sessions were run until all animals attained asymptotic performance. On experimental days, the animals received two intraperitoneal injections: propranolol HCl (5 mg/kg) (Sigma) was administered 60 min before the start of the session and d-amphetamine sulphate (0.5 mg/kg) (Smith, Kline and French) 30 min before. Control injections in both cases were distilled water (1 ml/kg). During experimental sessions, which were 30 min long, a computer recorded each response on the tray door, as described above. Each animal received all four treatment combinations in a counterbalanced order, at two-day intervals. On the intervening days, a 10-min session was run, with no drug treatments. Analysis of microstructural parameters of feeding was carried out as described above. Results were analysed by analysis of variance, supplemented by tests of simple main effects. The mean breakpoint in the four conditions varied between 16.8 and 18.3 sec; the differences were not significant (all F-ratios <1).

RESULTS

Amphetamine caused a small (13%) but highly significant ($p<0.001$) decrease in food intake (Fig. 4A), which was apparently blocked by propranolol pretreatment (interaction: $F(1,23)=3.2$, $0.05<p<0.1$). However, this conclusion would be seriously misleading. Total food intake may be broken down into eating rate and eating time (Figs. 4B and C), and propranolol actually increased the amphetamine-induced changes in both these parameters: eating rate was only very slightly increased by amphetamine alone, but a substantial increase was seen following propranolol pretreatment; eating time was decreased by amphetamine, and this effect was also somewhat greater following propranolol pretreatment. It is the combination of decreased eating time and increased eating rate, following propranolol pretreatment, which results in no significant net change in total intake.

A description of the distribution of behaviour within the session is given by the mean length of feeding bouts, the mean length of gaps between bouts, and the initial latency; these three parameters determine the total feeding time. Amphetamine did not significantly decrease bout length (Fig. 4D), but did significantly increase the length of gaps (Fig. 4E). Propranolol treatment blocked this effect (Fig. 4E). There were smaller, but insignificant effects on latency (Fig. 4F) (see Note 1).

Propranolol significantly increased bout length (Fig. 4D); this effect led to an increase in eating time (Fig. 4C), and is reflected in an increase in bout size (Fig. 4G), and a decrease in the number of bouts (Fig. 4H). As was the case for eating rate, propranolol increased the effect of amphetamine on bout length (Fig. 4D), bout size (Fig. 4G) and the number of bouts (Fig. 4H): on each of these measures, significant effects of amphetamine were seen following propranolol pretreatment, but amphetamine alone produced small and insignificant effects.

In both pretreatment conditions, the effect of amphetamine on eating time was significantly correlated with the change in total food intake; after propranolol pretreatment, there was also a significant negative correlation between the decrease in food intake and the increase in eating rate (Table 2). In both conditions, a significant correlation

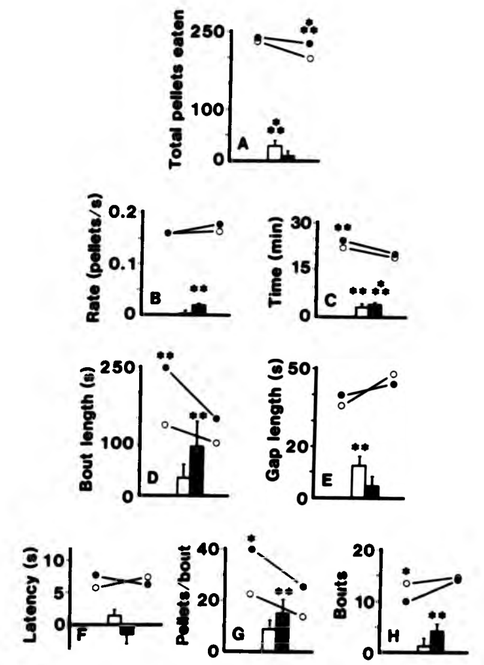

FIG. 4. Effect of amphetamine and propranolol on microstructural parameters. A: Total food intake; B: Local eating rate; C: Eating time; D: Bout length; E: Gap length; F: Latency; G: Bout size; H: Number of bouts. Circles show the scores in each condition: Left—control, right—amphetamine; white—control, black—propranolol. Bars show the difference brought about by amphetamine (mean + standard error): white—control, black—propranolol pretreatment. One star—$p<0.05$; two stars—$p<0.01$; three stars—$p<0.001$.

was seen between the increase in eating rate and the decrease in bout length (even though in the control condition there was no significant net change in either). However, changes in these parameters were uncorrelated (in one case, there was a significant negative correlation) with increases in gap length. In the control condition only, changes in gap length were significantly correlated with changes in total food intake.

GENERAL DISCUSSION

The apparent outcome of Experiment 2 was an attenuation of amphetamine anorexia by propranolol. However, it is clear from the microstructural analysis that this result is largely fortuitous, since propranolol, amphetamine and the propranolol-amphetamine combination each produced a different pattern of behavioural changes. The results reveal the wealth of information which is lost by restricting studies of feeding to measures of total food intake. The generality of the following discussion must obviously be qualified by the fact that only a single dose of each drug was tested. However, in view of the complexity of the behavioural data, it

TABLE 2

INTERCORRELATIONS BETWEEN AMPHETAMINE-INDUCED CHANGES IN MICROSTRUCTURAL PARAMETERS

	Total ↓	Rate ↑	*Time* ↓	Bout Length ↓	*Gap Length* ↑
Total ↓		−.28	.46*	.24	.47*
Rate ↑	−.45*		.63†	.45*	−.08
Time ↓	.50†	.46*		.80†	.12
Bout length ↓	.35*	.40*	.81†		
Gap length ↑	.09	−.18	−.12	−.44*	

The table shows correlations (Spearman rank-order correlation coefficients) between the changes induced by amphetamine in different microstructural parameters. *$p<0.05$; †$p<0.01$. The upper part of the table shows values obtained in control conditions; and the lower part shows values obtained following propranolol pretreatment. Arrows show the direction of change; italicized parameters were those in which significant net changes were seen.

might be noted in passing that a similar criticism could be levelled at the more standard design, in which a range of drug doses are tested against the single dependent variable, total food intake.

Propranolol did not affect eating rate, but increased bout length, and consequently, bout size and eating time. Whilst these effects did not cause a significant increase in food intake, it is clear that appropriate testing circumstances might reveal hyperphagia, and this has, in fact, been observed (see Note 2). This result is consistent with the finding that hyperphagia was caused by lesions to adrenergic systems innervating the perifornical hypothalamus [2, 20, 21], and with the concept of a beta-adrenergic satiety system.

It has been previously reported that the anorexic effect of a low dose of amphetamine (0.25 mg/kg) was caused by a selective effect on eating time with no change in eating rate [11]. The present study confirmed this observation; it was also found that the decrease in eating time was brought about primarily by an increase in the length of gaps, with no significant change in the length of eating bouts.

In contrast to the effect of amphetamine alone, after propranolol pretreatment, gap length was the only parameter (other than latency) which was not significantly altered by amphetamine. The animals showed, on the one hand, a different hypophagic effect (decreased bout length), and on the other, a hyperphagic effect (increased eating rate). As a result, there was no significant net change in food intake. Since the interaction of propranolol with amphetamine produces such contradictory effects, it is clear that, depending on the dose and specific experimental conditions, the outcome might be a decrease in the efficacy of amphetamine (the present study and ref. [27]), no change [13, 15, 17], or even an increase [13, 15, 28]. It is important, however, not to lose sight of the fact that in the present study, propranolol did block the effect underlying amphetamine anorexia, and also blocked the correlation between changes in gap length and changes in total food intake.

The starting point for the interpretation of these results is the observation that propranolol interferes with the metabolism of amphetamine [29]. To what extent may the effects of propranolol be understood as simply an increase in the dose of amphetamine? In this study, only a single dose of amphetamine, 0.5 mg/kg was tested. However, it is well established that amphetamine at 1 mg/kg significantly increases eating rate and decreases eating time [3, 4, 5, 11, 12]. It has also been reported (or it is possible to calculate from published figures) that bout length and bout size were decreased by amphetamine [4,5]. Data on the length of gaps have not previously been reported, but from published figures it is possible to calculate that amphetamine caused a substantial increase in gap length (see Note 3). The effects of propranolol are therefore consistent with a functional increase in the dose of amphetamine, with one exception: gap length. Propranolol blocked the effect of amphetamine on gap length, where an increase would be predicted from an increase in dose.

Not only was gap length the only parameter which was significantly altered by amphetamine alone, but also, this was the one parameter which was not significantly intercorrelated with all the others. The results therefore suggest the involvement of two separate mediating systems. At low doses, amphetamine induces anorexia by increasing gap length (i.e. reducing the tendency to begin eating), and at higher doses (assumed to result from propranolol pretreatment), a number of other mechanisms come into play. The anorexic effect of the low dose appears to be mediated by beta-receptors, since the increase in gap length was blocked by propranolol. The other effects appear to be dopaminergically mediated, since it has been reported that the changes in eating rate, bout length and bout size are antagonized by DA receptor blocking drugs [5,11]. It is of great relevance to the present argument that gap length was the one feeding parameter which was unaffected by the DA receptor blocker pimozide (see Note 3).

The relationship of the observed effects to the physiological control mechanisms for food intake is uncertain. The putative beta-receptor mediated effects of amphetamine and propranolol (decreases in the likelihood of starting and stopping eating, respectively), may represent direct effects on hunger and satiety mechanisms; the present methods are appropriate for further investigation of this problem. However, the putative DA-mediated effect appears less likely to be a direct satiety effect. A general theory of stimulant drug action has proposed that the effects of amphetamine may be described as an increase in the intensity of ongoing behaviour combined with an increased tendency to change behaviour [25]. This model is strongly suggested by the correlations observed in the present study between the amphetamine-induced increase in eating rate and shortening of bouts: increases in eating rate were significantly correlated with decreases in bout length, both in the control condition and also following propranolol pretreatment (Table 2). Thus, it may be that amphetamine has two anorexic effects, one genuine and the other an artefact of the stimulant effect, mediated respectively by beta-adrenergic and dopaminergic systems.

NOTES

Note 1

In general, the microstructural parameters reported in this study are comparable to those of other workers. The exception is eating latency; values reported here are some 10–25% of those in previous reports [4, 5, 10, 11, 12]. This difference might reflect the salience of the food dispenser in the present study, and also the subjects' long experience of the testing procedure. The short initial latency suggests a high degree of stimulus control by the food dispenser, which would tend to reduce disruptive drug effects.

Note 2

In most studies, propranolol reduces food intake at higher doses, probably by a non-specific sedative effect. In one study [13], however, a significant increase in food intake was observed at a dose of 4 mg/kg ($t(11)=3.2$, $p<0.01$, calculated from published figures).

Note 3

From values of latency, eating time and number of bouts, published by Blundell and Latham [5], it is possible to calculate the following figures for mean gap length: saline—76 sec; amphetamine—142 sec; pimozide—49 sec; pimozide + amphetamine—144 sec. In the present discussion, it is assumed that bout length and gap length are the primary variables, the values of which determine the number of bouts: the animal decides when to start eating and when to stop, but cannot control the number of bouts, even if it wanted to, since it does not know how long the session will last.

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REFERENCES

1. Abdallah, A. H., D. M. Roby, W. H. Boeckler and C. C. Riley. Role of DA in the stimulant effect of DITA in mice: comparisons with d-amphetamine. *Eur. J. Pharmac.* **35:** 29–34, 1976.
2. Ahlskog, J. E. Food intake and amphetamine anorexia after selective forebrain norepinephrine loss. *Brain Res.* **82:** 211–240, 1974.
3. Antelman, S. M., A. R. Caggiula, C. A. Black and D. J. Edwards. Stress reverses the anorexia induced by amphetamine and methylphenidate but not fenfluramine. *Brain Res.* **143:** 580–585, 1978.
4. Blundell, J. E. and C. J. Latham. Pharmacological manipulation of feeding behaviour: possible influences of serotonin and dopamine on food intake. In: *Central Mechanisms of Anorectic Drugs*, edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 83–109.
5. Blundell, J. E. and C. J. Latham. Characterization of adjustments to the structure of feeding behaviour following pharmacological treatment: effects of amphetamine and fenfluramine and the antagonism produced by pimozide and methergoline. *Pharmac. Biochem. Behav.* **12:** 717–720, 1980.
6. Burridge, S. L. and J. E. Blundell. Amphetamine anorexia: antagonism by typical but not atypical neuroleptics. *Neuropharmacology* **18:** 453–457, 1979.
7. Burton, M. J., S. J. Cooper and D. A. Popplewell. The effect of fenfluramine on the microstructure of feeding and drinking in the rat. *Br. J. Pharmac.* **72:** 621–633, 1981.
8. Carey, R. J. Effects of selective forebrain depletions of norepinephrine and serotonin on the activity and food intake effects of amphetamine and fenfluramine. *Pharmac. Biochem. Behav.* **5:** 519–523, 1976.
9. Carey, R. J. and E. B. Goodall. Attenuation of amphetamine anorexia by unilateral nigrostriatal lesions. *Neuropharmacology* **14:** 827–834, 1975.
10. Cooper, S. J. and R. L. Francis. Feeding parameters with two food textures after chlordiazepoxide administration, alone or in combination with d-amphetamine or fenfluramine. *Psychopharmacology* **62:** 253–259, 1979.
11. Cooper, S. J. and R. L. Francis. Interactions of chlordiazepoxide and anorectic agents on rate and duration parameters of feeding in the rat. *Psychopharmacology* **69:** 261–265, 1980.
12. Cooper, S. J. and K. F. Sweeney. Effects of spiperone alone and in combination with anorectic agents on feeding parameters in the rat. *Neuropharmacology* **19:** 997–1003, 1980.
13. Dobrzanski, S. and N. S. Doggett. The effect of propranolol, phentolamine and pimozide on drug-induced anorexia in the mouse. *Psychopharmacology* **66:** 297–300, 1979.
14. Fibiger, H. C., A. P. Zis and E. G. McGeer. Feeding and drinking deficits after 6-hydroxydopamine administration in the rat: similarities to the lateral hypothalamic syndrome. *Brain Res.* **55:** 135–148, 1973.
15. Frey, H. and S. Schulz. On the central mediation of anorexic drug effects. *Biochem. Pharmac.* **22:** 3041–3049, 1973.
16. Heffner, T. G., M. J. Zigmond and E. M. Stricker. Effects of dopamine agonists and antagonists on feeding in intact rats and 6-OHDA-treated rats. *J. Pharmac. exp. Ther.* **20:** 386–399, 1977.
17. Lehr, D. and W. Goldman. Continued pharmacological analysis of cosummatory behaviour in the albino rat. *Eur. J. Pharmac.* **23:** 197–210, 1973.
18. Leibowitz, S. F. Catecholaminergic mechanisms of the lateral hypothalamus: their role in the mediation of amphetamine anorexia. *Brain Res.* **98:** 529–545, 1975.
19. Leibowitz, S. F. Identification of catecholamine receptor mechanisms in the perifornical lateral hypothalamus and their role in mediating amphetamine and l-DOPA anorexia. In: *Central Mechanisms of Anorectic Drugs*, edited by S. Garattini and R. Samanin. New York: Raven Press, 1978, pp. 39–82.
20. Leibowitz, S. F. and L. L. Brown. Histochemical and pharmacological projections to the perifornical hypothalamus in relation to feeding inhibition. *Brain Res.* **201:** 315–345, 1980.
21. Leibowitz, S. F., N. J. Hammer and L. L. Brown. Analysis of behavioural deficits produced by lesions in the dorsal and ventral midbrain tegmentum. *Physiol. Behav.* **25:** 829–843, 1980.

22. Leibowitz, S. F. and C. Rossakis. Analysis of feeding suppression produced by periformical hypothalamic injection of catecholamines amphetamines and mazindol. *Eur. J. Pharmac.* **53:** 69–81, 1978.
23. Leibowitz, S. F. and C. Rossakis. Pharmacological characterisation of periformical hypothalamic β-adrenergic receptors mediating feeding inhibition in the rat. *Neuropharmacology* **17:** 691–702, 1978.
24. Leibowitz, S. F. and C. Rossakis. Mapping study of brain dopamine- and epinephrine-sensitive sites which cause feeding suppression in the rat. *Brain Res.* **172:** 101–113, 1979.
25. Lyon, M. and T. W. Robbins. The action of central nervous stimulant drugs: a general theory concerning amphetamine effects. In: *Current Developments in Psychopharmacology*, vol. 2, edited by W. B. Essman and L. Valzelli. New York: Spectrum, 1975, pp. 79–164.
26. Samanin, R., C. Bendotti, S. Bernasconi, E. Borroni and S. Garattini. Role of brain monomines in the anorectic activity of mazindol and d-amphetamine in the rat. *Eur. J. Pharmac.* **43:** 117–124, 1977.
27. Sanghvi, I. S., G. Singer, E. Friedman and S. Gershon. Anorexigenic effects of d-amphetamine and l-DOPA in the rat. *Pharmac. Biochem. Behav.* **3:** 81–86, 1975.
28. Schmitt, H. Influence d'agents interférant avec les catécholamines et la 5-hydroxytryptamine sur les effets anorexigènes de l'amphétamine et de la fenfluramine. *J. Pharmac.* **4:** 285–294, 1973.
29. Shoeman, D. W., C. R. Sirtori and D. L. Azarnoff. Inhibition of amphetamine tolerance and metabolism by propranolol. *J. Pharmac. exp. Ther.* **191:** 68–71, 1974.
30. Slater, P. J. B. The temporal pattern of feeding in the zebra finch. *Anim. Behav.* **22:** 506–515, 1974.
31. Wiepkema, P. R. Behavioural factors in the regulation of food intake. *Proc. Nutr. Soc.* **30:** 142–149, 1971.
32. Willner, P. and T. Montgomery. Attenuation of amphetamine anorexia in rats following subchronic treatment with tricyclic antidepressant. *Communs Psychopharmac.* **4:** 101–106, 1980.
33. Zis, A. P. and A. P. Fibiger. Neuroleptic-induced deficits in food and water regulation: similarities to the lateral hypothalamic syndrome. *Psychopharmacology* **43:** 63–68, 1975.

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CHANGES IN AMPHETAMINE-INDUCED ANOREXIA AND STEREOTYPY DURING CHRONIC TREATMENT WITH ANTIDEPRESSANT DRUGS

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Amphetamine-induced anorexia and stereotyped behaviour were studied in rats, following pretreatment with the antidepressants DMI, inprindole and mianserin. A complex drug-dependent and dose-dependent pattern of results was obtained. Acute pretreatment with DMI and iprindole enhanced amphetamine anorexia and stereotypy; at high doses only, the enhancement of anorexia disappeared during chronic treatment. Mianserin had no effects acutely, but chronic treatment with high doses attenuated anorexia and enhanced stereotypy. High doses of all three drugs attenuated anorexia and enhanced stereotypy during withdrawal. The most parsimonious account of these results is that the acute affects of DMI and iprindole are artefactual, and that chronic administration of all three antidepressants increased dopaminergic function and decreased β-adrenergic function.

Catecholamine hypothesis of depression
Stereotyped behaviour
Dopamine
Amphetamine
Iprindole
Desmethylimipramine
Mianserin
Noradrenaline
Anorexia

1. Introduction

As originally formulated, the catecholamine hypothesis of depression proposed that depression resulted from a deficiency of noradrenaline (NA) at certain functionally important synapses in the central nervous system, and that the action of antidepressant treatments was to increase noradrenergic activity (Schildkraut, 1965). This view was based in part on the well-established observation that tricyclic antidepressant drugs enhance NA function, by, blocking reuptake of NA from the synapse. The development of two new non-tricyclic antidepressant drugs challenged this concept: mianserin was found to have only slight effects on NA uptake, whilst iprindole appeared to be almost totally ineffective (Zis and Goodwin, 1979). Subsequently, however, it was established that these drugs are antagonists at presynaptic α_2-receptors. They also, therefore, enhance NA activity, by disinhibiting NA neurons and thereby increasing NA release (Baumann and Maitre, 1976; Hendley, 1978).

A different challenge to the catecholamine hypothesis came from studies of chronic antidepressant treatment. It is now well-established that a number of parameters of NA function are reduced, rather than increased, by chronic antidepressant treatments, most notably, the sensitivity of postsynaptic β-receptors (Sulser, 1978). These findings have led to a reformulation of the catecholamine hypothesis, in terms diametrically opposed to the original: in this view, depression is seen to result from hyperactivity at NA synapses, which is 'downregulated' by antidepressants (Segal et al., 1974; Sulser, 1978).

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When attempting to adjudicate between these two approaches, it is essential to know the net effect of antidepressants at β-adrenergic synapses: since antidepressants have two opposed effects at NA synapses, the outcome might be an increase, a decrease or no change in the efficacy of the synapse, depending upon which effects predominate. A hypothesis concerning the mechanism of clinical action of antidepressants must incorporate the solution to this problem. However, it is clear that the problem cannot be solved by methods which assess single parameters of synaptic activity (such as receptor assays), but only by methods which assess the integrated activity of the synapse (Maas, 1981). We have previously suggested that one approach might be to use behaviour mediated by NA pathways for this purpose (Willner and Montgomery, 1980a, 1981; Willner et al., 1981).

Amphetamine-induced anorexia is a behaviour which appears to have a central noradrenergic component, since this effect of amphetamine is partially attenuated by lesions to fibres of the ventral NA bundle (Ahlskog, 1974; Leibowitz and Brown, 1980; Samanin et al., 1978). In previous studies, amphetamine anorexia was found to be enhanced by acute pretreatment with the tricylic antidepressant desmethylimipramine (DMI), and attenuated during withdrawal from chronic treatment. However, during the course of chronic treatment, neither enhancement nor attenuation were seen (Willner and Montgomery, 1980b, 1981). These findings suggested that during chronic DMI treatment, the NA-enhancing and NA-reducing effects of DMI exactly cancelled out. In the present paper, this conclusion is reassessed, using a wider range of drugs and doses than those used previously.

The use of amphetamine-induced behaviours to test for effects of antidepressants is problematic, owing to the fact that a large number of antidepressants, including DMI and iprindole (but excluding mianserin), which were used in the present study, impair the metabolism of amphetamine (e.g. Lemberger et al., 1970). This problem does not arise when tests are carried out during drug withdrawal following a wash-out period, but makes interpretation of results obtained during chronic drug treatment extremely difficult. Nonetheless, it seemed important to make the attempt, in view of the uncertainty over the net effects of antidepressants at NA synapses, the clinical significance of this controversy, and the absence of any other well-established behavioural test for β-receptor activation (Davis, 1982). The strategy employed has been to use effects observed during withdrawal to guide the interpretation of results obtained during drug treatment.

We have previously suggested (Willner and Montgomery 1980a, 1981) that the enhancement of amphetamine anorexia by acute antidepressants does not, in fact, result significantly from a metabolic interaction between the two drugs, as a result of the observation that DMI appeared to potentiate the maximal anorexic effect of amphetamine, but not to prolong the effect. This conclusion arose from an experiment in which no anorexia was seen in the final 2.5 h of a 3 h feeding session (Willner and Montgomery, 1981, experiment 4). In the light of results obtained in the present study, this issue was also re-examined.

After the experiments reported here were completed, a paper appeared which suggested that amphetamine anorexia at low doses might, in fact, be a peripheral phenomenon, possibly mediated by hepatic glycogenolysis, since the effect was blocked by interruption of the sympathetic nervous innervation of the viscera (Tordoff et al., 1982). However, this study used undeprived animals. It is unlikely that a hepatic effect would contribute markedly to amphetamine anorexia in deprived animals, since liver glycogen stores run down within the first few hours of fasting (Tordoff, pers. comm.) Moreover, it is well established that the anorexic effect of peripherally administered amphetamine may be abolished by hypothalamic lesions, (e.g. Russek et al., 1973), by lesions of the ventral NA bundle (see above), or by administration of the β-blocker propranolol to the lateral hypothalamus (Leibowitz, 1975). Since there is no evidence that peripheral effects do, in fact contribute to amphetamine anorexia in food-deprived animals, the observations of Tordoff et al. (1982) do not preclude the use of amphetamine anorexia as an index of central β-receptor activation.

2. Materials and methods

2.1. Subjects

Adult, male, Lister hooded rats (Olac, Bicester, England), weighing 300–400 g, singly housed in plastic cages, and maintained on a 12-h light/dark cycle (light period 09:00–21:00), were used in all experiments. They were allowed access to a weighed amount of lab chow (Fisons) from 13:00 to 16:00 daily; food was removed briefly for weighing at 13:30. Water was freely available at all times. Spillage of food appeared to be minimal; any spilled food which was noticed was also removed and weighed.

2.2. Drugs

The following drugs were used; desmethylimipramine hydrochloride (Geigy, Macclesfield, England); iprindole hydrochloride (Wyeth, Taplow, England); mianserin hydrochloride (Beecham, Epsom, England); d-amphetamine sulphate (Smith, Kline and French, Welwyn Garden City, England). Drugs were dissolved in distilled water, which was also used for control injections; doses were calculated as the salts. All injections were intraperitoneal, in a volume of 1 ml/kg.

2.3. Procedure

Antidepressants were administered at approximately 17.00 h. In feeding studies, with the exception of experiment 8, amphetamine (0.5 mg/kg) was administered 30 min prior to feeding (i.e. 19–20 h following antidepressant pretreatment). All food intake results refer to the first 30 min of the 3-h feeding session. In acute studies, animals received either a single antidepressant pretreatment followed by amphetamine, or the antidepressant followed by a control injection; both procedures were administered, in a counter-balanced order, with at least two drug-free days intervening. In chronic studies, antidepressants were administered daily, and the anorexic effect of amphetamine was assessed in relation to food intake on the preceding day. Anorexia was measured as food intake (g) on the control day minus food intake (g) on the test day. In some experiments, amphetamine stereotypy was also tested: amphetamine (3 mg/kg) was administered at approximately 09:30 h, and seven blind, half-hourly ratings of stereotypy were made, using the 7-point scale of Creese and Iversen (1973), beginning 30 min after the injection.

Amphetamine doses and experimental procedures were based on those used previously (Willner and Montgomery, 1980b, 1981). Under these experimental conditions, 0.5 mg/kg amphetamine produces an anorexic effect of approximately 30–40%, and 3 mg/kg produces a moderate stereotypy (3–4 on the Creese and Iversen scale) lasting 1.5–2.5 h. In each case, both increases and decreases in the response may be readily observed.

A total of seven experiments were carried out using these procedures (one DMI, four iprindole, two mianserin). Where both acute and chronic studies were carried out in the same animals, the acute tests were run first, and at least three drug-free days were allowed before beginning the chronic study. At least two amphetamine-free days intervened between a stereotypy test and a subsequent anorexia test. Chronic treatment lasted between two and three weeks, and tests were also carried out for up to two weeks following withdrawal. Full details of numbers, doses and the sequence of tests are shown in table 1.

In the final experiment (table 1, Expt. 8) the effects of varying the time of amphetamine administration were studied. Following pretreatment (19.5–20.5 h) with DMI or distilled water, amphetamine (0.5 mg/kg) or distilled water was administered to three groups of animals, either 30, 60 or 90 min before food presentation. All four drug combinations were presented, in a counterbalanced order, at three-day intervals.

Anorexia scores (see above), food intake on anorexia control days, and the means of the series of seven stereotypy ratings, were subjected to two-way analysis of variance (with drug doses as a between-groups factor and amphetamine tests as a within-groups factor), followed, where appropriate, by post-hoc t-tests; two-tailed tests were used throughout. We have consistently observed that taking the mean of a series of stereotypy ratings produces data which are approximately

TABLE 1

Experimental details.

Experiment	Drug	n	Doses (mg/kg)	Days of chronic treatment	Anorexia test days [a]		Stereotypy test days [a]
(1)	DMI	10	0, 2.5, 7.5	17	A 7, 12, 17	4W, 9W, 12W	A 14 6W
(2)	IPR	10	0, 15	–	A		
(3)	IPR	8	0, 7.5	14	7, 11, 14	4W, 7W	
(4)	IPR	12	0, 7.5, 20	14	7, 14	4W, 6W, 8W, 10W	
(5)	IPR	16	0, 15	20	14, 18	5W, 7W, 11W	20 8W
(6)	MIAN	12	0, 3, 10	17	A 7, 12, 17	4W, 9W, 12W	A 14 6W
(7)	MIAN	12	0, 15	21	A 7, 12, 14, 21	5W, 10W, 13W	A 6W
(8)	DMI	12	0, 7.5	–	A [b]		

[a] A, acute test; W, withdrawal. [b] Variable amphetamine pretreatment time.

normally distributed, and therefore suitable for parametric statistics. Four animals receiving the highest dose of iprindole stopped eating during chronic treatment, lost weight, and died; all data obtained from these animals were discarded. The results of this experiment were analyzed in two ways: in one analysis, data were estimated for the missing animals, while in the second, four animals were randomly discarded from each of the other groups; the two methods of analysis gave similar results.

3. Results

DMI and iprindole showed similar patterns of results. Amphetamine anorexia was enhanced by acute pretreatment with both drugs. At lower doses (2.5 mg/kg DMI and 7.5 mg/kg iprindole), the enhancement was maintained throughout chronic treatment, but absent during withdrawal. However, at higher doses (7.5 mg/kg DMI and 15–20 mg/kg iprindole), the enhancement of anorexia disappeared during chronic treatment, and significant attenuation of anorexia was seen during withdrawal (figs. 1 and 2: the results are expressed a little differently in the two figures owing to the need to combine the results of four separate experiments in fig. 2). Except on amphetamine days, food intake did not change significantly during the course of the experiments, and did not differ significantly between antidepressant-treated and untreated animals.

During the period of drug treatment, both drugs caused a large enhancement of amphetamine-induced stereotypy; at the higher doses, this effect, though substantially reduced, was also seen during withdrawal (table 2). Additionally, at the higher

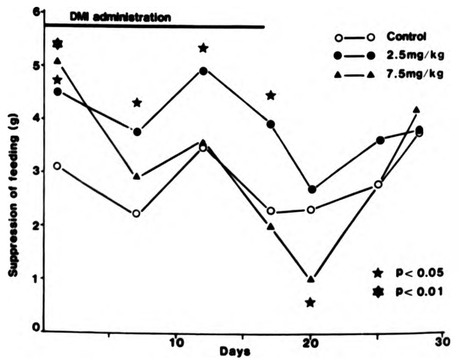

Fig. 1. Effects of DMI on amphetamine anorexia (n = 10). The period of DMI administration is shown by the bar above the figure. Stars refer to significant differences between DMI-treated and control animals.

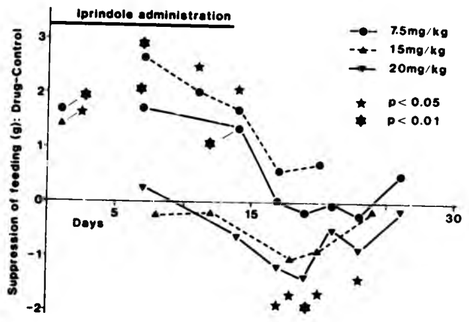

Fig. 2. Effects of iprindole on amphetamine anorexia. Four separate experiments were carried out: the acute (one-day) experiment (n = 10), and three chronic experiments denoted by the solid (n = 12), dashed-circles (n = 8) and dashed-triangles (n = 16) lines. In order to combine the four sets of results without loss of clarity, the results have been expressed as the difference between the anorexic effects shown by drug-pretreated and control animals (i.e. the corresponding control mean has been subtracted from every iprindole mean). Stars refer to significant differences between iprindole-treated and control animals (i.e. significant deviations from zero). The period of iprindole administration is shown by the bar at the top of the figure. In one experiment (dashed line-triangles) there were an extra 6 days of drug treatment (not shown) prior to the first amphetamine test.

dose of DMI, chronic pretreatment caused significantly more stereotypy than acute pretreatment; this comparison could not be made for iprindole, as no acute test was run. As previously observed (Willner and Montgomery, 1981), examination of the frequency distribution of each of the half-hourly stereotypy ratings did not reveal any qualitative differences between untreated and antidepressant-pretreated animals.

A different pattern of results was seen with mianserin. Acute mianserin pretreatment had no significant effects on amphetamine anorexia, but at the higher doses (10 and 15 mg/kg), a significant attenuation of anorexia developed during chronic treatment, and carried over into withdrawal (fig. 3). As these results differ from those previously described, the results of the two experiments are presented in full, rather than combining them into a single figure, and expressed both as absolute scores and difference scores, to facilitate comparison with fig. 1 (fig. 3, solid lines) and fig. 2 (fig. 3, dashed lines). Again, there were no significant changes in food intake on control days. Amphetamine stereotypy did not differ significantly between untreated and mianserin-pretreated animals. However, the higher doses did

TABLE 2

Effect of antidepressant pretreatment on amphetamine-induced stereotypy

Values are the means of 7 half-hourly stereotypy ratings. The figures above each panel refer to: length of treatment (days); day of chronic test; day of withdrawal test; number of subjects.

	DMI			Iprindole	
See caption	17;14;6;10			20;20;8;16	
Dose (mg/kg)	0	2.5	7.5	0	15
Acute	1.87	3.09 [a]	3.83 [a]	–	–
Chronic	1.80	3.37 [a]	4.21 [a,c]	2.23	3.62 [a]
Withdrawal	1.93	2.43	2.64 [a]	1.88	3.13 [a]

	Mianserin				
See caption	17;14;6;12			21;–;6;12	
Dose (mg/kg)	0	3	10	0	15
Acute	1.70	1.75	1.59	2.08	1.99
Chronic	1.81	1.89	1.87 [b]	–	–
Withdrawal	1.83	1.83	1.93 [b]	2.29	2.56 [c]

[a] Increased at $P < 0.01$, relative to control. [b] Increased at $P < 0.05$, relative to acute test. [c] Increased at $P < 0.01$, relative to acute test.

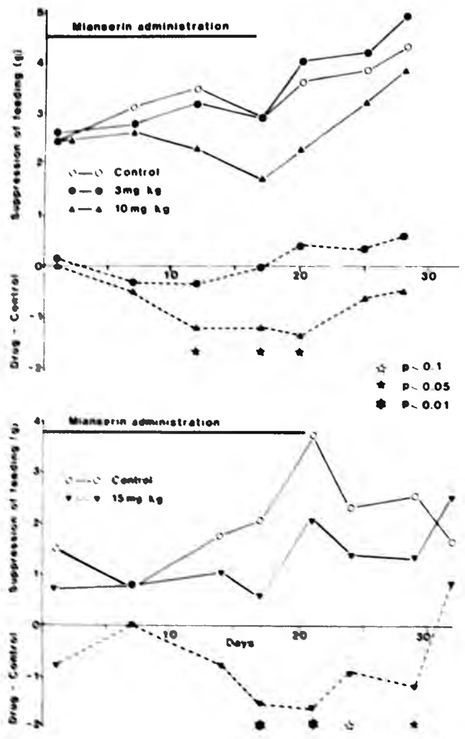

Fig. 3. Effects of mianserin on amphetamine anorexia (n = 12). The results of two separate experiments are shown. The upper part of each figure (solid lines) shows the anorexic effect of amphetamine, as in fig. 2; the lower part (dashed lines) shows the differences between drug pretreated and control animals, as in fig. 3. The period of mianserin administration is shown by the bar at the top of each figure. Stars refer to significant differences between mianserin-treated and control animals.

produce a small, but significant increase in amphetamine stereotypy during chronic treatment and withdrawal, in relation to the effect of acute pretreatment (table 2).

As previously observed (Willner and Montgomery, 1980b) the anorexic effect of amphetamine was variable within a group of animals from trial to trial. In one experiment, untreated animals showed a small increase in sensitivity to amphetamine with repeated testing (fig. 3, upper panel). In general, however, no consistent changes were observed in untreated animals (fig. 1; fig. 3, lower panel; iprindole experiments not shown). Similarly, no significant or consistent change was shown by untreated animals in their stereotypy response to the higher dose of amphetamine, on repeated testing (table 2, all four panels).

Except at the lowest dose of each antidepressant, chronically treated animals receiving DMI or iprindole lost weight relative to controls, and by the end of drug treatment were 10–15% lighter. No weight changes were seen in mianserin-treated animals.

When the time of amphetamine administration was varied (expt. 8), acute DMI pretreatment was found to enhance the anorexic effect significantly at all amphetamine pretreatment intervals. At the longest interval (90 min), DMI pretreated animals showed a substantial anorexic effect (mean 2.1 g. cf. figs. 1 and 3), compared with a very slight anorexia (0.6 g) in untreated animals (difference: $P < 0.01$).

4. Discussion

Results obtained with the higher dose of DMI confirm our previous finding of attenuated amphetamine anorexia during DMI withdrawal (Willner and Montgomery, 1980b, 1981); this observation is now extended to iprindole and mianserin. As drugs were administered on a mg/kg basis, it is conceivable that the difference in weight between drug-treated and control animals might account in part for the observed attenuation of amphetamine anorexia. We have addressed this question in a previous study, and shown that anorexia was attenuated during DMI withdrawal even after an experimental manipulation which eliminated the weight differential (Willner and Montgomery, 1980b, expt. 3). In the present study, attenuation of anorexia was also seen in mianserin-treated animals, in the absence of any significant weight loss. Moreover, since the attenuation of amphetamine anorexia is accompanied by an increased stimulation of locomotor activity, (Willner and Montgomery, 1981, expt. 2) it is unlikely

that this effect results from a reduction in competing behaviours. Increased stereotypy, observed during withdrawal from all three drugs, has been previously reported by a number of authors, following chronic antidepressant drugs or ECT, though this effect has not been universally observed (see Willner, 1983 for review).

The major new observations to emerge from the present study are the differences between low and high doses of DMI and iprindole, in their effects on amphetamine anorexia, and the attenuation of amphetamine anorexia which developed during the course of chronic mianserin administration.

A framework for interpreting the results of these experiments is given by the models shown in fig. 4. The models have two components: the acute components (enhancement of anorexia and stereotypy) are present during the period of drug administration; the chronic components (attenuation of anorexia and enhancement of stereotypy) develop slowly during chronic treatment, and persist following withdrawal; the combination of these two components results in there being no significant net change in anorexia and a further increase in stereotypy during chronic treatment. Within this framework, the results may be summarised as follows: high doses of DMI and iprindole show both components, low doses of DMI and iprindole show the acute components only, and high doses of mianserin show the chronic components only.

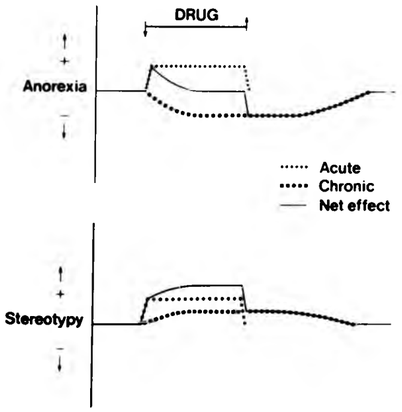

Fig. 4. Two-component models of the effects of antidepressants on amphetamine-induced anorexia and stereotypy. The acute components are present during the period of drug administration; the chronic components develop slowly, and persist following withdrawal.

In the case of amphetamine stereotypy, identification of the two components is relatively straightforward. A number of effects of amphetamine are known to be enhanced by acute pretreatment with DMI and iprindole, but not mianserin (e.g. Van Riezen, 1972), and it has been demonstrated many times that the mechanism of these effects is the impairment of amphetamine metabolism by the liver, which effectively increases the dose of amphetamine reaching the brain. In other words, the acute component is artefactual. This artefact cannot, however, explain the chronic component, which is shared by DMI and mianserin. Amphetamine stereotypy is known to be mediated by the dopaminergic (DA) nigrostriatal pathway (Kelly et al., 1975); the chronic component probably represents a change in DA function. In addition to increases in stereotyped behaviour, a number of other behavioural and physiological changes suggesting that chronic antidepressant treatments increase DA function have also been reported recently (see Willner (1983) for review). The present results are consistent with these findings, and extend them by the observation that in addition to the effects during withdrawal, increased stereotypy was also present during the course of chronic treatment. It is not yet clear whether putative antidepressant-induced increases in DA function represent direct effects on DA neurons or indirect effects, mediated perhaps by concomittant changes in NA (Antelman and Caggiula, 1977; Green and Deakin, 1980). There is considerable evidence from binding studies that antidepressants do not increase the sensitivity or number of DA receptors (Charney et al., 1981).

In view of the fact that DA function appears to be increased by chronic antidepressant administration, whereas amphetamine anorexia has been shown to be attenuated by treatments which specifically reduce DA function (e.g. Burridge and Blundell, 1979; Leibowitz, 1975; Leibowitz and Rossakis, 1978; Samanin et al., 1977), it seems most unlikely that the chronic component of the

anorexia model is mediated by DA. Since it is well established that chronic treatment with many antidepressants, including DMI, iprindole and mianserin, induces subsensitivity of central β-receptors (Sulser, 1978; Wolfe et al., 1978), it seems more likely that this component represents a β-receptor-mediated decrease in the functional efficacy of the ventral NA bundle, although the possibility of a peripheral effect cannot be excluded (Tordoff et al., 1982). Amphetamine anorexia has been shown to be attenuated by lesions to the ventral NA bundle (Ahlskog, 1974; Garattini et al., 1978; Hoebel, 1979; Leibowitz and Brown, 1980; Samanin et al., 1978), and by peripheral (Sanghvi et al., 1978; Willner and Towell, 1982) and central (Leibowitz, 1975, 1978; Leibowitz and Rossakis, 1978) administration of β-blockers. An alternative hypothesis, which would also be compatible with the present results is that antidepressant-induced changes in amphetamine anorexia might reflect an increase in postsynaptic α-adrenergic function (Charney et al., 1981; Davis, 1982).

The acute component of the anorexia model has previously been taken to represent the enhancement of β-adrenergic transmission by acute antidepressant treatment (Willner and Montgomery, 1981). However, two aspects of the present results suggest that this may not be the case. Firstly, since the 'down-regulation' of β-receptors by antidepressants has been shown to be an indirect effect, mediated by an increase in NA levels (e.g. Wolfe et al., 1978), it would be expected that similarly sized increases in NA on acute treatment would result in similar chronic compensatory effects. A similar enhancement of anorexia was seen at low and high doses of DMI and iprindole. However, only the high doses caused the subsequent amelioration and reversal of this effect. Secondly, although mianserin is more potent that iprindole in blocking α_2-receptors, and thereby enhancing NA release (Baumann and Maitre, 1975), iprindole did enhance anorexia, whereas mianserin did not. An alternative hypothesis to explain the acute enhancement of anorexia by DMI and iprindole, but not mianserin, is that, like stereotypy, the enhancement of anorexia results from the metabolic artefact described above. This possibility has previously been discounted (Willner and Montgomery, 1981), on the grounds that typically, the inhibition of amphetamine metabolism results primarily in a prolongation of the effects of amphetamine, which did not appear to be the case for the enhancement of anorexia. However, this conclusion of our previous study now appears to have arisen from the use of an inappropriate method: the results of expt. 8 show clearly that amphetamine anorexia was not only increased, but also prolonged by DMI pretreatment. The hypothesis that the enhancement of amphetamine anorexia by acute antidepressant administration is primarily a metabolic artefact is supported by a further observation: potentiation of anorexia by DMI was minimal when amphetamine was applied directly to the perifornical hypothalamus (Towell and Willner, in preparation).

The most parsimonious account of the present results would therefore appear to be that both the acute components of the model shown in fig. 4 are artefactual, whilst the chronic components represent an increase in DA function (stereotypy) and a decrease in β-adrenergic function (anorexia). If it is accepted that the acute effects on amphetamine-induced anorexia are artefactual, it may be concluded that during the course of two to three weeks treatment at high but not low doses, DMI, iprindole and mianserin all reduced amphetamine anorexia. The results are therefore consistent with the hypothesis that chronic antidepressant treatment may cause a net reduction in the output of the relevant β-adrenergic synapses. A similar conclusion was reached by studies which have addressed the problem electrophysiologically, by recording the activity of cells in hippocampus and cerebellum which receive a β-adrenergic input (Bloom et al., 1979; Huang, 1979; Huang et al., 1980). The relevance of these findings to the clinical effect of antidepressants is, of course, another question.

Clearly, the results obtained in these experiments provide very indirect and inferential evidence concerning brain function, which could be subject to a number of alternative explanations. The interpretation presented here is offered as a parsimonious explanation, which is consistent with the literature. Further, more direct, insight into whether chronic antidepressants produce a net decrease in β-adrenergic function should be forthcoming from experiments employing intracranial amphetamine administration, which are currently in progress.

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References

Ahlskog, J.E., 1974, Food intake and amphetamine anorexia after selective forebrain norepinephrine loss, Brain Res. 82, 211.

Antelman, S.M. and A.R. Caggiula, 1977, Norepinephrine-dopamine relations and behaviour, Science 195, 646.

Baumann, P.A. and L. Maitre, 1976, Effects of alpha receptor blockers and antidepressant drugs on the presynaptic alpha receptor of rat cerebral cortex, Naunyn-Schmiedeb. Arch. Pharmacol. 293, Suppl.

Bloom, F.E., G.R. Siggins, J.E. Schultz and S.J. Henriksen, 1979, Catecholamine transmitter systems, in: The Kinetics of Psychiatric Drugs, J.L. Schoolar and J.L. Claghorn (eds.), p 63, Brunner/Mazel, New York.

Burridge, S.L. and J.E. Blundell, 1979, Amphetamine anorexia: antagonism by typical but not atypical neuroleptics, Neuropharmacol. 18, 453.

Charney, D.S., D.B. Menkes and G.R. Heninger, 1981, Receptor sensitivity and the mechanism of action of antidepressant treatment: implications for the etiology and treatment of depression, Arch. Gen. Psychiat. 38, 1160.

Creese, I. and S.D. Iversen, 1973, Blockade of amphetamine-induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine, Brain Res. 55, 369.

Davis, M., 1982, Agonist-induced changes in behaviour as a measure of functional changes in receptor sensitivity following chronic antidepressant treatments, Psychopharmacol. Bull. 18, 137.

Garattini, S., E. Borroni, T. Mennini and R. Samanin, 1978, Differences and similarities among anorectic agents, in: Central Mechanisms of Anorectic Drugs, S. Garattini and R. Samarin (eds.), p. 127, Raven Press, New York.

Green, A.R. and J.F.W. Deakin, 1980, Brain noradrenaline depletion prevents ECS-induced enhancement of serotonin- and dopamine-mediated behaviour, Nature 285, 232.

Hendley, E.D., 1978, Iprindole is a potent enhancer of spontaneous and KCl-induced efflux of norepinephrine from rat brain slices, Soc. Neurosci. Abstr. 4, 494.

Hoebel, B.G., 1979, Hypothalamic self-stimulation and stimulation escape in relation to feeding and mating, Fed. Proc. 36, 2454.

Huang, Y.H., 1979, Chronic desipramine treatment increases activity of noradrenergic postsynaptic cells, Life Sci. 25, 709.

Huang, Y.H., J.W. Maas and G.H. Hu, 1980, The time course of noradrenergic pre- and postsynaptic activity during chronic desipramine treatment, European J. Pharmacol. 68, 41.

Kelly, P.H., P.W. Seviour and S.D. Iversen, 1975, Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum, Brain Res. 24, 507.

Leibowitz, S.F., 1975, Catecholaminergic mechanisms of the lateral hypothalamus: their role in the mediation of amphetamine anorexia, Brain Res. 98, 529.

Leibowitz, S.F. and L.L. Brown, 1980, Histochemical and pharmacological analysis of catecholaminergic projections to the lateral hypothalamus in relation to feeding inhibition, Brain Res. 201, 315.

Leibowitz, S.F. and C. Rossakis, 1978, Analysis of feeding suppression produced by perifornical hypothalamic injection of catecholamines, amphetamine and mazindol, European J. Pharmacol. 53, 69.

Lemberger, L., E. Sernatinger and R. Kuntzman, 1970, Effect of desmethylimipramine, iprindole and DL-erythro-α (3,4-dichlorphenyl)-β-(t-butyl amino) propanol HCl on the metabolism of amphetamine, Biochem. Pharmacol. 19, 3021.

Maas, J.W., 1979, Neurotransmitters and depression: too much, too little or too unstable?, Trends Neurosci. 2, 306.

Russek, M., A.M. Rodriguez-Zendejas and P. Teitelbaum, 1973, The action of adrenergic anorexigenic substances on rats recovered from lateral hypothalamic lesions, Physiol.Behav. 10, 329.

Samanin, R., Bendotti, C., Bernasconi, S. and R. Pataccini, 1978, Differential role of brain monoamines in the activity of anorectic drugs, in: Central Mechanisms of Anorectic Drugs, S. Garattini and R. Samanin (eds.), p. 233, Raven Press, New York.

Samanin, R., C. Bendotti, E. Borroni and S. Garattini, 1977, Role of brain monoamines in the anorectic activity of mazindol and d-amphetamine in the rat, European J. Pharmacol. 43, 117.

Sanghvi, I.S., G. Singer, E. Friedman and S. Gershon, 1975, Anorexigenic effects of d-amphetamine and l-DOPA in the rat, Pharmacol. Biochem. Behav. 3, 81.

Schildkraut, J.J., 1965, The catecholamine hypothesis of affective disorders: a review of supporting evidence, Am. J. Psychiatry 130, 509.

Segal, D.S., R. Kuczenski and A.J. Mandell, 1974, Theoretical implications of drug-induced adaptive regulation for a biogenic amine hypothesis of affective disorder, Biol. Psychiatry 9, 147.

Sulser, F., 1978, Mode of action of antidepressant drugs, Biochem. Pharmacol. 27, 257.

Tordoff, M.G., J. Hopfenbeck, L.L. Butcher and D. Novin, 1982, A peripheral locus for amphetamine anorexia, Nature 297, 148.

Van Riezen, H., 1972, Different effects of the 5-HT antagonists mianserin and cyproheptadine, Arch. Int. Pharmacolyn. Ther. 198, 256.

Willner, P., 1983, Dopamine and depression: A review of recent evidence. III. The effects of antidepressant treatments, Brain Res. Rev. 6, 237.

Willner, P. and T. Montgomery, 1980a, Neurotransmitters and depression: too much, too little, too unstable – or not unstable enough?, Trends Neurosci. 3, 201.

Willner, P. and T. Montgomery, 1980b, Attenuation of amphetamine anorexia in rats following subchronic treatment with a tricyclic antidepressant, Commun. Psychopharm. 4, 101.

Willner, P. and T. Montgomery, 1981, Behavioural changes during withdrawal from desmethylimipramine (DMI). I. Interactions with amphetamine, Psychopharmacology 75, 54.

Willner, P., T. Montgomery and D. Bird, 1981, Behavioural changes during withdrawal from desmethylimipramine (DMI). II. Increased resistance to extinction, Psychopharmacology 75, 60.

Willner, P. and A. Towell, 1982, Microstructural analysis of the involvement of beta-receptors in amphetamine anorexia, Pharmacol. Biochem. Behav. 17, 255.

Wolfe, B.B., K. Harden, J.R. Sporn and P.B. Molinoff, 1978, Presynaptic modulation of beta-adrenergic receptors in rat cerebral cortex after treatment with antidepressants, J. Pharmacol. Exp. Ther. 207, 446.

Zis, A.P. and F.K. Goodwin, 1979, Novel antidepressants and the biogenic amine hypothesis of depression. The case for iprindole and mianserin, Arch. Gen. Psychiat. 36, 1097.

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