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Studies into Amphetamine-Induced Unconditioned Behaviour in the Rat.

McHale, Susan Lesley

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S. L. McHALE

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Studies into Amphetamine-Induced Unconditioned Behaviour in the Rat.

by

Susan Lesley McHale

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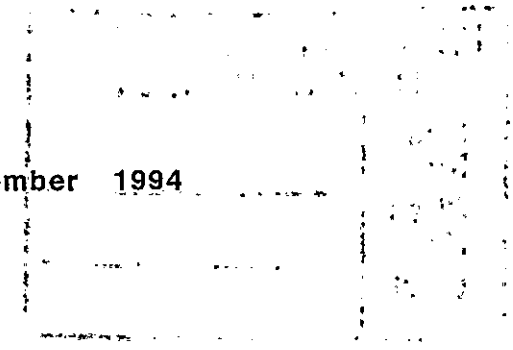

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Susan Lesley McHale.

Abstract.

Previous work on the unconditioned effects of amphetamine in rats has examined qualitative changes in behaviours which become stereotyped and quantitative changes in locomotion. Stereotyped behaviours have been adopted as a model of raised caudate-putamen function whilst locomotion has been adopted as a model of raised mesolimbic dopamine function. These models have been used to study drugs which are effective in the treatment of schizophrenia. Only locomotion is reliably antagonised by all classes of antipsychotic drugs, although it has been hypothesised that, under some doses of amphetamine, locomotion may also become stereotyped. The Lyon-Robbins hypothesis of the behavioural effects of amphetamine predicts competition between the output of the mesolimbic and caudate-putamen, and would predict that stereotyped locomotion represents a 'blending' of mesolimbic and caudate-putamen behavioural output.

An experiment was conducted to test the Lyon-Robbins hypothesis using contrast-based image analysis to determine the spatio-temporal characteristics of open-field locomotion. A further four experiments examined the effects of a classic antipsychotic (haloperidol), the atypical antipsychotics (clozapine and sulpiride) and a putative antipsychotic (a 5-HT3 antagonist, ondansetron) on open-field locomotor routes taken by rats following treatment with 3.5mg/kg amphetamine.

Measures of stereotyped locomotion derived from image analysis were supported by a novel form of behavioural analysis based on multi-dimensional scaling which provided an integrated analysis of behavioural change following drug treatment. Haloperidol blocked locomotion and stereotyped behaviours including stereotyped locomotion, whereas clozapine, sulpiride and ondansetron blocked locomotion but not stereotyped locomotion and in some cases increased stereotyped behaviours. This suggests that stereotyped locomotion represents synergistic functioning of both mesolimbic and caudate-putamen systems, when the output from the caudate-putamen is insufficient to over-ride that of the mesolimbic system. Antagonism of a 5-HT3 enhancement of mesolimbic locomotor activity by ondansetron allowed latent 5-HT and dopamine mediated behaviours to be expressed. This effectively mimicked a leftwards shift of the amphetamine dose response curve, hypothesised as amplification of the caudate-putamen output. These findings lend support to the Lyon-Robbins hypothesis of the behavioural effects of amphetamine.

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Chapter One
Introduction

Preface

Animal experiments on the functional correlates of brain dopaminergic transmission have resulted in the rapidly growing area of psychopharmacology. The use of specific and selective chemical agents to manipulate normal brain function has provided the basis for research into dopamine and the changes in behaviour that result from this selective interference. Animal models based on these manipulations has been of great value in the development of new drugs for the treatment of Parkinsonism and schizophrenia.

Studies into the treatment of schizophrenia have examined the antagonism of amphetamine-induced behaviours in animals as a model of raised striatal and mesolimbic dopamine function to study the underlying mechanisms of antipsychotic drug treatment, and to detect novel antipsychotic agents.

In the past attention has focused on amphetamine-induced behaviours such as sniffing, licking and gnawing because these behaviours are reliably antagonised by classic antipsychotics used in the treatment of schizophrenia, such as haloperidol and chlorpromazine (Szechtman et al. 1988). However, it is now clear that a group of clinically effective drugs - so-called atypical antipsychotics - do not antagonise all the components of the amphetamine syndrome. Both classic and atypical antipsychotics share the ability to antagonise amphetamine-induced hyperactivity, and this may form the basis of a more appropriate test for antipsychotic potential (Rebec and Bashore, 1984; Ljungberg and Ungerstedt, 1985).

The development of a model based on amphetamine-induced increases in locomotion (so-called hyperactivity) may not adequately address the problem of providing a clear, well defined animal model for antipsychotic drug screening, as

all aspects of this amphetamine-induced behaviour have not been adequately investigated. To date much of the characterisation of drug effects on locomotor activity in rats is based solely on quantitative increases and pays scant regard to the qualitative changes which occur. It has been known for some time that locomotion may have stereotyped characteristics under some doses of amphetamine therefore the development of a model which incorporates the spatio-temporal changes in locomotion which occur following treatment with amphetamine may provide better insight into the underlying mechanisms of antipsychotic drug action, and the amphetamine response in rats. Such a model may also be better able to discriminate effective antipsychotic agents including those that may act via different mechanisms than the dopamine antagonists already identified. This research programme sets out to examine the nature of the qualitative changes which occur following administration of amphetamine and the way in which antipsychotic drugs acting via several different mechanisms behave within the model system.

This introductory chapter considers amphetamine-induced behaviours from a broad historical perspective and discusses some of the unresolved problems which have led to the current series of experiments. Initially, I will describe the nature of schizophrenia and the dopamine hypothesis of this disease, which suggests that dopaminergic over-activity is an inherent feature of schizophrenia. I shall examine the role that amphetamine has played in this theory, which although by no means unequivocal, has dominated scientific enquiry since its formulation by Randrup and Munkvad in 1965. I will then turn to the role amphetamine has played in the formulation of animal models of this illness, a difficult task in view of the fact that the major manifestations of the disease are those related to dysfunction of thought and information processing. I will examine the nature of the behaviour exhibited by rats treated with amphetamine, and the manner in which these behaviours have helped to identify some of the underlying mechanisms relating to the biochemistry and morphology of dopamine neurotransmission in rat brain, whilst at the same time providing much of the paradox inherent in the dopamine theory of schizophrenia. In particular I shall examine stereotyped

behaviours, which in addition to being induced after centrally active stimulant drug administration, are also present in clinical conditions. I will propose that far from being a convenient way in which to categorise the effectiveness of stimulant drugs, stereotyped behaviours induced after drug administration - when adequately investigated - have the capacity to provide rich information with regard to the function of brain systems and the organisation and manifestation of behaviour. Finally it is suggested that hyperlocomotion induced after amphetamine administration has stereotyped properties and the study of this behaviour in greater depth will provide a powerful model which will support and develop an understanding of the theoretical issues arising from the effects of stimulant drugs on behaviour, and in addition provide a better model for selecting new drugs for the treatment of schizophrenia, perhaps acting via different mechanisms from those drugs already in use.

1.1 Schizophrenia: Disease and Treatment

The diagnosis of schizophrenia has existed for over a century and the massive and extreme disruptions of thoughts, perceptions, emotions and behaviour manifest in this disease are the epitome of what we perceive to be *'madness'*. Of all the psychiatric disorders it is perhaps the most interesting and elusive in terms of gaining an understanding of the underlying mechanisms of this extremely complex disorder.

Schizophrenia is the most common of the psychoses, afflicting approximately 1 percent of the population. The concept of schizophrenia proposed by Kraeplin (1913), which he termed dementia praecox, had at its core psychotic symptoms such as delusions and hallucinations. Since this original definition the concept has gradually broadened. Bleuler (1911), in contrast to Kraeplin's emphasis on a deteriorating course of the illness, emphasised the importance of a variety of symptoms and stressed those that were on a spectrum with normality, such as ambivalence in thoughts and emotions, and abnormalities of affect. Bleuler

reduced the emphasis on psychotic symptoms and expanded the definition to include latent and non-psychotic forms. The resulting over-diagnosis of schizophrenia in the United States led to an attempt to redefine the concept of schizophrenia in the DSM IIIR (1987) classification which removed schizoaffective disorder and latent and non-psychotic forms from the definition, in addition to requiring the symptoms to persist for longer than 6 months, with a deterioration in functioning.

At our current level of understanding, schizophrenia is characterised by a multiplicity of symptoms that represent a broad range of cognitive dysfunctions. Patients with schizophrenia suffer from dysfunction of perception, attention, communication, affect, cognition and motor function. The clinical 'perspective' can be very different over a broad range of patients, and the disease is characterised by a clustering of symptoms, none of which are specific to the disorder, or necessarily all present in any one patient, or any one time or indeed over the course of the illness (see Andreasen, 1987).

In an attempt to define schizophrenic symptoms in a more coherent manner several attempts have been made to group symptoms together in a logical and meaningful manner and currently the classification which has gained most support is to place the symptoms into two major groups: positive symptoms which include abnormality, distortion or exaggeration of normal functioning and include delusions and hallucinations and abnormalities in language and behaviour, and negative symptoms which represent a deficit or loss of function and include such features as poverty of speech and content (alogia), affective blunting, anhedonia and loss of will (Andreasen, 1983, 1985). There is some evidence to suggest that positive symptoms occur more frequently in the early stages of schizophrenia, whereas negative symptoms are more prevalent in the later stages and are persistent (Pfoh and Winokur, 1982, 1983).

The discovery of the antipsychotic effect of chlorpromazine (Delay et al, 1952) heralded the beginning of modern psychopharmacology and a new understanding of the mechanisms and aetiology of schizophrenia. Antipsychotic drugs are primarily effective against the positive symptoms and are much less

effective against the negative symptoms of schizophrenia (see Tamminga and Gerlach, 1987). There are also some patients with positive symptoms who are refractory to treatment with these drugs. The greatest problem posed by long-term treatment with antipsychotic drugs is the induction of unwanted side-effects. Many of the antipsychotics produce sedation, but this effect does not account for the symptom remission they produce, as other sedative drugs eg the benzodiazepines, barbiturates and antidepressants, are not clinically effective antipsychotic agents.

Other side effects are collectively called the extrapyramidal syndrome (EPS) as they are similar to symptoms seen with dysfunctions in the extrapyramidal system of the brain (the caudate nucleus, the putamen and the globus pallidus). The symptoms include motor restlessness, rigidity and tremor similar to that seen in Parkinson's disease, as well as muscle spasms and loss of muscle tone. These side effects occur in approximately 50% of patients and like Parkinson's disease can be treated with anticholinergic agents, which do not reduce the clinical effectiveness of antipsychotic drugs. During long term treatment a chronic, disfiguring and often permanent movement disorder known as tardive dyskinesia may develop in 10-15% of patients (see Casey 1987).

A newer class of antipsychotic drugs have been classified as 'atypical' as they retain antipsychotic action with a relative absence of EPS. These include the substituted benzamides eg sulpiride and the dibenzazepines eg clozapine which despite having a low incidence of EPS has many side-effects, notably sedation, cardiovascular effects and in some patients a pathological state in which there is a marked decrease in the number of granulocytes in the blood known as agranulocytosis (see Levinson and Simpson 1987).

The dopamine hypothesis of schizophrenia came into existence primarily as a result of increased understanding of the mechanisms of action of antipsychotic drugs.

1.2 The Dopamine Hypothesis of Schizophrenia

The dopamine hypothesis of schizophrenia is based almost entirely on pharmacological evidence, and a disturbed dopamine function has not yet been established beyond doubt in schizophrenia. It is hypothesised that enhanced cerebral dopaminergic activity exacerbates the symptoms of schizophrenia whilst a reduction in dopaminergic activity is associated with an amelioration of the illness. The hypothesis is supported by two lines of pharmacological evidence; dopamine receptor antagonists are often effective antipsychotics whereas dopamine agonists induce psychosis in non-psychotic subjects and are known to exacerbate psychosis in schizophrenic patients (Youngs and Scoville, 1938).

Psychosis associated with the use of amphetamine, an indirect dopamine agonist, was first described by Youngs and Scoville (1938). With increasing recreational use of amphetamine in the 1950s cases of amphetamine psychosis increased and Connell (1958) proposed that the clinical features of amphetamine psychosis were indistinguishable from acute or chronic schizophrenia. Ellinwood (1967) provided a detailed behavioural description of amphetamine psychosis in humans. In addition amphetamine was shown to induce paranoid delusions in an experimental setting (Griffith et al, 1968) and large doses were able to induce hallucinations and thought disorder in addition to paranoia (Angrist et al, 1974) This work provides the most convincing parallel of the two conditions. Further evidence was provided by the fact that small doses, which failed to induce psychotic symptoms in normal volunteers, intensified existing psychotic symptoms in patients with schizophrenia (Davis, 1974; Segal and Janowsky, 1978). Although a major premise of the dopamine hypothesis of schizophrenia is that dopamine agonists induce psychotic states which resemble schizophrenia, the evidence is not conclusive. There are reports that some volunteers given high doses of amphetamine do not develop psychotic symptoms (Kornetsky, 1976), and paradoxically, chronic schizophrenics maintained on neuroleptics may show additional improvement if L-dopa is administered concurrently (Flemming et al, 1970), such a response to L-dopa seems incompatible with the dopamine hypothesis of schizophrenia. The preponderance of delusions and hallucinations in amphetamine psychosis are visual and tactile, whereas in schizophrenia, auditory

hallucinations seem to be more common. As visual hallucinations are prevalent in unrelated drug states and fever this has led some researchers to suggest that amphetamine psychosis is merely a toxic drug response (see Jenner et al, 1989). Slater (1959) and Bell (1965) put forward the view that amphetamine psychosis can be distinguished from schizophrenia on three criteria: a lack of thought disorder; frequency of visual hallucinations; and quick affective response, although Angrist and Gershon (1970) describe auditory hallucinations and formal thought disorder in some of their amphetamine treated volunteers. Despite reservations, these observations and experimental findings have provided one of the major foundations for the dopamine theory of schizophrenia and encouraged the study of amphetamine in laboratory animals.

Amphetamine is known to release the catecholamines dopamine and noradrenalin from nerve terminals and to inhibit reuptake processes. Biochemical tools (eg. alpha-methyl-para-tyrosine and dopamine ß-hydroxylase) have lacked the sensitivity to examine the relative action of dopamine or noradrenalin in the behavioural effects of amphetamine (see review by Moore, 1978). Observation of the uptake of the d- and l-isomers of amphetamine in the cerebral cortex synaptosomes, rich in noradrenalin compared with striatal synaptosomes predominantly dopamine initially suggested that the d-isomer was 10 times more potent than the l-isomer in blocking catecholamine uptake into cerebral cortex synaptosomes (Taylor and Snyder, 1971), indicating that behaviours mediated by noradrenalin should be more affected by the d-isomer. Failure to replicate these findings (Harris and Baldessarini, 1973; Holmes and Ruttledge, 1976) eroded what originally seemed to be a clear pharmacological tool for determining the catecholamine system responsible for mediating certain behaviours. Lesion studies have played an important role in defining the roles of dopamine and noradrenalin in the amphetamine response. Results from lesion studies examining the 'running behaviours' following administration of low doses of amphetamine and the stereotyped motor responses seen following higher doses have indicated that there is strong evidence that dopamine rather than noradrenalin is involved (see Iversen, 1986). These studies implicating the role of dopamine in both locomotor

and stereotyped behaviours will be discussed later in greater detail (see section 1.4).

The second line of evidence to support the dopamine hypothesis of schizophrenia is the action of dopamine antagonists. The mechanism of action common to effective antipsychotic drug action is thought to be dopamine receptor blockade, usually at the post-synaptic receptor sites (Carlsson and Lindquist, 1963). It is now accepted that there are several dopamine receptor subtypes, a distinction being made between D1 'type' linked to adenylate cyclase and D2 'type' inhibitory or not coupled to adenylate cyclase (Kebabian and Calne, 1979; Leff and Creese, 1983). It is widely believed, but by no means universally accepted, that the effect of antipsychotic drugs result from D2 receptor blockade. This is based on evidence that a significant correlation exists between the therapeutic potency of antipsychotic drugs and their relative ability to inhibit dopamine release and displace radiolabelled haloperidol from dopamine receptor binding sites in vitro (Seeman et al, 1976) and specifically for the D2 receptor (Creese, Burt and Snyder, 1978). The majority of antipsychotic dopamine receptor antagonists show a higher affinity for D2 sites (Carlsson, 1988). Recent positron emission tomography (PET) scan studies have demonstrated that a large number of antipsychotic agents with varying chemical structure, given to schizophrenic patients at therapeutic doses, caused displacement of the highly selective dopamine D2 receptor ligand raclopride from striatal binding sites (Farde et al, 1988). The regional localisation of mRNA for the D2 receptor has been determined by histochemical methods and the areas of highest expression correspond to major dopamine projection areas such as the caudate-putamen, nucleus accumbens and the olfactory tubercle. D2 receptor mRNA is also found in the dopaminergic cell bodies within the substantia nigra and ventral tegmental areas, indicating a pre-synaptic, as well as a post-synaptic, role for the D2 receptor. It is also clear that drugs interfering with the function of dopamine in other ways have antipsychotic properties. Drugs which cause depletion of dopamine from transmitter stores (eg reserpine) are effective antipsychotic agents, and drugs which inhibit the rate-limiting step in dopamine synthesis (eg alpha-methyl-para-tyrosine), have been

shown to potentiate the antipsychotic action of receptor blocking antipsychotic drugs (Carlsson, 1987).

Until the introduction of specific D1 agonists and antagonists it was thought that the D1 receptor had no behavioural function (Laduron, 1983; Seeman, 1980; Creese et al, 1983). This is no longer thought to be the case, there is now convincing evidence that the two receptor types cooperate and that a 'complete' behavioural dopaminergic response requires activation of both receptor types (see Longini et al, 1987).

Although pharmacological evidence for a role of dopamine in schizophrenia is convincing, the evidence for dopamine dysfunction in this disease has not been shown. Post-mortem analysis of brains of patients suffering from chronic schizophrenia have shown an increased number of D2 receptors. It is unclear whether this increase is the result of antipsychotic drug treatment, or a primary effect of the disease (Henn, 1987). The data from PET scan studies has provided contradictory evidence. Farde et al, (1987) found no difference in D2 density between drug-naive schizophrenics and age-matched controls, whilst Wong et al, (1986) claim to have observed an increase in D2 receptors in drug naive schizophrenics.

It has been established that changes in the firing rates of dopamine neurons, either electrochemically or pharmacologically, are correlated with local changes in the major extracellular dopamine metabolite, homovanilic acid (HVA) (Post et al, 1975). Attempts to discover differences in cerebrospinal fluid (CSF) HVA levels between groups of schizophrenia patients and normal control subjects have largely resulted in negative findings (Berger et al, 1980; Bowers 1973; Post et al, 1975) although, high CSF HVA has been found in schizophrenics with a family history of schizophrenia (Sedvall and Wode-Helgodt, 1980). As the majority of centrally produced HVA appears to originate in the nigrostriatum, especially the caudate (Portig and Vogt 1968; Wood 1980), this method may be unable to detect overactivity of dopamine systems in other areas of the brain. Another source of information concerning dopamine activity is measures of plasma HVA (pHVA) levels. Administration of dopamine receptor agonists or antagonists

produce parallel changes in brain HVA and pHVA in primates and rodents (Kendler et al, 1981, 1982). Unmedicated severely ill schizophrenic patients have been shown to have higher pHVA concentrations than less severely ill patients (Davis et al, 1985).

Currently dopamine is the main target for both typical and atypical antipsychotic treatment, although dysfunction of other neurotransmitter systems is postulated as taking a role in schizophrenia. The majority of these suggestions have evolved from the dopamine theory of schizophrenia which has provided, over the last thirty years, a useful working hypothesis to explain the symptoms of the disease but has not fulfilled its promise in providing convincing explanations of the aetiology of schizophrenia. Because the cost of drug development remains high it remains all the more difficult to test drugs dependent on novel non-dopaminergic principles.

Amphetamine is known to release noradrenalin as well as dopamine, although lesions to the forebrain noradrenalin pathway did not block the motor effects of amphetamine (Creese and Iversen 1975). Iversen herself claims that 'one cannot feel entirely confident in ruling out a component of noradrenalin dysfunction in schizophrenia' (Iversen 1986, p 91).

Serotonin (5HT) is found in high concentrations in dopamine terminal areas of the brain. Cell bodies containing 5HT are found clustered in the midline region of the pons and upper brain stem and the raphe nuclei, from where they ascend to innervate the basal ganglia, hypothalamus, thalamus, hippocampus, limbic forebrain and neocortex, and also the cerebellum. Prior to the dopamine hypothesis, serious consideration was given to the possibility that abnormal 5HT activity was involved in schizophrenia (Gaddum 1954, Woolley and Shaw 1954). More recent evidence suggests that serotonin may indeed be involved, as raised 5HT turnover has been found in familial schizophrenia (Sedvall 1980). In addition, Leysen et al, (1978) reported that some antipsychotics act as antagonists at 5-HT receptors as well as dopamine receptors. Clozapine, for example, is a potent 5-HT_2 antagonist, but a weak D2 antagonist, a profile which is claimed lowers EPS liability (see Meltzer, 1988). It has also been shown that

5-HT_3 antagonists inhibit dopamine release in the nucleus accumbens (Imperato and Angelucci, 1989, Carboni et al, 1989a), and block nicotinic or morphine-induced place preference which has been shown to depend on mesolimbic dopamine systems (Carboni et al, 1989b), since these systems may be involved in psychosis, it is speculated that 5-HT_3 antagonists may have antipsychotic potential. Recent studies in rats and marmosets show that 5-HT_3 antagonists are effective at antagonising behaviours thought to be mediated by dopaminergic systems (Brittain et al, 1987, Costall et al, 1987). Drugs such as ondansetron (GR 38032F), a potent selective antagonist at 5-HT_3 receptors may represent a new class of antipsychotic agents, with the absence of side effects normally associated with antipsychotic drug treatment.

Cortical glutamatergic neurons project to presynaptic dopamine terminals and exhibit modulatory effect on dopamine release (Maura et al, 1988). It has been suggested that glutamate antagonism has potential as an antipsychotic treatment (see Neilsen and Andersen 1991). Glutamate receptors exist in several subtypes which include NMDA, quisqualate, kainate (Honore 1989), and a modulatory site to NMDA sensitive to glycine (White et al, 1989). In addition there are suggestions that glutamate antagonists may provide protection against the putative ischemic process in schizophrenia (Deakin 1988).

The interactions between dopamine systems and sigma receptors is complex, and to date it has been difficult to evaluate to what extent sigma antagonism is a valid therapy in the treatment of psychosis (see Tamminga and Gerlach 1987; van Kammen and Gelernster 1987).

Recently there has been considerable interest in the role of peptides as neurotransmitters or neuromodulators. Cholecystokinin (CCK) is found to co-exist with dopamine neurons in the mesencephalon, and for this reason it is suggested that it may take a role in schizophrenia (see Nair et al, 1985). CCK has been suggested as having an antagonistic effect on dopamine mediated neurotransmission and thus may prove to have therapeutic effects in the treatment of schizophrenia (Fuxe et al, 1986). Neurotensin (NT) is another peptide which has been linked to modulation of dopamine activity (see Nemeroff, 1983), with

preference for the mesolimbic system. Several researchers have suggested that NT possesses a pharmacological profile resembling antipsychotic drugs (Quirian, 1983, Nemeroff et al, 1984, Nemeroff and Cain 1985).

Some antipsychotic drugs are potent antagonists of calcium (Ca^{2+}) channels (Deutsch et al, 1988). Meltzer et al, (1986) have speculated that this may relate to antipsychotic potential, particularly on negative symptoms. Reports by Pilebald and Carlsson (1978) Fadda et al, (1989) suggest that Ca^{2+} blockade may lead to a decrease in dopamine synthesis.

These varied and interesting approaches to antipsychotic drug treatment have largely been developed from pharmacological discoveries made using animal models.

1.3 Animal Models of Schizophrenia

Animal models are one of the most important tools for studying disease processes. The development of animal models for psychiatric disorders is by necessity a compromise between experimental simplicity and the complexity of the disorder. Lack of understanding of the process underlying the disease leads to an inability to develop good animal models, which in turn leads to an inability to fully elucidate the underlying mechanisms of the disease. Despite these limitations, animal models have proved to be of great value in elucidating dopaminergic mechanisms, and evaluating novel treatments. The ideal animal model should resemble the disease it models in its symptomology, aetiology, biochemistry and treatment (McKinney and Bunney, 1969). No such ideal model exists of course, and although there are numerous ways in which animal models can be categorised eg drug-induced versus non-drug-induced models or categorisation by neurotransmitter, a more theoretical approach to describe animal models of psychiatric disorder is appealing as it possibly leads to a more appropriate set of validating criteria and ultimately to a better way of evaluating the various models. The theoretical approach of examining the validity of a model was introduced for models of depression by Willner (1984). Such an approach can be directly applied to animal models of schizophrenia (Ellenbroek and Cools

1990), which of all the psychiatric disorders has proved the most difficult to model.

If the validity of a model is taken in terms of an increasing hierarchy from predictive to face to construct validity, then those models with predictive validity have the lowest level of validity, as the actual behaviour displayed in the model is generally totally unrelated to the symptoms of the disease. In the study of schizophrenia such models are based on the effectiveness of antipsychotic drugs, and are therefore unlikely to uncover drugs with a mechanism of action completely different from that of the current antipsychotics, but such models have value as a screening technique for developing more effective antipsychotic drugs and to give insight into the neuronal mechanisms underlying the effects of existing antipsychotic treatments. Drugs such as haloperidol, a butyrophenone, clozapine, a dibenzazepine and sulpiride, a benzamide, all differ greatly in their chemical structure but share antipsychotic action and should act similarly in a good animal model. Thus, an animal model with predictive validity should satisfy the following criteria: antipsychotic drugs of various chemical classes should be effective, and there should be no false negative or false positive drugs.

All drugs have multiple effects. One of these effects may be a beneficial reduction of symptoms of a disorder that defines a particular class of drugs eg antipsychotic action or anxiolytic action. The other effects are secondary to this activity, often unwanted and termed 'side effects'. Those drugs which reduce symptoms can be classified as *type I*. Those drugs which do not produce symptom reduction but can produce the same secondary effects as the *type I* drugs are termed *type II* drugs whilst a further category of drugs, *type III*, have effects of their own which include neither symptom reduction nor the secondary effects seen in *type I* and *type II* drugs. An effective model should therefore select *type I* drugs as being effective and should not select either *type II* or *type III* drugs. The model should discriminate drugs in the same way that the clinical response (symptom reduction) differentiates them. The difference between *type I* and *type II* drugs lies in their ability to reduce symptoms not in their secondary effects. Therefore the model should select drugs on the basis of their symptom reduction and not on the

basis of their secondary effects (see Table 1). This is essentially a restatement of the term power, in which the cell in the upper right is the probability of rejecting H0 when it is actually false (p=1-ß); the upper left cell constitutes a type II error (p=ß), and the lower right cell a type I error (p=a).

Table1.1 Effects of various classes of drugs tested in an animal model.

Clinical Change	No Response	Response
YES (Type I drug)	False Negative	Effective Drug
NO (Type II and Type III drug)	Correct Rejection	False Positive

Adapted from Carlton 1983.

In addition, anticholinergic drugs should not reduce the therapeutic effects of antipsychotic drugs, although anticholinergic drugs reduce the extrapyramidal side effects of antipsychotic treatment. The same effect occurs with chronic antipsychotic therapy. The effects of anticholinergic drugs and chronic antipsychotic treatment indicate that the EPS and therapeutic effects of a treatment can be separated pharmacologically.

There should also be a relationship between the clinical potency of the drug and its potency in the model. A confounding factor is that the potency of the drug depends on a number of non-disease-related but species-specific phenomena (eg ability to penetrate the blood brain barrier) so that a perfect correlation between potencies in the model and in treatment would not necessarily be expected, but a general agreement between potencies of disease control and efficacy in the model should exist. Animal models of schizophrenia which satisfy the criteria for predictive validity are the conditioned avoidance response (Arnt 1982), intra-cranial self stimulation (Wanquier, 1979) and catalepsy (Janssen et al, 1965).

The second category of animal models are those with face validity. These models exhibit a phenomenological similarity between the disease and the animal model. As these models are hierarchically higher than models with predictive validity, models with face validity should also obey the criteria for predictive

validity. In addition the animal model should resemble schizophrenia in a number of respects, which should be specific to the disease. The similarities should coexist in a specific subtype of schizophrenia, but not show features unrelated to schizophrenia. Animal models of schizophrenia which have face validity are difficult to develop as most symptoms are related to thought disorder and perception and are therefore very difficult to model in an animal. In addition, symptoms which appear phenomenologically similar need not necessarily result from similar underlying mechanisms. For these reasons the number of animal models with face validity is small. These include amphetamine and apomorphine-induced stereotypy (Janssen et al, 1967). As amphetamine-induced hyperactivity and stereotyped behaviours are the main subject of this study an evaluation of these animal models is not appropriate at this point and will be discussed in detail throughout the remainder of the introduction although a discussion of the relevance of stereotypy to schizophrenia is relevant to the face validity of this model.

Stereotypy in schizophrenic patients and following amphetamine use in humans has been well documented. Bleuler considered that motor stereotypy was a fundamental symptom of schizophrenia (see Iversen 1986). A form of stereotyped behaviour frequently reported in amphetamine addicts is a repetitive scratching of the skin (Sudilovsky 1975), similar to the repetitive grooming behaviours seen in amphetamine treated rhesus monkeys (Ellinwood et al, 1973). Complex patterns of stereotyped behaviours following amphetamine have been variously described as 'punding' (Rylander 1971), obsessive compulsive tendencies (Ellinwood 1967; Kramer et al, 1972) and 'hung up activity' (Scher 1966). There is strong evidence that behavioural stereotypy is a characteristic feature of the response to amphetamine both in experimental animals and humans.

The highest level of validating criteria for animal models are those models which possess construct validity. These are models which attempt to model the psychopathological construct underlying the disease process. In order to develop models of schizophrenia which reflect accurately the hypothetical constructs underlying the disease it is important to understand the pathological constructs

relevant to this disorder. At the present time there is no clear consensus, and the constructs underlying schizophrenia are the subject of much debate. Many researchers have argued that a dysfunction in information processing lies at the core of schizophrenia, and have sought to interpret deficits in terms of the information processing theory of Broadbent (1971). Indeed, many studies have shown that schizophrenic patients are more easily distracted than normal controls (Green and Walker 1984; Cornblatt et al, 1985, 1989; Walker and Harvey 1986; Harvey and Pedley 1989). Therefore it is hypothesised that schizophrenic patients suffer from disruptive selective attention (Nuechterlein and Dawson, 1984; Ellenbroek and Cools 1990).

Animal models which have been suggested as relevant to the study of attentional processes in relation to schizophrenia are amphetamine-induced disruption of latent inhibition and blocking (Solomon et al, 1981, Lubow et al, 1987, Crider et al, 1982), and phencyclidine-induced disturbances of the startle response (Braff et al, 1978). Schizophrenic patients have been shown to perform differently from normal control subjects in a latent inhibition task, with schizophrenia patients pre-exposed to a stimulus performing better than the pre-exposed control subjects, thus for schizophrenic patients pre-exposure to the stimuli did not interfere with subsequent learning, as is normally the case. Animal models have examined the role of dopamine agonists on latent inhibition. Several studies, using a variety of methods have shown that amphetamine disrupts latent inhibition in a similar manner to that seen in schizophrenic patients (Solomon et al, 1981, Weiner et al, 1988, Crider et al, 1982). The majority of these studies showed that chronic rather than acute injections of amphetamine disrupt latent inhibition. In addition several studies have examined the effects of antipsychotic drugs on latent inhibition. Solomon et al, (1981) and Crider et al, (1982) showed that acute doses of chlorpromazine and haloperidol prevented amphetamine-induced disruption of blocking, whilst several studies have shown that chronic rather than acute administration of both classical and atypical antipsychotic drugs improved latent inhibition. Interestingly, clozapine was found to exhibit no influence on amphetamine-induced latent inhibition and as such

would represent a false negative in this model (Dunn et al, 1989). As the ability to prevent amphetamine disruption of latent inhibition by the majority of antipsychotic drugs occurs after chronic rather than acute treatment, this mirrors treatment in clinical settings and the validating criteria for assessing the predictive validity of the model. Amphetamine injected into the nucleus accumbens, but not neostriatum can disrupt latent inhibition (Solomon and Statton, 1982). Furthermore, lesions to the hippocampus disrupt latent inhibition (Ackil et al, 1969, Kaye and Pearce 1987) and blocking (Solomon 1977, Richert et al, 1978), whilst lesions to the 5HT innervation of the hippocampus disrupt latent inhibition (Cassaday et al, 1990). Further studies into the neuronal mechanisms underlying latent inhibition are required before the construct validity for latent inhibition and blocking are fully established.

The second model which has been suggested as having construct validity for schizophrenia is phencyclidine-induced disturbance of the startle response. The startle response is a reaction to a novel intense stimulus. The most commonly used are either acoustic (about 100dB burst of white noise) or tactile (usually a puff of air directed at the head of the animal). The response is measured as either a whole body response, or an increase in EMG activity in certain muscles. It has been suggested that the startle response is a useful model for studying sensorimotor integration and as such it may well represent an animal model with construct validity for schizophrenia. The startle response occurs if the stimulus reaches a certain intensity, therefore if the subjects threshold for external stimuli is lowered subjects should show a response to a lower intensity stimulus or an increased response to the normal startle stimulus. In addition, habituation occurs to a repeatedly occurring startle stimulus and the startle response is subject to prepulse inhibition, whereby the response is reduced if the stimulus is preceded by a comparable stimulus of lower intensity. Schizophrenic patients have been shown not to have a lowered threshold for external stimuli but to exhibit a reduced rate of habituation (Geyer and Braff 1982). In addition, unlike control subjects, schizophrenic patients did not show a diminished response when an acoustic stimuli was preceded by a less intense stimulus (Braff et al, 1978).

Several explanations have been put forward to explain the changes in the startle response, habituation and prepulse inhibition in schizophrenic patients. The disruption of pre-pulse inhibition has been explained as a loss of inhibitory control (Adler et al, 1982), whilst the decrease in the rate of habituation is suggested to result from a general slowness in information processing (Geyer and Braff, 1987). Many drugs have been shown to effect the acoustic startle response (see Davis 1980, 1984), very few induce the same deficits as those observed in schizophrenic patients. (Mansbach et al, 1988, Geyer and Braff, 1987). Phencyclidine retards habituation and prepulse inhibition without lowering the threshold of responding (Geyer et al, 1984, Mansbach and Geyer 1989), suggesting that phencyclidine-induced changes in the startle response exhibit face validity for schizophrenia. There have been few studies on the effects of antipsychotic drugs on this animal model.

Haloperidol has been shown to block the effects of phencyclidine on prepulse inhibition (Adler et al, 1986). Ellenbroek and Cools (1990) suggest that the morphology of the startle response in humans and animals is very similar and consequently models of the startle response in animals relate to the constructs underlying the deficits in schizophrenia. Whilst the neuronal mechanism underlying the startle response has been partly elucidated, in particular the primary startle circuitry (Davis 1984), further studies are required to assess the validity of the startle response as a model for schizophrenia, in particular the ability of antipsychotic drugs to antagonise the response within this model.

Clearly, these two models are based on the hypothetical construct that schizophrenic patients have a diminished capacity to distinguish relevant from irrelevant stimuli, and as such these patients are easily distracted. Ellenbroek and Cools (1990) point out that these models do not account for the negative symptoms (anhedonia, flat effect and social isolation) seen in the chronic phase of the disease, and that the construct hypothesised to underlie latent inhibition and the startle response - disruption of selective attention, and an increased propensity for distractibility - does not apply to the negative symptoms of schizophrenia. Hemsley (1988) proposed a compensatory mechanism in

schizophrenic patients which protects them from 'sensory flooding'. Chronic treatment with amphetamine or phencyclidine may lead to a comparable compensatory mechanism in animals. Certainly in rats (Gambill and Kornetsky 1976) and monkeys (Haber et al, 1977; Ridley et al, 1979) chronic administration of amphetamine leads to social withdrawal and isolation. In addition, amphetamine treatment induces stereotyped behaviour in monkeys, often with more individual variability than that seen in rodents (Ellenbroek et al, 1989) and as such has face validity for the positive symptoms of schizophrenia, since stereotypy of movement, speech and thought occur in schizophrenia patients (Bleuler, 1911). It would appear that the validity of all these models is not without attendant problems, nevertheless they represent a more systematic and focused approach to the development of animal models for schizophrenia enabling an increase in understanding of the neuronal structures underlying information processing dysfunction.

1.4 Amphetamine-Induced Behaviours as an Animal Model of Schizophrenia

Angrist and Gershon (1970) claim that amphetamine psychosis mirrors important aspects of paranoid schizophrenia including such conditions as thought disorder, delusions, paranoia and auditory hallucinations. Amphetamine psychosis - like schizophrenia - can be treated with antipsychotic drugs, reinforcing the view that amphetamine psychosis and schizophrenia have some common neurobiological element. In addition amphetamine worsens schizophrenic symptoms regardless of the subtype of schizophrenia. In view of these facts, amphetamine-induced behaviours in animals after chronic and acute exposure to the drug have been used to study the underlying mechanisms , and also the effects of antipsychotic drug treatment.

Amphetamine produces dose-dependent changes in the expression of individual types of behaviour in the rat (Rebec and Bashore, 1984). D-amphetamine sulphate injected subcutaneously in the range of 0.3 - 1.5 mg/kg

produces an increase in forward locomotion accompanied by sniffing and head bobbing. The locomotion persists for 40 - 90 minutes, depending on the dose, and is followed by a period of sleep. Higher doses (2 - 10 mg/kg) produce a multiphasic response that consists of early and late phases of locomotion and an intermediate phase of focused stereotyped behaviours during which locomotion is absent. Stereotypy describes the characteristics of a behaviour rather than a specific response. Ellinwood (1967) defined stereotyped behaviour as the performance of an invariant sequence of movements repetitively which is inappropriate with respect to its environmental context. The focused stereotypy phase is characterised by sniffing, repetitive head and limb movements and oral behaviours, which include licking, biting and gnawing; all expressed in a small area of the open field. These behaviours, although mainly restricted to the focused stereotypy stage, can also be observed intermittently during the other phases. The time course for each phase is dose-dependent, and with increasing doses the animal spends less time in forward locomotion and more time in focused stereotypy.

Lyon and Robbins (1975) put forward a hypothesis to explain the behavioural effects seen following amphetamine treatment which states that: 'amphetamine with increasing dose produces an increasing response rate within a progressively narrowing response repertoire'. Amphetamine stimulation leads initially to reductions in pausing, and at higher doses to enhanced behavioural competition among the different response sequences. The performance of behaviour, especially complete sequences is hindered by competition, even amongst the elements constituting a behavioural sequence. Behaviour as a consequence becomes dominated by elements, which are performed irrespective of sequence and the animal becomes immobilised as a result of over-stimulation. The resulting behavioural stereotypy is the culmination of a process of increased activation, mediated by dopamine release in the basal ganglia, whereby responses are elicited at an increasing rate.

Many of the behaviours in the animal's response repertoire compete for expression. Evidence for behavioural competition is derived from three lines of evidence: particular responses take place over a shorter time with the occurrence

of abortive behavioural sequences; there is enhanced switching between behavioural elements; there is an enhanced rate of performance when competing behavioural responses are blocked. The most striking example of behavioural competition is that seen between the locomotor effects of amphetamine and the intense head movements and sniffing stereotypies (see Robbins et al, 1990).

The Lyon-Robbins hypothesis would predict that amphetamine will not induce any form of behaviour not already present in the animals behavioural repertoire, and that those behaviours which occur at high rates have peak effects at much lower doses than those behaviours occurring at low rates. Robbins et al, (1990) state that this is essentially a representation of the Yerkes-Dodson (1908) principle relating the optimal performance of tasks to different levels of arousal, such that difficult tasks are performed optimally at lower levels of arousal than easy ones. The Lyon-Robbins hypothesis also postulates that animals under amphetamine do not suffer from deficits of sensory input. In fact, environmental influences can either disrupt the behavioural response, lead to behavioural competition resulting in a blending of behavioural patterns, or lead to stereotyped responses of learned or conditioned behaviours. For example, novel stimuli can disrupt amphetamine stereotypy (Sahakian and Robbins 1975), and escape behaviour from a water maze is sufficiently strong to overcome stereotypy, at least temporarily (Mittleman as quoted in Robbins et al, 1990).

Chronic treatment with amphetamine can cause sensitisation of certain behaviours (increased response output), whilst producing apparent tolerance to others (Eichler et al, 1980, Segal and Schuckit, 1983, Mittleman et al, 1985). Sniffing, head and limb movements tend to increase following repeated treatment, licking and gnawing decline in frequency. It is not clear whether the sensitisation effects following repeated administration of amphetamine result from neuropharmacological factors or the influence of conditioning. The basic premise of Lyon and Robbins to account for acute effects of increasing doses of stimulants would appear to account for the cumulative effects of repeated treatment (Robbins et al, 1990).

It is now clear that stereotyped behaviours are mediated by the caudate-putamen whilst hyperactivity is mediated by the mesolimbic system, in particular the nucleus accumbens. Creese and Iversen (1975) showed that selective dopamine depletion from the caudate-putamen in the rat reduced the stereotyped head movements produced by amphetamine, and subsequently Kelly et al, (1975) showed that locomotor responses following treatment with amphetamine resulted from the mesencephalic dopamine projection from the ventral tegmental dopamine pathway to the nucleus accumbens. Pjinenburg et al, (1976) demonstrated that infusions of amphetamine into the nucleus accumbens and also into the olfactory tubercle, elicited hyperactivity in the rat. The caudate-putamen and nucleus accumbens can be considered as part of the dorsal and ventral striatum respectively, with independent outputs through the dorsal and ventral palladium. As a consequence of the neuroanatomical relationship of these two structures the nucleus accumbens is in a position to alter functioning in the nigrostriatal projection, whereas the reciprocal interaction is not possible. Thus there is a possibility of competition between parallel independent output pathways and direct interactions whereby interruption of nigrostriatal activity may occur, or of boosting or amplifying the nigrostriatal output. The locomotor and overtly stereotyped effects following amphetamine do appear to compete for expression, and it would appear that stereotypy masks further dose-dependent increases in locomotion. The hypothesis put forward by Lyon and Robbins predicts this competition between the two systems.

Mogenson et al, (1984) have suggested that the nucleus accumbens represents a functional interface between the limbic system and the motor system and as such provides a link between motivational and motor processes. This view is supported by (Cools et al, 1991). In addition, Cools (1980) suggested that striatal dopamine is involved in the sequencing and selection of behavioural responses. Dopamine in the striatum and accumbens has been implicated in the perseveration and switching of behaviour (Evenden and Robbins, 1983, Koob et al, 1978, Oades 1985).

Rotation seen following amphetamine administration and unilateral depletion of dopamine in the caudate can be suggested as a form of stereotyped locomotion with evidence that both structures participate. Kelly and Moore (1976) showed that unilateral depletion of dopamine from the head of the caudate determined the direction of rotation, whilst additional depletion of dopamine from the nucleus accumbens determined its rate. This led to the proposal that the accumbens projection 'gain amplified' the bias produced by the caudate imbalance. This hypothesis is compatible with the suggestion that the intensity of stereotyped behaviour is determined by dopamine projections throughout the striatum, including the nucleus accumbens itself. Fink and Smith (1980) and Winn and Robbins (1985) have shown that amphetamine hyperactivity depends on dopamine projections to the anteroventral head of the caudate nucleus as well as in the nucleus accumbens, and that amphetamine locomotor response depends on the combined action of the central dopamine systems (Fink and Smith, 1980).

In summary, stereotypy is a complex pattern of behaviour which when analysed at the behavioural and neural level can provide much information about CNS mechanisms of behaviour and psychological process. The induction of stereotypy in animals can be seen as a convincing model of psychopathology, including aspects of schizophrenia, and that models with greater face and construct validity (as stated earlier) will emerge when the full complexity of this behavioural response is taken into account.

1.5 Locomotion as a More Appropriate Measure of the Amphetamine Syndrome

Considerable attention has been placed on amphetamine-induced focused stereotyped behaviours because they are reliably antagonised by classic antipsychotics such as haloperidol or chlorpromazine (Szechtman et al, 1988). This observation was the foundation for an important preclinical screening technique for putative antipsychotic drugs. It is now clear that a group of

clinically effective drugs, so called 'atypical' antipsychotics do not antagonise all the components of amphetamine-induced stereotyped behaviour.

Atypical antipsychotics are not a homogeneous class of compounds. They show a wide spectrum of biochemical effects on dopaminergic mechanisms and the incidence of EPS are claimed to be less with atypical antipsychotic drugs (Tamminga and Gerlach, 1987). In addition, they vary in their ability to antagonise specific amphetamine-induced stereotyped behaviours (Tschanz and Rebec, 1989). Ljungberg and Ungerstedt (1978) reported that sulpiride, clozapine and thioridazine predominantly antagonised apomorphine-induced hyperactivity without antagonising the stereotyped gnawing induced under this drug, and that clozapine and sulpiride antagonised only the locomotion induced by amphetamine (Ljungberg and Ungerstedt, 1985). There are several problems relevant to these studies. The holeboard apparatus did not adequately measure gnawing, and this is probably responsible for the anomalous results obtained for the largest dose of clozapine tested (50mg/kg). In addition, the 2.5 cm holes are sensitive to all parts of the rat's body, including limbs and tail, which may enter and break the photobeam, thus locomotor activity may artificially inflate gnawing measures. The measures of activity used were ill-defined and unnecessarily complex, relying on a predetermined distance, and locomotion in two 'arms' of the apparatus as a definition of forward locomotion. The use of visible light for the photobeams could act as a confounding factor, changing the characteristic nature of the locomotor behaviour. As stated earlier, amphetamine- induced behaviour is sensitive to sensory input, and high levels of illumination are known to induce lower levels of activity in the rat (Montenaro and Babbini, 1965). In studies with apomorphine (Ljungberg and Ungerstedt, 1978) and amphetamine (Ljungberg and Ungerstedt,1985) the number of animals tested in each condition was extremely small (4-6 at each dose) The variability of these data are not quoted, but in view of the fact that the antipsychotic drugs were administered in large volume (5ml/kg body weight), it would be expected that the variability between individual rats would be high. Locomotion and gnawing are mutually exclusive behavioural categories and cannot be exhibited at the same time within

this apparatus. Ljungberg and Ungerstedt (1978) report that the peak activity for both behaviours occur at different times following administration of apomorphine (0-30min for locomotion and 30-60min for gnawing), yet in a later study (Ljungberg and Ungerstedt, 1985) animals were tested for a short 10 minute period 50-60 min following amphetamine and 90 minutes following antipsychotic pre-treatment, this timing would allow for a sensitive test of the effects of drugs on gnawing behaviour but would be less sensitive to effects on locomotor behaviours. In addition this short test period would maximise the effects of handling stress and habituation procedures. The description of gnawing behaviour following treatment with apomorphine by Ljungberg and Ungerstedt (1978) implies that pre-treatment with clozapine actually potentiated this behaviour, although the apparatus appears to be unable to detect this. A paradoxical finding was reported by Robertson and MacDonald (1984, 1985) who found that the atypical antipsychotics, sulpiride, clozapine and thioridazine *enhanced* some stereotyped behaviours (repetitive head movements, sniffing and gnawing).

The paradoxical effects of atypical antipsychotics on stereotyped behaviour, and the observation that atypical antipsychotics are less likely to produce the Parkinson-like side effects which are associated with classic antipsychotic drugs (for review see Tamminga and Gerlach, 1987), has cast doubt on the utility of amphetamine antagonism as a screening technique for antipsychotic potential (Robertson and MacDonald, 1985, Tschanz and Rebec, 1989), and led to the suggestion that antagonism of amphetamine-induced stereotypy predicts a compounds potential to produce unwanted extrapyramidal side effects in humans (Ljungberg and Ungerstedt, 1985). This suggestion means that a fundamental reassessment of antagonism of amphetamine - induced stereotyped behaviours as a preclinical screening test for antipsychotic potential is urgently needed. Recently it has been argued that the shared ability of classic and atypical antipsychotics to reduce amphetamine-induced hyperactivity may form the basis of a more satisfactory test of antipsychotic potential (Ljungberg and Ungerstedt, 1985; Rebec and Bashore, 1984).

It is important to recognise that hyperactivity, or a general increase in motor activity, is not a single class of behaviour, but depending on the recording technique may consist of many different forms of motor movement. This has often led to a reliance on measures of locomotor rather than motor behaviour (Geyer, 1990). Locomotor activity can be defined as movement from place to place, and is a specific measure because with either observational or automated monitoring, movements can be defined in units, often termed 'crossings' or 'crossovers' and invariably require ambulation by the animal. A limited number of different measurement techniques have been applied to the detection of locomotor activity in rodents, in particular the use of photobeams which have the added advantage in that they can be used to detect rearings and to monitor holepokes as an explicit measure of exploratory behaviour.

Although these provide an accurate quantification of locomotor activity they do not allow for the description of any *patterns* inherent in the ambulation.

1.6 A Distinction Between Amphetamine-Induced Locomotion and Stereotyped Locomotion

Traditionally the view was held that at certain doses amphetamine produces a three phase behavioural response in rats consisting of an enhanced locomotor phase followed by a stereotypy phase followed by an afterphase of enhanced locomotion (Schiorring, 1971; Segal 1975), with a clear distinction drawn between amphetamine-induced locomotion and focused stereotyped behaviours (Szechman et al, 1988). Several authors have argued that amphetamine-induced forward locomotion has stereotyped properties. Lat (1965) described the repetition of a 8-shaped locomotor route restricted to only part of the cage following treatment with amphetamine whilst Segal (1975) observed perseveration in the pattern of locomotion with doses as low as 0.5 mg/kg d-amphetamine.

Schiorring (1979) used plots of locomotor movements to demonstrate the perseverative nature of routes taken by amphetamine treated rats in an open field

divided into a number of equal sized squares. He used the term 'trip' to define the distance moved by the rat between two turns. If the rat made a tour of the open field apparatus, without any turns, this was registered as a trip. Schiorring classified three different types of trip: a complete repetition whereby the rat entered exactly the same squares as it did on the trip before; a partial repetition, in which the rat entered some of the squares it did on the previous trip, without including any new squares; and finally a complete repetition of the previous trip but including new squares. Amphetamine increased the number of complete repeats by about 10% and this indirectly implies perseverative or stereotyped patterns of locomotion in the open field. Schiorring also noted that trips along the whole periphery of the open field were seen in over 50% of the amphetamine treated rats and termed this stereotyped locomotion. This work was hampered by the difficulty of quantifying locomotor stereotypy. Schiorring divided the locomotion into two phases: before and after the first complete tour of the perimeter; which seems an unnecessarily clumsy way of quantifying these data. As a consequence it is difficult to determine the number and proportion of trips around the periphery of the apparatus. The study also employed high illumination (2x100 w) which as already stated would be likely to reduce open-field hyperactivity. Despite these limitations, Schiorring's work is pivotal in laying the groundwork for more detailed studies into amphetamine-induced hyperactivity and the perseverative nature of open-field locomotion. The strength of this study lies in the large size of the test field (3 x 3.5 m^2) which allowed him to observe the spatial pattern of amphetamine-induced locomotion in detail, and the long (3.5 hour) test session which enabled him to fully document the pre, stereotypy and after phase of the amphetamine response (Schiorring, 1971).

Mueller et al, (1989a, 1989b) attempted to refine Schiorring's method and quantify the repetition of any given pattern by converting the animal's path through the open field into a series of trips. A trip started in the centre of the open field, was terminated when the animal changed direction or completed a tour of the perimeter, or entered the centre area. A statistic, "gamma", was calculated to quantify repetitive locomotor patterns or locomotor stereotypy. Gamma is the

maximum likelihood estimate of the probability that the animal would repeat the trip it had just exhibited, "If the second trip is the same as the first, a 'repeat' (R) is recorded; if the third trip is the same as the second, another 'repeat' is recorded and so on. If any trip is different from the preceding trip a 'change' (C) is recorded. Gamma is defined as the number of repeats divided by the number of repeats plus changes: Gamma= R/(R+C)" (Mueller et al, 1989a, p75).

Using this method to quantify locomotor stereotypy, Mueller et al, (1989a) report that amphetamine produced a dose-related increase in 'gamma', whilst caffeine, which produced dose-related increases in locomotion, did not increase 'gamma'. In a second study (Mueller et al, 1989b) 'gamma' did not increase in a dose-related manner. Interestingly, in both studies Mueller and colleagues, like Schiorring, reported that circling the perimeter of the open field was associated with the highest doses of amphetamine " a particular type of locomotor stereotypy (circling the perimeter of the open field) tended to be produced in the vast majority of animals." (Mueller et al, 1989a, p78), and; "after 1 and 2 mg/kg amphetamine trips of '1' were most frequent. But after 3 and 4 mg/kg amphetamine, at some time periods trips of '4' were as frequent as trips of '1'. A subset of rats repeated trips of '4' exclusively." (Mueller et al, 1989b, p505). It should be noted that the open field used by Schiorring was exceptionally large, 3 x 3.5 metres divided into 42 0.5 x 0.5 metre squares, whereas the open field used by Mueller et al, was much smaller, a little under a square metre divided into four areas with a central portion. Like Schiorring, Mueller's work was hampered by difficulties in quantifying the exact nature of perseverative locomotor routes and gamma does not consistently measure the dose effects of amphetamine-induced locomotion. The study reported by Mueller et al, (1989a) is beset with methodological problems. The animals were not tested until 25 minutes after the injection of amphetamine. As peak locomotor effects occur at different time periods for different doses, the peak drug effects of the higher doses may well have been missed. The spatial distribution of amphetamine-induced locomotion is likely to change with time, with animals displaying different trips at different times. This would not necessarily, and indeed would be unlikely, to take

an identical course for all doses. Thus in this experiment measures of 'gamma' were likely to be confounded by omitting to test during the first 25 minutes following drug administration. An additional confounding factor is that animals were not habituated to the open field prior to testing; rats and guinea pigs show reduced levels of amphetamine-induced stereotypy when tested in a novel environment (Einon and Sahakian, 1979; Sahakian and Robbins, 1975).

In the later study (Mueller et al, 1989b), animals were habituated in the open field prior to treatment with 0, 1, 2, 3, or 4mg/kg amphetamine, and animals were observed for 100 minutes immediately following amphetamine administration. These refinements go some way towards addressing the faults of the earlier study. These authors report a high 'gamma' score for animals treated with 1 and 2 mg/kg amphetamine and a decrease in 'gamma' for 3mg/kg. Additionally, they were unable to calculate gamma for the most relevant time intervals following treatment with 4mg/kg. The results reported in this study appear to contradict the earlier findings which reported a dose-related increase in 'gamma'.

Work in our laboratory (Kenyon et al, 1992) has shown that 'gamma' correlates well with length 1 trips, and consequently high gamma scores reflect a high number of length 1 trips (eg saline and low dose amphetamine treated animals)and that a decline in 'gamma' reflects a decline in length 1 trips. Such a decline occurs in animals treated with higher doses of amphetamine when length 4 trips emerge (Kenyon et al, 1992). These findings suggest that 'gamma' does not quantify stereotyped locomotion accurately. As locomotor patterns become more thigmotaxic, 'gamma' declines. The higher 'gamma' scores for amphetamine-treated animals in the first study can perhaps be accounted for by the lack of habituation, which perhaps suppressed perseverative locomotor patterns in the novel surroundings, and by the short testing period (20 minutes).

Geyer and colleagues have demonstrated perhaps the most convincing quantification of locomotor patterns in the open field using the behavioural pattern monitor (BPM), which is designed to combine the features of activity and holeboard chambers (Adams and Geyer, 1982; Flicker and Geyer, 1982; Geyer

1982). Each chamber consists of a 30.5 x 60 cm black Plexiglas box connected to a metal 'home cage'. The chamber contains three floor and seven wall holes equipped with infra-red beams and a wall touch plate to detect rearings. Several experiments have demonstrated the sensitivity of this system in the study of locomotor and investigatory behaviour. The use of the BPM has revealed a remarkably consistent structure of the behaviour of untreated rats. There is a general tendency for virtually all untreated rats to avoid the centre region and to stay near a corner of the chamber. Untreated rats make short excursions from the home corner and back following their own particular preferred pattern of movement.

Geyer (1982) and Geyer et al, (1986b) have developed a measure termed 'spatial coefficient of variation (CV)'. Originally a measurement was made of the transitions between any five areas, subsequent applications of this approach have involved the calculation of transitions between any nine areas. Relative transition frequencies are calculated as percent of the total permissible cell entries, and the spatial coefficient of variation is derived from this set of numbers. To the extent that an animal repeated certain transitions, the spatial CV increases. A more random distribution of these spatial transitions produces a low spatial CV.

The effects of drugs on locomotor patterns have been studied in the BPM. At low doses amphetamine disrupts the normal structure of locomotion by producing highly varied patterns of directional change (Geyer et al, 1986). At higher doses, perseverative locomotor routes are produced following treatment with amphetamine (Lat, 1965; Schiorring, 1979; Mueller et al, 1989a). Other stimulant drugs essentially replace the normal pattern of locomotion with new, even more highly structured patterns. For example apomorphine-treated rats make circular trips around the perimeter of the chamber consistently in one direction for most of the session. Scopolamine treated rats also circulate the perimeter of the chamber but in addition frequently change directions and pause to investigate holes, and rear against the walls (Geyer et al, 1986). Stimulants such as caffeine and nicotine do not disrupt the normal structural pattern of locomotion,

locomotor patterns under these stimulant drugs are similar to those exhibited by untreated or saline animals. Rats in an enclosed arena have a tendency to remain close to the walls, referred to as thigmotaxis (Barnett, 1963). This trait is thought to be related to the need to avoid predators and the importance of vibrissae contact for rodents. This behaviour is obviously potentiated under treatment with some stimulant drugs eg scopolamine and apomorphine (Geyer et al, 1986), and higher doses of amphetamine (Lat 1965; Schiorring 1979; Mueller et al, 1989a,1989b).

Measures of centre entries have proved to be extremely sensitive to the effects of drugs. For example hallucinogens decrease entries into the centre of the chamber (Adams and Geyer, 1985). These studies indicate there is much to be gained measuring both peripheral and central movements in the analysis of the behavioural effects of drugs, and endorses the fact that the open field should be large enough to elicit thigmotaxis and to enable the detection of peripheral and central movement. Drugs such as MDMA (Ecstasy) which combine hallucinogenic activity with the classical stimulant actions of amphetamine (Beck and Morgan, 1986), and MDE (Eve) show a combination of the behavioural effects of traditional stimulants and those of hallucinogenic drugs such as LSD. MDMA and MDE are similar to the hallucinogens in producing an avoidance of the centre, unlike LSD, MDMA produces increases in perserverative and thigmotaxic patterns of locomotion reflected by increases in 'spatial CV '.

In contrast to the methods described above the use of metrics derived from ergodic theory of dynamical systems have been applied to locomotor movements of rats in an enclosed arena (Paulus et al, 1990). Such an approach aims to describe the statistical behaviour of stochastic as well as deterministic systems and allows for a comparison that has not been specified a priori but is chosen relative to the data set in question and allows that the animal has constructed its own specific pattern of movement in space. Such an approach to quantifying locomotor patterns following drug treatment, although at an early stage of development, holds much promise.

Behaviours induced by stimulant drugs are inter-related, even those behaviours which seem independent such as sleeping and locomotion because they compete with each other for expression. In order to draw conclusions about any one aspect of behaviour other contributions to the measured behaviour must be excluded, controlled or monitored. Changes in an unmeasured behavioural category could be responsible for an observed change in the measured behavioural category. Many behavioural actions are mutually exclusive and therefore will compete with each other for expression, thus the characterisation of drug-effects on behaviour will require the measurement of all behaviours that are induced under the drug as well as those behaviours that are in the normal repertoire of the animal in the test situation. This is particularly relevant in the use of measures of locomotion as indicators of constructs such as arousal and exploration. In many instances, the correct interpretation of the data obtained from automated measures of locomotor behaviour will depend on the use of direct observational techniques and the utility of a behavioural inventory. For example, under amphetamine, stereotypy interferes with the manifestation of locomotor activity. An interpretation of the possible underlying mechanisms requires measurement of both locomotor and the stereotyped behaviours. Using a multivariate approach to assess drug-induced unconditioned behaviour provides an opportunity to assess the validity of hypothetical constructs proposed to underly the behaviour, to make comparisons with findings reported in the literature, to examine the generality or specificity of the observations, and to identify the role of response competition as well as to detect and eliminate artefacts.

A totally different approach has been adopted by Szechtman et al, (1985) who have measured behaviour following apomorphine by decomposing the observed motor activity into kinematic variables. Using the Eshkol-Wachmann movement notation (Eshkol and Wachmann,1958) which allows for the measurement of behaviour as a spatiotemporal process without defining behavioural acts a priori, they have identified three relevant variables - snout contact, progression and turning - and claim that seemingly unrelated acts such as sniffing, keeping the head down, forward locomotion, pivoting and head swaying are the result of an

interplay of these three independent processes, each with a unique time of peak action amplitude and rate of change.

1.7 Studies into Amphetamine-Induced Stereotyped Locomotion

The problem as defined above is to develop an effective animal model of schizophrenia with predictive, face and construct validity which can effectively discriminate antipsychotic drugs regardless of classification, and with the capability to evaluate novel antipsychotic drugs possibly acting via different mechanisms from those already identified. The characterisation of drug effects on locomotor activity in rodents which is based not only on quantitative increases in the amount of locomotion per se but also on the spatiotemporal characteristics of the hyperactivity seems to be one model which presents itself as worthy of investigation and further development. Clearly, models involving stimulant-induced hyperactivity will become powerful tools only when the statistical assessment of behavioural sequences is adequately addressed. Certainly the measurement of 'spatial CV' and 'trips' go someway towards quantifying the repetitive locomotor patterns, and could be most successful when used in conjunction with behavioural analysis using a rating scale specifically designed to assess behaviours-induced by the stimulant drug under study. For example, the method proposed by Fray et al, (1980) for the assessment of amphetamine-induced stereotypy exemplifies this approach. Whilst novel approaches derived from non-linear dynamics (Paulus et al, 1990) and movement notation (Szechtman et al, 1988) have provided interesting and novel approaches to the characterisation of drug effects in rodents.

This research programme takes as its starting point the effective description and quantification of the spatial characteristics of amphetamine-induced open field hyperactivity. Using computer assisted 'contrast' image analysis to describe the spatial distribution of amphetamine-induced movement in an open field, these studies set out to evaluate the utility of examining changes in the

nature and proportion of thigmotaxic locomotion as an effective quantification of amphetamine-induced changes in spatial patterns of locomotion.

Recent work (Kenyon et al, 1992) has described the animals movements between quadrants in the open field as a series of 'trips'. The first 'trip' begins when the animal leaves the centre of the open field, and is completed when the animal reverses direction, completes a tour of the perimeter or moves back to the centre of the open field. Trips can be classified according to the number of quadrants entered. Thus a length 4 trip involves the animal entering each of the four quadrants in turn before returning to the quadrant from which it started; a length 1 trip takes the animal from one quadrant to an adjacent quadrant ; whilst a length 2 trip and length 3 trip involve the animal entering 2 and 3 new quadrants respectively. Initial studies with the D2 receptor specific 'atypical' antipsychotic, sulpiride, have shown that a dose of 20 mg/kg reduced the total distance animals travelled in the test session, but failed to reduce the proportion of amphetamine-induced thigmotaxic length 4 trips (Kenyon et al, 1992). The implication that hyperactivity and the stereotyped nature of locomotion can be dissociated by an 'atypical' antipsychotic reflects an animal model worthy of further study. The initial aim is to examine the reliability and validity of 'trip length' as a quantifier of the spatial characteristics of amphetamine-induced hyperactivity in an open field. Further studies will examine the manner in which antipsychotic drugs (of various classes) change the spatial characteristics of amphetamine-induced behaviour within this model system.

Chapter Two
Materials and Methods

Preface

Locomotor activity can be defined as movement from place to place, and the measurement of this behaviour in open field studies is a much used technique in behavioural pharmacological research. Most of these measures rely on absolute values of locomotor activity such as distance moved, beam breaks, lines crossed and ignore the actual position of the animal in space. Although the history of examining the spatiotemporal characteristics of locomotion in an open field goes back to the work of Hall and Ballachey (1932) who reported tracings of the path taken by rats in a circular open field, and following drug administration to Lat (1965) and Schiorring (1979), the advent of new technologies such as video tracking systems and associated computer image analysis alongside the expanding knowledge of non-linear dynamics means that there is now considerable potential to gain an understanding of the effects of drugs on the spatiotemporal characteristics of spontaneous locomotor activity.

This chapter discusses some of the methodological considerations related to this task, and sets out the methods that will be employed to examine 'trip length' as a quantifier of the spatial characteristics of amphetamine induced hyperlocomotion in an open field.

2.1 Reliability of Open Field Measures

The open field is a commonly used experimental tool for studying animal behaviour in a wide variety of settings and disciplines. Originally introduced by Hall (1932; 1934) for studying defecation and timidity, it has gained widespread

acceptance, because of the simplicity of the apparatus and the ease and rapidity of measurement of clearly defined behaviours.

Behaviour in an open field represents an interaction of various factors including genetic background, maturation, biological rhythms, rearing, experience immediately prior to testing, stimulation by the test environment, previous experience of the test apparatus and method of measurement. Obviously this places limitations on the generalisations that can be made between experiments conducted in different laboratories. In addition it emphasises the importance that changes made at any stage of the experimental studies contribute to the findings. This led Henderson (1970) to pessimistically note that because of early environmental interactions with genotype were likely to limit the validity of findings uniquely to the laboratory of testing.

The factors which influence the manifestation of behaviour in the open field can best be classed under the headings of environmental, experiential and internal factors.

Environmental factors. Of all the environmental factors which influence behaviour the open field itself is perhaps of paramount importance. If the behaviour under study is locomotion, then the open field should be large enough to elicit this behaviour. Generally open fields are either 0.75 -1 metre square or circular in nature, but some researchers (notably Schiorring, 1971) have used considerably larger open fields. Locomotion in rats is known to increase with increasing field size (Broadhurst, 1957; Montgomery, 1951), whilst a large open field size has been reported to produce a disproportionately large increase in locomotion under low levels of illumination (Blizard, 1971). There appears to be no reports in the literature of the effect of open field shape on behaviour, despite the fact that avoidance of the centre of the apparatus and thigmotaxis are an important aspect of the behavioural response in an open field.

Testing animals in the light or dark portion of the day/night cycle is an important variable influencing motor activity. Ivinskis (1970) has shown that open-field measures are influenced by the amount of illumination. High levels of

illumination produce low levels of activity, and stimulant drugs administered and tested in the dark produce a rate-dependent 'ceiling effect' of high control levels of activity (Montenaro and Babbini, 1965). Experiments should be designed to optimally test in the dark portion of the diurnal cycle with the use of red illumination, as the retina of the rat is relatively insensitive to this wavelength of light.

Noise interference, extraneous to the test environment, can markedly affect activity levels in an open field (Ivinskis, 1970). High levels of noise have been shown to be aversive to the rat (Campbell and Bloom, 1965), and abrupt loud noise has been shown to markedly inhibit locomotion and induce prolonged periods of immobility (Hofer, 1970; Walsh and Cummins, 1976). White noise has often been used to mask ambient noise, however its use has been noted to paradoxically reduce (Bindra and Spinner, 1958), or increase (Livesy and Egger, 1970), locomotion.

Environmental odours are present in the test environment from both the previous test subject and the experimenter. Therefore it would seem to be a wise precaution to wash the open field between tests to exclude possible biasing effects of odour trails left by previous test subjects. The exclusion of the experimenter from the immediate test environment would also seem to be relevant.

Experiential factors. Experiential factors refer to the experience of the animal in the same test situation, previous experience of the drug by the animal, and handling experience immediately prior to the experiment.

The animal's prior experience with the test environment can exert a profound influence on the nature of open field locomotion. Experience with the test apparatus leads to habituation and a consequent decrease in levels of locomotion. In order to avoid masking drug-induced effects by a ceiling effect induced by a novel situation, animals should be habituated to the open field before treatment, though Battig (1969), claims that levels of activity were unaffected by exposure to the apparatus more than 2 hours before the experiment.

Previous experience with both apparatus and drug can affect behaviour over long periods (Rushton et al, 1963;1968). The studies to be outlined in the following chapters employed a 'single shot' method, thus there would seem to be little to be gained from an exhaustive discussion here of the interaction of previous experience with the test environment and drug, other than to record that it is highly significant.

Details of the animal's removal from the home cage and transfer to the open field are seldom reported. Nevertheless, handling 'stress' is an important confounding factor in any test situation. Subjects which have been habituated to the transport procedure prior to testing show increased levels of locomotion at the beginning of an open field trial (Abel, 1971). Rats placed against a retaining wall have a tendency to remain on that side (Satinder, 1969). Therefore it would seem logical to standardise handling procedures, and to preferentially place animals in the centre of the open field at the start of a trial.

The behaviour induced by a drug following injection varies as a function of the time preceding the test and the experience of the animal in this intervening period. Keeping the animal in its home cage for a period before testing produced different results on exploratory behaviour from those seen in animals placed in the experimental situation for the same period following methylphenidate (Hughes, 1972a), d-amphetamine and morphine (Sparber et al, 1973). In addition the route of drug administration will also effect behaviour, typically amphetamine is administered intra-peritoneally (IP) (see Dews 1972).

Internal factors. The species and strain used in experimental studies places constraints on the extrapolation, generalisation and comparison with results reported in the literature, as strain differences in locomotor activity are well documented (Robbins, 1977). In addition to strain differences, age is another factor which is known to influence open-field locomotor activity. In general, older animals are less responsive to both stimulant and depressant drugs. Both sex hormonal changes and circadian rhythms have been shown to influence drug-

induced effects. Female rats are more active than males in a variety of situations including photobeam cages (Watzman et al, 1967).

The nutritional state of the animal is also an important factor in influencing activity levels following drug treatment. Food deprivation is known to potentiate locomotor activity following treatment with d-amphetamine (Campbell and Fibinger, 1971., Simpson, 1974) and following treatment with apomorphine (Sahakian and Robbins, 1975)

In some previous studies measuring locomotor activity in open fields along with concurrent visual observation of behaviour, test sessions were as brief as 5 to 10 minutes (eg Ljungberg and Ungerstedt, 1985). Such short test sessions maximise the influences of factors such as handling, familiarity with the test environment and escape related behaviour, all of which can be influenced by drug manipulation in a similar manner to the behaviour under study. The guidelines set out by the United States Environmental Protection Agency (EPA) indicate that the test session should be long enough for motor activity to reach asymptotic levels by the final 20 percent of the session for the majority of treatments and that activity measures should be collected in equal time periods not longer than 10 minutes duration (Federal Register, 1986). These considerations suggest that sessions examining unconditioned amphetamine effects should be at least 30 and preferably longer than 60 minutes in duration (see Schiorring, 1971 and Rebec and Bashore, 1984).

2.5 Validity of Open Field Measures

So far the discussion of the open field and its relevance to behavioural pharmacological studies has hinged on the reliability of the methods employed and rigorous procedural constraints required to ensure inter- and intra- laboratory reliability of measurements. If the open field measure is examined in terms of its underlying constructs then the position is far from clear. The construct of "emotionality" has been widely represented in open field studies (Denenberg, 1969). Ivinskis (1970), using a factor analytic design, attempted to test the

validity of a number of open field measures as indices of 'emotionality' in the rat. Of the seven parameters selected only defecation and latency to leave the starting area gave effective measures of 'emotionality', and then only as a consequence of changes in open field measures following changes in ambient auditory and visual stimuli. Clearly the evidence for any of the open field measures representing the intervening variable 'emotionality' is somewhat tenuous. In fact the term 'emotionality' appears to be rooted in anthropomorphic interpretations.

The act of placing a rodent in a novel open space from which it is prevented from escape by a surrounding wall can be viewed as a form of stress, rather than inducing emotionality. Several theorists have sought to emphasise the conflict between fear and exploration that occurs in rodents when placed in a novel environment (Hayes, 1960; McReynolds, 1962; Montgomery, 1955). The concept of a general stress response was developed by Selye (1950) and includes hormonal, metabolic and cytological changes which constitute the general response to environmental change, to this list of adaptive capability should be added behavioural change. Kinne (1964) considered the time course of adaptive responses in marine organisms to environmental change, and distinguished between the immediate response, the stabilisation of the response and the new steady state of performance. The immediate response to environmental change occurs over a time course of seconds to hours and may often involve 'overshoot or 'undershoot' phenomena in altered rates of either behavioural or physiological processes. Such an approach can readily be applied to the open field situation.

Antelman and Chiodo (1983) proposed that stress is an important factor in any model of schizophrenia, and went as far as to propose that the interchangeability of stress for amphetamine can account for a number of observations. Certainly acute psychosis can be induced by environmental stressors (Gardos and Cole, 1978), whilst stress can re-induce amphetamine psychosis in patients during remission (Utena, 1974). In addition the behavioural response to amphetamine is enhanced if the animal is exposed to unavoidable mild stress. Tail pinch has been studied as a non-specific arousing stimulus which activates dopamine neurones (Antleman et al, 1980), whilst rats

reared in isolation show both enhanced response to amphetamine and to tail pinch-induced oral behaviour (Sahakian et al, 1975; Sahakian and Robbins, 1977). Whilst one of the major assumptions of Antleman and Chiodo (1983) is that the presence of a stressor should move the dose response curve for amphetamine-induced stereotypy to the left, this need not necessarily be the case. As Kinne (1964) has pointed out, the immediate response to a stressor may involve either 'overshoot' or 'undershoot' in altered responses such that movement in the dose response curve could be expected in either direction. In fact Robbins et al, (1990) state that 'Increments in "arousal" and "stress" do not always act in the same direction as increasing doses of amphetamine.' It is clearly over-simplistic to assert that environmental change acts upon a single hypothetical construct such as stress. The response of the organism to environmental change can readily be seen as falling into three broad categories: the components of a general response to stress; the specific behavioural and/or neuronal changes seen following individual stressors; and finally the change in 'fitness' that results from these responses to environmental stimuli. These aspects of change to environmental stressors are inter-related and measurement of any or all of these aspects may contribute to the open field dependent measure, and thereby allow for a mixture of interoceptive and exteroceptive cues.

Any behavioural test represents an interaction of the subject with the experimental situation, and exploration is defined as a broad category of behaviour which provides the animal with information about its external environment (Berlyne, 1960; Fowler, 1965), either by bringing the organism into contact with distant stimuli (inquisitive exploration) or by directed examination of proximal stimuli (inspective exploration) (Berlyne, 1960). Many behaviours are proposed to be indicative of exploration, including sniffing, rearing, and most commonly locomotion. Denenberg (1969) suggests that locomotion is both a measure of 'emotionality' in animals as well as indicative of exploratory behaviour, although the relationship between these two constructs remains unclear. Lat and Gollova-Hemon (1969) have described the behaviour in terms of arousal-habituation and constructs such as excitability, inhibition and

lability. Berlyne (1960) cites novelty and complexity of stimuli as eliciting exploratory behaviours, Robbins (1977) concludes that, of these, novelty is the more important. Lore (1968) proposes that in any particular test situation general activity levels consist of 'pure locomotor' behaviour alongside an exploratory component which is driven by the novelty and complexity of the test environment. Leyland et al, (1976) claim that they were able to dissociate 'pure' locomotor activity and exploration using both novel and complex stimuli and amphetamine to obtain a double dissociation of the behavioural components. However, the use of visual stimuli seems somewhat incongruous in an animal with poor visual acuity.

Plots of locomotor routes taken by an animal in an open field following drug administration do not rely on specific exploratory variables and therefore any interpretation of these results in terms of exploratory behaviour must be viewed with caution. Such studies are useful for determining drug effects on general activity levels, but they do not distinguish between exploratory behaviour directed towards environmental stimuli and behaviour which is motivated by internal states or stimuli. These caveats should not deter or diminish the value of studying locomotor routes following drug administration but should provide the incentive to study this behaviour, not only in terms of its quantitative properties but also to gain an increasing level of understanding of the underlying constructs involved.

In an attempt to comply with the above recommendations for the optimal testing of open-field locomotor behaviour, in particular with respect to its spatiotemporal characteristics, the procedures outlined below were adopted. Where these were not optimal, due to constraints placed on experimental design by factors outside the control of the experimenter, explanations are given.

2.6 Method

Apparatus. All experiments used an open field constructed from black perspex (30cm in height), which was either square (60 x 60cm) or circular (diameter

75cm). A closed circuit TV camera (Panasonic Colour WVP 200E), was mounted approximately five feet above each open field. In a separate room the camera picture was analysed by an HVS image systems VP112 unit which converted the video signal into a stream of XY co-ordinate pairs, 3 per second. The digital tracking device used foreground/background contrast (white rat against black perspex) to determine the animal's position in the open field from the video image, by locating the first pixel group in the scanning pattern that met the pre-set criterion for contrast (Renner et al, 1990). The apparatus was placed in a laboratory adjacent to the animal holding room which was devoid of ambient noise. Each apparatus was lit using dim red light, sufficient to allow for contrast-based image analysis (see figure 2.1).

Subjects . Male Wistar rats (300-450g), the descendants of stock supplied by Olac, Bicester, Oxon. were used. Animals were bred in the University of Plymouth animal house facility and were available for use once they had reached the age of 6 weeks. A minimum of 48 hours prior to an experiment, animals were moved to the experimental holding facility to acclimatise, where they were housed in groups of six with free access to food and water. Rats were maintained on a 12 hour light/dark cycle with testing taking place in the light portion of the cycle. It would have been preferable to maintain animals on a 12 hour reverse light/dark cycle with testing taking place in the dark portion (Robbins, 1977; Geyer, 1990), but as the animal house facilities were not exclusively for the use of the Department of Psychology this was not possible. The room temperature was maintained at 22 +- 3 degrees Celsius.

Procedure. Animals were removed from the home cage, and if no pre-treatment drug was required, they were placed in the open field to habituate for 30 minutes. If a pre-treatment drug was part of the experimental protocol, then animals were weighed, injected and then placed in the open field for the thirty minute habituation period. Immediately prior to injection animals were removed from the open field, weighed if this had not been done previously, and injected intra-

peritoneally (IP) with stimulant drug. Animals were immediately replaced in the centre of the open field, for an experimental period of 105 minutes. The apparatus was washed with warm soapy water and dried with absorbent paper between test subjects. The open fields were not washed between the habituation period and the test session.

Testing took place between 0900 - 1900 hours with the order of testing randomised for condition, time of day and cohort of animals. Weighing and injection took place in the animal holding facility, which was a room adjacent to the experimental laboratory. Both rooms were maintained at the same ambient temperature (22 +-3 degrees celsius) and once the interconnecting door was closed were completely separated in terms of light, noise and odours. Analysis was conducted in a third room adjacent to the animal holding facility, where the experiment was monitored via video screens, enabling the experimenter to be absent from the test area for the entire experimental period.

Behavioural observation . Animals were exposed to the open field for 30 minutes prior to injection of a psychostimulant drug (for exact details see individual experiments) and replaced immediately in the centre of the open field following injection of drug. The duration of each experimental trial was 105 minutes subdivided into 5 minute session intervals. This was deemed to be long enough to capture the entire preliminary locomotor phase (Schiorring 1971) for all doses of psychostimulants tested.

Data Collection and Analysis . During the 105 minute test session the output from the camera and the HVS image analyser was monitored on a TV screen. The path taken by the subject was displayed on the computer screen by TRACKER software (Kenyon, 1990a), an IBM PC package written in Microsoft QuickBasic 2.0. The screen showed a representation of the floor of the open field divided into four equal-sized areas. The software tracked the animals movements between these quadrants. Horizontal movement was recorded as distance (in cm) moved by an animal in 5 minute time intervals.

The subjects sequence of movements between adjacent quadrants was subdivided into a series of trip lengths using STEREO software (Kenyon, 1991). A single trip consists of a sequence of movements between adjacent regions, and is terminated when the animal reverses direction, or completes a tour of the perimeter. Trip length is defined as the number of regions entered (lines crossed) during a period of forward locomotion without a turn (figure 2.2). STEREO assumes the floor of the open field has been divided into four regions (labelled 1,2,3,4), and that a complete trip in a clockwise direction around the field, starting and finishing in region 1, would consist of the sequence: 1,2,4,3,1. This trip would be classified as a length 4 trip (figure 2.3). A centre trip is scored whenever the subject crosses between any of the regions of the open field that are diagonally opposite each other (eg from region 1 to region 4), and is classified as a length 1 trip.

The program, written in Microsoft QuickBASIC 4.0, reads the sequence of region entries from DATA statements. This allows a word processor to prepare data for analysis. STEREO stores values for the number of trips, number of consecutive repeat trips for each subject in comma separated value (CSV) files. These files were then read into an Excel spreadsheet program and converted from IBM PC/XT (DOS) to Macintosh format.

Figure 2.1

Components of the video image analysing system.

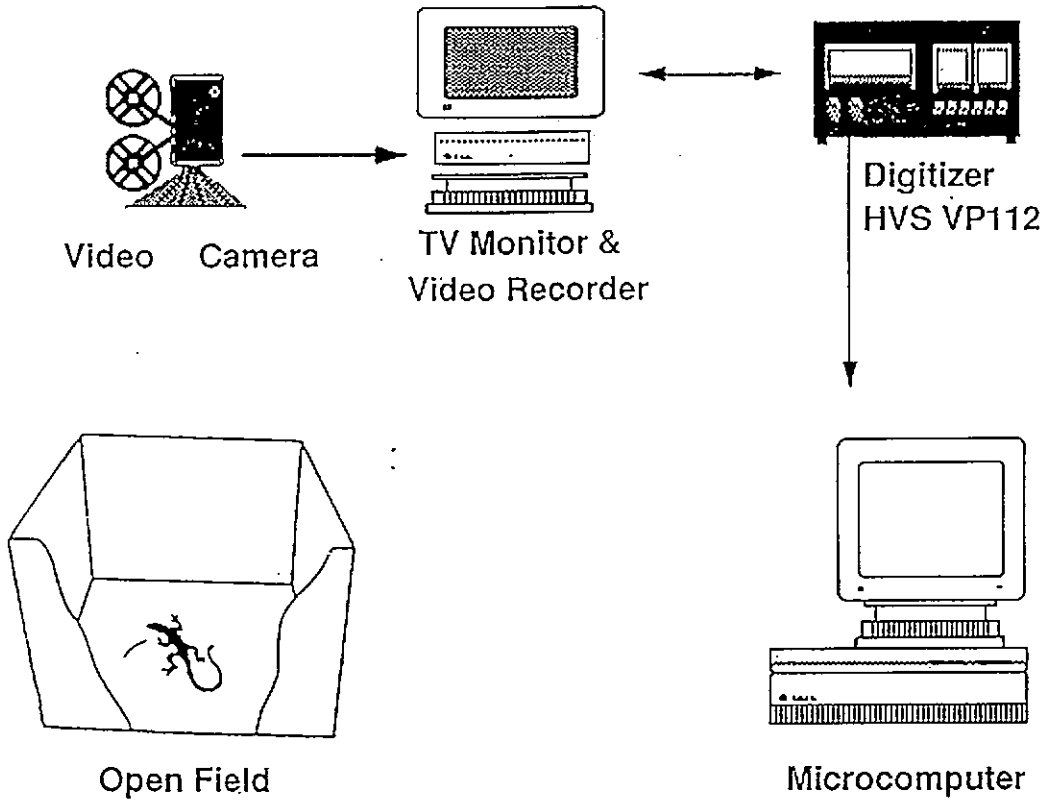

Video Camera
TV Monitor &
Video Recorder
Digitizer
HVS VP112
Open Field
Microcomputer

Figure 2.2

Forward locomotion is divided into various 'trip lengths'. This sequence begins with a length 4 trip, the next trip is a length 3, this is followed by a length 2 trip, the last trip is length 1.

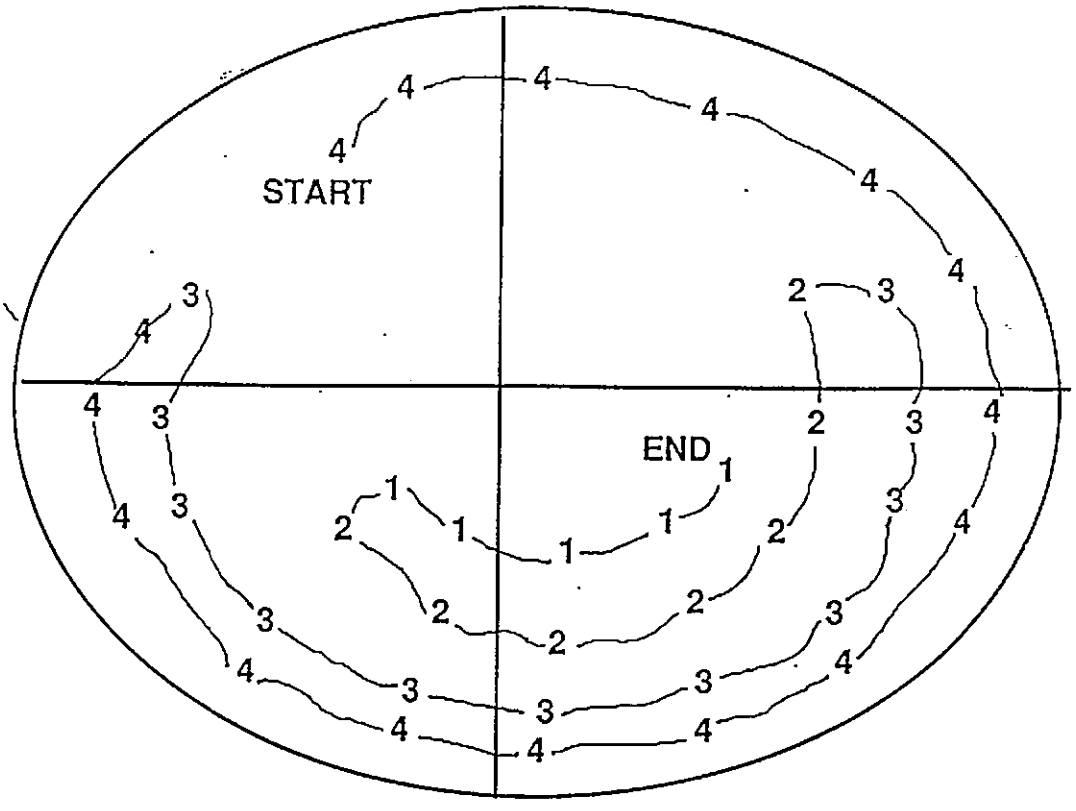
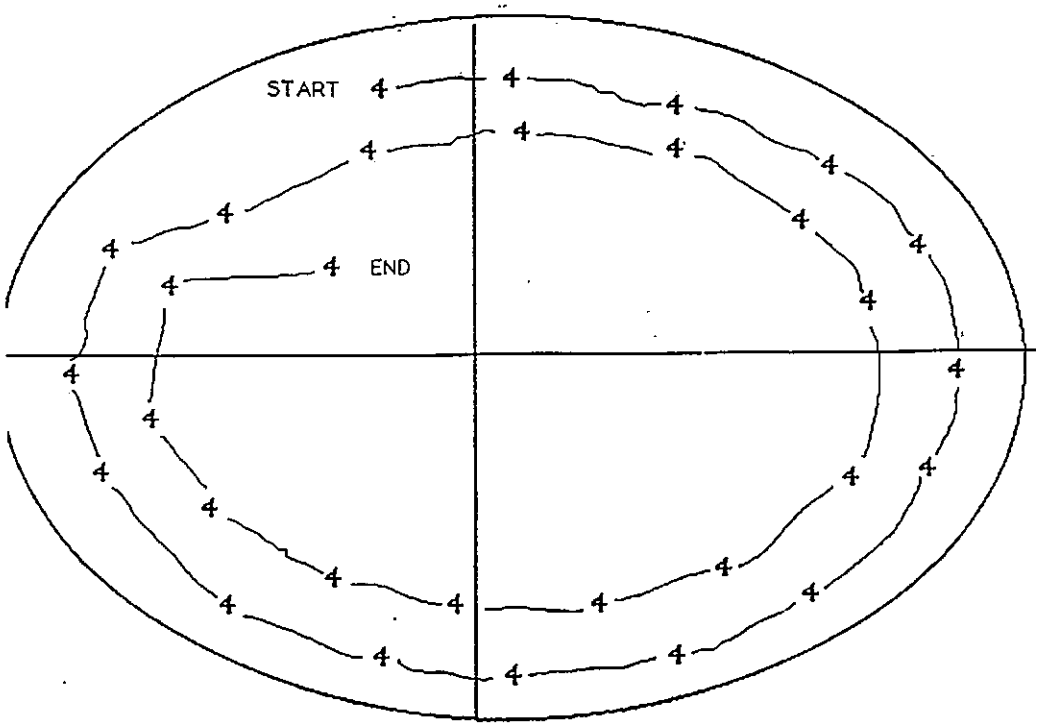

START
END

Figure 2.3

Example of length 4 trips. This sequence begins with a length 4 trip, the next trip is also a length 4 trip.


START
END

Chapter Three

A Comparison between Caffeine- and Amphetamine-Induced Hyperactivity and Stereotyped Locomotion in Open Fields with Different Shaped Perimeters.

3.2 Introduction

It is well documented that amphetamine not only increases locomotion in rats, but also produces a qualitative change in the nature of open field hyperactivity (Lat, 1965; Segal, 1975; Schiorring, 1979; Mueller et al, 1989a, 1989b). Several investigators have defined the perseverative nature of locomotor routes following administration of amphetamine as a form of stereotyped behaviour (eg Schiorring, 1979; Mueller et al, 1989a, 1989b). The work of Geyer and colleagues has indicated that quantification of the perseverative nature of open field locomotion following administration of a psychostimulant drug has the potential to discriminate drugs which would otherwise be indistinguishable when using conventional methods of quantifying open field locomotor behaviour (see Geyer, 1990). To date, quantification of locomotor patterns following stimulant drug administration remains only partially successful (see Chapter 1). The increasing sophistication of computer-based tracking and image analysis systems presents the opportunity to remedy this shortfall, and to develop novel techniques for the measurement of open field activity following drug administration, which will enable a more complete description of the amphetamine response in rats and ultimately lead to a more powerful tool at the disposal of behavioural pharmacologists.

In our laboratory we have used a measure (termed 'trip length') as a means of quantifying perseverative patterns following amphetamine administration (Kenyon et al, 1992). For a complete description see Chapter 2. The purpose of the present study was to examine the reliability and validity of

'trip length as a quantifier of locomotor patterns following amphetamine administration. Schiorring (1979) examined the locomotor patterns following a single (5mg/kg) dose of amphetamine and noted a change in the spatial characteristics of the resulting hyperactivity. Mueller et al, (1989a, 1989b) examined the changes in locomotor patterns following administration of amphetamine over a range of doses between 0.75 and 6.5 mg/kg, but were unable to find a dose-related increase in locomotor stereotypy, reporting that - contrary to expectations - animals treated with 2mg/kg exhibited the highest and most sustained increase in locomotor stereotypy (Mueller et al, 1989b). In this study we examined three hypotheses; that thigmotaxic length 4 trips would increase with increasing dose of amphetamine; that length 4 trips would decline as the dose of amphetamine was increased to a dose at which a 'switch' between locomotor behaviour and 'focused' stereotyped behaviours occurred, and finally that the increase in 'length 4 trips' would be unrelated to increases in activity levels *per se.*

Caffeine, a methylxanthine and centrally active phosphodiesterase inhibitor, is often used in comparative psychopharmacological studies. It is a psychostimulant which produces increases in forward locomotion without the concomitant stereotyped behaviours seen following treatment with amphetamine. Caffeine does not seem to affect dopamine activity directly (Modrow et al, 1981; Katims et al, 1983), and it is claimed that caffeine stimulates locomotor activity independent of neural mechanisms associated with amphetamine-induced hyperactivity (Swerdlow and Koob, 1984). The mechanism of action of caffeine is thought to be a result of inhibition of the cyclic nucleotide phosphodiesterases (Butcher and Sutherland, 1962), which is responsible for the breakdown of cyclic AMP. Thus for systems using cyclic AMP as a second messenger, the effect of these transmitters will be potentiated and prolonged. Furthermore, caffeine potentiates responses in the autonomic nervous system which are mediated by ß1 and ß2 adrenoceptors. It was hypothesised that caffeine would increase open field hyperactivity but would not increase the proportion of 'trip lengths' associated with thigmotaxic patrolling of the perimeter.

Kenyon et al, (1992), used a circular open field and it is unclear whether the thigmotaxic nature of length 4 trips which they reported is an artefact of this environment. Clearly open field behaviour in drug treated animals can be influenced by environmental factors. In Chapter 2 evidence was reviewed which suggests that levels of locomotion in rats is affected by illumination and the size of the open field. In view of the fact that spatiotemporal characteristics of locomotion show a marked change following amphetamine administration there have been no reports in the literature of the effect of open field shape on the patterns of locomotion seen following psychostimulant drugs. Robbins et al, (1990) claims that stereotyped behaviours induced under amphetamine are under a degree of environmental control. Oades (1985) reviewed the available evidence which claims a functional role for dopamine in facilitating the 'switching' between inputs and outputs of information to specific brain regions. He concluded that environmental feedback could play an important role in the unfolding of the amphetamine response in the open field. Szechtman et al, (1982) reported different patterns of stereotypy from two substrains of rats placed in the same environment. All the stereotyped behaviours exhibited contained one invariant feature: the maintenance of snout contact with a surface.

The studies conducted by Schiorring (1971; 1979) and by Mueller et al, (1989a, 1989b) both used a square shaped open field. A square field contains corners which could possibly break snout contact, and as a consequence impede the thigmotaxic nature of amphetamine-induced hyperactivity. In contrast, the open field used by Kenyon et al, (1992) was circular and it remains unclear whether the nature of the unbroken perimeter contributed to the increase in thigmotaxic length 4 trips they observed under a dose of 3.5mg/kg amphetamine, or indeed whether these were purely an artefact of the circular nature of the environment.

This present study examined the effects, in rats, of a wide dose range of amphetamine on the measure termed 'trip length'. In addition it was proposed to examine the effects of caffeine, a psychostimulant thought not to induce stereotyped behaviours, on 'trip lengths'. To provide an insight into the effect that properties of the perimeter exert on the stimulant response, animals

were tested in square or circular open fields with an equal perimeter length. The circular open field was identical to the one used by Kenyon et al, (1992). The square open field had an equivalent length perimeter, but contained corners which could act to break snout contact with the surface, and possibly impede the animals thigmotaxic progression around the apparatus. In addition to providing information on the reliability and validity of 'trip length' as a quantification of the spatiotemporal characteristics of amphetamine induced locomotion, it was also expected that this study would provide information on both the optimum dose of amphetamine and the nature of the open field in which to study the spatial characteristics of amphetamine-induced hyperactivity.

3.3 Materials and Methods

Design. A 2 (field shape) x 9 (drug dose) x 21 repeated measures (session interval) experiment was conducted to examine the effects of field shape and differing psychostimulants on hyperactivity and trip types. Recordings were made (between subjects) in a square or circular open field during 105 minute test period, divided into 5 minute intervals. Each field was divided into a matrix of 4 equal quadrants and the movement between these quadrants was recorded using an image analysis system. Distance moved (cm), and the number and proportion of each trip type (1-4) was recorded for each 5 minute interval.

Animals. Seventy two male Wistar rats (3-4 months of age, 300 +- 74g) were housed in groups of six in a temperature regulated room (22+-3 ^{0}C) adjacent to the laboratory for a minimum of 48 hours prior to being tested. They were maintained on a 12 hour light/dark cycle, lights on at 08:00, with food and water continuously available.

Drugs. Amphetamine was dissolved in phosphate buffered saline at doses of 1, 2, 3, 4 or 5 mg/kg and injected IP in a volume of 1 ml/kg. Caffeine was

dissolved in phosphate buffered saline at a dose of 10 or 30 mg/ml. Caffeine was injected IP in a volume of 1 ml/kg (10, 30 mg/kg) and 2 ml/kg (60 mg/kg).

Apparatus. The square open field (60 x 60 cm) and the circular open field (diameter 75 cm) had a perimeter of equal distance. Each apparatus was lit using dim red light. A video camera mounted above the open fields relayed an image to a monitor, image analyser and microcomputer, which sampled the animals position and stored it as XY co-ordinate pairs at a rate of 3 per second. The pattern of movement was analysed using TRACKER and STEREO programmes (Kenyon, 1990, Kenyon, 1991). A video recording of each experimental session was made.

Procedure. 72 rats were randomly assigned to saline (SAL), amphetamine 1, 2, 3, 4, or 5 mg/kg (AMPH), caffeine 10, 30, or 60 mg/kg (CAFF): square (SQ) or circular (C) open field groups (n = 8).

Animals were habituated in the open fields for 30 minutes immediately prior to injection. Amphetamine or caffeine was injected IP and the animal immediately returned to the open fields. Testing took place between 09:00 - 19:00 hours on each test day; both square and circular open fields were run concurrently with the test order randomised for drug dose and time of day. Each animal was used once only. Between each test the apparatus was thoroughly cleaned and dried to remove scent trails.

Data Collection and Analysis. The computer-assisted image analysis system recorded distance moved, and analysed the sequence of movements (trips) between quadrants in the open fields during a 105 minute test session divided into 5 minute intervals. Trip length was defined as the number of regions entered (lines crossed) during a trip. A trip was terminated when the rat changed direction, or completed a tour of the perimeter of the apparatus.

Amphetamine and caffeine data were analysed separately, using a mixed design Analysis of Variance (ANOVA) with treatment (field shape, drug dose) the between subjects factors and 5 minute session intervals the repeated within

subjects factor. Specific comparisons were made using the Newman-Keuls multiple range test (Winer, 1971). All ANOVA's were carried out using the STATVIEW package for Apple Macintosh and the follow up analyses were conducted manually.

3.4 Results

Total distance moved.

The ANOVA indicated no significant main effect of field shape on the total distance moved in cm across the test session for any of the doses tested ($F(1,84) = 0.001$ $p < 0.9729$. Figure 3.1 shows the total distance moved across the session for all the drug doses administered.

Amphetamine. Amphetamine (1-4 mg/kg) significantly increased the total distance moved across the session above that of saline treated animals in a dose related manner ($F(5,84) = 39.37$ $p < 0.0001$.). Animals treated with 4 mg/kg amphetamine achieved the greatest increase in distance moved over the 105 minute test session. Animals treated with the highest dose of amphetamine (5 mg/kg) showed a significant increase in the total distance moved above that of saline treated animals, but this treatment group covered a significantly shorter total distance across the session than animals treated with lower doses of 2, 3 or 4 mg/kg. of the drug. $p < 0.05$

Caffeine, 10 & 30 mg/kg. Caffeine significantly increased the total distance moved across the session ($F(3,56) = 10.32$ $p < 0.0001$). Animals treated with 10 and 30 mg/kg caffeine travelled approximately equivalent distances to those exhibited by animals treated with either 1 or 5 mg/kg amphetamine ($ps < 0.05$).

Caffeine, 60 mg/kg. Animals treated with 60 mg/kg caffeine did not increase the total distance moved across the session above that exhibited by saline treated animals. It is possible that this lack of hyperactivity was a result of this dose being at the threshold of the maximum dose which these animals could tolerate. Many of the rats remained immobile during the entire test session, although complete recovery from the effects of this drug was evident 2 - 3 hours after injection. These data will not be discussed further.

Distance moved across the session

As expected there was a significant effect of time on the level of hyperactivity shown by animals treated with amphetamine ($F(5,20) = 25.16$, $p < 0.0001$). Figure 3.2 shows the activity over the test session for all amphetamine and caffeine treated groups. All groups treated with amphetamine were most active in the first hour of the test session, with only those animals treated with 1 mg/kg returning to the level of activity exhibited by saline treated animals, 50 minutes after injection.

In addition the ANOVA indicated a significant Drug Dose x Session Interval interaction. ($F(5,100) = 4.44$ $p<0.0001$). As the dose of amphetamine increased the latency to maximum level of activity was shortened, with animals treated with 5 mg/kg having the shortest latency time of 20 minutes post-injection to reach maximum activity levels.

Animals treated with caffeine (10 or 30 mg/kg) also showed a significant effect of time on the level of hyperactivity, with animals being most active in the first 45 minutes of the test session ($F(2,20) = 10.78$ $p< 0.0001$). Caffeine, unlike amphetamine, did not show a Dose x Session Interval interaction ($F(2,40) = 1.35$ $p > 0.05$).

Effect of Field Shape on Distance Moved

The ANOVA showed that field shape did not significantly affect the total distance moved across the session for any of the doses tested. Field shape had no

significant effect on any of the measures recorded for any of the caffeine treatment groups. Interestingly, the ANOVA revealed that animals treated with amphetamine exhibited a significant Field Shape x Drug Dose x Session Interval interaction for distance moved ($F(100,1680) = 1.35$ $p < 0.05$). Examination of this interaction revealed that animals treated with 4 or 5 mg/kg and tested in the square field showed a reduction in the peak level of activity, whilst the duration of hyperactivity continued further into the test session than the same treatment groups tested in the circular field (see figure 3.2).

Figure 3.3 shows the cumulative percentage of the total distance moved for each of the 5 minute session intervals. It is clearly evident that the development of the hyperactive response in animals treated with 1, 2 and 3 mg/kg amphetamine follows an identical time course when tested in either a square or circular open field. In marked contrast, the development of amphetamine-induced hyperactivity following treatment with either 4 or 5 mg/kg amphetamine exhibited a markedly different time course depending on whether the animals were tested in an open field with a square or circular perimeter. Animals treated with 4 mg/kg amphetamine had covered a significantly greater distance in the circular field compared to this group tested in the square field after 60 minutes of the test session had elapsed. Those animals treated with 5 mg/kg amphetamine covered significantly greater distances 45 minutes into the test session in a circular open field. This significant increase in hyperactivity was maintained for the remainder of the test session in both groups.

Proportion of length 4 trips.

Treatment with amphetamine changed the pattern of movement made by animals in the open field. Amphetamine treated animals took a locomotor path which became increasingly repetitive, remaining close to the perimeter wall (thigmotaxis). The qualitative change in the spatial distribution of movement exhibited by amphetamine treated animals was reflected in a change in the proportions of trip lengths over the session.

Figure 3.4 shows an example of relative proportions of length 1 and length 4 trips made by a typical group of Vehicle-Saline treated animals, and a typical group of Vehicle-Amphetamine (3.5mg/kg) treated animals.

.Figure 3.4A shows that the majority of trips made by Vehicle-Saline treated animals were length 1, with a significantly smaller proportion of length 4 trips ($p < .01$).

Figure 3.4B shows that treatment withan intermediate dose of amphetamine increased the proportion of length 4 trips. In addition, amphetamine resulted in a significant change in the proportions of trip lengths as a function of time interval across the session. At time intervals 15 to 90 minutes the proportion of length 4 trips was increased and the proportion of length 1 trips was decreased under amphetamine ($p < .01$). The increase in length 4 trips after treatment with amphetamine reflected a tendency for animals to take a path around the perimeter of the open field which became increasingly repetitive, particularly during the period of peak drug response.

Number of length 4 trips

Figure 3.5 shows the total number of length 4 trips made by each of the treatment groups. Saline treated animals made relatively few thigmotaxic perimeter trips, usually less than 10. In marked contrast, the total number of length 4 trips increased following treatment with amphetamine. Groups treated with 3 or 4 mg/kg amphetamine made between 120 - 190 perimeter tours of the open field during the 105 minute test session.

Proportion of length 4 trips

Figure 3.6 shows the effect of amphetamine on the proportion of complete trips around the perimeter (length 4 trips) in a square or circular open field over time. An ANOVA conducted on this measure revealed that there was no significant main effect of field shape on the total proportion of length 4 trips over

the entire105 minute test session for any of the treatment groups ($F(1,84) = 1.204$ $p < 0.2756$. The ANOVA confirmed a significant main effect of amphetamine dose ($F(5, 84) = 13.93$, $p < 0.0001$) on this behaviour. Comparisons using Newman-Keuls multiple range test revealed that animals treated with 2, 3, 4 or 5mg/kg amphetamine showed a significant increase in the proportion of length 4 trips, and that those animals treated with 3 or 4mg/kg amphetamine showed significantly more length 4 trips than any other treatment group ($p < 0.05$). Treatment with caffeine (10 & 30mg/kg) did not result in a significant increase in the proportion of length 4 trips above those of saline treated animals ($F(2,42) = 2.519$, $p > 0.05$).

Effect of field shape on the proportion of length 4 trips

The ANOVA conducted on the proportion of length 4 trips data also revealed a significant Field Shape x Drug Dose x Session Interval interaction ($F(100,1680) = 1.41$, $p < 0.005$). Figure 3.6 shows that animals treated with 4 or 5 mg/kg amphetamine exhibited a time course for the emergence of length 4 trips which was varied according to field shape. Animals treated with 4 mg/kg amphetamine and tested in the square field continued to make thigmotaxic length 4 trips longer into the test session than in the circular field. This group made proportionally more length 4 trips at session interval '65 minutes' (Mean = 0.39, SEM = 0.14) than in the circular open field (MEAN = 0.18, SEM = 0.07) ($p < 0.025$). This significant increase in the proportion of thigmotaxic trips was maintained until 90 minutes into the test session (Circular Field Mean = 0.07, SEM = 0.02; Square Field Mean = 0.21, SEM = 0.35 $p < 0.025$). During the final 10 minutes of the session the proportion of length 4 trips remained equivalent in both the square and circular shaped open fields.

Figure 3.7 shows the cumulative percentage of the number of length 4 trips made for each of the 5 minute session intervals. These illustrate the development of length 4 trips, and show quite clearly the stepwise progression of saline treated animals touring the perimeter of the square open field. In marked

contrast, treatment with amphetamine (1 to 3mg/kg) removed this stepwise movement and resulted in an identical development of length 4 trips in both types of open-field. Treatment with the higher doses of 4 or 5 mg/kg amphetamine resulted in a marked facilitatory effect on this measure for animals tested in a circular field. Rats treated with the maximum dose of 5mg/kg, tested in a circular open field made 100% of the length 4 trips by 45 minutes compared with similar treated animals tested in a square field, who made length 4 trips over the entire test session.

3.4 Discussion

As expected, amphetamine and caffeine increased hyperactivity in the open field, resulting in an increase in the distance moved over the 105 minute test session. In contrast, amphetamine but not caffeine, resulted in an increase in the proportion of thigmotaxic length 4 trips. This increase was most marked for groups treated with 3 or 4 mg/kg amphetamine, doses which are also associated with the greatest increase in distance moved across the test session. The initial locomotor phase is known to decrease in duration at doses greater than 5mg/kg (see Rebec and Bashore, 1984), and with increasing dose the animal will spend more time engaging in focused stereotyped behaviours. This effect was replicated in this experiment; animals treated with 5mg/kg amphetamine covered less distance, and made fewer length 4 trips, than animals administered 2-4 mg/kg of the drug. The increase in the proportion of length 4 trips exhibited by amphetamine - but not caffeine-treated groups - would tend to support the suggestion that thigmotaxic patrolling of the perimeter of the open field measured as length 4 trips is an aspect of amphetamine-induced hyperactivity rather than the stimulant properties of the drug *per se.* These results remain far from unequivocal. The magnitude of the hyperactive response induced by either 10 or 30 mg/kg caffeine was only equivalent to the increase in activity exhibited by animals treated with 1 mg/kg amphetamine. It should be noted that treatment with this dose of amphetamine did not result in an increase in the proportion of length

4 trips. Caffeine was administered at a dose of 60 mg/kg in an attempt to obtain increased levels of hyperactivity, but these animals remained hypoactive for reasons which are not clear, although one likely explanation is that this high dose exerted a toxic, rather than stimulatory, effect on these animals.

Perhaps the most interesting aspect of these results is the effect of field shape on the time course of the amphetamine response. The nature of the perimeter had no effect on the time course of hyperactivity exhibited by animals treated with caffeine or low doses of amphetamine. Animals treated with the highest doses of amphetamine (either 4 or 5 mg/kg) exhibited markedly different patterns of response over time when tested in a square or circular open field. Animals tested in a circular field covered greater distances at the time of peak drug response and progressed more rapidly from the locomotor phase of the amphetamine response to other static behaviours (see Chapter 5) than animals treated with equivalent doses of amphetamine tested in a square open field. Those animals tested in a square field exhibited lower peak levels of activity and spent a longer time engaged in forward locomotion and thigmotaxic patrolling. At the end of the 105 minute test session there was no significant difference in the total distance either group of animals had covered: the time course rather than the total amount of forward locomotion was influenced by field shape. Surprisingly, the corners present in the square perimeter did not impede forward locomotion and force the animal to engage in static focused behaviours but actually increased the time spent in forward locomotion and thigmotaxic progression around the perimeter. The hypothesis put forward by Lyon and Robbins (1975) states that administration of amphetamine leads to reductions in pausing and enhanced behavioural competition among the different response sequences. These results lend support to this hypothesis. The effect of the perimeter on the time course of forward locomotion and length 4 trips is relevant only to those doses of amphetamine close to the dose at which the dominant response shifts from locomotory behaviours to focused stereotyped behaviours, indicating that at these doses the animal is experiencing competition for expression between the locomotor effects of the drug and focused stereotyped behaviours. It would appear

that far from being an artefact of the circular open field, thigmotaxic length 4 trips are a link between locomotory and static focused behaviours, with the locomotory path taken by the animal becoming increasingly stereotyped leading eventually to focused stereotyped behaviours. Presumably at this time the animal experiences increasing competition for the expression of behavioural elements of the amphetamine response which are open to sensory input and individual expression.

Szechtman et al (1982) have claimed an important role for the maintenance of snout contact in the amphetamine response, particularly linked to forward locomotion. The nature of the perimeter could serve to either maintain snout contact (circular open field) or disrupt snout contact (square open field) which may be an important factor in the unfolding of the behavioural sequence of events. The expression of amphetamine-induced forward locomotion could be dependent upon snout contact. The findings in this experiment suggest that neither open field encouraged more or less forward locomotion, the influence was merely on the time course of the behavioural sequence.

The finding that thigmotaxic patrolling of the perimeter measured as an increase in the proportion of length 4 trips increases with increasing dose of amphetamine between 2-4 mg/kg, and that a maximum increase in the proportion of length 4 trips was obtained in animals treated with 4 mg/kg amphetamine indicates that future studies should utilise a dose within this range. Ideally, activity levels should be elevated to such an extent that both inhibitory and facilitatory effects of pre-treatment with antipsychotic drugs could be detected. These experimental findings indicate that measures of thigmotaxic patrolling obtained from animals administered amphetamine at a dose close to that at which the dominant response shifts from locomotory behaviours to focused stereotyped behaviours are less robust, and that the time course of this behavioural response can be readily altered by environmental input. In the light of these findings it would be advisable to use a dose of amphetamine which gives near maximum increases in the proportion of length 4 trips, but the time course remains invariant regardless of open field shape. Kenyon et al, (1992) used 3.5mg/kg

amphetamine and pre-treatment with 20mg/kg sulpiride and were able to show a decrease in hyperactivity, without entirely eliminating forward locomotion or the associated increase in the proportion of length 4 trips. The findings of this present experiment, and the findings of Kenyon et al, (1992) would support the use of 3.5mg/kg amphetamine in studies designed to examine the effects of pre-treatment with antipsychotic drugs on amphetamine-induced hyperactivity and the associated increase in the proportion of length 4 trips.

Open field shape had no effect on the total number of length 4 trips, although groups treated with 3 or 4 mg/kg amphetamine and tested in the square open field showed increased variability in the number of trips around the perimeter of the open field, this may have masked a trend for animals to make more length 4 trips in the square field at these higher doses. What remains clear from these findings is that testing in a circular field produces a sharper dose response, with animals reaching higher numbers of length 4 trips at the time of peak drug response. It could be argued that this test environment eliminates the exploratory component of the locomotor response. Preliminary studies into stereotyped forms of locomotor behaviour induced under amphetamine should use a circular open field, allowing more explicit measures of exploratory behaviour (eg holepokes) to be introduced once measures of the stereotyped component of hyperactivity have been well documented.

Summary

This experiment tested the hypothesis that thigmotaxic perimeter trips (length 4 trips) are a discrete stereotyped component of amphetamine-induced hyperactivity. Rats were treated with a range of doses of amphetamine, or caffeine, and then tested in either a square or a circular open field. An automated tracking system was used to record distance moved and sequences of movements between quadrants in the open fields. The results showed that amphetamine (all doses) and caffeine (10 or 30 mg/kg) significantly increased the total distance moved across the 105 minute test session. Amphetamine (2 to 5 mg/kg), but not

caffeine, increased the proportion of length 4 trips above those of saline treated animals. Field shape did not influence either the total distance moved, or the proportion of length 4 trips over the session at any of the doses tested. Interestingly, animals treated with 4 or 5 mg/kg amphetamine exhibited a significant Field Shape x Drug Dose x Session Interval interaction for both distance moved and the proportion of length 4 trips. Analysis of this interaction revealed that the duration of hyperactivity and thigmotaxic patrolling was prolonged in rats tested in the square field. This experiment also indicated that using a dose of amphetamine between 3 and 4 mg/kg and then testing animals in a circular open field provides the optimum conditions for studying amphetamine-induced hyperactivity and trip lengths.

Figure 3.1

Mean (+-SEM) distance moved (cm) during the 105-min observation period by rats (n = 8 per group) injected with saline, amphetamine (1-5 mg/kg) or caffeine (10-60mg/kg) and tested in a square or circular open field.

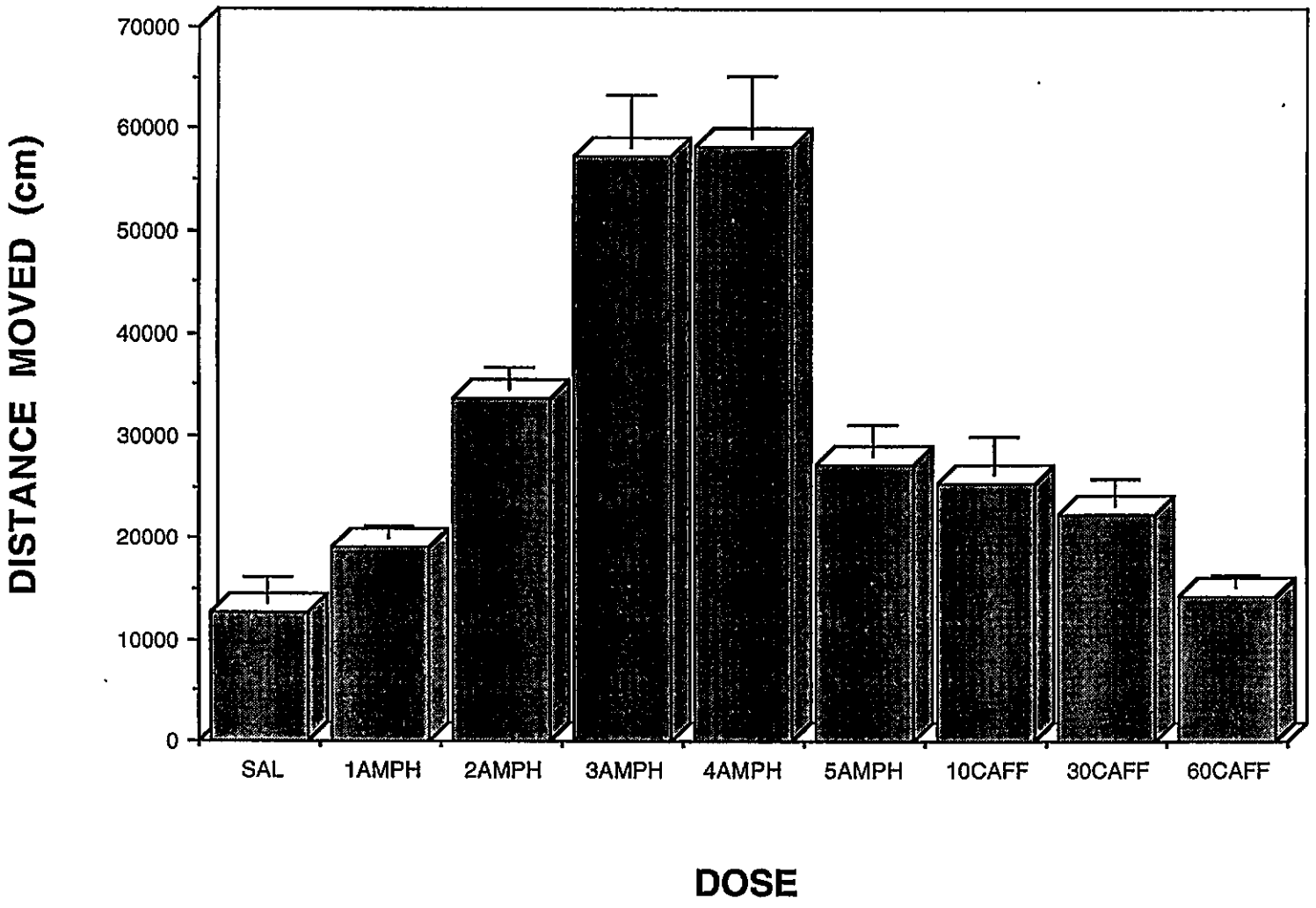
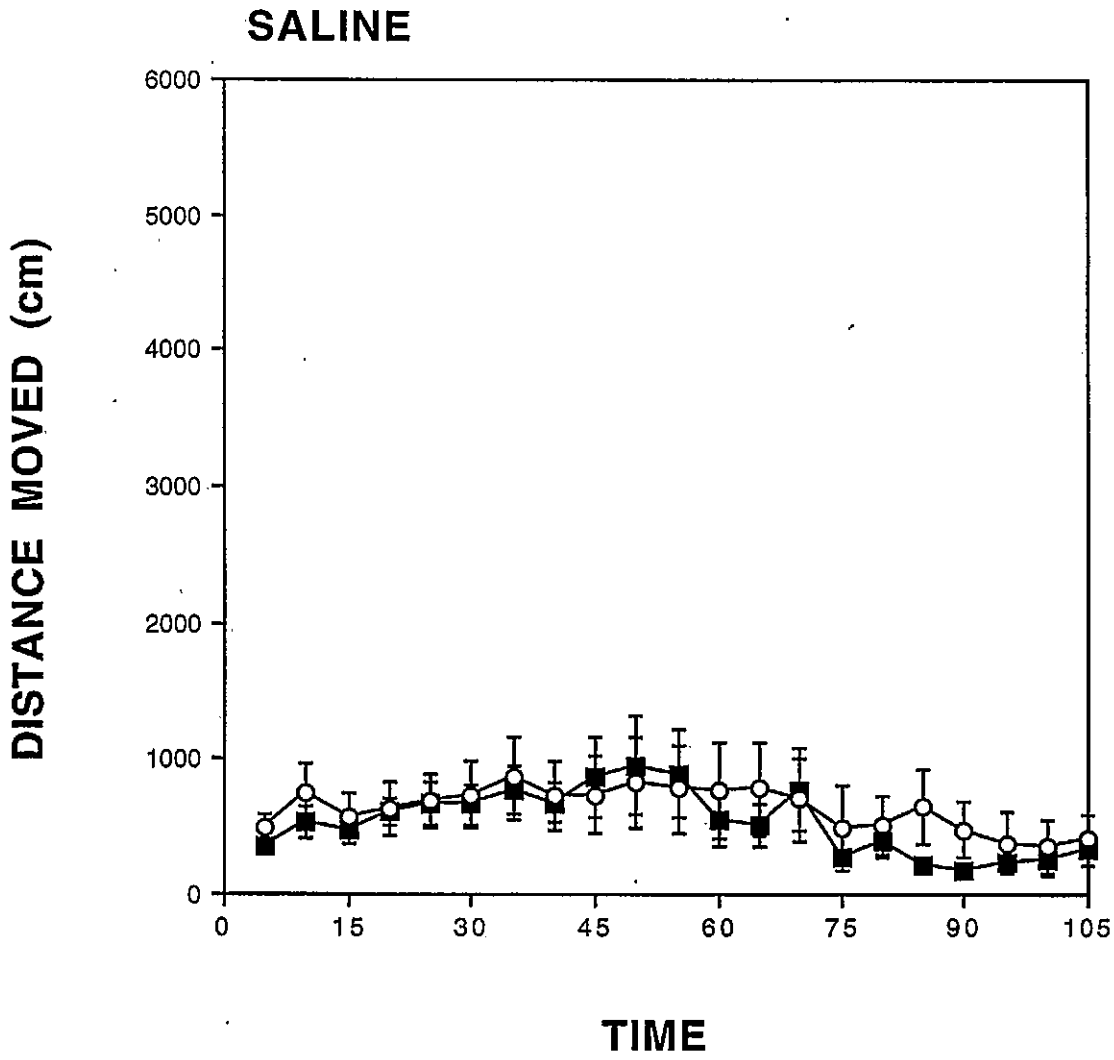
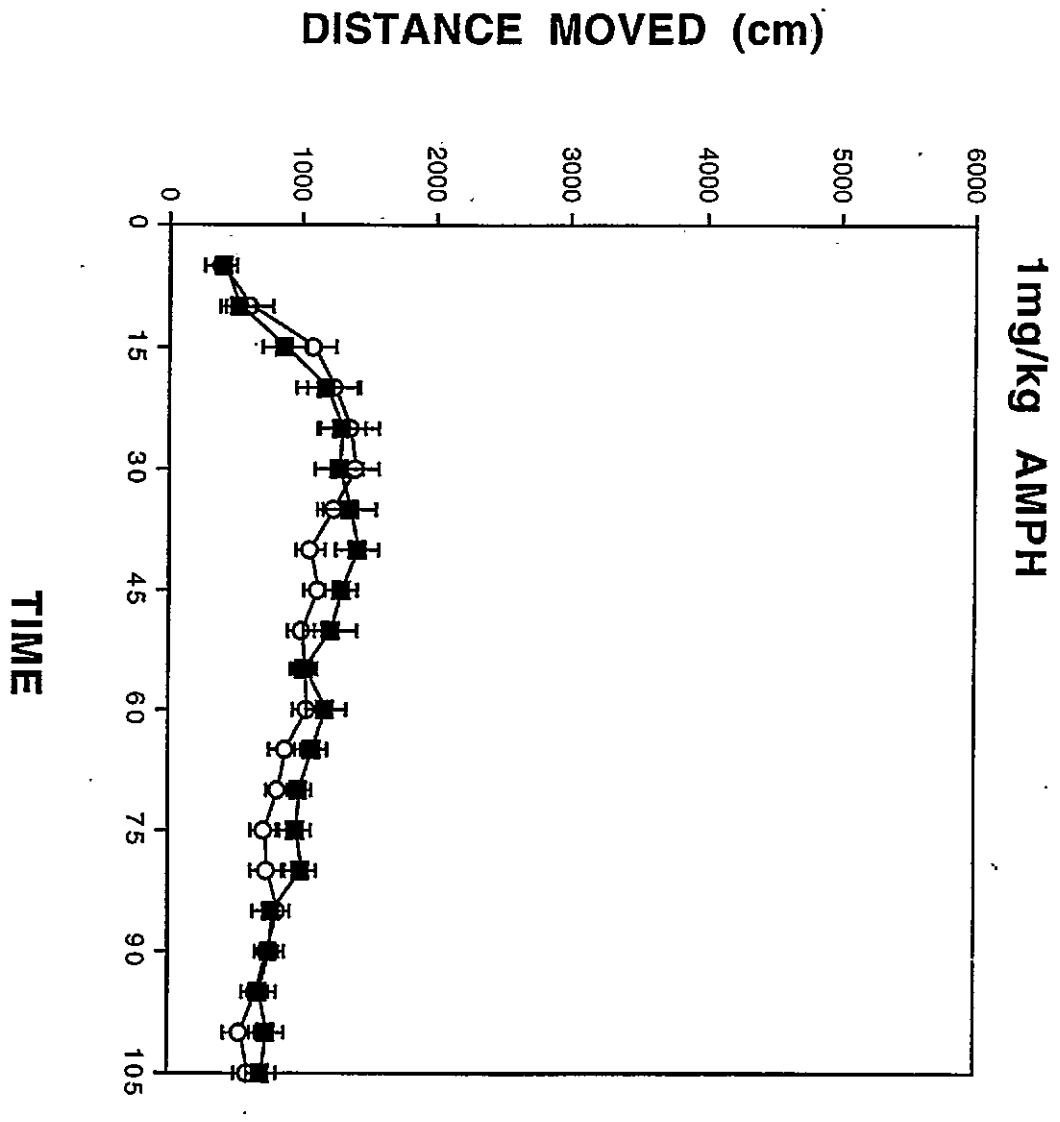
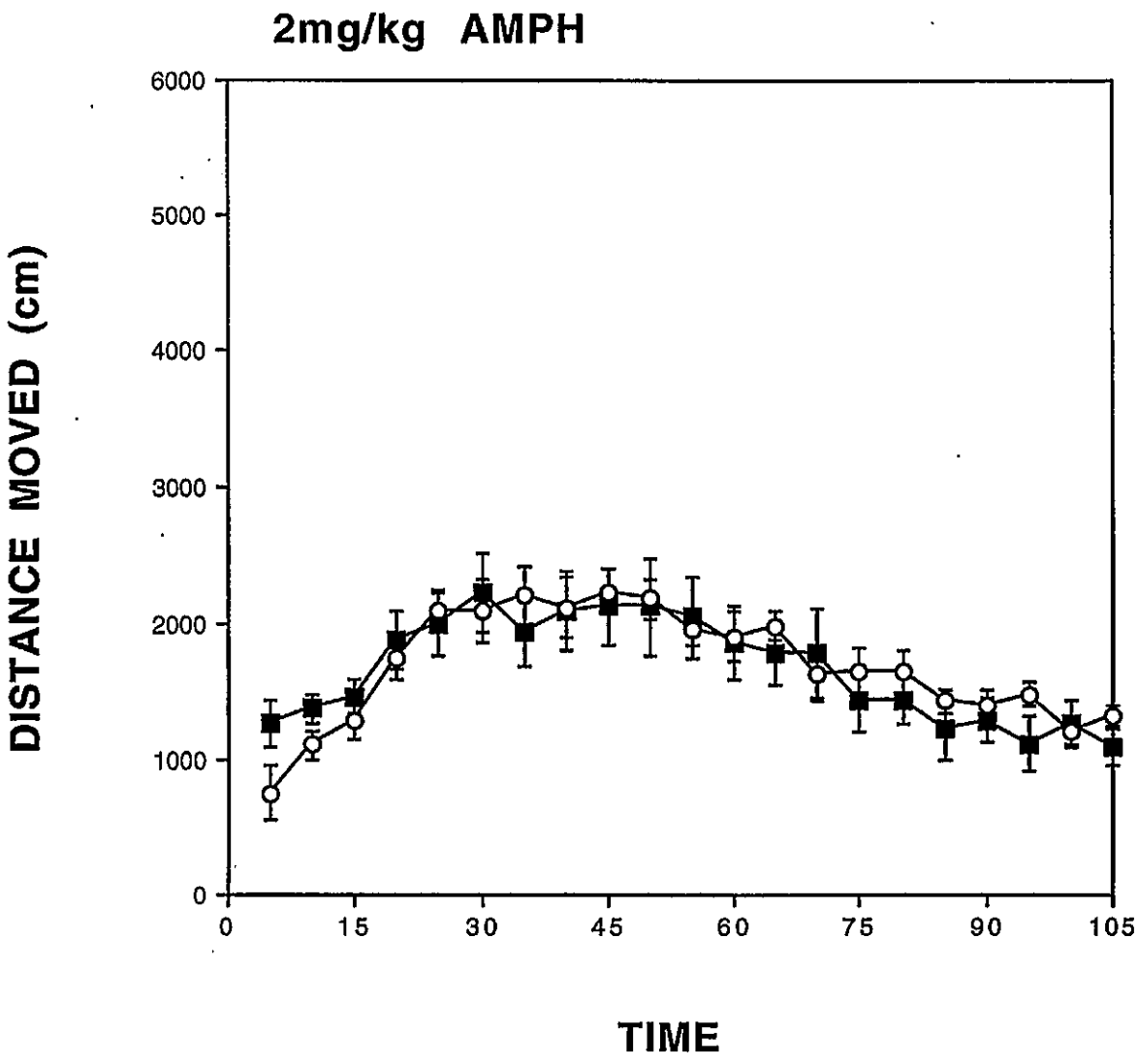

DISTANCE MOVED (cm)
70000
60000
50000
40000
30000
20000
10000
0
SAL
1AMPH
2AMPH
3AMPH
4AMPH
5AMPH
10CAFF
30CAFF
60CAFF
DOSE

Figure 3.2

Mean (+-SEM) distance moved (cm) per 5 min during the 105-min observation period by rats (n = 8 per group) treated with saline, amphetamine (1-5 mg/kg) or caffeine (10-60 mg/kg), and tested in a square (closed squares) or circular (open circles) open field.


SALINE
DISTANCE MOVED (cm)
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60
75
90
105
TIME


DISTANCE MOVED (cm)
0
1000
2000
3000
4000
5000
6000
0
15
30
45
60
75
90
105
TIME
1mg/kg AMPH


2mg/kg AMPH
DISTANCE MOVED (cm)
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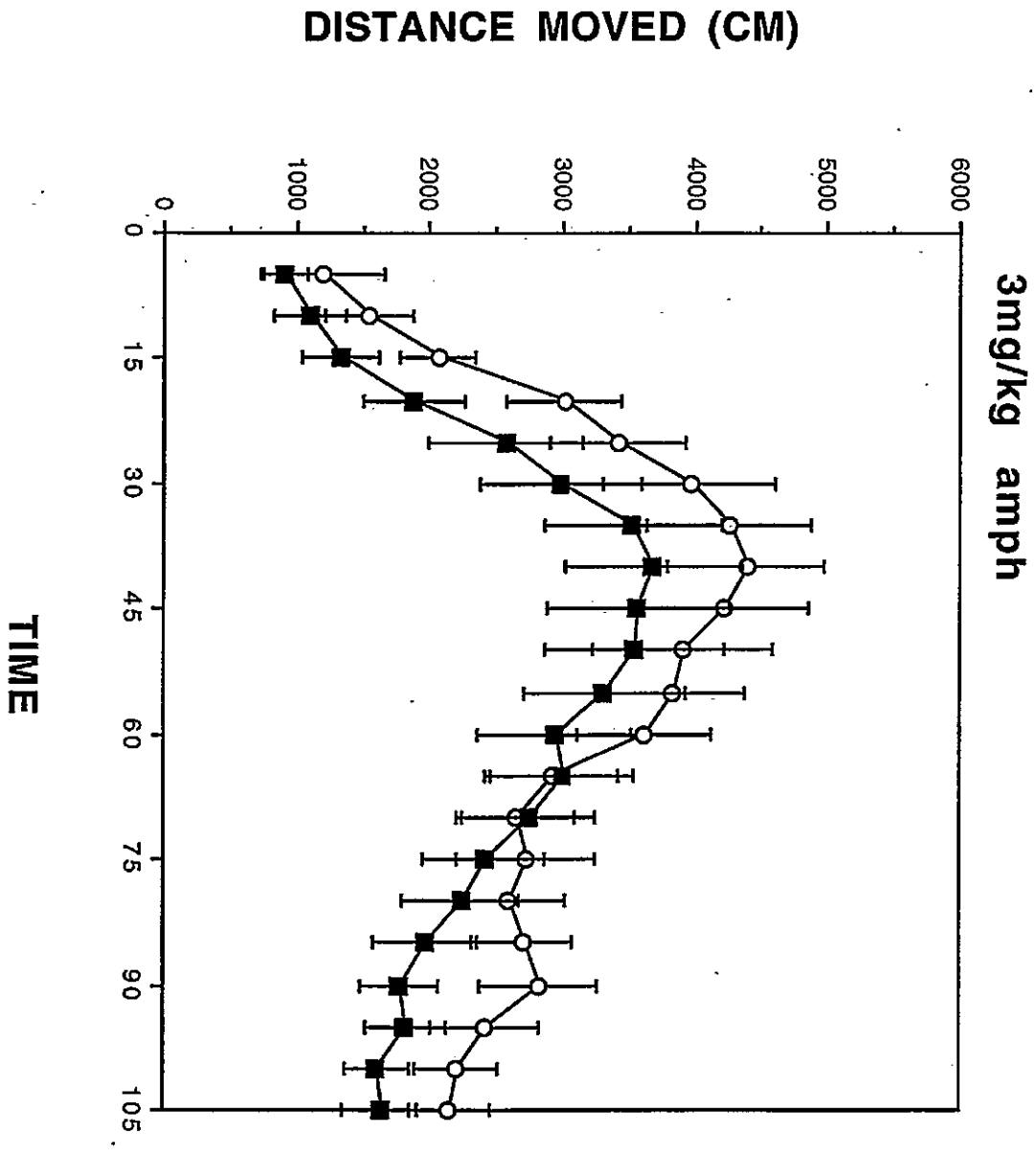
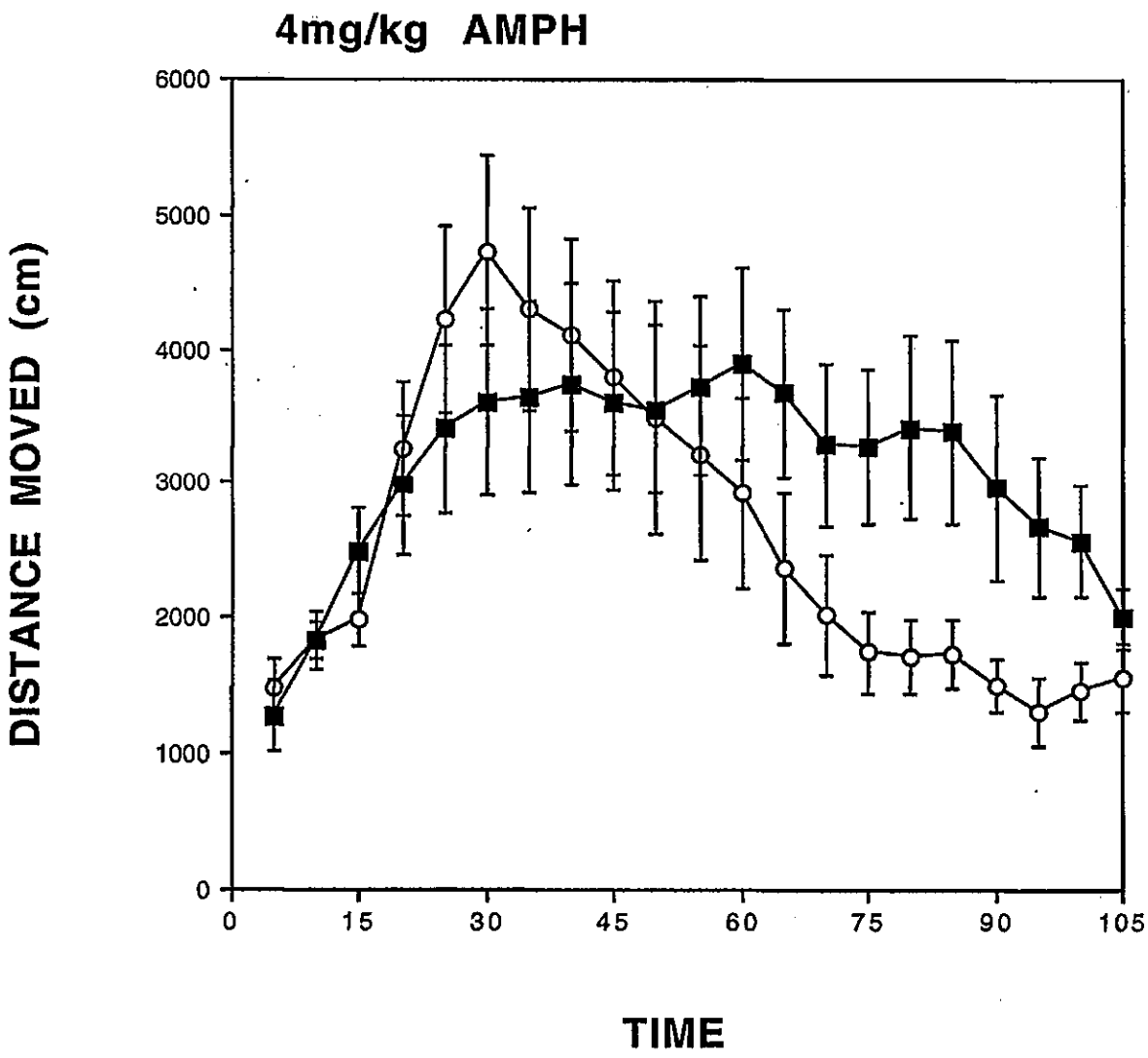
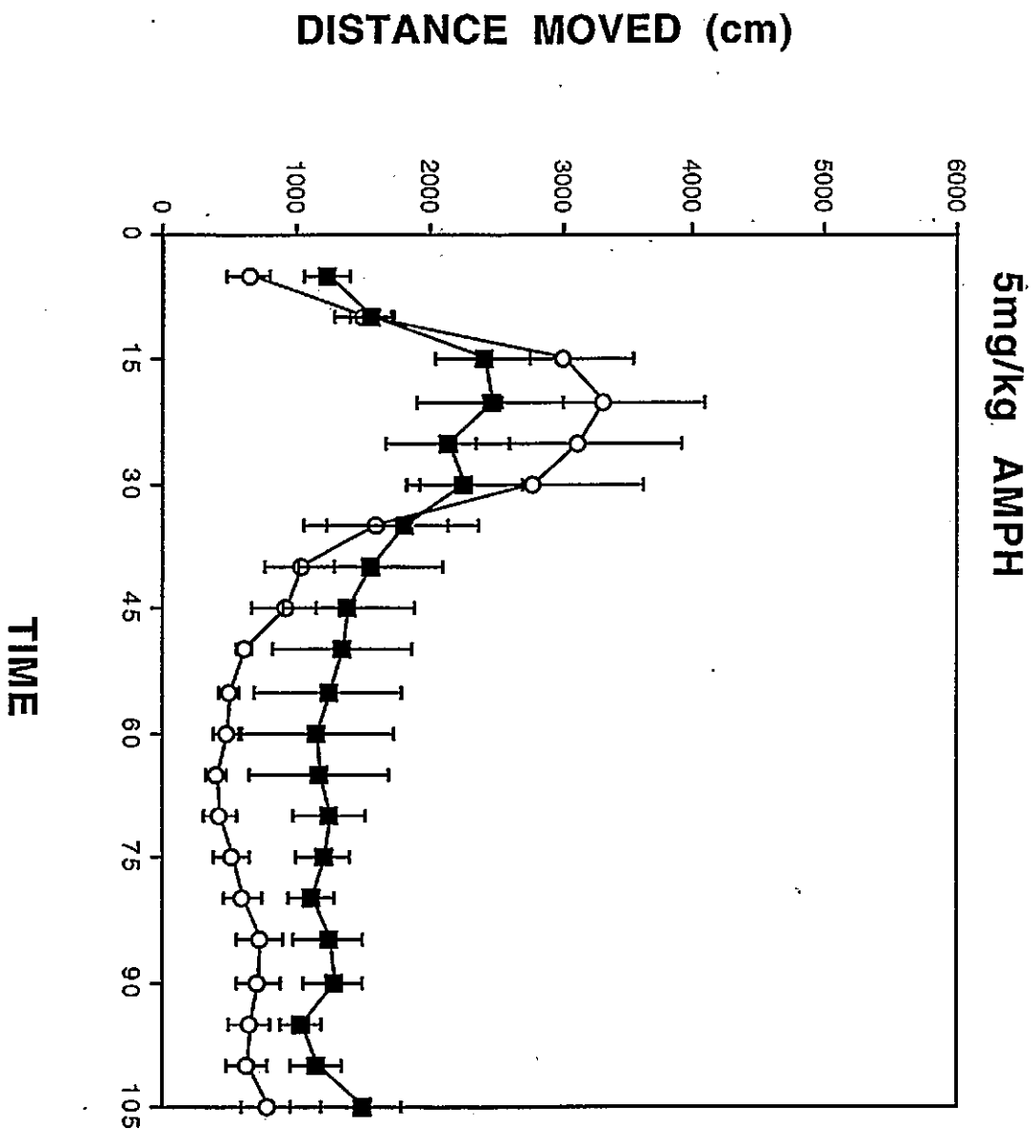
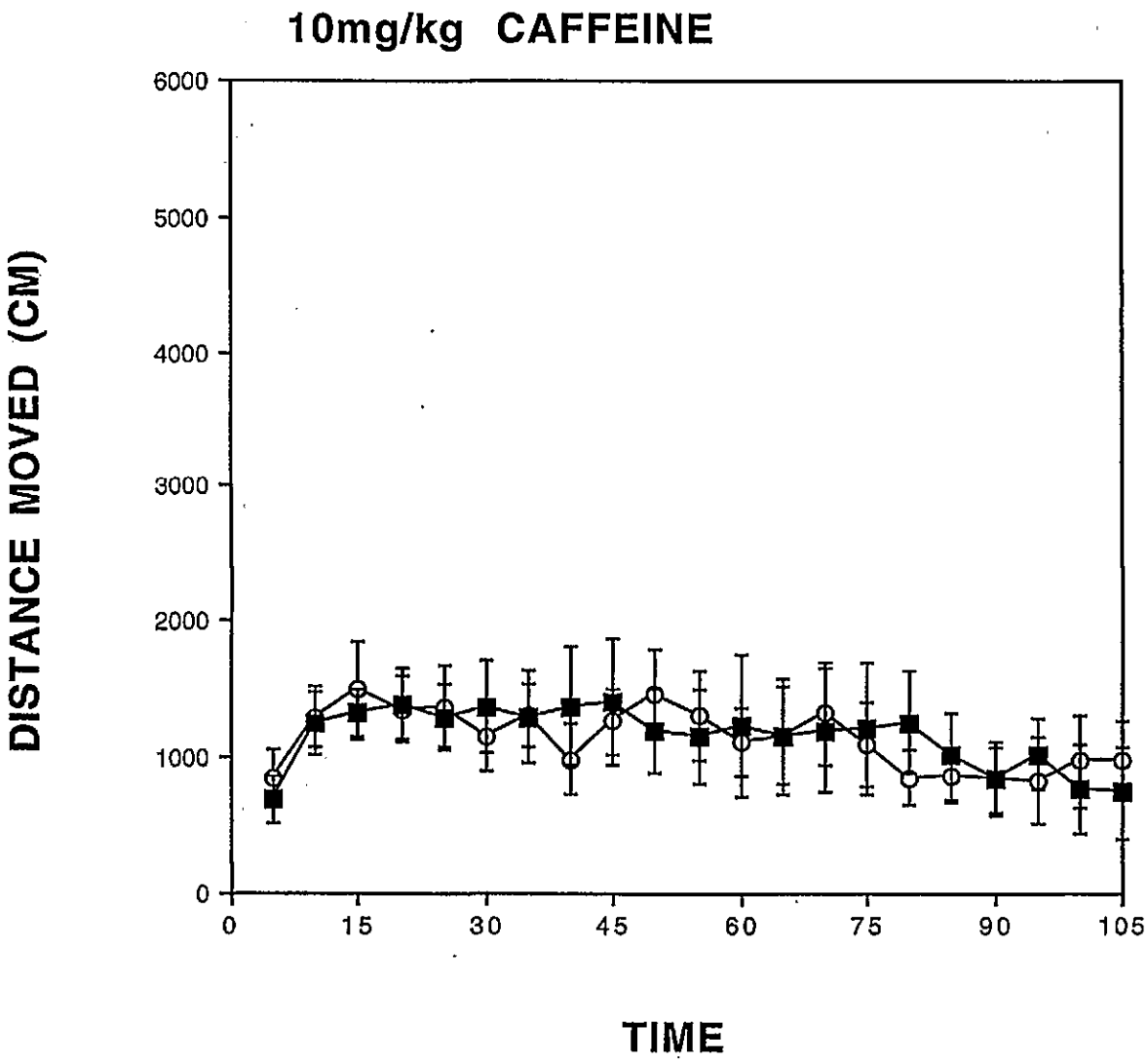
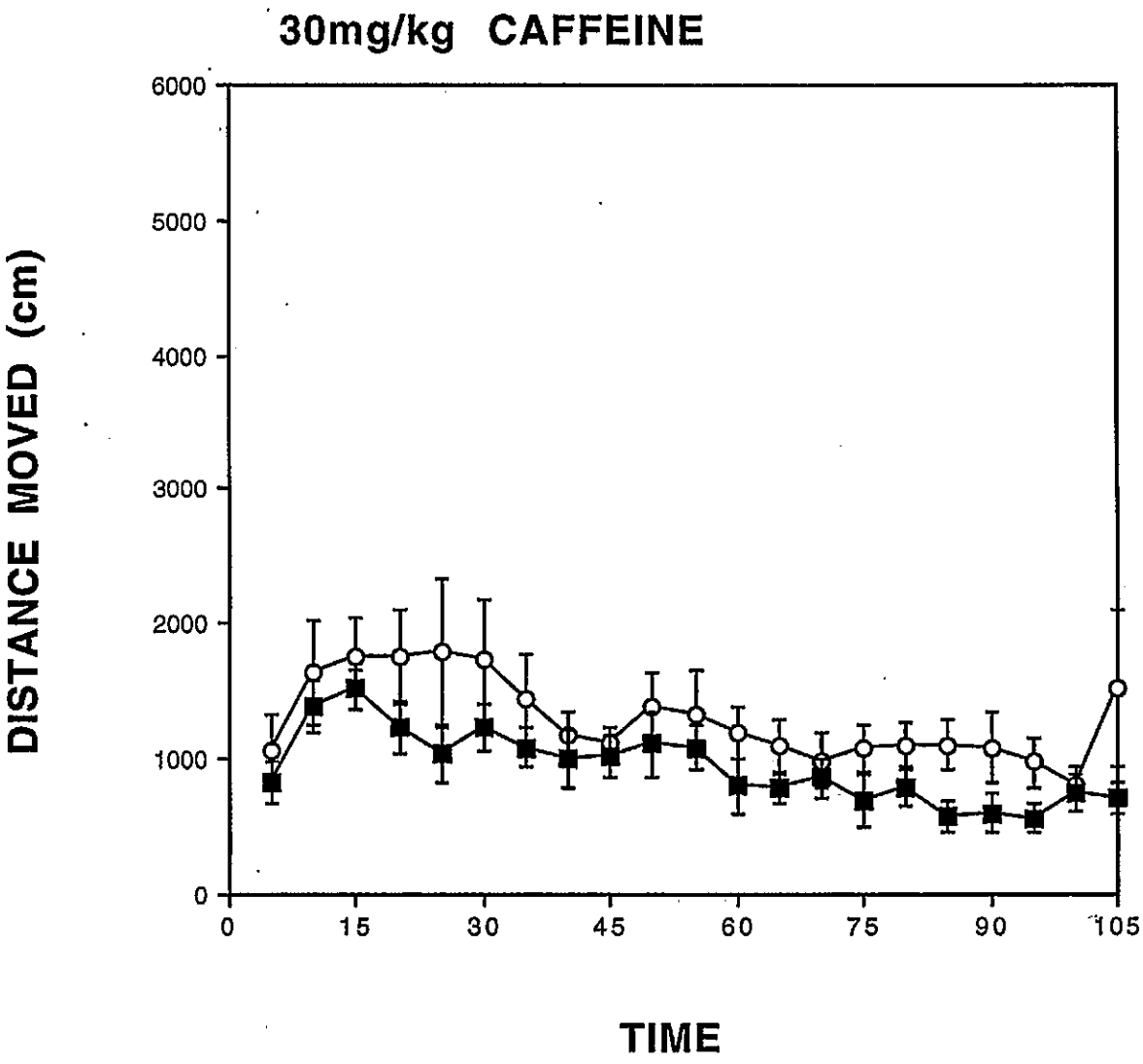
5000
4000
3000
2000
1000
0
0
15
30
45
60
75
90
105
TIME


DISTANCE MOVED (CM)
0
1000
2000
3000
4000
5000
6000
0
15
30
45
60
75
90
105
TIME
3mg/kg amph


4mg/kg AMPH
DISTANCE MOVED (cm)
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60
75
90
105
TIME


DISTANCE MOVED (cm)
0
1000
2000
3000
4000
5000
6000
0
15
30
45
60
75
90
105
TIME
5mg/kg AMPH


10mg/kg CAFFEINE
DISTANCE MOVED (CM)
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60
75
90
105
TIME


30mg/kg CAFFEINE
DISTANCE MOVED (cm)
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60
75
90
105
TIME

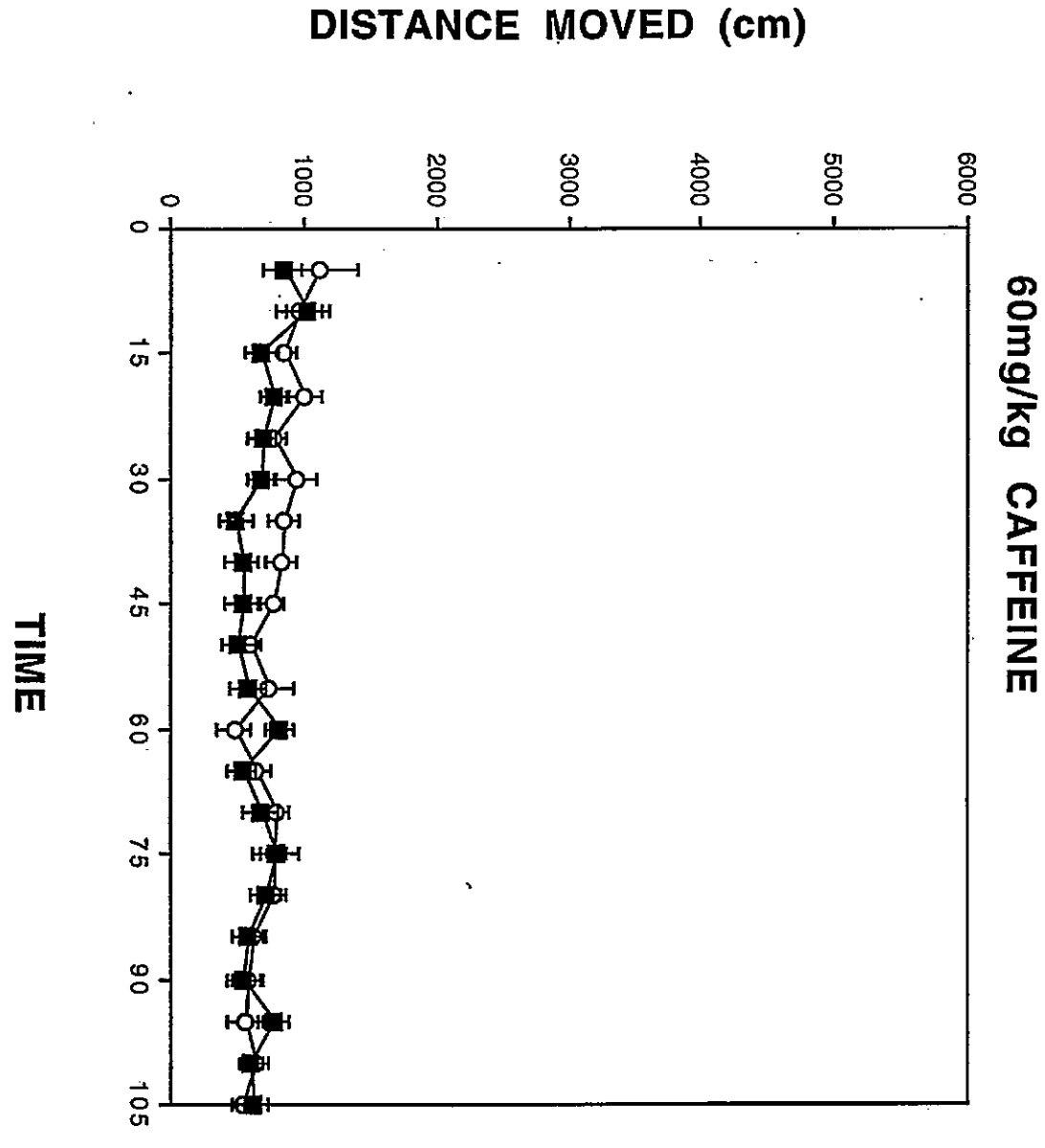
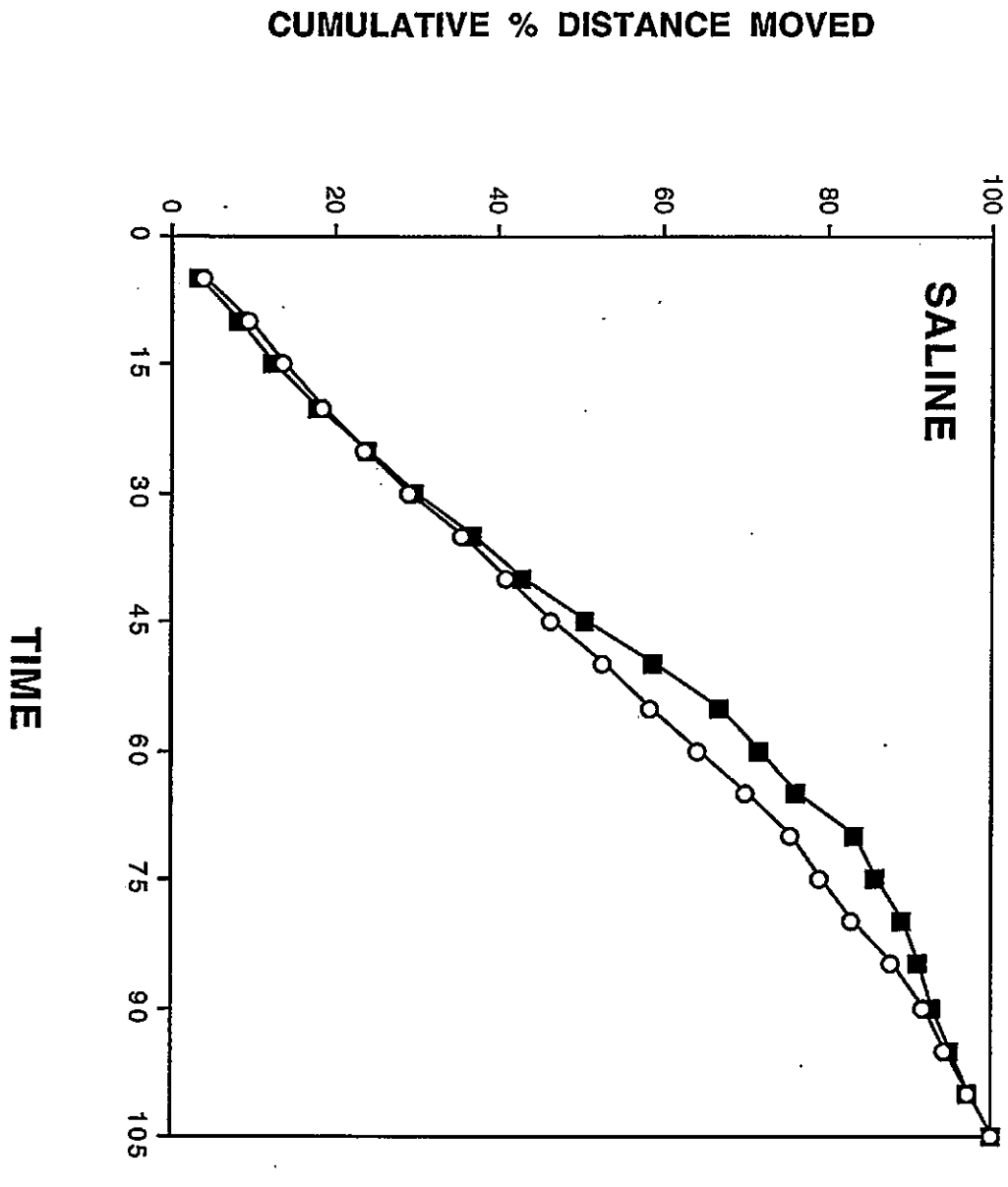
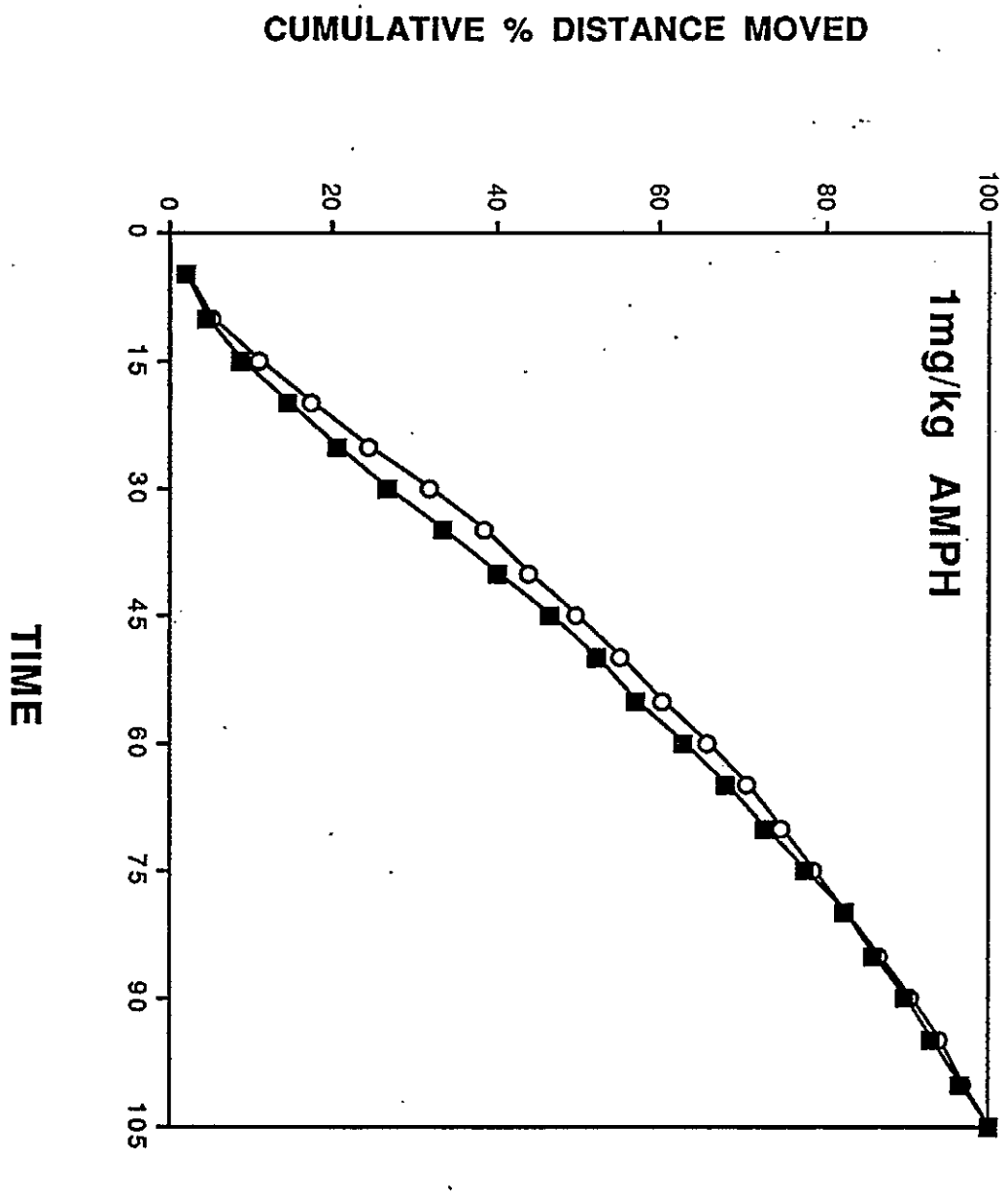
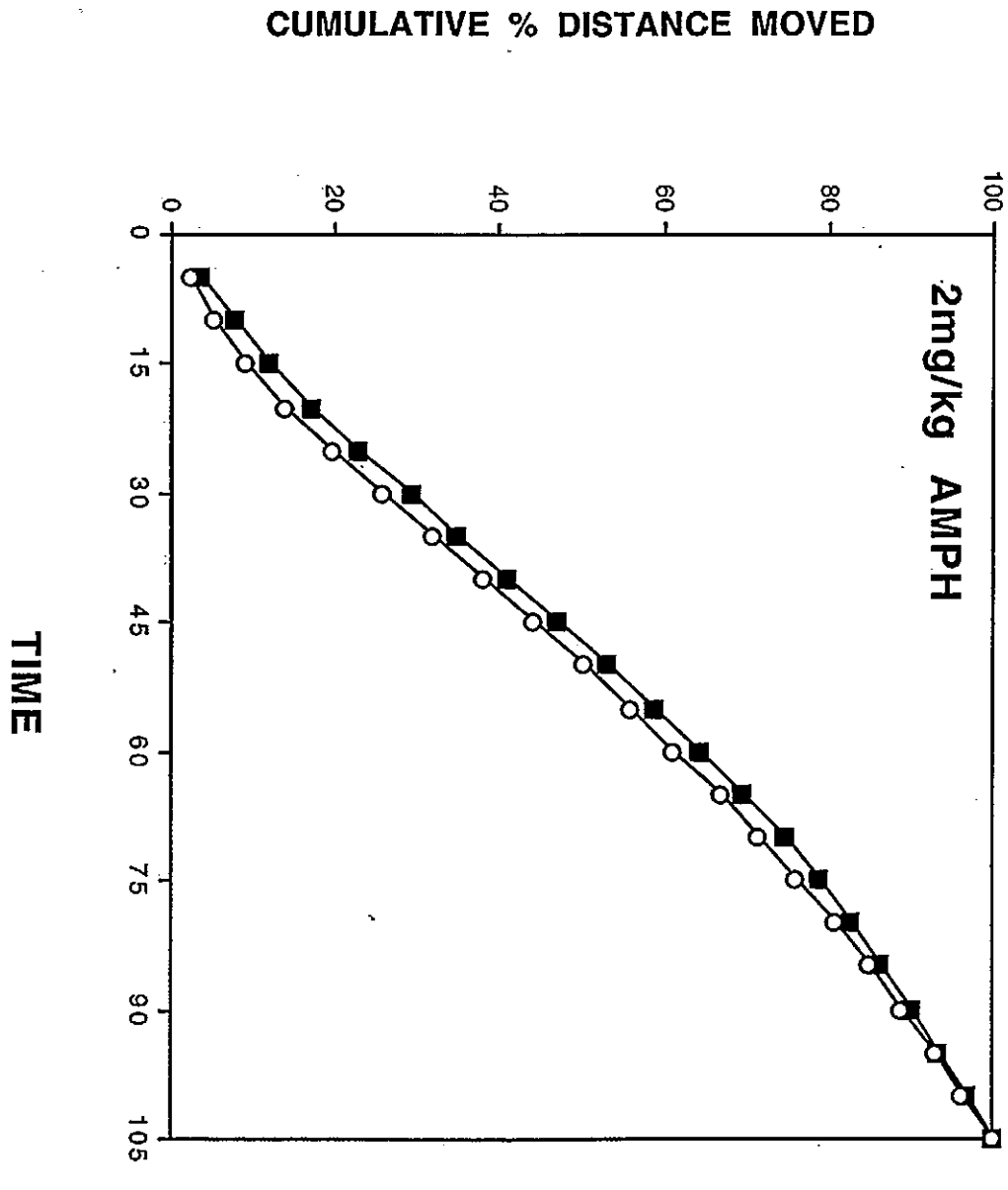

DISTANCE MOVED (cm)
0
1000
2000
3000
4000
5000
6000
0
15
30
45
60
75
90
105
TIME
60mg/kg CAFFEINE

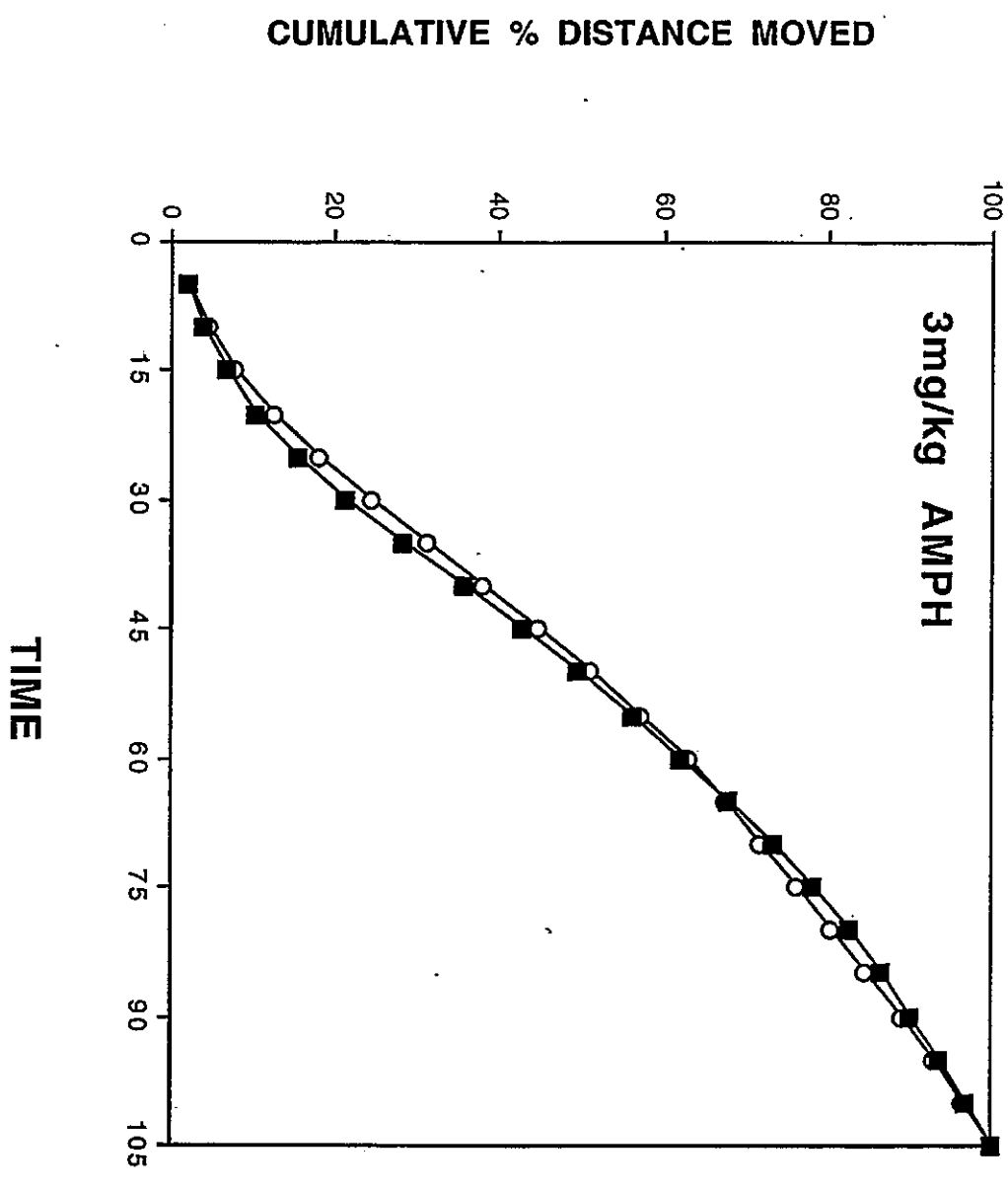
Figure 3.3

Cumulative percentage of the total distance moved by rats (n = 8 per group) at each of the 5 min session intervals. Rats were treated with saline, amphetamine (1-5 mg/kg) or caffeine (10-60 mg/kg) and tested in a square (closed squares) or circular (open circles) open field.


CUMULATIVE % DISTANCE MOVED
SALINE
0
20
40
60
80
100
0
15
30
45
60
75
90
105
TIME


CUMULATIVE % DISTANCE MOVED
0
20
40
60
80
100
1mg/kg AMPH
0
15
30
45
60
75
90
105
TIME


CUMULATIVE % DISTANCE MOVED
2mg/kg AMPH
0
20
40
60
80
100
0
15
30
45
60
75
90
105
TIME


CUMULATIVE % DISTANCE MOVED
3mg/kg AMPH
0
20
40
60
80
100
0
15
30
45
60
75
90
105
TIME

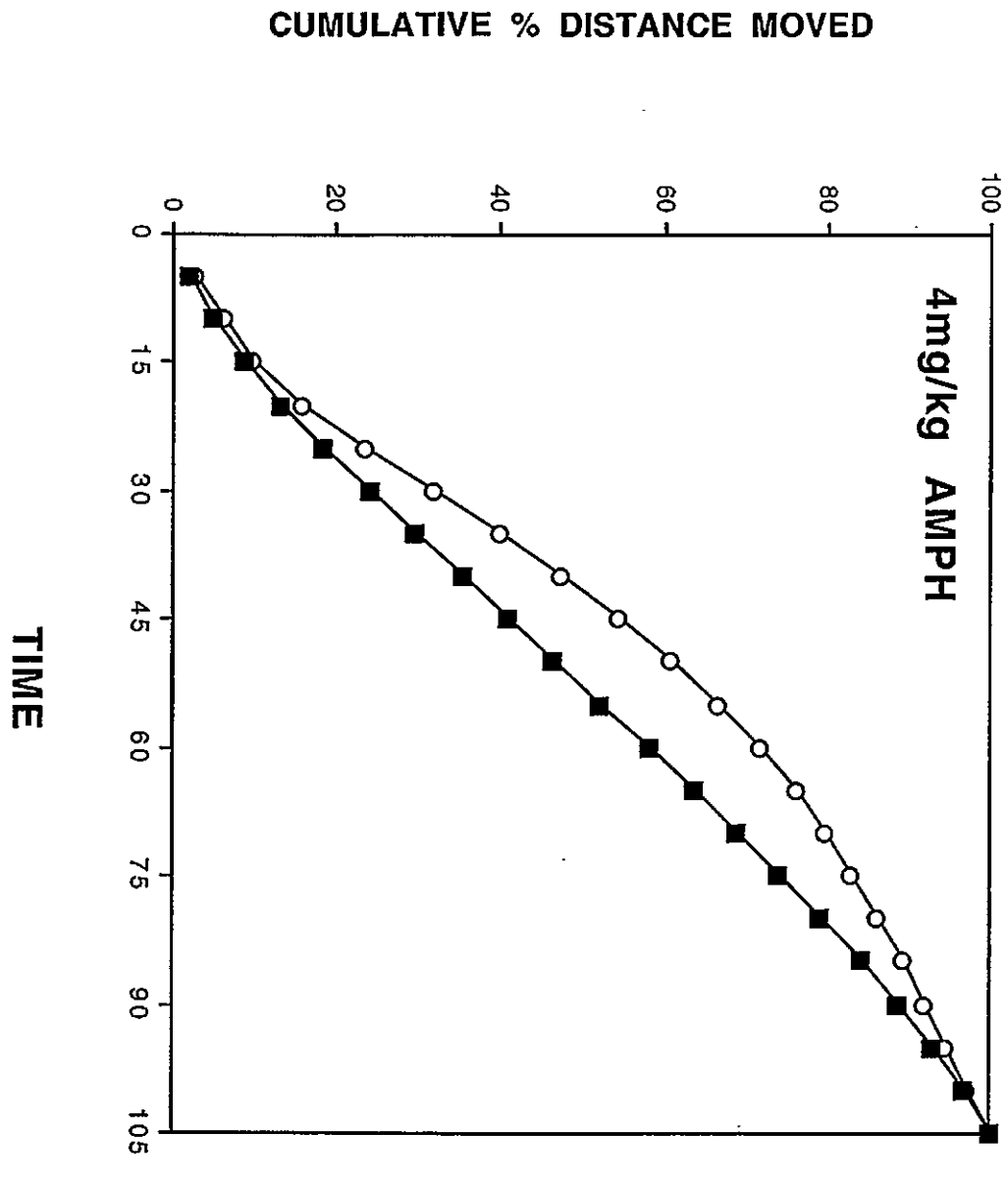
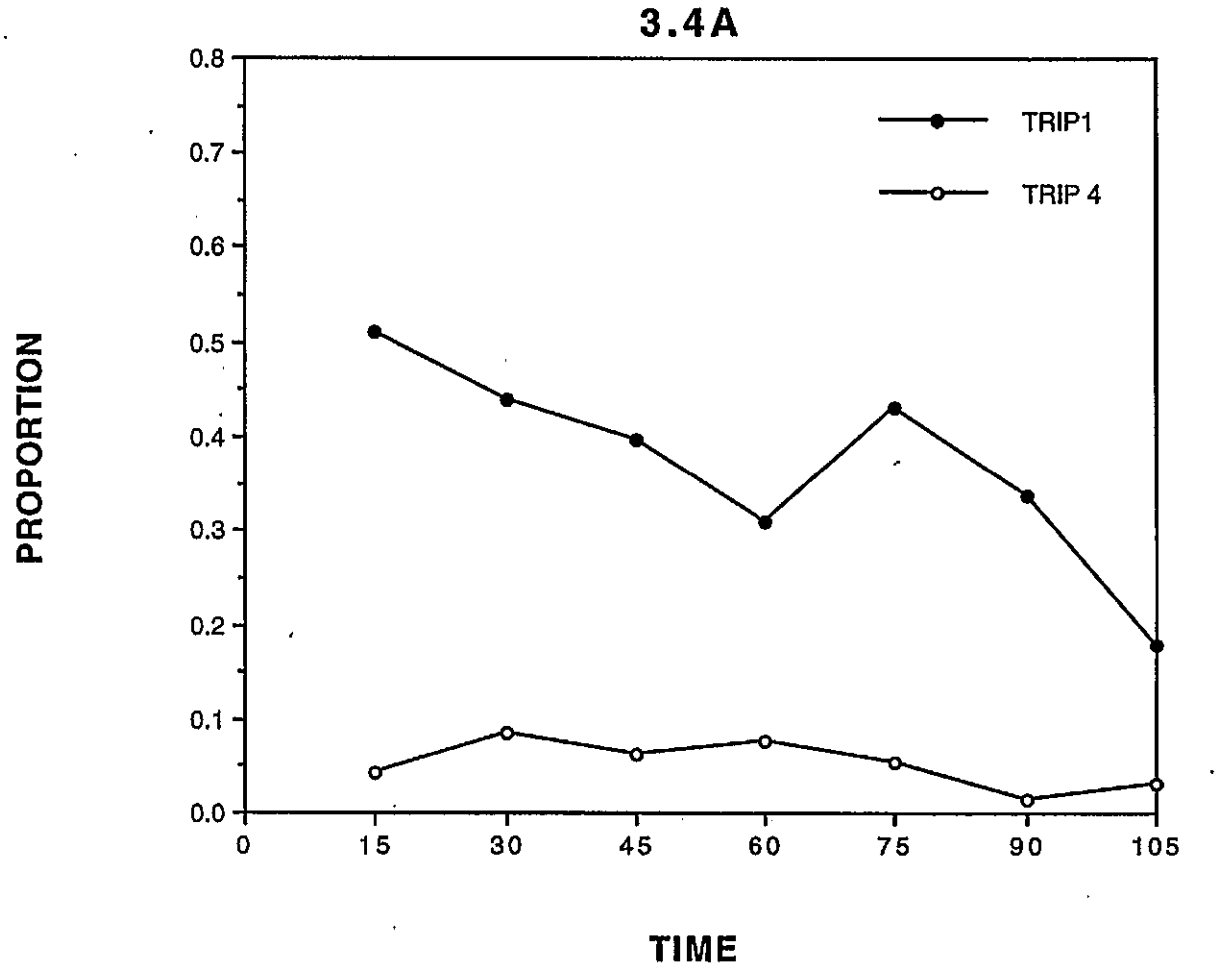

CUMULATIVE % DISTANCE MOVED
4mg/kg AMPH
0
20
40
60
80
100
0
15
30
45
60
75
90
105
TIME

Figure 3.4 A-B.

Mean (+-SEM) proportion of length 1 and length 4 trips per 15 min over 105 min test session (n=8 per group). Length 1 trips (closed circles); Length 4 trips (open circles). (A) Vehicle-Saline; (B) Vehicle-Amphetamine.


3.4A
PROPORTION
TIME
TRIP1
TRIP 4
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
15
30
45
60
75
90
105

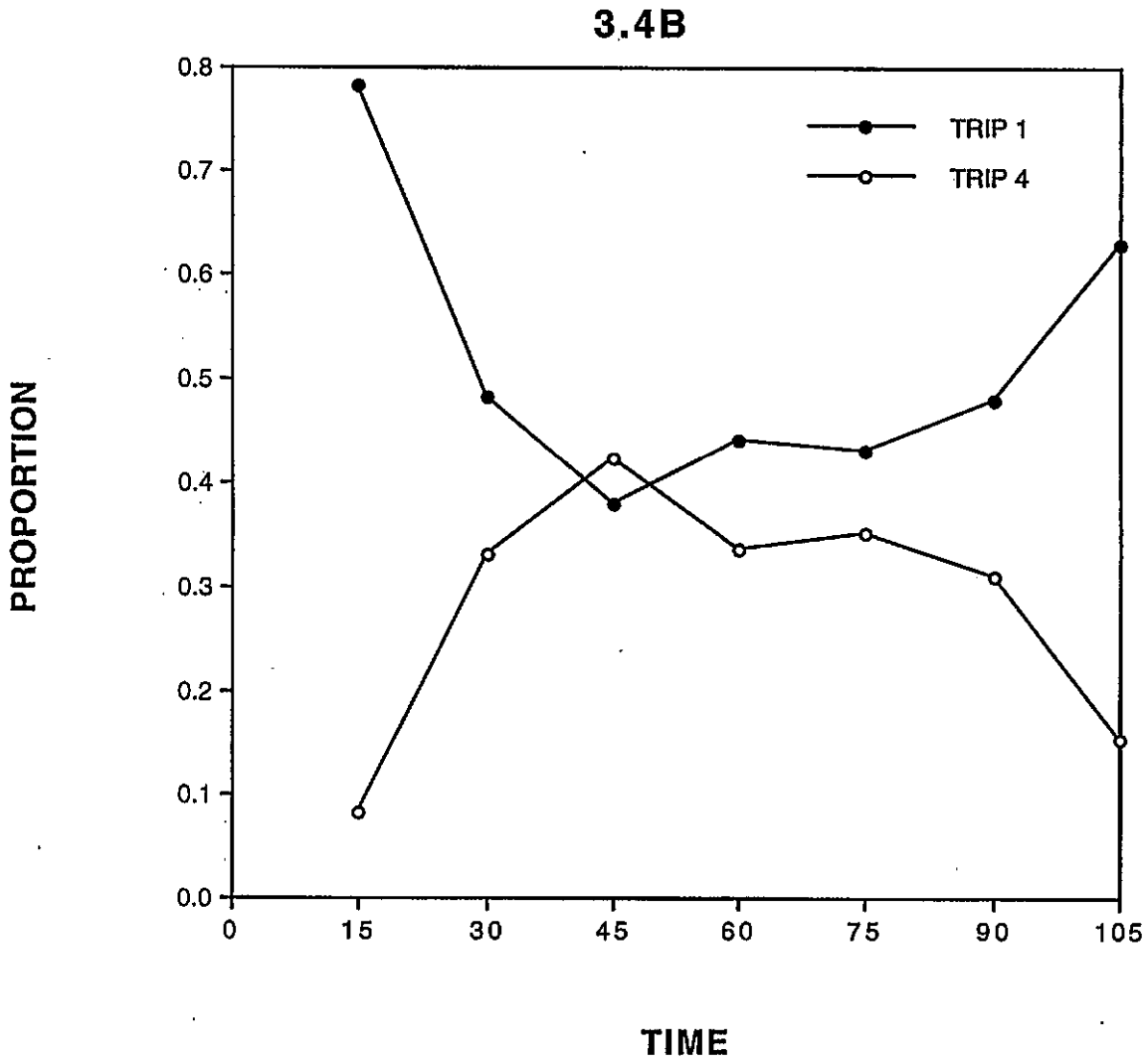
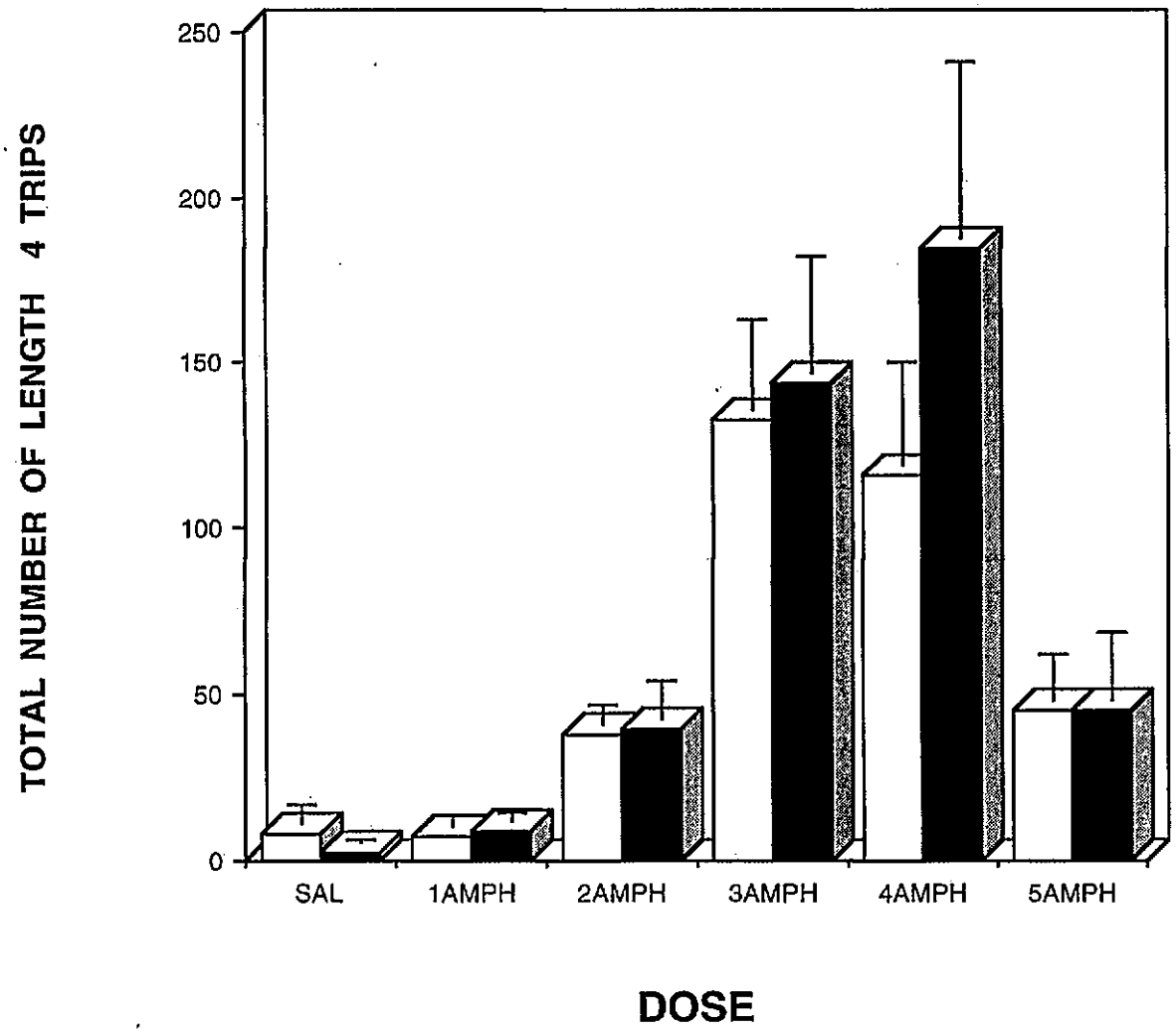

3.4B
TRIP 1
TRIP 4
PROPORTION
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
15
30
45
60
75
90
105
TIME

Figure 3.5

Mean (+-SEM) number of length 4 trips made over the 105-min observation period by rats (n = 8 per group) treated with saline, amphetamine (1-5 mg/kg) or caffeine (10-60 mg/kg), and tested in a square (dark bars) or circular (light bars) open field.


TOTAL NUMBER OF LENGTH 4 TRIPS
250
200
150
100
50
0
SAL
1AMPH
2AMPH
3AMPH
4AMPH
5AMPH
DOSE

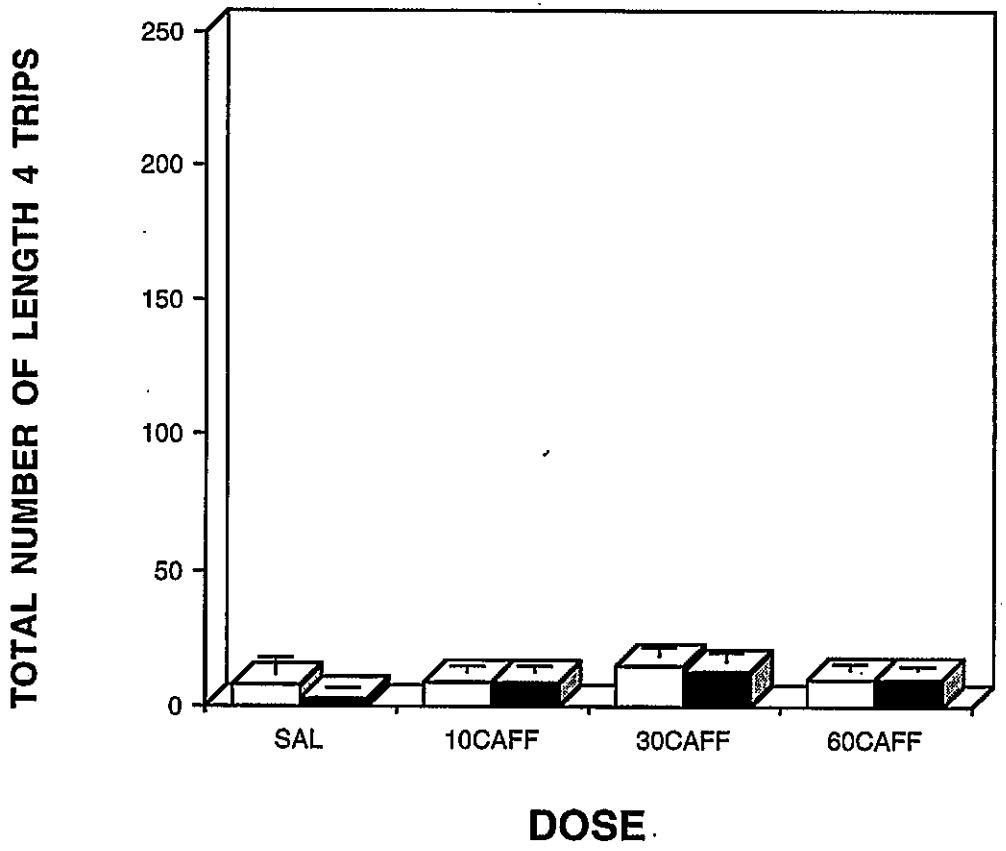
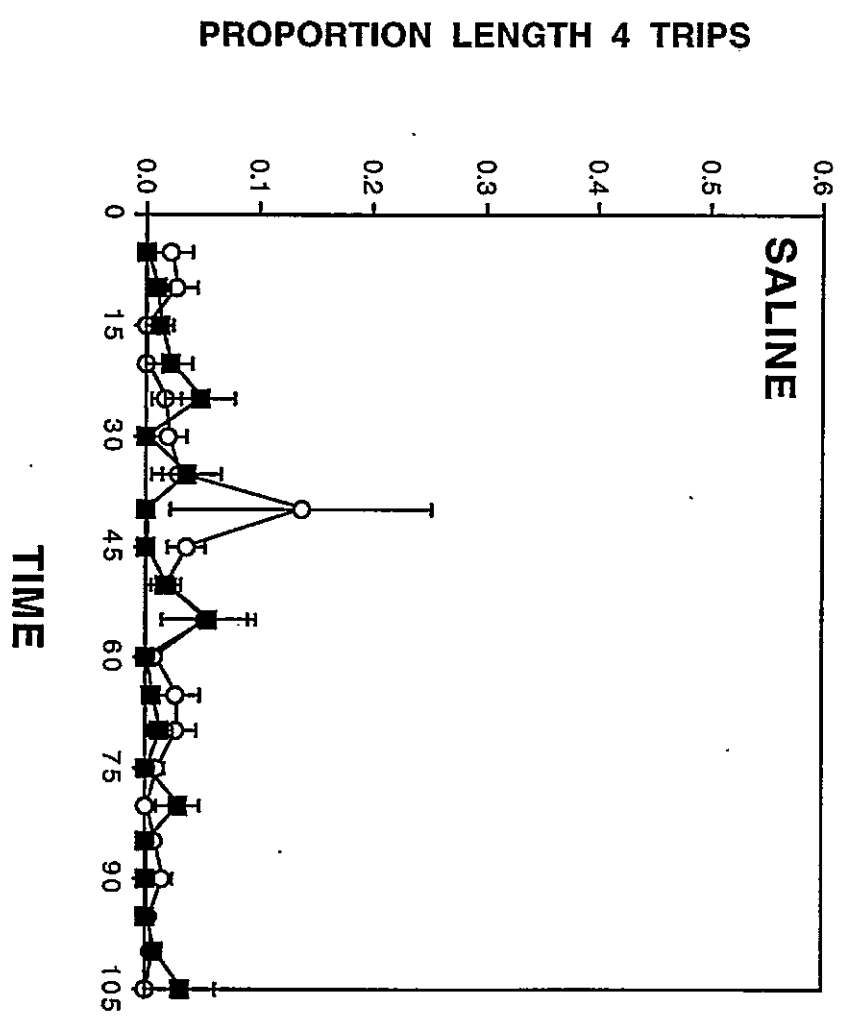
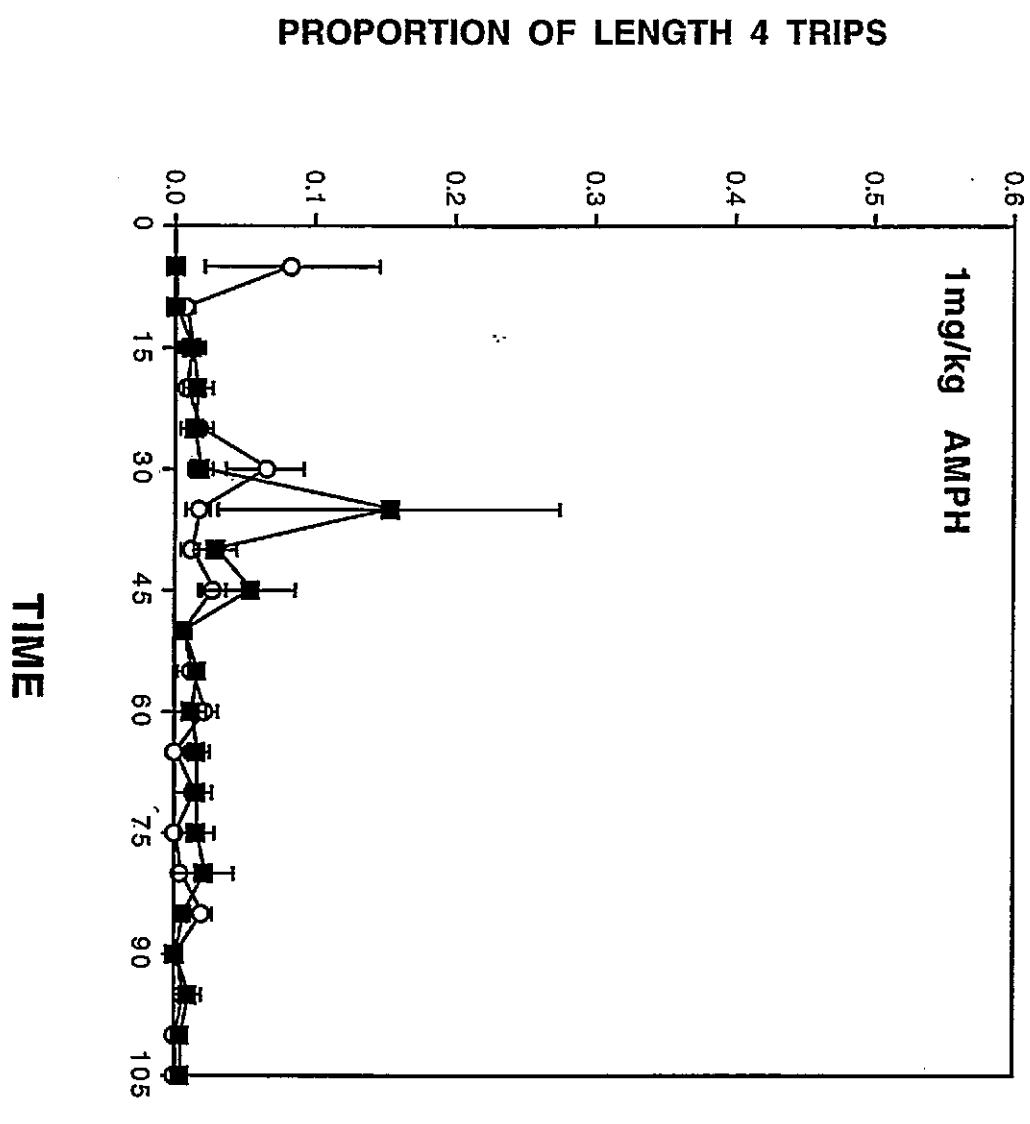
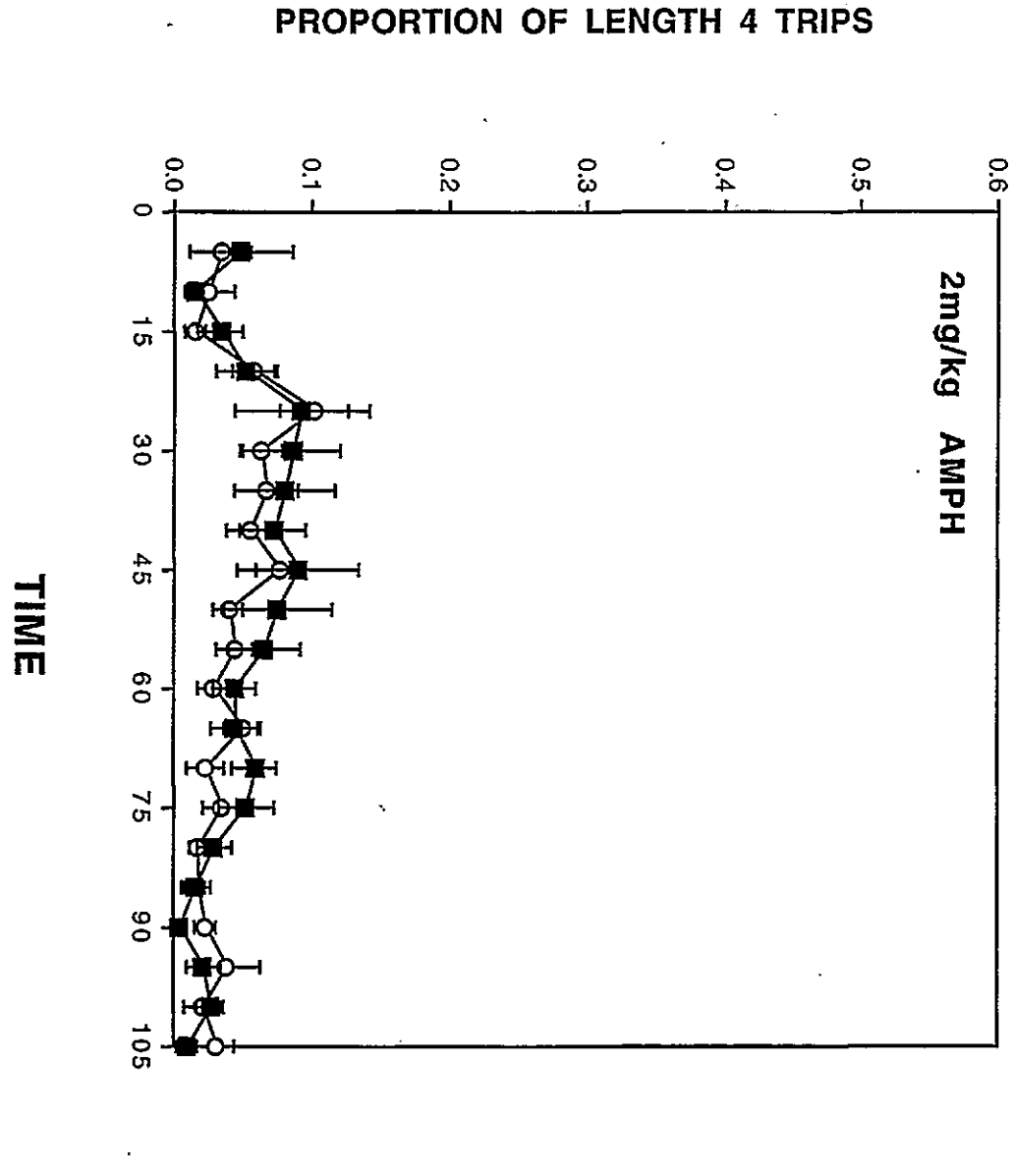

TOTAL NUMBER OF LENGTH 4 TRIPS
250
200
150
100
50
0
SAL
10CAFF
30CAFF
60CAFF
DOSE

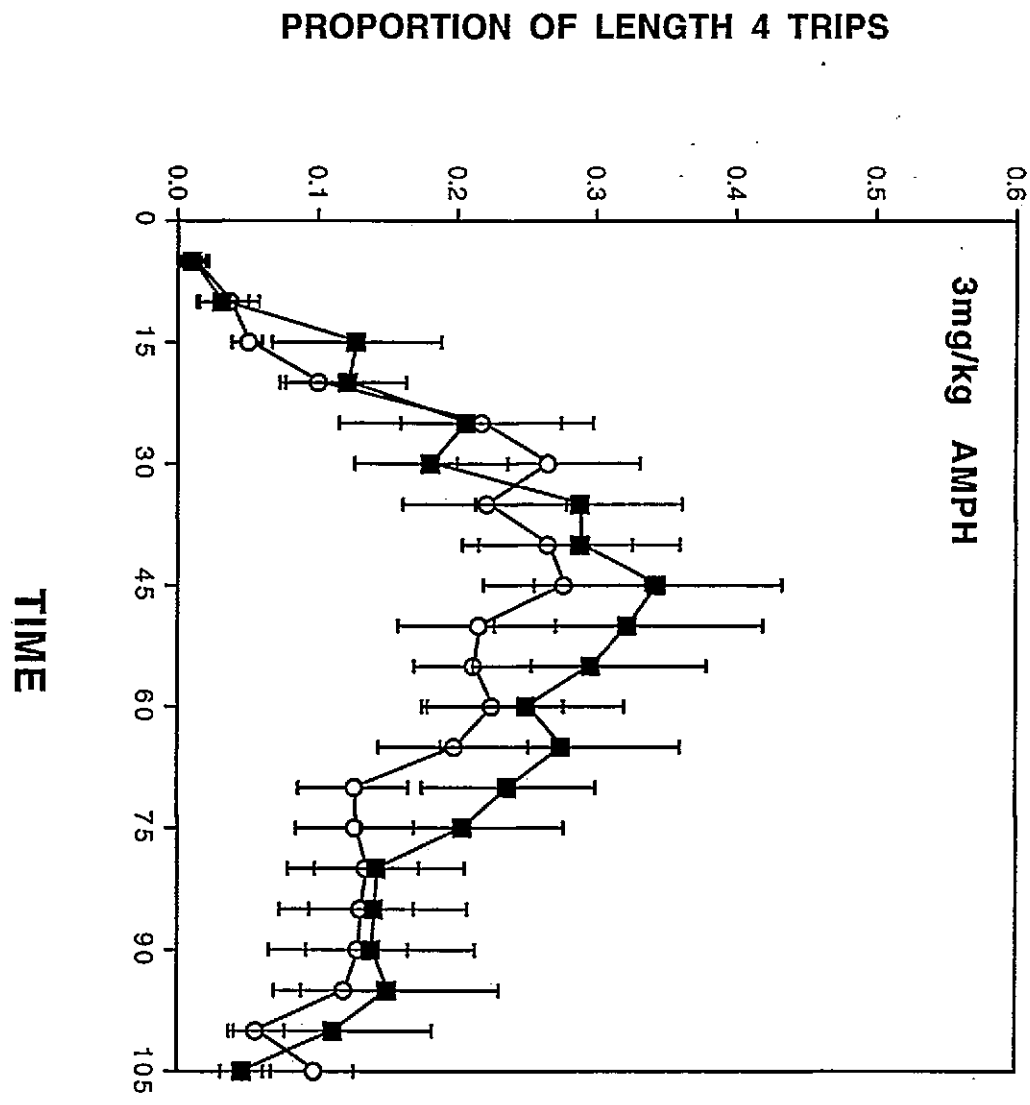
Figure 3.6

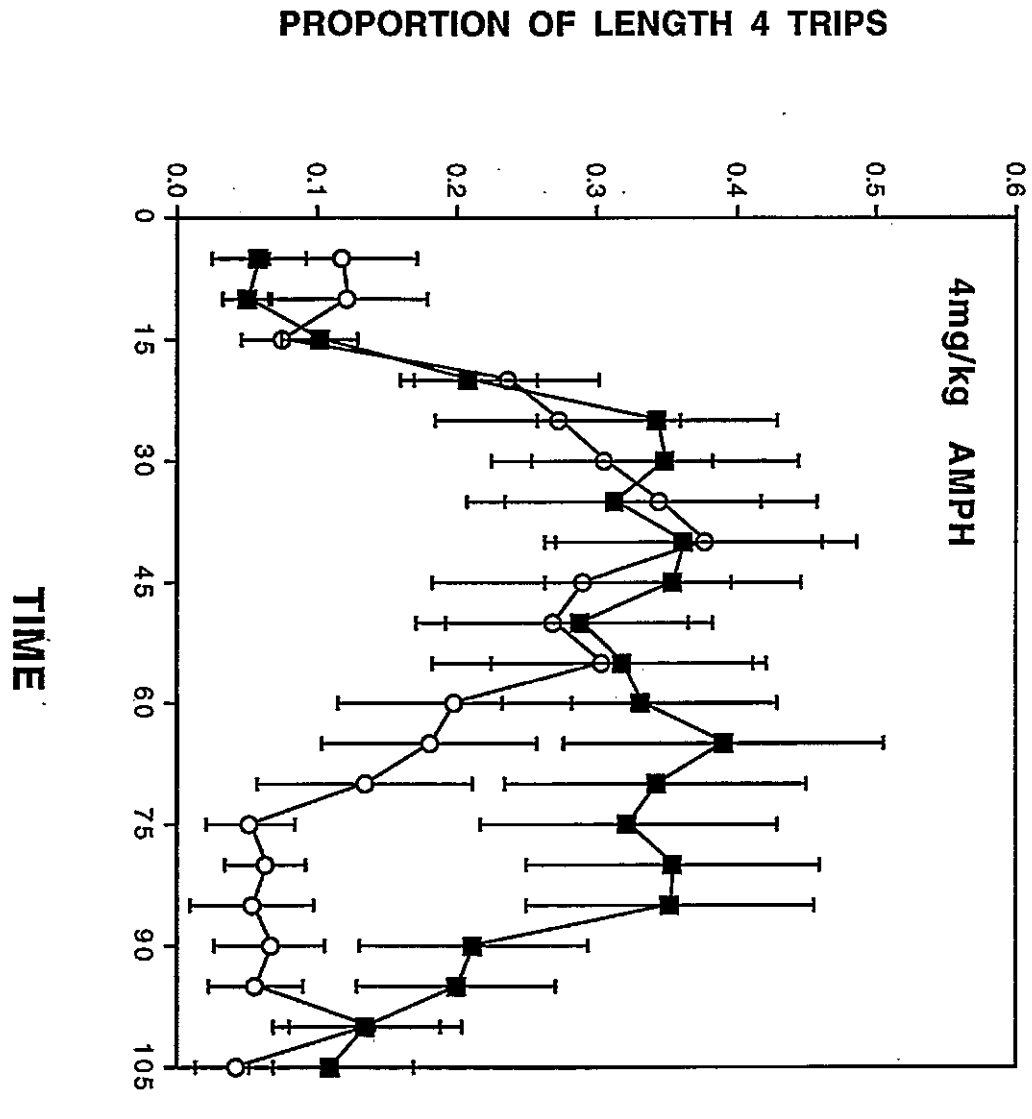
Mean (+-SEM) proportion of length 4 trips per 5 min during the 105-min observation period by rats (n = 8 per group) treated with saline or amphetamine (1-5 mg/kg), and tested in a square (closed squares) or circular (open circles) open field.

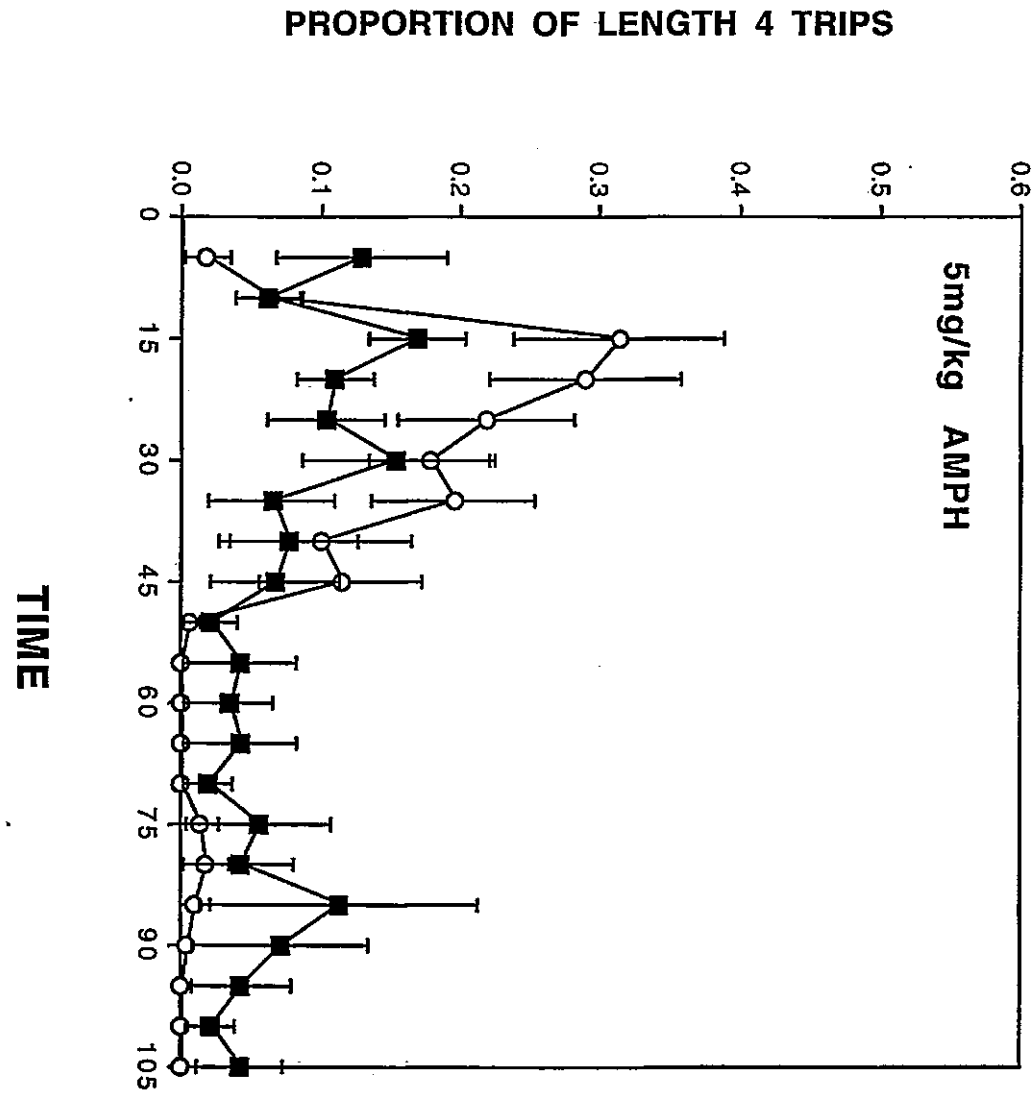

PROPORTION LENGTH 4 TRIPS
SALINE
TIME
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
15
30
45
60
75
90
105

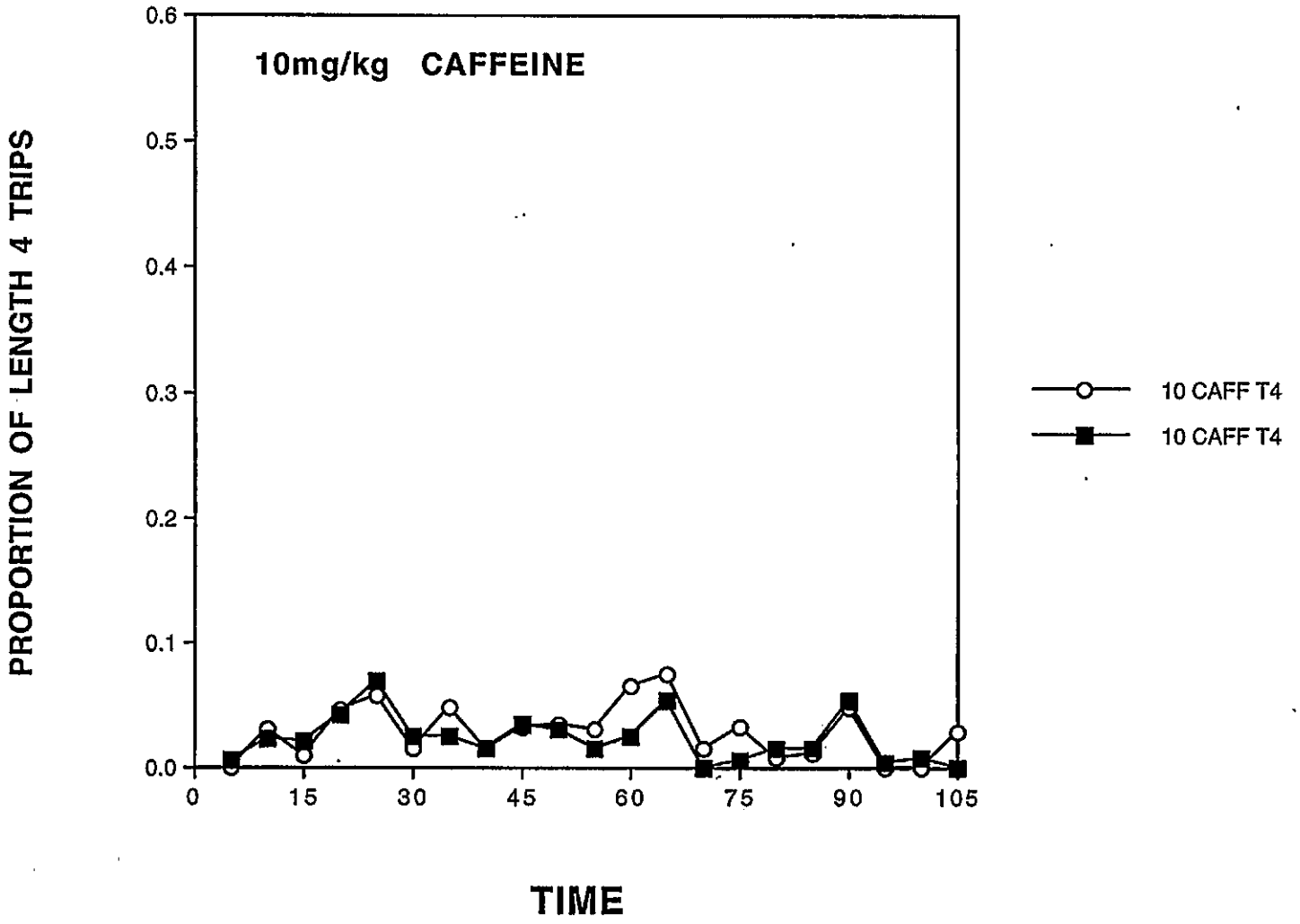

PROPORTION OF LENGTH 4 TRIPS
1mg/kg AMPH
TIME
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
15
30
45
60
75
90
105

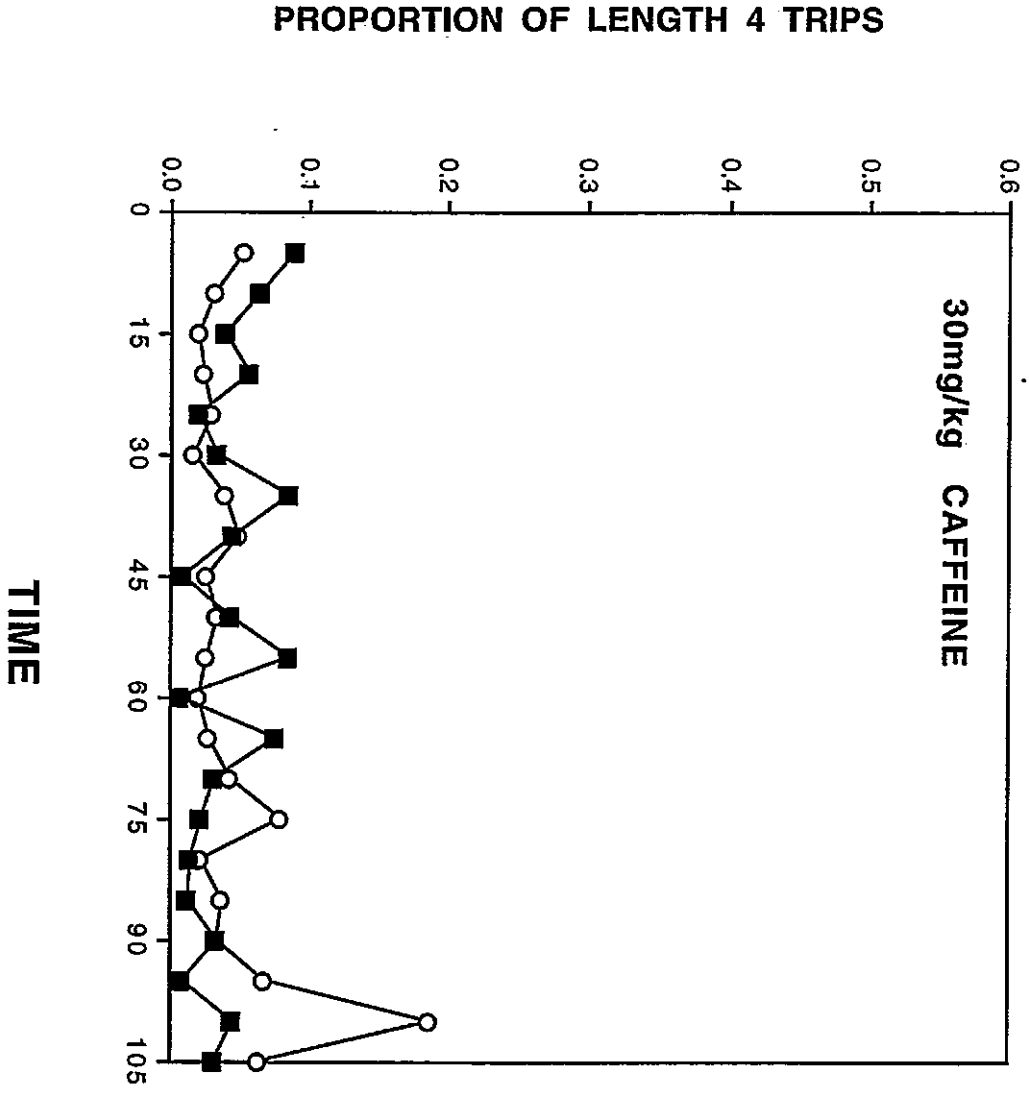

PROPORTION OF LENGTH 4 TRIPS
0.0
0.1
0.2
0.3
0.4
0.5
0.6
2mg/kg AMPH
0
15
30
45
60
75
90
105
TIME


PROPORTION OF LENGTH 4 TRIPS
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
15
30
45
60
75
90
105
TIME
3mg/kg AMPH


PROPORTION OF LENGTH 4 TRIPS
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
15
30
45
60
75
90
105
4mg/kg AMPH
TIME


PROPORTION OF LENGTH 4 TRIPS
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
15
30
45
60
75
90
105
TIME
5mg/kg AMPH


10mg/kg CAFFEINE
PROPORTION OF LENGTH 4 TRIPS
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
15
30
45
60
75
90
105
TIME
10 CAFF T4
10 CAFF T4


PROPORTION OF LENGTH 4 TRIPS
30mg/kg CAFFEINE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
15
30
45
60
75
90
105
TIME

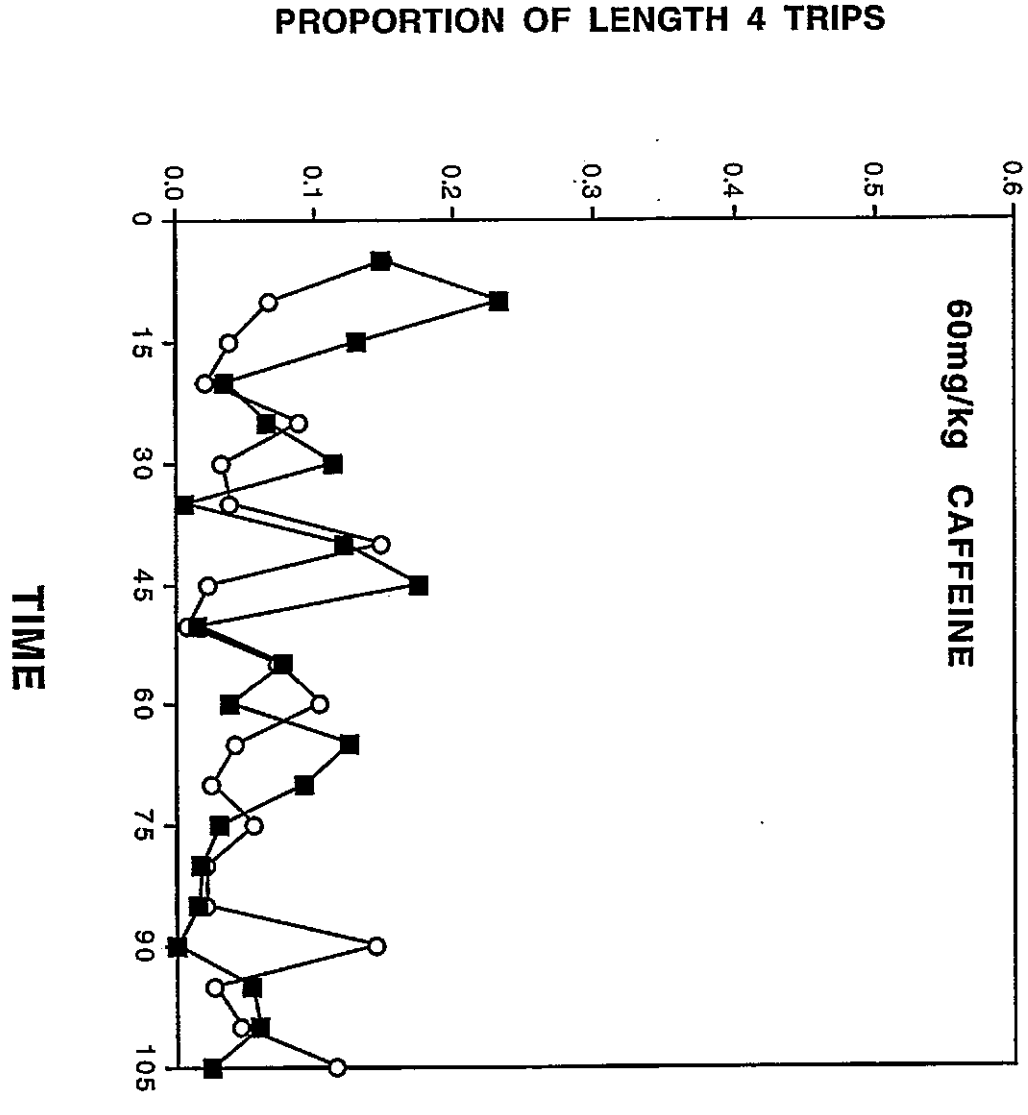
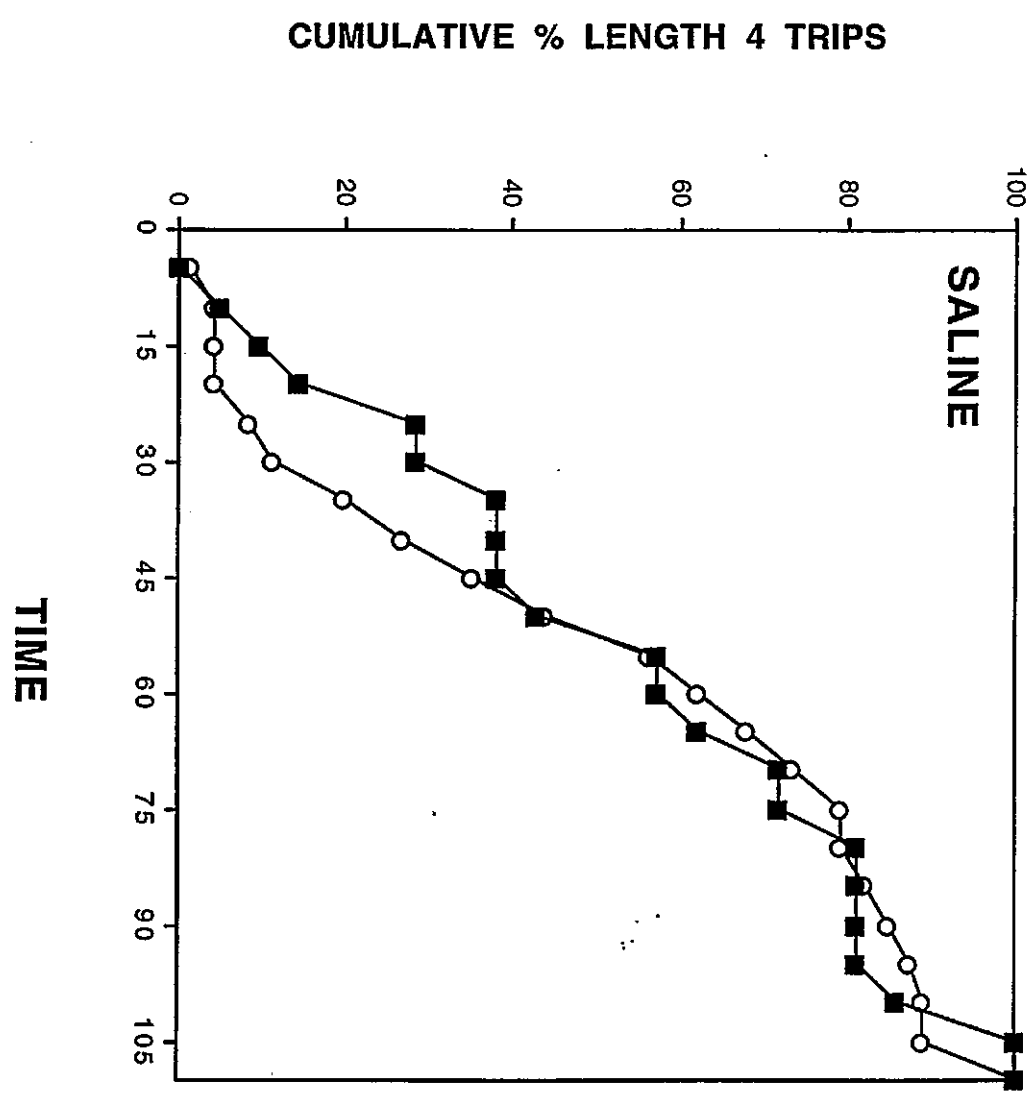
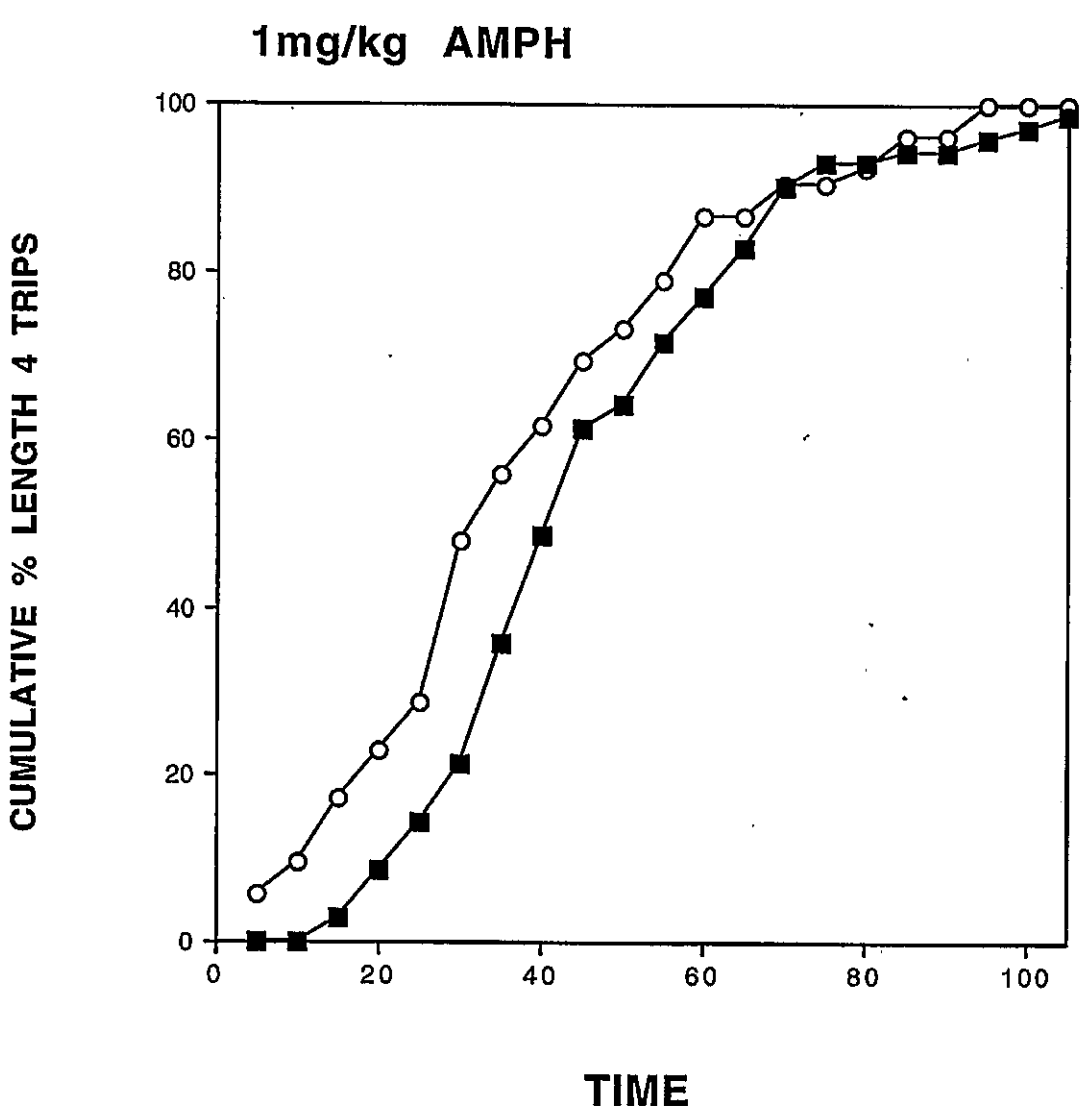
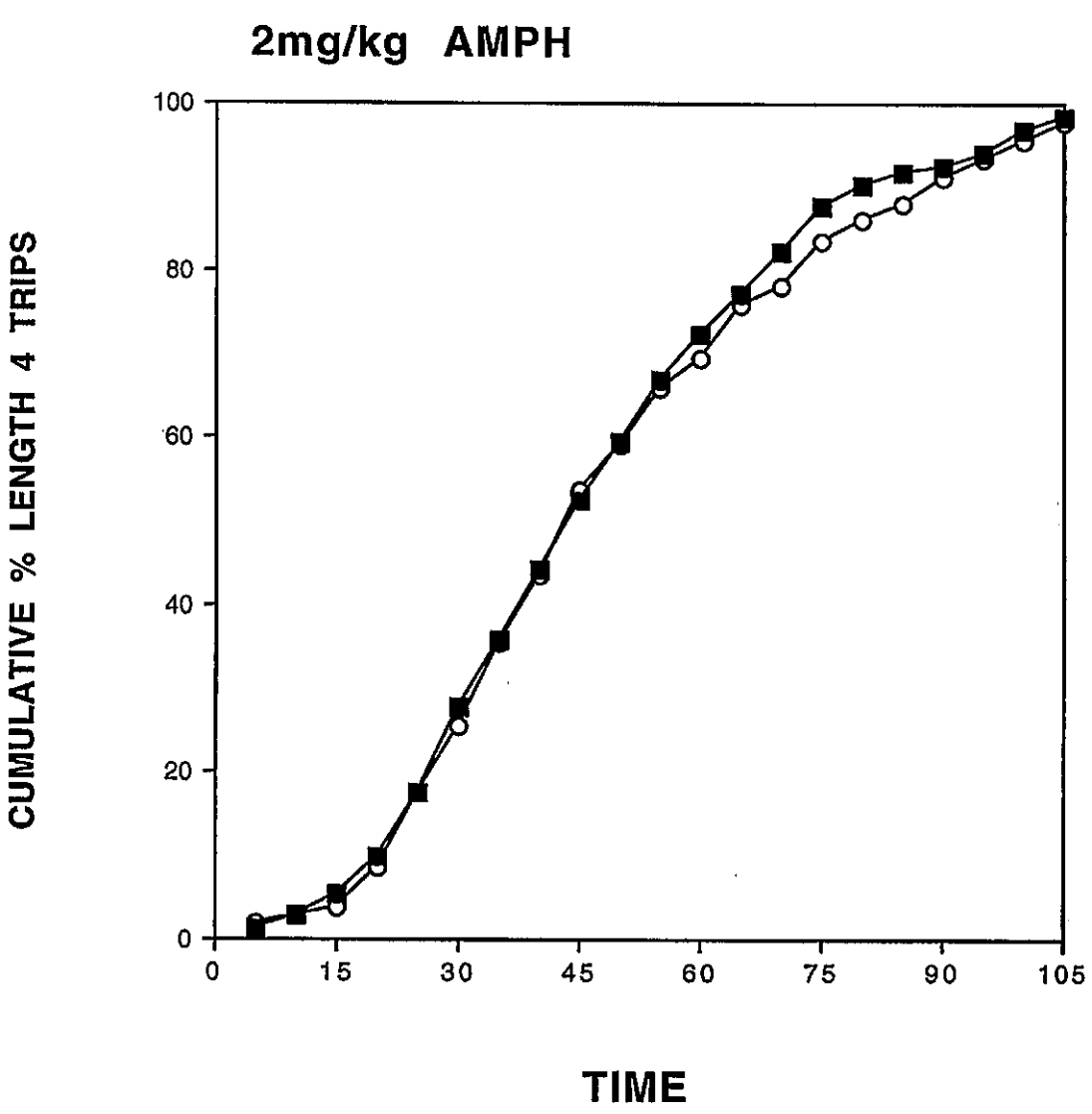

PROPORTION OF LENGTH 4 TRIPS
60mg/kg CAFFEINE
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
15
30
45
60
75
90
105
TIME

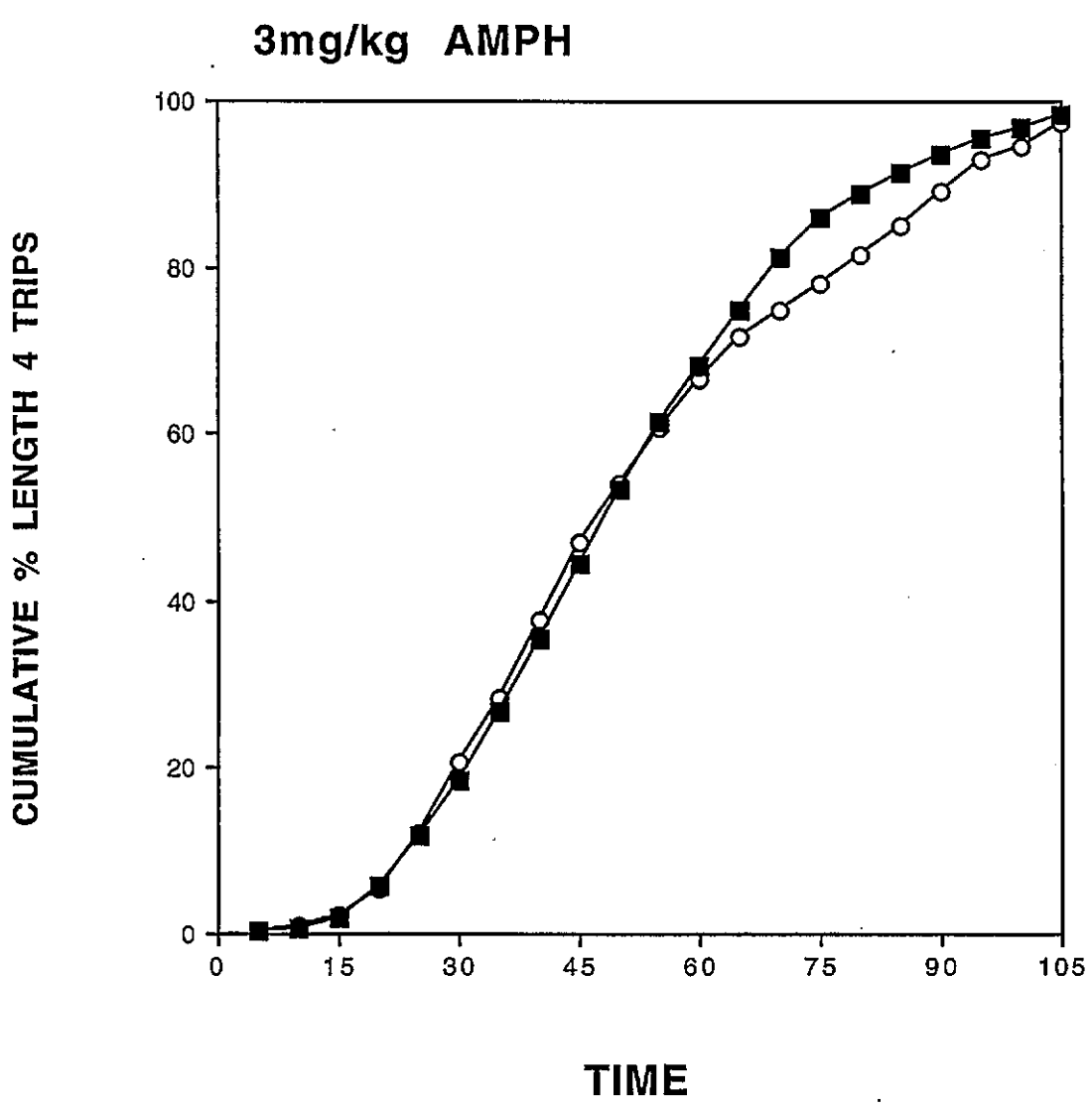
Figure 3.7

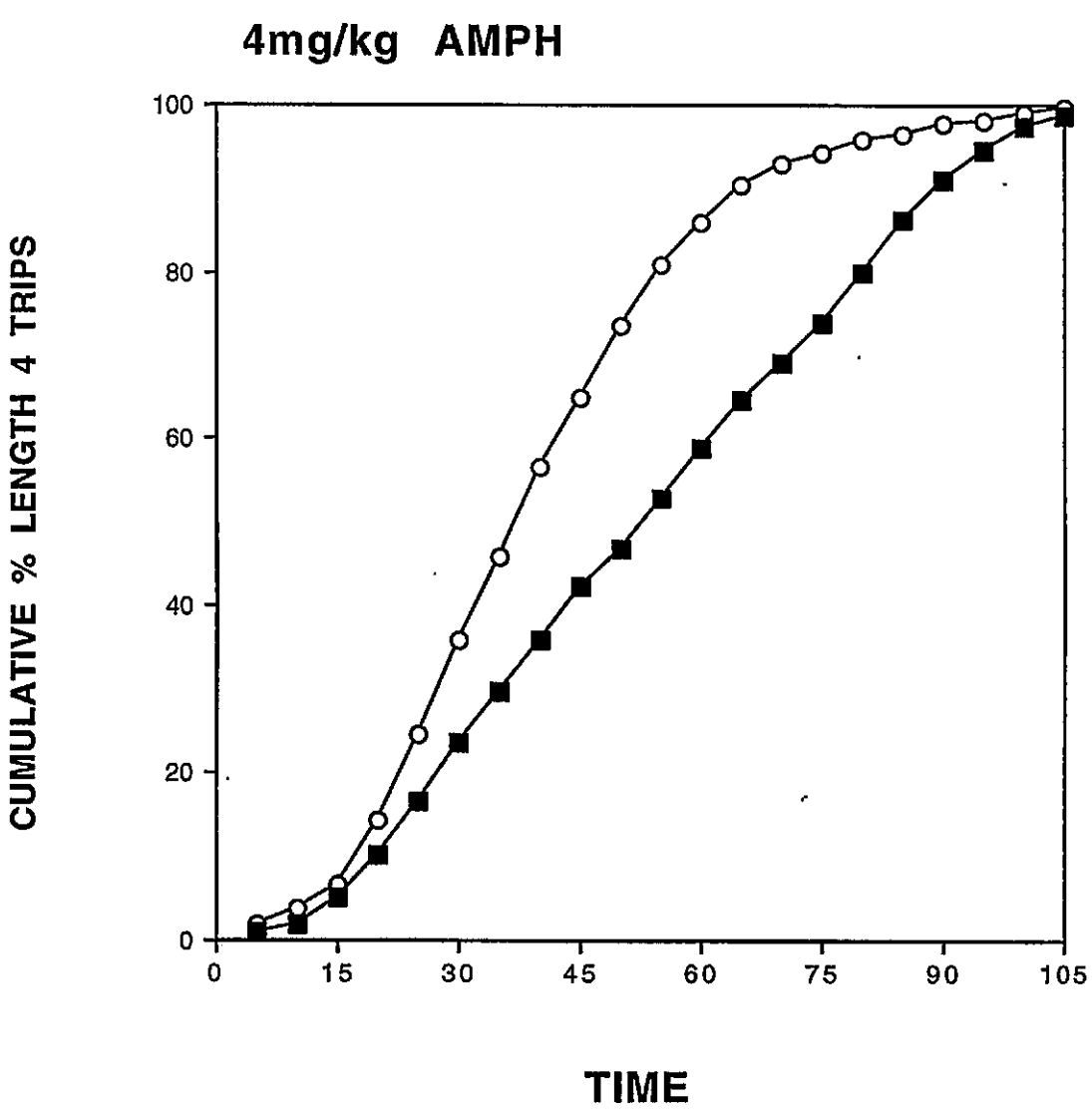
Cumulative percentage of the total number of length 4 trip made by rats (n = 8 per group) at each of the 5 min session intervals, treated with saline or amphetamine (1-5 mg/kg) and tested in a square (closed squares) or circular (open circles) open field.

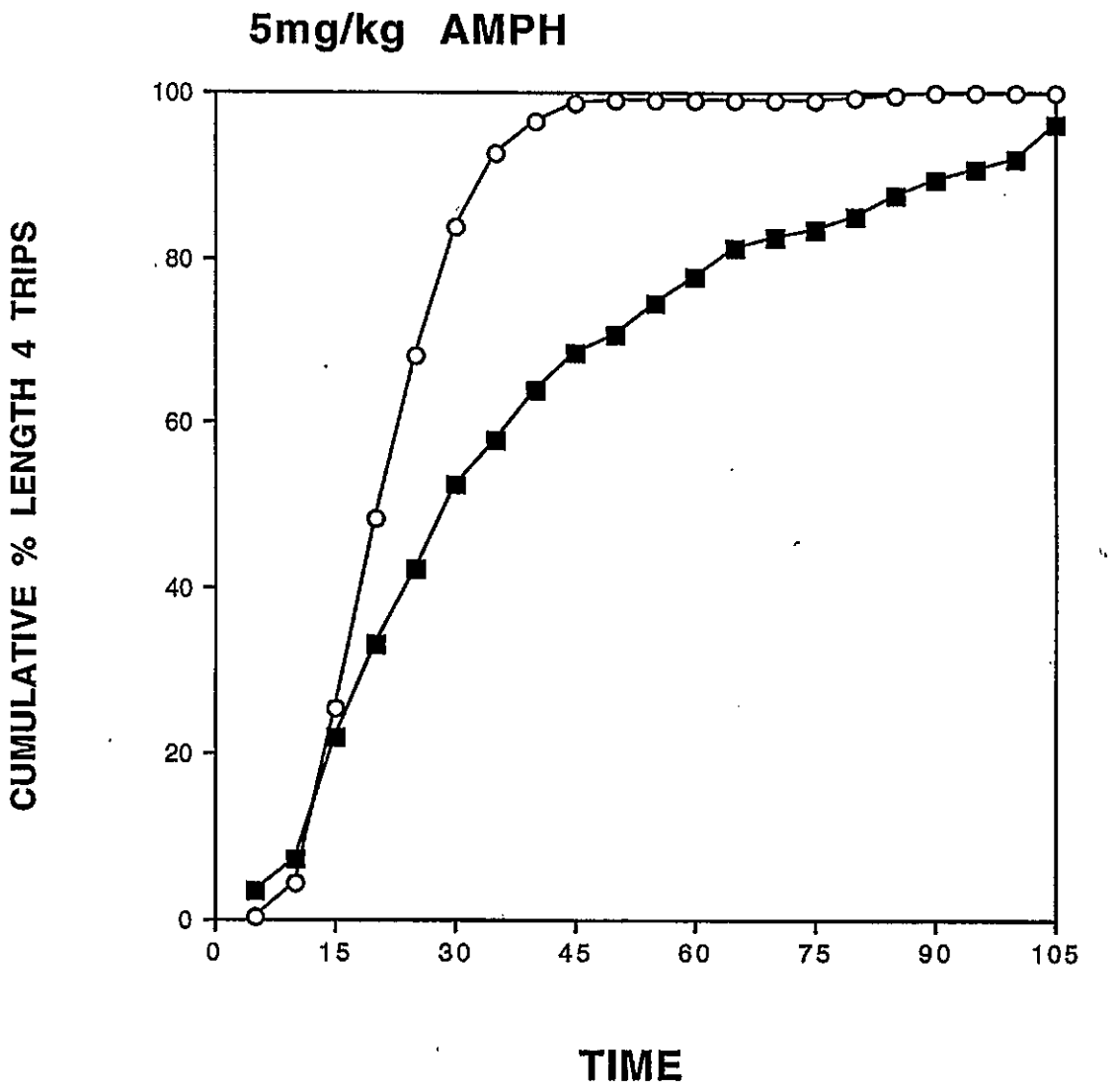

CUMULATIVE % LENGTH 4 TRIPS
SALINE
TIME
0
20
40
60
80
100
0
15
30
45
60
75
90
105


1mg/kg AMPH
CUMULATIVE % LENGTH 4 TRIPS
100
80
60
40
20
0
0
20
40
60
80
100
TIME


2mg/kg AMPH
CUMULATIVE % LENGTH 4 TRIPS
100
80
60
40
20
0
0
15
30
45
60
75
90
105
TIME


3mg/kg AMPH
CUMULATIVE % LENGTH 4 TRIPS
100
80
60
40
20
0
0
15
30
45
60
75
90
105
TIME


4mg/kg AMPH
CUMULATIVE % LENGTH 4 TRIPS
100
80
60
40
20
0
0
15
30
45
60
75
90
105
TIME


5mg/kg AMPH
CUMULATIVE % LENGTH 4 TRIPS
100
80
60
40
20
0
0
15
30
45
60
75
90
105
TIME

Chapter Four

Effects of Clozapine, Sulpiride and Haloperidol on Amphetamine-Induced Perseverative Locomotor Patterns.

4.1 Rationale for drug selection

Antipsychotic drugs used to treat schizophrenia are classified as either 'classical' or 'atypical' depending on their clinical profile. Both classes of drug ameliorate the thought disorders associated with the disease, and some atypical drugs (eg clozapine) appear to have a broader therapeutic range and are claimed to improve the negative symptoms (Meltzer, 1986). It has also been claimed that atypical antipsychotics are less likely to elicit extrapyramidal side effects (Burki, 1979). In animal tests of motor dysfunction atypicals, unlike classical antipsychotics, fail to produce catalepsy or elicit only a mild form of it. Classical and atypical antipsychotic drugs have also been shown to differ in their ability to block amphetamine-induced stereotyped behaviours in rats (Ljungberg and Ungerstedt, 1985; Tschanz and Rebec, 1988), but in contrast, locomotor activity produced under low doses of amphetamine is blocked by both classical and atypical antipsychotics (Ljungberg and Ungerstedt, 1985). This has been seen as evidence that atypical drugs fail to block striatal dopamine receptors which play a major role in the induction of stereotyped behaviours.

White and Wang (1983) found that prolonged treatment with classic antipsychotic drugs decreased dopamine activity in the substantia nigra and the mesolimbic dopamine system, whereas atypical drugs caused a time dependent inactivation of the mesolimbic dopamine system only, suggesting that the inability of atypical antipsychotics to decrease striatal dopamine activity may be related to their lowered potential to induce tardive dyskinesia, whilst the inactivation of the mesolimbic dopamine system may be associated with the delay in onset of the therapeutic effects. These findings have led to the suggestion that blockade of

stereotyped behaviours mediated via striatal dopamine reflect a compound's ability to induce possible motor side effects, whereas blockade of amphetamine-induced locomotor activity mediated via mesolimbic dopamine is an indication of therapeutic effect (Tschanz and Rebec, 1989).

What is becoming increasingly clear is that the hyperactivity and stereotyped behaviour seen following amphetamine administration are not discrete responses. Low doses of amphetamine increase locomotion but also increase repetitive sniffing, rearing and head swaying, all behaviours which occur under higher doses of amphetamine during the focused stereotypy stage when locomotor activity declines (Schiorring, 1971; Rebec and Bashore, 1984). Furthermore, there is evidence that perseverative motor patterns seen following administration of intermediate doses of amphetamine may constitute a stereotyped form of locomotion (Schiorring, 1979; Mueller et al, 1989a, 1989b; see Chapter 3). What has become increasingly clear is that locomotion does not occur independently of all stereotyped behaviours and that stereotypy is not a unitary response (Randrup and Munkvad, 1974; Rebec and Bashore, 1984). Indeed interesting results by Robertson and MacDonald (1984, 1985) have shown that three atypical antipsychotic drugs: clozapine, thioridazine, and sulpiride all potentiated some, but not all, amphetamine-induced stereotyped behaviours.

Kenyon et al, (1992) found that sulpiride, although reducing amphetamine-induced hyperactivity, had no effect on the proportion of thigmotaxic trips around the perimeter of the open field. In view of the fact that atypical antipsychotic drugs fail to antagonise all aspects of amphetamine-induced stereotyped behaviours this finding lends support to the claim that aspects of amphetamine-induced open field locomotion may be stereotyped. In order to evaluate this claim it is necessary to examine the effects of a wide range of doses of both classical and atypical antipsychotic drugs on the measure termed 'trip length' to determine whether this behaviour is differentially affected by different classes of antipsychotic drug.

Atypical and classical antipsychotic drugs are not a homogeneous group of compounds, and the major method of classification for such a disparate group of drugs, irrespective of their ability to induce extrapyramidal symptoms in treatment, is in

terms of their chemical structure. The first category of antipsychotic drugs to be developed were the phenothiazines, (see Bradley and Hirsch 1986), which includes chlorpromazine. In addition to antipsychotic action the phenothiazines exhibit cardiovascular effects, and anti-emetic action. In experimental animals the phenothiazines reduce motor activity, induce catalepsy and block conditioned avoidance responses. All the phenothiazines, with the exception of the piperidine group (eg thioridazine), induce extrapyramidal symptoms, which closely resemble Parkinson's disease. Consequently the phenothiazines constitute a major group of the classic antipsychotic drugs.

Another major category of classic antipsychotic drugs are the butyrophenones which - unlike the phenothiazines - do not possess tricyclic structure. The first of these drugs to be used clinically was haloperidol. Butyrophenones have strong antipsychotic action but lack some of the properties of the phenothiazines, having little or no antihistaminic, anticholinergic or antiadrenergic activity and as a consequence lack sedative properties associated with histamine antagonism, and have a reduced tendency to cause autonomic disturbances when compared with the phenothiazines. All the butyrophenones have a marked tendency to produce extrapyramidal side effects.

A relatively new class of antipsychotic drugs, classified as atypical, are the substituted benzamides, of which the drug sulpiride has been used as an effective antipsychotic agent (Borenstein et al, 1969). Sulpiride binds to a subgroup of D2 receptors which are sodium dependent (Jenner and Marsden, 1982), and are located pre-synaptically (Verhoeven et al, 1979). Sulpiride does not induce catalepsy even at high doses, and at relatively low doses it inhibits apomorphine induced locomotion. With long-term treatment sulpiride does not induce an increase in D2 receptors, but does produce a significant increase in D1 receptors in contrast to antipsychotics such as haloperidol which elevate D2 but not D1 receptors (Jenner et al, 1980). Sulpiride has been shown to induce extrapyramidal side effects, but the incidence of these effects are reported to be less than for other antipsychotic drugs (see Tamminga and Gerlach, 1987). Because of the selective action of sulpiride at D2 receptors there are very few autonomic and cardiovascular side effects, hormonal side effects such as galactorrhea

and amenhorrhea resulting from an increase in prolactin levels are relatively common (Gerlach 1991). Since sulpiride shows only part of the activity in animal models common to the antipsychotic drugs they are classed as atypical, although Meltzer et al, (1989c) has suggested that the substituted benzamides along with thioridazine, should not be classified with clozapine as atypical.

The dibenzazepine derivatives are compounds of immense theoretical interest, the principle antipsychotic drug in this group is clozapine. Interest has focused on clozapine because although it has weak D2 receptor blockade, it is an extremely effective antipsychotic agent (Meltzer et al, 1989a), and has been shown to be more effective than the classical antipsychotics in the treatment of schizophrenia (Kane et al, 1988). It produces little or no tardive dyskinesia, and few extrapyramidal side effects.

Behavioural studies suggest that clozapine is mainly active at the mesolimbic sites, and that the striatal system is relatively unresponsive to clozapine. Clozapine produces little or no catalepsy in rats (Bartholini et al, 1972; Burki et al, 1975a; De Maio, 1972; Honma and Fukushima 1978), which is a classic test of neostriatal dopamine receptor blockade (Carlsson, 1978). Amphetamine-induced locomotion resulting from enhanced dopaminergic transmission in the nucleus accumbens (Kelly and Iversen, 1976; Pijnenberg et al, 1976) is inhibited by acute clozapine whereas amphetamine-induced stereotyped gnawing, sniffing and grooming is unaffected by this drug (Iversen and Koob, 1977). Ljungberg and Ungerstedt (1978) have also shown that clozapine blocks apomorphine-induced locomotion but not stereotypy. In addition, hyperactivity induced by direct injection of dopamine into the nucleus accumbens is blocked by lower doses of clozapine than are required to block behaviours elicited by striatal dopamine injections (Costall and Naylor, 1976). Robertson and MacDonald (1984) report that clozapine potentiated some amphetamine-induced stereotyped behaviours.

Clozapine has been reported to increase the firing rate of mesolimbic but not striatal dopamine neurones, whereas acute haloperidol increased the activity of both mesolimbic and striatal neurones (Hand et al, 1987), an effect entirely consistent with the concept that clozapine exhibits mesolimbic selectivity. Contradictory

evidence was provided by several researchers who found that both clozapine and haloperidol increased the firing rate of both striatal and mesolimbic neurones (Sauto et al, 1979; Rebec et al, 1980; Chiodo and Bunney, 1983, 1985). This has led Meltzer (1991) to conclude that there is some evidence to suggest that clozapine exhibits mesolimbic selectivity and that this may account for the drugs lack of extrapyramidal symptoms and tardive dyskinesia. Limbic selectivity would be unlikely to account for the drugs increased efficacy in treatment. In addition, Meltzer (1991) claims that depolarisation inactivation following chronic treatment with antipsychotic drugs (Chiodo and Bunney, 1983, 1985; White and Wang, 1983) are unlikely to provide an explanation of the mechanism of action because release of dopamine from nerve terminals may be more independent of cell firing than was originally thought (Abercrombie and Zigmond 1990).

The selectivity of clozapine for the mesolimbic dopamine system may well be the result of interactions with other neurotransmitter systems which would compensate for dopamine blockade in the striatum. Clozapine's antagonistic effects on cholinergic or adrenergic receptors may account for the drug's mesolimbic selectivity. Clozapine has moderately potent antagonistic effects on several types of adrenergic receptor (Gross and Schumann, 1982), and there is some evidence that enhanced noradrenergic activity contribute to the intensification of negative and positive symptoms of schizophrenia (van Kammen et al, 1990), therefore blockade of adrenergic receptors may have some clinical relevance (Meltzer, 1991). Clozapine is a potent antimuscarinic agent, and this high potency may account for the low extrapyramidal symptoms seen in clozapine treated patients, although administering large doses of the antimuscarinic drug, cogentin, with haloperidol or chlorpromazine did not achieve the same effects as clozapine with respect to extrapyramidal side effects, positive symptoms or negative symptoms (Kane et al, 1988).

A more likely explanation of clozapine's unique clinical profile is its effect on serotonin systems. Clozapine has been shown to be an effective 5HT antagonist in vivo (see Meltzer, 1991), although the data to support an effect on 5HT metabolism in vitro in rat brain are inconclusive (Ichikawa and Meltzer, 1991). Meltzer et al, (1989c) have shown that atypical antipsychotics produce two orders of magnitude

more 5HT2 receptor blockade than the classical drugs produce. The importance of 5HT2 receptors in schizophrenia is supported by evidence that 5HT2 receptor density is decreased in frontal cortex in patients with schizophrenia (Mita et al, 1986; Meltzer, 1991). Meltzer (1991) speculates that serotonergic activity in schizophrenia leads to a down regulation of 5HT2 receptors, and that clozapine and other atypical drugs may lead to further decreases in 5HT transmission, as a consequence of their potent antagonism at 5HT2 receptors.

There is also evidence that 5HT3 receptor stimulation may increase dopamine release (Blandina et al, 1988). There is therefore some expectation that 5HT3 antagonists may exhibit antipsychotic action as a result of decreasing dopamine release, somewhat analogous to the development of autoreceptor agonists as a mechanism of decreasing dopamine release. Clozapine exhibits moderate 5HT3 antagonism (Ashby et al, 1989; Watling et al, 1990), although how this relates to the therapeutic action of clozapine is unknown.

In summary clozapine, in contrast to the classic antipsychotics, exhibits low in vitro affinity for the striatal D2 receptors and relatively greater affinity for striatal D1 receptors, cortical and subcortical 5HT2 and 5HT3 receptors, and central adrenergic, histamine and muscarinic receptors (see review by Fitton and Heel, 1990). It is unlikely that D2 receptor antagonism can fully account for the drugs superior antipsychotic action, and it has been suggested that the drugs efficacy may be related to D1, or a combination of D1 and D2, antagonism (Alter et al, 1988; Andersen and Braestrup, 1986; Coward et al, 1989; Criswell et al, 1989), with 5HT2 antagonism playing a supplementary role (Meltzer, 1989), whilst the low incidence of extrapyramidal symptoms seen following treatment with clozapine result from its selective action on mesolimbic dopaminergic mechanisms (Borison and Diamond, 1983).

Recently the D3 receptor (Sokoloff et al, 1990) has been identified. Limbic regions are rich in D3 mRNA, whilst high concentrations of D3 receptors on the cell bodies of both striatal and mesolimbic dopamine neurones suggest that the D3 receptor may be an autoreceptor. In addition dopamine has been shown to have a twenty fold greater affinity for the D3 than for the D2 receptor. Although all antipsychotic drugs

are more potent at the D2 than D3 receptor, drugs classified as atypical such as clozapine and (-) sulpiride are only two to three times more potent at the D2 receptor. Sokoloff et al, (1990) suggest that D3 antagonism may be an important aspect of antipsychotic activity.

In studies into the putative mechanisms of dopaminergic and non-dopaminergic drugs which are proposed as antipsychotic agents a strong note of caution should be made, which is best illustrated by the drug savoxepine. The possibility that the hippocampus may be abnormal in schizophrenia has been intensively examined. Studies into patients post-mortem (Stevens, 1973), and using magnetic resonance imaging techniques, have found hippocampal abnormalities and these could be relevant to the memory dysfunction associated with schizophrenia. This led Bischoff (1986) to suggest that selective blockade of dopamine receptors in the hippocampus, without striatal dopamine receptor blockade, might produce an antipsychotic drug with an atypical profile. Savoxepine is a novel tricyclic compound with strong D2 blocking effect with a tenfold higher affinity for hippocampal than striatal D2 receptors (Bischoff et al, 1986). Furthermore, savoxepine has a profile similar to clozapine in that it blocks D1, 5HT2, adrenergic and histaminergic H1 receptors (Gerlach, 1991). In behavioural tests savoxepine has been shown to antagonise dopamine agonist-induced locomotion and stereotyped behaviours at relatively low doses, but much higher doses are required to induce catalepsy (see Gerlach, 1991). This action in animal models led to trials of savoxepine as an antipsychotic agent which, it was claimed, would not induce extrapyramidal symptoms at therapeutic doses.

In three open studies it has been shown that savoxepine has therapeutic effects at doses between 0.25 and 2 mg/day (Butler and Bech, 1987; Moller et al, 1989; Wetzel et al, 1991). In the last two studies, savoxepine induced Parkinson's symptoms, akithisia and acute dystonia. Although these studies were not conducted as blind trials it would appear that affinity for hippocampal D2 receptors, and the differential doses required to induce catalepsy and block dopamine agonist-induced behaviours did not predict a drug with a reduced risk of extrapyramidal side effects at therapeutic doses.

4.3 *Introduction*

Antagonism of amphetamine-induced behaviours in animals has been used to study the underlying mechanisms of antipsychotic drug treatment, and to detect novel antipsychotic agents. Considerable attention has focused on amphetamine-induced stereotyped behaviours such as sniffing, licking and gnawing because these behaviours are reliably antagonised by classic antipsychotics such as haloperidol and chlorpromazine (Szechtman et al, 1988). It is now clear that a group of clinically effective drugs - so called atypical antipsychotics - do not antagonise all the components of the amphetamine-induced syndrome.

Tschanz and Rebec (1989) reported that atypical antipsychotics vary in their ability to antagonise specific amphetamine-induced behaviours. For example, clozapine blocks sniffing but not the rearing or head bobbing produced by the drug. Paradoxically several researchers have reported that clozapine and other atypical antipsychotics actually enhance the stereotyped head movements and sniffing induced by 2 - 5 mg/kg amphetamine (Robertson and MacDonald, 1985; Sharp et al, 1986).

Under amphetamine, locomotion often takes the form of thigmotaxic patrolling of the open field boundary and this behaviour is exhibited repetitively under higher doses of the drug (Schiorring 1979; Mueller et al, 1989a, see chapter 3). Previous studies have reported that approximately 30 - 40% of trips were around the perimeter of the apparatus, entering all four quadrants in turn (length 4 trip) during the period of peak drug response (25 - 45 minutes) after 3 - 3.5 mg/kg amphetamine (Mueller et al, 1989a; 1989b; Kenyon et al, 1992).

Recent work in our laboratory has shown that the atypical antipsychotic sulpiride (20mg/kg) reduced locomotor distance, but had no effect on the proportion of length 4 trips under amphetamine (3.5 mg/kg). We concluded that sulpiride reduced hyperactivity, but did not disrupt the perseverative nature of locomotion under amphetamine (Kenyon et al, 1992), suggesting that aspects of amphetamine-induced locomotion may indeed be stereotyped.

Recent findings have demonstrated that, in marked contrast to the action of the classic antipsychotic haloperidol, atypical neuroleptics do not elicit comparable effects on locomotor behaviours mediated via dopaminergic mechanisms. Specifically

sulpiride, thioridazine and clozapine antagonised the enhanced locomotion in apomorphine-treated rats which had been rendered selectively 6-OHDA 'supersensitive' in the nucleus accumbens (Schremmer et al, 1990). In a recent study by White et al, (1991), sulpiride, in contrast to the effects of haloperidol and clozapine, failed to impair lever release avoidance response rates even at doses that significantly reduced amphetamine-induced locomotion.

These findings, indicating that typical and atypical antipsychotics differentially affect various dopaminergic mediated behaviours, led us to investigate the hypothesis that classical and atypical antipsychotic drugs would exhibit different actions on amphetamine-induced length 4 trips; that only a classic antipsychotic would antagonise both hyperactivity and length 4 trips. We also considered the possibility that atypical antipsychotic drugs might even potentiate length 4 trips.

The present study examined the effects of haloperidol, clozapine and sulpiride on amphetamine-induced hyperactivity and perseverative locomotor patterns in an open field. Haloperidol is a clinically effective antipsychotic but is known to induce extrapyramidal side-effects (EPS; Green and Costain, 1981). Clozapine and sulpiride are known to elicit fewer EPS (Tamminga and Gerlach, 1987), and they do not exhibit typical antipsychotic properties in animal models (Ljungberg and Ungerstedt, 1985; Robertson and MacDonald, 1984; 1985; Tschanz and Rebec, 1989; Schremmer et al, 1990; White et al, 1991).

A single intermediate dose of amphetamine (3.5 mg/kg) was used in this study because it provides a suitable baseline for detecting either facilitatory or inhibitory effects of a wide dose range of antipsychotic drugs on locomotor patterns, without eliminating forward locomotion completely.

All three antipsychotic drugs antagonised amphetamine-induced hyperactivity; however only haloperidol significantly reduced the perseverative nature of hyperactivity under amphetamine.

4.4 Materials and methods

Animals. Male Wistar rats weighing 300 - 400 g (bred at the University animal house facilities) were used. Rats were housed in groups of six on a 12h light/dark cycle and allowed free access to food and water. The groups to be tested were allowed at least 3 days acclimation in a temperature regulated room (18 - 22^{o} C) adjacent to the laboratory. Each animal was tested only once in the open field.

Drugs. The following drugs were used and all doses are expressed as the salt: d-amphetamine sulphate (Sigma) 3.5 mg/kg dissolved in phosphate buffered saline; (+-) sulpiride (Sigma) 10, 20 and 50 mg/kg dissolved in 1% lactic acid; clozapine (donated by Sandoz Products Ltd) 5, 10 and 20 mg/kg dissolved in 0.2N HCl, final volume achieved with sterile distilled water, and the pH adjusted to 4.5; haloperidol (Sigma) 0.01, 0.025, 0.05 and 0.075 mg/kg dissolved in 1% lactic acid.

Amphetamine and saline were injected intra-peritoneally (IP) immediately before testing in the open field. All other drugs were injected subcutaneously (SC) 30 minutes prior to the injection of either amphetamine or saline.

Experimental procedure. Animals were randomly assigned to a treatment group (n = 8 per group). A Vehicle-Control and Vehicle-Amphetamine group was included with each of the three antipsychotic drugs tested. Rats received a SC injection of antipsychotic drug or vehicle before being placed in the open field. They were removed after 30 minutes, given amphetamine or saline IP and replaced immediately in the open field, where they remained for 105 minutes.

Apparatus. Four circular open fields (75 cm diameter, height 30 cm) constructed from black Perspex were used. A video camera was mounted above each field. In a separate room the camera picture was analysed by an HVS image systems VP112 unit which converted the video signal into a stream of XY co-ordinate pairs representing the position of the animal. The subject's pattern of movement was analysed using software, described in detail elsewhere (Kenyon, 1990; 1991), which was modified

to accept input from the VP112 unit. All data are stored permanently in disk files for subsequent data analysis.

Data collection. During the 105 minute test session the output from the camera and the HVS image analyser was monitored on a TV screen. The path taken by the subject was displayed on the computer screen by the TRACKER software (Kenyon, 1990). The screen showed a representation of the floor of the open field subdivided by lines into four equal-sized areas. The software tracked the animal's movements between these quadrants. Horizontal movement was recorded as distance (in cm) moved by the animal in each 5 minute time interval, with the total distance moved resulting from the sum of the 21 five minute intervals recorded over the test session.

The subject's sequence of movements between adjacent quadrants of the apparatus was subdivided into a series of trip lengths using STEREO software (Kenyon, 1991). A single trip consists of a sequence of movements between adjacent quadrants of the open field. A trip is terminated when the rat changes direction, or completes a tour of the perimeter of the apparatus. Trip length can take values of 1, 2, 3 or 4 in an open field divided into four regions. In reality, very few trips exhibited by either saline- or amphetamine-treated animals are of length 2 or 3 (less than 10%), therefore in subsequent analysis length 2 and 3 trips were discarded. The proportion of length 1 and length 4 trips were calculated as a proportion of all trip types, and used in the statistical analysis.

Data analysis. Logarithmic transformations were performed on the distance moved data, and arcsin transformations were performed on the proportional data, as recommended by Howell (1992).

Data were analysed separately for each antipsychotic drug, using analysis of variance (ANOVA) with treatment (drug dose) the between subjects factor, and 5 minute intervals the repeated within subjects factor. Differences were evaluated using the Newman-Keuls multiple range test (Howell 1992). Vehicle-Amphetamine groups were compared with Vehicle-Saline groups and all antipsychotic pre-treatment groups were compared with Vehicle-Amphetamine groups.

4.5 Results.

Amphetamine.

Distance Moved. In each phase of the study, treatment with amphetamine (3.5 mg/kg) increased locomotor activity. During the 105 minute test session rats treated with amphetamine moved a significantly greater distance (Mean Vehicle-Saline = 11750 cm, SEM +- 5576 cm [n=24]; Mean Vehicle-Amphetamine = 63560 cm, SEM+- 2071 cm [n=24]; $p < .01$; Newman-Keuls post hoc test).

Pre-treatment with antipsychotic drug.

Distance moved. Figure 4.1A shows that haloperidol had a significant effect on amphetamine-induced hyperactivity ($F(4,35) = 12.21$, $P < .0001$). Pre-treatment with .025, .05, .075 mg/kg haloperidol resulted in a significant decrease in the total distance moved by amphetamine treated animals ($p < .05$). Pre-treatment with the lowest dose of haloperidol (.01 mg/kg) did not significantly reduce amphetamine-induced hyperactivity.

Figure 4.1B shows that sulpiride also had a significant effect on amphetamine-induced hyperactivity ($F(3,28) = 3.329$ $p < .03$). Pre-treatment with 20 and 50 mg/kg sulpiride significantly reduced the distance moved by amphetamine treated rats over the test session ($p < .05$).

Clozapine (Fig. 4.1C) had a significant effect on the hyperactivity produced by amphetamine ($F(3,28) = 8.76$ $p < .0004$). Pre-treatment with 10 and 20 mg/kg clozapine reduced the distance amphetamine treated animals moved over the test session ($p < .01$). Rats pre-treated with the lowest dose of clozapine (5 mg/kg), did not differ significantly from Vehicle-Amphetamine treated rats on this measure over the 105 minute test session; during the first 30 minutes of the test session this group showed increased levels of hyperactivity, thereafter, activity levels in this treatment group remained comparable to those exhibited by Vehicle-Amphetamine treated animals (ps < .05 for first 30 minutes).

Proportion of length 4 trips. Figure 4.2A shows that haloperidol had a significant effect on the increased proportion of length 4 trips associated with amphetamine treatment ($F(4,35) = 7.68$ $p < .0001$). Pre-treatment with .025, .05, .075 mg/kg haloperidol significantly reduced the proportion of length 4 trips under amphetamine (ps <.01). The lowest dose of haloperidol (.01 mg/kg) did not significantly reduce the proportion of length 4 trips induced by amphetamine.

Figure 4.1B and 4.1C show that sulpiride ($F(3,28) = 1.092$ $p < .369$). and clozapine ($F(3,28) = 2.136$ $p < .118$) did not result in a significant decrease in the proportion of length 4 trips exhibited after treatment with amphetamine.

4.6 Discussion.

In agreement with previous findings, an intermediate dose of amphetamine increased open-field locomotion and the proportion of length 4 trips around the periphery of an open field (Mueller et al, 1989; Kenyon et al, 1992, chapter 3). Pre-treatment with haloperidol, clozapine and sulpiride, antagonised amphetamine-induced locomotion. These findings are in agreement with reports that a consistent feature of both typical and atypical antipsychotic drugs is their ability to antagonise amphetamine-induced hyperactivity. (Ljungberg and Ungerstedt, 1985; Rebec and Bashore, 1984).

In addition to reducing forward locomotion, haloperidol (.025, .05, .075mg/kg) also reduced the proportion of length 4 trips associated with amphetamine-induced hyperactivity. Although both clozapine and sulpiride antagonised hyper-locomotion, neither of these atypical drugs significantly reduced the proportion of length 4 trips under amphetamine. This suggests that following pre-treatment with an atypical antipsychotic - whilst hyperactivity is reduced - the perseverative nature of the remaining locomotion remains unchanged. It should be noted that the finding that sulpiride did not antagonise length 4 trips is by no means unequivocal. Animals pre-treated with sulpiride did show a reduction in the proportion of length 4 trips, but the large variability in this measure for these

groups may account for the lack of statistical significance, specifically for animals pre-treated with 10 mg/kg sulpiride.

Pre-treatment with .025mg/kg haloperidol, 20mg/kg sulpiride or 10mg/kg clozapine reduced the distance moved by amphetamine treated animals by an equivalent amount over the entire test session (total distance moved approx. 3000cm.). Only this dose of haloperidol (.025mg/kg) resulted in a significant reduction in the proportion of length 4 trips, suggesting that although all three antipsychotic drugs are equipotent in reducing hyperactivity at these doses, only the classic antipsychotic, haloperidol, blocks the repetitive boundary patrolling associated with hyperactivity.

The results reported in this study replicate the previous observation that 20mg/kg sulpiride did not disrupt amphetamine-induced perseverative locomotor paths in hyperactive rats and broadens the findings to include the atypical antipsychotic clozapine. These findings support the gathering evidence that clozapine and sulpiride do not exhibit actions similar to haloperidol on all forms of amphetamine-induced locomotor behaviours (Schremmer et al, 1990; Kenyon et al, 1992), and that locomotion cannot be defined as a unitary behavioural response to amphetamine.

These studies have found consistent repetitive patterns of movement exhibited by animals treated with 3.5 mg/kg amphetamine, which are manifest as repetitive thigmotaxic boundary patrolling, resulting in a quantifiable increase in the proportion of length 4 trips. There was no evidence for a systematic increase in any other type of trip length under this dose of amphetamine. The results reported here add weight to the suggestion that hyperactivity induced by intermediate doses of amphetamine has stereotypic features by way of its invariance and repetition of route around the open field (Ljungberg and Ungerstedt, 1978; Schiorring, 1979; Kenyon et al, 1992). The Lyon-Robbins hypothesis (1975) suggests that stereotypy is the end point of a continuous process of psychomotor stimulation and behavioural competition. This would predict competition and blending between locomotor effects of amphetamine, mediated by the ventral striatum in particular the nucleus accumbens and stereotyped behaviours mediated, at least in part, by the caudate putamen (Pijnenburg et al, 1973; Kelly et al, 1975, Creese and Iversen, 1975). The

dissociation of length 4 trips from locomotor effects *per se* by the atypical antipsychotics clozapine and possibly sulpiride which is reported here suggest that perseverative patterns of hyperactivity constitute a 'blending' of behavioural responses in an attempt to resolve competition between amphetamine-induced locomotion and more overt stereotyped behaviours.

This study has examined only a small number of classic and atypical antipsychotic drugs, and the results are not entirely unequivocal, particularly with respect to sulpiride. Clearly further investigation of length 4 trips as a proposed measure of repetitive perseverative patterns of hyper-locomotion are required. It is not clear whether the inability of clozapine and sulpiride to block perseverative patterns in amphetamine-induced hyper-locomotion would generalise to other atypical antipsychotics, or the 5-HT_3 antagonists which have been suggested as putative antipsychotic agents because of their selectivity in blocking the mesolimbic dopamine system (Costall et al, 1987).

It is now evident that much additional information can be gained by examining drug-induced effects on the spatial patterns inherent in open field activity (Geyer et al, 1987; Mueller et al, 1989; Paulus et al, 1988; Paulus and Geyer, 1991; Kenyon et al, 1992). The analysis of locomotion using computer imaging techniques has revealed consistent repetitive patterns of movement exhibited by amphetamine treated rats in an open field, and the use of trip length as a method of quantifying the perseverative patterns in amphetamine-induced locomotion has provided a useful and sensitive tool which may discriminate classic and atypical antipsychotics, in much the same way that antagonism of hyperactivity and antagonism of stereotyped behaviours discriminates these drugs.

The rate and duration of length 4 trips may provide additional information concerning the spatial distribution of perseverative patterns in amphetamine-induced hyper-locomotion and may also discriminate antipsychotic drugs (personal observations).

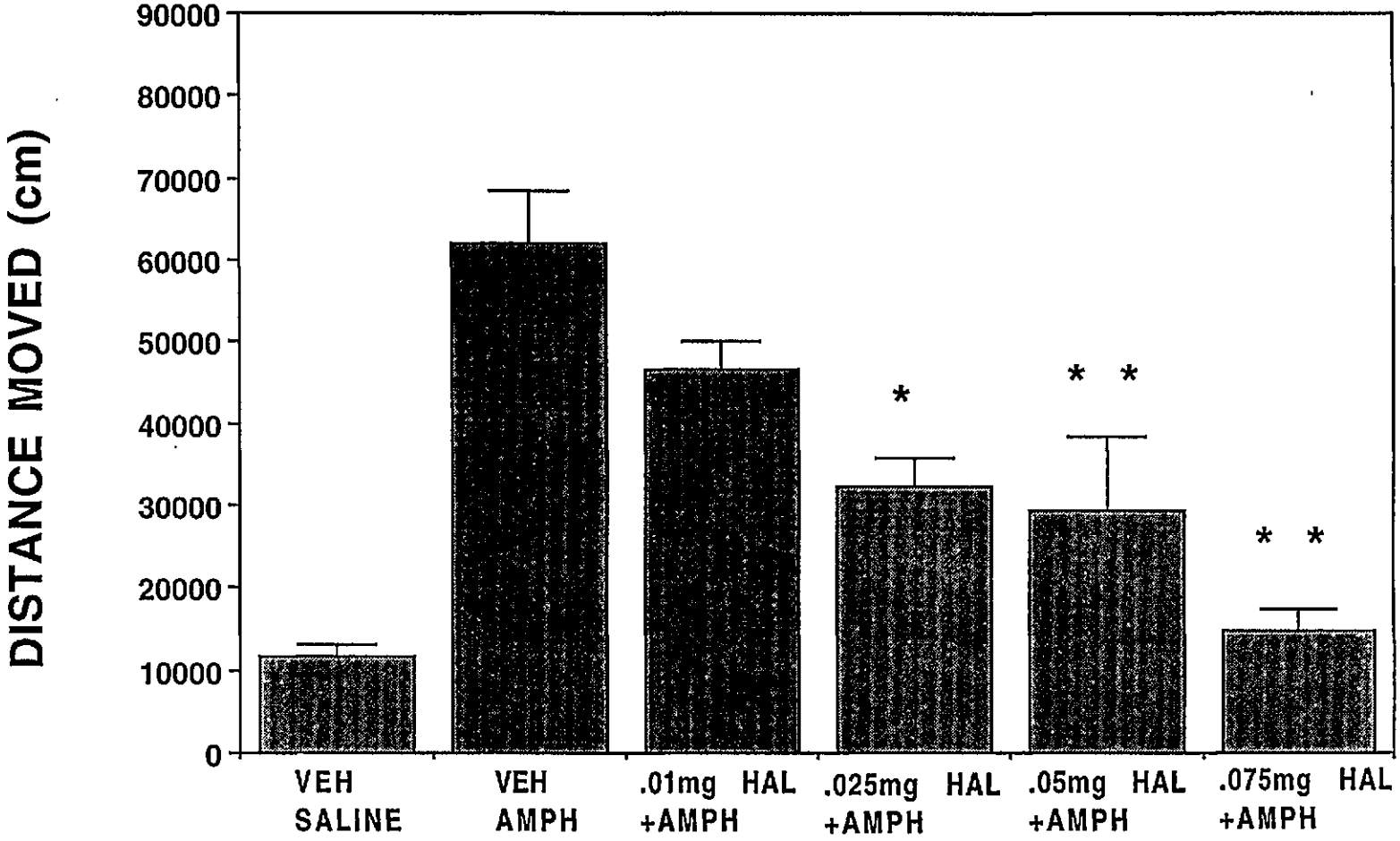
Summary

An automated tracking system was used to analyse stereotyped locomotion in amphetamine-treated rats. Amphetamine (3.5mg/kg) increased the horizontal distance moved and the number and proportion of thigmotaxic trips around the perimeter of the apparatus (length 4 trips). The ability of the classic antipsychotic, haloperidol, and the atypical antipsychotics, clozapine and sulpiride, to block amphetamine-induced length 4 trips was investigated. The results showed that the classic antipsychotic haloperidol antagonised both hyperactivity and the increased proportion of length 4 trips. In marked contrast, the atypical antipsychotics clozapine and sulpiride antagonised hyperactivity but did not reduce the proportion of length 4 trips. The inability of atypical antipsychotics to reduce the repetitive boundary patrolling associated with amphetamine-induced hyperactivity is consistent with the action of these drugs on other forms of amphetamine-induced stereotyped behaviour, and indicates that locomotor routes under amphetamine are stereotyped. The measurement of trip lengths provides a sensitive tool for examining drug action on the spatial distribution of open field locomotion.

Figure 4.1 A-C.

Mean (+-SEM) distance moved (cm) during the 105 min test session (n=8 per group). (A). 3.5mg/kg amphetamine pre-treated with vehicle, 0.01, 0.025, 0.05 or 0.075 mg/kg haloperidol. (B). 3.5mg/kg amphetamine pre-treated with vehicle, 10, 20 or 50 mg/kg sulpiride. (C). 3.5mg/kg amphetamine pre-treated with vehicle, 5, 10 or 20 mg/kg clozapine (*p< 0.05, **p< .01. Antipsychotic pre-treatment versus Vehicle-Amphetamine).

4.1A


DISTANCE MOVED (cm)
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80000
70000
60000
50000
40000
30000
20000
10000
0
*
* *
* *
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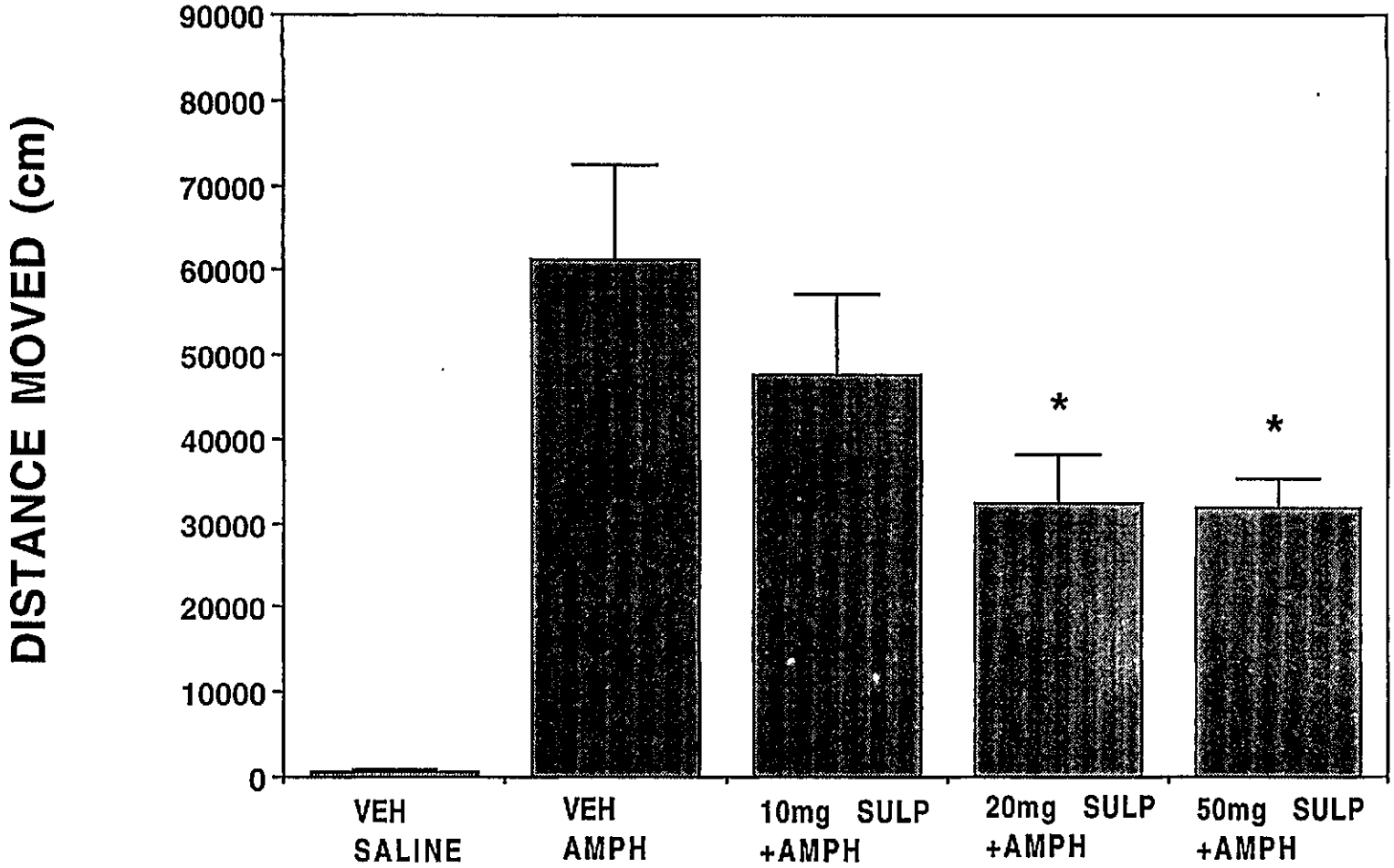
VEH AMPH
.01mg HAL +AMPH
.025mg HAL +AMPH
.05mg HAL +AMPH
.075mg HAL +AMPH

4.1B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70000
60000
50000
40000
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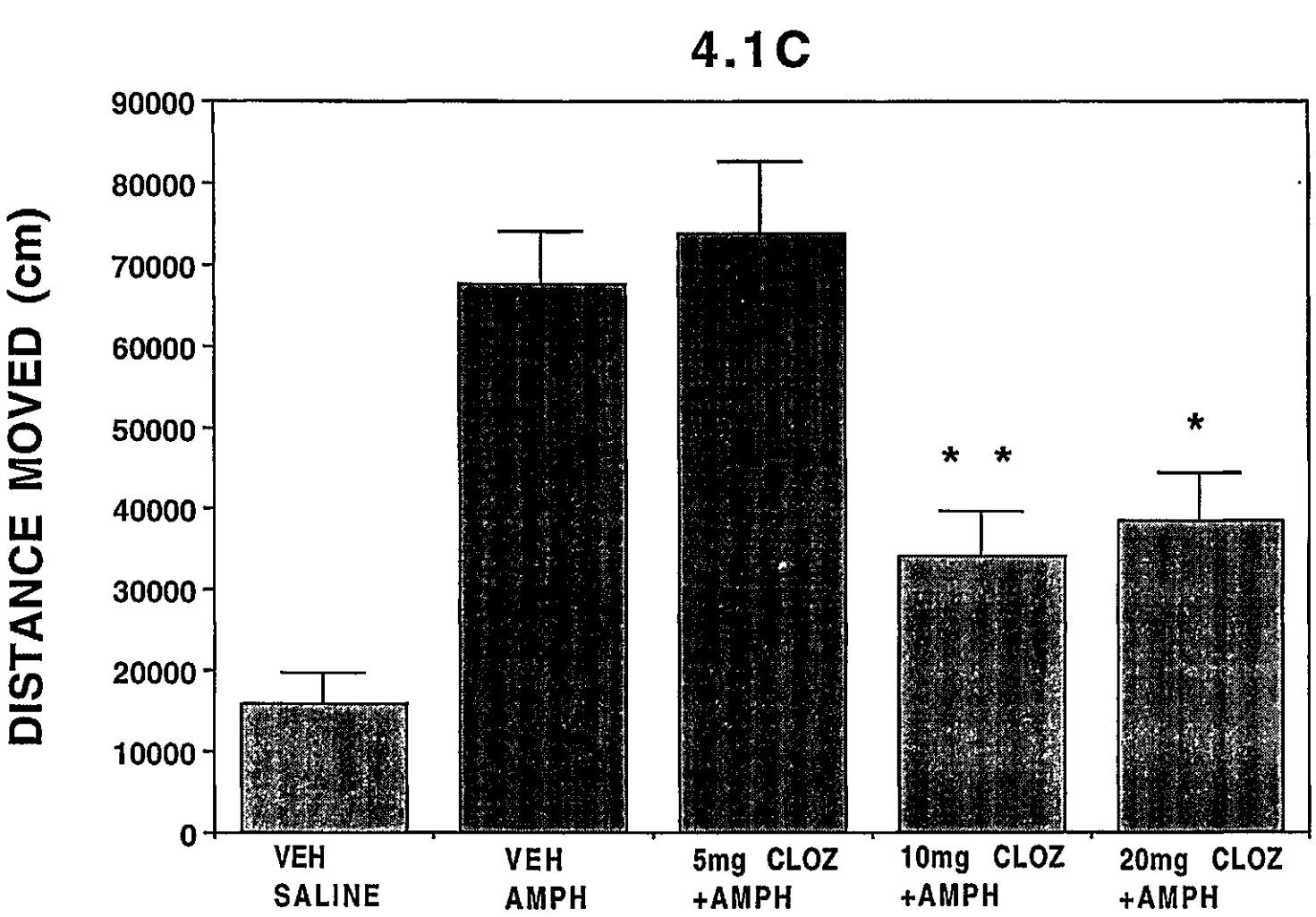
4.1C
DISTANCE MOVED (cm)
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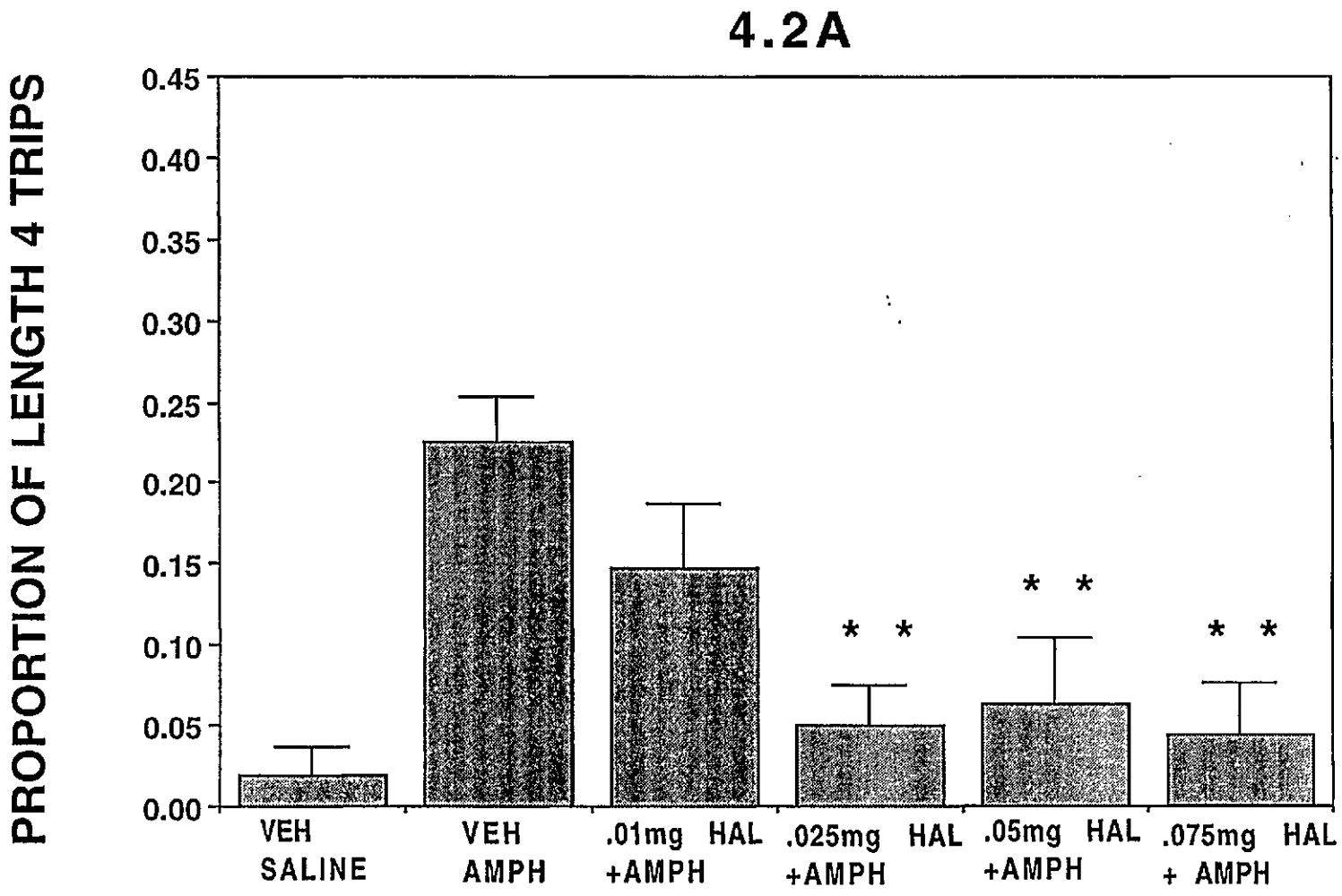
80000
70000
60000
50000
40000
30000
20000
10000
0
* *
*
VEH SALINE
VEH AMPH
5mg CLOZ +AMPH
10mg CLOZ +AMPH
20mg CLOZ +AMPH

Figure 4.2 A-C.

Mean (+-SEM) proportion of length 4 trips during 105 min test session (n=8 per group). (A). 3.5mg/kg amphetamine pre-treated with vehicle, 0.01, 0.025, 0.05, or 0.075 mg/kg haloperidol. (B). 3.5mg/kg amphetamine pre-treated with vehicle, 5, 10 or 50 mg/kg sulpiride. (C). 3.5mg/kg amphetamine pre-treated with vehicle, 5, 10 or 20mg/kg clozapine. (**p< 0.01. Antipsychotic pre-treatment versus Vehicle-Amphetamine).


4.2A
PROPORTION OF LENGTH 4 TRIPS
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0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
* *
* *
* *
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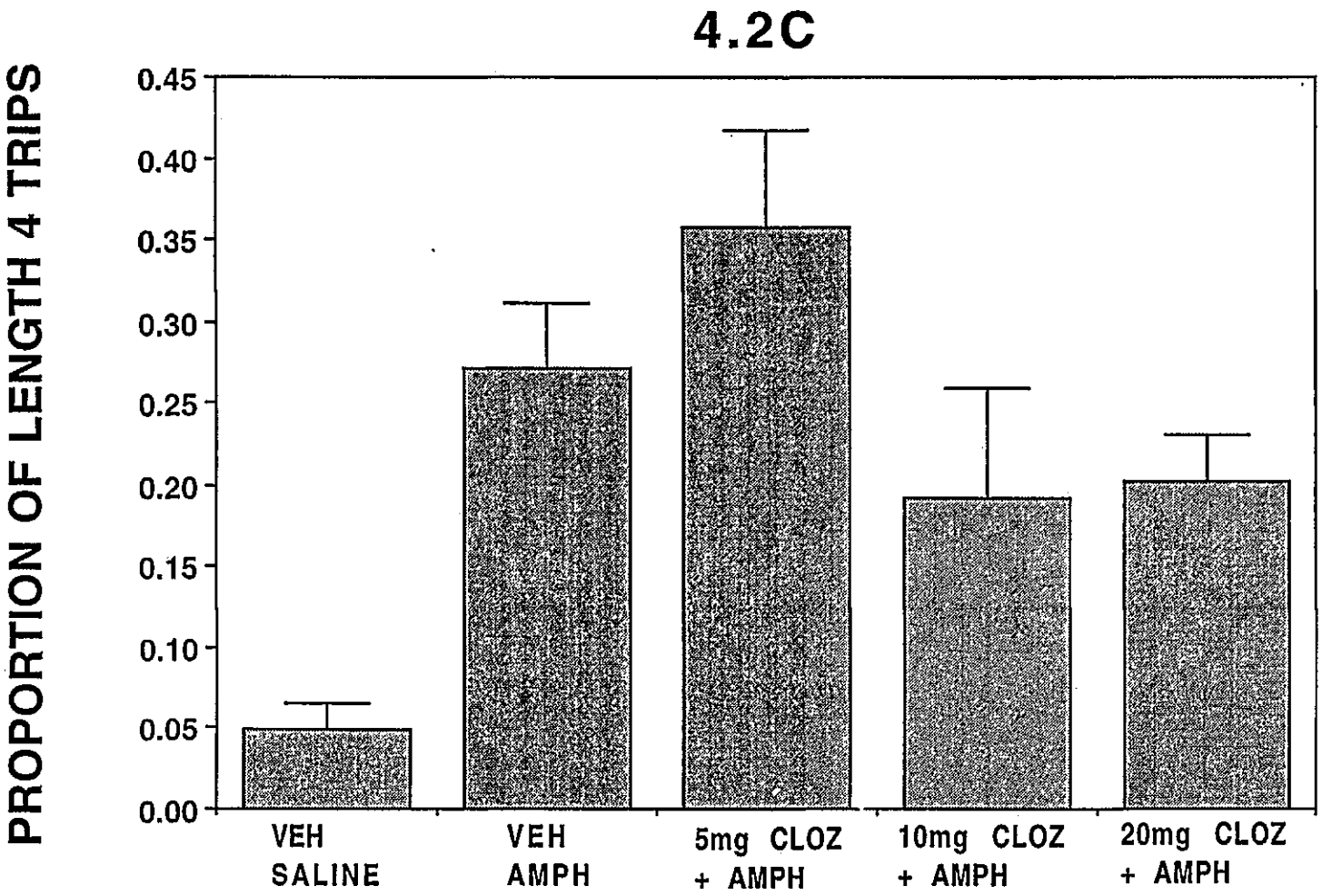
VEH AMPH
.01mg HAL +AMPH
.025mg HAL +AMPH
.05mg HAL +AMPH
.075mg HAL + AMPH


4.2C
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0.30
0.25
0.20
0.15
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0.00
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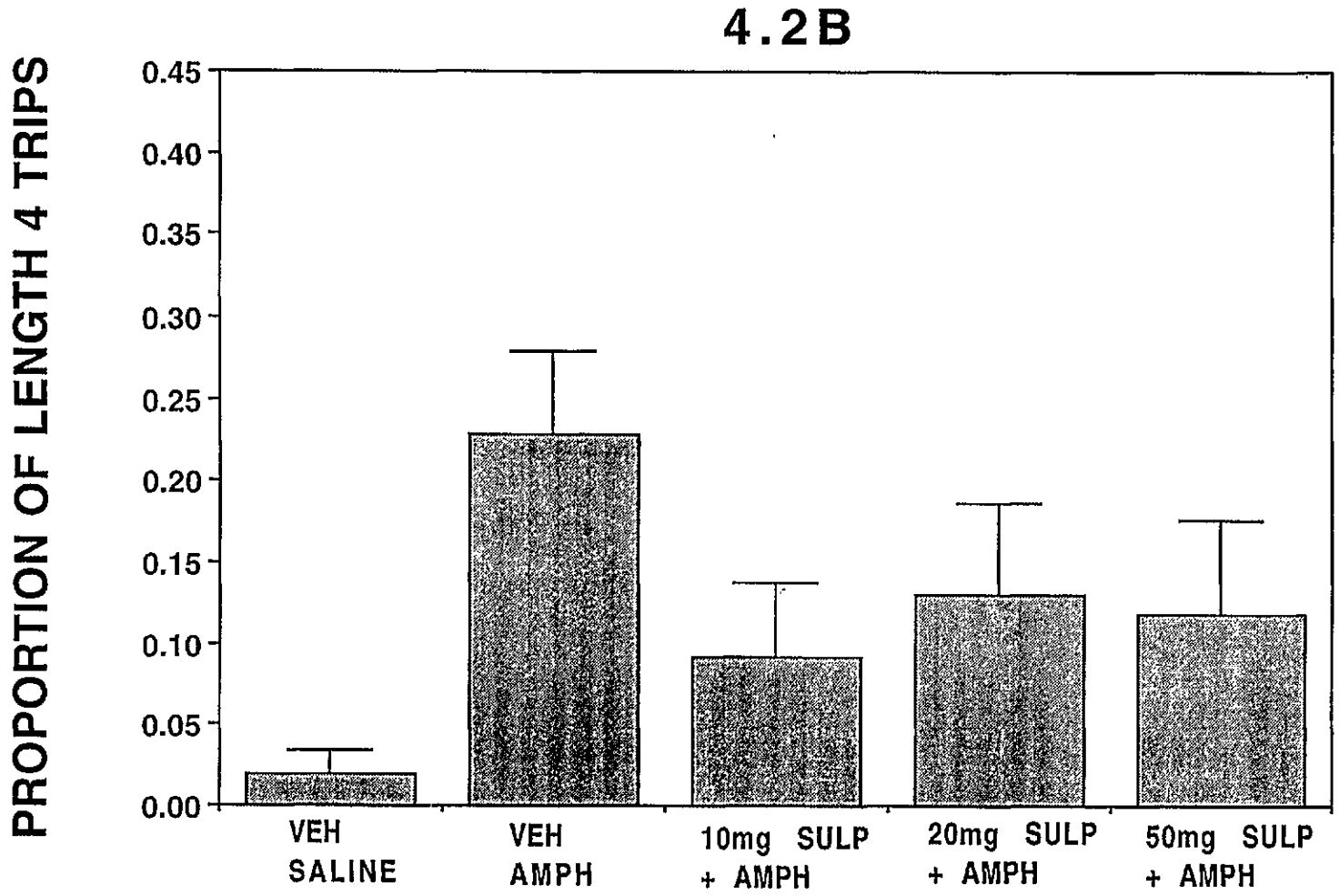
VEH AMPH
5mg CLOZ + AMPH
10mg CLOZ + AMPH
20mg CLOZ + AMPH


4.2B
PROPORTION OF LENGTH 4 TRIPS
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
VEH SALINE
VEH AMPH
10mg SULP + AMPH
20mg SULP + AMPH
50mg SULP + AMPH

Chapter Five
Behavioural analysis

5.1 Introduction

Rebec and Bashore (1984) state that 'the mechanisms of action of amphetamine and the antipsychotic drugs will not be fully understood nor will new light be shed on the neuropathology of paranoid schizophrenia until the behavioural syndrome produced by amphetamine and the specific components antagonised by the antipsychotic drugs are precisely delineated' (p154). This view is entirely consistent with the ideas put forward in the preceding chapters concerning the adequate description and quantification of amphetamine-induced locomotor behaviour. Locomotion is only one aspect of the amphetamine response, dose dependent changes in other behaviours also occur (see Chapter 1 section 1.4). Many reviewers of the methodological considerations to be taken into account in the assessment of motor activity recommend the assessment of multiple aspects of unconditioned behaviour following drug manipulations (Lat, 1965; Reiter and MacPhail, 1979; Robbins, 1977; Geyer, 1990). Geyer, (1990) claims that in addition to automated recording of locomotor activity, the use of direct observation should also be utilised. Some behaviours following treatment with amphetamine will be mutually exclusive, and will compete for expression, therefore an effective characterisation of the drug effect will require the measurement of all behaviours which occur under the drug. It may well be that correct interpretation of the data obtained from automated measures of locomotor activity is dependent upon the use of observational techniques. The use of multivariate assessment provides an opportunity to assess the validity of the hypothetical constructs, to make comparisons with results reported in the literature, to examine the relative specificity of the effect, to identify aspects of response competition and to detect artefacts (Geyer, 1990).

In an earlier experiment (see Chapter 3) conducted to examine the dose response characteristics of amphetamine on the spatial distribution of open-field locomotion and the influence that field shape made to the behavioural response, findings suggested that animals treated with 4 or 5 mg/kg amphetamine and subsequently tested in a circular open field progressed more rapidly from the locomotor phase to the stereotypy phase. Without concurrent behavioural observations it is impossible to determine whether this is a correct interpretation of these data, or indeed what behaviours animals tested in a circular open field were performing during those session intervals when their counterparts in the square field continued to engage in locomotor behaviours. In further experiments (Chapter 4), examining the effects of antipsychotic drugs on the stereotyped nature of hyperactivity, none of the drugs tested potentiated stereotyped locomotion. Without measuring the action of these drugs on all aspects of the amphetamine syndrome it is impossible to draw conclusions concerning the relative contribution of stereotyped behaviours. The aim of this current investigation was to provide an adequate description of those behaviours which occurred in conjunction with hyperactivity. What follows is a detailed discussion of the methodological considerations which were met to achieve this.

Stereotypy has been defined as the performance of an invariant sequence of movements in a repetitive manner (eg Fray et al, 1980; Rebec and Bashore, 1984). Rating scales have frequently been used to assess the intensity of stereotyped behaviours (eg Costall et al, 1972; Creese and Iversen, 1973; Sahakian et al, 1975). Although these rating scales have proved useful in the study of drugs and behaviour, there are problems associated with rating scales. The degree to which a behaviour is stereotyped is somewhat subjective and human observers do not always record behavioural types accurately or consistently (see Fray et al, 1980; Jacobs, 1988), this can create difficulties for both inter- and intra-laboratory reliability even when studies are rated blind. Both behavioural rating scales and automated recordings of locomotor activity (typically using photobeams) fail to distinguish the individual components of the amphetamine response (see Fray et al, 1980), and

many rating scales confuse stereotypy with specific behaviours, summing separate behavioural components together to obtain a global stereotypy score, resulting in a loss of information with respect to the individual behavioural elements of the response. For example Costall and Naylor (1977) showed that selective damage to dopamine neurones in the neostriatum abolished licking and biting but not sniffing. Lesion studies have demonstrated that dopamine projections to various brain regions including the olfactory tubercle, nucleus accumbens and the amygdala are responsible for various behavioural responses seen under amphetamine (Costall et al, 1977; Costall and Naylor, 1977; 1975). Rating scales which combine behavioural categories are unable to make this type of distinction. In addition, not all aspects of the amphetamine response have been shown to be enhanced by multiple injections of amphetamine, suggesting that not all amphetamine-induced behaviours provide an adequate model of clinical psychosis (Rebec and Bashore, 1984). Rebec and Bashore (1984) also argue that photobeam analysis of locomotor behaviour can lead to inaccurate interpretations. Fink and Smith (1979; 1980) observed behaviour directly and reported that neostriatal lesions reduced amphetamine-induced hyperactivity, but the effect was not apparent when photocell beam breaks were automated, as the lesions reduced the length of locomotion rather than its frequency. Some researchers have noted that photocell counts lead to exaggerated measures of locomotor activity (eg Krsiak et al, 1970 and Fray et al, 1980).

Fray et al, (1980) developed a method for scoring the presence or absence of behavioural response categories without rating the stereotyped nature of the behaviour in an attempt to overcome some of these problems. Using this method these authors were able to provide a detailed comparison between the unconditioned behavioural effects of d-amphetamine and apomorphine in the rat. They were able to describe the different threshold doses required to elicit each behaviour and also the temporal aspects of the behavioural response.

In an adaptation of the method described by Fray et al, (1980) video tapes of all experimental subjects were analysed in order to provide a detailed behavioural description to address specific questions relating to the findings reported in Chapter

3 and Chapter 4. First, is there a difference in snout contact resulting from differences in open field shape, or amphetamine dose? Second, does locomotor activity occur persistently or do the animals pause between bouts of locomotion, and how does this relate to drug treatment and environmental factors? Finally, what behaviours occur concurrently with locomotion and how are these affected by environmental factors or antipsychotic drug pre-treatment?

The information statistic employed by Fray et al, (1980) used planned contrasts and did not allow for direct pairwise comparisons. In addition, this statistical test is biased towards behaviours which occur infrequently. Recently a non-parametric analysis of changes in community structure was introduced into marine ecology (see Clarke, 1993; Field et al, 1982). This approach uses multivariate methods to incorporate formal hypothesis-testing without sacrificing the 'distribution-free' nature of analyses based on rank similarities. Such an approach has direct parallels with the study of drug-induced behavioural change and can be directly applied to records of behaviour similar to those described by Fray et al, (1980).

The data are organised into an 'abundance array' whose columns represent individual animals and whose rows are the full set of behaviours observed for each animal. The behavioural relationship between any two animals is distilled into a coefficient measuring similarity or dissimilarity in behavioural composition. The resulting triangular matrix of similarities between each pair of animals is used to classify them into groups using either a dendrogram (Clifford and Stephenson, 1975) or by ordination to 'map' the interrelationships between animals by non-metric multi-dimensional scaling (MDS eg Kruskal and Wish 1978). Briefly the main features of MDS can be best illustrated using the example given by Clarke (1993), using the analogy of reconstructing a map of the world . Starting from the triangular matrix of distances between every pair of major cities in the world a map of the world can be reconstructed by placing the cities in their correct location; the result is an almost perfect map of the world. The algorithm works by initially placing the cities in three dimensional space at arbitrary locations, and then refining their

relative positions in an iterative cycle. Thus the rank order of the inter-city distances gets closer to the rank order of the original triangular matrix. The extent to which the ordination and triangular matrix disagree is reflected in the 'stress' coefficient, where this value tends to zero the rank orders reach perfect agreement, although this will rarely actually be achieved. Clarke (1993) suggests that practical experience with ecological data indicates the following rule of thumb for Kruskal's stress formula 1 (Kruskal and Wish 1978).

Stress < 0.05 gives an excellent representation with no prospect of misinterpretation.

Stress < 0.1 corresponds to good ordination with no real risk of drawing false inferences; though in a tightly clustered situation the fine structure of individual groups might bear separate examination.

Stress < 0.2 values at the upper end of this range may have a tendency to mislead but nevertheless this can still lead to a utilisable picture.

Stress > 0.2 is likely to lead to plots which are misleading. By the time stress reaches 0.35-0.4 the samples are effectively randomly placed, bearing little relation to the original similarity matrix (see Clarke, 1993, for a more detailed description of stress and its interpretation).

It is possible to determine which behaviours are responsible for grouping animals together, using Bray-Curtis dissimilarities (∂jk) between any two samples j and k defined as

$$\delta_{jk} = \sum_{i=l}^{p} = \delta jk(i) \qquad \text{(equation 1)}$$

where

$$\delta jk(i) = 100 lyij - \quad yikl / \Sigma_{i=l}^{p} (yij + yik) \qquad \text{(equation 2)}$$

equation 2 is the contribution of the ith behaviour and yij is the transformed abundance of the ith behaviour in the jth animal. Averaging ∂jk over all sample pairs (jk) with j in the first group and k in the second group gives the overall average dissimilarity (∂) between the two groups. The same averaging over each ∂jk(i) gives the average contribution to ∂i from the ith behaviour to the overall dissimilarity

(∂). As there are many pairs of samples (jk) making up the average ∂i a useful measure of how consistently a behaviour contributes to ∂i is the standard deviation SD(∂i) of the ∂jk(i) values. If ∂i is large and SD(∂i) small (the ratio of ∂i/SD(∂i) is also large), then the ith behaviour contributes consistently to the dissimilarity between the two groups. In much the same way one can examine the contribution each behaviour makes to the average similarity within a group (S). The average contribution of the ith behaviour (Si) is defined by taking the average over all pairs of rats (jk) within a group, of the ith behaviour Sjk(i) in the alternative definition of the Bray-Curtis similarity:

$$sjk=\Sigma_{i=1}^{p} sjk(i) \quad \text{(equation 3)}$$

where

$$sjk(i)=200\min(yij,yik)/\Sigma_{i=1}^{p}(yij+yik) \quad \text{(equation 4)}$$

the more a behaviour occurs within a group the more it will tend to contribute to the intra-group similarities. Therefore one would expect the ratio of Si/SD(si) to be high. These similarity-dissimilarity breakdowns are termed the 'similarity percentages' or SIMPER procedure. So far the explanation has relied upon *a posteriori* grouping of the animals in examining which behaviours are principally responsible for an observed clustering. In experimental work the groups are determined *a priori* and the need is for a more theoretical statistical framework within which to test hypotheses concerning differences in behaviour and responses between groups. Such a test is based on the ranked similarities between animals within the triangular similarity matrix. If rw is defined as the rank similarities between replicates within treatments and rB is the average of rank similarities arising from all pairs between treatments then the following test statistic may be used.

$$R=\left(\bar{r}B-\bar{r}W\right)/(M/2) \quad \text{(equation 5)}$$

where M= n(n-1)/2 and n is the total number of animals under consideration.

The denominator constant has been chosen so that R can never lie outside the range (-1,1); R=1 only if all replicates within groups are more similar to each other than any replicates from different groups; R=0 if the null hypothesis is true so that similarities within and between treatments are the same on average. R will generally fall between 0 and 1 indicating some degree of discrimination between treatments. An R value substantially less than zero is an unlikely event since it would correspond to similarities across different treatments being smaller than those within treatments, an occurrence most likely to indicate incorrect labelling of animals. Under the null hypothesis H0: ' no differences between treatments', there will be little effect on average to the value R by arbitrarily assigning animals to different treatments, as animals are merely replicates of a single treatment group if H0 is true. This is essentially the rationale for permutation test of H0, using the randomisation principle (Hope 1968). Essentially the labels to each animal are randomly shuffled and the R statistic re-calculated for each random reshuffle. There are

$$(kn)! \quad [(n!)^k \; k!] \text{ (equation 6)}$$

ways of permutating the labels for n animals in each of k treatments. The full set of permutations is often extremely large, even with few animals in each treatment group so the full set of random permutations is randomly sampled tp give the null distribution of R, giving the range of likely values of R if Ho is correct. These can be compared with the true value of R derived from the correct labelling of animals within treatment groups.

Again for a full detailed explanation of the rationale and iterative procedure see Clarke (1993). This test (ANOSIM) was carried out using a specially written FORTRAN program.

Behavioural data from all experiments were analysed using this novel non-parametric analysis to test for differences between treatments and to determine the relative contribution of various behavioural categories to between group differences.

5.2 Materials and Methods

Subjects. The subjects were male Wistar rats whose experimental data has already contributed to the findings reported in Chapter 3 and Chapter 4. Video recordings were made of each test session and stored until required for behavioural analysis.

Apparatus. The open field and computer imaging system have been described in detail in Chapter 2. A video-player was calibrated to determine 5 minute session intervals. Video tapes were viewed on a black and white monitor (Hitachi 900E/K) with the rater in full control of the stop/start mechanism. At each 5 minute interval of the 105 minute test session each rat was observed on the monitor for 10s by the rater (author). Timing was achieved using a hand held stopwatch.

Procedure. Video tapes were randomised and all treatments were analysed blind, with the exception of field shape. The occurrence of any behaviour in Table 5.1 was recorded; any combination of these categories could be exhibited by a rat in the 10s observation period with the exception of continuous locomotion (**LOCO**), which could not occur in conjunction with locomotion with pause (**LOCO+P**) or with **STILL.** A random subset of video tapes were resampled and the percentage agreement determined between the first and second sample for each behavioural category.

Table 5.1a Definition of behavioural categories based on 10s observation periods.

Category	Definition
STILL (still)	Asleep or not moving with the occasional sniff.
LOCO (continuous locomotion)	All four legs moving without a pause for the entire 10s period.
LOCO+P (locomotion with pause)	All four legs moving but the animal may pause for longer than 3s.
REARO (rearing in the open)	Both front feet off the floor.
REARW (rearing against a wall)	Both front feet raised against the wall, only one of which may be touching the wall surface.
SNIFF (sniffing)	Sniffing for longer than 3s.
HEAD-D (head down)	Snout in contact with floor for longer than 3 s.
HEAD-S (head swaying)	Head swaying fom side to side for longer than 3s.
GROOM (grooming)	Grooming for more than 3s.
MISC (miscellaneous)	Any category of behaviour not already defined that occurs for more than 3s. Noted in detail.

Table 5.1b Mean percentage agreement between the first and second sample for each behavioural category.

BEHAVIOUR	PERCENTAGE AGREEMENT
STILL	98.4
LOCO	96.5
LOCO+P	97.2
REAR-O	86.5
REAR-W	87.8
SNIFF	94.6
HEAD-D	88.4
HEAD-S	83.2

Drugs. The drugs and doses have been described in detail in Chapter 3 and Chapter 4. A summary of experiments and treatment groups is provided in Table 5.2

Data analysis. Each experiment (see Table 5.2) was analysed separately. The data were arranged into an array whose columns represented animals and whose rows represented individual behaviours. The data were then analysed using PRIMER (Plymouth Routines in Multivariate Ecological Research; Carr et al, 1993), a program written specifically for IBM-compatible PCs running under DOS.

Table 5.2 Experiments and treatment groups.

Experiment	Variable	Treatment/Dose (mg/kg)					
Exp. 1	Field shape	**SF3**	**SF4**	**SF5**	**CF3**	**CF4**	**CF5**
	d-amphetamine	3.0	4.0	5.0	3.0	4.0	5.0
Exp. 2		**V-AMPH**		**5CLOZ**	**10CLOZ**	**20CLOZ**	
	d-amphetamine	3.5		3.5	3.5	3.5	
	clozapine	0		5.0	10.0	20.0	
Exp. 3		**V-AMPH**		**10SULP**	**20SUIP**	**50SULP**	
	d-amphetamine	3.5		3.5	3.5	3.5	
	sulpiride	0		10.0	20.0	50.0	
Exp.4		**V-AMPH**	**.01HAL**	**.025HAL**	**.05HAL**	**.075HAL**	
	d-amphetamine	3.5	3.5	3.5	3.5	3.5	
	haloperidol	0	.01	.025	.05	.075	

5.3 Results.

Experiment 1. (field shape)

Figures 5.1 and 5.2 show the percentage of animals exhibiting each of the behavioural categories for groups treated with 0, 3, 4, or 5 mg/kg amphetamine tested in the square and circular field respectively.

The results of the two-way ANOSIM indicated that there was no significant difference between field shape groups [(Global R) = 0.054, p> 0.1]. There was an overall effect of dose averaged across field shape groups, [(Global R) = 0.585, p< .0001]. A series of pairwise tests between the doses of amphetamine indicate those behaviours which contributed most to the significant differences between the groups.

Pairwise tests.

3mg/kg amph. v 4mg/kg amph.

Table 5.3 shows behaviours in order of their contribution to ∂i to the average dissimilarity ∂(=48.44) between groups treated with 3mg/kg and 4mg/kg amphetamine with a cut-off ($\Sigma\partial(i)\%$) = 20%; The behaviours which contributed most to the difference between these two doses were locomotion (**LOCO+P**) and snout contact (**HEAD-D**). Animals treated with the higher dose of 4 mg/kg showed an increase in locomotion at the beginning of the test session between intervals 10-15 minutes and an increase in head-down posture between session intervals 70-105 minutes. Interestingly, 50 minutes into the session animals treated with the lower dose of 3mg/kg amphetamine were moving much faster, with 63% of animals moving without a pause (**LOCO**), compared with only 38% of animals treated with 4 mg/kg amphetamine. Therefore increasing the dose of amphetamine to 4 mg/kg resulted in increased locomotion early in the session followed by a decline in locomotion in session intervals accompanied by an increase in head down posture.

Table 5.3. Percentage (ABUND) of behaviours in group A (4mg/kg) and group B (3mg/kg). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(= 48.44) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 20%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
70	rearo	0.00	0.75	.70	.41	1.70	1.45
80	head-d	0.75	0.00	.69	.41	1.69	2.87
10	still	0.13	0.75	.64	.44	1.46	4.20
95	head-d	0.75	0.13	.63	.43	1.45	5.50
10	loco+p	0.75	0.25	.58	.46	1.27	6.70
5	sniff	0.63	0.13	.56	.47	1.19	7.85
85	head-d	0.63	0.13	.54	.46	1.19	8.97
90	head-d	0.63	0.13	.54	.45	1.19	10.09
100	head-d	0.63	0.13	.54	.45	1.19	11.20
105	head-d	0.63	0.13	.54	.45	1.19	12.31
55	loco+p	0.75	0.38	.52	.47	1.12	13.39
35	loco+p	0.38	0.75	.52	.47	1.12	14.47
70	head-d	0.62	0.25	.52	.47	1.12	15.54
75	head-d	0.63	0.25	.51	.46	1.11	16.60
25	loco+p	0.63	0.38	.49	.47	1.05	17.62
50	loco+p	0.63	0.38	.49	.47	1.05	18.64
50	loco	0.38	0.63	.49	.47	1.05	19.66
15	still	0.00	0.50	.47	.48	0.99	20.64

3mg/kg amph. v 5mg/kg amph.

Table 5.4 shows behaviours in order of their contribution to ∂(i) to the average dissimilarity ∂ (=57.74) between groups treated with 3mg/kg and 5mg/kg amphetamine with a cut-off when the cumulative % contribution (∑∂(i)%) to ∂ reaches 20%. Behaviours which contributed most to the difference between these two doses were locomotion with pause (**LOCO+P**)and snout contact (**Head-D**). Animals treated with 5 mg/kg amphetamine were less active between session intervals 50-75 minutes. In addition, animals treated with 5mg/kg amphetamine showed an increase in head-down posture (**Head-D**) betwen session intervals 45-90 minutes.

Table 5.4. Percentage (ABUND) of behaviours in group A (5mg/kg) and group B (3mg/kg). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(= 57.74) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 20%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
70	still	0.88	0.00	.74	.29	2.57	1.29
90	rearo	0.00	0.86	.73	.31	2.38	2.55
60	head-d	0.88	0.14	.66	.37	1.78	3.68
45	head-d	0.88	0.14	.66	.37	1.78	4.82
65	head-d	0.88	0.14	.66	.37	1.78	5.96
55	still	0.88	0.14	.66	.37	1.78	7.09
60	still	0.88	0.14	.65	.37	1.78	8.22
55	loco+p	0.00	0.71	.63	.40	1.55	9.30
50	head-s	0.75	0.00	.63	.37	1.70	10.39
50	loco+p	0.25	0.86	.59	.42	1.43	11.42
40	loco+p	0.25	0.86	.59	.41	1.43	12.43
55	head-d	0.75	0.14	.58	.41	1.43	13.44
50	head-d	0.75	0.14	.58	.41	1.43	14.44
75	still	0.75	0.14	.58	.40	1.43	15.44
80	head-d	0.75	0.14	.58	.40	1.43	16.44
75	head-d	0.75	0.14	.58	.40	1.43	18.43
75	still	0.75	0.14	.58	.40	1.43	19.43
90	head-d	0.75	0.14	.58	.40	1.43	20.42

4mg/kg amph v 5mg.kg amph.

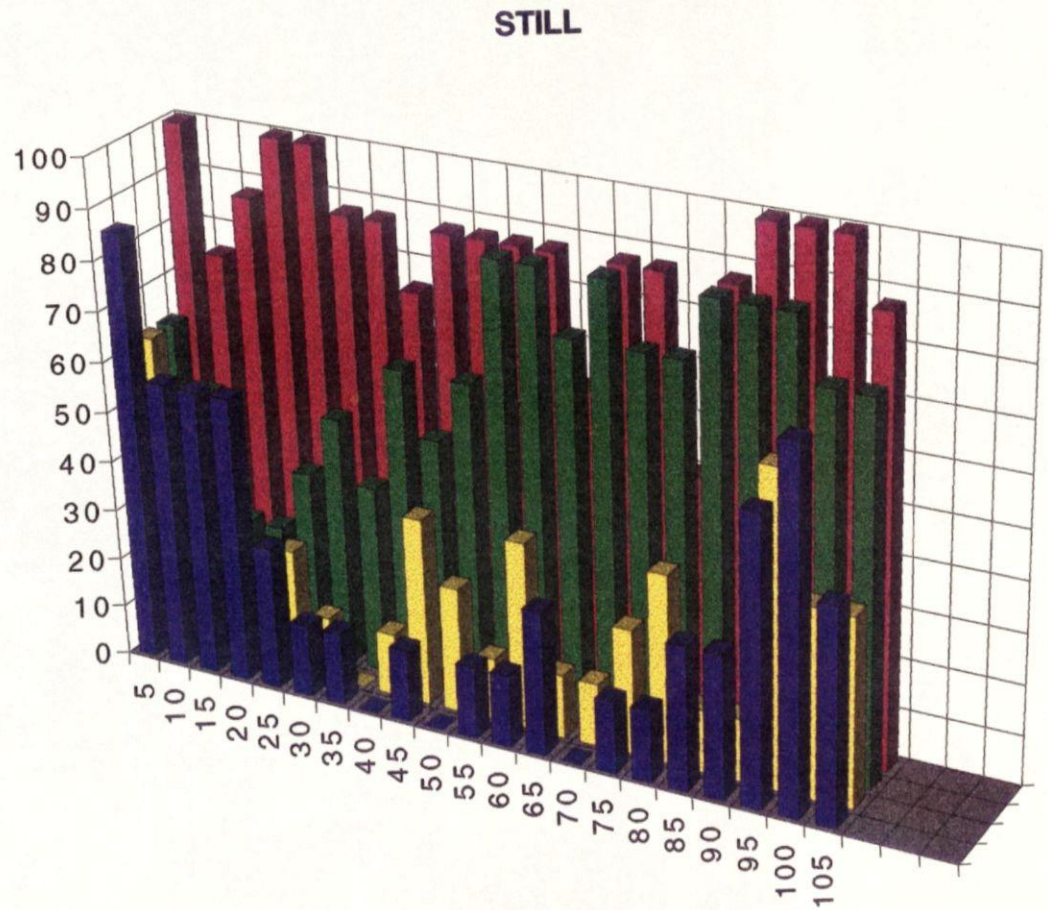
Table 5.5 shows behaviour in order of their contribution to ∂(i) to the average dissimilarity ∂(=43.27) between groups treated with 4mg/kg and 5mg/kg with a cut off (∑∂(I)%) = 30%. Behaviours which contributed most to the difference between the two doses were locomotion with pause (**LOCO +P**) and snout contact (**HEAD-D**). Animals treated with the higher dose of 5 mg/kg amphetamine were less active between session intervals 55-105 minutes, and showed an increase in head-down posture (**HEAD-D**) between session intervals 45-50 minutes. In addition, by session intervals 70 & 75 minutes, 62% of animals treated with 5 mg/kg amphetamine were engaged in head-swaying behaviour (**HEAD-S**) compared with only 13% of animals treated with 4mg/kg amphetamine. Thus increasing the dose of amphetamine to 5 mg/kg increased head down posture and head swaying whilst reducing locomotion.

Table 5.5. Percentage (ABUND) of behaviours in group A (5mg/kg) and group B (4mg/kg). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(=43.27) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 30%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
60	still	1.00	0.00	.87	.07	12.83	2.02
65	still	1.00	0.13	.78	.30	2.57	3.81
55	still	0.88	0.00	.77	.30	2.56	5.58
70	still	1.00	0.25	.67	.39	1.70	7.13
80	still	1.00	0.25	.67	.39	1.70	8.67
60	loco+p	0.00	0.75	.65	.38	1.70	10.18
50	still	0.75	0.00	.65	.38	1.70	11.67
45	head-d	0.88	0.25	.61	.42	1.46	13.09
55	loco+p	0.13	0.75	.60	.41	1.46	14.47
75	loco+p	0.00	0.63	.55	.44	1.27	15.57
75	still	1.00	0.38	.55	.44	1.27	17.03
70	loco+p	0.00	0.63	.55	.43	1.27	18.30
100	still	0.75	0.25	.55	.43	1.27	19.57
95	loco+p	0.25	0.75	.55	.43	1.27	20.83
70	head-s	0.63	0.00	.55	.43	1.27	22.09
65	loco+p	0.00	0.63	.55	.43	1.27	23.35
50	head-d	0.88	0.38	.53	.44	1.19	24.57
10	still	0.63	0.13	.53	.44	1.19	25.79
105	loco+p	0.13	0.63	.52	.44	1.19	27.00
105	still	0.88	0.38	.52	.44	1.19	28.20
75	head-s	0.62	0.13	.51	.43	1.19	29.38
10	loco+p	0.38	0.75	.50	.44	1.12	30.53

Figure 5.1.

The percentage of animals exhibiting each of the behavioural categories during the 105-min observation period by rats (n = 8) per group) injected with vehicle-saline (**red**), vehicle + 3mg/kg amphetamine (**blue**), vehicle + 4mg/kg amphetamine (**yellow**), or vehicle + 5mg/kg amphetamine (**green**) and tested in a **square** open field.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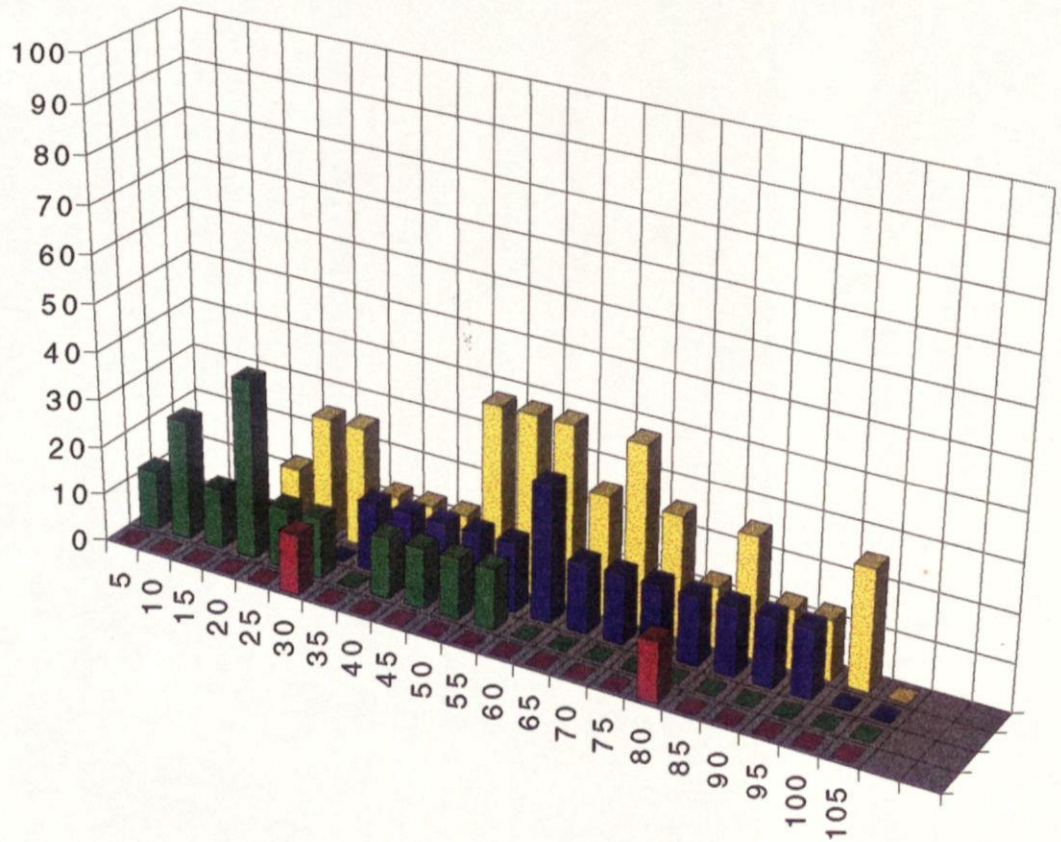
LOCOMOTION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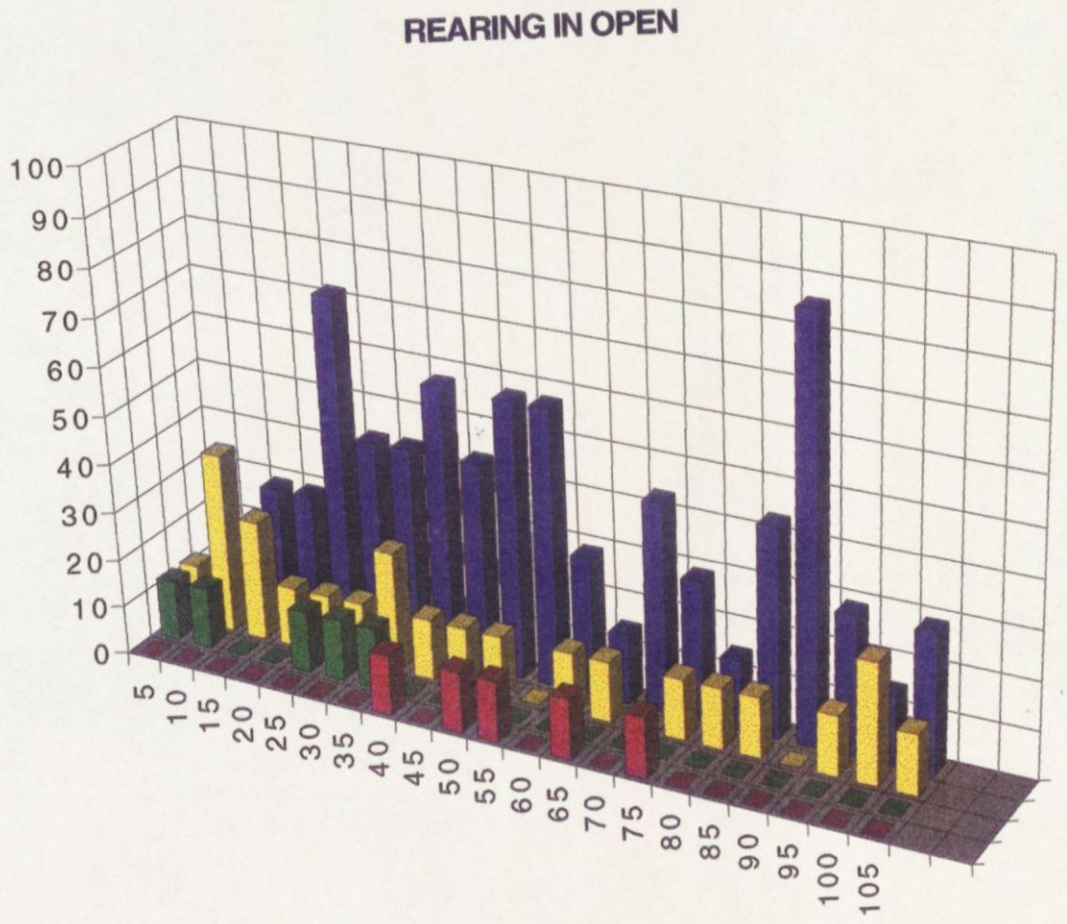
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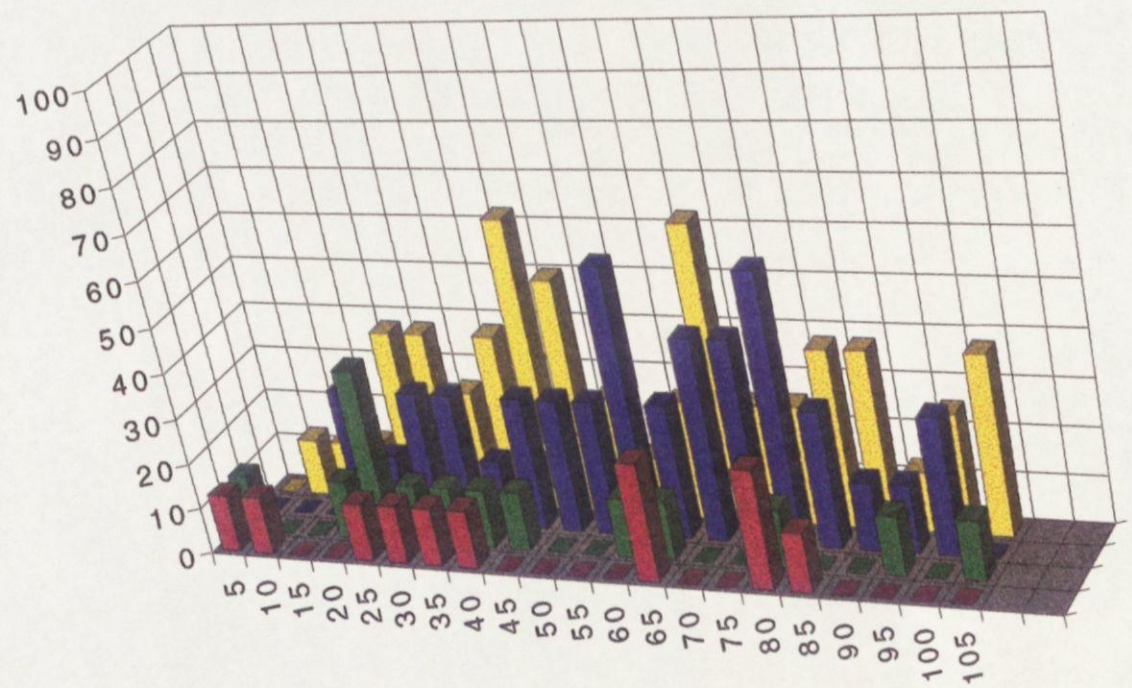
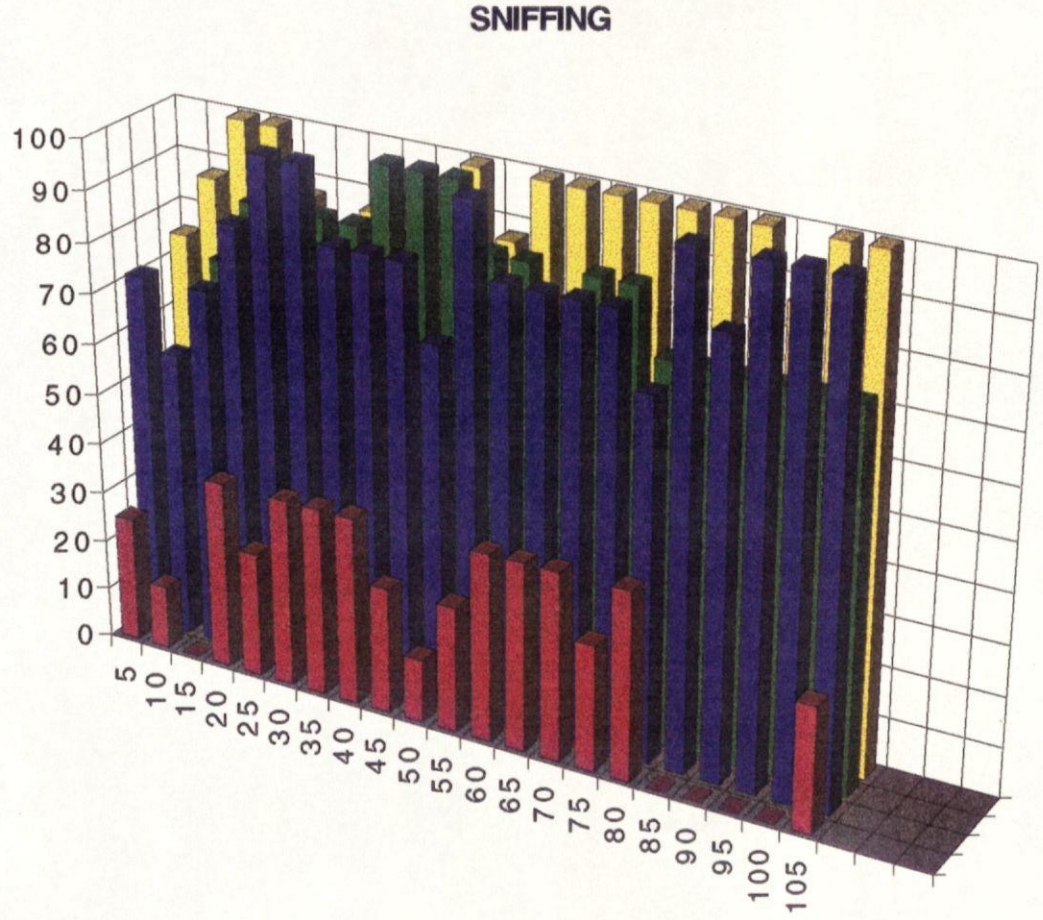
REARING AGAINST WALL

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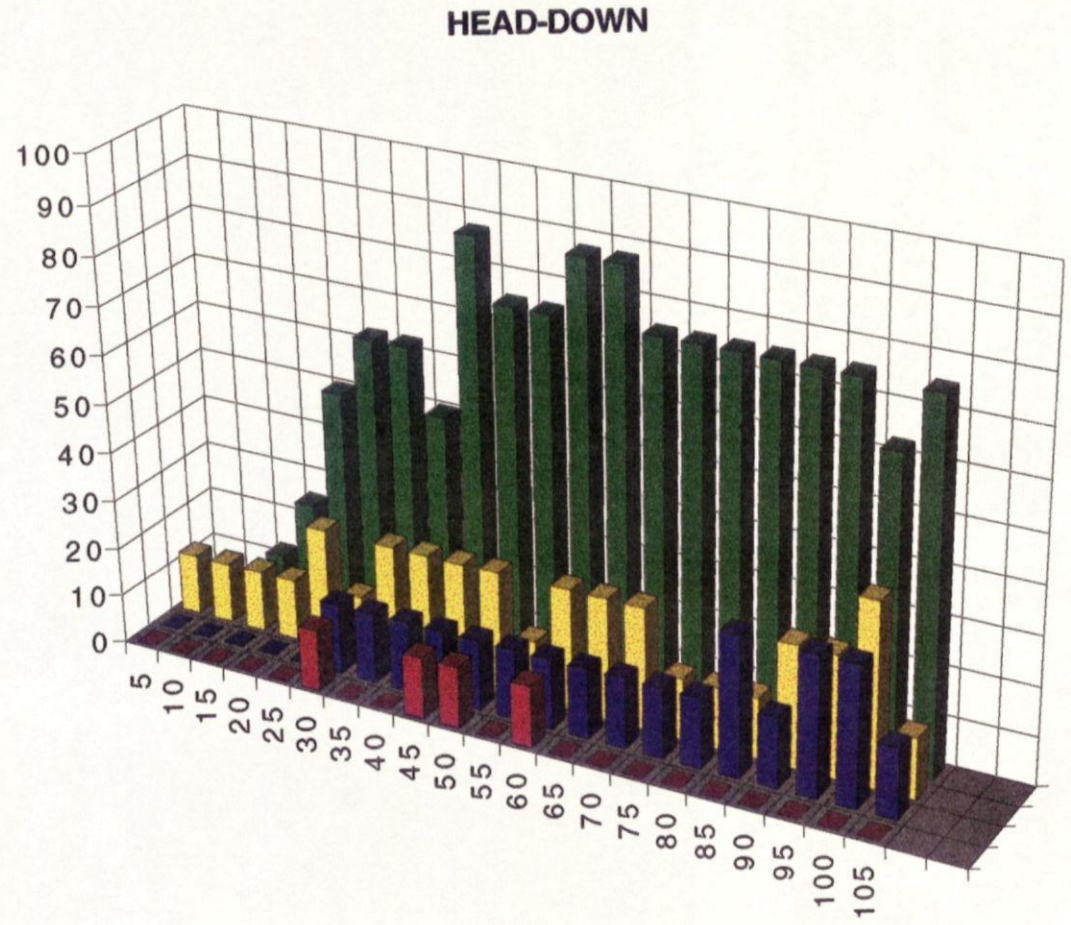
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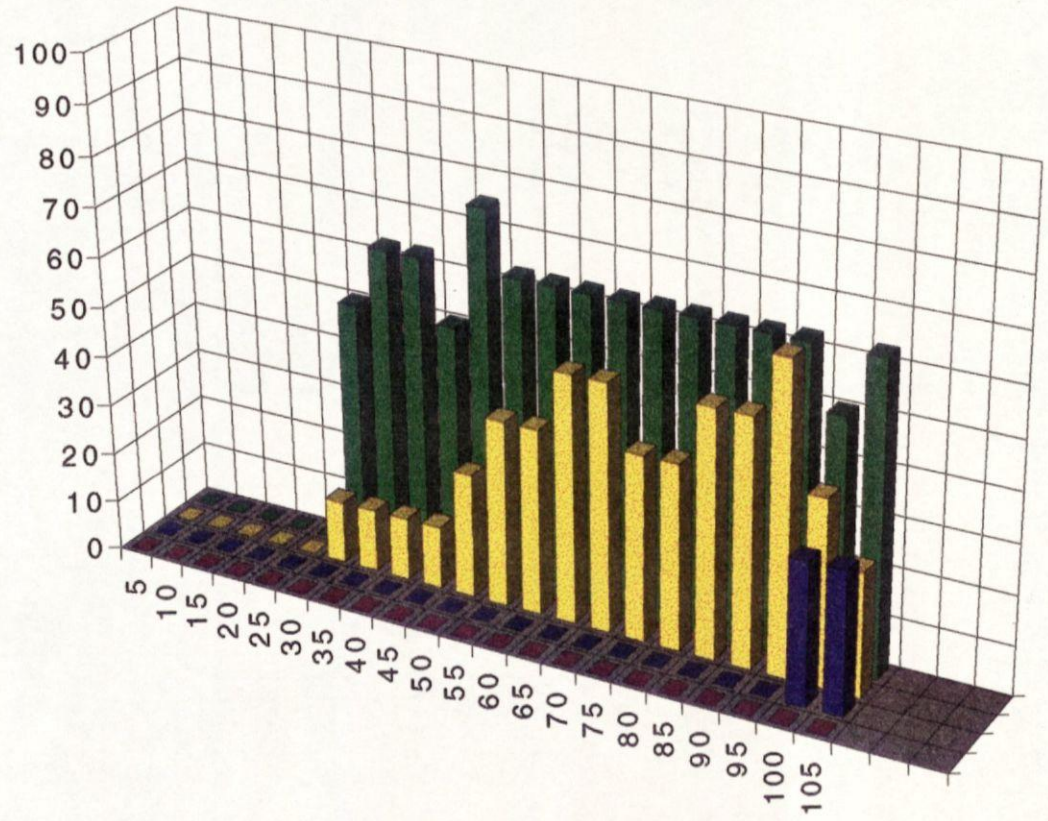
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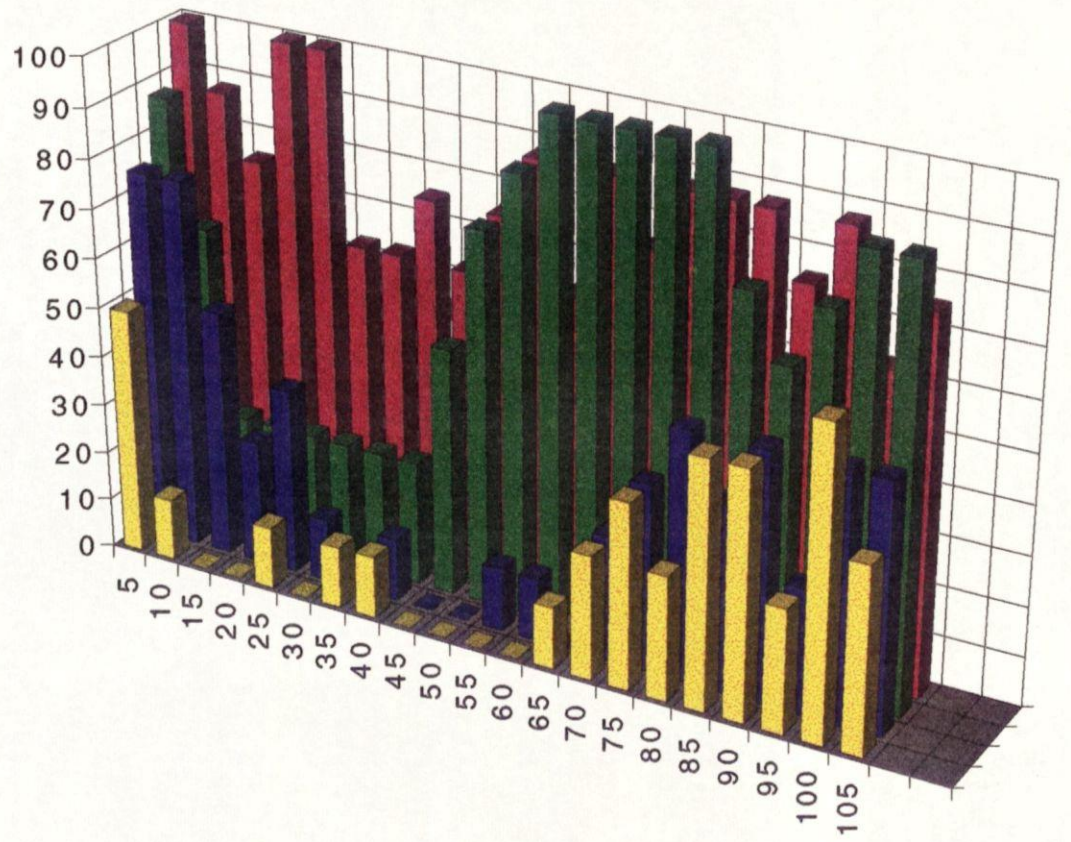
Figure 5.2.

The percentage of animals exhibiting each of the behavioural categories during the 105-min observation period by rats (n =8) per group) injected with vehicle-saline (**red**), vehicle + 3mg/kg amphetamine (**blue**), vehicle + 4mg/kg amphetamine (**yellow**), or vehicle + 5mg/kg amphetamine (**green**) and tested in a **circular** open field.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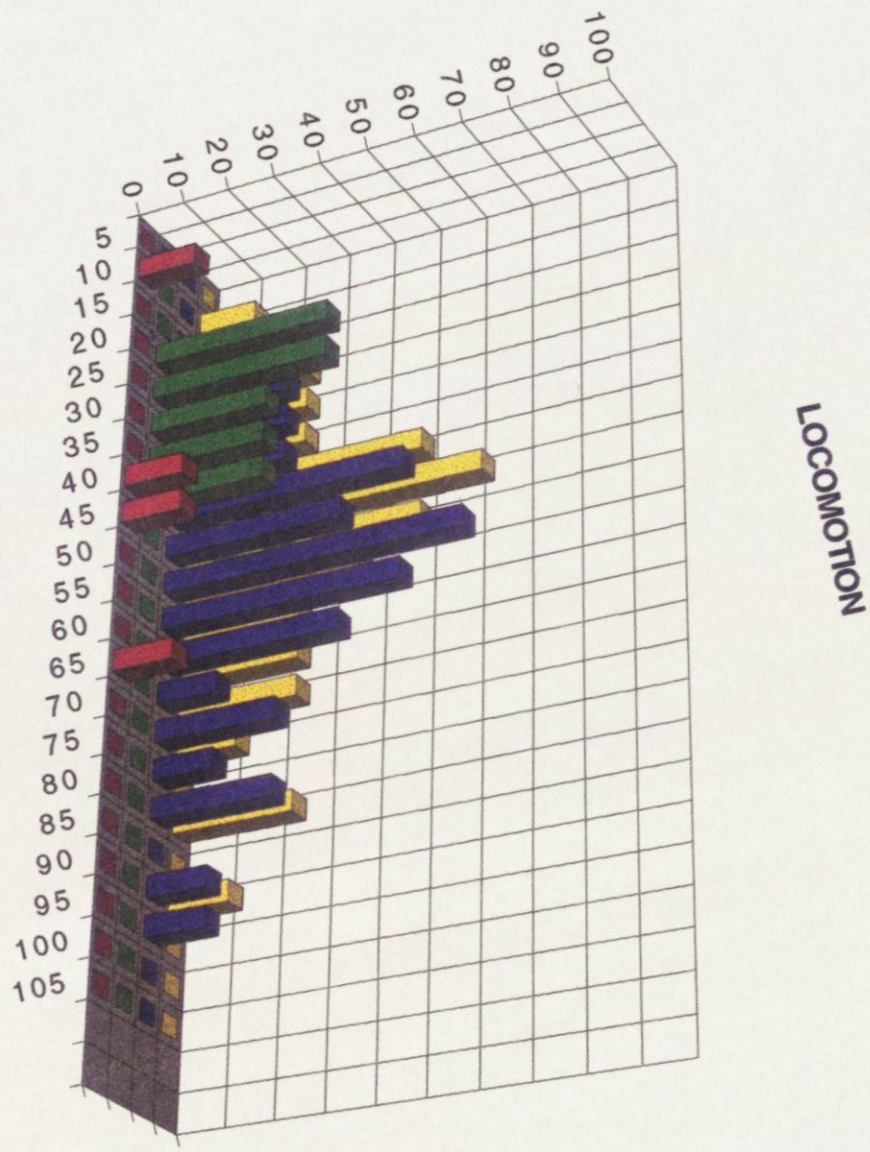
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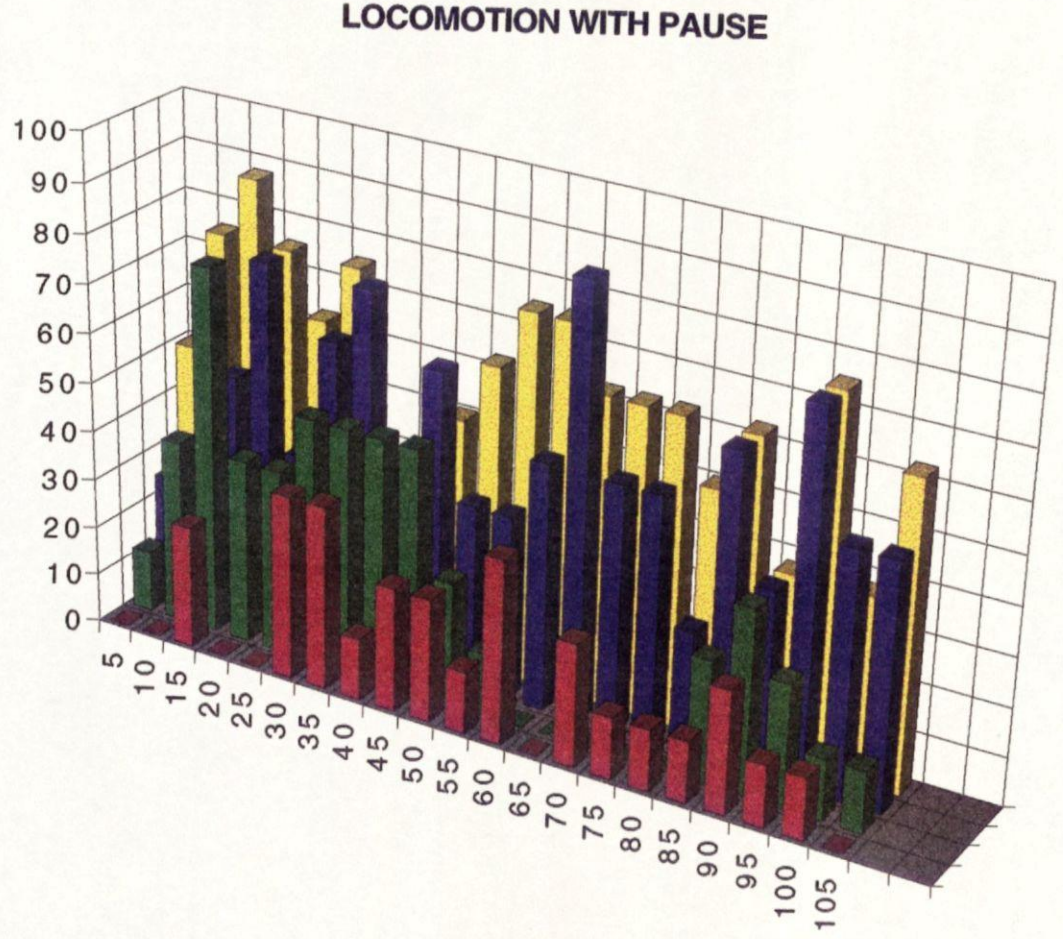
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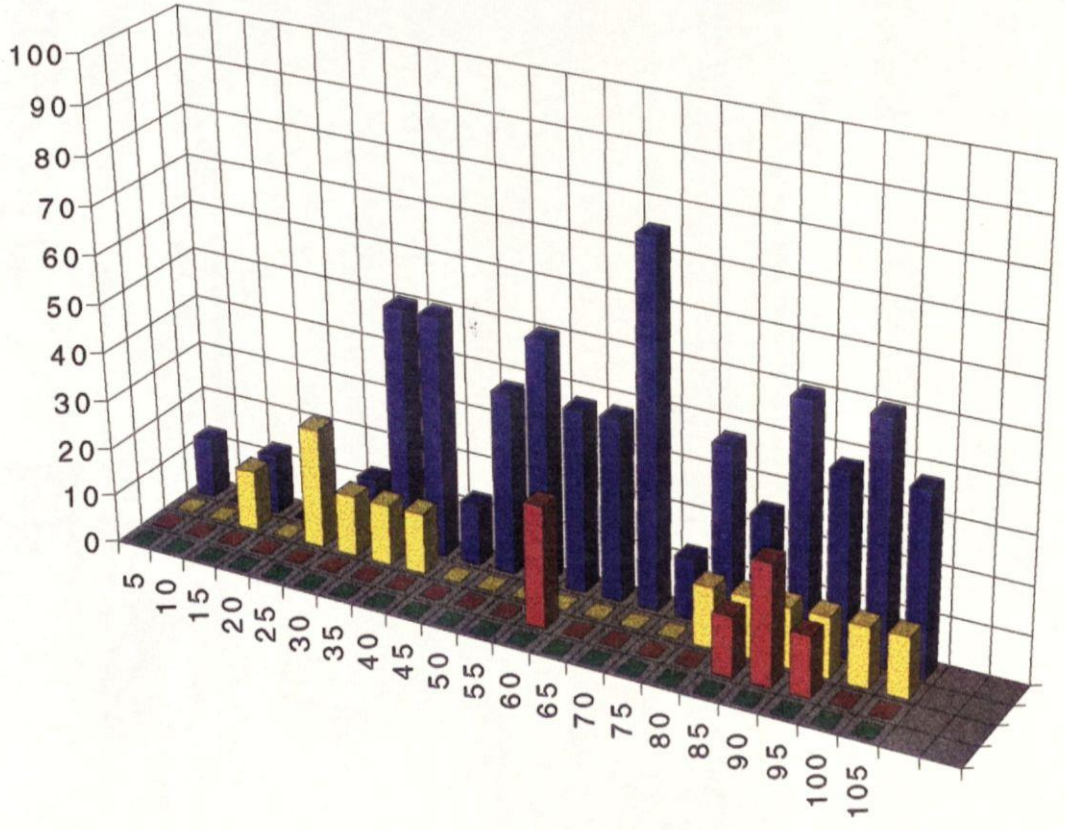
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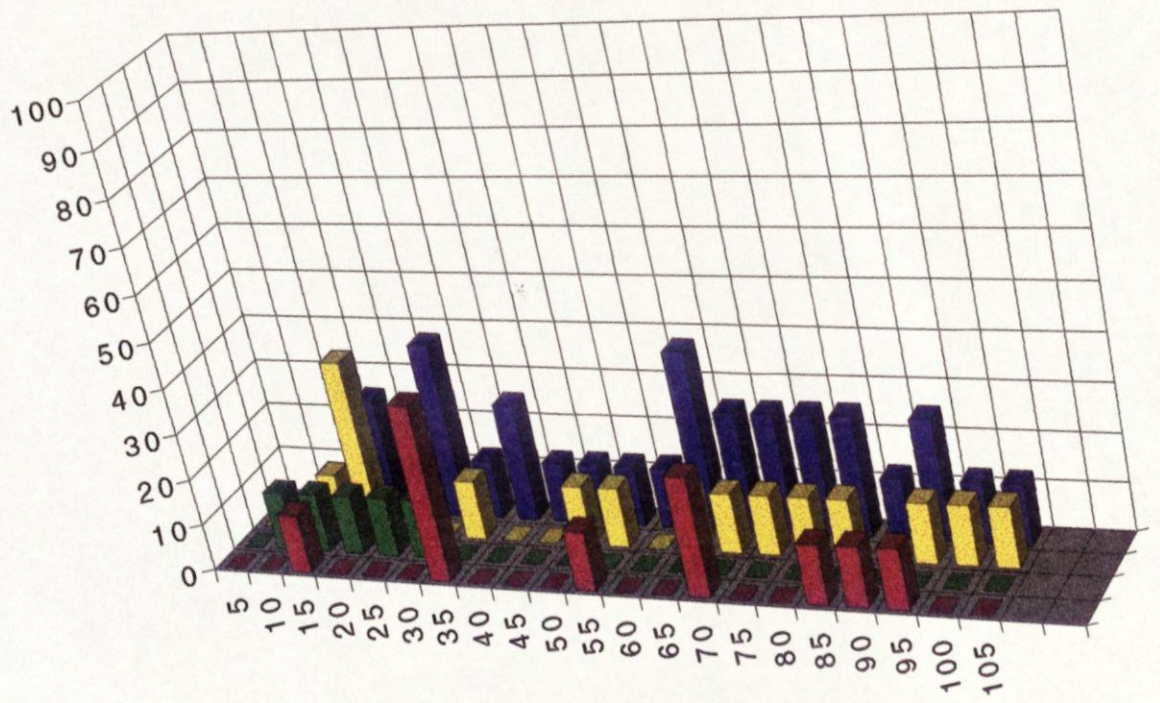
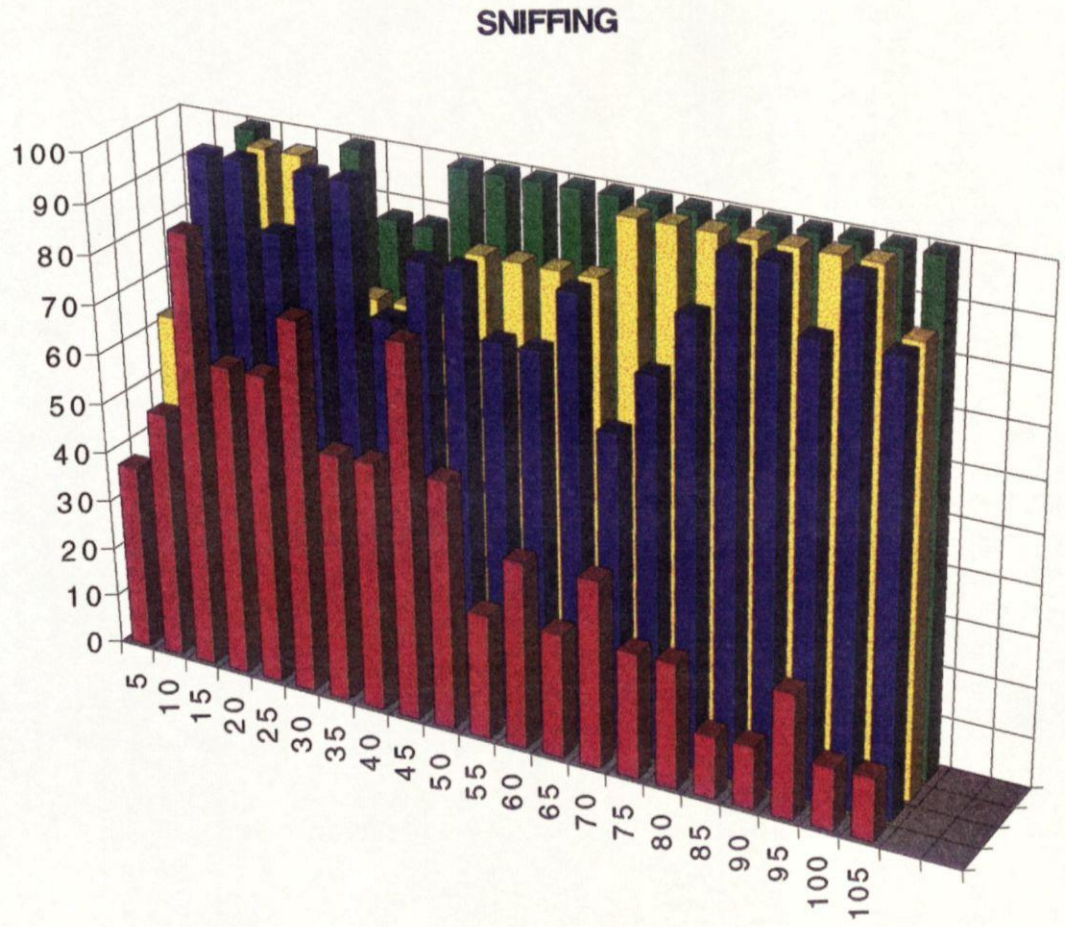
REARING AGAINST WALL

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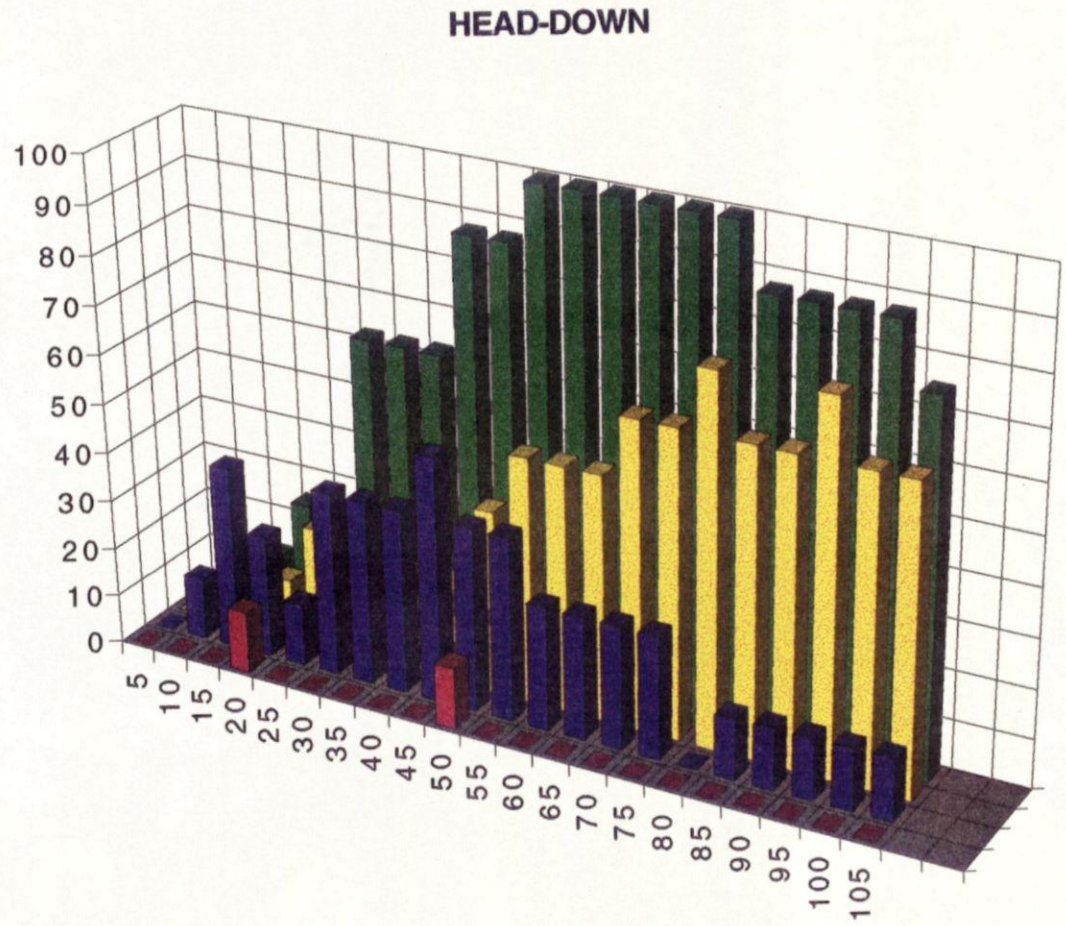
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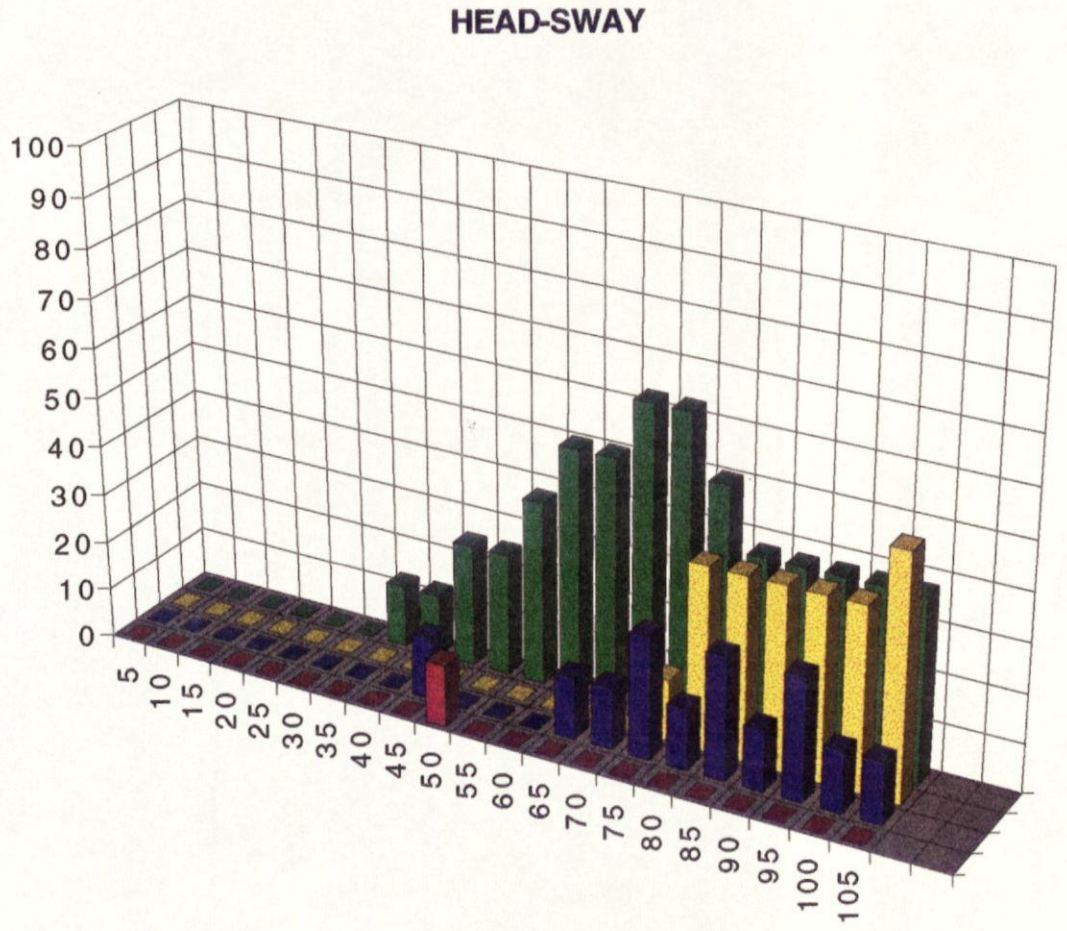
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Experiment 2. *(3.5 mg/kg amphetamine pre-treated with clozapine).*

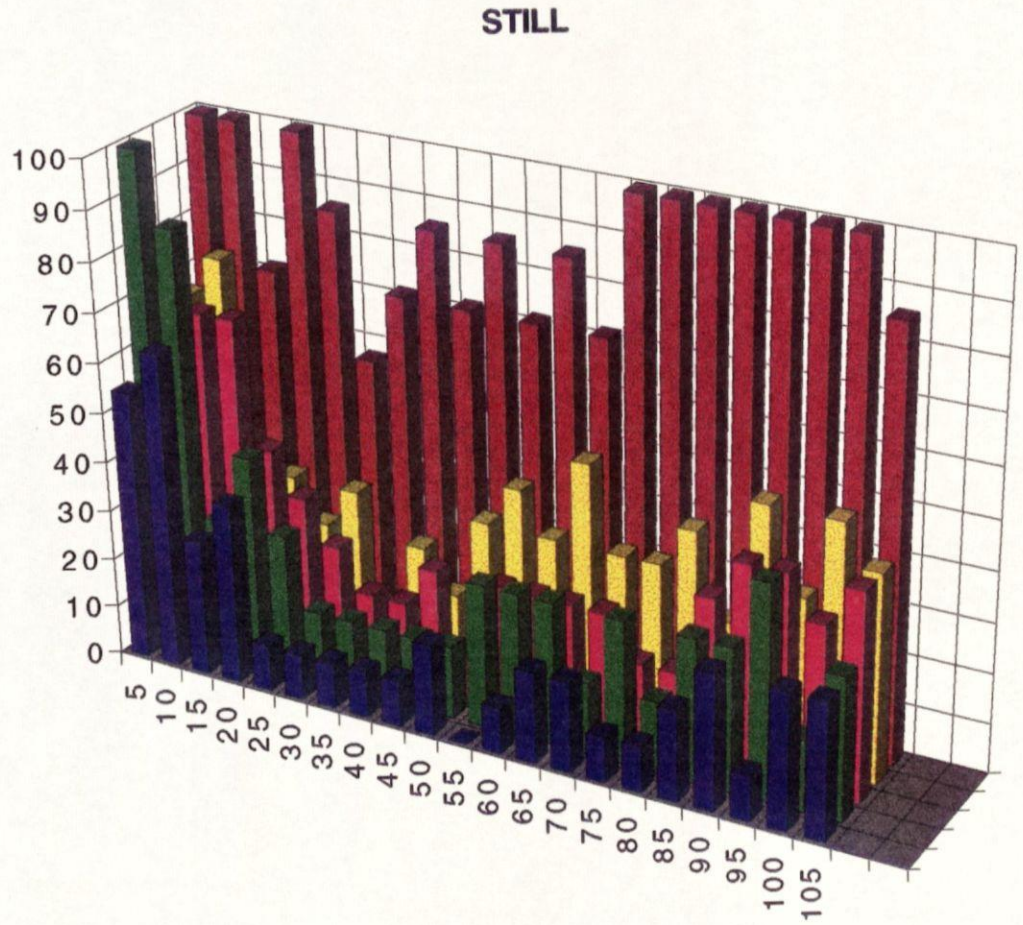
Figure 5.3 shows the percentage of animals exhibiting each of the behavioural categories for animals treated with 3.5 mg/kg amphetamine, pre-treated with 0, 5, 10 or 20mg/kg clozapine. The results of the one-way ANOSIM indicated that there was no significant difference in the behaviours exhibited by the treatment groups. The sample statistic (Global R) = 0.023, (not significant NS). Table 5.6 shows the significance levels for the pairwise tests between animals treated with vehicle-amphetamine and each of the clozapine pre-treatment doses following the one-way ANOSIM and the value of ∂ derived from the similarities terms analysis.

Table 5.6. Significance levels of pairwise tests following one-way ANOSIM, and the average dissimilarity (∂) between vehicle-amphetamine and clozapine pre-treated groups.

Veh-Amph. versus	5mg/kg clozapine	10mg/kg clozapine	20mg/kg clozapine
∂	37.86 (NS)	43.71 (NS)	41.61 (NS)

Figure 5.3.

The percentage of animals exhibiting each of the behavioural categories during the 105-min observation period by rats (n =8) per group) injected with vehicle-saline (**red**), vehicle + 3.5mg/kg amphetamine (**green**), 5mg/kg clozapine + 3.5mg/kg amphetamine (**blue**), 10mg/kg clozapine + 3.5mg/kg amphetamine (**yellow**) or 20mg/kg clozapine + 3.5mg/kg amphetamine (**magenta**) and tested in a circular open field.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Experiment 3. *(3.5 mg/kg amphetamine pre-treated with sulpiride).*

Figure 5.4 shows the percentage of animals exhibiting each of the behavioural categories for animals treated with 0, 10, 20 or 50 mg/kg sulpiride before receiving 3.5mg/kg amphetamine.

The results of the one-way ANOSIM indicated that there was a significant difference in the behaviours exhibited by the treatment groups. The sample statistic (Global R) = 0.366, $p < .0001$. Examination of the differences between treatment groups revealed that pre-treatment with 10, 20, or 50 mg/kg sulpiride resulted in a significant difference in behavioural categories compared to vehicle-amphetamine treated animals see table 5.7.

Pairwise tests

Veh-Amph v 10SULP

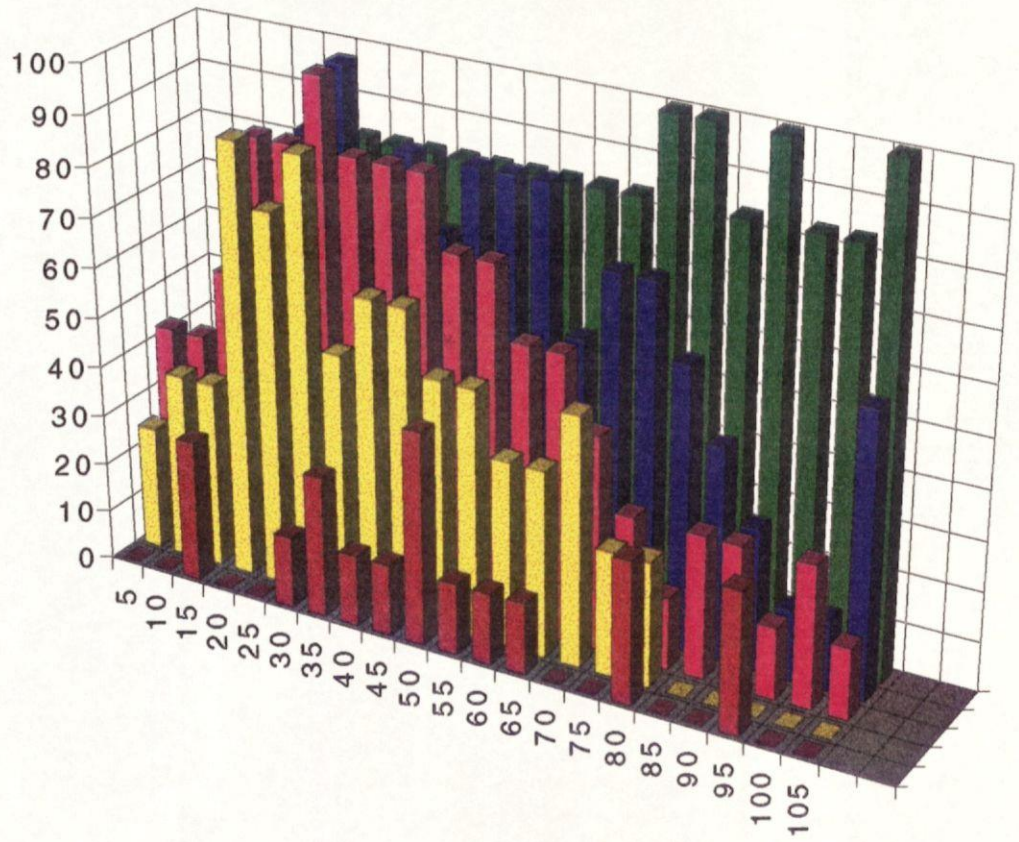
Table 5.8 shows the behaviours in order of their contribution to the average dissimilarity $\partial(=39.89)$ between vehicle-amphetamine and animals pre-treated with 10mg/kg sulpiride with a cut-off $(\sum\partial(i)\%) = 30\%$.

Behaviours which contributed most to the difference between the two groups were locomotion with pause (**LOCO+P**), and head-swaying (**HEAD-S**). Animals pre-treated with 10mg/kg sulpiride were less active 15-20 min following administration of amphetamine. There was an increase in the percentage of animals not moving (**STILL**) during session intervals 85-100 min accompanied by an increase in head-swaying (**HEAD-S**) between 65-105 min, there was also a decrease in the percentage of animals engaged in rearing against the wall (**REARW**) at session intervals 70 and 80 min.

Figure 5.4.

The percentage of animals exhibiting each of the behavioural categories during the 105-min observation period by rats (n = 8) per group) injected with vehicle-saline (**red**), vehicle + 3.5mg/kg amphetamine (**green**), 10mg/kg sulpiride + 3.5mg/kg amphetamine (**blue**), 20mg/kg sulpiride + 3.5mg/kg amphetamine (**yellow**) or 50mg/kg sulpiride + 3.5mg/kg amphetamine (**magenta**) and tested in a circular open field.

LOCOMOTION WITH PAUSE



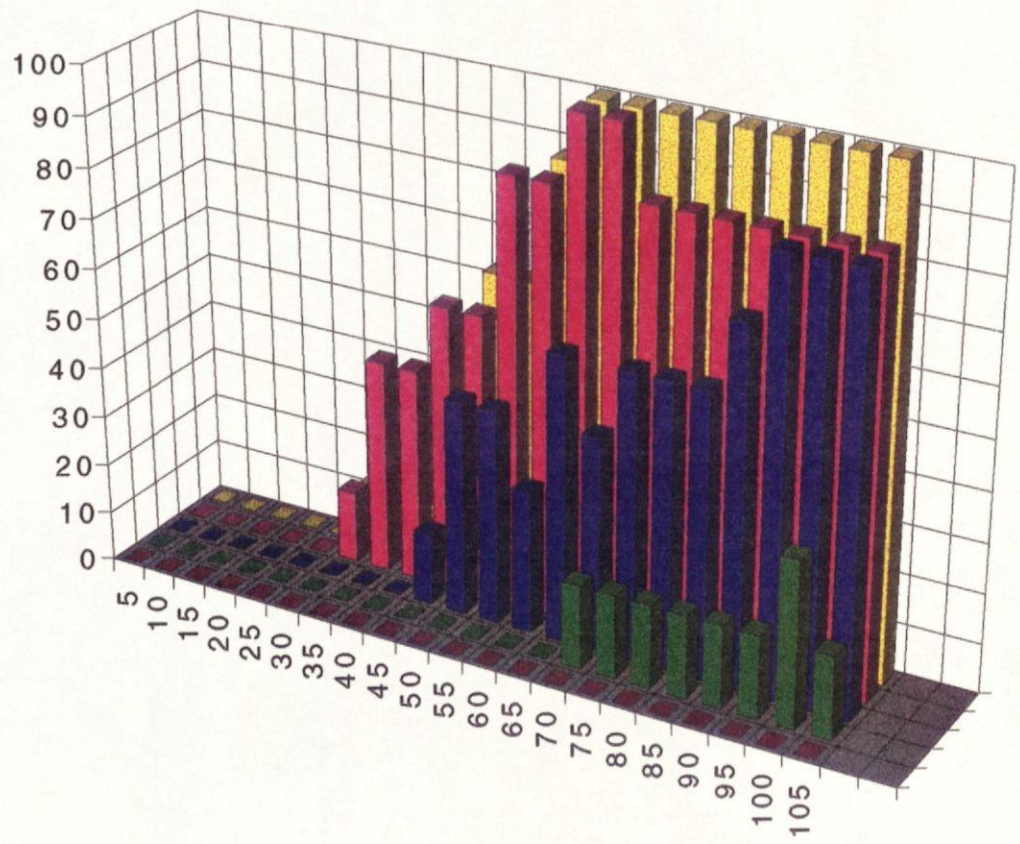

HEAD-SWAY
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Table 5.7. Significance levels of pairwise tests following one-way ANOSIM, and the average dissimilarity (∂) between vehicle-amphetamine and sulpiride pre-treated groups.

Veh-Amph versus	10mg/kg Sulpiride	20mg/kg Sulpiride	50mg/kg Sulpiride
∂	39.89	48.64	42.82
	(p< 0.016)	(p<0.0001)	(p<0.0001)

Table 5.8. Percentage (ABUND) of behaviours in group A (10SULP) and group B (veh-amph). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(=39.89) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 32%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
100	loco+p	0.14	0.83	.70	.44	1.60	1.75
95	loco+p	0.14	0.83	.70	.44	1.60	3.51
100	still	0.86	0.17	.70	.44	1.60	5.26
95	still	0.86	0.17	.70	.44	1.60	7.02
90	loco+p	0.29	1.00	.68	.45	1.51	8.72
90	still	0.71	0.00	.68	.45	1.51	10.43
105	head-s	0.86	0.17	.68	.42	1.62	12.31
95	head-s	0.86	0.17	.68	.42	1.62	13.82
80	rear-w	0.00	0.67	.61	.44	1.37	15.34
90	head-s	0.71	0.17	.59	.44	1.30	16.82
100	head-s	0.86	0.33	.58	.47	1.24	18.27
70	rear-w	0.14	0.67	.57	.46	1.24	19.70
20	loco+p	0.71	0.33	.54	.48	1.12	21.05
20	still	0.29	0.67	.54	.48	1.12	22.40
15	loco+p	0.57	0.17	.53	.49	1.07	23.72
15	still	0.43	0.83	.53	.49	1.07	25.04
85	still	0.57	0.17	.52	.49	1.06	26.34
85	loco+p	0.43	0.83	.52	.49	1.06	27.65
65	head-s	0.57	0.00	.51	.45	1.13	28.92
80	head-s	0.57	0.17	.49	.46	1.07	30.16
75	head-s	0.57	0.17	.49	.46	1.07	31.39
85	head-s	0.57	0.17	.49	.46	1.07	32.63

Veh-Amph v 20SULP. Table 5.9 shows the behaviours in order of their contribution to the average dissimilarity ∂(=48.64) between vehicle-amphetamine and animals pre-treated with 20mg/kg sulpiride with a cut off ($\sum\partial(i)\%$) = 35%. Behaviours which contributed most to the differences between these two groups were locomotion with pause (**LOCO+P**), head-down (**HEAD-D**) and head-swaying (**HEAD-S**) in the latter half of the test session. An increased percentage of animals showed head-down posture (**HEAD-D**) and head-swaying (**HEAD-S**) following pre-treatment with 20mg/kg sulpiride during session intervals 60-105 min. In addition, pre-treatment with 20mg/kg sulpiride increased the percentage of animals who were immobile (**STILL**) between session intervals 85-105 min.

Table 5.9. Percentage (ABUND) of behaviours in group A (20SULP) and group B (veh-amph). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(=48.64) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 35%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
65	head-s	1.00	0.00	.84	.07	12.9	1.73
105	loco+p	0.00	1.00	.84	.07	12.9	3.47
90	loco+p	0.00	1.00	.84	.07	12.9	5.20
105	still	1.00	0.00	.84	.07	12.9	6.93
90	still	1.00	0.00	.84	.07	12.9	8.67
60	head-s	0.88	0.00	.73	.29	2.56	10.17
70	head-d	0.88	0.00	.73	.29	2.56	11.67
75	head-d	0.88	0.00	.73	.29	2.56	13.18
100	head-d	0.88	0.00	.73	.29	2.56	14.68
105	head-d	0.88	0.00	.73	.29	2.56	16.18
70	head-s	1.00	0.17	.71	.33	2.17	17.64
85	loco+p	0.00	0.83	.71	.33	2.17	19.10
100	loco+p	0.00	0.83	.71	.33	2.17	20.55
100	still	1.00	0.17	.71	.33	2.17	22.01
95	still	1.00	0.17	.71	.33	2.17	23.47
95	loco+p	0.00	0.83	.71	.33	2.17	24.92
85	still	1.00	0.17	.71	.33	2.17	26.38
75	head-s	1.00	0.17	.71	.32	2.17	27.83
80	head-s	1.00	0.17	.71	.32	2.17	29.28
85	head-s	1.00	0.17	.71	.32	2.17	30.73
90	head-s	1.00	0.17	.71	.32	2.17	32.18
95	head-s	1.00	0.17	.71	.32	2.17	33.63
105	head-s	1.00	0.17	.71	.32	2.17	35.08

Veh-Amph v 50SULP. Table 5.10 shows the behaviours in order of their contribution to the average dissimilarity ∂(=42.82) between vehicle-amphetamine and animals pre-treated with 50mg/kg sulpiride with a cut-off ($\Sigma\partial$(i)%) = 36%. Behaviours which contributed to the difference between these two groups were locomotion with pause (**LOCO+P**), (**STILL**) and head-swaying (**HEAD-S**) during the latter half of the test session. The percentage of animals engaged in head-swaying (**HEAD-S**) behaviour increased following pre-treatment with 50mg/kg sulpiride, during session intervals 55-105 min, particularly at interval 65 min, where 100% of sulpiride pre-treated animals were engaged in head swaying behaviour compared with none of the vehicle-amphetamine treated animals. Pre-treatment with 50mg/kg sulpiride also increased the percentage of animals who were not moving (**STILL**) between session intervals 80-105 min.

Table 5.10. Percentage (ABUND) of behaviours in group A (50SULP) and group B (veh-amph). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(=42.82) between the two groups, with a cut-off when the cumulative % contribution ($\sum\partial i\%$) to ∂ reaches 35%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	$\partial(i)$	$SD(\partial i)$	$\partial(i)/SD(\partial i)$	$\sum\partial i\%$
65	head-s	1.00	0.00	.91	.09	9.27	2.12
105	loco+p	0.14	1.00	.79	.34	2.34	3.97
80	loco+p	0.14	1.00	.79	.34	2.34	5.81
70	head-s	1.00	0.17	.77	.36	2.14	7.60
55	head-s	0.86	0.00	.76	.33	2.35	9.39
60	head-s	0.86	0.00	.76	.33	2.35	11.17
105	still	0.86	0.00	.76	.33	2.35	12.96
80	still	0.86	0.00	.76	.33	2.35	14.74
95	loco+p	0.14	0.83	.68	.42	1.63	16.34
75	head-s	0.86	0.17	.67	.41	1.62	17.90
80	head-s	0.86	0.17	.67	.41	1.62	19.46
85	head-s	0.86	0.17	.67	.41	1.62	21.02
90	head-s	0.86	0.17	.67	.41	1.62	22.58
95	head-s	0.86	0.17	.67	.41	1.62	24.14
105	head-s	0.86	0.17	.67	.41	1.62	25.70
100	still	0.86	0.17	.67	.41	1.63	27.26
95	still	0.86	0.17	.67	.41	1.63	28.81
75	loco+p	0.29	1.00	.66	.43	1.53	30.35
90	loco+p	0.29	1.00	.66	.43	1.53	31.88
90	still	0.71	0.00	.63	.41	1.54	33.36
85	loco+p	0.29	0.83	.59	.45	1.30	34.74
70	rearw	0.00	0.67	.59	.42	1.38	36.12

Experiment 4. *(3.5mg/kg amphetamine pre-treated with haloperidol).*

Figure 5.5 shows the percentage of animals exhibiting each of the behavioural categories for amphetamine treated animals pre-treated with 0.01, 0.025, 0.05 or 0.075 mg/kg haloperidol.

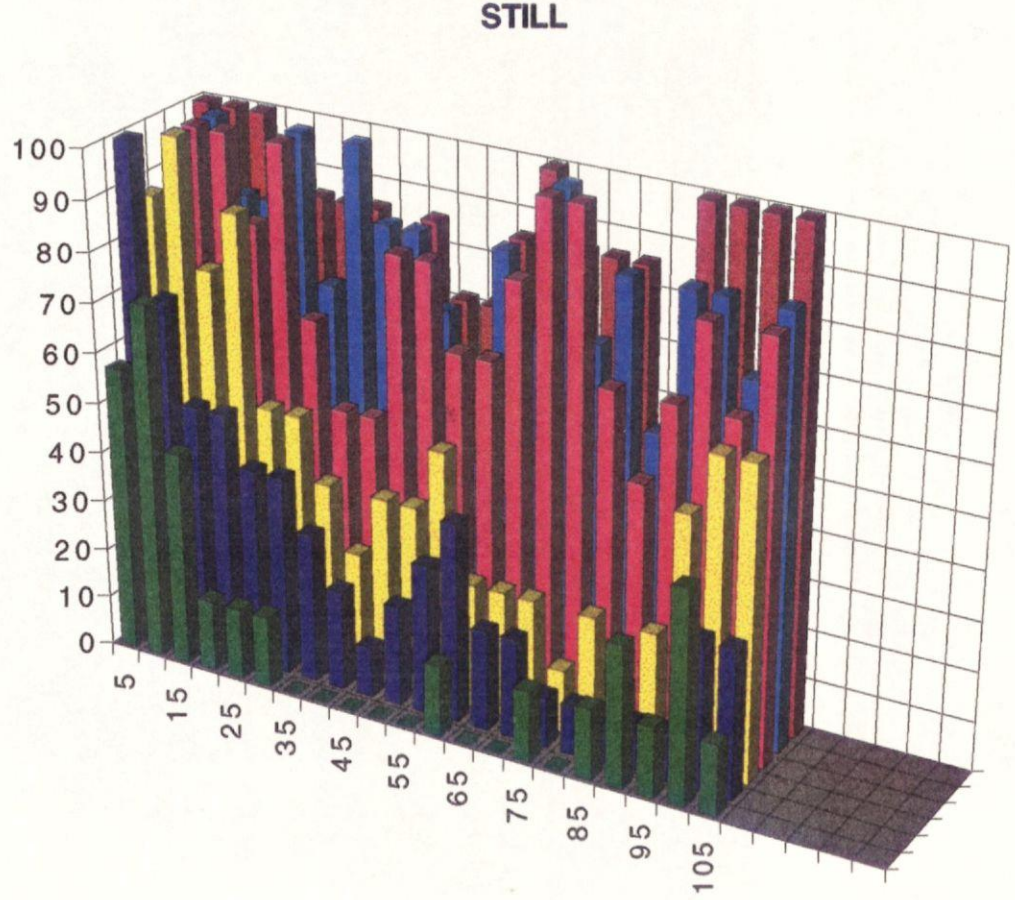
The results of the one-way ANOSIM indicated that there was a significant difference in the behaviours exhibited by the treatment groups. The sample statistic (Global R) = 0.478, $p < 0.0001$. Examination of the difference between treatment groups revealed that pre-treatment with haloperidol (0.025, 0.05, or 0.075 mg/kg) resulted in a significant difference in behavioural categories compared with vehicle-amphetamine treated animals, see Table 5.11

Pairwise tests

Veh Amph v .025HAL. Table 5.12 shows the behaviours in order of their contribution to the average dissimilarity $\partial(=31.30)$ between vehicle-amphetamine and animals pre-treated with .025mg/kg haloperidol with a cut-off $(\sum\partial(i)\%) = 10$. Those behaviours which contributed to the first 10% of the difference between the two groups contributed consistently, (the ratio $\partial i/SD(\partial i)$ is large), behaviours which contributed thereafter had a lower ratio and are not reported. Behaviours which contributed most to the difference between the two groups were rearing against the wall (**REARW**) and **STILL**. Animals pre-treated with 0.025mg/kg haloperidol showed an increase in rearing against the wall (**REARW**) at session intervals 75-90 min. This group also showed an increase in the percentage of animals who were **STILL** at session interval 20 min.

Figure 5.5.

The percentage of animals exhibiting each of the behavioural categories during the 105-min observation period by rats (n = 8) per group) injected with vehicle-saline (**red**), vehicle + 3.5mg/kg amphetamine (**green**), 0.01mg/kg haloperidol + 3.5mg/kg amphetamine (**dark blue**), 0.025mg/kg haloperidol + 3.5mg/kg amphetamine (**yellow**), 0.05 mg/kg haloperidol + 3.5mg/kg amphetamine (**magenta**) or 0.075mg/kg haloperidol + 3.5mg/kg amphetamine (**light blue**) and tested in a circular open field.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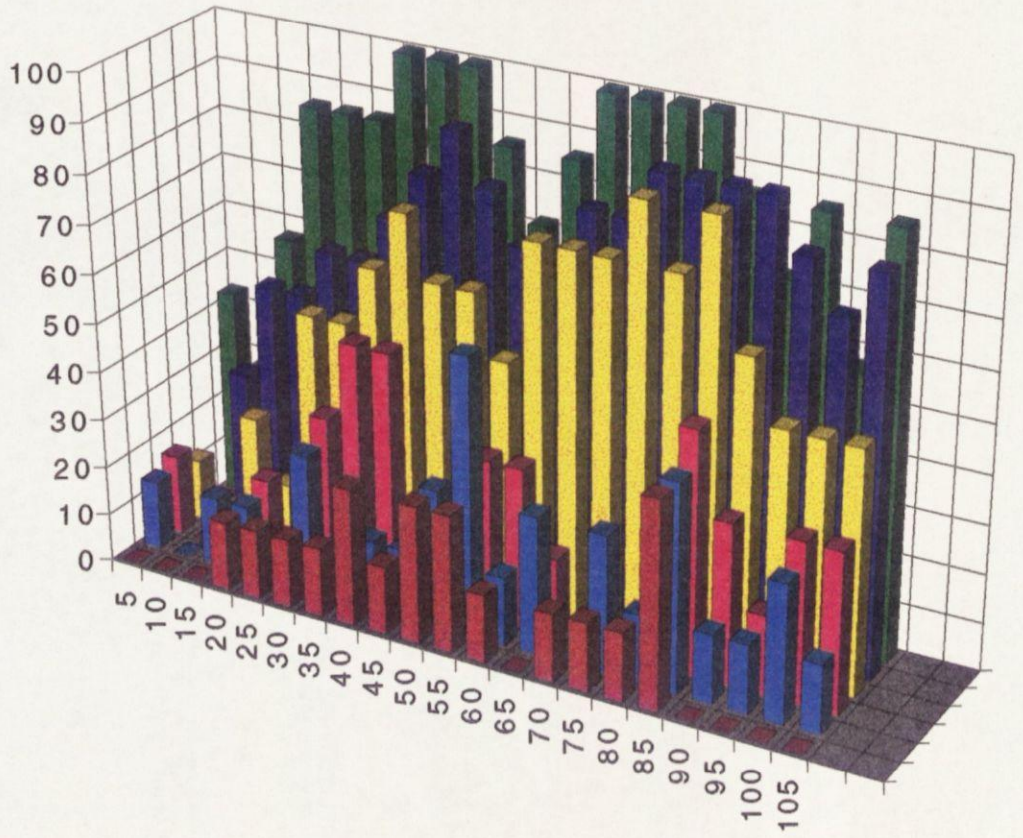
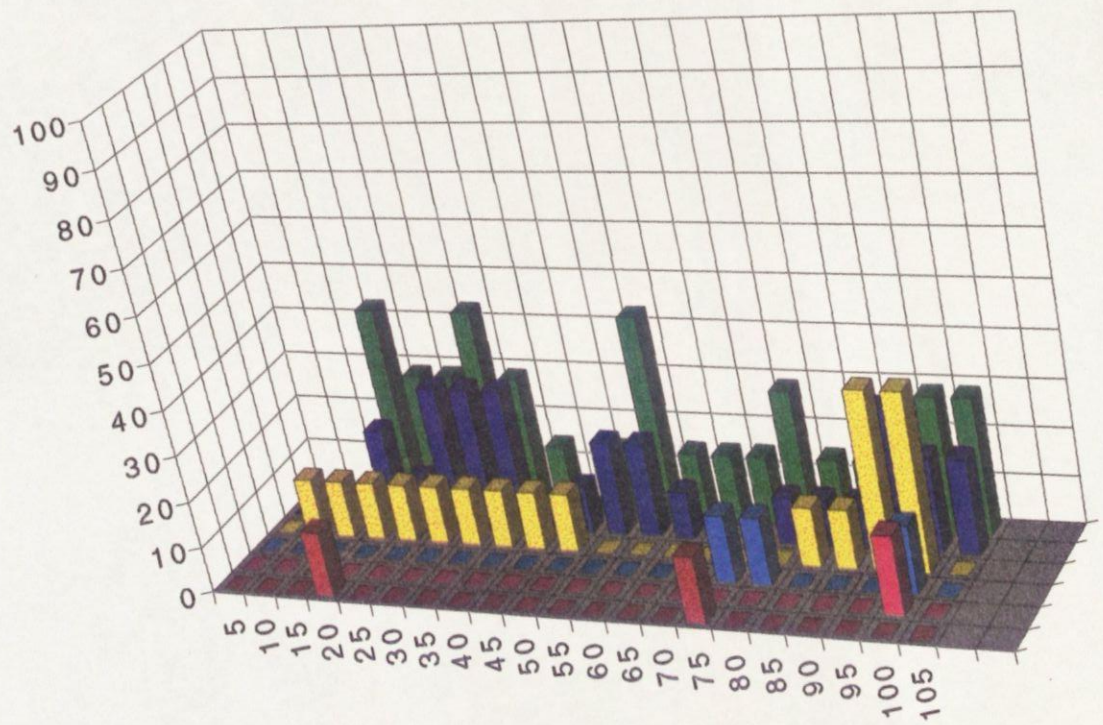
LOCOMOTION WITH PAUSE



REARING IN OPEN



HEAD-SWAY

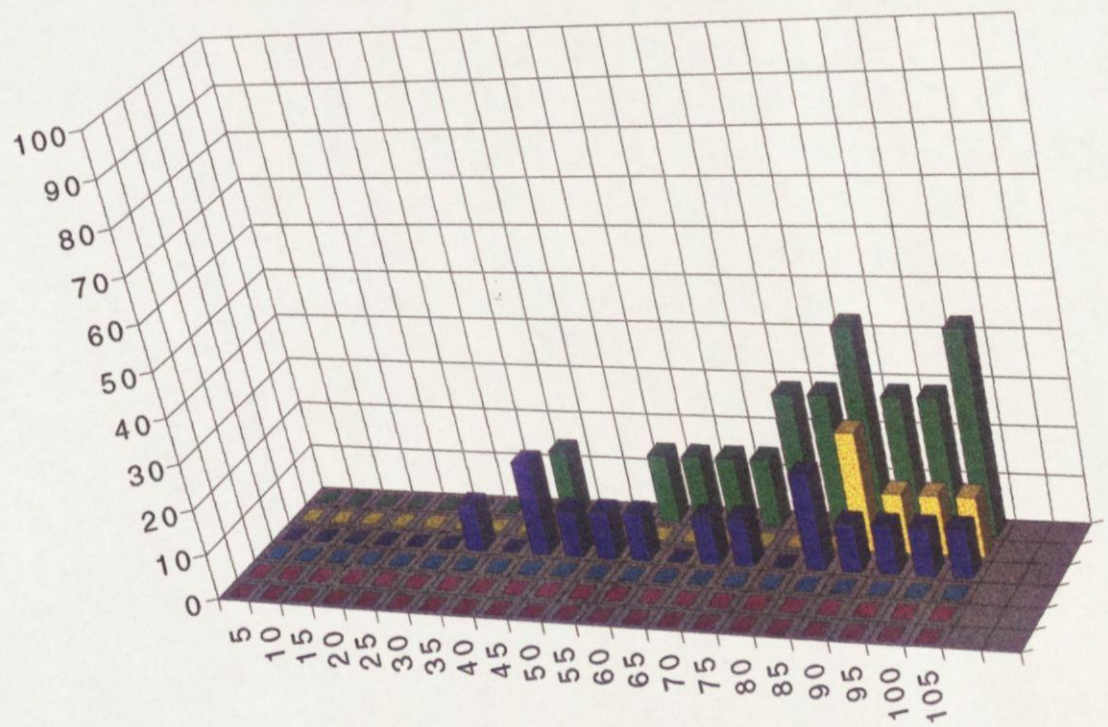

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Table 5.11. Significance levels of pairwise tests following one-way ANOSIM, and the average dissimilarity (∂) between vehicle-amphetamine and haloperidol pre-treated groups.

Veh-Amph versus	0.01mg/kg Haloperidol	0.025mg/kg Haloperidol	0.05mg/kg Haloperidol	0.075mg/kg Haloperidol
∂	34.30 (NS)	31.30 (p<0.008)	35.56 (p<0.0001)	60.13 (p<0.001)

Table 5.12. Percentage (ABUND) of behaviours in group A (.025HAL) and group B (veh-amph). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(=31.30) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 10%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
90	rearw	0.70	0.14	.60	.46	1.33	1.93
80	rearw	0.60	0.29	.51	.48	1.08	3.57
75	rearw	0.60	0.29	.51	.48	1.08	5.21
10	sniff	0.70	0.43	.51	.49	0.99	6.85
20	still	0.50	0.14	.48	.49	0.99	8.39
20	loco+p	0.50	0.86	.48	.49	0.99	9.93

Veh-Amph v .05HAL. Table 5.13 shows the behaviours in order of their contribution to the average dissimilarity ∂(=35.36) between vehicle-amphetamine and animals pre-treated with .05mg/kg haloperidol with a cut-off (∑∂(i)%) = 12%. The behaviour which contributed to the first 12% difference between the two groups was locomotion with pause (**LOCO+P**), (**STILL**). Animals pre-treated with 0.05mg/kg showed an increase in the percentage of animals who were **STILL** at session intervals 15-20 and 100-105min.

Veh-Amph v .075HAL. Table 5.14 shows the behaviours in order of their contribution to the average dissimilarity ∂(=60.13) between vehicle-amphetamine and animals pre-treated with .075mg/kg haloperidol with a cut-off (∑∂(i)%) = 30%. Behaviours which contributed to the first 30% difference between the two groups were locomotion with pause (**LOCO+P**), (**STILL**) and **SNIFF**. Animals pre-treated with 0.075mg/kg haloperidol showed a marked decrease in locomotion (**LOCO+P**) between session intervals 20-25 and between session intervals 45-105, with 100% of the haloperidol treated animals **STILL** from session interval 70 to the end of the session compared with 0% of the vehicle-amphetamine treated animals. Animals pre-treated with this dose of haloperidol also showed a decrease in sniffing (**SNIFF**) between session intervals 15-25 min.

Table 5.13. Percentage (ABUND) of behaviours in group A (.05HAL) and group B (veh-amph). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(=35.36) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 12%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂ i)	∑∂i%
20	loco+p	0.13	0.86	.81	.45	1.78	2.27
20	still	0.88	0.14	.81	.45	1.78	4.54
105	still	0.63	0.14	.61	.52	1.18	6.25
15	still	0.75	0.43	.56	.53	1.06	7.83
15	loco+p	0.25	0.57	.56	.53	1.06	9.41
10	sniff	0.63	0.43	.54	.53	1.02	10.93
100	still	0.63	0.43	.54	.53	1.02	12.45

Table 5.14. Percentage (ABUND) of behaviours in group A (.075HAL) and group B (veh-amph). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(=60.13) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 30%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
70	loco+p	0.00	1.00	1.19	.10	11.5	1.97
75	loco+p	0.00	1.00	1.19	.10	11.5	3.95
15	sniff	0.00	1.00	1.19	.10	11.5	5.92
20	sniff	0.00	1.00	1.19	.10	11.5	7.90
25	sniff	0.00	1.00	1.19	.10	11.5	9.87
70	still	1.00	0.00	1.19	.10	11.5	11.85
75	still	1.00	0.14	1.02	.43	2.36	13.55
25	still	1.00	0.14	1.02	.43	2.36	15.25
25	loco+p	0.00	0.86	1.02	.43	2.36	16.95
65	sniff	0.17	1.00	1.00	.46	2.16	18.61
45	still	0.83	0.00	1.00	.46	2.16	20.27
45	loco+p	0.17	1.00	1.00	.46	2.16	21.93
65	still	0.83	0.00	0.99	.46	2.16	23.58
65	loco+p	0.17	1.00	0.99	.46	2.16	25.23
50	still	0.83	0.00	0.99	.46	2.16	26.87
20	loco+p	0.17	0.86	0.88	.54	1.63	28.33
20	still	0.83	0.14	0.88	.54	1.63	29.79
105	still	0.83	0.14	0.87	.53	1.64	31.24

Discussion.

Administration of amphetamine has been shown to induce changes in open-field behaviour in the rat in a dose dependent manner (eg Fray et al, 1980, Rebec and Bashore, 1984). In the present study it was found that amphetamine increased snout contact (**HEAD-D**), head swaying (**HEAD-S**) and sniffing (**SNIFF**) in a dose dependent manner, and also rearing in the open (**REARO**), rearing against the open field wall (**REARW**) and locomotion (**LOCO+P**). Snout contact, head swaying and locomotion contributed most to the significant differences between the doses tested in this study. With increasing dose of amphetamine the percentage of animals with head down (**HEAD-D**) and engaging in head swaying (**HEAD-S**) behaviour increased. Animals treated with 5mg/kg amphetamine showed a decrease in the duration of the locomotor phase, at the same time as exhibiting an increase in focused stereotyped behaviours.

Although a significant difference was found between animals treated with 4 or 5 mg/kg amphetamine when tested in a square or circular open field on automated measures of locomotor activity, there was no significant difference in behavioural categories between the two open fields at any of the doses tested. There appeared to be a trend for animals tested in a circular open field to make snout contact (**HEAD-D**) at a lower threshold dose, 4mg/kg compared to 5mg/kg, than animals tested in the square field.

A significant proportion of rats treated with 3.5 mg/kg amphetamine did not engage in either **HEAD-D** or **HEAD-S** behaviour. Pre-treatment with sulpiride (10, 20 or 50mg/kg) produced an increase in both of these behaviours, suggesting that in agreement with the findings of Robertson and MacDonald (1985) sulpiride administered at these doses potentiates some aspects of stereotyped behaviour. Somewhat surprisingly, pre-treatment with clozapine did not significantly change the behavioural 'profile' of animals treated with 3.5mg/kg amphetamine.

In this study locomotor activity was divided into locomotion which took place over the entire 10s observation period without a pause (**LOCO**), and locomotion which contained pauses lasting longer than 3s (**LOCO+P**). It was anticipated that this

might distinguish the period of peak locomotor activity and that this peak might vary following antipsychotic drug treatment. In only one comparison, that between 3 and 4mg/kg amphetamine, did locomotion without a pause over the 10s observation period (**LOCO**) contribute to the difference between groups. As might be expected, measurement of locomotor activity using behavioural categories provided a less sensitive measure of locomotion than measures of distance moved and trip length using automated computer-assisted image analysis. The behavioural categorisation of locomotion failed to find significant differences between vehicle-amphetamine and clozapine pre-treated animals, although significant differences were found on automated measures of distance moved following pre-treatment with 10 or 20mg/kg clozapine. In contrast, behavioural categorisation of differences in locomotion (**LOCO+P**) contributed to the significant difference between vehicle-amphetamine and sulpiride pre-treated groups and to the significant difference between vehicle-amphetamine and haloperidol pre-treated groups, findings which support the significant decreases in locomotor activity found on automated measures of distance moved. In the case of haloperidol a decline in **LOCO+P** was the major behavioural category which contributed to the difference between haloperidol pre-treated groups and vehicle-amphetamine groups.

The behavioural analysis conducted on experiments 1-4 was made as an adjunct to the area of main interest, which was to examine the effects of environmental factors and antipsychotic drug pre-treatment on measures of stereotyped locomotion. Therefore doses of amphetamine were chosen which would elicit locomotion in the 105 minute test session. Chapter 3 described in detail the duration of the initial locomotor phase for each of the doses tested and the finding that the maximum duration of locomotion was observed following a dose of 4mg/kg amphetamine.

It was anticipated that the difference between behaviours elicited under doses of amphetamine that were very similar would be minimal, with the possible exception of animals treated with 5mg/kg amphetamine where computer image analysis had shown that the duration of the locomotor phase declined, and it was

hypothesised that at this dose animals were engaging in focused stereotyped behaviours rather than ambulation. The findings of the observational study support this claim showing that the percentage of animals engaging in head down and head swaying markedly increased following treatment with 5 mg/kg amphetamine. It was also noted that differences were found between all the doses of amphetamine on several of the behavioural categories as noted above. Fray et al, (1980) found no significant difference in locomotor activity between animals treated with 3 and 5 mg/kg amphetamine, and reported only minimal differences between these groups on measures of rearing and head down. As most of the behaviours which contributed to the difference between animals treated with 3, 4 and 5mg/kg amphetamine in the present study occurred between session intervals 60-105 minutes it is likely that the study undertaken by Fray et al, (1980) was too short to detect differences in behavioural response under these doses of amphetamine.

The multivariate analysis provided a detailed 'picture' of the behaviours which contributed to the between group differences. The advantage of such an analysis is that it does not examine behaviours in isolation but compares the overall behavioural profile. Behavioural effects following treatment with amphetamine are seen as exhibiting different thresholds for response activation, with each behaviour having its own characteristic inverse U-shaped function (Robbins et al, 1990). The present analysis examined all the behaviours measured as a complete and distinct expression of the effects of a specific dose, subsequently examining the contribution of behavioural categories to the differences between treatments rather than examining differences in behavioural categories *per se*. Such an approach can be seen as 'slicing through' all the behaviours present at any given dose and comparing 'slices'. This analysis provides a novel way of examining the differences between drug treatments and can be seen as an adjunct, rather than a replacement, to testing for differences in specific behavioural categories between treatment groups. This approach is directly in accordance with the view that examining behaviour as inter-related acts gives greater insight into the patterns of behaviour which develop in drug treated animals. Clearly, the emphasis in these studies has been on a more integrated

approach to behavioural analysis rather than to measuring drug-induced behaviour as isolated components of the response. For example the approach taken by Teitelbaum et al, (see Teitelbaum et al, 1990), using the Eshkol-Wachman movement notation (Eshkol and Wachman, 1958) to analyse movement in rats, has shown that the expression of apparently unrelated stereotyped behaviours relies on values of three component variables: snout contact; forward progression and turning. The current study has found that an increase in snout contact and a decrease in forward progression is a major contributing factor to the significant difference between increasing doses of amphetamine, and are seen as support for the findings (see Teitelbaum et al, 1990) that an interaction between snout contact and forward locomotion contribute to the development of stereotyped behaviour with increasing dose of amphetamine.

Summary

A number of behaviours induced following administration of amphetamine were examined using a distribution-free analysis based on rank similarities of behaviours and non-metric multi-dimentional scaling (MDS). With increasing dose of amphetamine from 3 to 5 mg/kg, there was a significant decrease in locomotion accompanied by an increase in two forms of stereotyped head movements: head-down and head-swaying behaviour.

Following treatment with 3.5mg/kg amphetamine, rats showed high levels of locomotion without displaying head-swaying or head-down stereotyped behavours. Pre-treatment with the classic antipsychotic haloperidol reduced locomotor activity without inducing stereotyped head movements. The atypical antipsychotic, clozapine, had no significant effect on any of the behavioural measures. In contrast pre-treatment with the atypical antipsychotic, sulpiride, decreased locomotor activity and significantly increased stereotyped head movements.

Chapter Six.

The Effects of Ondansetron on Behaviours Exhibited Following Treatment with 3.5mg/kg Amphetamine.

6.1 Introduction

Serotonin (5HT) is thought to play a role in various types of pathological conditions including anxiety, depression, aggressiveness, panic, obsessive-compulsive disorders, suicidal behaviour, neurodegenerative disorders such as Alzheimer's disease, Parkinsonism and Huntington's Chorea, as well as migraine, emesis and alcoholism. It is not surprising that serotonin has been suggested as playing a role in the aetiology of schizophrenia and in dopamine mediated behaviours (see review by Zifa and Fillion 1992). However, studies which have evaluated the role of 5HT transmitter systems in schizophrenia show varying and often contradictory results (For review see Bleich et al, 1988).

Stimulation of post synaptic 5HT receptors in rodents by various pharmacological agents results in a complex behavioural syndrome. The behaviours elicited include hind limb abduction, 'wet dog' shakes, side to side head swaying, reciprocal forepaw treading, tremor, Straub tail and increased reactivity to stimuli. Head swaying and forepaw treading are stereotyped in that they are repetitive and apparently meaningless fragments of normal behaviour (see Curzon, 1990). Hyperactivity induced following 5HT changes is in part mediated by associated activation of dopaminergic systems (Crow and Deakin, 1977; Jenner et al, 1980; Marsden, 1980; Deakin and Dashwood, 1981).

The 5HT receptors can be subdivided into three families: the 5HT1 family consisting of the 5HT1a, 5HT1b and 5HT1d receptors, the 5HT2 family consisting of 5HT1c, 5HT2a and 5HT2b receptors and 5HT3 receptors. The relevant

psychopharmacological agents with antipsychotic potential are 5HT1 agonists, 5HT2 antagonists and 5HT3 antagonists (for review see Zifa and Fillion 1992).

The behavioural role in rodents of many of the 5HT receptor types have been evaluated. 5HT1a agonists induce hyperlocomotion, headswaying, reciprocal forepaw treading and flat body posture (Tricklebank, 1987; Yamada et al, 1988; 1989), whilst it is thought that pre-synaptic autoreceptors in the median raphe are more likely to induce hypolocomotion, especially of horizontal movements (Mittman and Geyer, 1989). 5HT1a activity is thought to play a role in sexual behaviour, although the mechanism is unknown. This receptor also plays an important role in feeding behaviour as 5HT1a agonists have been shown to increase food intake in rats (Dourish et al, 1985). These receptors are implicated in psychiatric disorders such as anxiety and depression. 5HT1a agonists have been shown to have anxiolytic properties which are very different from those of the benzodiazepines, and also exhibit antidepressant properties in animal models of depression.

The 5HT2 receptor was initially identified as the site that could bind [3H] spiroperidol and that had specific serotonergic pharmacological properties (Leysen et al, 1978). These receptors are involved in motor behaviour in rodents. Head twitch can be induced by 5HT2 agonists and blocked by selective antagonists eg Ketanserin (Ogren and Fuxe, 1989). 'Wet dog shake' is also inhibited by 5HT2 antagonists (Fone et al, 1989) The number of 5HT2 receptors has been shown to decrease in schizophrenic patients (Mita et al, 1986). Therefore on the basis of this and experimental findings, 5HT2 antagonists are being developed as antipsychotic agents (Ortmann et al, 1982; Gelders et al, 1986; Wander et al, 1987; Lowe et al, 1988; Van der Heyden, 1989). The antipsychotic activity of 5HT2 antagonists has not yet been clearly demonstrated in the clinical setting, although many antipsychotic drugs have considerable potency as 5HT2 antagonists eg thioridazine and clozapine. Recently much attention has focused on combining 5HT2 and D2 antagonistic effects in the development of new antipsychotic agents. There is considerable evidence to suggest that clozapine is a more effective antipsychotic agent than classical antipsychotic drugs for both schizophrenic patients (Claghorn,

1987) and treatment resistant schizophrenic patients (Kane et al, 1988; Meltzer et al, 1989). Indeed, Meltzer et al. (1989) has suggested that clozapine's ability to alter both dopamine and serotonin in an integrated manner reflects an abnormality in the interaction of the dopaminergic and serotonergic system in the aetiology of schizophrenia, rather than in either of the systems alone.

The ability of clozapine to act as a 5HT antagonist (Lai et al, 1980; Fink et al, 1984; Freidman et al, 1985) has been suggested as an explanation of its low induction of extrapyramidal symptoms. In support of this claim lesions to the serotonergic systems or inhibition of 5HT synthesis with PCPA has been shown to decrease neuroleptic-induced catalepsy in rodents (Kostowski et al, 1972). Meltzer (1989) postulates that dorsal and median raphe serotonergic activity on frontal cortical, mesolimbic and mesostriatal dopaminergic systems could lead to decreased dopaminergic activity in frontal cortex, and increased dopaminergic activity in the mesolimbic and mesostriatal system, and that the primary abnormality in schizophrenia is in dopaminergic-serotonergic interactions with the possibility that if the ratio of serotonergic to dopaminergic activity is high, negative symptoms predominate, while in the opposite case positive symptoms would predominate. In addition clozapine has been shown to have affinity for the 5HT3 receptor (Ashby et al, 1989; Barnes et al, 1990; Hoyer et al, 1989; Watling et al, 1989).

The synthesis of selective and potent receptor antagonists has made possible the detailed study of the behavioural pharmacology of 5HT3 receptors: eg granisetron (Sanger and Nelson, 1989), ICS 205-930 (Richarson et al, 1985), LY278584 (Robertson et al, 1990), MDL 72222 (Fozard, 1984), MDL 73147 (Sorenson et al, 1989), ondansetron (Butler et al, 1988), renzapride (Sanger, 1987), Zacopride (Smith et al, 1988). The 5HT3 receptor is different from the other 5HT receptor subtypes in that it is not linked to a G protein but is activated by an ion channel which increases the conductance of monovalent cations (Peters and Lambert, 1989). Radioligand binding studies using tritiated [H3] 5HT3 receptor antagonists have shown that 5HT3 receptors are distributed in the cortex, amygdala, hippocampus, accumbens, septum, thalamus and hypothalamus, whilst the

cerebellum is almost devoid of specific 5HT3 sites. Also when compared with the other 5HT receptor subtypes, the 5HT3 receptors exhibit a ten times lower density in the brain (Barnes et al, 1990, 1989; Kilpatrick et al,1987; Peroutka and Hamik 1988; Robertson et al, 1990; Waeber et al, 1988, 1990; Watling et al, 1988, 1989).

Costall et al, (1987) have shown that a 5HT3 receptor antagonist GR 38032F [ondansetron] injected into the nucleus accumbens or administered peripherally, inhibited the hyperlocomotion induced by acute intra-accumbens injection of 10µg amphetamine in the rat, and also hyperactivity induced following persistent intra-accumbens infusion of dopamine into the rat or marmoset. Ondansetron has no known affinity for other neurotransmitter sites and does not interact with dopamine receptors (Brittain et al, 1987), therefore Costall et al, (1987) attribute the blocking of hyperactivity by this drug to 5HT3 receptor antagonism. Ondansetron and amphetamine were administered bilaterally to the nucleus accumbens of the rat and it would seem likely that 5HT3 receptor blockade in this area was responsible for the decrement in the amphetamine-induced hyperactive response. In addition, ondansetron was administered peripherally and this also reduced the hyperactivity caused by the intra-accumbens injection of amphetamine or the infusion of dopamine. This may also have resulted from 5HT3 receptor blockade in the nucleus accumbens although other regions could not be ruled out. Injection of ondansetron into either the left or right amygdala in rats receiving unilateral dopamine infusion to the left amygdala inhibited hyperactivity, a finding which demonstrates inter-hemispheric communication in the regulation of locomotor activity in the limbic system and provides evidence of 5HT involvement in the hyperactive response. Thus a specific, potent 5HT3 antagonist, when administered into the amygdala, nucleus accumbens or peripherally, blocked either amphetamine or dopamine induced hyperactivity in a manner similar to that of a dopamine antagonist. This study (Costall et al, 1987), demonstrated important differences between the action of ondansetron and proven antipsychotic agents such as fluphenazine, haloperidol and sulpiride. Ondansetron did not reduce activity levels

in normal untreated animals, nor did it reduce raised limbic dopamine activity to levels below control values, which is in marked contrast to the depressant effects of dopamine antagonist antipsychotic agents which depress all forms of motor activity. Such a profile might indicate that ondansetron may be able to restore dopamine activity to normal levels. Another distinction between the action of ondansetron and antipsychotic agents relates to the prolonged increases in locomotor activity seen on discontinuation of neuroleptic-dopamine treatment which appear to reflect dopamine receptor sensitivity (Rupniak et al, 1983). After discontinuation of ondansetron-dopamine treatment activity levels remained at control levels. In addition, ondansetron reduced the 'rebound' hyperactivity seen following the discontinuation of haloperidol-dopamine treatment. Taken together these findings indicate a role for 5HT3 in the modulation of dopamine or amphetamine-induced locomotor responses. Interestingly and paradoxically, ondansetron failed to antagonise hyperactivity following the peripheral administration of amphetamine (Costall et al, 1987; Van der Hoek and Cooper ,1990). Furthermore, higher doses of many of the 5HT3 antagonists show a reduced ability to reverse intra-accumbens dopamine-induced hyperactivity (Costall et al, 1990). Costall at al, (1987) attribute the failure of ondansetron to antagonise peripherally administered amphetamine-induced behaviours to its inability to affect striataly-mediated behaviours. The mesolimbic system has been shown to be involved in the activation of amphetamine-induced locomotor behaviours (Pijnenburg and Van Rossum 1973) and peripherally administered ondansetron has been shown to block hyperactivity following injection of amphetamine into the nucleus accumbens, this would indicate that some reduction in locomotor activity might have been expected. Costall et al, (1987) do not report the amphetamine dose administered peripherally. The findings reported would be expected if the dose administered was high enough to initiate stereotyped behaviours, the results are ambiguous particularly with respect to lower doses of amphetamine where the predominant behaviours are locomotor.

Certainly the ability of ondansetron to reduce locomotor activity following central administration of either dopamine or amphetamine indicates a facilitatory

role for 5HT on dopamine transmission, and supports the findings that injections of 5HT into the nucleus accumbens increases locomotor activity (Pijnenburg et al, 1975; Costall et al, 1979; Makanjuola et al, 1980). In addition, the 5HT3 receptor agonist 2-methyl-5-HT increased amphetamine-induced hyperactivity, but not normal activity, and this effect was antagonised by ondansetron (Costall et al, 1987). As few overt behavioural changes are noted following the administration of 5HT3 receptor antagonists to normal undrugged animals (Hagan et al, 1987) it would appear that the normal activity of this system is low, but that this may change as a consequence of dysfunction within the mesolimbic dopamine system (Costall et al, 1987).

It would seem that there is convincing evidence in both rats and primates that the 5HT3 receptor antagonists can exhibit a modulatory effect on the hyperactivity response following raised mesolimbic dopamine activity. How this relates to effective antipsychotic activity remains to be seen. Ondansetron has been reported to possess antipsychotic action in uncontrolled trials (De Veaugh-Geiss et al, 1990), although results from the only double-blind placebo-controlled study in acute schizophrenia, to date, were inconclusive (Meltzer, 1991).

In view of the fact that strong evidence suggests that 5HT3 receptors are strongly implicated in taking a modulatory role in the hyperactivity following raised mesolimbic dopamine activity and the intriguing reports that 5HT3 antagonists do not block hyperactivity following peripherally administered amphetamine, the aim of this present study was to use a selective 5HT3 antagonist, ondansetron (Brittain et al, 1987), and investigate its action on the spatiotemporal aspects of open-field locomotion and on behaviours induced following treatment with 3.5mg/kg amphetamine.

6.2 Materials and methods

Animals. Male wistar rats weighing 350 - 450 g (bred in the University animal house facilities) were used. Rats were housed in groups of six on a 12 hour

light dark cycle and allowed free access to food and water. The groups to be tested were allowed at least three days acclimation in a temperature-regulated room adjacent to the laboratory. Each animal was tested only once in the open field.

Drugs. The following drugs were used and all doses are expressed as the salt: d-amphetamine sulphate (sigma) 3.5 mg/kg dissolved in phosphate buffered saline; ondansetron (donated by Glaxo) 0.05, 0.1, 0.5, 1.0 mg/kg. dissolved in phosphate buffered saline and stored in neutral glass containers. Amphetamine and saline were injected intraperitoneally (IP) immediately before testing in the open field. Ondansetron or vehicle was injected subcutaneously (SC) 30 minutes prior to the injection of either amphetamine or saline.

Experimental procedure. Animals were randomly assigned to a treatment group (n=9 per group). Rats received a SC injection of vehicle or ondansetron before being placed in the open field. They were removed after 30 minutes, given amphetamine or saline IP and replaced in the open field where they remained for 105 minutes.

Apparatus. The experiment used 4 circular open fields (75cm diameter x 30cm in height, see Chapter 2 for a detailed description). The apparatus used for the analysis of behaviour is described in Chapter 5.

Data collection. Chapter 2 provides a detailed description of the procedure used to obtain automated measures of locomotor activity. Chapter 5 provides information on the methods employed in the behavioural analysis.

Data analysis. Data obtained from the image analysis of locomotor activity were analysed using analysis of variance (ANOVA) with treatment (drug dose) the between subjects factor and 5 minute session intervals the repeated within subjects factor. Differences were evaluated using the Newman-Keuls multiple range test (Howell 1992). Vehicle-amphetamine groups were compared with vehicle-saline

groups and ondansetron pre-treated groups were compared with vehicle-amphetamine groups. Data derived from the behavioural analysis of video tapes were analysed using PRIMER (Carr et al, 1990) - See Chapter 5.

6.3 Results

Computer assisted image analysis of locomotor behaviour.

Distance moved The total distance moved during the 105 minute test session was not significantly affected by pre-treatment with ondansetron ($F(4,41) = 2.007$ $p > .1$). Figure 6.1 shows the total distance moved across the session by all amphetamine treated groups and by all vehicle treated groups. Figure 6.2 shows the distance moved by amphetamine treated animals pre-treated with ondansetron (0 - 1.0mg/kg). This figure clearly demonstrates a significant main effect of time on the distance moved for all groups ($F(20,800) = 36.668$ $p < .0001$). Examination of this effect revealed that although pre-treatment with ondansetron tended to increase the distance moved under amphetamine between session intervals 5-40 minutes post-injection with amphetamine, the effect was not significant. Between session intervals 55-95 minutes animals pre-treated with 0.1, 0.5, & 1.0 mg/kg ondansetron made significantly less horizontal movement than vehicle amphetamine treated rats. During the final 10 minutes of the test session these animals travelled a similar distance to vehicle-amphetamine treated animals. In marked contrast, animals pre-treated with the lowest dose of ondansetron (0.05mg/kg) covered a significantly greater distance than vehicle-amphetamine treated rats during some session intervals. At session interval 55 minutes animals treated with the lowest dose of ondansetron moved a significantly greater distance than vehicle amphetamine treated animals, however, between session intervals 60-80 minutes there was no significant difference between these two groups on this measure. At session intervals 85-105 pre-treatment with 0.05 mg/kg ondansetron again resulted in a significant increase in distance moved (Newman-Keuls $p < .05$).

Proportion of length 4 trips. Figure 6.3 shows the proportion of length 4 trips made by all amphetamine treated groups. Pre-treatment with ondansetron did

not significantly affect the total proportion of length 4 trips made by amphetamine treated animals ($F(4,40) = 0.497$ NS).

Effect of time in the proportion of length 4 trips. There was a significant effect of time on the proportion of length 4 trips ($F(20,800) = 13.276$ $p < .0001$). Figure 6.4 shows the proportion of length 4 trips for all amphetamine treatment groups across the 105 minute test session. Newman-Keuls multiple range tests conducted on the means at each session interval, revealed that there was no significant difference in the proportion of length 4 trips made by any of the treatment groups between session intervals 5-35 minutes. Between session intervals 40-45 minutes, and also at session interval 55 minutes, animals pre-treated with an intermediate dose of ondansetron (0.5mg/kg), made a significantly increased proportion of length 4 trips. Paradoxically, at session interval 65 minutes, animals pre-treated with ondansetron approximately 0.5mg/kg above or below the intermediate dose made significantly less length 4 trips than vehicle-amphetamine treated animals.

In addition the ANOVA revealed a significant Dose x Session Interval interaction ($F(80,800) = 1.417$ $p<0.01$). Figure 6.5 and figure 6.6 show the cumulative percentage for both distance moved and the proportion of length 4 trips over the entire test session. Both graphs show clearly the facilitatory effect of pre-treatment with ondansetron in the early session intervals on both measures.

Figure 6.1

The total distance moved across the 105 min test session by rats (n = 9) treated with 3.5 mg/kg amphetamine and pre-treated with vehicle or 0.05-1.0 mg/kg ondansetron.

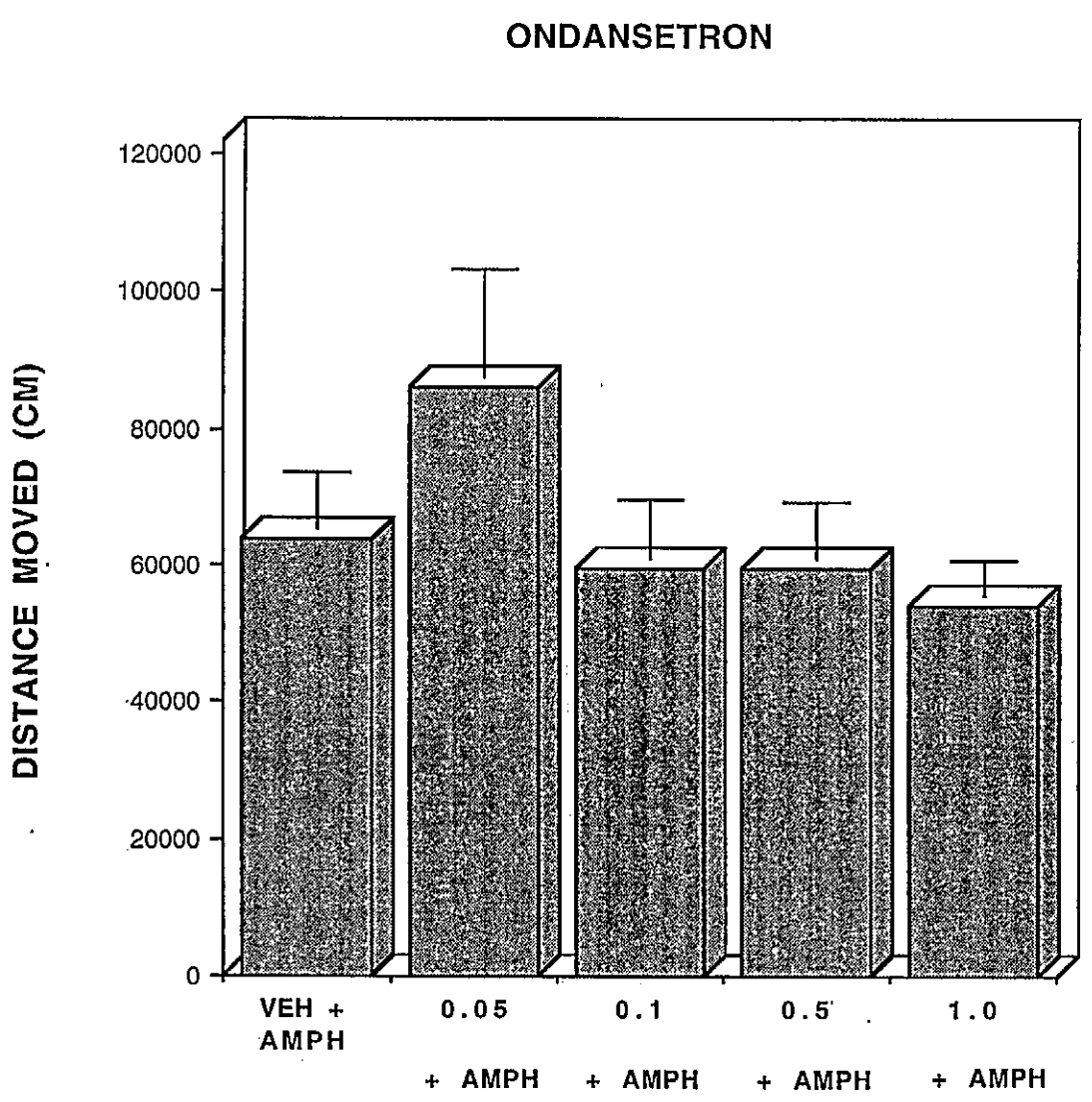

ONDANSETRON
DISTANCE MOVED (CM)
120000
100000
80000
60000
40000
20000
0
VEH + AMPH
0.05 + AMPH
0.1 + AMPH
0.5 + AMPH
1.0 + AMPH

Figure 6.2

Distance moved at each of the 5 min time intervals by rats (n = 9) treated with 3.5 mg/kg amphetamine and pre-treated with vehicle or 0.05-1.0 mg/kg ondansetron.

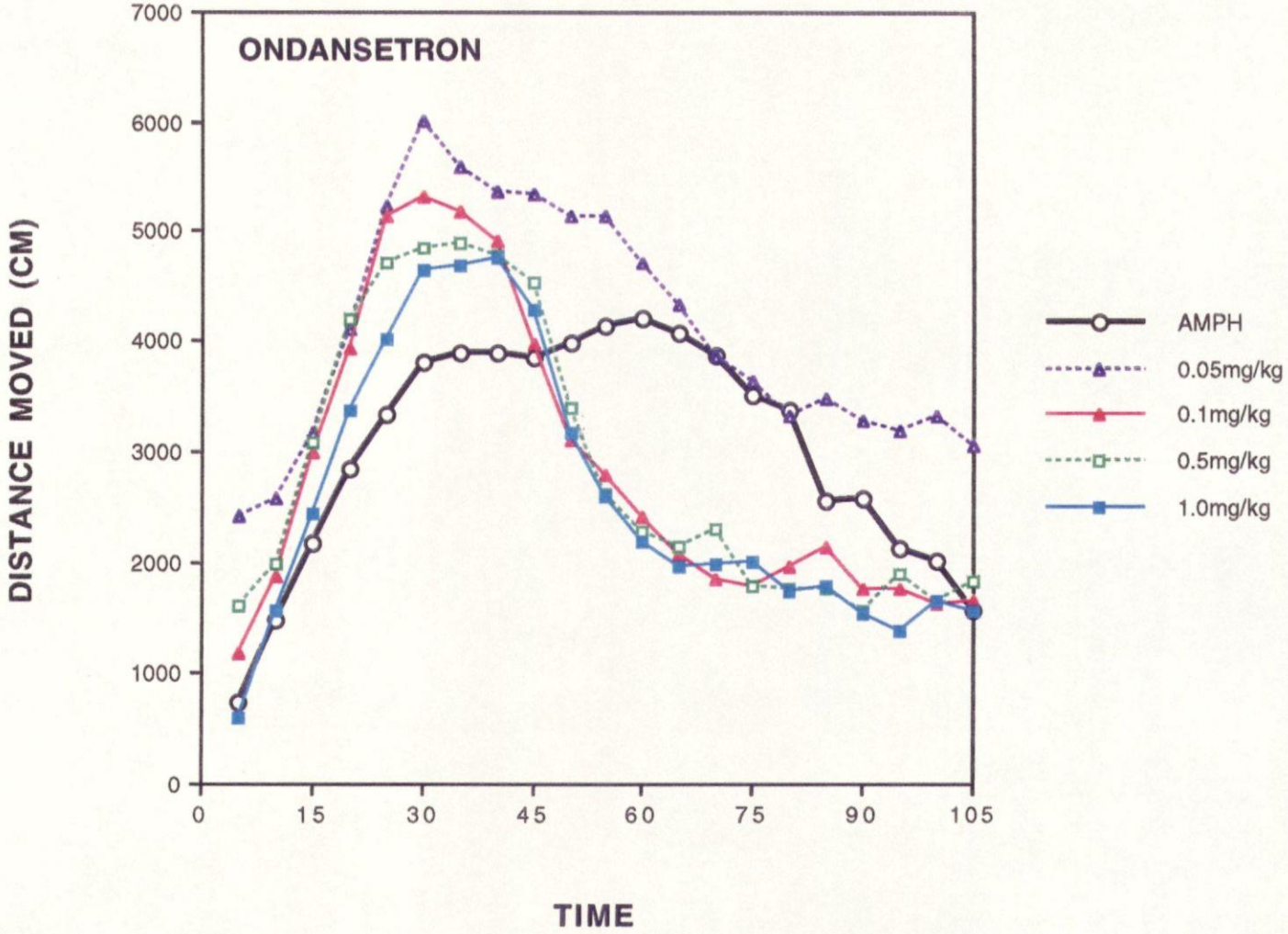

ONDANSETRON
DISTANCE MOVED (CM)
7000
6000
5000
4000
3000
2000
1000
0
0
15
30
45
60
75
90
105
TIME
AMPH
0.05mg/kg
0.1mg/kg
0.5mg/kg
1.0mg/kg

Figure 6.3

Proportion of length 4 trips over the 105 min test session by rats (n = 9) treated with 3.5 mg/kg amphetamine and pre-treated with vehicle or 0.05-1.0 mg/kg ondansetron.

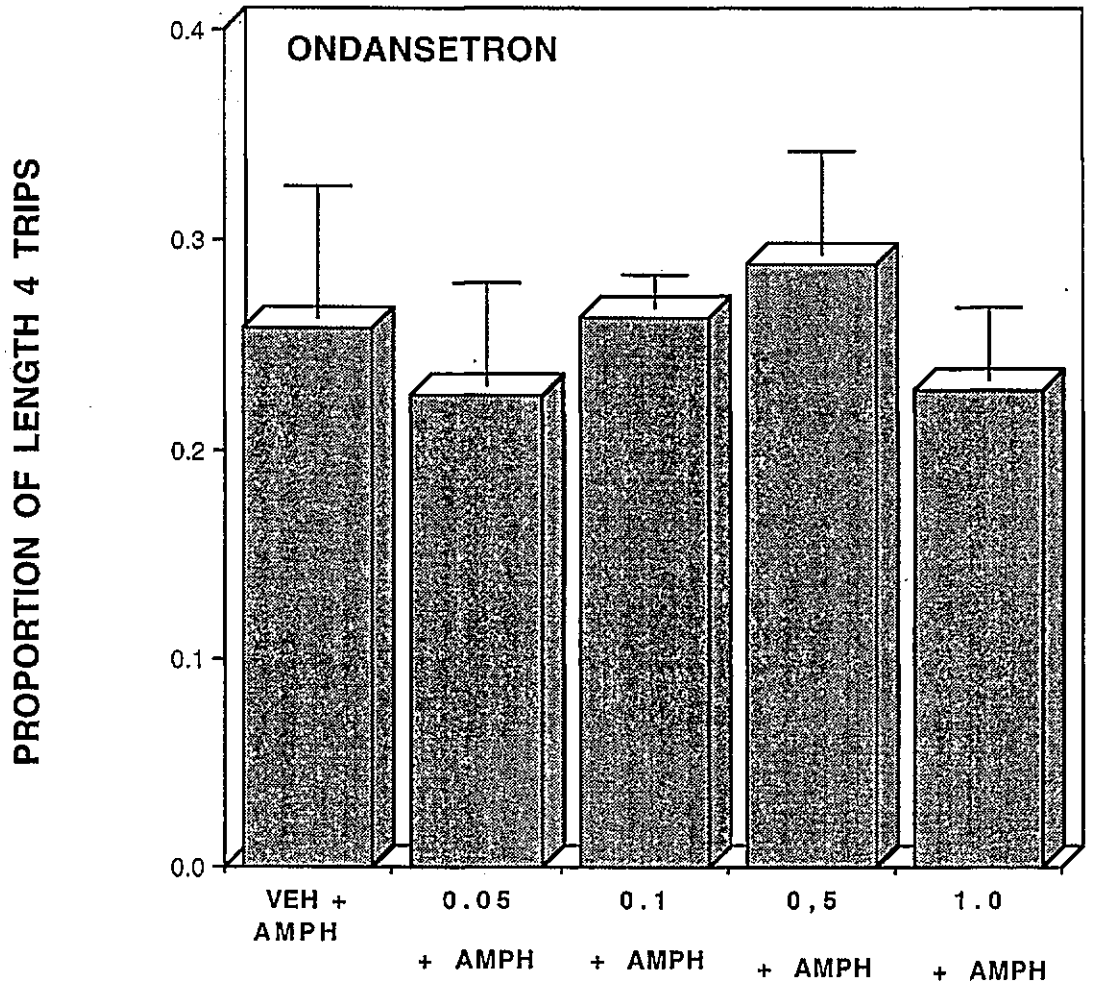

ONDANSETRON
PROPORTION OF LENGTH 4 TRIPS
0.4
0.3
0.2
0.1
0.0
VEH + AMPH
0.05 + AMPH
0.1 + AMPH
0,5 + AMPH
1.0 + AMPH

Figure 6.4

Proportion of legth 4 trips made at each 5 min session interval by rats (n = 9) treated with 3.5 mg/kg amphetamine and pre-treated with vehicle or 0.05-1.0 mg/kg ondansetron.

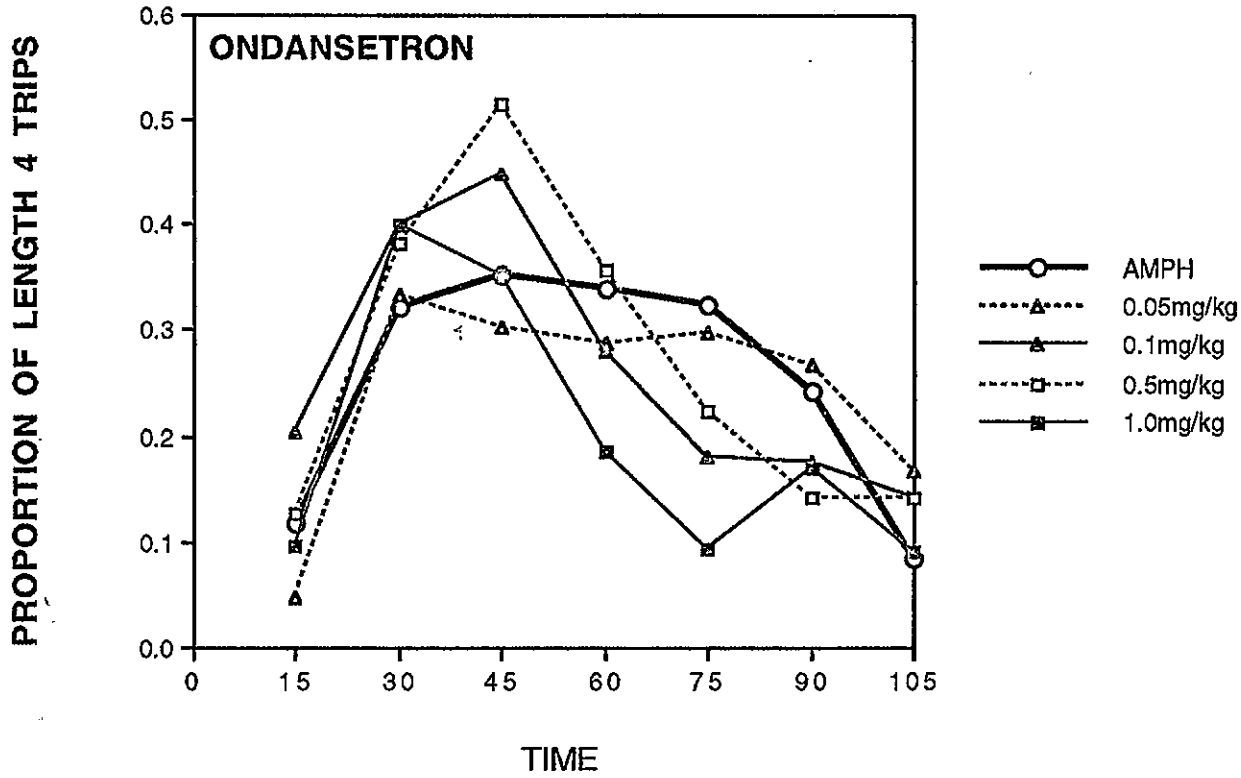

ONDANSETRON
PROPORTION OF LENGTH 4 TRIPS
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
15
30
45
60
75
90
105
TIME
AMPH
0.05mg/kg
0.1mg/kg
0.5mg/kg
1.0mg/kg

Figure 6.5

Each session interval shows the cumulative percentage of the total distance moved by rats (n = 9) treated with 3.5 mg/kg amphetamine and pre-treated with vehicle or 0.05-1.0 mg/kg ondansetron.

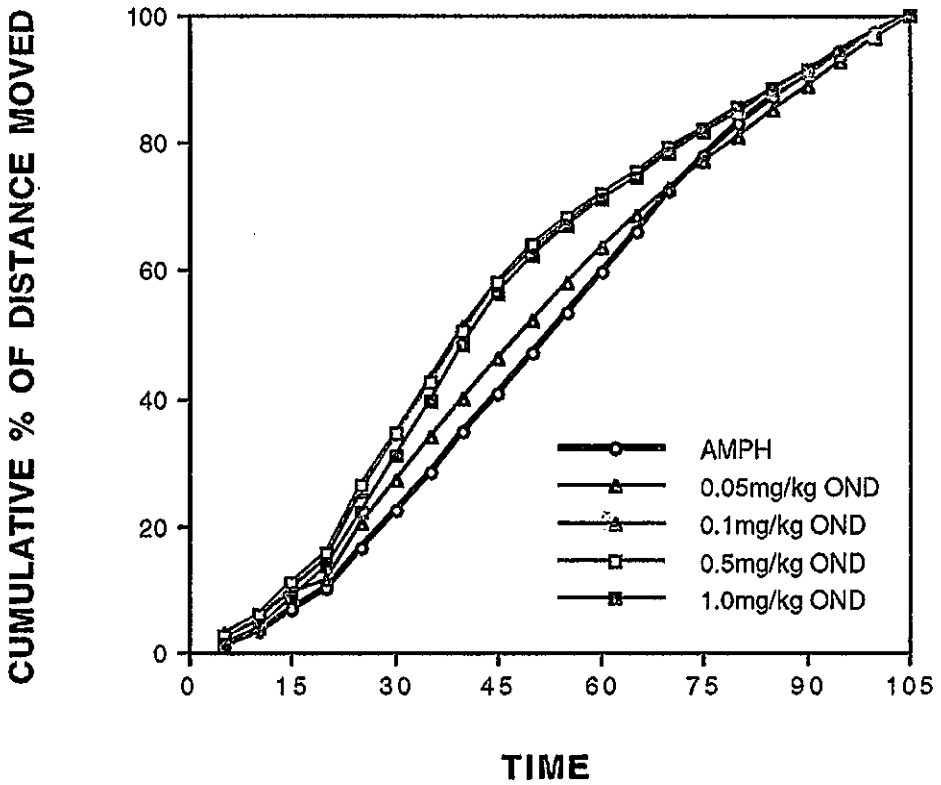
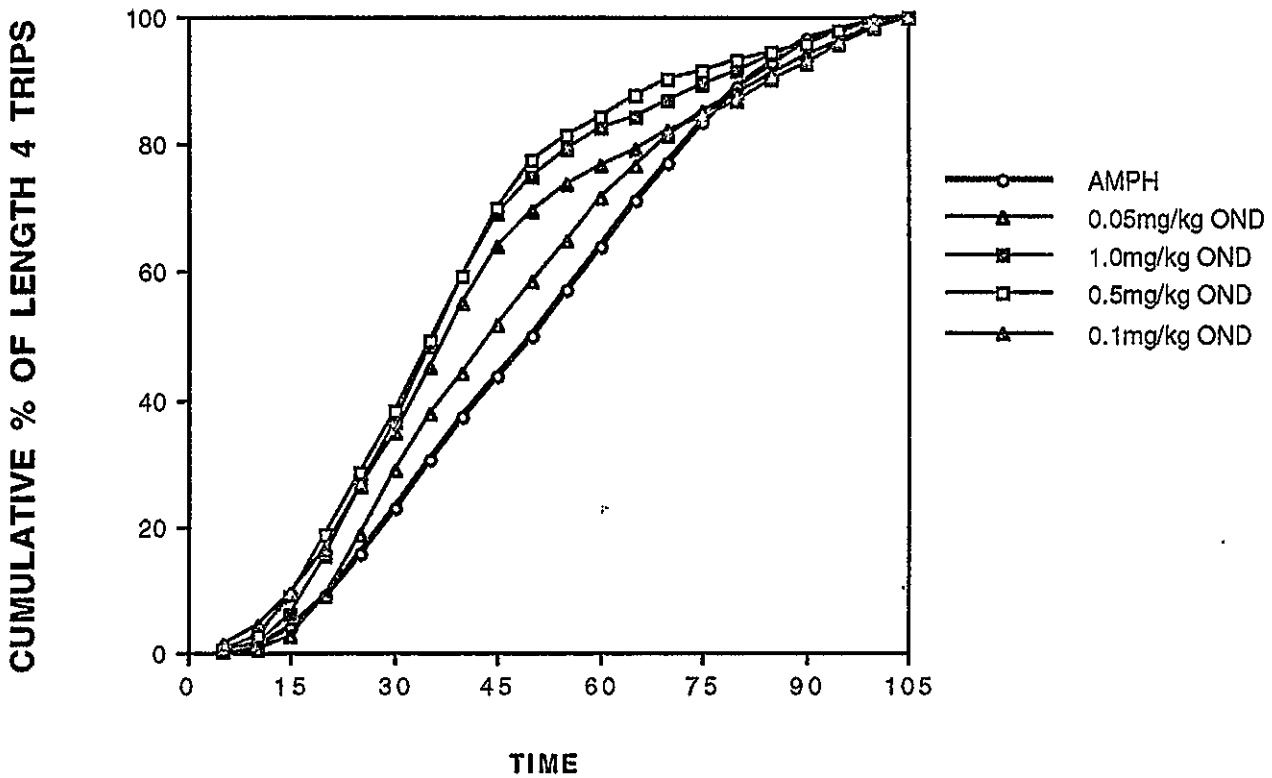

CUMULATIVE % OF DISTANCE MOVED
100
80
60
40
20
0
0
15
30
45
60
75
90
105
TIME
AMPH
0.05mg/kg OND
0.1mg/kg OND
0.5mg/kg OND
1.0mg/kg OND

Figure 6.6

Each session interval shows the cumulative percentage of the total number of length 4 trips by rats (n = 9) treated with 3.5 mg/kg amphetamine and pre-treated with vehicle or 0.05-1.0 mg/kg ondansetron.


CUMULATIVE % OF LENGTH 4 TRIPS
100
80
60
40
20
0
0
15
30
45
60
75
90
105
TIME
AMPH
0.05mg/kg OND
1.0mg/kg OND
0.5mg/kg OND
0.1mg/kg OND

Behavioural analysis.

Figure 6.7 shows the effect of ondansetron pre-treatment on each of the behavioural categories. The results of the one-way ANOSIM indicated that there was a significant difference in the behaviours exhibited by treatment groups. The sample statistic (Global R) = 0.108 p< .023. Examination of the differences between treatment groups revealed that pre-treatment with 0.1, 0.5, or 1.0 mg/kg ondansetron resulted in a significant difference in behavioural categories compared with vehicle-amphetamine treated animals. See Table 6.1.

Table 6.1. Significance levels of vehicle-amphetamine groups compared with ondansetron pre-treated groups.

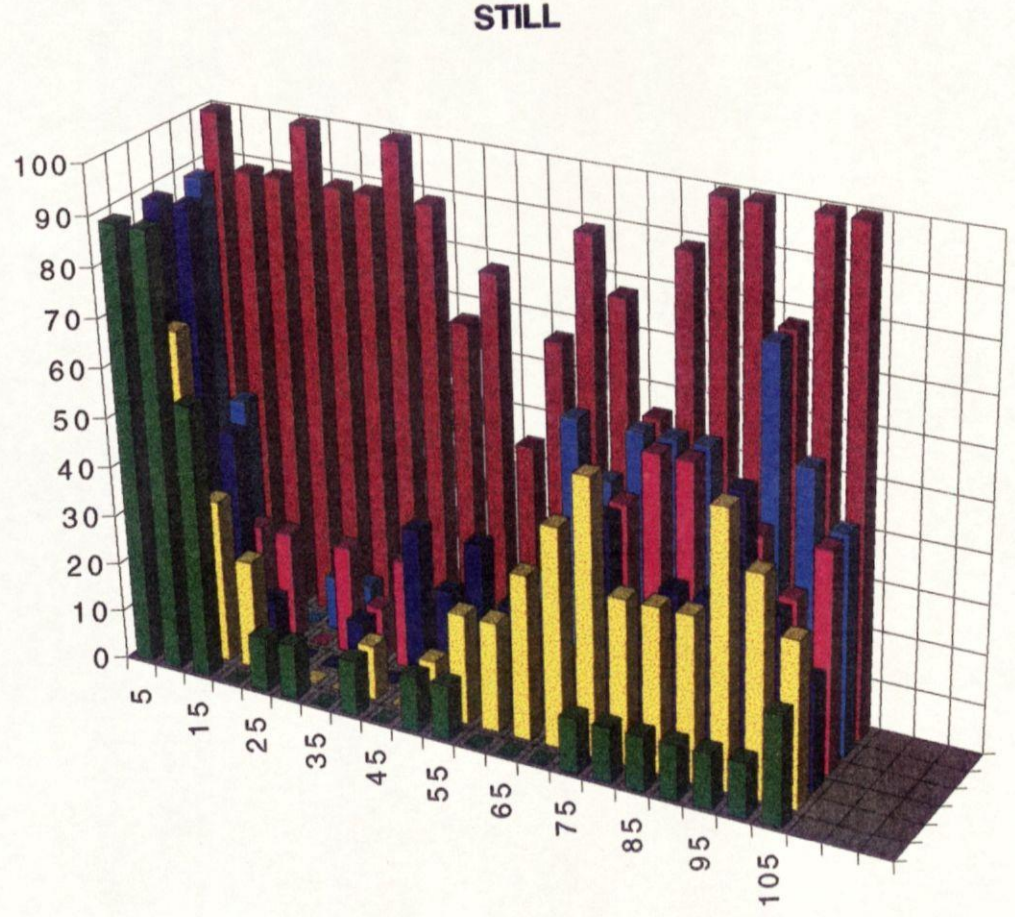
Veh-Amph versus	**0.05mg/kg Ondansetron**	**0.1mg/kg Ondansetron**	**0.5mg/kg Ondansetron**	**1.0 mg/kg Ondansetron**
∂		37.16	36.80	38.76
	NS	p<0.003	p<0.01	p<0.01

Veh-Amph v 0.1mg/kg ondansetron. Compared with vehicle-amphetamine treated animals, pre-treatment with 0.1mg/kg ondansetron resulted in an increase in **Locomotion without Pause** between session intervals 30-40 minutes, but by session interval 45 min there was a decline in locomotion. **Rearing in the Open** was unaffected by pre-treatment with 0.1mg/kg ondansetron. **Rearing against the Wall** occurred earlier in the test session than for vehicle-amphetamine treated animals and appeared to be associated with the period at which maximum levels of locomotor activity occurred. Pre-treatment with 0.1mg/kg ondansetron increased **Head-Swaying** behaviour from session interval 45 min and was markedly increased in these animals between session intervals 45-90 min. In addition to an increase in **Head-Swaying** behaviour these animals also made snout contact earlier in the test session (30 min), and maintained an increase in **Head-Down** posture throughout the remainder of the test session. Table 6.2 shows the results of the

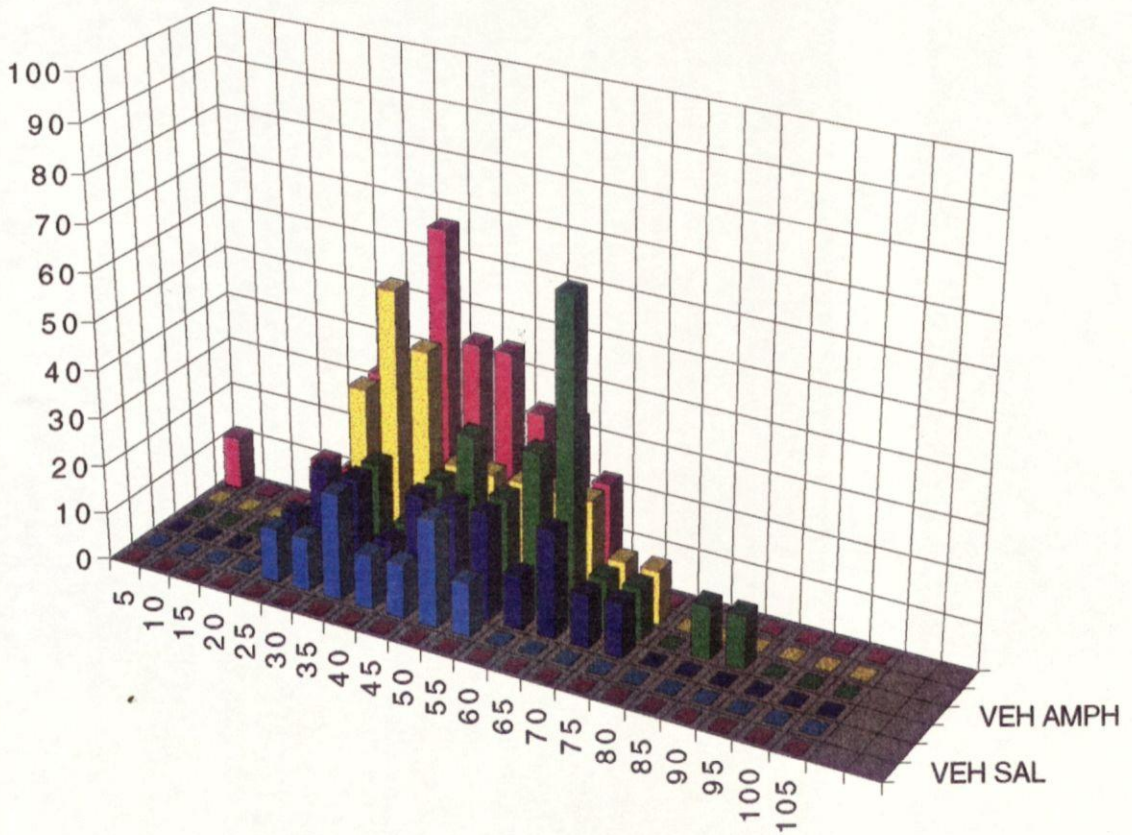
similarities terms analysis (SIMPER), which revealed that an increase in **Head-Swaying** behaviour between session intervals 50-70 min contributed to the first 30% difference between these two treatment groups. An increase in snout contact between session intervals 60-100 min also contributed to the first 30% difference between the two groups. The SIMPER also revealed that a decrease in pausing during the early locomotor phase of the amphetamine response occurred following pre-treatment with 0.1 mg/kg ondansetron, and this contributed to the significant difference between the two groups. The commencement of locomotor activity for animals pre-treated with ondansetron occurred 15 minutes into the test session, earlier than for animals treated with vehicle-amphetamine. This earlier onset of locomotor activity also contributed to the first 30% difference between the two groups.

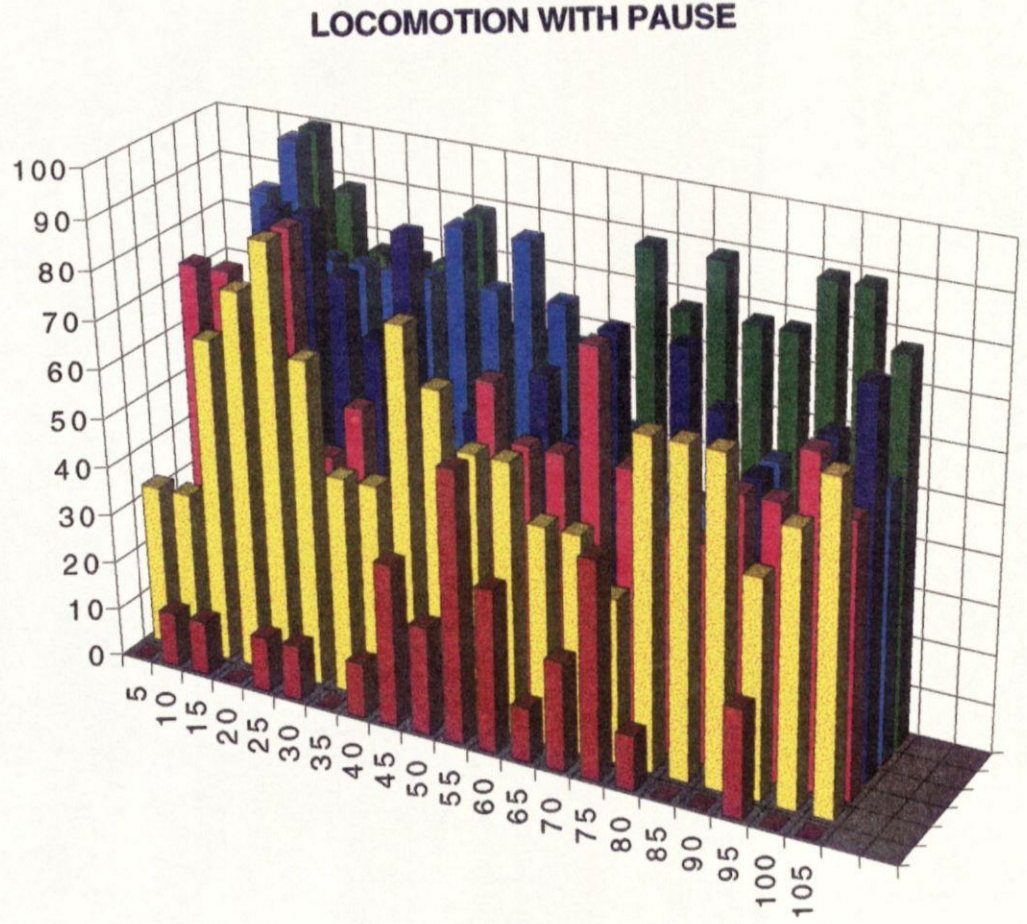
Figure 6.7

The percentage of animals exhibiting each of the behavioural categories during the 105-min observation period by rats (n = 9 per group) injected with vehicle-saline (**red**), Vehicle-amphetamine (**green**), 0.05 mg/kg ondansetron + 3.5 mg/kg amphetamine (dark blue), 0.1 mg/kg ondansetron + 3.5 mg/kg amphetamine (yellow), 0.5 mg/kg ondansetron + 3.5 mg/kg amphetamine (magenta), 1.0 mg/kg ondansetron + 3.5 mg/kg amphetamine (light blue).

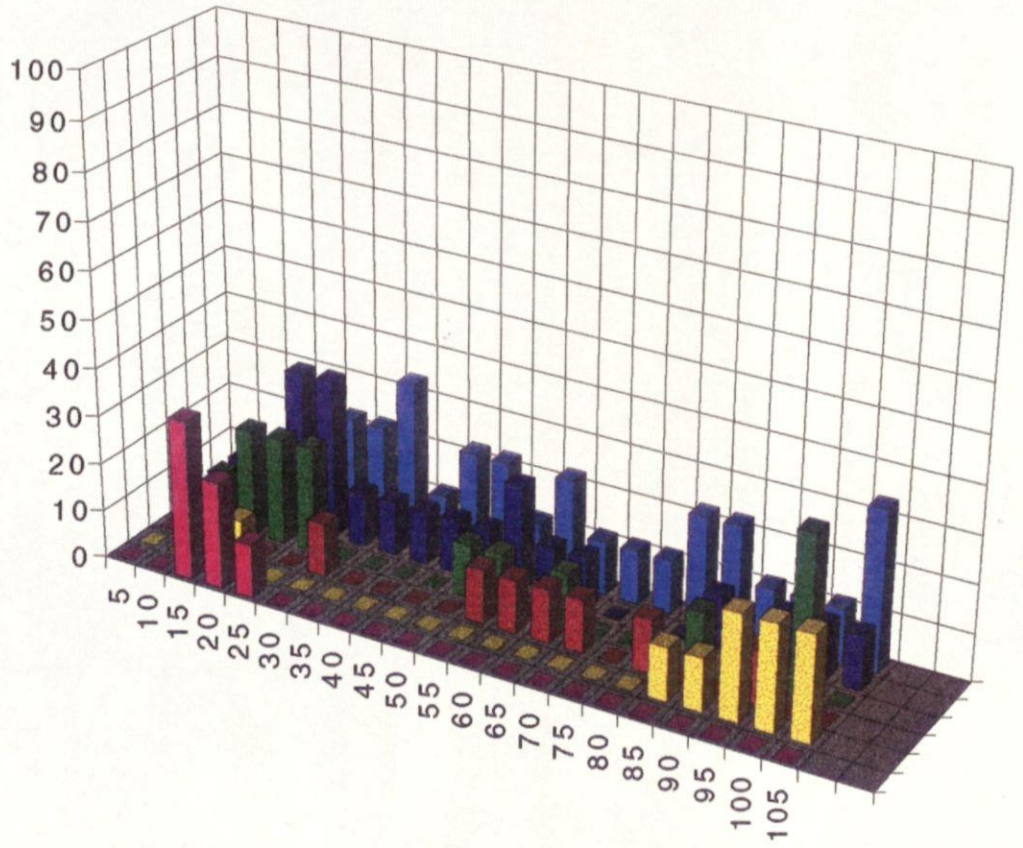

STILL
100
90
80
70
60
50
40
30
20
10
0
5
15
25
35
45
55
65
75
85
95
105

LOCOMOTION

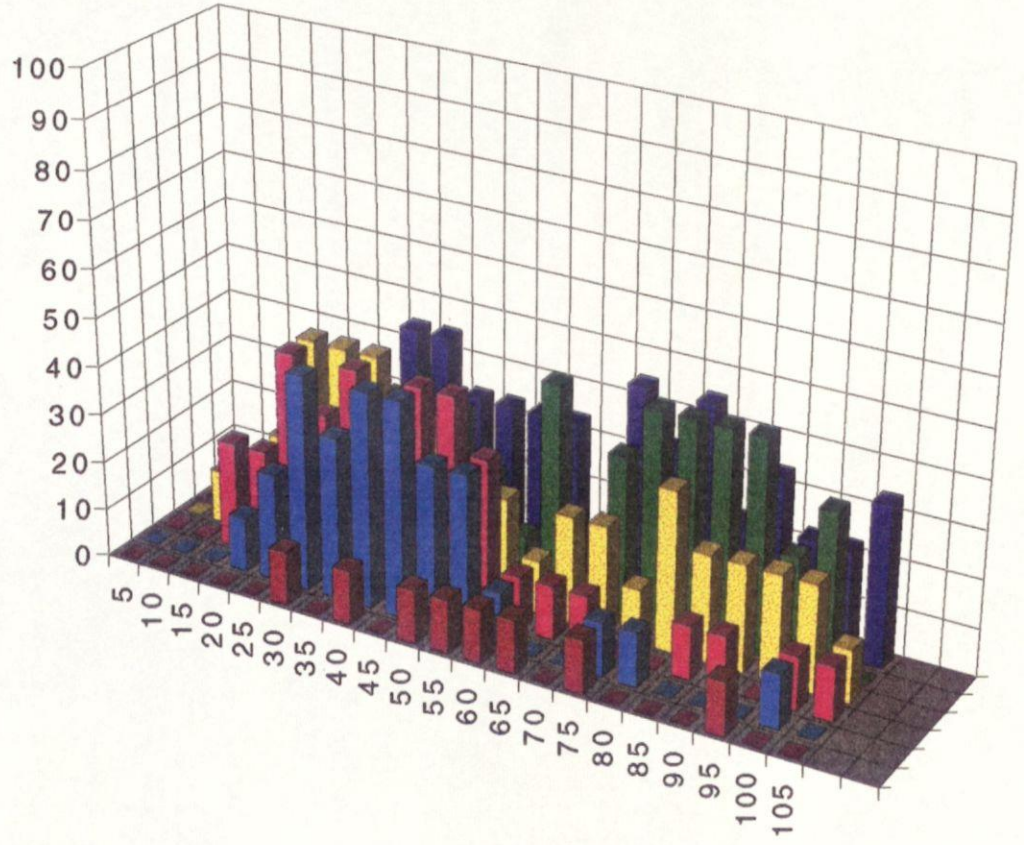

100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
VEH AMPH
VEH SAL

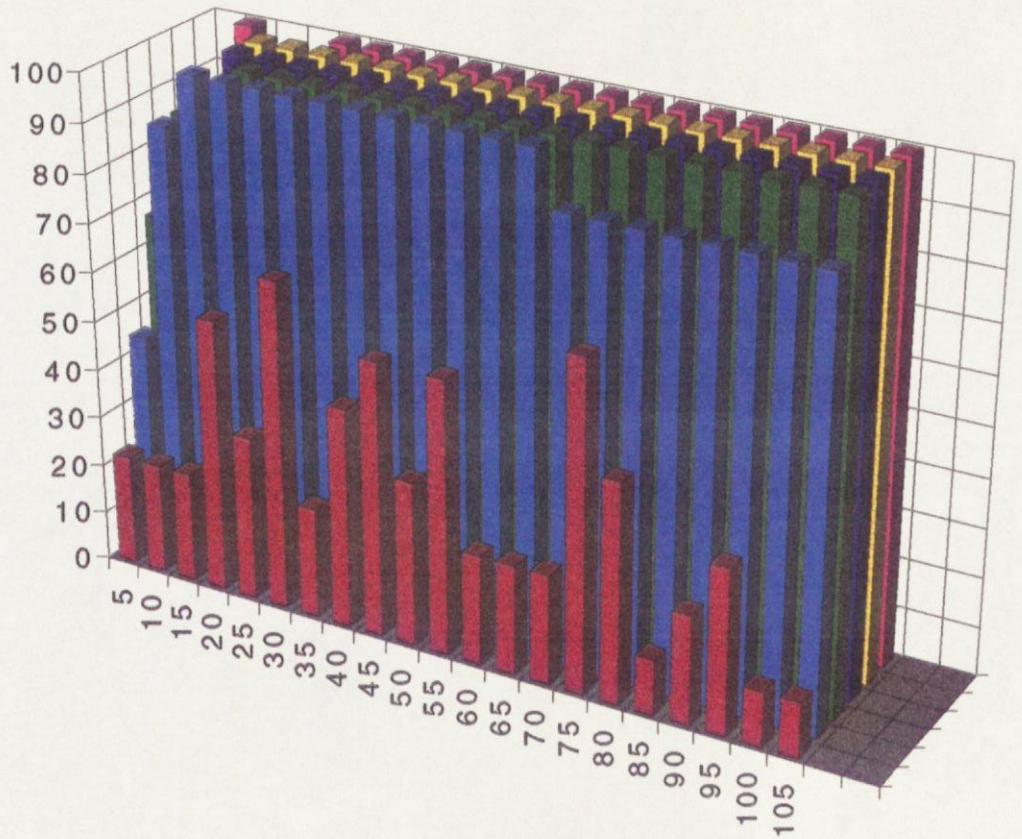

LOCOMOTION WITH PAUSE
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105

REARING IN OPEN

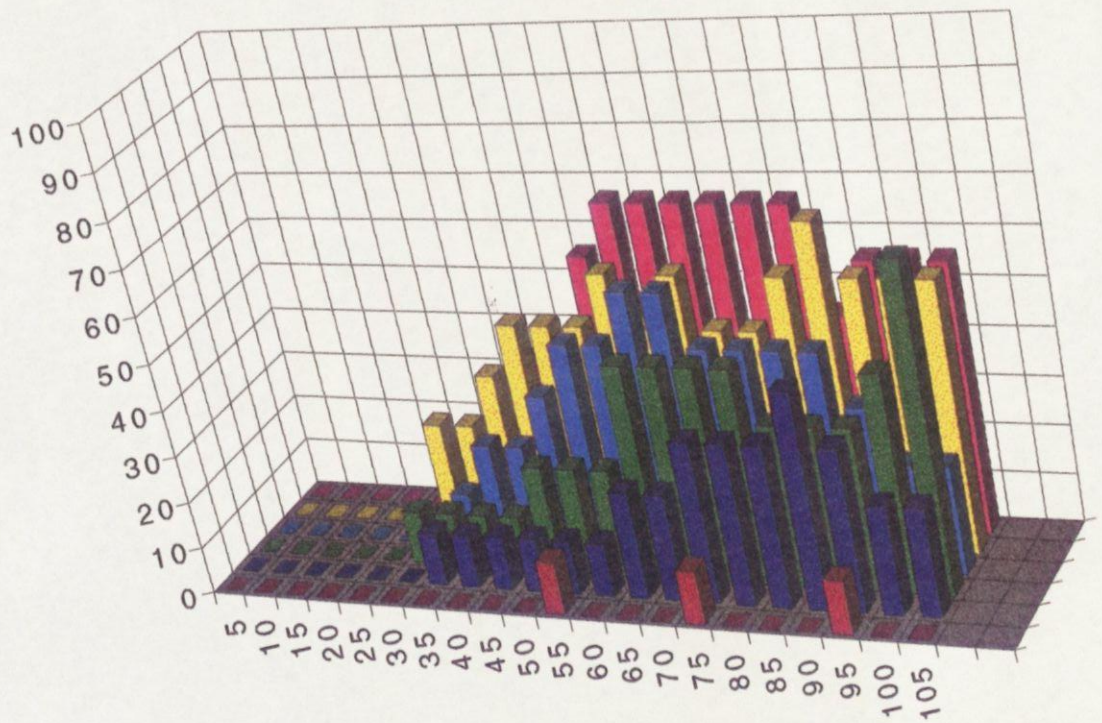


REARING AGAINST WALL




SNIFFING
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105

HEAD-DOWN


100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105

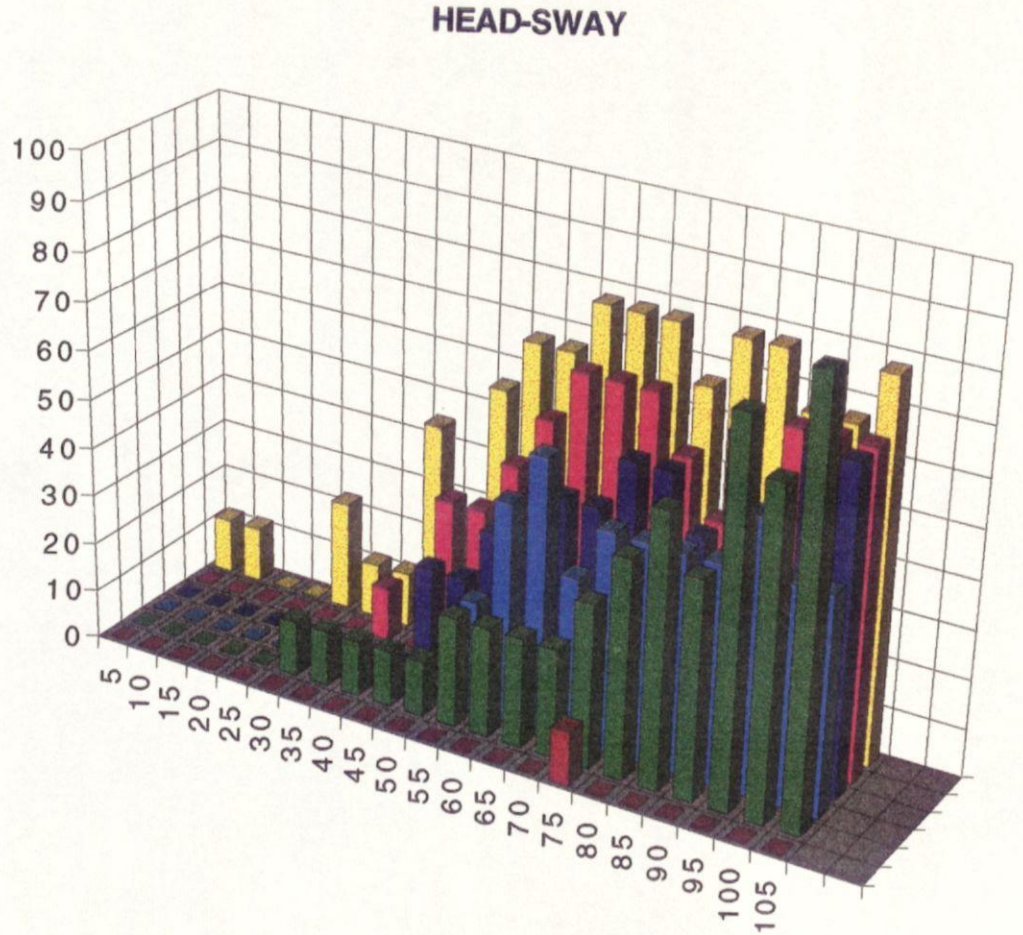

HEAD-SWAY
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105

Table 6.2. Percentage (ABUND) of behaviours in group A (0.1mg/kg ondansetron) and group B (vehicle-amphetamine). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(= 37.16) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 30%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
65	head-s	0.78	0.22	.55	.41	1.36	1.49
70	head-s	0.78	0.22	.55	.41	1.36	2.97
75	head-s	0.78	0.33	.51	.43	1.19	4.33
65	loco	0.22	0.67	.50	.42	1.19	5.68
60	head-s	0.67	0.22	.50	.42	1.19	7.01
55	head-s	0.67	0.22	.50	.42	1.19	8.35
75	loco+p	0.33	0.78	.49	.42	1.18	9.68
90	head-d	0.67	0.33	.48	.43	1.10	10.96
70	loco+p	0.44	0.89	.46	.43	1.07	12.20
90	head-s	0.78	0.44	.46	.44	1.05	13.44
40	loco+p	0.44	0.78	.45	.43	1.05	14.66
95	loco+p	0.44	0.89	.45	.42	1.07	15.88
95	still	0.56	0.11	.45	.42	1.07	17.10
35	loco	0.56	0.22	.45	.43	1.04	18.32
35	loco+p	0.44	0.78	.45	.43	1.04	19.54
50	head-s	0.56	0.11	.45	.42	1.08	20.75
75	still	0.56	0.11	.45	.42	1.07	21.96
15	loco+p	0.67	0.44	.44	.44	1.02	23.16
60	head-d	0.56	0.22	.44	.42	1.05	24.35
85	head-d	0.56	0.33	.44	.43	1.02	25.44
95	head-d	0.56	0.33	.44	.43	1.02	26.72
80	head-s	0.67	0.44	.44	.43	1.02	27.91
70	head-d	0.56	0.44	.43	.43	1.00	29.07
100	head-d	0.56	0.44	.43	.43	1.00	30.23

Veh-Amph v 0.5mg/kg ondansetron. Pre-treatment with 0.5mg/kg ondansetron increased locomotor activity earlier in the test session, with the majority of ondansetron treated animals engaging in **Locomotion** at session interval 10 min. In addition pre-treatment with 0.5 mg/kg ondansetron resulted in a marked decrease in pausing between session intervals 35-65 min compared with vehicle-amphetamine treated animals. **Rearing in the Open** was unaffected by treatment with ondansetron. Like animals treated with 0.1 mg/kg ondansetron, **Rearing against the Wall** appeared to be associated with the period of peak locomotor activity and consequently occurred earlier than for vehicle-amphetamine treated animals. Pre-treatment with 0.5 mg/kg ondansetron resulted in an increase in **Head-Swaying** between session intervals 50-75 min. There was also a marked increase in snout contact between session intervals 55-85 min, with this group displaying the greatest increase in **Head-Down** posture of all the treatment groups. Table 6.3 shows the results of the SIMPER, which revealed that an earlier onset of locomotion, and an associated decrease in pausing during locomotor bouts following pre-treatment with ondansetron accounted for the first 6% difference between the two groups. An increase in **Head-Swaying** and snout contact between session intervals 55-85 min also accounted for the first 30% difference between these two treatment groups.

Table 6.3. Percentage (ABUND) of behaviours in group A (0.5mg/kg ondansetron) and group B (vehicle-amphetamine). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(= 36.80) between the two groups, with a cut-off when the cumulative % contribution ($\sum \partial i\%$) to ∂ reaches 30%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	$\partial(i)$	$SD(\partial i)$	$\partial(i)/SD(\partial i)$	$\sum \partial i\%$
10	loco+p	0.78	0.11	.63	.41	1.54	1.72
40	loco+p	0.11	0.78	.61	.39	1.56	3.37
40	loco	0.67	0.11	.55	.43	1.28	4.87
65	loco	0.22	0.67	.52	.44	1.18	6.27
60	head-d	0.67	0.22	.52	.43	1.19	7.67
65	head-s	0.67	0.22	.51	.43	1.19	9.06
70	head-s	0.67	0.22	.51	.43	1.19	10.44
85	head-d	0.67	0.33	.49	.45	1.10	11.77
75	head-s	0.67	0.33	.49	.44	1.10	13.10
15	loco+p	0.78	0.44	.47	.45	1.04	14.38
85	still	0.56	0.11	.47	.44	1.07	15.66
80	loco+p	0.44	0.89	.47	.44	1.07	16.93
80	still	0.56	0.11	.47	.44	1.07	18.20
30	loco+p	0.44	0.78	.46	.44	1.04	19.46
65	head-d	0.67	0.44	.46	.45	1.02	20.72
70	head-d	0.67	0.44	.46	.45	1.02	21.97
75	head-d	0.67	0.44	.46	.45	1.02	23.22
80	head-d	0.67	0.44	.46	.45	1.02	24.47
55	head-d	0.56	0.22	.46	.44	1.05	25.72
85	loco+p	0.44	0.78	.46	.44	1.04	26.97
60	head-s	0.56	0.22	.45	.43	1.05	28.21
50	loco+p	0.44	0.67	.45	.44	1.02	29.44
95	head-s	0.56	0.33	.45	.44	1.00	30.66

Veh-Amph v 1.0 mg/kg ondansetron. The onset of locomotor activity occurred earlier following pre-treatment with 1.0 mg/kg ondansetron, unlike groups treated with the two lower doses of ondansetron, pre-treatment with 1.0 mg/kg did not result in a decrease in pausing during locomotor bouts. Animals pre-treated with the maximum dose of ondansetron showed an increase in **Rearing against the Wall** during the early session intervals, unlike other treatment groups, a small proportion of animals continued to **Rear in the Open** throughout the 105 minute test session. Pre-treatment with 1.0 mg/kg resulted in increased **Head-Swaying** behaviour between session intervals 60-65 min, thereafter **Head-Swaying** occurred in fewer animals than in the vehicle-amphetamine treatment group. Pre-treatment with 1.0 mg/kg ondansetron also resulted in an increase in **Head-Down** posture (snout contact) between session intervals 35-65 min. Table 6.4 shows the results of the SIMPER analysis. An increase in locomotor activity early in the test session, and a decline in locomotor activity between session intervals 65-100 min contributed to the first 30% difference between the two treatment groups. In addition, an increase in **Head-Down** (snout contact) and **Head-Swaying** between session intervals 65-70 min and a subsequent decrease in both these behaviours in the final 20 minutes of the test session contributed to the first 30% difference between these two groups.

Table 6.4. Percentage (ABUND) of behaviours in group A (1.0mg/kg ondansetron) and group B (vehicle-amphetamine). Behaviours are listed in order of their contribution (∂i) to the average dissimilarity ∂(= 38.76) between the two groups, with a cut-off when the cumulative % contribution (∑∂i%) to ∂ reaches 25%.

TIME	BEHAVIOUR	$ABUND_A$	$ABUND_B$	∂(i)	SD(∂i)	∂(i)/SD(∂i)	∑∂i%
95	loco+p	0.22	0.89	.64	.42	1.54	1.65
95	still	0.78	0.11	.64	.42	1.54	3.31
65	loco	0.00	0.67	.61	.44	1.38	4.87
105	head-d	0.22	0.67	.53	.45	1.18	6.24
65	still	0.56	0.00	.51	.46	1.09	7.55
15	loco+p	0.89	0.44	.50	.47	1.07	8.83
105	head-s	0.44	0.89	.50	.46	1.07	10.11
100	loco+p	0.44	0.89	.49	.46	1.07	11.37
100	still	0.56	0.11	.49	.46	1.07	12.63
10	loco+p	0.56	0.11	.48	.45	1.07	13.87
75	still	0.56	0.11	.48	.45	1.07	15.11
85	still	0.56	0.11	.48	.45	1.07	17.57
75	loco+p	0.44	0.78	.47	.45	1.04	18.81
85	loco+p	0.44	0.78	.47	.45	1.04	20.02
100	head-s	0.44	0.67	.47	.46	1.02	21.23
5	sniff	0.44	0.67	.47	.46	1.02	22.44
65	head-s	0.56	0.22	.46	.44	1.05	23.64
65	head-d	0.56	0.44	.45	.45	1.00	24.84
70	head-d	0.56	0.44	.45	.45	1.00	26.01
85	head-s	0.44	0.44	.45	.45	1.00	27.18

6.4 Discussion

In agreement with previous reports (Costall et al, 1987; Van der Hoek and Cooper, 1990) ondansetron failed to reduce the total distance moved over the 105 minute test session following peripheral administration of amphetamine. Interestingly, administration of a 5HT3 antagonist 30 minutes before peripherally administered amphetamine was not entirely without effect. All doses of ondansetron tested tended to increase locomotor behaviour in the first 45 minutes of the session in an inverse dose-related manner, although this increase in activity was not significant at the time of peak drug action, differences between the means approached significance. It is apparent that ondansetron administered at a dose between 0.1-1.0 mg/kg facilitated the expression of locomotor activity 30-60 minutes following administration of amphetamine. These findings are somewhat in contrast to the action of peripherally administered ondansetron (0.1-1.0 mg/kg) on amphetamine administered directly to the nucleus accumbens, which significantly reduced hyperactivity over a 100 minute test period

More notably, in the latter half of the test session animals pre-treated with 0.1-1.0 mg/kg ondansetron showed a significant reduction in the distance moved during each of the session intervals. Perhaps of greatest interest is the effect of ondansetron (0.05-1.0mg/kg) on length 4 trips. In common with the atypical antipsychotics, clozapine and sulpiride, tested within this animal model, ondansetron failed to reduce the thigmotaxic perimeter circling associated with hyperactivity. In marked contrast to clozapine and sulpiride, ondansetron (0.5mg/kg) succeeded in potentiating the proportion of length 4 trips during session intervals 40-45 minutes and at 55 minutes, an increase unlikely to be related to a general increase in locomotor activity as during these session intervals locomotor activity was declining to levels below those exhibited by vehicle-amphetamine treated animals. One explanation for the increase in thigmotaxic patrolling seen following pre-treatment with an intermediate dose of ondansetron is that a decline in locomotor activity caused by possible 5HT3 receptor antagonism may allow latent stereotyped behaviours to be elicited. This appeared to be manifest initially as an increase in

stereotyped forms of locomotion, which was followed by an increase in head-swaying behaviour, and finally resulted in an increase in head-down posture (snout contact) during the final part of the test session. It would appear that amphetamine animals pre-treated with ondansetron at a dose between 0.1 -1.0mg/kg exhibit an increase in some forms of stereotyped behaviours which appear sequentially, firstly as a decrease in pausing resulting in increased levels of locomotion, followed by a short period in which the locomotor route taken by the animal became increasingly stereotyped, then locomotor activity diminished, to be replaced by head-swaying behaviour, and finally the head posture of the animal changed and the snout dropped to make contact with the floor surface of the open field. The blockade of a 5HT facilitatory effect on locomotor behaviours, may allow other subtypes of 5HT receptors to exert a greater influence on dopamine mediated behaviours. For example, head swaying is mediated by both 5HT1a receptors and striatal dopamine (see Curzon, 1990), and it seems that this behaviour is 'unmasked' following 5HT3 antagonism of an amphetamine-induced locomotor response.

The effect of 5HT receptors on locomotor behaviours is complex and experimental findings have often been contradictory. Evidence supports either a facilitatory or inhibitory action. For example 5HT receptor antagonists have been shown to potentiate dopamine-induced locomotor behaviour (Dourish, 1982), or inhibit dopamine dependant locomotion (Warbritton et al, 1978; Jones et al, 1981). 5HT3 antagonists have been clearly shown to block amphetamine-induced locomotion when amphetamine is administered solely to the nucleus accumbens. A more general distribution of amphetamine within brain structures appears to allow for more complex interactions between 5HT and dopamine to influence response output. It is interesting to note that at higher doses many 5HT3 receptor antagonists show reduced effectiveness, resulting in a bell shaped dose response curve (Costall et al, 1990). The reasons for the loss of activity are not clear but Barnes et al, (1992) speculate that the loss may be a consequence of additional pharmacological interactions, a view entirely consistent with the speculation that changing 5HT3 'tone' on locomotor behaviours may allow other 5HT-dopamine interactions to

influence behaviour. In the current study the bell shaped dose response curve which has been reported by several researchers was clearly evident. Animals pre-treated with the lowest dose of ondansetron (0.05mg/kg) showed no significant effect on amphetamine-induced behaviours, whilst animals treated with the highest dose of ondansetron showed less effect on amphetamine-induced behaviours than animals treated with an intermediate dose of 0.1 - 0.5 mg/kg ondansetron. Van der Hoek and Cooper (1990) found no effect following pre-treatment with 0.03 mg/kg ondansetron in animals treated with 3 mg/kg amphetamine and this would agree with the present findings in which a similar dose (0.05mg/kg) had no significant effect on amphetamine-induced behaviours.

Examining locomotor behaviour in greater detail has provided evidence that although the net amount of amphetamine-induced hyperactivity does not change, the temporal characteristics of locomotion are altered following 5HT3 antagonism. Several authors have reported that photocell beam counts fail to detect changes in the length of locomotion (Fink and Smith 1979, 1980: Fray et al, 1980; Krsiak et al, 1970) and this could well account for studies using photocell beam measures of locomotor activity failing to detect changes in the locomotor response following treatment with ondansetron and peripherally administered amphetamine. The current method of measuring locomotion also detected an increase in the stereotyped nature of amphetamine-induced locomotion, midway through the test session. Clearly further work is required to examine the effect of 5HT3 antagonists on locomotor behaviours, although the findings of the present study reinforce the view that the study of both the temporal and spatial aspects of amphetamine-induced open field locomotion provide a more accurate 'picture' of this behaviour and has the potential to detect more subtle changes in the response following pre-treatment with compounds which interact with amphetamine-induced hyperactivity.

Summary

This experiment examined the effect of the 5HT3 receptor antagonist, ondansetron, on amphetamine-induced behaviours. The findings showed that animals pre-treated with ondansetron demonstrated an increase in some forms of stereotyped behaviours which appeared sequentially. This was manifest first as a decrease in pausing resulting in increased levels of locomotion, followed by a period mid-session in which the locomotor route taken by the animal became increasingly stereotyped. Finally, locomotor activity diminished to be replaced by an increase in head-swaying and head-down posture (snout contact).

It would appear that latent behaviours are 'unmasked' following 5HT3 antagonism of an amphetamine-induced locomotor response. This is seen as an example of behavioural competition whereby blocking a facilitatory effect of 5HT3 on a dopamine-mediated behaviour allows other subtypes of 5HT receptors to exert a greater influence on amphetamine-induced behaviours.

Chapter Seven.
General discussion and conclusions.

7.1 Amphetamine-induced stereotyped locomotion.

The findings of the current series of experiments show that rats given amphetamine at doses between 1 and 4 mg/kg became hyperactive, and that with increasing dose, the route taken by the animal became perseverative. The categorisation of forward movement into a series of trip-lengths using contrast based image analysis, and specially written software (Kenyon, 1991) enabled a complete quantification of the distinctive changes that occurred in the spatial distribution of locomotion seen under each dose of the drug (see Chapter 3). This is in direct contrast to previous research which had been unsuccessful in fully quantifying these locomotor changes (eg Lat, 1965; Schiorring, 1979; Mueller et al, 1989a, 1989b). In the current series of experiments it was found that maximum levels of locomotion were induced following 4 mg/kg amphetamine, and that animals given the drug at doses higher than this produced less locomotion. The increase in perimeter circling measured as an increase in length 4 trips paralleled the increase in hyperactivity, with maximum levels of length 4 trips also observed following 4 mg/kg amphetamine. Indicating that contrary to the findings of Mueller et al, (1989b) maximum levels of stereotyped locomotion were observed under 4 mg/kg amphetamine. The proportion of length 4 trips declined in animals given amphetamine at the higher dose of 5 mg/kg.

It is hypothesised that the stereotyped nature of locomotion seen following intermediate doses of amphetamine reflects a link between the locomotor output mediated by the nucleus accumbens and stereotyped behaviours which are mediated by the caudate-putamen. As the dose of amphetamine increases the behavioural output of these two systems becomes increasingly antagonistic, and it would appear

that stereotyped locomotion, measured as an increase in length 4 trips, is an attempt by the animal to resolve this conflict when the output from the caudate-putamen is insufficient to override the locomotor activity mediated by the nucleus accumbens.

7.2 Stereotyped locomotion and snout contact.

Categorisation of the behaviours elicited in rats following administration of 1-5 mg/kg amphetamine showed that a change in head-posture occurred with increasing dose of amphetamine. The head of the rat dropped below a horizontal plane until the snout came into contact with the open field floor. This abnormal posture was associated with a decline in forward progression which occurred following treatment with the highest doses (4 or 5 mg/kg) of the drug.

The categorisation of behavioural elements was made in such a way as to support the proposed model of locomotion, thus the main interest focused on snout contact, rearing and locomotion (see Chapter 5), using an adaptation of the method proposed by Fray et al. (1980). An attempt was made to examine the role of snout contact on the development of length 4 trips by manipulating contact with the surface of the open field by altering the shape of the perimeter. The findings indicated that an open field with a circular perimeter wall allowed for the development of locomotor activity more quickly, and for peak levels of activity to be achieved earlier in the test session, than when these animals were tested in a field with a square perimeter wall.

Despite finding significant differences in locomotion and stereotyped locomotion in open fields with different shaped perimeters there was no significant difference in any other behaviours measured, although there was some evidence to suggest that there was a trend for animals treated with 4 mg/kg amphetamine to engage in head-down posture earlier in the circular field. The square wall surface periodically broke the animal's snout contact with the floor of the arena. Certainly future work should investigate this aspect of behaviour, particularly in relation to the link between snout contact and forward progression. Furthermore, it is clear

that an increase in the proportion of length 4 trips associated with amphetamine-induced hyperactivity was not an artefact of the circular nature of the perimeter wall, as dose-related increases in this measure of stereotyped locomotion were found in both types of open field. The findings suggest that the unbroken circular perimeter wall facilitated the expression of stereotyped locomotion and led to an earlier induction of perseverative locomotor routes.

It is interesting to note that many researchers have reported the influence that environment plays in the expression of behaviour following administration of amphetamine (eg Oades, 1985; Szechtman, 1982; Robbins et al, 1990). It is probable that the differences in locomotor responses seen in different shaped fields would not have been detected using conventional photocell beam measures, and was detected as a direct consequence of the sensitivity of a contrast based image detection system, which tracked the exact position of the animal.

As a consequence of these studies into the dose response characteristics of amphetamine-induced locomotion and stereotyped locomotion it was decided that 4 or 5 mg/kg of the drug led to a less robust measure of length 4 trips, which was readily disrupted by factors such as field shape. Subsequent studies used a dose of 3.5mg/kg amphetamine. Furthermore, this dose (3.5mg/kg) was close to the dose at which maximum levels of locomotion were observed and it was clear that this would allow for the detection of a facilitatory or inhibitory effect of drug interactions within this model.

7.3 Interaction of antipsychotic drugs and stereotyped locomotion.

Treatment with the atypical antipsychotics clozapine and sulpiride 30 min before administration of 3.5mg/kg amphetamine had no significant effect on measures of stereotyped locomotion, despite showing a considerable capacity to reduce amphetamine-induced hyperactivity (see chapter 4). This finding is in agreement with the actions of atypical antipsychotic drugs on other amphetamine-induced behaviours where they have been shown not to antagonise stereotyped behaviours, and in some reports to even potentiate some stereotyped behaviours

(Robertson and MacDonald, 1984,1985). In contrast, the classical antipsychotic drug haloperidol brought about a marked reduction in both locomotion and stereotyped locomotion, again an effect which is entirely consistent with this drug's ability to block other amphetamine-induced behaviours. Furthermore, it seems unlikely that a decline in length 4 trips following haloperidol was associated with a general decline in activity. The dose of amphetamine was chosen specifically to ensure that not all hyperactivity was eliminated following pre-treatment with antipsychotic drugs, and that the locomotion remaining following pretreatment with haloperidol showed a noticeable decline in the proportion of length 4 trips in marked contrast to equivalent levels of locomotion following pretreatment with both clozapine and sulpiride, which remained stereotyped in nature.

In addition to focusing attention on dopamine receptor antagonists with established antipsychotic action it was necessary to examine the action of putative antipsychotic agents within the proposed model system. It is increasingly evident that many non-dopaminergic compounds have antipsychotic potential, and serotonin (5HT) as well as dopamine influences the behavioural response following treatment with amphetamine. Many 5HT antagonists are known to block amphetamine-induced hyperactivity, and this is often seen as support for their antipsychotic potential (see Chapter 6). Currently much interest has focused on the influence of 5HT3 receptors on hyperactivity seen following stimulation of the mesolimbic system with either dopamine or amphetamine (Costall et al. 1987). A 5HT3 antagonist seemed an appropriate drug with which to further examine the potential of this proposed model system, particularly as a 5HT3 antagonist (ondansetron) had been found to antagonise hyperactivity following direct administration of amphetamine into the mesolimbic system but not following peripheral administration of amphetamine (Costall et al, 1987, Van der Hoek and Cooper, 1990).

A study examining the effects of the 5HT3 antagonist ondansetron on hyperactivity and stereotyped locomotion following intra-peritoneal injection of amphetamine was able to shed light on some of these paradoxical findings and at the

same time provide support for the claim that this model system provides a more sensitive and complete measurement of locomotor activity, providing information not detectable using conventional photocell beam measures.

The results showed that pre-treatment with ondansetron facilitated locomotor activity early in the test session resulting in a decline in pausing and potentiated stereotyped locomotion, this drug was also found to increase head swaying and the head down posture seen following treatment with higher doses of amphetamine but rarely seen in animals treated with 3.5 mg/kg amphetamine. The D2 specific antagonist, sulpiride, was also found to potentiate head swaying and the head down posture adopted by amphetamine treated animals, confirming other reports that sulpiride potentiates some aspects of amphetamine-induced stereotyped behaviour (Robertson and MacDonald, 1985; Sharp et al, 1986).

The current studies have shown that locomotion is not a unitary behaviour, rather it is a complex phenomenon which changes both quantitatively and qualitatively following treatment with amphetamine, and has the capacity to become stereotyped in a manner similar to other behaviours which are seen to 'fragment' following treatment with amphetamine.

It is hypothesised that the measurement of increases in locomotion and stereotyped locomotion can be seen as a model of both raised mesolimbic and caudate-putamen function and as such has the capacity to identify drugs which interact with either one or both of these systems. Drugs which fail to antagonise the stereotyped aspects of amphetamine-induced locomotion, despite a marked capability to reduce locomotion are most likely to have a reduced capacity to induce extrapyramidal side-effects. Further work is clearly required to examine the nature of open field hyperactivity following direct intracerebral administration of either amphetamine or dopamine to various regions particularly the nucleus accumbens, amygdala, and caudate-putamen. Fink and Smith (1980) suggest that amphetamine-induced locomotor activity depends on the 'mass action' of the central dopamine systems whilst Kelly and Moore (1976) produced a behaviour analogous to stereotyped locomotion by unilateral depletion of dopamine from the head of the

caudate, which determined the direction of rotation of locomotion in amphetamine treated rats whilst depletion of dopamine from the nucleus accumbens determined its rate. This is similar to the current proposal that stereotyped aspects of amphetamine-induced locomotion depend on both structures. Robbins et al, (1990), describe an interesting unpublished study whereby they injected amphetamine into the nucleus accumbens and the head of the caudate-putamen and compared a full dose to either structure with half the dose administered to both structures. Their findings are similar to those of the current investigation in that the strongest effects were obtained when amphetamine was administered to both structures and the behaviours which seemed to be most affected were head swaying, head down posture and locomotion. It would be interesting to examine the effects of combined nucleus accumbens and caudate-putamen bilateral micro injection on components of unconditioned behaviour, particularly with respect to the spatial distribution of open-field locomotion as described in the present model system.

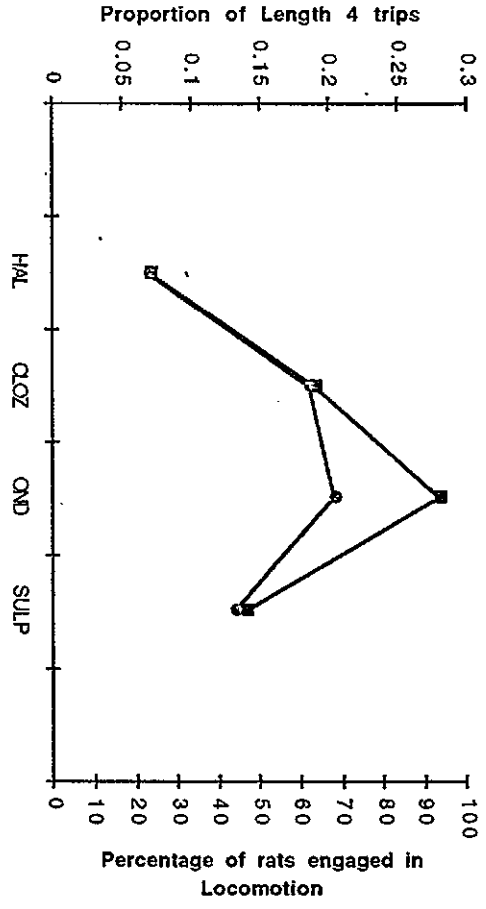
Another explanation for the finding that both ondansetron and sulpiride bring about a reduction in amphetamine-induced locomotion whilst at the same time increasing the amount of stereotyped head behaviours, and in the case of ondansetron, potentiating the stereotyped nature of locomotion for a short period before stereotyped head movements were induced, could be that pre-treatment with either of these drugs brings about a leftwards shift of the behavioural dose response curve.

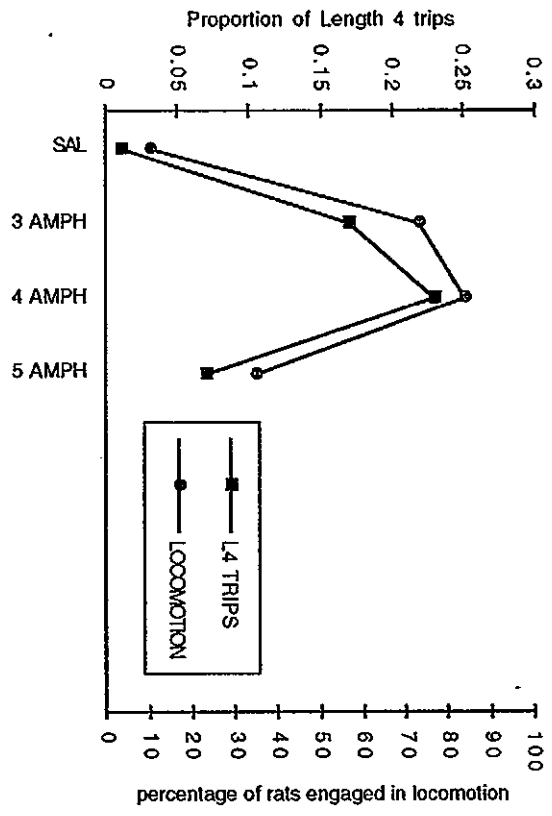
Figure 7.1(a) shows the behavioural dose response curve for 3 to 5 mg/kg amphetamine, and (b) for each intermediate dose of antipsychotic tested. The behavioural profile seen in animals given 3.5mg/kg amphetamine and pre-treated with either ondansetron or sulpiride resembles the behavioural profile of animals given a higher dose of amphetamine. Certainly the profile of animals pre-treated with 20mg/kg sulpiride (see Figure 7.1b) is very similar to that of animals treated with the higher dose of 5mg/kg amphetamine (see Figure 7.1a). Sharpe et al, (1986) argue that the prolongation of the dopamine releasing effects of amphetamine following pretreatment with sulpiride does not correspond to that of a

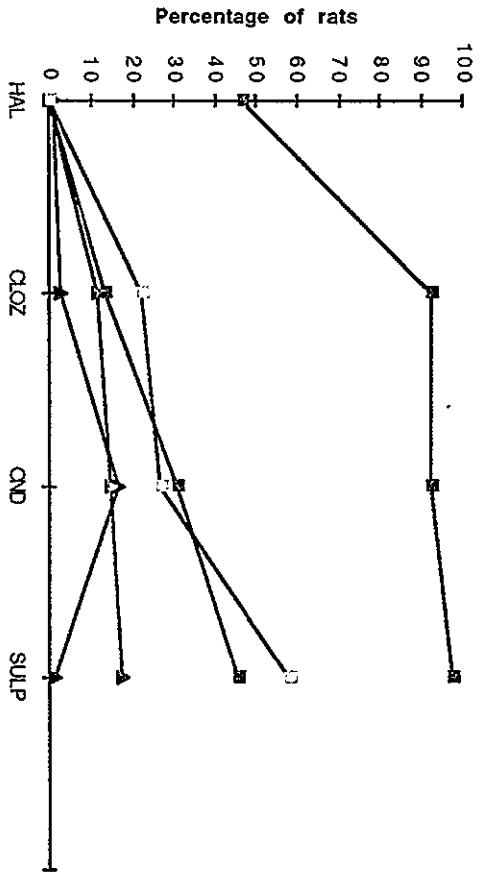
Figure 7.1

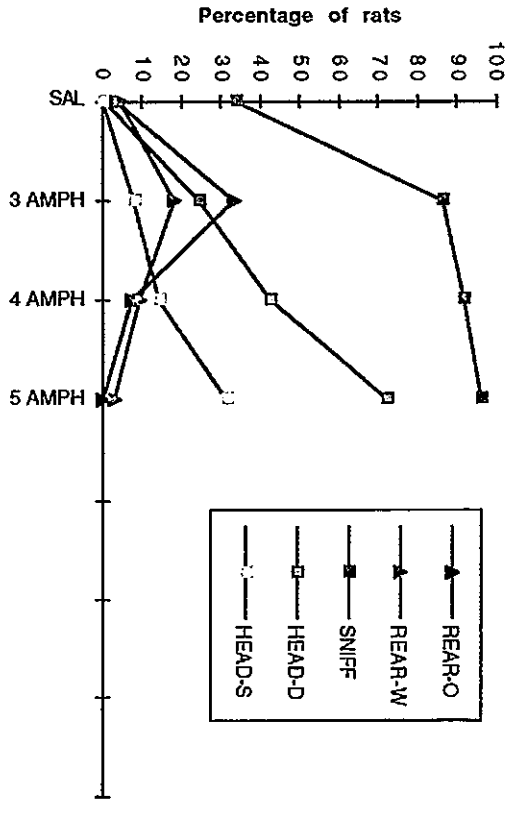
(a) Behaviours elicited in rats treated with saline (SAL) or 3 - 5mg/kg amphetamine.

(b) Behaviours elicited in rats treated with an intermediate dose (see chapters 4 & 6) of haloperidol (HAL), Clozapine (CLOZ), Ondansetron (OND), or sulpiride (SULP).


Proportion of Length 4 trips
0
0.05
0.1
0.15
0.2
0.25
0.3
HAL
CLOZ
CND
SULP
0
10
20
30
40
50
60
70
80
90
100
Percentage of rats engaged in Locomotion


Proportion of Length 4 trips
0
0.05
0.1
0.15
0.2
0.25
0.3
SAL
3 AMPH
4 AMPH
5 AMPH
L4 TRIPS
LOCOMOTION
0
10
20
30
40
50
60
70
80
90
100
percentage of rats engaged in locomotion


Percentage of rats
0
10
20
30
40
50
60
70
80
90
100
HAL
CLOZ
CND
SULP


Percentage of rats
0
10
20
30
40
50
60
70
80
90
100
SAL
3 AMPH
4 AMPH
5 AMPH
REAR-O
REAR-W
SNIFF
HEAD-D
HEAD-S

higher dose of amphetamine. Findings from the current investigation suggest that this may well be the case, indicating that the behavioural effects of amphetamine-treated rats pre-treated with sulpiride or ondansetron represent a change in the relative outputs of the ventral and dorsal striatum rather than a blocking of behaviour per se.

The current series of experiments examined the interaction of antipsychotic drugs on rats given 3.5mg/kg amphetamine. At this dose both maximum levels of locomotion and perseverative locomotor routes were observed. Thus an enhancement of the effects of amphetamine led to stereotyped head behaviours. Treatment with a lower dose of amphetamine (eg 2mg/kg), where perseverative locomotor routes are minimal, may possibly lead to induction of stereotyped locomotion, resulting in an increase in the proportion of length 4 trips.

Treatment with the low dose of 5mg/kg clozapine prior to 3.5mg/kg amphetamine did in fact lead to an increase in both locomotion and length 4 trips for some of the session intervals, reinforcing the view that these antipsychotic drugs enhance, rather than block, the action of amphetamine.

7.5 Proposal for future studies not adequately addressed by the current investigation.

Temporal aspects of contrast based image analysis: The animal model of amphetamine-induced unconditioned behaviour has focused on the spatial characteristics of open-field locomotion yet personal observations have noted that temporal aspects of locomotor behaviour are an important component of the amphetamine response and readily manipulated by drug treatment. Direct observation and classification of locomotor behaviour into ambulation which occurred continuously and forward progression which occurred with frequent pauses lasting longer than 3 seconds, showed quite clearly that pre-treatment with ondansetron significantly reduced pausing between bouts of locomotion early in the test session. The classification of locomotor behaviour by direct observation is

somewhat unsatisfactory. An aim of the present investigation was to incorporate measures of temporal, in addition to spatial, aspects of amphetamine-induced locomotor behaviour using contrast based image analysis, time did not permit analysis of temporal measures derived from the tracking system. Furthermore, rats were unavailable for further studies although much of the information required to undertake this task had already been collected and was stored both on video tape and on computer. The future development of trip lengths as a measure of stereotyped locomotion should attempt to quantify both the temporal and the spatial aspects of the behaviour. Paulus and Geyer (1991) found that both temporal and spatial scaling exponents used to assess the acute behavioural effects of various psychoactive substances on unconditioned motor behaviour provided a dose-dependent, sensitive and distinctive 'fingerprint' for each of the substances tested. Personal observations in the present series of experiments suggest that temporal aspects of interactions between antipsychotic drugs and amphetamine-induced locomotion do indeed discriminate drugs.

Snout contact: Past work in our laboratory (Kenyon et al, 1981, 1983) examined the disruption of maternal retrieving in rodents following perioral anaesthesia. These authors injected lidocaine into the mystacial pads of rats. The mystacial pads are an area of the snout innervated by the infra-orbital nerve (Greene, 1955; Vincent, 1912, 1913), which is a principal branch of the trigeminal nerve serving the snout and lips, and consists of the external nasal and superior labial branches of the trigeminal nerve, which innervate the side of the nose, the snout and the lips (Greene, 1955). Injection of lidocaine into the mystatial pads renders the snout anaptic (Thor and Ghisselli, 1975). The majority of the cells in the main sensory nucleus of the trigeminal system send their axons to the contralateral side of the brain where they join with the medial lemiscus and terminate in the medial part of the ventral posterior medial nucleus of the thalamus (see Kelly, 1985). This proprioceptive information serves as the initial step in generating a sensory perception of the world, whereby these perceptions from several sensory systems,

including that outlined above, are integrated and related to higher motor behaviours.

The work of Teitelbaum et al, (see Cooper and Dourish, 1990) has shown that apparently unrelated stereotyped acts produced by apomorphine in rats consist of a mixture of snout contact, forward progression and turning and are established as a result of changing levels of these three behaviours.

Future work sets out to extend these findings and examine the relationship between forward progression, turning and snout contact on the development of amphetamine-induced behaviours particularly in relation to locomotion and stereotyped locomotion.

The procedure adopted by Kenyon et al, (1981, 1983) of inducing perioral anaesthesia by injecting lidocaine into the mystacial pads could be adapted for use in the study of the interaction of snout contact and forward progression following treatment with amphetamine. Experiments would be conducted to examine the function of sensory input from the snout area via the trigeminal system on the development of locomotion and turning behaviour following treatment with amphetamine. The development of amphetamine-induced hyperactivity could be assessed following unilateral or bilateral injection of lidocaine into the mystacial pads of the rat prior to treatment with amphetamine.

Construct validity: In the introduction (section 1.3) it is proposed that animal models which address issues of predictive, face and construct validity offer a better way forward in the development of models of schizophrenia (see Ellenbroek and Cools, 1990). Despite this claim the present study has failed to examine the construct validity of the proposed model system and has examined only problems relating to the reliability, predictive and face validity of amphetamine-induced locomotion and stereotyped locomotion. Clearly future studies should attempt to examine the underlying constructs relating to open-field measures. The environmental stimulus provided by placing animals in an open field following

treatment with amphetamine could be examined in terms of both 'stress' and 'exploration'.

There is evidence that stressful environmental conditions produce stereotypy in laboratory animals (Meyer-Holzapel, 1968; Ridley and Baker, 1982; Fentress, 1983), although Robbins et al, (1990) argue that stereotyped behaviours are most likely to be elicited under conditions involving high levels of arousal having no external focus or cause. For example, exposure to stressors such as electric shock or changes in temperature do not appear to lead to stereotyped behaviour whereas treatments such as rearing or housing in isolation, mild tail pinch and even amphetamine all lead to the induction of stereotyped behaviour yet lack strong exteroceptive properties.

It is important to develop an understanding of schizophrenia alongside models which include genetic and environmental factors which pre-dispose an individual to the chemical imbalance which is suggested to be the neurological correlate of psychosis. Antelman et al, (1980) propose that stress is an important aspect in any model of schizophrenia, including amphetamine. Therefore the aim of future research into open-field locomotion would examine the effect of stress on the spatial distribution of open-field locomotion. It is likely that contrast based image analysis has the capacity to detect subtle changes in open-field locomotor activity which would be missed using photocell beam measurement techniques.

Several researchers (eg Sahakian et al., 1975; Sahakian and Robbins 1977)have used animals reared in isolation as a model of raised dopamine function, therefore it would be possible to compare open field measures obtained from socially isolated animals with animals administered amphetamine.

Serotonin and dopamine: The current study examined the effects of a 5HT3 antagonist (ondansetron) on amphetamine-induced open-field locomotor behaviour. The findings of this study indicated that 5HT involvement in locomotor activity induced by amphetamine is complex and that blocking a 5HT3 synergistic effect on dopamine may allow other 5HT receptor mediated behaviours to be expressed.

Certainly 5HT1A receptors in addition to dopamine are known to be involved in head swaying (Luki et al, 1984), a behaviour which in the current investigation was shown to increase following antagonism of 5HT3 sites by ondansetron. Head swaying is known to significantly decrease following 6-OHDA lesions to the substantia nigra, ventral tegmentum and striatum but not the nucleus accumbens, suggesting that 5HT-dependent stereotyped head swaying is also dependent on dopamine (Andrews et al, 1982). Both 5HT1a and 5HT1b agonists have anxiolytic and anti-aggressive properties and there is some evidence to suggest that in combination they may have antipsychotic properties, particularly if administered with a D2 blocking antipsychotic treatment such as sulpiride (see Gerlach, 1991). As both agonists and antagonists are available for 5HT1a receptors (see review by Middlemiss and Tricklebank, 1992, Luki, 1992), it may be fruitful to explore the effects of these in addition to studies involving 5HT3 and D2 receptor antagonism within this proposed model system.

7.6 Conclusions

The experiments reported in this thesis (Chapters 3-6), provide convincing evidence that contrast based image analysis of drug-induced locomotion coupled with direct observation of behaviour analysed in terms of its similarity matrix (Clarke, 1993) is a powerful model of amphetamine-induced behaviours, which afford a detailed and integrated picture of the amphetamine response in rodents.

It is proposed that measures which encompass this complexity will have greater utility in the study of drug interactions with amphetamine-induced behaviours. The findings relating to antipsychotic drug action and locomotion are interesting in that they have shown that stereotyped aspects of locomotion can be dissociated from locomotion by different classes of antipsychotic drug, supporting the claim that measures of stereotyped locomotion, for example length 4 trips, have the potential to be developed as an animal model of locomotor activity which will identify unsatisfactory antipsychotic drugs which have the capacity to

antagonise stereotyped locomotion and are therefore likely to produce extrapyramidal side effects. This model system is simple in that it is fully automated and measures a single behaviour (locomotion), which has been accepted by the majority of researchers as a model of raised mesolimbic dopamine activity and therefore a better animal model for schizophrenia and for the detection of dopamine receptor antagonists which are antipsychotic agents, without extrapyramidal side effects.

Glossary

Acetylcholine A neurotransmitter, found in autonomic ganglia, postganglionic parasympathetic nerve terminals, the neuromuscular junction, the adrenal medulla, the central nervous system (CNS) and postganglionic sympathetic nerve terminals at sweat glands. Drugs which mimic the actions of acetylcholine are *cholinomimetics* and drugs which prevent the action of acetylcholine are called *anticholinergics*. Receptors are known as Muscarinic and Nicotinic.

Adenylate cyclase An effector enzyme regulated by G proteins which catalyses the production of the secondary messenger, cyclic 3'5-adenosine monophosphate (cyclic AMP).

Alpha-methyl-para-tyrosine Is used experimentally to inhibit tyrosine hydroxylase, and thus to prevent the synthesis of all the catecholamines (noradrenalin, dopamine and adrenalin).

(+)-Amphetamine Is a potent dopamine-releasing substance.

Apomorphine Is a direct dopamine-receptor agonist.

Benzamides The substituted benzamides are a relatively new class of antisychotic drugs, chemically related to the antiemitic metoclopromide. The parent substance is procainamide.

Benzodiazepines A group of chemically related hypnotics, sedatives, anxiolytics and anticonvulsants, eg Diazepam.

Butyrophenone A class of antipsychotic drugs not possessing tricyclic structure, chemically related to pethidine. These include, haloperidol, benperidol and droperidol.

Caffeine A powerful CNS stimulant, which blocks the action of adenosine receptors which are located on cell membranes in the CNS and peripheral nervous system.

Chlorpromazine An antipsychotic drug which is the prototype of the phenothiazines. In addition to its antipsychotic action it possesses cardiovascular effects, anti-emetic action, induction of catatonia, and blocking of conditioned avoidance responses. Chlorpromazine also produces iatrogenic parkinsonism, an unwanted side effect of this drug.

Cholecystokinin (CCK) A neuroactive peptide which acts as a neurotransmitter. CCK has a heterogeneous distribution in the mammalian CNS, with a very high concentration in the cerebral cortex, hippocampus, the amygdala and septum. Significant amounts are also found in the hypothalamus, dorsal raphe nucleus, the caudate-putamen and ventral tegmentum.

Clozapine Is a dibenzazepine with a tricyclic structure, having a piperazine sidechain. Clozapine does not cause cataleptic activity in animals and does not produce extrapyramidal side effects in man. Clozapine produces agranulocytosis in some patients.

Cyclic AMP A second messenger, which leads to the activation of kinases by phosphorylation. The phosphorylated kinases alter the activity of enzymes and structural proteins leading to cellular response.

Dibenzazepines Antipsychotic drugs with a tricyclic structure, although the centre ring differs from that of the phenothiazines and thioxanthines. The principle drug of this class is clozapine.

Dopamine A catecolamine neurotransmitter in the CNS and at some ganglia in the autonomic nervous system. Dopamine is a precursor of noradrenaline and adrenalin. Dopamine is made from the amino acid L-tyrosine which is hydroxylated by the enzyme tyrosine hydroxylase to L-dopa.

Dopamine-Beta-hydroxylase Dopamine is hydroxylated to form noradrenalin (norepinephrine) by the enzyme dopamine-beta-hydroxylase, which is associated with noradrenalin storage vesicles. It is a copper containing enzyme which requires molecular oxygen and ascorbic acid as a cofactor.

Fluphenazine An antisychotic drug of the phenothiazine class, possessing a piperazine sidechain. Amongst the most powerful of antipsychotic drugs, however, fluphenazine has marked anti-emetic function and readily produces extrapyramidal side effects.

Glutamate A dicarboxylic amino acid, associated with a number of metabolic processes within the cell. It is an important constituent of the diet. L-glutamic acid which is known to act as a neurotransmitter can be synthesised from glutamine by phosphate-activated glutaminase.

Granisetron A potent 5-HT3 antagonist.

Haloperidol An antisychotic drug of the butyrophenone class. Haloperidol lacks sedative properties. but has a marked propensity to cause extrapyramidal side effects.

Homovanilic acid (HVA) A major metabolite of dopamine.

Ketanserin A 5-HT2 antagonist.

L-dopa The precursor of dopamine. The rate limiting step in the synthesis of dopamine is the conversion of tyrosine into L-dopa. L-dopa is actively taken up into dopamine neurones in the CNS. where it is converted into dopamine by DOPA decarboxylase.

Lysergic acid diethylamine (LSD) LSD is an ergot alkaloid which acts as a dopamine agonist. It is a non-selective antagonist at 5-HT2 receptors.

MDE (Eve) An amphetamine derivitive.

MDMA (Ecstasy) (Methylenedioxymethamphetamine). An amphetamine derivative.

2-methyl-5-HT A 5-HT3 agonist.

Morphine An agonist at the mu-opiate receptor, distributed with particular high density in the brainstem, trigeminal nuclei, spinal cord, periaqueductal grey region, caudate-putamen, amygdala and cerebral cortex.

Muscarinic Receptors One of the two main types of cholinergic receptor. Muscarine was found to mimic the effect of parasympathetic-nerve stimulation, and the receptors on neuroeffector tissues with a parasympathetic nerve supply are known as muscarinic receptors.

Neurotensin (NT) Is a 13 amino acid residue peptide which was originally identified as a potent vasodilator. Areas of high concentration include the hypothalamus, basal ganglia, the interstitial nucleus of the stria terminalis, the limbic system and the dorsal region of the spinal cord. NT is proposed as having a

close association with dopamine neurones and the peptide can modify the effects of dopamine agonists on motor activity. It has been suggested to exhibit a modulatory function in the mesolimbic system.

Nicotinic Receptors. One of the two main types of cholinergic receptor. Nicotine mimics acetylcholine at ganglia in the autonomic nervous system, at the adrenal medulla and in parts of the CNS.

Noradrenalin Noradrenalin (norepinephrine, norarternol) is a catecholamine neurotransmitter in post ganglionic sympathetic nerves and the CNS. Noradrenalin is released from the adrenal medulla.

6-hydroxydopamine (6-OHDA) Is a neurotoxin which is selectively taken up into catecholaminergic neurones. It causes degeneration of the neurones and is used experimentally to make selective lesions in neuronal systems which use catecholamines as neurotransmitters.

Ondansetron (GR 38032F) A potent selective 5-HT3 antagonist.

Phenothiazines The first agent to be successfully used as an antipsychotic agent (chlorpromazine).The phenothiazines have a wide range of pharmacological actions which include antihistamine action, weak antagonism of 5-HT and acetylcholine.

Phencyclidine (PCP) Commonly known as *angel dust* it is an antagonist at glutamate NMDA receptors. These are found in the hippocampus, basal ganglia, limbic system cerebral cortex, superior colliculus and vestibular nuclei.

Phosphodiesterase Enzyme which when inhibited leads to effects similar to beta-adrenoceptor stimulation.

Raclopride A substituted benzamide like sulpiride, but more potent and causing less prolactin release.

Reserpine Rauwolfia derivitive. Reduces sympathetic tone by noradrenalin depletion. Depletes brain noradrenalin, dopamine and 5-HT. Used in the treatment of hypertension and as an antipsychotic. Induces parkinsonism.

Savoxepine A novel tricyclic compound with a higher affinity for hippocampal D2 than striatal D2 receptors.

Scopolamine A psychedelic drug which acts by blocking post synaptic acetylcholine receptors.

Serotonin (5-HT) 5-hydroxytryptamine (5-HT, serotonin) is a neurotransmitter in the CNS and in the myenteric plexus of the gut. 5-HT is a monoamine synthesised from the aromatic amino acid L-tryptophan, which is hydroxylated to 5-hydroxytryptophan by the enzyme tryptophan hydroxylase, found only in the cytoplasm of 5-HT neurones, and is the rate limiting enzyme in the synthesis of 5-HT.

Sulpiride One of the substituted benzamides. When tested in animals sulpiride shows only part of the spectrum of activity common to most antipsychotics. and was classed as an 'atypical' antipsychotic. The drug does not induce catalepsy in animals and does not antagonise the stereotyped behavioural effects of amphetamine and apomorphine. Sulpiride does produce Parkinson-like effects but these appear to be less than with other antipsychotic drugs.

Thioridazine An antipsychotic drug of the phenothiazine class, having a piperidine sidechain. This drug produces more sedative activity than the other phenothiazines and is claimed to produce fewer extrapyramidal side effects.

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