SOME STUDIES ON DRUG-INDUCED BEHAVIOUR IN YOUNG CHICKS.

BY

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THESIS APPROVAL

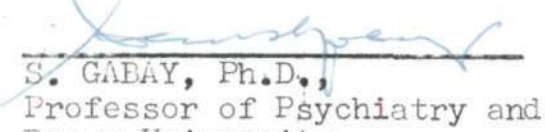
This thesis by Elijah Ndajiya Sokomba meets the requirements governing the degree of Doctor of Philosophy of Ahmadu Bello University and it is approved for its contribution to scientific knowledge and literary presentation.

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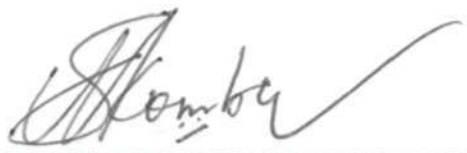
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This is to certify that I carried out the work reported in this thesis in the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria under the supervision of Prof. G.E. OSUIDE. The work of other investigators is acknowledged and referred to accordingly. I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.



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DEDICATION

This thesis is dedicated to my dear wife, Mercy,my son, Ndawusa and daughter, Nnadzana, for their love, understanding and moral encouragement.

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I am very grateful to God for His grace that has sustained me throughout the period of this project.

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Osuide, G. & Sokomba, E.N. (1978) Recording of photic-evoked response in conscious young chicks, Journal of Pharmacological Methods Vol. 1. 3: 203-212.

ABBREVIATIONS

AM	amphetamine
AP	apomorphine
ATR	atropine
CB	cerebellum
CPH	cyproheptadine
DA	dopamine
DDC	diethyldithiocarbamate
DOPS	3,4-dihydroxyphenyl-serine
ECC	excitation, chorea, circling
EEG	electroencephalogram
EMG	electromyogram
ET495	1-(2-pyrimidyl)-4-piperanyl-piperazine methanesulphate
FC	frontal cortex
FLA-63	bis(1-methyl-4-homopiperazinyl thiocaruamate)disulphide
g	gramme
GABA	gamma aminobutyric acid
GHB	gamma hydroxybutyrate
HA	haloperidol
HS	hyperstriatum
5-HT	5-hydroxytryptamine
HYO	Hyoscine
IDPN	β,β'-iminodipropionitrile
i.p	intraperitoneally
Kg	Killogramme
L-dopa	3,4-dihydroxyphenylalanine

LSD	lysergic acid diethylamide
MBRF	mid brain reticular formation
Mg	milligramme
Ml	millilitre
NA	noradrenaline
OC	optic cortex
6-OHDA	6-hydroxydopamine
OT	optic tectum
PBZ	phenoxybenzamine
PER	photic-evoked response
PHYSOS	physostigmine
PM	pons-medulla
PMZ	pimozide
QUIP	quipazine
RF	reticular formation
S.C	subcutaneous
Ug	microgramme.

SUMMARY

Some behavioural and electrophysiological studies were undertaken in chicks and rats using observational, EEG, EMG and evoked potential techniques.

Some dopamine (DA) agonists (apomorphine, d-amphetamine, amantadine, and piribedil) induced stereotyped pecking activity and aggressive behaviour of varying intensities in chicks. On the other hand, DA, L-dopa and ergometrine did not induce characteristic stereotyped activity in the chicks. D-amphetamine and ergometrine induced distinct postural changes in chicks. Pretreatment of chicks with ergometrine and piribedil antagonized the behavioural effects of apomorphine and d-amphetamine. The results show that apomorphine d-amphetamine, amantadine and piribedil induce a similar pattern of stereotyped activity in young chicks. The results further suggest that

ergometrine exerts a predominant inhibitory influence on stereotyped behaviour , while piribedil has both DA agonistic and antagonistic effects in young chicks.

Pretreatment of chicks with reserpine and 6-hydroxydoparaine (6-OHDA) potentiated apomorphine and piribedil-induced stereotyped behaviour while the effects of d-amphetamine and amantadine were inhibited. The behavioural effect of apomorphine were potentiated when chicks were pretreated with dihydroxy-phenylserine, while d-amphetamine and amantadine effects were antagonised. FLA--63 potentiated d-amphetamine and amantadine-induced stereotyped behaviour and inhibited apomorphine effects. Propranolol antagonized the behavioural effects of apomorphine and d-amphetamine, while phenoxybenzamine slightly potentiated the effects. Pimozide and haloperidol antagonized apomorphine, d-amphetamine, amantadine and piribedil-induced stereotyped behaviour.

These observations indicate a primary role for

dopaminergic system in drug-induced stereotyped behaviour in young chicks as in other species.

Serotonin and quipazine antagonized apomorphine and d-amphetamine-induced stereotyped behaviour. On the other hand, cyproheptadine and parachloro-phenylalanine (PCPA) potentiated the effects of apomorphine and d-amphetamine. These results suggest that serotonergic system plays an inhibitory role in drug-induced stereotyped behaviour in chicks.

Desipraraine, imipramine and amitriptyline induced biphasic locomotor effects in chicks. Aporaorphine-induced locomotor activity was potentiated when the chicKs were pretreated 30 rain, previously with amitriptyline and imipramine. When the three antidepressants were administered 1 h previously, apomorphine-induced locomotor activity was inhibited. The results suggest that tricyclic antidepressants produce a time-dependent effect on apomorphine-induced locomotor activity in

chicks.

Repeated administration of j3,i$'-imino-dipropionitrile(IDPN) for five days induced specific behavioural changes in chicks. These behavioural changes were characterized by excitation which was accompanied by loud chirping, jerky head and neck movements, and circling motion (i.e. ECC-syndrome). Amphetamine potentiated the syndrome while apomorphine and piribedil enhanced the head and neck movements with minimal effect on the circling behaviour. Pimozide antagonized the ECC-syndrome and also inhibited amphetamine-induced potentiation of the syndrome. Atropine and hyoscine enhanced the ECC-syndrome and also potentiated the effects of d-amphetamine, while physostigmine antagonised the syndrome. Quipazine, in low doses, abolished and in high doses enhanced the ECC-syndrome. The latter effect was antagonised by both cyproheptadine and pimozide. Such results suggest that DA and

serotonergic mechanisms may be involved in IDPN-induced effects in chicks. It is also possible that cholinergic system may exert an inhibitory influence on the ECC-syndroioe.

Evoked responses to photic stimulation recorded under artificial light and dark adaptation from three different brain areas in conscious young chicks were compared. The magnitude of the evoked responses varied from one experiment to another, but for a particular chick consistent responses were obtained under the same behavioural state, stimulus intensity, and recording conditions. The evoked responses recorded under dark adaptation were larger in magnitude and more reproducible when compared with those recorded..under artificial light. The photic-evoked responses under dark adaptation were very sensitive to drug effects. These observations suggest that photic-evoked response (PER) could probably be utilized in the elucidation of the actions of some

Psychoactive drugs.

The influence of acute and chronic administration of phenobarbitone and ethanol on PER was studied in rats. Both phenobarbitone and ethanol produced behavioural depression. EEG synchronization and varying effects on the averaged PER recorded from the frontal cortex (FC) optic cortex (OC) and midbrain reticular formation (MBRF). In phenobarbitone- and ethanol- dependent animals, amplitude of the PER recorded from the FC and OC were enhanced while the recordings from the MBRF were reduced. On the other hand, the amplitudes of the PER from all the three brain areas were enhanced during withdrawal. The results suggest that both phenobarbitone and ethanol produce similar effects on PER in rats and that the effects may probably be characteristic of the sedative - hypnotic group of drugs.

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CHAPTER ONE

1. LITERATURE REVIEW.

1.1. General Introduction.

The need to understand more precisely the aetiology of diverse mental illnesses has continued to stimulate interest in the study of drugs which influence behaviour. Despite considerable efforts, it has not yet become possible to identify a biochemical or morphological factor which could be specifically linked with mental disorders (e.g. schizophrenia, depression, etc.). The discovery that the clinical pictures of schizophrenia and depression can be mimicked by some drugs in both laboratory animals and man greatly promoted research in psychopharmacology.

The main aim of animal experimentation with psychotropic drugs is to attempt to establish a rational basis for the development and use of such drugs through animal models. Presently, experimental work in this field can be divided into two broad types.

(i) the detailed study of psychotropic drugs utilising electrophysiological, pharmacological and biochemical approaches. The objective of these investigations is to improve our understanding of the mechanisms underlying the activity of such drugs.

(ii) the development of simple laboratory methods to detect new substances with possible useful psychotropic activity. In addition, the study of drugs in animals may also contribute to a fundamental understanding of animal behaviour.

It is a sobering thought however, that inspite of the considerable amount of experimental work in psychopharmacology, the majority of new drugs for treating mental disorders have arisen from clinical observations rather than from pharmacological prediction. For example, chlorpromazine and the antidepressants were initially developed from clinical observations.

Inspite of this limited success in its application, it is encouraging that some progress has been made regarding the study of the mode of action of some therapeutic agents using laboratory animals.

1.2. Theories of behaviour.

Several theories have been put forward to explain behaviour. For example Stellar (1954) proposed that the amount of motivated behaviour is a direct function of the amount of activity in certain excitatory centres in the hypothalamus. These centres were believed to receive inputs from internal and external environments, cortical and thalamic centres and a related inhibitory centre which integrated various negative inputs and depressed the activity of the excitatory centre. The algebraic sum of these inputs determine the output along the final common path to the effectors. Based on this model, a theory of

behavioural centres was developed to explain all types of behaviour. This theory postulated that there were excitatory and inhibitory centres throughout the brain which controlled the pattern of response (Morgan, 1965) and the magnitude of any response was thought to depend on the balance between excitating and inhibiting influences. The second theory of behaviour emphasizes the outcome of a pattern of activity in networks of neural units which are aligned serially or parallel to each other (Morgane, 1964). The final output in such a case does not depend on a single nucleus. According to this concept, there are multiple interacting pathways which combine to produce a specific behavioural pattern. These networks give the brain considerable resistance to disruption by malfunction of an individual pathway such that damage to a single pathway would not necessarily impair function radically. Functional impairment would only result from

changes in the pattern of activity in the whole network.

This broader view of behaviour had its origin in the ideas of Flourens (1824). Flourens believed that although specific regions had certain functions and properties, there was a unity in the central nervous system, so that loss of one part reduced the energy of the other parts but did not abolish the function. This theory implies that there is a specific function to each anatomical nucleus in the sense that it enables integration of new information in the system, but that it also has a joint function in the output of the network.

As a consequence of the concepts of functional control of behaviour by centres in the brain, theories of the biochemical basis of behaviour followed the same logic. It was believed that drugs modified behaviour by changing the levels of excitation and inhibition at specific sites in the brain. Thus, chlorpromazine was believed to

act on the hypothalamus to reduce its level of activation while [illegible] amphetamine activated it (Duffy, 1962).

This idea of drugs modifying levels of activity in neural centres was carried over to the neurochemical studies of the brain where extensive work has been done to determine the levels of neurochemical substances at specific sites and correlate these with definite behavioural patterns. The basic assumption underlying these studies is that the level of neural activity at any given site may be directly or indirectly related to the concentration of chemical agents within the locality. In particular, research has focussed on presumed central nervous system transmitter substances which may underlie drug-induced changes (increase or decrease) in the output along some final common pathways for behaviour.

1.3 Biochemical Basis of Behaviour.

1.3.1 The role of catecholamines in behaviour.

Several studies have implicated biogenic amines in the modulation of behaviour. In 1915, Cannon proposed that the hormone of the adrenal medulla was involved in mammalian adaptation to stress. Thus, when noradrenaline (NA) and adrenaline were found to be present in mammalian brain, it was considered that they might be involved in regulating responses such as fear, hunger or mood within the central nervous system.

The catecholamines thus far identified in the brain are DA, NA and adrenaline. Adrenaline has been less studied in the brain than the other two catecholamines and its role, if any,in brain function is not yet clear.

In many areas of the brain, DA functions as an intermediate precursor of NA. Until recently, this precursor role has been considered by many to be its primary function in the brain. Evidence has

however accumulated which suggests that in certain neural systems, DA is, in its own right, a neural transmitter. Blaschko (1957) and Carlsson, Lindqvist and Magnusson (1957) were the first to suggest that DA may have an important and differentiable physiological function. Since that time, a number of findings have been reported which indicate some differences between the effects of NA and DA.

Histochemical, biochemical and pharmacological evidence have been accumulated which support the existence of specific DA containing neurons in the central nervous system (Carlsson, 1959; Andén, Carlsson, Dahlstrom, Fuxe, Hillarp and Larsson, 1964). For example, Bertler and Rosengren (1959), showed that DA was present in high concentrations in the basal ganglia and the substantia nigra. In 1960, Ehringer and Hornykiewicz observed a decreased DA content in the corpus striatum of patients with parkinson's disease. This finding indicated the patho-pysiological role for brain DA in psychomotor

function (Hornykiewicz, 1966).

There is good reason to believe that most of the DA found in the striatum has physiological function. Although NA is also present in the striatum, it has been found that the concentration of this amine is relatively low when compared to DA. Goldstein, Anagnoste, Owen and Battista, (1967) have shown that certain brain lesions produce a greater loss of caudate DA than of NA. This evidence has been taken to indicate the existence of separate DA and NA neurons since the lesion appears to primarily affect the dopaminergic neurons in the caudate. Snyder and his co-workers (1970) studied the uptake of catecholamines into nerve ending particles (synaptosomes) which were obtained from the striatum and the hypothalamus (contains a relatively greater proportion of NA neurons). They found that while the striatal synaptosomes were equal in their ability to accumulate d or l - noradrenaline, synaptosomes from the

hypothalamus showed four times the affinity for l-noradrenaline than the d-isomer. These differences in uptake preference have been attributed to fundamental differences in the membranes of the two types of neurons.

There has been a great deal of research concerning the influence of catecholamines on various behavioural patterns. For example, Leimdofer, (1948) reported that intracisternal injection of NA into man produced a normal and deep sleep. Similar results have been reported by Felberg (1954) in cats, through an intra-ventricular route. Also, Mandell and Spooner (1968) observed sedation after administration of NA into the brain of various species of experimental animals. In contrast, Goodwin, Murphy, Brody and Bunney (1970) have shown that administration of large doses of dihydroxyphenylalanine (DOPA), which is the immediate precursor of DA, elicited both behavioural and locomotor activation with

accompanying EEG pattern of arousal. Everett and Wiegand (1962) speculated that DA, and not NA, is the principal catecholamine subserving such functions as the arousal state. In support of this speculation, Crevelling, Daly, Tokuama and Witkop (1968) showed that dihydroxyphenylserine (DOPS), which selectively replenishes brain NA in reserpine-treated mice, did not reverse reserpine-induced sedation. They suggested, therefore, that the reversal of reserpine-induced sedation by DOPA was due to the conversion of the later to DA, and not to NA, in the central nervous system. In a similar study, cocaine-induced behavioural stimulation which had been antagonised by pretreatment with reserpine was restored following DOPA administration, while the effect was not restored by DOPS (Van Rossum, 1968).

Further experimentation led to the suggestion that DA and NA may mediate different behavioural patterns. The NA system was associated with gross spontaneous activity while the DA system was

associated with stereotyped behaviour (Randrup and Jonas, 1967). Randrup and Jonas (1967) suggested that stereotyped behaviour and locomotor activation induced by amphetamine may be related to increased brain level of DA and NA respectively.

Snyder, Taylor, Coyle, and Meyerhoff, (1970) attempted to separate the behavioural role of brain NA and DA by the use of d- and l-amphetamine. These workers showed that although d- and l- amphetamine were equally potent as inhibitors of catecholamine uptake in the corpus striatum, d-amphetamine was ten times as potent an inhibitor in noradrenergic areas of the brain. Thus, it was assumed that behaviour primarily influenced by noradrenergic system would be affected more by d-amphetamine than by l- amphetamine.DA mediated behaviour was considered to be equally affected by both isomers of amphetamine. The results of these studies showed that the effects on locomotor

activity paralled the differential effects of these isomers on uptake inhibition.

D-Amphetamine was found to be approximately ten times more effective in increasing locomotor activity while there was no difference in the effects of d- and l- amphetamine on compulsive gnawing. These findings are compatible with the evidence that ablation of the corpus striatum eliminates amphetamine-induced gnawing, and that direct injection of DA into this area can induce this behaviour (Ernst, 1967; Randrup and Munkvad, 1967).

1.4. Catecholamines and behavioural disorders.

1.4.1. Schizophrenia.

Dysfunction of catecholaminergic neurotransmission has been implicated in many behavioural abnormalities. Interest in the catecholamines in schizophrenia was greatly stimulated by the discovery of their role in the pathogenesis of parkinsonism.

Several studies have associated schizophrenia with disturbances in dopaminergic mechanism in the brain. Carlsson and Lindqvist (1963) proposed that the therapeutic action of phenothiazine drugs was probably due to blockade of dopamine receptors and this has been linked with comparisons of therapeutic potencies of neuroleptic drugs and the production of extrapyramidal symptoms. Discrepancies between these two parameters in both clinical and laboratory tests of some phenothiazines and butyrophenones (Crow and Gillbe, 1973) have threatened the DA hypothesis of schizophrenia. However, in 1974, Snyder and his co-workers reported that the affinities of a range of these drugs for central muscarinic receptors varies inversely with their ability to produce extrapyramidal symptoms. Thus, a potent antagonist of DA could be a very effective antischizophrenic drug producing marked extrapyramidal effects which were suppressed by its own antimuscarinic actions.

Another line of evidence implicating DA in schizophrenia is the apparent link between the syndrome of amphetamine-induced stereotypy in animals and amphetamine psychosis in man.

1.4.2. Parkinson's Syndrome.

Disturbances in catecholamine function, particularly DA, have been implicated in both naturally occurring parkinsonism and that produced by antipsychotic drugs (Hornykiewicz, 1966).

The symptoms of Parkinson's disease include akinesia, rigidity and tremor. These are primarily disorders of motor function. The outstanding neuropathology of Parkinson's disease is a depigmentation and loss of the melanin-containing cell bodies of the pars compacta of the substantia nigra and other pigmented areas of the brain. There is also a consistent chemical pathology involving mainly DA (Hornykiewicz, 1966). The decrease in DA is highly significant and is specific for the caudate nucleus, putamen and

substantia nigra. The DA content of the striatum is less than 10% of normal and in idiopathic parkinson's disease this decrease is significantly more severe in the putamen than in the caudate (Bernheimer and Hornykiewicz, 1973). Associated with the loss of brain DA is a parallel deficiency in its synthetic enzymes i.e. tyrosine hydroxylase and DOPA decarboxylase, the specific uptake system for DA and the level of homovanillic acid (Hornykiewicz, 1966; Bernheimer et al, 1973).

After the discovery of the specific destruction of the nigro-striatal DA pathway in parkinson's disease, it was proposed that this was the neuropathological basis for the clinical symptomatology of this disorder. This proposition was made on the basis of the following observations:

(i) the degree of DA deficiency correlated with the cell loss in the substantia nigra, but not with changes found in other basal ganglia nuclei (Bernheimer et al 1973);

(ii) in patients with hemi-parkinsonism there was

a more severe loss of DA in the striatum in the hemisphere contralateral to the side of symptoms (Borolin, Bernheimer and Hornykiewicz, 1964). (iii) the immediate precursor of DA, L-dopa, was found to be a very efficacious agent for the treatment of parkinsonian akinesia and rigidity (iv) in the brains of parkinsonian patients with L-DOPA, the DA concentrations of the striatum were much higher than those of the non-DOPA treated patients and in some cases approached normal levels (Davison, Lloyd, Dankova and Hornykiewicz, 1971).

Apart from DA, the levels of NA and 5-HT have been observed to be subnormal in parkinsonian patients. Though the functional significance of these low levels of NA and 5-HT is not clearly understood, it has been associated with the increased incidence of severe depression that is observed in patients suffering from Parkinson's disease (Ajuriaguerra, Constantinidis, Eisenring, Yanniotis and Tissot, 1972). Gumulka, Ramirez, Samanin and Valzelli, (1970) have accumulated

data to show that some of the symptoms of human parkinsonism may be due to degeneration of serotonergic fibres which terminate in the striatum.

1.4.3. Huntington's Disease.

Huntington's disease, in many aspects, is the clinical opposite of parkinsonism. The symptoms include muscle hypotonia with involuntary hyperactivity and this results in facial grimacing, writhing and twisting of the trunk and limbs. These symptoms may be due to hyperactivity of the DA system of the putamen as compared to the DA content of the caudate. In support of this hypothesis, L-dopa excerbates while AMPT modulates the dyskinesias. (Gerstenbrand, Pateisky & Prosenz, 1963)

1.5. Animal models for studying behaviour.

1.5.1. Stereotyped behaviour.

Drug-induced stereotyped behaviour has been defined as a perseverative, continuous, apparently purposeless activity, during which there is an

absence of otherwise normal behaviour (Randrup and Munkvad, 1966). The pattern of stereotyped behaviour depends on the animal species (variations exist among individuals of the same species) and the dose of drug. Examples of stereotyped behaviour include the continuous biting, sniffing and licking observed in guinea-pigs, rats and mice respectively (Randrup and Munkvad, 1966; Schelkunov, 1964), constant purposeless searching head movements of cats (Emele, Schanaman and Warren 1961), continuous twitter movements and pecking in pigeons and chicks (Dhawan and Saxena, 1960; Osuide and Adejoh, 1973).

Various approaches have been taken so as to improve our understanding of the mechanisms involved in drug-induced stereotyped behaviour. Most of these studies have implicated central dopaminergic system as the mediator of this behaviour. The dopaminergic neurons which have received the greatest attention are those found in the extrapyramidal areas of the brain. The

striatum and related areas have been found to contain the highest concentration of DA in the mammalian and avian brain, (Hornykiewicz, 1966). Most of the drugs that produce stereotyped behaviour affect this dopaminergic system either directly or indirectly (Van Rossum, 1970).

Amsler (1923) investigated the effect of ablation of the various parts of the brain upon apomorphine and found that its induction of compulsive gnawing in rats and guinea-pigs is mediated through the corpus striatum. Ablation of the other brain areas, including the cortex, had no effect while suctioning or high frequency electric current (Randrup and Munkvad, 1968) has shown that bilateral ablation of only the corpus striatum is necessary to block stereotypy.

1.5.2. Catecholaminergic agents and stereotyped behaviour.

A number of drugs have been shown to induce stereotyped behaviour in various species.

These include amphetamine and amphetamine-like drugs (Randrup and Munkvad, 1966) apomorphine (Amsler, 1923) Ernst, 1967; Andén, Rubenson, Fuxe and Hokfelt, 1967; Osuide and Adejoh, 1973), tryptamine and cocaine (Randrup and Munkvad, 1964). Because of this observation with several stimulant drugs, it had been thought that stereotyped behaviour was related to the action of stimulant drugs (Randrup and Munkvad, 1966). However, there are evidences which show that the manifested behaviour was not simply the property of every stimulant drug since after heavy sedation following meprobamate, amphetamine-induced stereotyped behaviour in rats still persisted (Munkvad and Randrup, 1966).

Amphetamine has been shown to act indirectly by releasing DA presynaptically, inhibiting its reuptake and inhibiting monoamine oxidases (Weissman, Koe and Tenen, 1966; Glowinski, Axelrod and Iversen, 1966). In studies in which reserpine was used to deplete neuronal stores of catecholamines, amphetamine-induced stereotyped

behaviour was not abolished (Van Rossum, Vander Schoot and Hurkmans, 1962, Smith, 1963). This observation led to the suggestion that amphetamine may directly stimulate catecholamine receptors (Smith, 1963). However, the synthesis of DA appears to persist after reserpine pretreatment (Anden et al, 1964; Person and Waldeck, 1969). It was therefore suggested that amphetamine could act by releasing newly synthesized catecholamines. Diethyl dithiocarbamate (DDC) inhibits the synthesis of NA and does not abolish amphetamine-induced stereotypy (Randrup and Scheel Kruger, 1966) or that produced by DOPA (Scheel-Kruger and Randrup, 1967). Noradrenergic beta receptor blocking drugs are also ineffective antagonists of stereotypy (Randrup, Munkvad and Udsen, 1963; Herman, 1967).. These studies therefore implicate dopaminergic neurons in amphetamine-induced stereotyped behaviour.

Amantadine is also believed to release DA from extragranular stores in the dopaminergic

neurons (Fernebo, Fuxe, Goldstein, Hamberger and Ungerstedt, 1971) and also block reuptake of dopamine (Heimans, Rand and Fennessy, 1972). But Heikkila and Cohen (1972) reported that neither release of endogenous catecholamines nor inhibition of reuptake specifically in the striatum accounts for the actions of amantadine and that further work was needed to elucidate its precise mechanism of action.

Several studies have indicated that apomorphine acts directly on dopamine receptors (Andén et al, 1967; Ernst, 1967; although a presynaptic mechanism may be involved (Costall and Naylor, 1973a). Other drugs which have been reported to act directly on DA receptors include piribedil and its metabolite (Corrodi, Fuxe and Ungerstedt, 1971), ergot alkaloids (Corrodi, Fuxe, Hokfelt, Lidbrink and Ungerstedt, 1973). However, a presynaptic component has also been postulated for ergometrine, bromocritpine and piribedil (Fuxe, 1973; Schnieden and Cox, 1976).

L-DOPA is believed to produce its central action by being converted to DA in the brain (Bartholini and Pletcher, 1968).

Consistent with the view that stereotypy is mediated by a dopaminergic mechanism, many of these drugs have been shown to increase the release of DA into a ventriculocisternal perfusate or to act as DA agonists in vitro (McKenzie and Szerb, 1968).

1.5.3. Lesion Studies:

Mechanical, electrolytic and 6-OHDA-induced lesions have been attempted to elucidate the anatomical basis of drug-induced stereotyped behaviour. Most authors agree that amphetamine-induced stereotypy is reduced by bilateral lesions of the neostriatum (Fuxe and Ungerstedt, 1970; Naylor and Olley, 1972), although Divac (1972) found no effect. It has also been reported that bilateral lesions of the neostriatum abolish apomorphine-induced stereotyped behaviour (Fuxe

and Ungerstedt, 1970), although others found no effect (Divac, 1972; Costall and Naylor, 1973a).

Selective destruction of catecholamine neurons by various types of 6-OHDA treatment has produced clearer evidence that nigrostriatal DA neurons are involved in drug-induced stereotyped behaviour. When 6-OHDA treatments which selectively deplete either NA or DA are compared (Hollister, Breese, and Cooper, 1974) only rats with destruction of DA neurons show blockade of amphetamine-induced stereotypy suggesting a specific involvement of DA neurons in this action.

Intranigral injections of 6-OHDA also blocked amphetamine-induced stereotypy (Creese and Iversen, 1972). Such administrations of 6-OHDA did not only destroy the nigrostriatal DA neurons but also caused some damage to the mesolimbic dopamine innervation of the nucleus accumbens and olfactory tubercle (Ungerstedt, 1971a). However, no blockade of amphetamine

stereotypy has been observed due to destruction of mesolimbic dopaminergic neurons (Kelly, Seviour and Iversen, 1975). In constrast, 6-OHDA lesions of the caudate nucleus produced a reduction of amphetamine-induced stereotypy (Creese and Iversen, 1974) implicating the nigro-striatal DA system in stereotypy.

Recently, Costall, Naylor and Olley (1972) observed that two weeks after bilateral electrolytic lesions of the substantia nigra, apomorphine-induced stereotyped behaviour was initially abolished but the ability of amphetamine to induce stereotypy in rats was not significantly changed. These workers thus concluded that the nigro-neostriatal dopaminergic pathway is not essentially involved in amphetamine-induced stereotypy and that apomorphine stereotyped behaviour is not mediated by a direct action on central DA receptors. These results differ remarkably from those of Ungerstedt (1971a) as mentioned previously.

However, Price and Fiberger (1974) made observations similar to those of Ungerstedt (1971a) after 6-OHDA-induced lesions of the substantia nigra of rats.

1.5.4. Rotational Model:

Perhaps the most extensively used test of dopamine function is the turning behaviour following unilateral lesion of the nigro-striatal pathway. Using this model direct acting DA receptor agonists, (e.g. apomorphine) cause contralateral rotation presumably due to development of supersensitivity on the lesioned side (Ungerstedt, 1971b). On the other hand, drugs which act indirectly by releasing DA (e.g. amphetamine) cause rotation of the animal towards the operated side, since no DA release will occur on the denervated side. This preparation has been widely used as a behavioural model for determining the mode of action of drugs interacting with DA mechanism.

1.6 Stereotyped behaviour and DA receptor blockers.

The neuroleptics, phenothiazines and butyrophenones have been observed to inhibit stereotyped behaviour induced by apomorphine, amphetamine and other DA agonists (Randrup, Munkvad and Udsen, 1963; Munkvad and Randrup, 1966; Janssen, Niemegeers and Shellenkens, 1965). Haloperidol and pimozide have been particularly considered as specific DA receptor antagonists (Carlsson and Lindqvist, 1963; Anden, Butcher, Corrodi, Fuxe and Ungerstedt, 1970). This has led to the use of stereotyped behaviour as a laboratory model for screening compounds for antipsychotic property (Janssen et al, 1965). Carlsson and Lindqvist (1963) suggested that the therapeutic action of neuroleptic drugs might be due to blockade of DA receptors. In addition to receptor blockade, the same workers also observed an increase in DA turnover and attributed this to a feed-back mechanism induced by the DA receptors. They proposed that when the

transmission mechanism is blocked, the activity of DA neurons is elevated by a positive feed-back mechanism. Since there is increased release and metabolism at the same time, the levels stay normal and they concluded that there was also stimulation of synthesis. These observations have been extensively confirmed by other workers (Ande et al, 1970). These drugs have also been shown to be potent antagonists of DA in vitro (Kebabian, Petzold and Greengard, 1972; Miller et al, 1974).

In contrast to the effects of DA agonists, neuroleptics administered directly into the striatum, produce catalepsy (Tseng, Wei and Loh, 1973) and abolish drug-induced stereotyped behaviour (Fog, Randrup and Pakkenberg, 1968).

1.7. Stereotyped behaviour and other neurotransmitters.

1.7.1. Acetylcholine:

Several workers have reported data consistent with an antagonistic cholinergic influence on drug-induced stereotyped behaviour (Arnfred and Randrup, 1968; Osuide and Adejoh,

1973). Anticholinergic drugs potentiate stereotyped behaviour induced by both amphetamine and apomorphine in rats, mice and chicks (Arnfred and Randrup, 1968; Osuide and Adejoh, 1973). Cholinergic agents enhance the blockade of amphetamine-induced stereotypy produced by a low dose of alpha-methyl-p-tyrosine (AMPT) (Arnfred and Randrup, 1968).

1.7.2. Serotonin (5-HT).

The role of serotonin in drug-induced stereotyped behaviour is not very clear and conflicting data have been reported by various workers. Randrup and Munkvad (1964) did not observe any influence of methysergide (Serotonin antagonist) on amphetamine-induced stereotypy in rats. Also, in rats, a study by Rotrosen, Angrist, Wallach and Gershon (1972) showed that the stereotypy produced by apomorphine was unaltered by PCPA pretreatment or by methysergide. Neither was it affected by the 5-HT precursor, L-tryptophan.

Breese, Cooper and Mueller (1974) observed no effect of PCPA pretreatment on amphetamine-induced stereotyped behaviour in rats. However, in guinea-pigs, Weiner, Goetz, and Klawans (1973) have reported data consistent with an inhibitory action of 5-HT on apomorphine and amphetamine stereotypy. Several other workers have reported data supporting this observation (Chen and Long, 1974; Saxena, Chawla, Johri and Iqbal, 1977). Electrolytic lesions of the raphe nuclei have been reported to attenuate the stereotypy produced by amphetamine, apomorphine, methylphenindate and piribedil (Costall and Naylor, 1974b).

1.7.3. Noradrenaline (NA).

Deplection of NA by the administration of DDC did not influence amphetamine-induced stereotypy (Randrup and Scheel-Kruger 1966). Destruction of NA neurons by 6-OHDA also does not affect amphetamine-induced stereotypy (Hollister et al, 1974). Thus, from these

observations it appears as if NA has no significant effect on drug-induced stereotypy.

1.8. Stereotyped behaviour as a model for screening antiparkinsonian agents.

Most of the current drugs used for the treatment of parkinsonism are either able to induce a stereotyped behaviour or enhance that induced by amphetamine and other drugs (Costall, Naylor, and Pinder, 1974). The relation between stereotypy and therapeutic activity against parkinsonism can be understood since stereotypy is considered to result from a stimulation of the nigro-striatal dopaminergic system (Fog, 1972) whilst parkinsonism is associated with its degeneration (Hornykiewicz, 1966). However, the mechanism by which drugs induce stereotyped behaviour is of critical importance, since a drug which mimics nigrostriatal stimulation by an action at presynaptic sites may only be of value in disease states characterized by incomplete degeneration of nigrostriatal pathway, whereas a

drug acting directly on the striatal DA receptors may have a wider application in the treatment of parkinsonism (Costall and Naylor, 1974a).

1.9. Stereotyped behaviour as a model of psychosis.

Amphetamine elicits stereotyped behaviour in humans as in other mammals (Randrup and Munkvad 1967; Ellinwood, Sudilousky and Nelson, 1973) and such a behaviour may be mediated by brain DA. Many items of behaviour can be selectively stimulated and become stereotyped in humans, e.g. cleaning of the house or a particular item, repeating the same words or sentences for hours, walking up and down the streets continuously and purposelessly (Rylander, 1969; Ellinwood et al, 1973). The abnormal states elicited by amphetamines in humans (amphetamine psychosis) often appear similar to schizophrenia, so much that misdiagnosis have been made by psychiatrists (Randrup and Munkvad, 1972).

Neuroleptics antagonize the symptoms of both schizophrenia and amphetamine psychosis

(Cole and Davis, 1968; Angrist, 1974). Since blockade of DA seems to be the only common and characteristic property of neuroleptics (Carlsson et al, 1972), this indicates an association of brain DA with the symptoms of schizophrenia. Based on these observations, Munkvad & Randrup (1970) proposed that absolute or relative overactivity of dopaminergic system in the brain is an important feature in the pathogenesis of schizophrenia. It cannot be ruled out, however, that abnormalities in other areas of the brain could elicit schizophrenic symptoms.

While many cases of amphetamine psychosis show resemblance to schizophrenia, some authors have reported cases which appear to resemble manic-depressive psychosis or have mixed manic-depressive and schizophrenia symptoms (Rylander, 1969) and this has led to further implication of DA in manic-depressive psychosis.

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1.10. Locomotor activity.

A variety of pharmacological procedures have been used in an attempt to relate locomotor activity to a particular neuronal system. These procedures include pretreatment with inhibitors of synthesis, storage and reuptake of transmitters as well as lesioning of discrete brain areas. There has been much debate about the possible role of central catecholamines in motor activity. The results of pharmacological manipulation of the amine systems has led some authors to claim that NA enhances certain aspects of motor activity Svensson, 1971; Maj, Grabowsky, and Gajda, 1972) although others have denied this (Thornburg and Moore, 1973).

On the other hand, there is a strong support in the literature of a role for DA in motor activity. Though several studies have been undertaken to elucidate the mechanism by which spontaneous and drug-induced locomotor activity is mediated, the results have been inconsistent.

The use of alpha methyl-para-tyrosine has provided convincing evidence that amphetamine-induced locomotor activity is mediated by release of catecholamines. It has been shown that pretreatment with AMPT, (inhibits the synthesis of NA & DA) blocks the locomotor stimulant-effect of amphetamine (Weissman, Koe and Tenen, 1966). However, depletion of NA and DA in the brain by pretreatment with reserpine does not block the amphetamine response (Van Rossum, Vander Schoot and Hurkmans, 1962; Smith, 1963). Although these early results with reserpine were taken as evidence for a direct action of amphetamine on catecholamine receptors (Van Rossum et al, 1962; Smith, 1963), it is now generally accepted that they can be explained by the hypothesis that amphetamine-induced locomotor activity is mediated by a small reserpine-insensitive functional pool of transmitter which is rapidly repleted by synthesis.

Further experimentation using FLA-63 (a dopamine B-hydroxylase inhibitor) has indicated a primary role for DA in spontaneous and drug-induced locomotor activity in rats (Corrodi, Fuxe, Ljungdahl, and Ogren, 1970; Thornburg and Moore, 1973).

1.10.1. Mesolimbic DA system and locomotor activity.

Injection of 6-OHDA into the nucleus accumbens or caudate nucleus selectively destroys mesolimbic or nigrostriatal dopaminergic terminals respectively (Kelly et al, 1975). It has been observed that animals with a 6-OHDA lesion of the nucleus accumbens showed a reduced locomotor response to d-amphetamine, but an enhanced response to apomorphine, probably due to denervation supersensitivity. On the other hand, those with lesion of the caudate nucleus showed neither of these changes, but showed changes in drug-induced stereotypy. These results suggest that the release of DA from the neurons of the mesolimbic system rather than those of the nigrostriatal

system is important for the stimulation of locomotor activity by amphetamine (Kelly et al, 1975). In addition to apomorphine, rats with 6-OHDA lesion of the nucleus accumbens also show enhanced locomotor activity response to ergometrine and other direct acting DA agonists, (Woodruff, Kelly and Elkhawad, 1976).

Injection of dopamineceptor agonists into the nucleus accumbens stimulate locomotor activity (Pijnenburg, Woodruff and Van Rossum, 1973). NA injected into the same area or DA injection into the caudate nucleus were significantly less effective (Pijnenburg and Van Rossum, 1973).

Dopaminoceptor antagonists administered either peripherally or directly into the nucleus accumbens antagonized the locomotor stimulation produced by dopaminoceptor agonists (Pijnenburg et al, 1973). These observations indicate that the mesolimbic DA system may be involved in locomotor activity.

1.11. The role of some other neurotransmitter systems.

Apart from the mesolimbic DA system, several studies have implicated other neurotransmitter systems as possible modulators of drug-induced locomotor activity.

1.11. 5-HT.

Decreases in functional brain serotonin, produced either through raphe lesions (Kostowki Giacolone, Garattini and Valzelli, 1968) or inhibition of synthesis with PCPA, resulted in a marked increase in locomotor activity in the rat. Conversely, procedures that increase 5-HT levels such as administration of tryptophan and 5-hydroxytryptophan (Brown, 1960) decrease locomotor activity in rats. These observations suggest a central inhibitory role of 5-HT on locomotor activity.

1.11.2. Acetylcholine.

Administration of cholinomimetic drugs reduced spontaneous and drug-induced locomotor activity (Fiberger, Lytle and Campbell, 1970).

Conversely, antimuscarinic drugs have been shown to increase locomotor activity (Payne and Anderson, 1967) and potentiate amphetamine-induced activity (Fiberger et al, 1970). Thus, the muscarinic effects of acetylcholine appear to exert an inhibitory influence on locomotor activity.

1.11.3. Tricyclic antidepressants and locomotor activity.

Several efforts have been made to develop experimental models for detecting compounds with antidepressant property. However, such efforts have been hampered by a poor understanding of the mechanisms of action of the currently available antidepressants. With an improved understanding of the mechanisms of their antidepressant action, appropriate animal models based upon these mechanisms could be developed. The use of such models might then result in the discovery of antidepressants with better properties than the currently available ones.

Among the many procedures which are currently used to identify antidepressant activity are, the blockade of NA and 5-HT uptake (Lidbrink, Johnson and Fuxe, 1971), the potentiation of noradrenergic activity (Sigg, Soffer and Gyermek, 1963) and

dopaminergic effects (Everett, 1966), the inhibition of cyclic nucleotide phosphodiesterase enzyme activity (Palmer, 1976), the increase in NA turnover (Schild-Kraut, Winokur and Applegate 1970), the depression of REM sleep (Khazan and Brown, 1970) and the depletion of S-adenosyl-methionine (Taylor and Randall, 1975). However, none of these parameters correlates as well as might be expected with clinical antidepressant activity. The main inconsistencies are the failure of many of these tests to predict the antidepressant effects of some tricyclics (such as iprindole, opiramol and trimipramine) and the failure to predict the delayed onset of activity of the standard tricyclic antidepressants (Oswald, Brezinova and Dunleavy, 1972). These observations suggest that further work is required to develop more sensitive tests for proper identification of agents with antidepressant action.

Imipramine and some other tricyclic antidepressants have been found to induce marked behavioural changes in chicks (Mandell, Spooner, Winters, Cruishank and Sabbot, 1963; Schrold, 1970). This is characterized by an initial period of decreased locomotor activity followed by a period of excitation and aggressive behaviour. Since catecholamines have been implicated in locomotor activity, these observations suggest a possible interaction between catecholamines and tricyclic antidepressants in locomotor activity.

1.12. Excitement, Chorea and Circling Behaviour (EEC-syndrome).

A variety of neurological symptoms may be induced by the administration of β,β'-immodipropionionitrile (IDPN) to rats and mice (Delay, Thuillier and Marquiset, 1952; Selye, 1957). IDPN has been shown to elicit a permanent hyperkinetic syndrome that is characterized by excitation with fine tremors,

choreiform head and neck movements and circling and this has led to the syndrome being called the ECC-syndrome (Seyle, 1957). Other abnormalities seen in IDPN-treated rats include kyphosis, ataxia, paresis of the hindlimbs, a tendency towards retropulsion, inability to swim (Gabay, 1966) and blindness (Forgacs, 1959). Similar behavioural patterns have been observed following the administration of chlorinated tertiary amines (Goldin, Noe, Landing, Shapiro and Goldberger, 1948), hexose reductase (Cutting, Furst, Read, Read and Parkman, 1960) arsilinic acid (Selye, Kourounakis and Szabo, 1972). The toxic effects of β-aminopropionitrile (Schilling and Strong, 1954) and methyleneaminoacetonitrile (Gabay, Vivanco, Ramos, and Jimenez-Diaz, 1961) which are structurally related to IDPN, have also been studied. These compounds induce gross skeletal deformities with connective tissue abnormalities in experimental animals. It has been

observed that pretreatment with thyroxine protects animals against the toxic effects of IDPN and B-aminopropionitrile.

Several studies have been performed on the brain of IDPN-treated animals in the search for the underlying causes of the ECC-syndrome. Histological lesions occuring after the drug (Hartmann et al, 1968) have been described in many regions of the central nervous system of IDPN-treated animals (Chou and Hartmann, 1964). Such studies have demonstrated a swelling of the proximal segment of axons (Chou and Hartmann, 1964). Histochemical and biochemical analyses of these axonal balloons have detected an increased axoplasmic protein content (Diezel and Ule, 1963) with no apparent change in RNA content. The various biochemical determinations performed on whole brain tissue include measurement of free amino acids, (Vivanco, Ramos and Jimenez-Diaz, 1966), GABA and GABA transaminase activity

(Gabay, 1966) aromatic aminotransferase activities (Gabay and Huang, 1969), adenine nucleotides (Williams, Paterson and Heath, 1968), and overall metabolism (Brownlow and Heath, 1969). However, in spite of all these studies, the primary anatomical and neurochemical bases for this syndrome remain unknown.

The biogenic amines, (DA, NA and 5-HT) as putative neurotransmitters, play an important role in the regulation of central nervous system functions (Hornykiewicz, 1966; Chase and Murphy, 1973). Neurological disturbances caused by brain damage due to injury or exposure to toxic substances are often accompanied by changes in the endogeneous levels of biogenic amines. The levels of these brain amines have been determined in various brain regions of IDPN-treated rats (Langlais, Huang and Gabay, 1975). Significant alterations were observed in the levels of 5-HT and 5-HIAA in several regions of the brain and these workers suggested that 5-HT-containing

neurons may be involved in IDPN neurotoxic effects. Evidence has also been reported on the possible involvement of dopaminergic mechanisms in the ECC-syndrome (Gabay, Langlais and Huang, 1974).

1.13. Evoked Response Studies.

An evoked response may be defined as that change in electrical activity within a particular part of the nervous system which is triggered by an incoming signal or stimulus. The stimulus may be:

a) simulated natural stimulus, e.g., flash, click, flicker, noise, joint manipulation, tactile pressure, etc,

b) an electrical stimulation of a specific fibre tract or nucleus, e.g. single shocks, paired shocks, or multiple shocks,

c) induced by the application of chemicals to neural tissue.

Using these various techniques, it has been observed that the study of these evoked responses,

have yielded important information about the input pathways involved in a given sensory modality, (Goldstein, 1961). Such an approach has also been found useful for mapping neuronal connections within the central nervous system.

1.13.1 Theories of Evoked Potentials generation.

Evoked potentials are generated by neuronal aggregates in response to a sensory stimulus. These potentials are thought to be generated in part by summation of excitatory and inhibitory postsynaptic potentials and to a lesser extent by action potentials (Schlag, 1973). However, under certain conditions, presynaptic activity, glial potentials and volume conduction of dipoles created by local changes in ionic levels may also contribute to the generation of evoked potentials (Schlag, 1973). This complexity makes interpretation of specific neuronal mechanism based on evoked potential changes rather difficult. Nevertheless, changes in sensory evoked potentials do provide a measure of possible changes in sensory

processing by a fairly large neural aggregate within the brain.

For many years it was believed that axon spikes were responsible for the generation of cerebral slow waves (Adrian and Matthews, 1934). However, in 1940, Renshaw and Co-workers demonstrated that there was no relationship between slow waves and axon spikes recorded from small groups of neurons. It was also later observed that axon spikes could be suppressed while slow wave activity persisted (Amassian, Waller and Macey, 1964). Hence this first hypothesis of slow wave generation has become unpopular.

In 1966, Creutzfeldt's and others proposed that the generation of EEG results from volume conduction of the synchronous activity of pyramidal cells. According to these workers these cells contribute uniformly to the EEG because of their common vertical orientation relative to the surface of the cortex.

1.13.2. Analysis of Evoked responses.

One of the major problems of evoked response studies is that of analysis, and this is due to the fact that the response is often very small, because it originates from a relatively few neurons, and the majority of adjacent neurons generate differing and random potentials which mask the evoked response.

The original technique of analysis consisted of photographing a series of evoked waves displayed on an oscilloscope (Dawson, 1947). The same author subsequently devised an instrument for automatic summation to detect small evoked potentials (Dawson, 1951).

Recently, special purpose digital computers with built-in programmes have been developed to perform the function of analysis. Although these computers differ in detail, the basic scheme is about the same. The computer is activated by a triggering pulse to commence EEG

sampling and the analog-to-digital converter in the computer samples the EEG at various specified times and deposits in the memory electronic pulses that are proportional to the sampled voltage. As each additional stimulus is presented to the animal, results are again recorded at the identical intervals and are added to the results already in the memory. These sums are displayed sequentially on the computer oscilloscope screen. Thus, the sum of the responses can be viewed at all times.

As the number of responses increases, all random variations tend to average towards zero, and the time-locked repetitive pattern, the evoked response, will add and become magnified. Random activity cancels because of algebraic addition of voltages of opposite polarity; there is an equal probability for the voltage to be positive or negative at a given instant. Thus, an increase in the number of responses analyzed improves the cancellation of background "noise" and

thereby improves the magnification of the evoked response.

Evoked responses data can be 'read out' of the memory in various ways either by viewing on the osciloscope screen, by typed digital data, by plotting on a strip-chart recorder, or by magnetic or paper tape that can be fed into other computers.

1.13.3. Clinical Applications of EPs.

Evoked potential methods are widely used in the diagnosis of various diseases of the nervous system. One of the major clinical applications of evoked potentials is in sensory testing especially in infants and young children. The aim of such an exercise is to determine whether a particular sensory dysfunction (such as deafness) is due to (a) peripheral damage, or (b) brain damage, mental retardation or psychosis. Informations obtained from such procedures allow vital early decisions to be made about the handling of young children and

infants.

Evoked potential methods have also been found useful for identifying lesions of peripheral nerves, for locating structures within the brain, and in psychiatry.

1.14. Addiction Studies.

Drug-dependence has customarily been defined as a state, caused by exposure to drug, such that cessation of exposure produces a disturbance that can be stopped by readministering the drug or one with which it is cross-dependent. Many psychoactive drugs are self administered by otherwise normal individuals because of their ability to induce pleasurable changes in perception and affect. Commonly abused drugs include arousal changing drugs such as the amphetamines and barbiturates, mood changing drugs such as the opiates, and hallucinogenic drugs such as lysergic acid derivatives and cannabis.

Experimental studies of the reinforcing properties of drugs were first reported in the 1950s

Nichols and his co-workers demonstrated that rats could be induced to consume increasing quantities of a morphine solution even when their usual drinking water was available, or to move their heads in a particular way when this movement was followed by infusion of morphine (Nichols et al, 1956). Particularly important contributions were also made by Thompson and Schuster (1964), who reported that rats and monkeys greatly increased the frequency with which they pressed a bar when such responses were followed by intravenous infusion of morphine. Yanagita et al, (1965) developed a technique that made possible intravenous self administration in relatively unrestrained rhesus monkeys. They reported reinforcing propertieis for a variety of drugs

including ethanol, pentobarbitone, amphetamine, and cocaine (Denau et al, 1969). It has been observed that prior exposure of rats to ethanol in a free choice situation results occasionally in the development of preferences for ethanol. Such rats will bar-press in order to obtain small oral doses of the ethanol solutions (Sinclair, 1974). In general, the taste of ethanol seems to be aversive to rats and monkeys. This aversion has led to some studies employing the intragastric and intravenous routes (Mello, 1973). However, the extremely aversive taste of morphine solutions does not inhibit rats from learning to ingest such solutions in preference to water. Indeed, Stolerman and Kumar (1970) have reported evidence indicating that the flavour of morphine serves as a discriminative stimulus or secondary reinforcer. Thus it seems that ethanol and sedative-hypnotic drugs are less powerful reinforcers of behaviour than are opoids.

Though, these techniques have served as useful animal models for identifying addictive drugs and have led to a better understanding of the behavioural influences underlying addiction, the neurophysiological basis of drug dependence have not been clearly understood.

Abrupt discontinuation after chronic administration of ethanol and barbiturates have been shown to precipitate withdrawal symptoms with EEG correlates. Guerrero-Figueroa, Rye, Gallort and Bishop (1970) reported an increase of neocortical and subcortical fast EEG activity in cats submitted to large oral doses of ethanol for 2 to 5 months, and a relative slowing of the EEG with superimposed epileptiform discharges upon withdrawal from such treatment. Walker and Zornetzer (1974) observed seizure activity in the EEG of mice upon abstinence from a 6-day exposure to a liquid diet containing a large amount of ethanol. Hunter, Boast, Walker, and Zornetzer (1973) reported similar findings in rats made

alcoholic for 15 days with a similar procedure. In man, Wikler, Prescor, Frazer and Isbell (1956) observed only minor alterations of the scalp EEG of chronic alcoholics, both before and during abstinence and even when the latter involved delirium tremens and convulsions.

1.15 Objectives of this project

1.15.1. Stereotyped behaviour.

Stereotyped behaviour is widely used as an index of dopaminergic stimulation and as an indicator of antiparkinsonian potential (Costall, Naylor and Pinder, 1974). Stereotyped behaviour is also widely used as a behavioural model for screening drugs for antipsychotic properties. In the light of these various applications, one of the objectives of this project is to study the influence of various DA agonists in young chicks and to compare the effects of these drugs with those of other drugs which have been observed to produce definite behavioural effects in this species. Such a study would further enable us to

establish whether these DA agonists induce a consistent pattern of stereotyped behaviour in chicks and the possibility of using this as a behavioural model for screening purposes.

Though activity at central DA receptors is generally considered to be responsible for the induction of stereotyped behaviour, the involvement of other neurotransmitter systems has been implicated. Using drug interaction studies it is the aim of this project to elucidate the influence of some neurotransmitters other than DA in drug-induced stereotyped behaviour in this species.

1.15.2. Locomotor activity.

Both apomorphine and tricyclic antidepressants have been reported to induce increased locomotor activity in various species. This action of apomorphine is however usually accompanied by stereotyped activity. Imipramine and other tricyclic antidepressants also induce a dose-dependent aggressive behaviour in young

chicks. It is therefore intended to establish the dose of apomorphine which increases locomotor activity without stereotyped behaviour in chicks. The second aspect of this study is to investigate the interaction of this dose of apomorphine with tricyclic antidepressants with the aim of establishing whether these drugs would produce a consistent locomotor pattern and the possibility of using this as a behavioural model for screening compounds for antidepressant properties.

1.15.3. Excitement - chorea and circling (ECC) Syndrome.

β, β′ - iminodipropionitrile (IDPN) has been shown to elicite a permanent hyperkinetic syndrome that is characterized by excitation with fine tremors, choreiform head and neck movements and circling and this has led to the syndrome being called the ECC - syndrome (Seyle, 1957). Though several studies have been carried out in IDPN-treated animals in the search for the underlying causes of the syndrome, the primary anatomical and neurochemical basis for this syndrome remains

unknown. One of the objectives of this project is to study the behavioural effects of IDPN in chicks and to compare these effects with those that have been reported in other species. It is also intended to study the influence of various groups of drugs on IDPN - induced effects with the aim of elucidating the mechanism by which IDPN produces its behavioural effects in chicks. It is further intended to study the influence of IDPN administration on the levels of brain biogenic amines in syndromized chicks using fluorimetric procedures.

1.15.4. Evoked Response studies.

The recording of evoked responses to sensory stimulations has been established as a sensitive method for studying the effects of drugs on the central nervous system. Various techniques have been used to record evoked responses in chicks (Scholes and Roberts, 1964; Scholes and Gutnick, 1970; Spooner and Winters, 1967; Kramer, Sherman and Seifter, 1967). Some of these workers used

curarized or anesthetized chicks, and under such conditions it would not be possible to appreciate the behavioural correlates of the evoked responses. Furthermore, the anaesthetic conditions, the necessity for artificial respiration and the central effects of curare in the young chick (Scholes, 1965), place limitations on the interpretation of results obtained under such conditions. There has been a reluctance to use photic - evoked response in conscious unrestrained animal studies due to inconsistent results (Winters, 1968). One of the objectives of this project is to investigate the pattern and utility of photic - evoked response in young chicks. A further objective of this project is to study the influence of various centrally acting drugs on photic - evoked response with a view to using the PER alone, or in combination with other methods, in elucidating actions of psychotropic drugs.

1.15.5. Drug-dependent studies.

Several methods have established for detecting drugs with addictive properties. However, most of these methods are based on the self-administration of such drugs by laboratory animals, and the neurophysiological correlates of the dependent states are not usually evident using such methods. One of the objectives of this project is to investigate the pattern and utility of photic-evoked response in drug addiction studies in rats and the possibility of using this technique for screening purposes.

CHAPTER II

2. MATERIALS AND METHODS.

2.1. Experimental Animals.

White ranger cockerels (Warren strain) obtained locally from Arewa Agricultural Enterprises Ltd Zaria and albino rats (inbred in our Animal House) were used for this project.

2.2. Drug solutions.

2.2.1. Preparation of drug solutions.

(a) Apomorphine hydrochloride (British Drug Houses Ltd).

Apomorphine hydrochloride solution was prepared by dissolving the powder in boiled, air-free saline and kept in an amber-coloured air-tight bottle. 0.1% w/w sodium metabisulphite was added to the weighed quantity of apomorphine hydrochloride before the preparation of each solution to enhance stability, since apomorphine hydrochloride solution decomposes readily when exposed to air or light. A fresh

solution was prepared for each day's experiment.

b) Haloperidol (Janssen Pharmaceutica) and FLA-63 (Labkemi AB).

Both Haloperidol and FLA-63 were dissolved in 0.05 M HCl with the aid of gentle heating over a water bath. Appropriate dilutions were made with normal saline.

(c) Pimozide (Janssen Pharmaceutica) and parachlorophenylalanine (PCPA, AB Biotec). Pimozide and PCPA were suspended in 0.3% v/v Tween 80 and adjusted to the appropriate volume with normal saline.

(d) Reserpine (Ciba), Ergometrine maleate (Richter), lysergic acid diethylamide (LSD, Sandoz).

These drugs were supplied in ampoules and appropriate dilutions were made with normal saline.

(e) 6-hydroxydopamine hydrobromide (6-OHDA, Sigma Chemical Co.) and Dopamine hydrochloride (DA, Sigma Chemical Co.)

The solutions of 6-OHDA and DA were prepared by dissolving the powder in 0.05 M HCl. 0.1% w/w of sodium metabi-sulphite was added to the solutions to enhance stability. Solutions were always freshly prepared.

(f) L-dopa (Sigma Chemical Co.).

L-dopa was dissolved in 0.05 M hydrochloric acid solution with the aid of gentle heating over a water-bath. The solution was diluted with normal salin e before injection.

(g) β,β'-iminodipropionitrile Hydrochloride (IDPN).

The solution of IDPN was freshly prepared daily by dissolving the crystals in phosphate buffer, pH 7.0.

(h) d-Amphetamine sulphate (Smith Kline and French Laboratories), Amantadine hydrochloride (Ciba-Geigy), Piribedil (ET 495, Servier Laboratories), DL-threo-3,4-dihydroxyphenylserine (DOPS, AB Biotec), Imipramine hydrochloride (Ciba-Geigy Pharmaceuticals) Desipramine hydrochloride (Ciba-Geigy Pharmaceuticals), Amitriptyline

hydrochloride (Roche Products), Quipazine (Miles Laboratories), 5-hydroxytryptamine creatinin (Sigma Chemical Co.), Cyproheptadine hydrochloride (Merk Sharp and Dohme), Gamma aminobutyric acid (GABA, Koch Light), Gama hydroxybutyrate (GHB, Sigma Chemical Co.), pentobarbitone Sodium (British Drug Houses Ltd), Mescaline sulphate (Koch Light), Physostigmine salicylate (Burroughs Wellcome & Co.), atropine Sulphate (British Drug Houses Ltd.), hyoscine hydrobromide (Koch-Light), dl-Propranolol Hydrochloride (Sigma Chemical Co.) and phenoxybenzamine (Smith Kline & French Laboratories). Solutions of the drugs mentioned above were prepared with normal saline. The drug solutions were usually freshly prepared for each day's experiment and stored in the refrigerator until ready for use.

2.3. Routes of Administration.

Apomorphine, d-amphetamine, ergometrine piribedil and IDPN were administered subcutaneously

while all the other drugs used were administered intraperitoneally.

2.4. Controls.

The control animals were administered with the appropriate vehicles used in preparing the drug solutions.

2.5. Stereotyped Behavioural Studies using chicks.

Chicks of the same age and approximately the same weight were observed in pairs in a cage of the following dimensions, 0.6 x 0.45 x 0.23 m The observations were carried out for 1 - 2 h in a quiet room. The floor of the cage was always kept clean and free of specks resembling food particles. There was a receptacle inside the cage from which the chicks could feed without spilling food particles on the floor of the cage. A second receptacle contained drinking water. The chicks fed and drank water from similar receptacles from the time of hatching. With this arrangement, it was possible to distinguish between true feeding and pecking at non-food

objects. The stereotyped behaviour consisting of the following activities was recorded using hand tally counters. One counter was used for each of the following parameters:
(1) pecking at own body and feet (2) pecking at each other's body and feet (3) pecking at non-food objects and (4) fighting episodes. The experiments in which the influence of various drugs were tested on the drug-induced abnormal behaviour were performed as follows. For each day's experiment, five pairs of chicks were used. The first experiment served as a control in which either apomorphine, amphetamine, amantadine or piribedil was administered alone. This was followed by three consecutive experiments in which three different pairs of chicks were pretreated with the drug under test before injection of either apomorphine, amphetamine, amantadine or piribedil. At the end of the test experiment, another control experiment was done similar to the first one. These drug interaction studies were carried out on four

separate days. The mean control count and the count obtained with the test chicks (i.e. treated with appropriate drugs) were calculated using the four sets of results

2.6. Locomotor activity studies using chicks.

Two chicks of approximately the same weight were placed in an opened perspex cage of dimensions 47 x 27 x 24 cm. The chicks were provided with food and water ad libitum. The cage with its contents, was then placed on a previously cablibrated Animex activity meter Type S (Farad electronics) and the meter was rebalanced such that all locomotor activities by the chicks inside the cage were picked up. Counting was recorded by the Farad Electronics Animex counter Type 1 x X - 0 connected to the meter and positioned at a remote distance from it to avoid disturbance created by the counting and the click noise which the counter made every 10 min. The chicks were left in the cage for an acclimatization period of 30 min. during which the

spontaneous locomotor activities of the untreated chicks were recorded. After this period the counter was switched off and the chicks were treated with the drug to be studied Counting was subsequently continued for such lengths of time as had been found from direct observational studies to be required for behavioural manifestations. In the experiments in which the influence of tricyclic antidepressants on the effect of apomorphine was studied, the chicks were pretreated with the drugs under test for specific pre-determined pretreatment times before subsequent administration of apomorphine. Control experiments followed the same pattern except that the chicks were treated with normal saline.

2.7. ECC-syndromized chicks.

Chicks aged 2 — 3 weeks were used for this study. The chicks were injected daily with IDPN (75 mg of base /kg sc) for 5 days. The optimum dose of IDPN was determined after numerous

toxicity studies using various doses. When this dose was administered subcutaneously, the ECC-syndrome appeared on the 6th day. Control chicks injected with phosphate buffer, (pH 7.0) were maintained under identical conditions, The syndromized chicks were observed singly in a cage of dimensions 0.6 x 0.45 x 0.23 m., which contained food and drinking water. Control observations were done for 1 - 1½ h. before the chicks were treated with the drugs to be tested. After drug treatment the observations were continued for 1 - 1½ h. and circling and choreiform head movements were counted using hand tally counters in each case.

2.8. Fluorimetric determination of Biogenic Amines in Syndromized chicks.

2.8.1. Method.

The method of Jacobowitz and Richardson (1978) was used for the fluorimetric determination of noradrenaline (NA), Dopamine (DA), and serotonin (5-HT) in four regions of the brain of control and syndromized chicks. The brain regions were

hyperstriatum (HS), Optic tectum (OT), Cerebellum (CB) and Pons-medulla (PM).

2.8.2. Reagents:

The following reagents were used in the assay. n-Butanol (BDH chemicals Ltd), n-heptane (May & Baker Ltd), 0.1 M phosphate buffer (pH = 6.5) prepared fresh every 2 days by adding 32 ml of di-sodium phosphate solution (prepared by dissolving 7.1 g $Na_2 HPO_4$ in 500 ml of distilled water) and 68 ml of monobasic sodium phosphate solution (prepared by dissolving 6.9 g NaH_2PO_4 in 500 ml of distilled water), versene prepared by dissolving 4 g disodium ethylene-diamine tetra-acetic acid, EDTA, in 95 ml. of distilled water and adjusting the pH to between 6 and 6.5 with 10 N sodium hydroxide before making the volume up to 100 mls), iodine solution (prepared from 4.8 g potassium iodide plus 0.25 g iodine and made up to 100 mls with distilled water), alkaline sulphite solution (prepared fresh daily by dissolving 2.5 g sodium sulphate (Na_2SO_3) in 100 ml 4 N sodium hydroxide),

5 N acetic acid, 10 N HCl, 0.01 and 0.1 N HCl, ortho-phthaldialdehyde solution (OPT, prepared fresh daily by dissolving 50 mg of OPT in 100 ml of absolute methanol). Apart from butanol, heptane, methanol, acetic acid, and hydrochloric acid (10 N), all the other reagents were stored in the refrigerator until ready for use.

2.8.3. Regional disection of the chick brain:

The syndromized and control chicks were decapitated, the brains were quickly removed and the hyperstriatum, optic tectum, pons-medulla and cerebellum were placed in polyethylene vials and kept in liquid nitrogen until the time of assay.

2.8.4. Extraction.

NA, DA and 5-HT were assayed as follows. 5 ml of n-butanol was placed in a hand homogenizing tube immersed in ice water. Each tissue sample was rapidly weighed in such a way that the tissue remained frozen. The weighed tissue sample

was then added to the n-butanol and an a appropriate amount of 0.01N HCl (table 2.1) so that tissue water plus 0.01 N HCl amounted to 0.75 ml (assuming tissue weight is 70% water) in 5 ml of n-butanol. The tissue was homogenized with the tube in ice water until the tissue was uniformly distributed throughout the n-butanol. The homogenate was poured into a 12 ml conical centrifuge tube and centrifuged for 10 min to sediment the tissue debris and a clear supernatant was obtained. 2 ml of the n-butanol supernatant was placed into a 12 ml conical centrifuge tube which contained 1.5 ml of 0.IN phosphate buffer in ice water. For analysis of 5-HT, an additional 2 ml of n-butanol supernatant was placed in another centrifuge tube which contained 5 ml heptane and 0.5 ml of 0.IN HCl in ice water. Both mixtures were vortexed for 20 sec and this resulted in the extraction of NA and DA into the phosphate buffer while 5-HT was extracted into the 0.IN HCl. Both mixtures were centrifuged (3000 rpm) to separate the organic

TABLE 2.1

VOLUME OF 0.01 N HCl FOR TISSUES IN A GIVEN WEIGHT RANGE

Weight in g between	HCl in ml	Weight in g between	HCl in ml
0.0000 & 0.0071	0.75	0.2500 & 0.2643	0.57
0.0071 & 0.0214	0.74	0.2643 & 0.2786	0.56
0.0214 & 0.0357	0.73	0.2786 & 0.2929	0.55
0.0357 & 0.0500	0.72	0.2929 & 0.3071	0.54
0.0500 & 0.0643	0.71	0.3071 & 0.3214	0.53
0.0643 & 0.0786	0.70	0.3214 & 0.3357	0.52
0.0786 & 0.0929	0.69	0.3357 & 0.3500	0.51
0.0929 & 0.1071	0.68	0.3500 & 0.3643	0.50
0.1071 & 0.1214	0.67	0.3643 & 0.3786	0.49
0.1214 & 0.1357	0.66	0.3786 & 0.3929	0.48
0.1357 & 0.1500	0.65	0.3929 & 0.4071	0.47
0.1500 & 0.1643	0.64	0.4071 & 0.4214	0.46
0.1643 & 0.1786	0.63	0.4214 & 0.4357	0.45
0.1786 & 0.1929	0.62	0.4357 & 0.4500	0.44
0.1929 & 0.2071	0.61	0.4500 & 0.4643	0.43
0.2071 & 0.2214	0.60	0.4643 & 0.4786	0.42
0.2214 & 0.2357	0.59	0.4786 & 0.4929	0.41
0.2357 & 0.2500	0.58	0.4929 & 0.5000*	0.40

*Weights between 0.5 and 1 g, divide weight by 2, find HCl for 1/2 original weight, put twice this amount of HCl in 10 ml butanol (e.g. tissue weighs 0.7100 ÷ 2 = 0.3550, HCl for 0.3550 = 0.50 ml x 2 = 1 ml HCl in 10 ml butanol). For weights between 1 & 1.5 g, find HCl for 1/3 original weight, put 3 times this amount of HCl in 15 ml butanol (Jacobwitz and Richardson, 1978).

and aqueous layers and the top organic layer was aspirated off with a pipette. 1 ml of the phosphate extract was placed in a test tube while 0.3 ml of the 0.1N HCl extract was placed in another test tube. These aliquots were oxidized into the respective fluorophores.

2.8.5. Oxidation of NA and DA.

The NA and DA in the phosphate buffer extract were oxidized to fluorophores as follows. The mixture was vortexed briefly after the addition of each reagent. 0.25 ml of versene was added to the extract and the mixture was vortexed briefly. The following reagents were added at exactly 2 min intervals: 0.20 ml iodine followed 2 min later by 0.25 ml alkaline sodium sulphite and 2 min later 0.3 ml 5 N acetic acid. The test tube was placed in boiling water for 5 min and then in ice water for 1 min. NA fluorescence was read immediately in a Perkin-Elmer fluorescence spectrophotometer Model MPF 44A) at 385 nm excitation and 485 nm

emission wavelengths. DA fluorescence was read 25 min later at 320 excitation and 385 emission wavelengths.

2.8.6. Oxidation of 5-HT.

The 5-HT in the 0.1N HCl extract was converted to fluorophore by adding 0.2 ml of OPT solution followed immediately by 1.5 ml of concentrated (10 N) HCl and the mixture was vortexed briefly. The test tube was placed in boiling water for 10 min, then cooled to room temperature and the fluorescence was read at 360 nm excitation and 470 nm emission wavelengths.

2.8.7. Controls.

Control chicks that were kept under identical conditions as the syndromized chicks but received only injections of phosphate buffer (pH 7.0) were used. The chicks were decapitated, the brain regions were quickly removed and stored in liquid nitrogen. The whole process of extraction of NA, DA and 5 HT

was repeated as stated above. The only difference was that 4 ml of n-butanol supernatant and 3 ml of phosphate buffer were used in this case. 1 ml of the phosphate extract was transferred into each of two separate test tubes which were labelled to identify initial and duplicate aliquots. One aliquot was oxidized into a fluorophore as above, while the second was processed to provide a blank reading by adding at exactly 2 min intervals, first the alkaline sulphite, then the iodine and finally the acetic acid. By reversing the order in which the iodine and alkaline sulphite were added, the oxidation of NA and DA into fluorophores was prevented. The mixture was boiled, cooled and read as above. The blank readings for a particular brain area were averaged and these values were substracted from each oxidized reading of similar areas from the brains of the syndromized and control chicks to get the relative fluorescence values for NA and DA at

the corresponding sensitivities.

For 5-HT, the control determinations were carried out in a similar manner to obtain the relative fluorescence values for each brain region.

2.8.8. Calibration curves.

Various concentrations of authentic samples of NA, DA and 5-HT were oxidized following the same procedures as in the assay. The concentrations used ranged between 0.025 ug and 10 ug and gave reasonable fluorescence to cover all the sensitivities used in the assay. The experiments were repeated four times and the arithmetic means of such values were used to draw the graphs of the relative fluorescence/concentration. The graphs were used to estimate the concentration of biogenic amines extracted from the chicks.

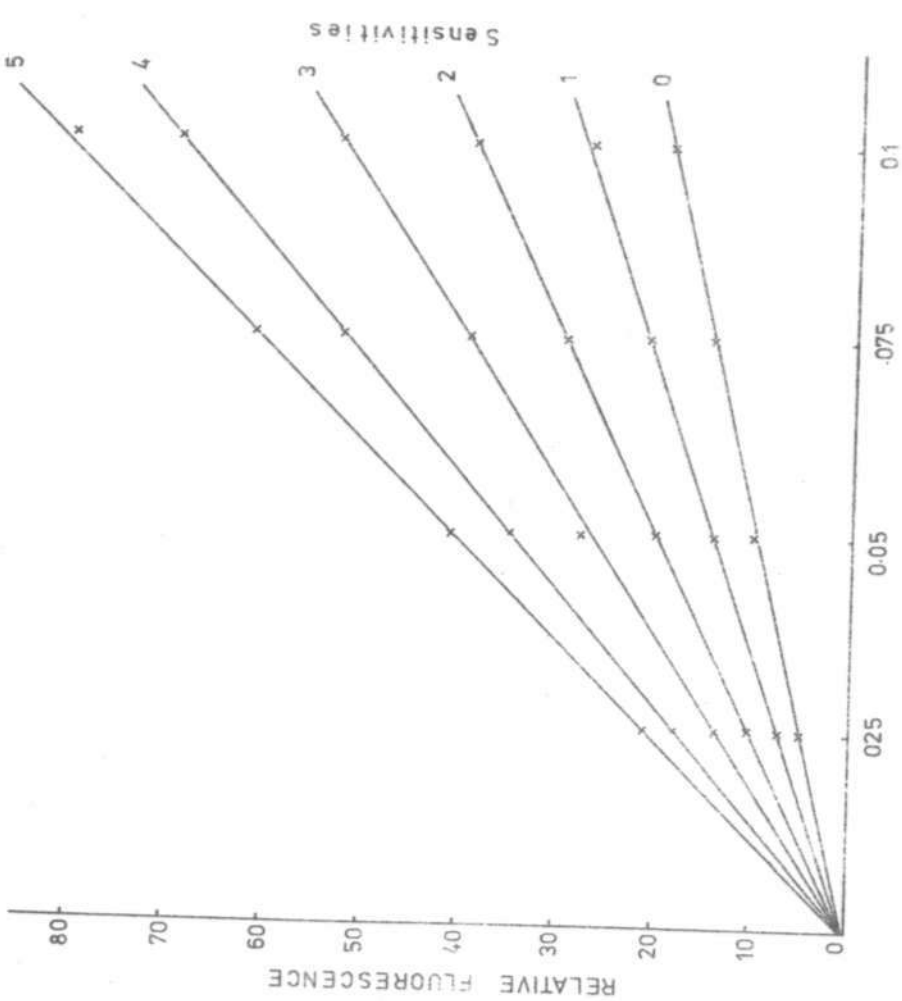


Fig. 2.1. Calibration curves for NA.

Coarse sensitivity of the amplifier = 10, while the fine sensitivities are shown on the right-hand panel.

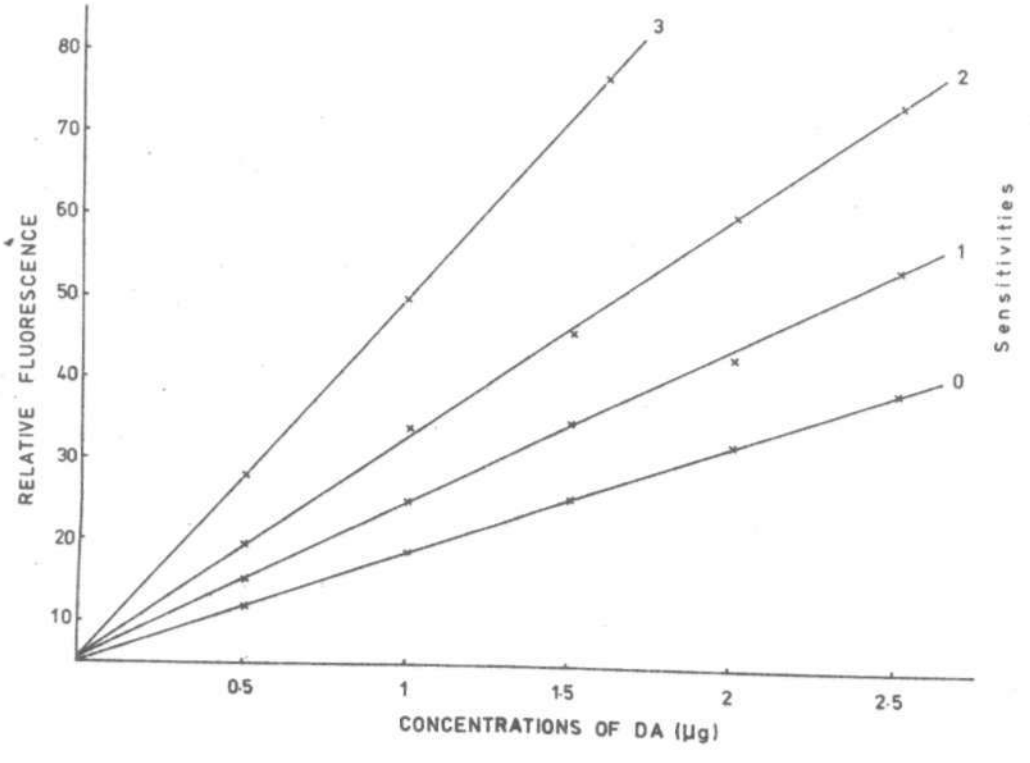


Fig. 2.2. Calibration curves for DA.

Coarse sensitivity of the amplifier = 10, while the fine sensitivities are shown on the right-hand panel.

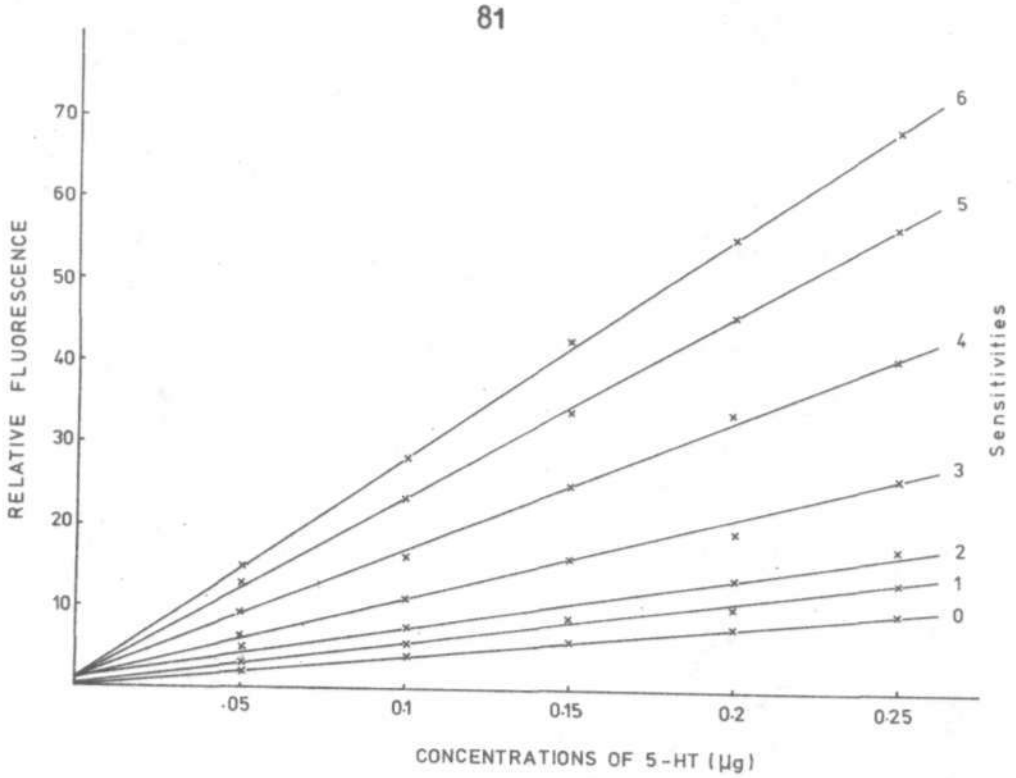


Fig. 2.3. Calibration curves for 5-HT.

Coarse sensitivity of the amplifier = 10, while the fine sensitivities are shown on the right-hand panel.

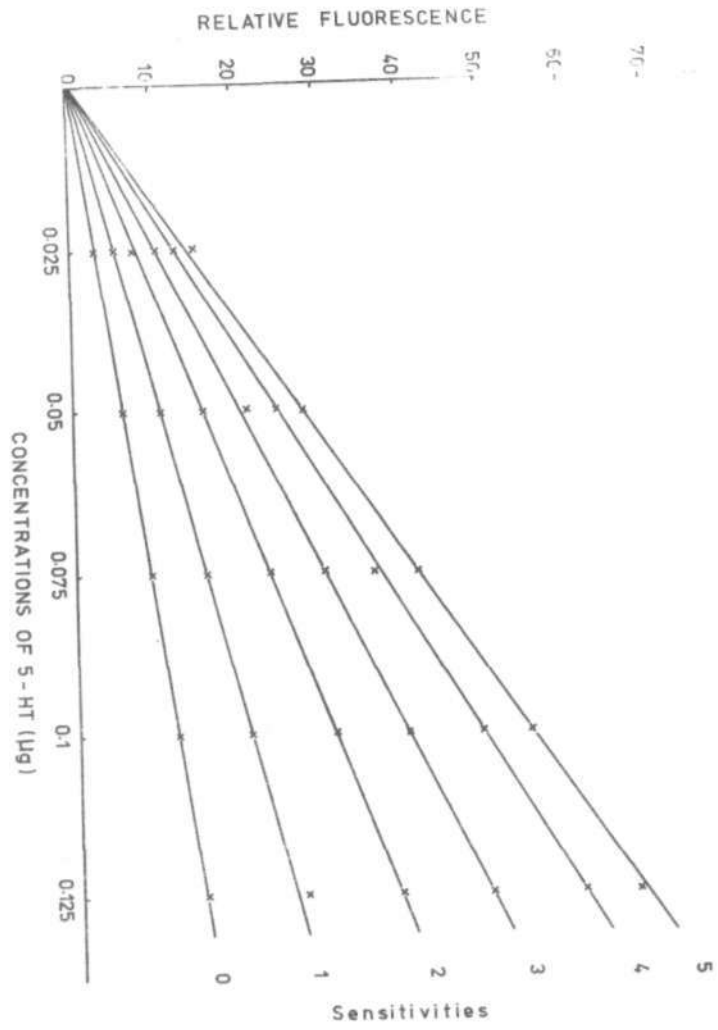


Fig. 2.4. Calibration curves for 5-HT.
Coarse sensitivity of the amplifier is = 30, while the fine sensitivities are shown on the right-hand panel.

CHAPTER III

3. STEREOTYPED BEHAVIOUR INDUCED BY DOPAMINE AGONISTS IN CHICKS.

3.1. Summary.

3.1.1. The behavioural effects of some DA agonists were studied in young chicks.

3.1.2. Apomorphine, d-amphetamine, amantadine and piribedil induced stereotyped pecking activity of varying intensities in chicks.

3.1.3. DA, L-dopa and ergometrine did not induce characteristic stereotyped pecking and aggressive behaviour in chicks.

3.1.4. d-Amphetamine and ergometrine induced distinct postural changes in chicks.

3.1.5. The behavioural effects of apomorphine and d-amphetamine were absent in chicks pretreated with ergometrine and piribedil.

3.1.6. The results show that apomorphine, amphetamine, amantadine and piribedil induce a similar pattern of stereotyped activity in young chicks. The results also indicate that ergometrine exerts a predominant inhibitory influence on stereotyped behaviour while piribedil has both DA agonistic and antagonistic effects in young chicks.

3.2. Introduction.

Stereotyped behaviour has been defined as perseverative, continuous, apparently purposeless activity, during which there is absence of otherwise normal behaviour (Randrup and Munkvad, 1966). The type of behaviour constituting stereotypy depends on the species and can also vary among individuals of the same species. Several drugs have been reported to induce stereotyped behaviour in experimental animals, e.g. amphetamine, apomorphine, tryptamine, ergometrine, piribedil).

The induction of stereotyped behavioural

patterns by various drugs, in experimental animals, is widely used as an index of dopaminergic stimulation and as an indicator of antiparkinsonian potentiality (Costall, Naylor and Pinder, 1974). Stereotyped behaviour is also used as a behavioural model for screening drugs for antipsychotic properties (Janssen et al, 1965 .

This work was designed to investigate the behavioural effects of some dopamine agonists in young chicks and to compare the effects with those of apomorphine which has been reported to produce definite behavioural effects in this species (Osuide and Adejoh, 1973).

3.3. Results:

3.3.1. Apomorphine (0.01 - 0.50 mg/kg).

With low doses of apomorphine (0.01 - 0.02 mg/kg), the chicks appeared slightly depressed but chirped intermittently. However, 0.03 mg/kg of apomorphine increased locomotor activity which was accompanied by loud chirping. Higher

doses (0.05 - 0.35 mg/kg) induced distinct behavioural effects in chicks. The stereotyped behaviour was characterized by loud chirping, rapid movements inside the cage, pecking at own body and feet, pecking at each other's body and feet, pecking at non-food objects (such as the floor and sides of the cage), fighting episodes which consisted of one chick grasping the other chick's foot with its beak and pulling the foot until the chick fell, or frontal attacks in which one chick pecked at the other's head in the region of the beak and eyes. In most of the experiments but not invariably, no one chick was dominant in these activities. Sometimes fighting was so intense that there was bleeding around the beak and also in some experiments the chicks pecked at their own feet so intensely that the feet bled. Also, on some occasions a chick would held on to its foot with its beak and role over on the cage floor until there was another urge to peck before the foot was released. During the hyperactive

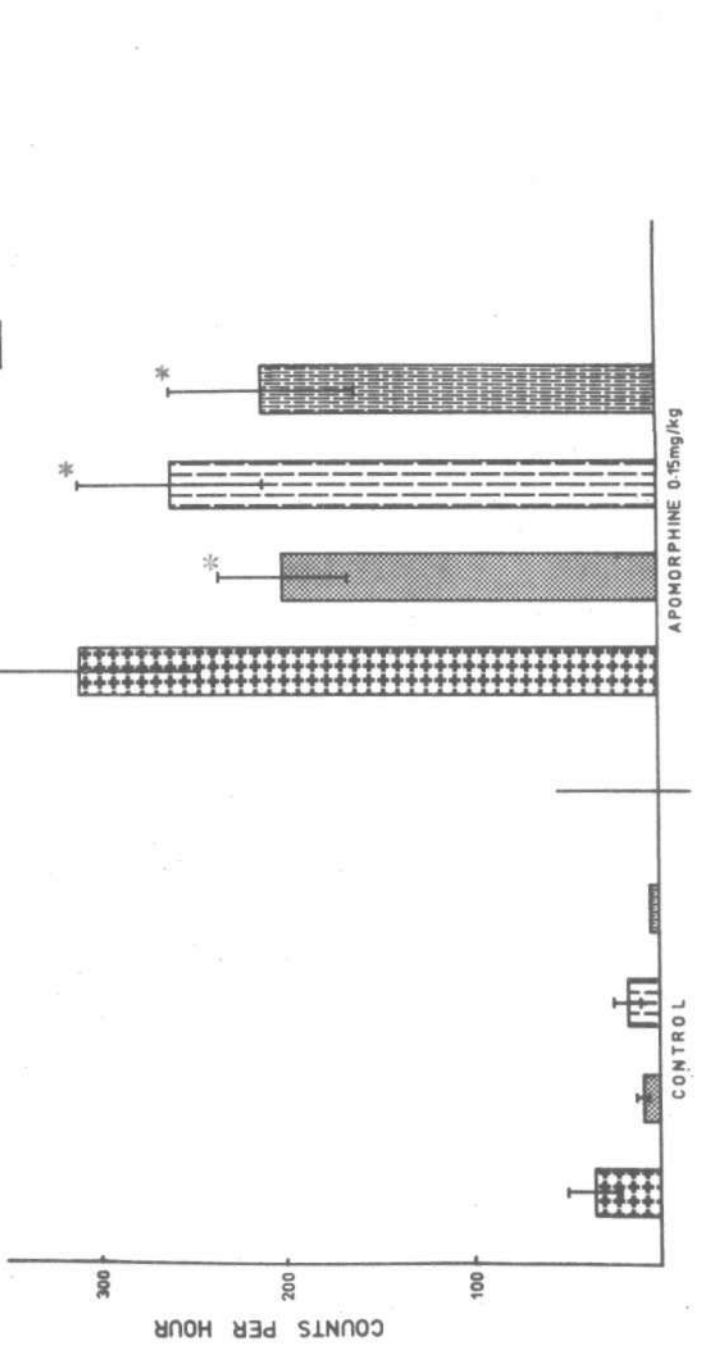


Fig. 3.1. Stereotyped activities induced by apomorphine in chicks. Vertical bars represent standard deviations. * $p < 0.001$ t - test. n = 9 pairs per histogram.

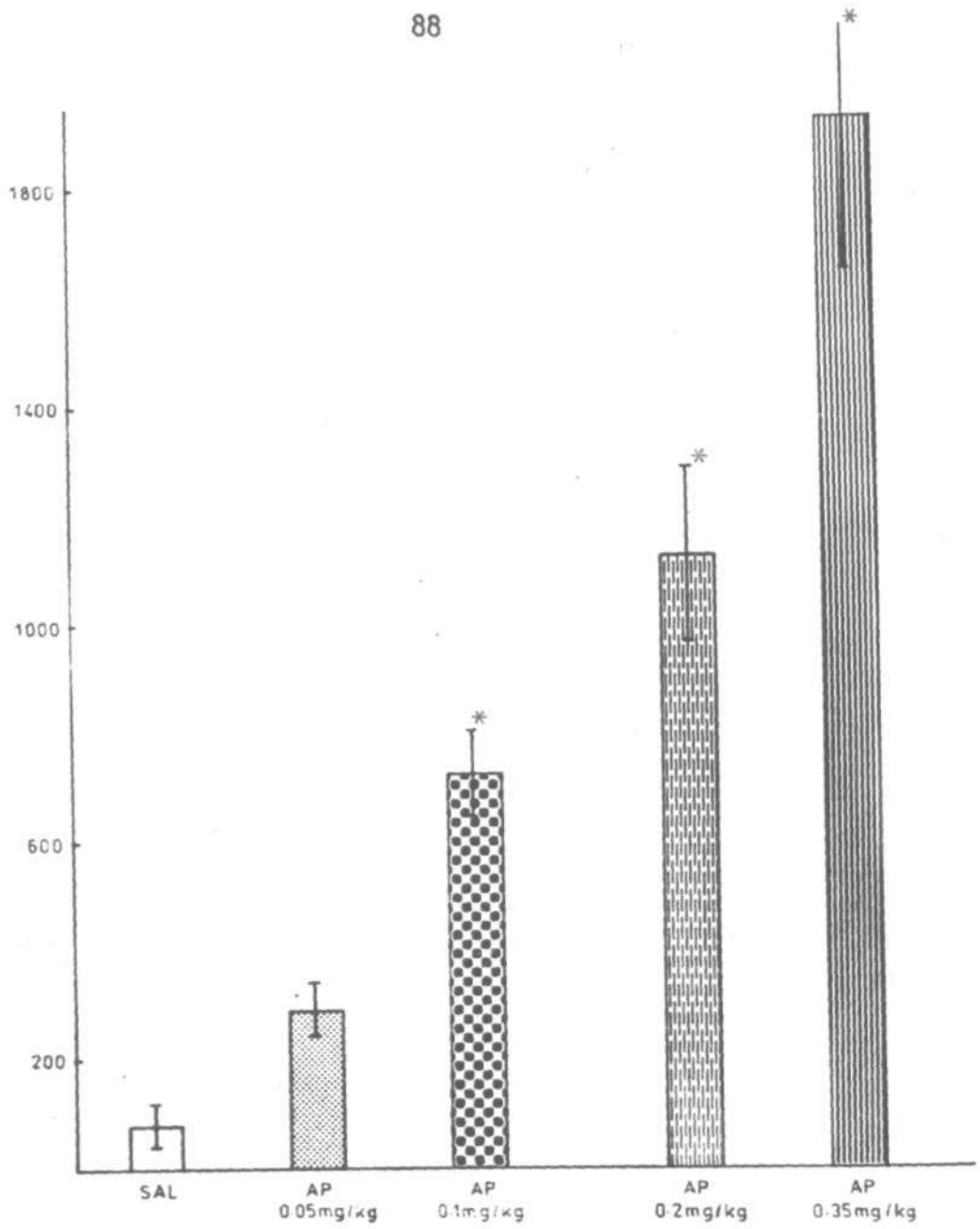


Fig. 3.2. Behavioural effects of various doses of apomorphine (AP) in young chicks. Each histogram is the mean of the total activity recorded. In this figure and the subsequent ones in this chapter, the total activity consists of (1) pecking at own body and feet, (2) pecking at each other's body and feet, (3) pecking at non-food objects and (4) fighting episodes. * $p < 0.001$, t - test. n = 9 pairs per histogram.

behaviour, food and water were disregarded. The hyperactive pecking behaviour usually started 3 - 5 min. after administration of apomorphine and usually lasted for 25 - 30 min.

The relative counts for the four parameters observed, varied widely between individual experiments. In most experiments, when one abnormal activity was increased, the others were suppressed. The degree of abnormal behaviour was assessed by calculating the sum of the four parameters of abnormal behaviour. The total count of abnormal behaviour increased as the dose of apomorphine was increased. With 5 mg/kg, the activity was so vigorous that counting became almost impossible. In older chicks (8 - 10 weeks), apomorphine did not induce aggressive behaviour. The most prominent activity observed in these cocks was pecking at non-food objects (figs. 3.1 and 3.2).

3.3.2 d-Amphetamine (0.01 - 10 mg/kg).

Low doses (0.01 - 0.1 mg/kg) of d-amphetamine did not produce any observable

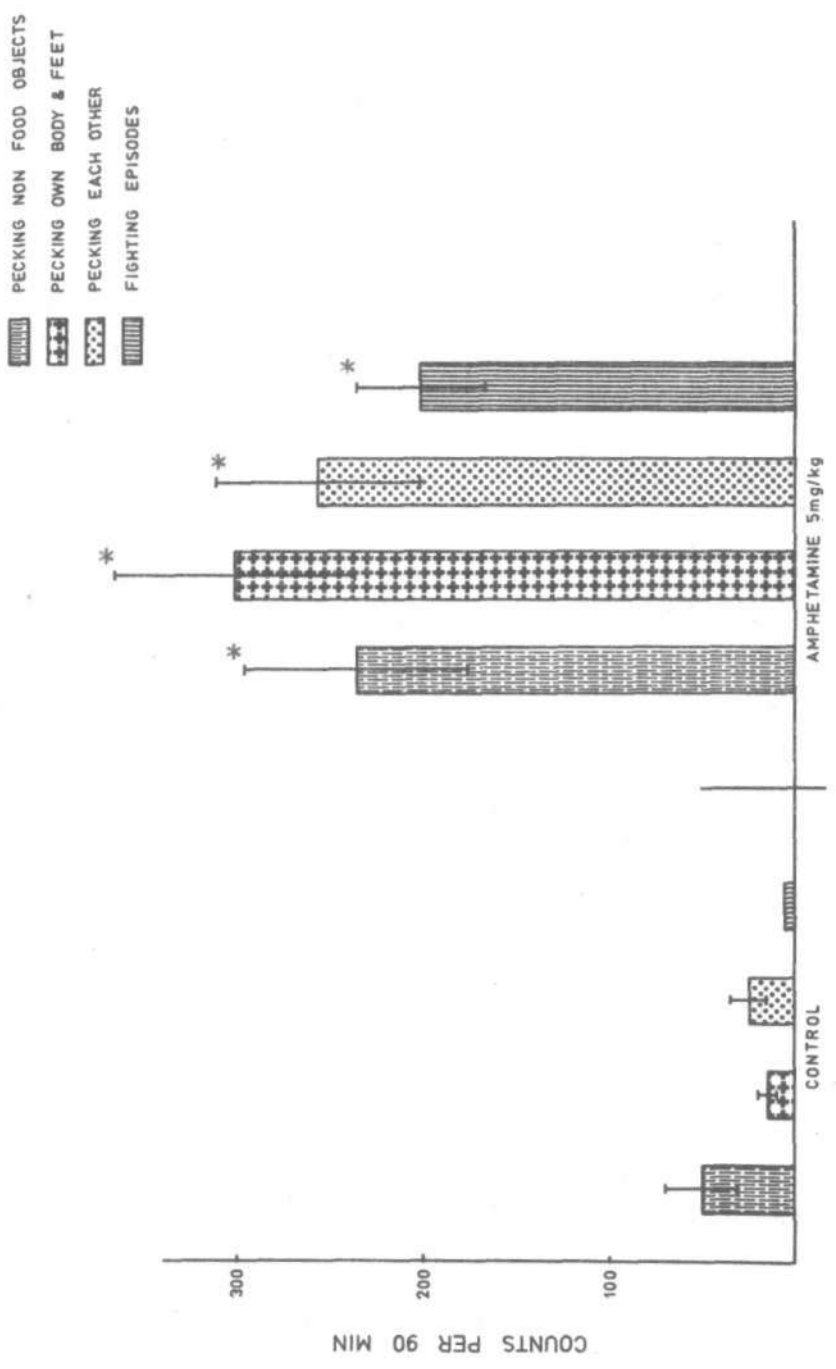


Fig. 3.3. Stereotyped activities induced by d-amphetamine in young chicks. Vertical bars represent standard deviations. * $P < 0.001$, t-test. n = 9 pairs per histogram.

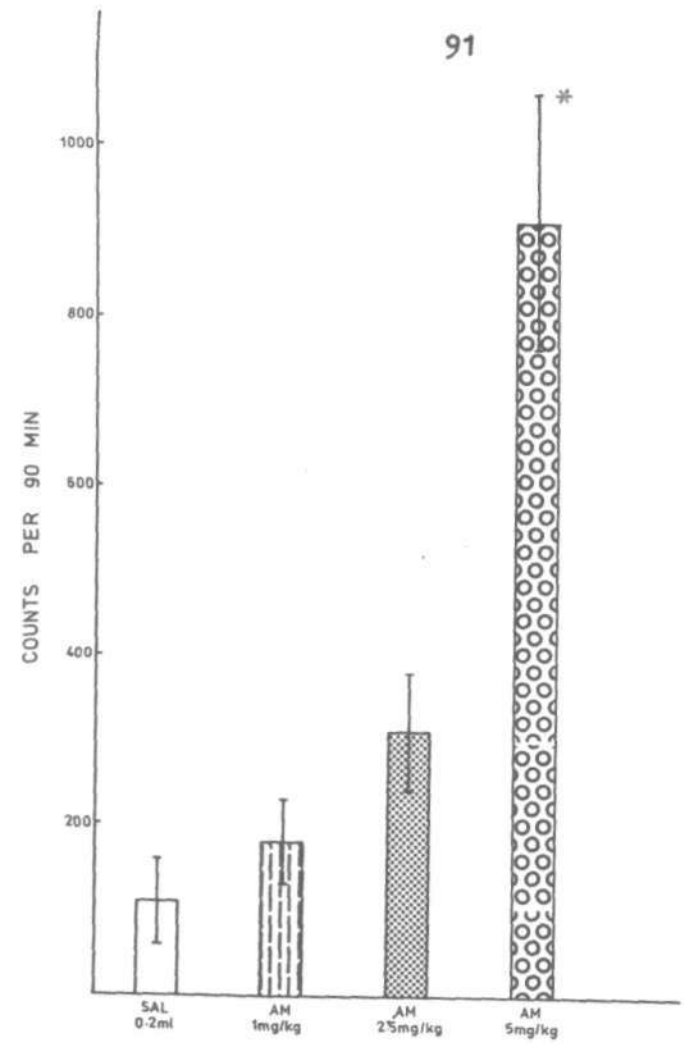


Fig. 3.4. Effect of various doses of amphetamine (AM) on pecking activities in chicks. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, t - test. n = 9 pairs per histogram.

effect on the behaviour of the chicks. With 0.5 - 1 mg/kg,there was a slight behavioural excitation. Higher doses (5 - 10 mg/kg) induced postural changes and stereotyped pecking activity in the chicks. The postural effect was characterized by spreading of the wings, sitting on the metatarsus with the trunk against the floor of the cage and continuous twittering while the chick pecked sporadically. The postural changes were more prominent in 1-5 day old chicks than in older ones and usually appeared 10-15 min after the administration of d-amphetamine. The pattern of compulsive pecking induced by d-amphetamine was similar to that of apomorphine but the intensity was less. Fighting episodes were mostly restricted to frontal attacks in which one chick pecked at the other's head on the region of the eyes and beak. On the whole, the intensity of hyperactive and aggressive behaviour induced by d amphetamine was less than with apomorphine. d-Amphetamine however, had a

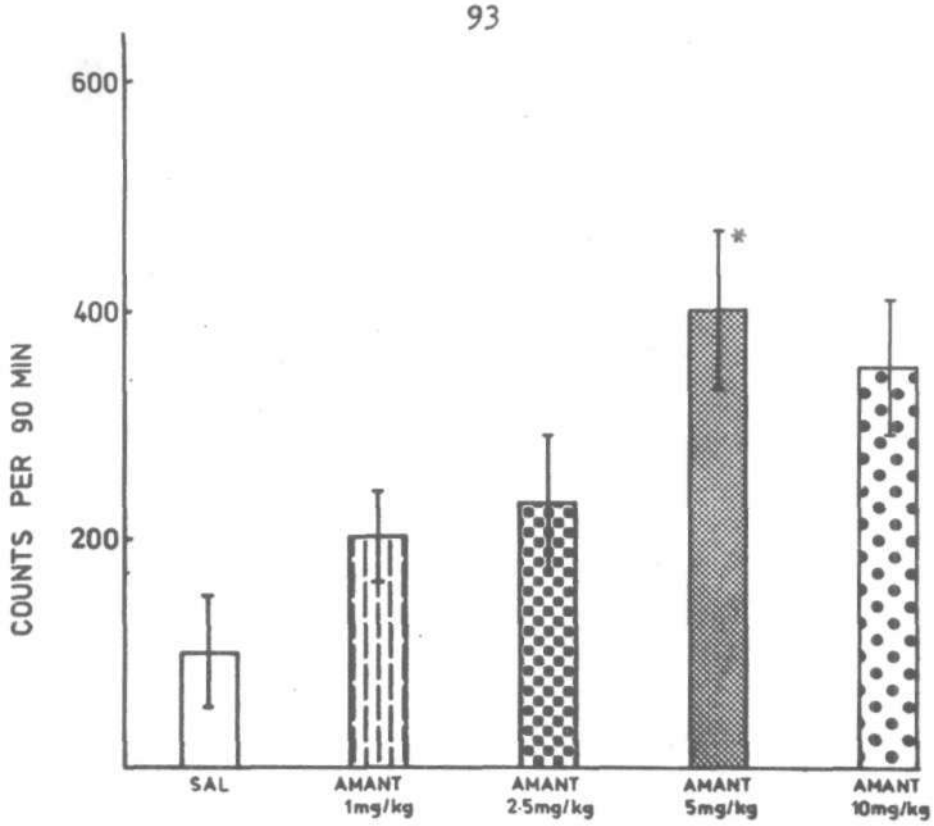


Fig. 3.5. Effect of various doses of amantadine (AMANT) on pecking activities in chicks. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.001$, t - test. n = 9 pairs per histogram.

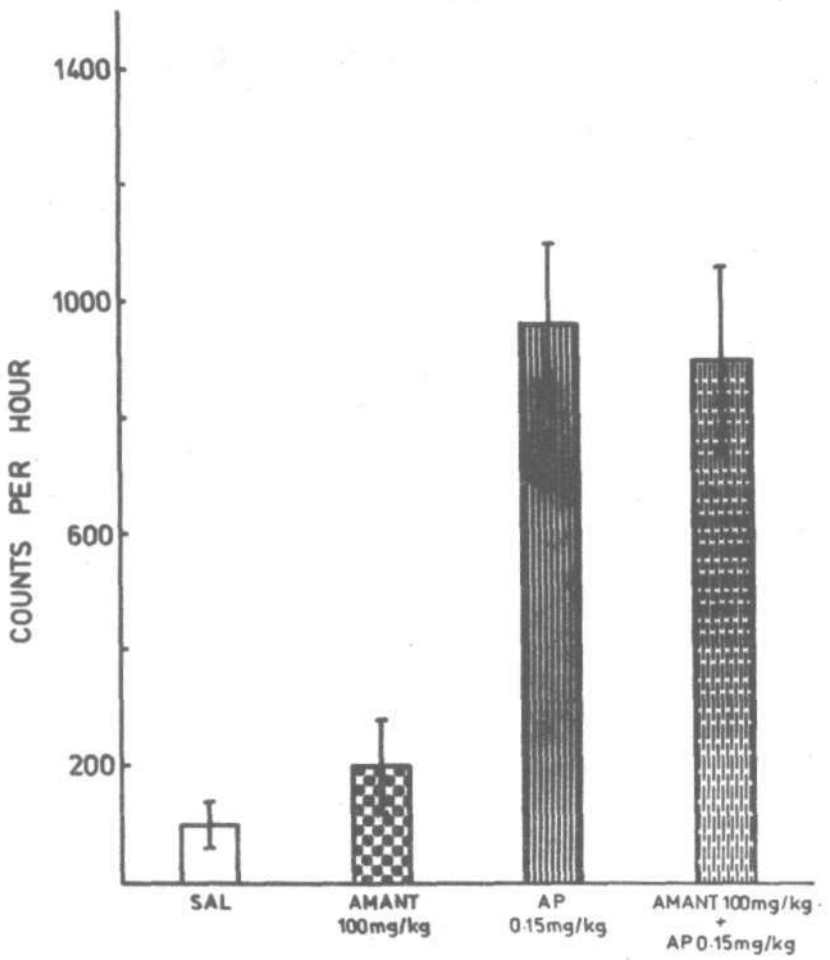


Fig. 3.6. Effect of amantadine (AMANT) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with amantadine 1 hr prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations

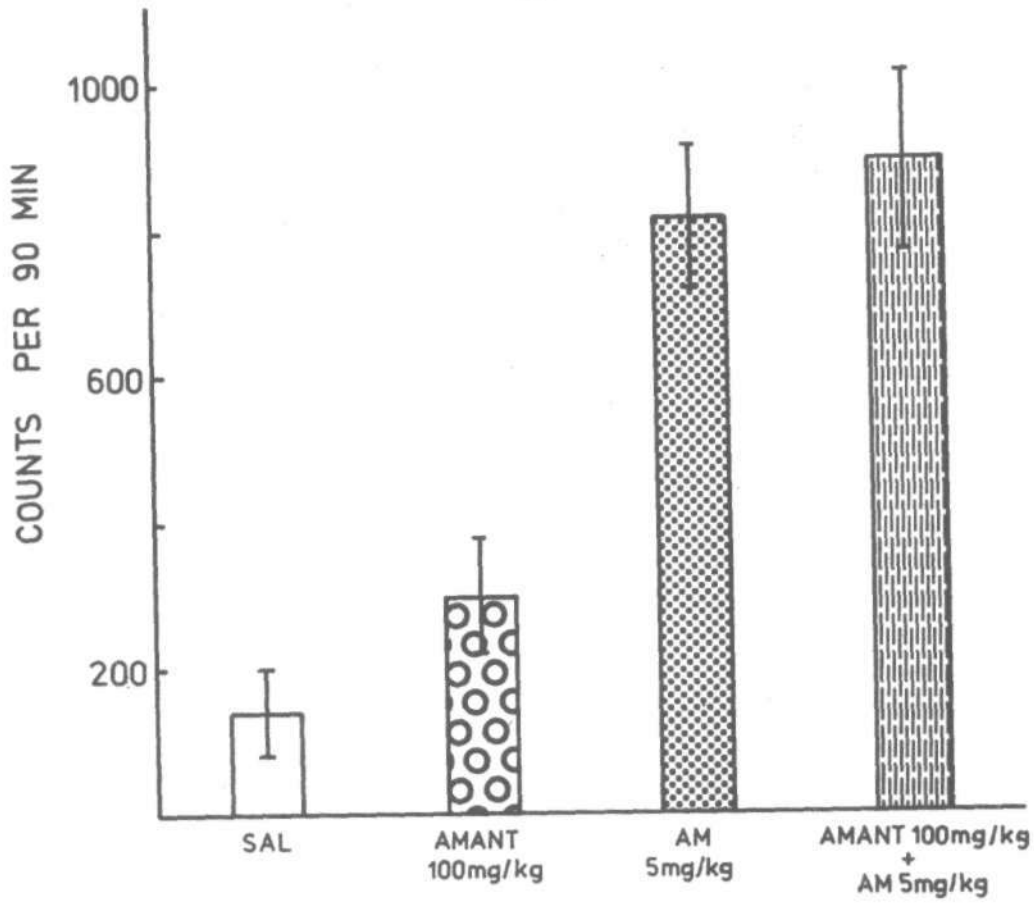


Fig. 3.7. Effect of amantadine (AMANT) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with amantadine 1 hr prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations.

longer duration of action than apomorphine (figs. 3.3 & 3.4).

3.3.4. Amantadine (0.5 - 100 mg/kg).

Low doses (0.5 - 2.5 mg/kg) of amantadine did not produce any significant change in the behaviour of the chicks, while moderate doses (5 - 10 mg/kg), induced a mild behavioural activation with an accompanying stereotyped pecking. Higher doses (50-100 mg/kg), induced shivering and spreading of the wings in the chicks, while the intensity of pecking was not markedly different from that observed with lower doses. The pattern of pecking was similar to that of apomorphine and amphetamine but the intensity was less. With amantadine, fighting episodes were restricted to frontal attacks. The onset of pecking was usually 10-15 min and lasted for 90 min. Pretreatment of chicks with amantadine (50 - 100 mg/kg) had no significant effects on apomorphine and d-amphetamine-induced stereotyped behaviour (figs. 3.5 - 3.7).

3.3.5. Ergometrine (0.10 - 10 mg/kg).

Low doses of ergometrine (0.1 - 1.5 mg/kg) produced behavioural sedation which was manifested by immobility, closing of eyes and intermittent chirping. Moderate doses (2.5-5 mg/kg) initially excited the chicks (characterized by loud chirping) but later depressed them (characterized by immobility accompanied with a squatting posture and spreading of wings). Even a dose of 10 mg/kg failed to induce compulsive pecking or twittering in the chicks as observed with apomorphine and amphetamine. Ergometrine (1.5 mg/kg) administered 15 min. previously, antagonized the behavioural effects of apomorphine (0.15 mg/kg) and d-amphetamine (5 mg/kg). The postural effects observed with ergometrine were partially antagonized by pretreating chicks with cyproheptadine (2.5 mg/kg). The depressant effect observed with ergometrine (1.5 mg/kg) was accompanied by EEG desynchronization (figs. 3.8-3.10).

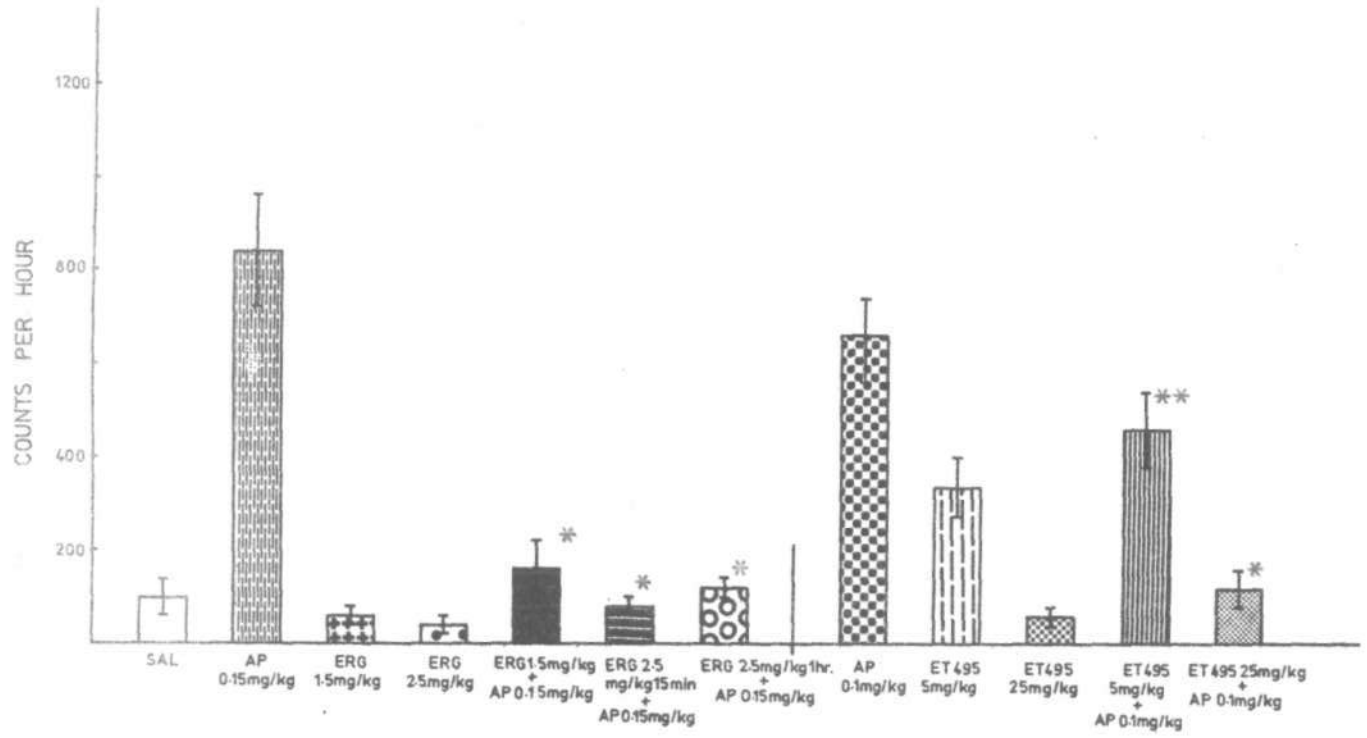


Fig. 3.8. Effects of ergometrine (ERG) and piribedil (ET 495) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with ergometrine or piribedil 15 min and 1 hr. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $P < 0.01$, t-test. n 12 pairs per histogram.
** $P < 0.05$

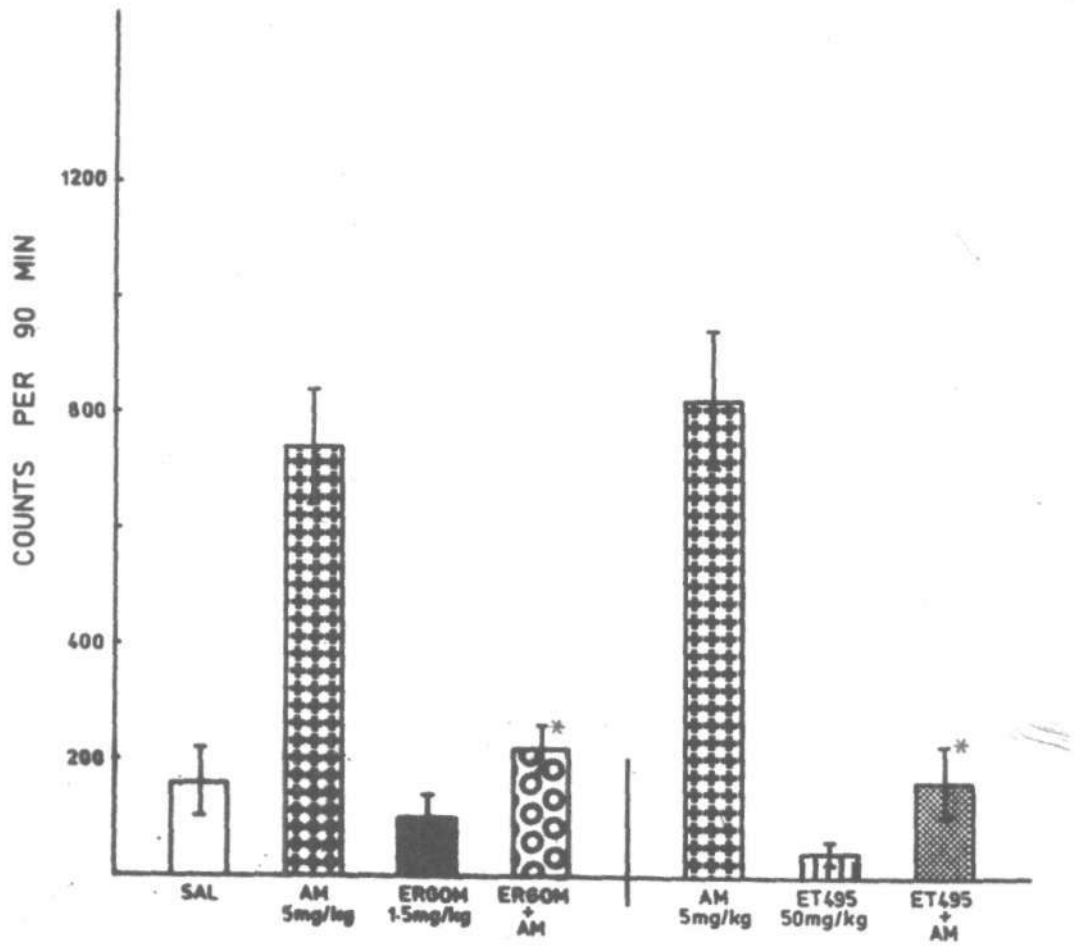


Fig. 3.9. Effects of ergometrine (ERGOM) and piribedil (ET495) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with piribedil or ergometrine 15 min prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $P < 0.001$, t-test. n = 12 pairs per histogram.

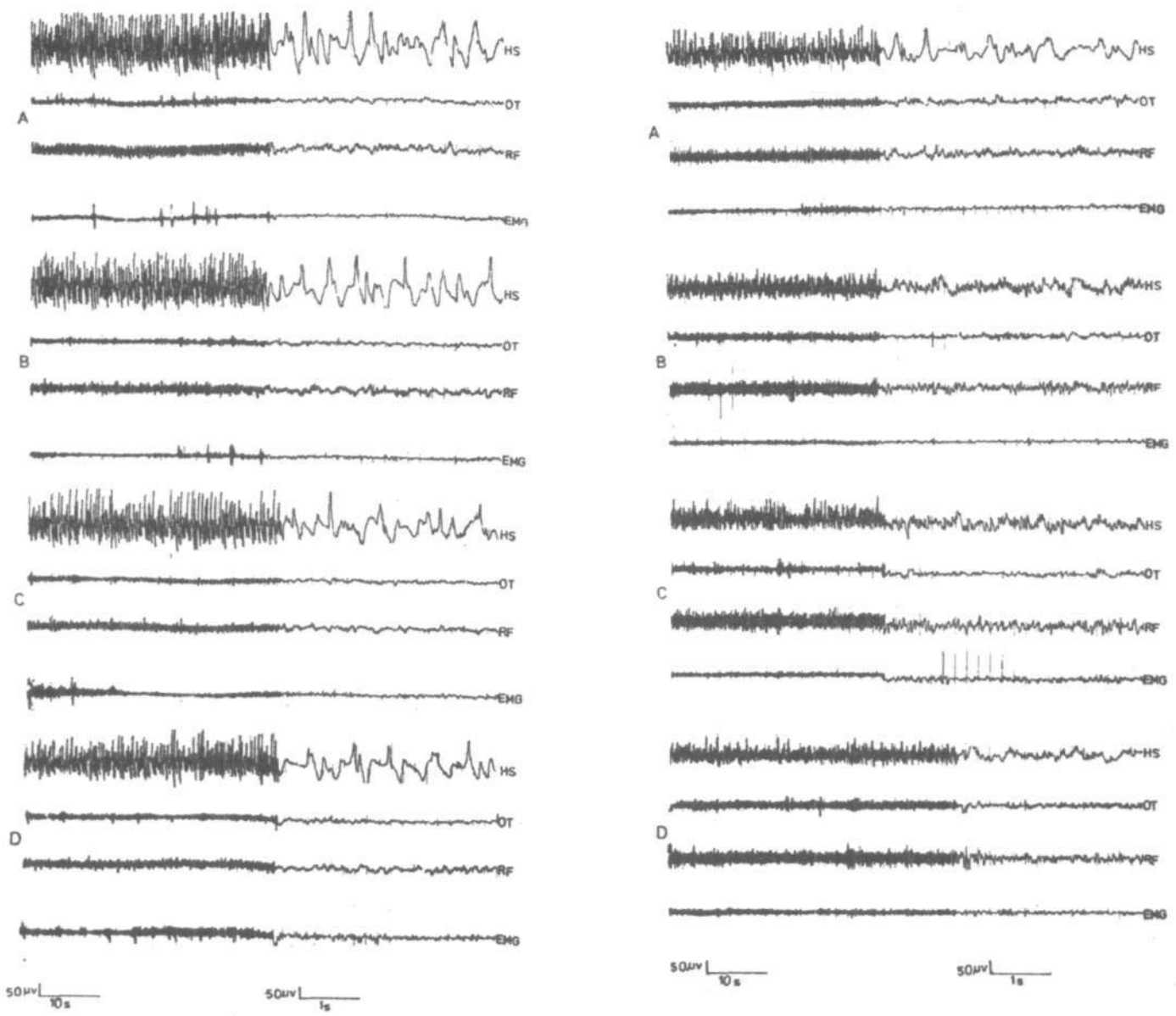


Fig.3.10. Effects of ergometrine (1.5 mg/kg; right tracing) and piribedil (5 mg/kg; left tracing) on EEG recording from the hyperstriatum (HS); optic tectum (OT); pontine reticular formation (RF) and electromyogram (EMG) activity in a 4-day old chick. A, is control recording while B,C, and D are recordings done at 15 min., 30 min and 1½ h. respectively, after drug administration.

3.3.6. Piribedil (ET 495, 0.1 - 100 mg/kg).

With low doses (0.1-0.5 mg/kg), the chicks appeared slightly sedated and occasionally pushed each other around the cage. With increase in dose (1-5 mg/kg), weak stereotyped pecking activity was observed (fig. 3.11). The intensity of compulsive pecking was not markedly affected when the chicks were given higher doses (10-20 mg/kg), but the degree of sedation increased. 100 mg/kg induced behavioural sleep in chicks. The behavioural effects of apomorphine (0.1 mg/kg) and d-amphetamine (5 mg/kg) were antagonized by piribedil (5 - 50 mg/kg), when administered 15 min. previously. Piribedil (5 - 25 mg/kg), produced EEG desynchronization and slightly increased EMG activity, showing central stimulation (figs. 3.8 - 3.11).

3.3.7. DA and L-Dopa (25 - 100 mg/kg).

Both DA and L-DOPA slightly increased locomotor activity in moderate doses (2.5 - 10 mg/kg). The chicks occasionally pushed

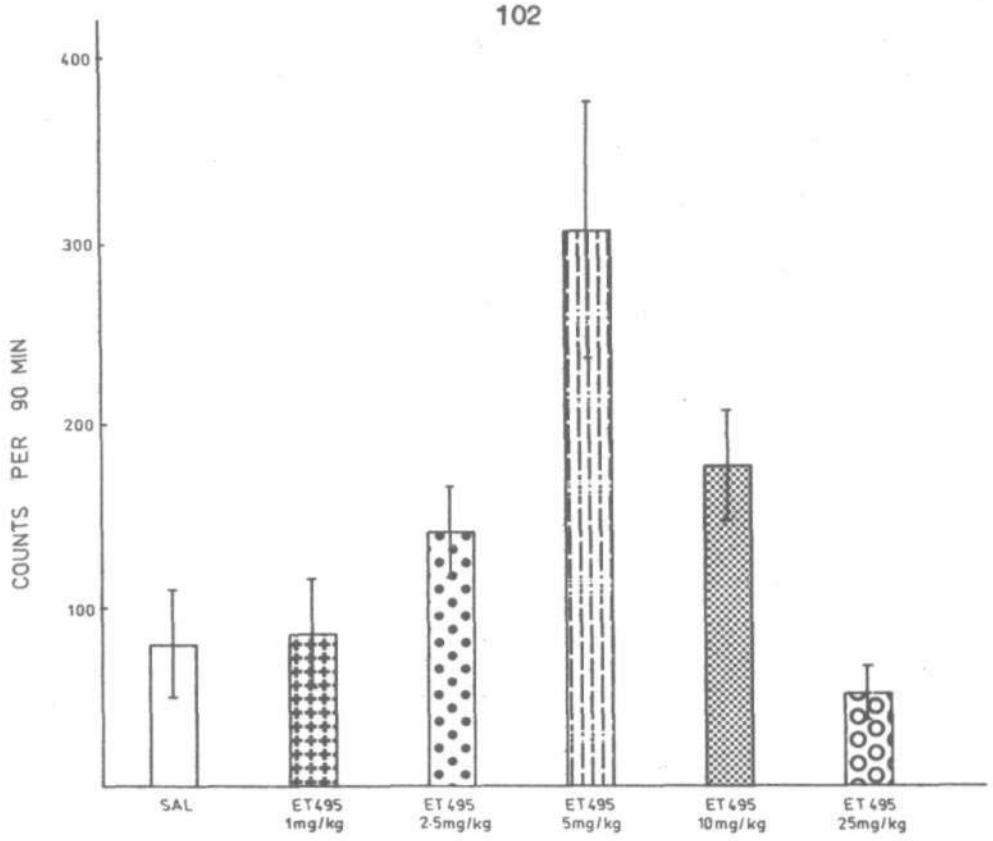


Fig. 3.11. Effect of various doses of ET495 on pecking activities in young chicks. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $P < 0.005$, t-test.
n = 9 pairs per histogram.

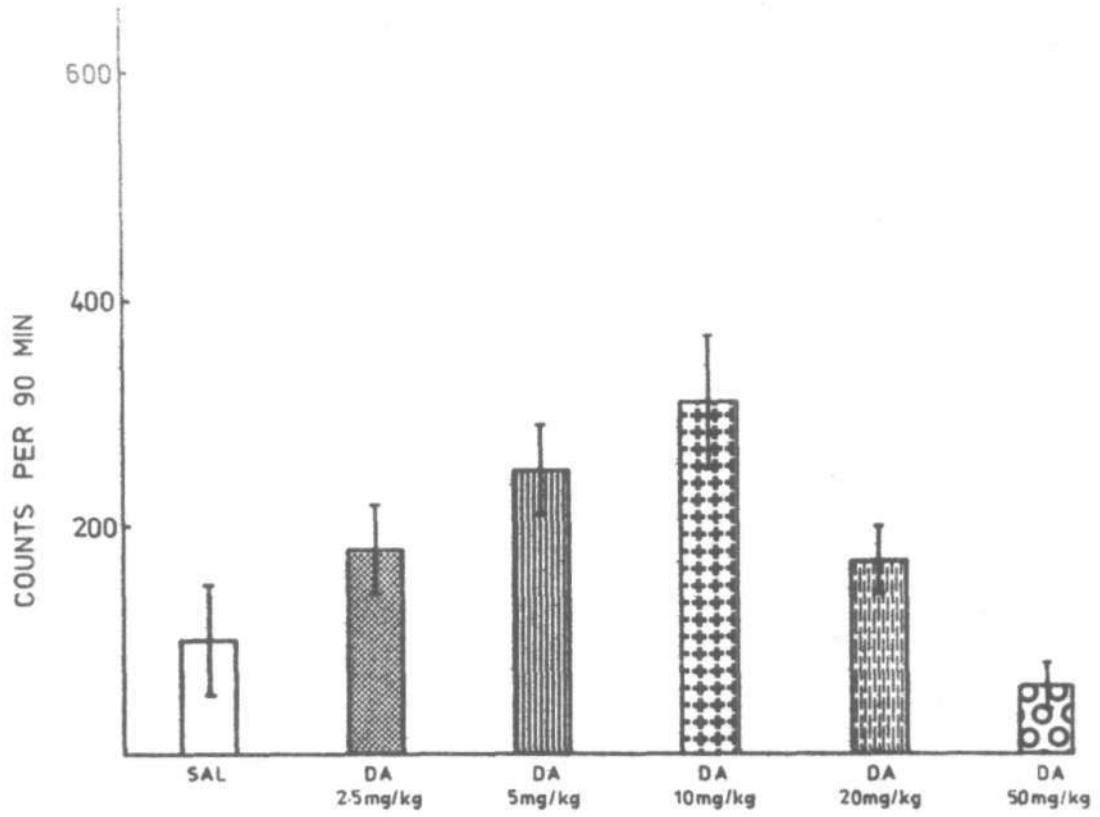


Fig. 3.12. Effect of various doses of dopamine (DA) on pecking activities in young chicks. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviation.

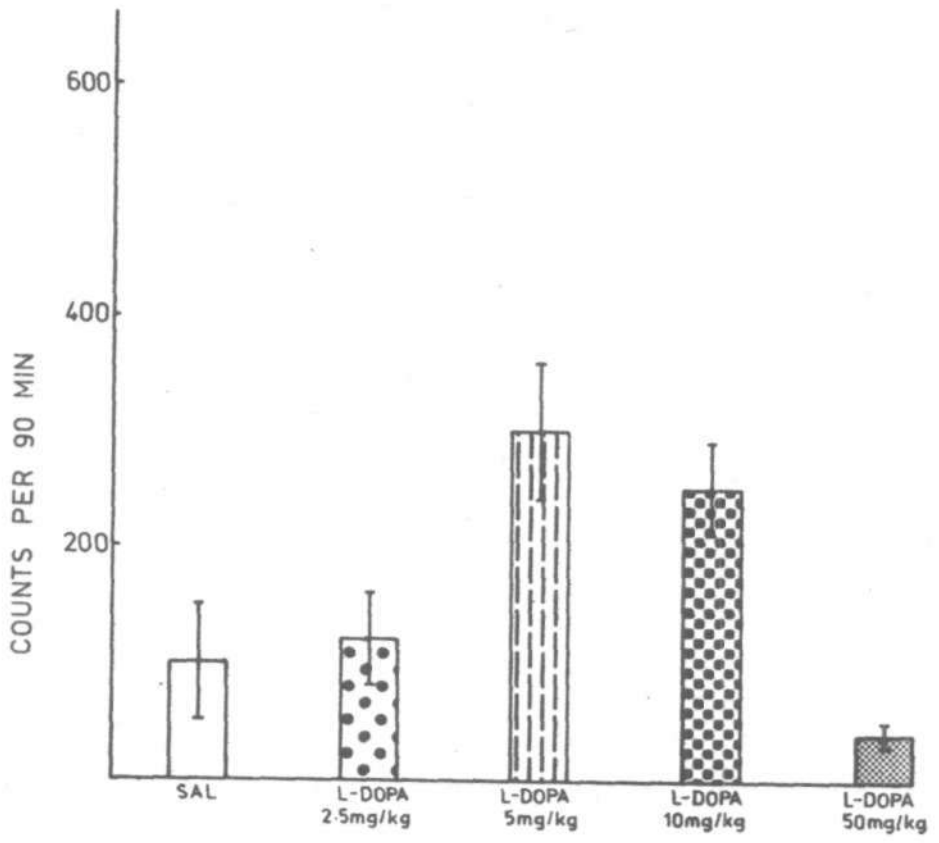


Fig. 3.13. Effect of various doses of L-dopa on pecking activities in young chicks. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations.

each other around the cage and occasionally pecked at non-food objects and chirped intermittently. The chicks later became sedated and remained calm at one corner of the cage. Higher doses (20-100 mg/kg), induced spreading of the wings and behavioural depression but there was no observable aggressive behaviour (figs. 3.12 & 3.13).

3.4. Discussion.

The observations made in this study with apomorphine are similar to those previously reported by Osuide and Adejoh (1973). Several studies have shown that apomorphine produces stereotyped behaviour in experimental animals by acting directly on DA receptors (Ernst, 1967; Andeń et al, 1967). The observations made with older chicks show that the ability of apomorphine to induce aggressive behaviour in chicks decreases with increase in age. This is in contrast to the observations of Senault (1970) and Carlsson and Almasi (1978), in which

apomorphine-induced fighting in rats and guinea-pigs increased with age of the animals. A possible explanation for the age-dependent effect of apomorphine on aggressive behaviour is that, pecking activity and aggressive behaviour may be mediated by different DA receptors. The existence of two types of DA receptors has been suggested by Cools and VAn Rossum (1976). Thus, it is probable that the effect of apomorphine on DA receptors mediating pecking activity becomes more predominant over its action on DA receptors mediating aggressive behaviour as the chicks became older. The apparent difference between the observations made in this study and those of Senault (1970) in rats, could be due to species variation.

The reaction of the young chick to d-amphetamine was first described by Selle (1940) and expanded by Clymer and Seifter (1947). Several other authors have also reported various effects of d-amphetamine in young chicks (Dewhurst and Marley, 1965; Schrold and Squires, 1971). In this study, d-amphetamine induced

stereotyped pecking and aggressive behaviour in chicks just like apomorphine. However, it is weaker in producing this effect than apomorphine. This is probably due to differences in the mechanism of action of the two drugs. D-amphetamine is claimed to produce its action indirectly by releasing catecholamines and inhibiting their reuptake (Weisman et al 1966; Glowinski et al, 1966), though some authors have also indicated that d-amphetamine stimulates catecholamine receptors directly (Van Rossum and Hurkmans, 1964; Feltz and De champlain, 1973).

The postural changes produced by d-amphetamine in this study were not observed with the doses of apomorphine used and this probably suggests that d-amphetamine is more potent in producing these effects than apomorphine. It is also probable that d-amphetamine produces these effects via mechanisms other than those involved in the

induction of stereotyped behaviour. Schrold and Squires (1971)) have shown that d-amphetamine produces its behavioural effects in young chicks via a predominantly serotonergic mechanism. The same authors also observed increase in 5-HT and 5-HIAA levels after d-amphetamine in chicks. However, reports of the effects of d-amphetamine on the metabolism of serotonin in the brain are inconsistent. Endogeneous levels of serotonin have been reported to be decreased (Paasonen and Vogt, 1956) and increased (Maclean and Mcartney, 1961) after d-amphetamine. These observations clearly indicate that d-amphetamine has a complex effect in the central nervous system.

Amantadine is believed to release DA from extra-granular stores in DA nerves (Farnebo, et al, 1971), and to prevent neuronal reuptake of DA (Heimans et al, 1972). The observations made in this study show that amantadine induces weak stereotyped pecking activity in young chicks.

Though, the pattern of pecking induced by amantadine was similar to that observed with d-amphetamine, no characteristic changes in posture were observed as with d-amphetamine. This probably suggests that amantadine does not share this property of d-amphetamine. The failure of amantadine to influence the actions of apomorphine and d-amphetamine in this study, is in constrast to its reported antagonism of apomorphine (Schnieden & Cox, 1976) and d-amphetamine (Menon et al, 1973) effects in rats using similar doses and pretreatment times. This probably shows that amantadine does not interact with these drugs in the chick.

Several studies have shown that ergometrine and some other ergot alkaloids have direct stimulant effects on central dopaminergic receptors (Woodruff et al, 1974, Corrodi et al, 1973). The doses of ergometrine used in the present study did not produce characteristic behavioural effects as observed with other DA

agonists. This probably suggests that ergometrine does not act at those sites which mediate stereotyped behaviour in the chick. In view of the similarity between the postural changes induced by ergometrine and d-amphetamine, it is tempting to suggest that both drugs produced these effects via a similar mechanism. Schrold and Squires (1971) reported that d-amphetamine produces its postural effects in young chicks via a serotonergic mechanism. Ergometrine has been reported to exert both agonist and antagonist actions on central 5-HT neurons (Corrodi et al, 1975). Thus, the observed changes in posture could probably be due to interaction of ergometrine with central serotonergic systems. This suggestion is supported by the partial antagonism of these effects when the chicks were pretreated with cyproheptadine, a 5-HT receptor antagonist.

Piribedil is believed to stimulate DA receptors directly and most of its behavioural

effects have been attributed to this action (Corrodi et al, 1971; Costall and Naylor, 1973b). The weak stereotyped pecking activity observed with piribedil in this study suggest that this drug exerts a weak effect on the central dopaminergic receptors of chicks.

The observed antagonism of d-amphetamine and apomorphine-induced stereotyped behaviour by both ergometrine and piribedil suggest that both drugs exert an inhibitory influence on central DA receptors in the chick. This inhibitory effect could be due to a direct blockade of postsynaptic DA receptors or stimulation of inhibitory DA pathways or due to activation of other neurotransmitter systems, such as serotonergic mechanisms, which exert an inhibitory effect on drug-induced stereotyped behaviour, as observed by other workers (Cheng and Long, 1974; Weiner et al, 1973). According to Cools and Van Rossum (1976) both ergometrine and piribedil exert antagonistic effects on

inhibitory DA receptors. Based on this suggestion, these drugs would have been expected to potentiate apomorphine and d-amphetamine effects. The observed antagonism therefore suggests that these drugs probably exert predominant agonistic effects on inhibitory DA receptors in the chick.

The observations made in this study show that both DA and L-dopa failed to induce characteristic stereotyped pecking and aggressive behaviour in chicks as observed with apomorphine. This probably suggests that in the chick, administration of these agents does not produce any marked behavioural excitatory effect on central dopamine receptors. It is also probable that injected dopamine has a greater affinity for inhibitory dopamine receptors than excitatory ones while apomorphine may have a higher affinity for the later. Such a proposal is consistent with hypothesis of Cools and Van Rossum (1976) that there might be two functional types of DA receptors.

These observations show that, as in other species, apomorphine and other DA agonists induced stereotyped pecking and aggressive behaviour in chicks but the intensity of the activity depends on the specific drug. The results of this study also suggest that in the chick, ergometrine has a predominant inhibitory influence on stereotyped behaviour while piribedil has both DA agonistic and antagonistic actions.

CHAPTER IV

4. EFFECT OF CATECHOLAMINERGIC AGENTS ON DRUG-INDUCED STEREOTYPED BEHAVIOUR IN CHICKS.

4.1. Summary.

4.1.1. The influence of catecholaminergic agents on drug-induced stereotyped behaviour was studied in chicks.

4.1.2. Pretreatment of chicks with reserpine and 6-hydroxydopamine (6-OHDA) potentiated apomorphine and piribedil-induced stereotyped behaviour while the effects of amphetamine and amantadine were inhibited.

4.1.3. Dihydroxyphenylserine (DOPS) potentiated apomorphine-induced stereotyped behaviour while d-amphetamine and amantadine-induced effects were antagonized.

4.1.4. FLA-63 potentiated d-amphetamine and amantadine-induced stereotyped behaviour while apomorphine effects were inhibited.

4.1.5. Propranolol antagonized the effects of apomorphine and d-amphetamine while phenoxybenzamine slightly

potentiated apomorphine and d-amphetamine-induced stereotyped behaviour.

4.1.6. Both pimozide and haloperidol antagonized apomorphine, piribedil and amantadine-induced stereotyped behaviour.

4.1.7. The results indicate a primary role for dopaminergic system in drug-induced stereotyped behaviour in young chicks, as in other species.

4.2. Introduction.

Various approaches have been used to elucidate the mechanisms involved in drug-induced stereotyped behaviour. Most of these studies have implicated central dopaminergic system as the mediator of this behaviour. The dopaminergic neurons which have received the greatest attention are those found in the extrapyramidal areas of the brain. The basal ganglia contain the highest concentration of DA in the mammalian brain (Hornykiewicz, 1966). Most of the drugs that produce stereotyped behaviour affect this

dopaminergic system either directly or indirectly or both (Van Rossum, 1970). Consistent with the view that stereotypy is mediated by a dopaminergic mechanism, many of the drugs that induce stereotyped behaviour have been shown to increase the release of DA into a ventriculocisternal perfusate or to act as DA agonists invitro (Mckenzie and Szerb, 1968). However, inspite of these evidences, the exact mechanisms by which some of these drugs interact with the dopaminergic system to induce stereotyped behaviour have not been clearly elucidated.

The present study is intended to investigate the effects of drugs which influence catecholamine levels and receptor activity on drug-induced stereotyped behaviour in chicks. Such a study would enable us to understand the mechanisms involved in the behavioural effects of these drugs in young chicks.

4.3. Results.

4.3.1. Reserpine (5 mg/kg).

Reserpine treated chicks appeared sedated

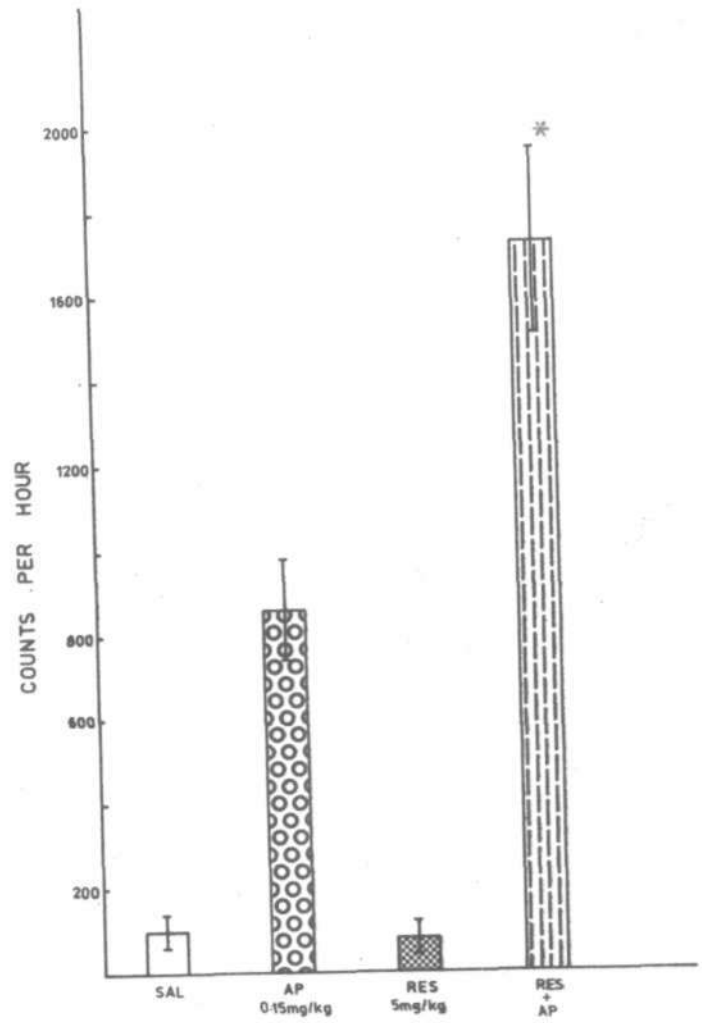


Fig. 4.1. Effect of reserpine (RES) on apomorphine (AP)-induced stereotyped behaviour in chicks. The chicks were pretreated with reserpine 21 hr. prior to apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, student's, t - test. (n = 12 pairs per histogram).

Note:

In this figure and the subsequent ones in this chapter, the total activity consists of (1) pecking at own body and feet, (2) pecking at each other, (3) pecking at non food objects and (4) fighting episodes.

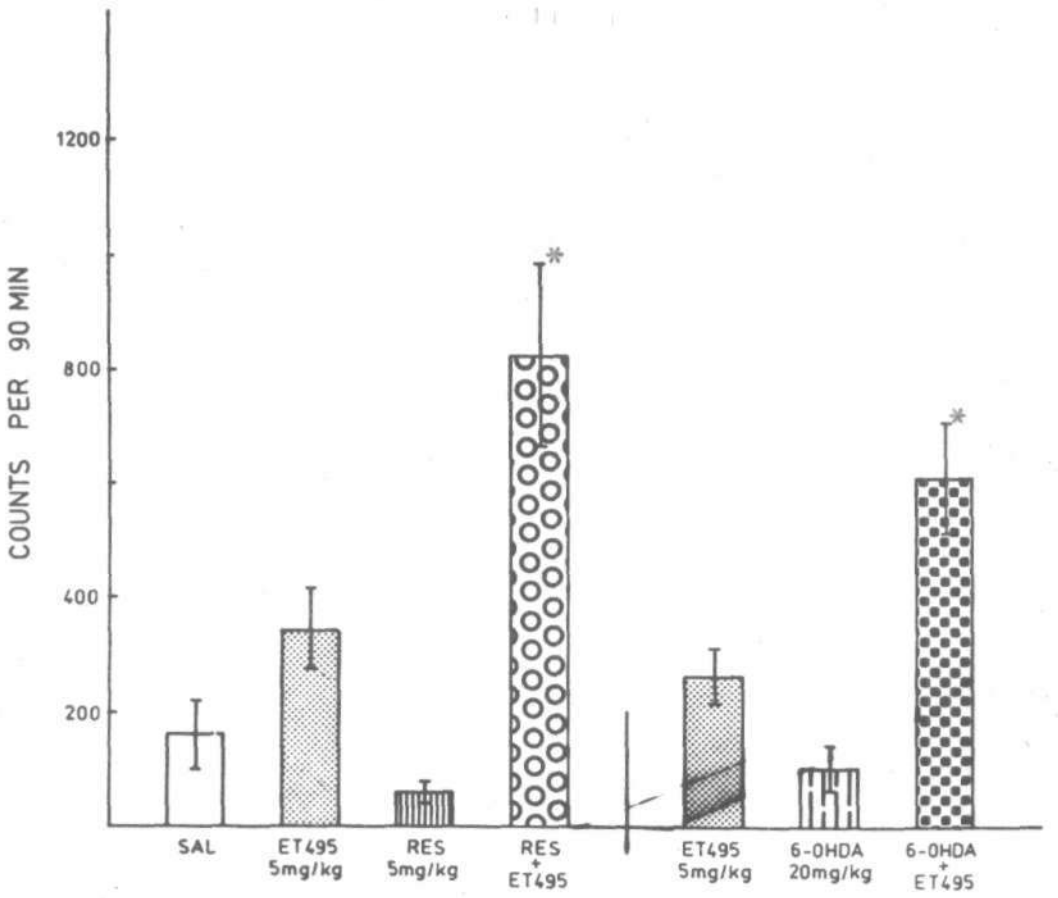


Fig. 4.2. Effect of reserpine (RES) and 6-hydroxydopamine (6-OHDA) on piribedil (ET495)-induced stereotyped behaviour in chicks. The chicks were pretreated with reserpine and 6-OHDA 21 hr. and 16 hr. respectively, prior to administration of piribedil. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.01$, t - test (n = 12 pairs per histogram).

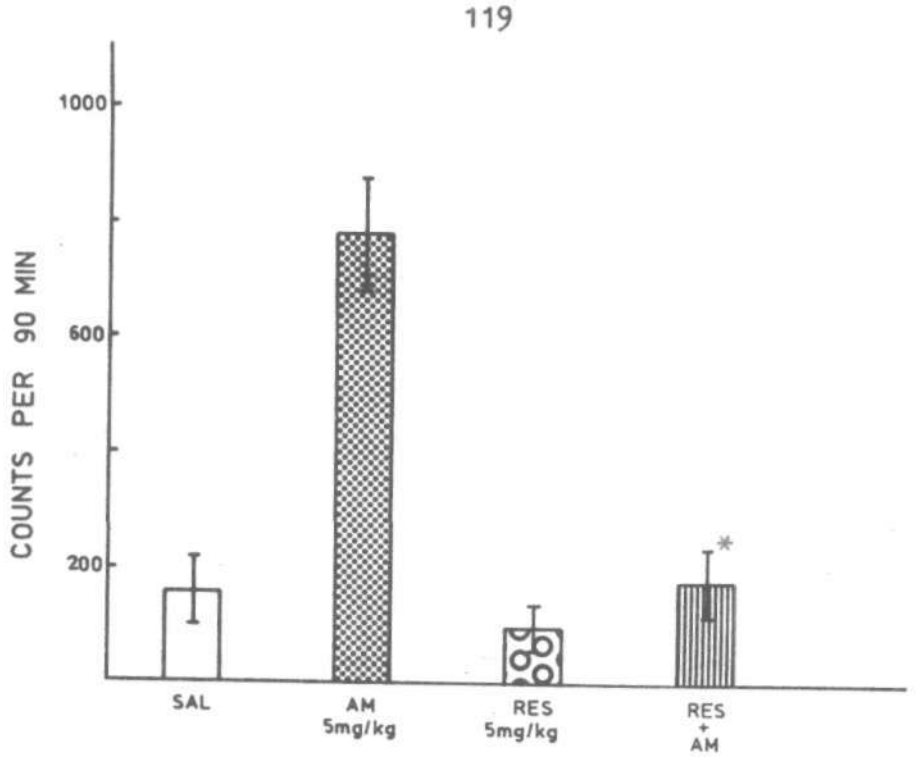


Fig. 4.3. Effect of reserpine (RES) on amphetamine (AM)-induced stereotyped behaviour in chicks. The chicks were pretreated with reserpine for 21 hr. prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, student's t - test (n = 12 pairs per histogram).

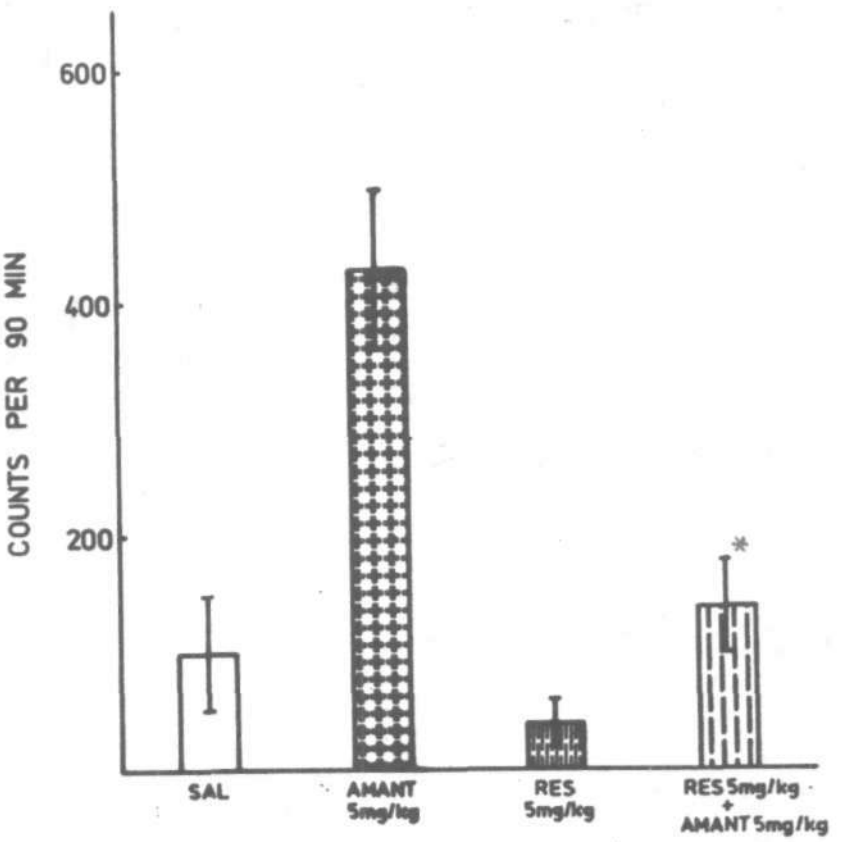


Fig. 4.4. Effect of reserpine (RES) on amantadine (AMANT)-induced stereotyped behaviour in chicks. The chicks were pretreated with reserpine for 21 hr. prior to administration of amantadine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$ t - test, (n = 12 pairs per histogram).

and inactive.

The behavioural effects of apomorphine (0.15 mg/kg) and piribedil (5 mg/kg) were potentiated in chicks pretreated 2 h. previously with reserpine. The onset of apomorphine and piribedil-induced effects was rapid and the intensity of aggressive behaviour was greater than in non-reserpinized chicks. On the other hand, pretreatment with reserpine, antagonized the stereotyped pecking activity induced by both d-amphetamine (5 mg/kg) and amantadine (5 mg/kg). The behavioural effects observed with ergometrine (1.5 mg/kg) were not significantly affected after reserpine pretreatment (Figs. 4.1 - 4.4).

4.3.2. FLA-63 (2.5 & 5 mg/kg).

5 mg/kg FLA-63 (a DA B-hydroxylase enzyme inhibitor) when administered 4 h previously, antagonized apomorphine-induced stereotyped behaviour. d-Amphetamine and amantadine effects were potentiated in chicks pretreated with FLA-63.

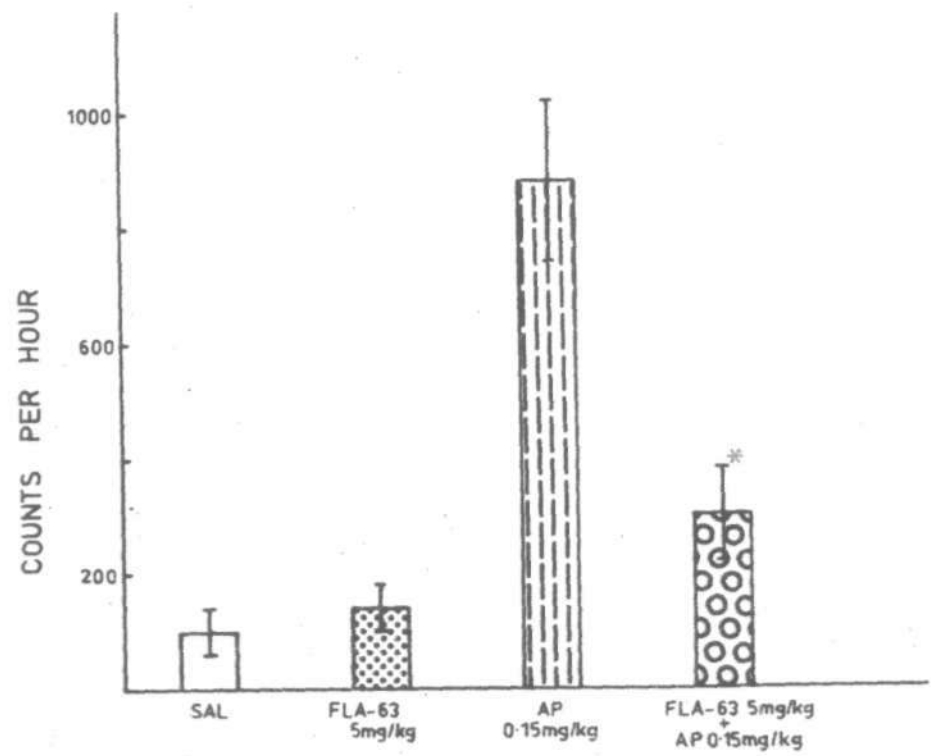


Fig. 4.5. Effect of FLA-63 on apomorphine (AP)-induced stereotyped behaviour in chicks. The chicks were pretreated with FLA-63 4 hr. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, t - test (n = 12 pairs per histogram).

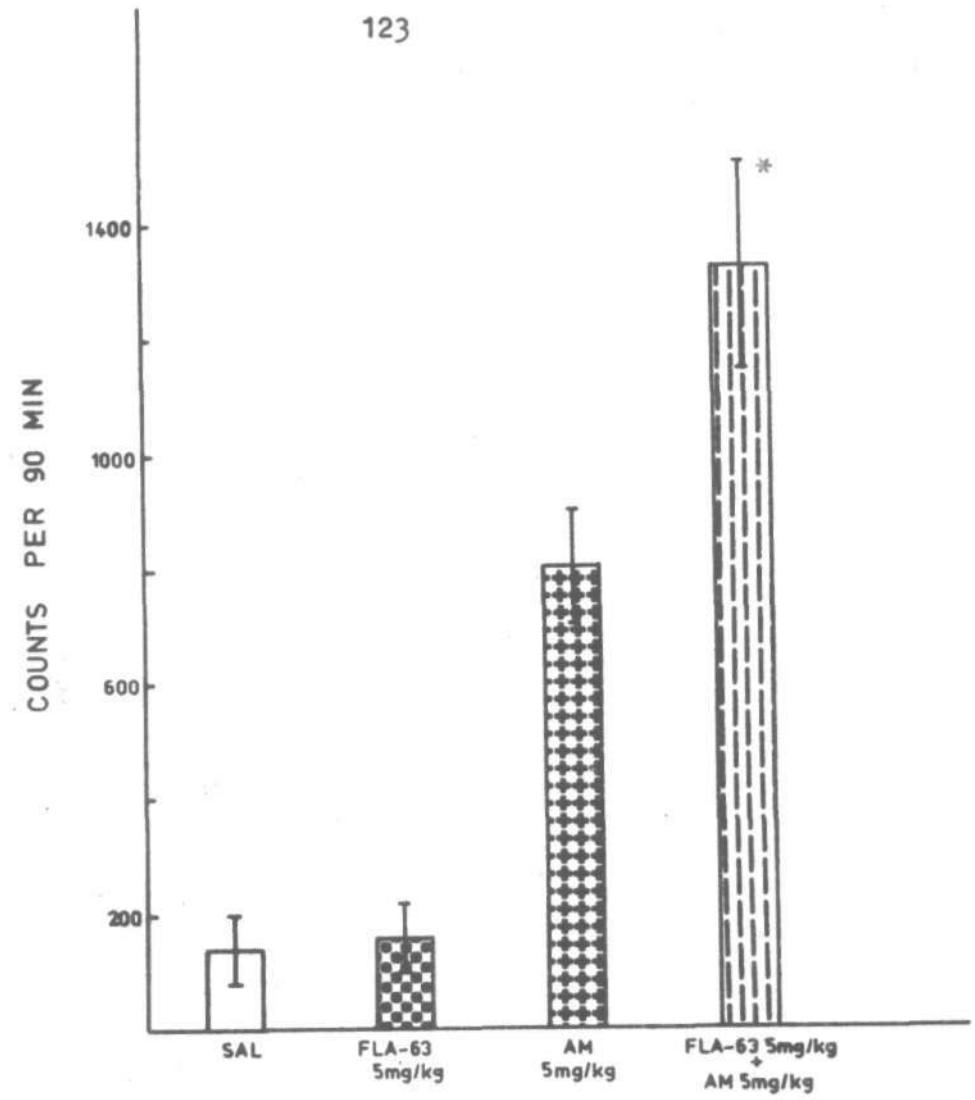


Fig. 4.6. Effect of FLA-63 on amphetamine (AMP)-induced stereotyped behaviour in chicks. The chicks were pretreated with FLA-63 4 hr. prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.001$, t - test n = 12 pairs per histogram.

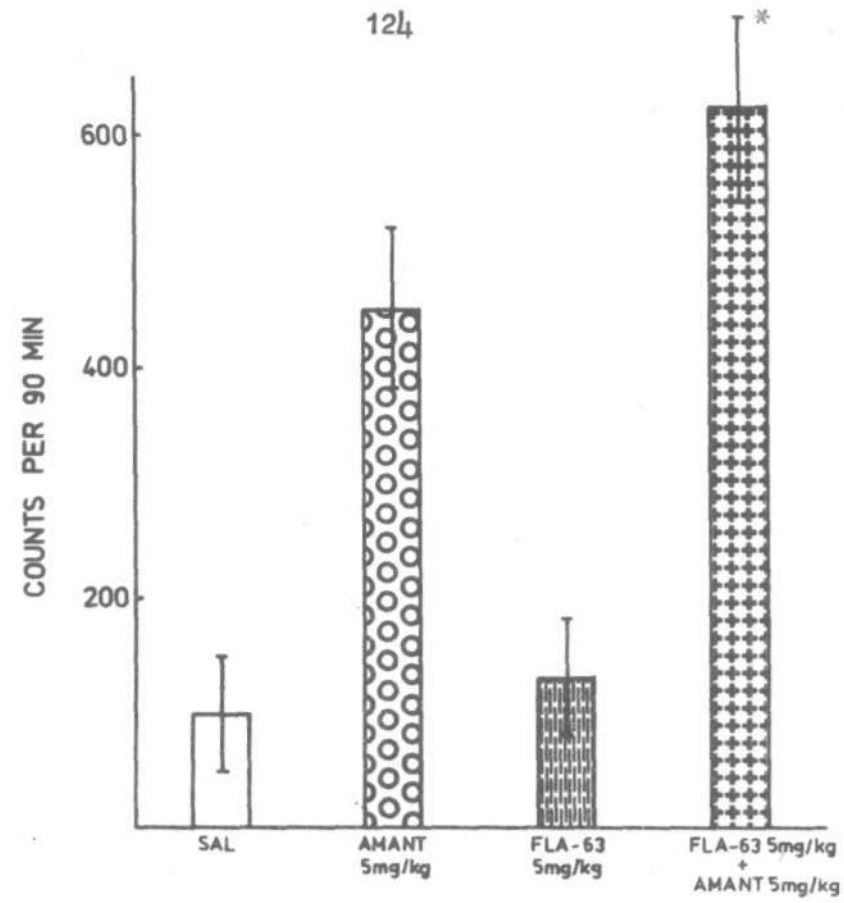


Fig. 4.7. Effect of FLA-63 on amantadine (AMANT)-induced stereotyped behaviour in chicks. Chicks were pretreated with FLA-63 for 4 hr. prior to administration of amantadine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.05$, t - test, (n = 12 pairs per histogram).

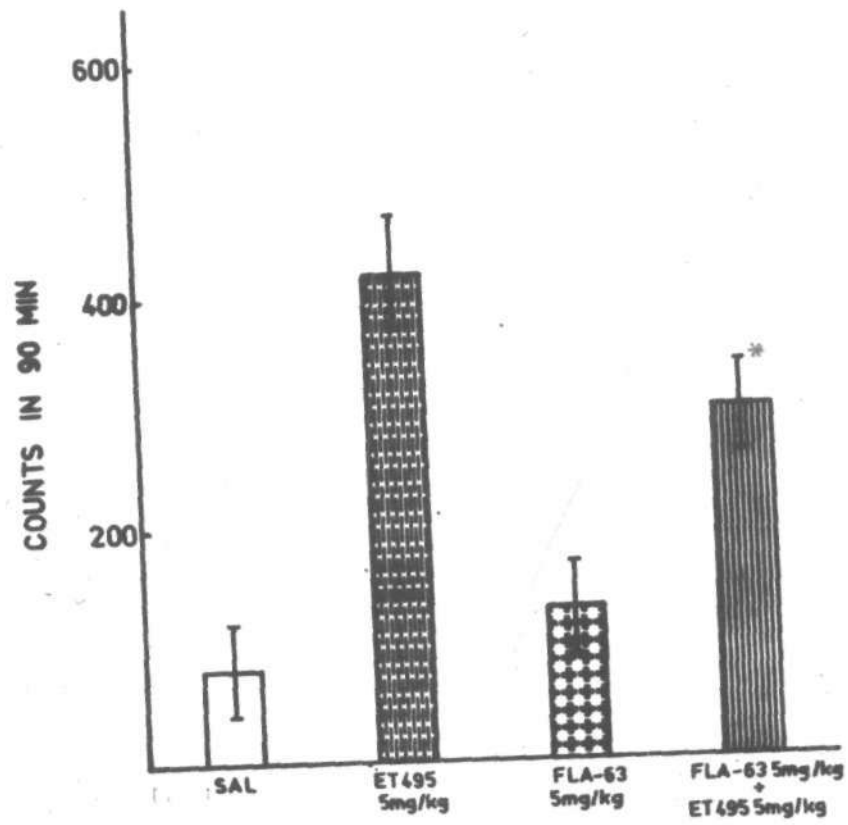


Fig. 4.8. Effect of FLA-63 on piribedil (ET495)-induced stereotyped behaviour in chicks. Chicks were pretreated with FLA-63 for 4 hr. prior to administration of piribedil. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviation. * $p < 0.05$, t - test. n = 12 pairs per histogram).

The weak stereotyped activity induced by piribedil was slightly antagonized after pretreating chicks with FLA-63, while there was no observable change in ergometrine-induced behavioural effects. With FLA-63 (5 mg/kg) alone, the chicks initially appeared sedated and stayed quietly at one place. They subsequently became slightly active and pushed each other around the cage and also occasionally pecked at non-food objects (figs. 4.5 - 4.8).

4.3.3. Dihydroxyphenylserine (DOPS, 5 - 10 mg/kg).

DOPS is believed to be converted directly to NA in the body and when administered alone, the chicks were slightly depressed. DOPS (5 mg/kg) given 16 h previously, potentiated apomorphine - induced stereotypy (fig. 4.9). On the other hand, d-amphetamine and amantadine effects were inhibited after pretreating chicks with DOPS (5-10 mg/kg; fig. 4.10). The effects of piribedil and ergometrine were not significantly changed after pretreating chicks

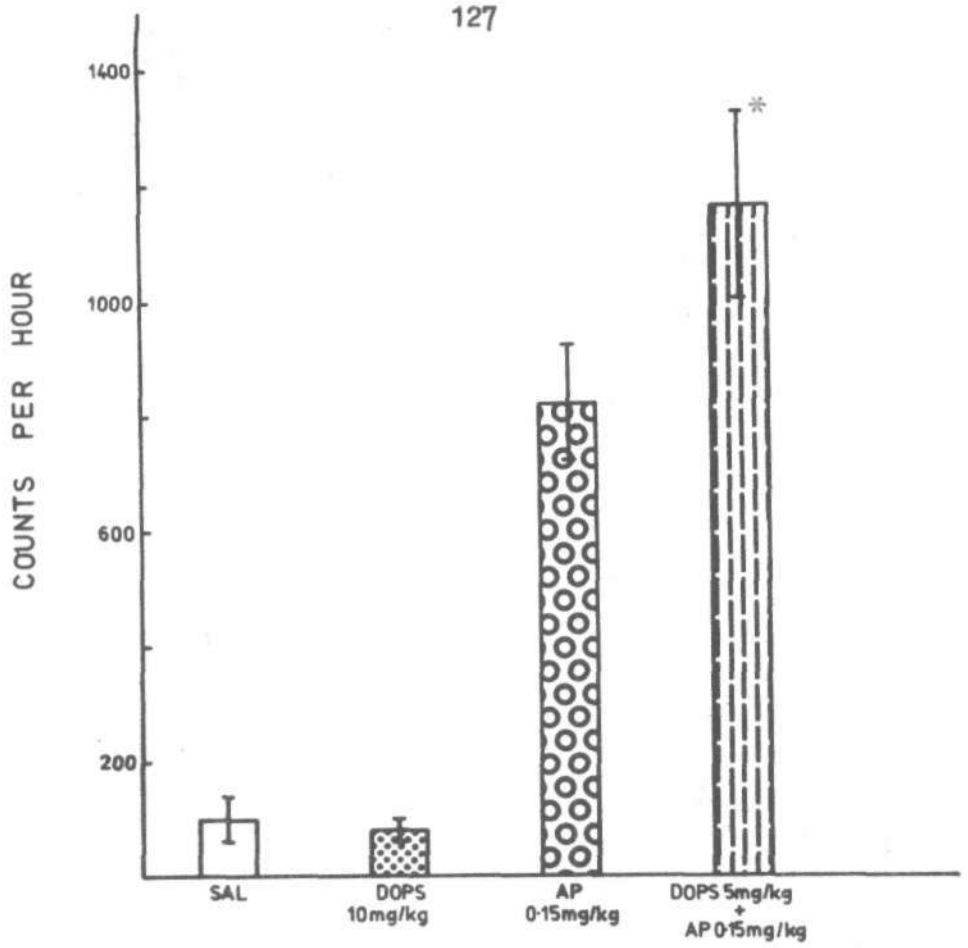


Fig. 4.9. Effect of dihydroxyphenylserine (DOPS) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with DOPS for 16 hr. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. * $p < 0.05$, t - test, (n =12 pairs per histogram).

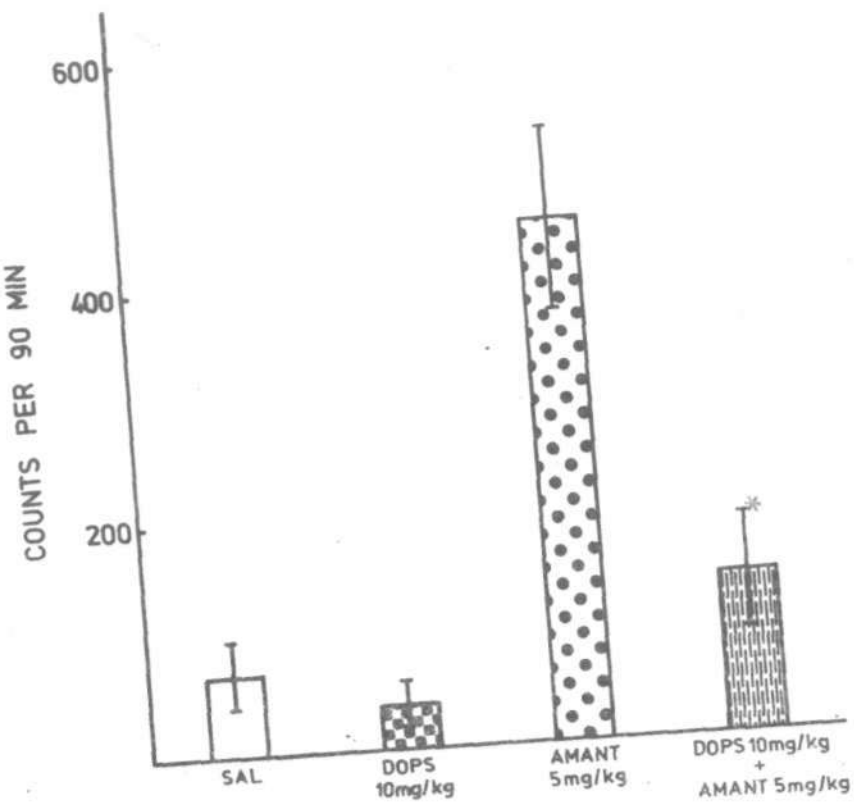


Fig. 4.10. Effect of dihydroxyphenylserine (DOPS) on amantadine (AMANT)-induced stereotyped behaviour in chicks. Chicks were pretreated with DOPS 16 hr. prior to administration of amantadine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.001$, t - test. n = 12 pairs per histogram.

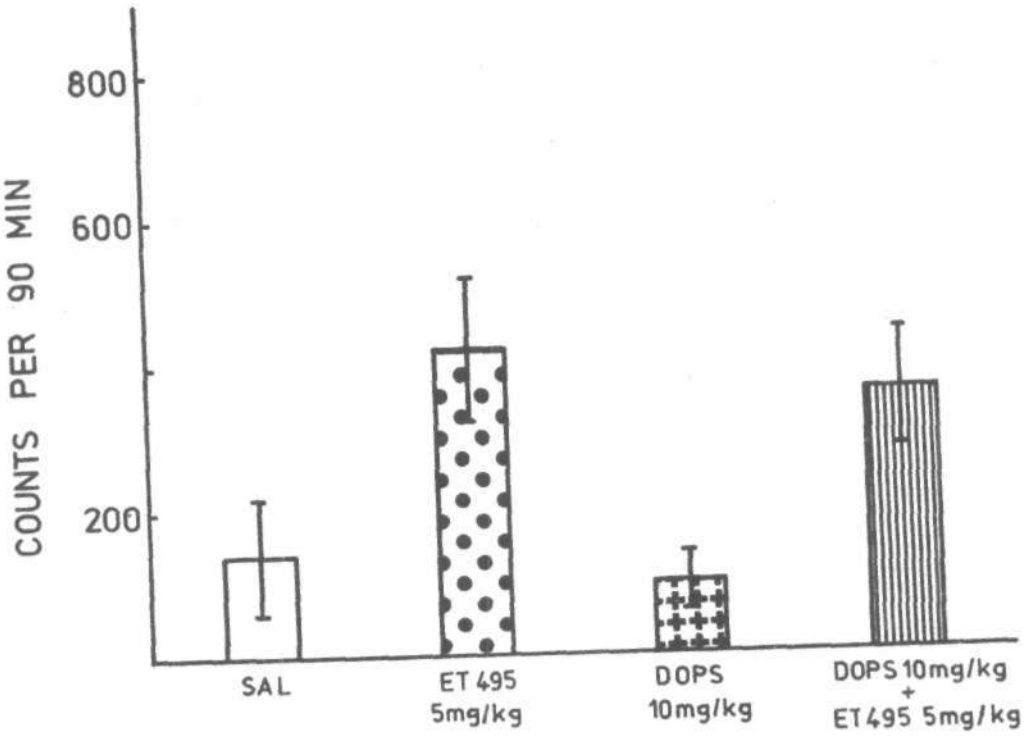


Fig. 4.11. Effect of dihydroxyphenylserine (DOPS) on piribedil (ET495)-induced stereotyped behaviour in chicks. Chicks were pretreated with DOPS 16 hr. prior to administration of piribedil. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations.

with DOPS (5 - 10 mg/kg, fig. 4.11).

4.3.4. 6-Hydroxydopamine (6-OHDA, 20 - 40 mg/kg).

6-OHDA selectively destroys catecholamine neurons (Ungerstedt, 1971a). When administered alone, the chicks showed head shaking and occasionally pushed each other round the cage. There was also occasional pecking of non-food objects. Pretreatment of chicks with 6-OHDA (20 - 40 mg/kg), potentiated apomorphine and piribedil-induced stereotyped behaviour (figs. 4.2 & 4.12). The chicks became more excited and the intensity of aggressiveness increased. With d-amphetamine and amantadine, there was a decrease in the intensity of pecking activity after 6-OHDA pretreatment (figs. 4.13 & 4.14). The effects of ergometrine (1.5 - 2.5 mg/kg) were not significantly altered after 6-OHDA (20 mg/kg).

4.3.5. Pimozide and Haloperidol.

Pimozide and haloperidol are specific DA receptor blockers (Anden et al, 1970;

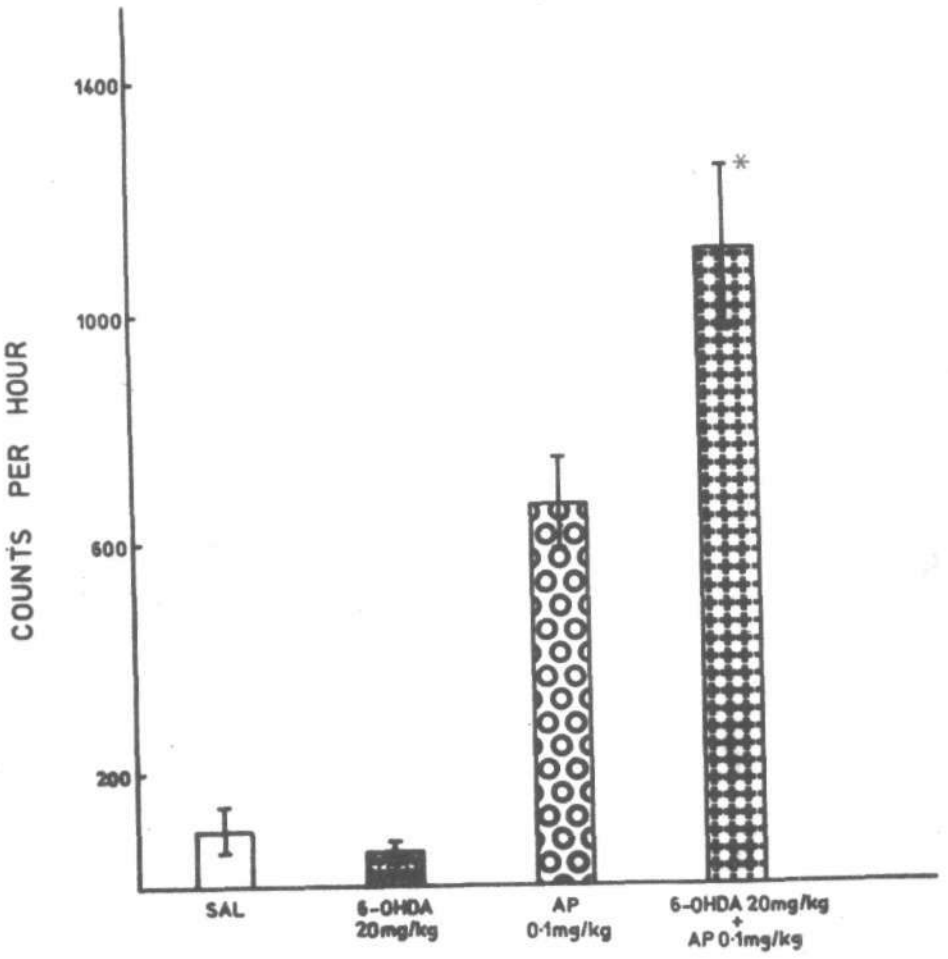


Fig. 4.12. Effect of 6-hydroxydopamine (6-OHDA) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with 6-OHDA 16 hr. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.001$, student's t - test. n = 12 pairs per histogram.

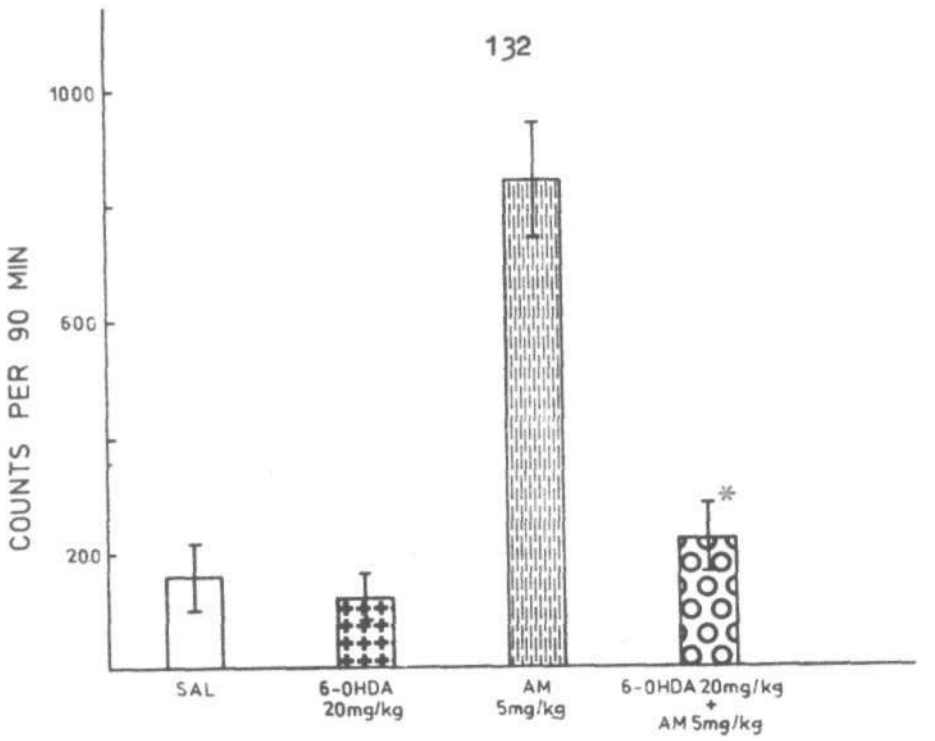


Fig. 4.13. Effect of 6-hydroxydopamine (6-OHDA) on amphetamine (AMP)-induced stereotyped behaviour in chicks. Chicks were pretreated with 6-OHDA 16 hr. prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.001$, t - test. n = 12 pairs per histogram.

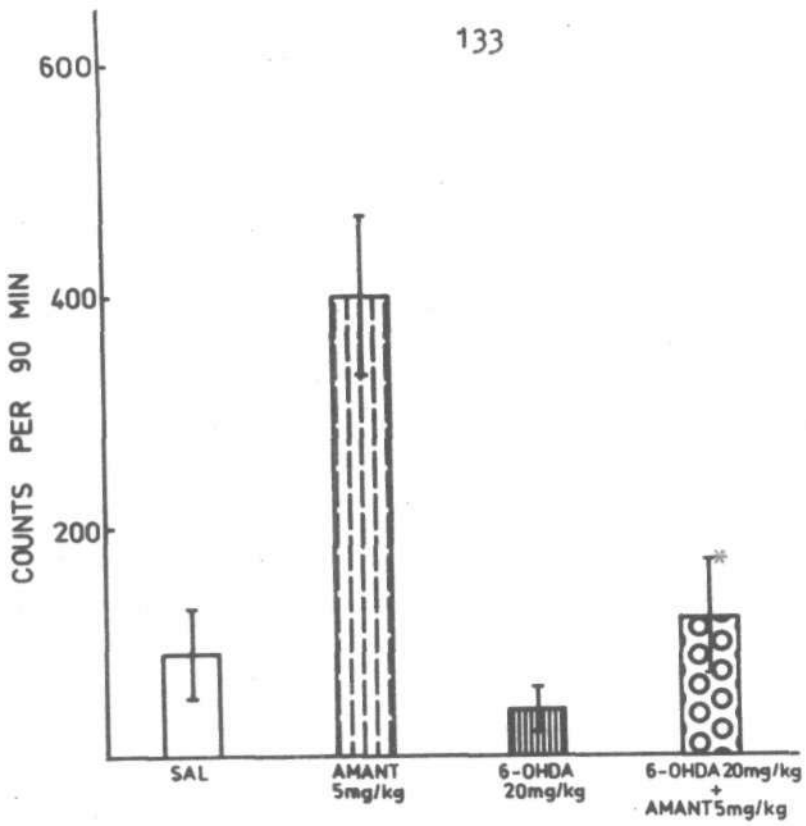


Fig. 4.14. Effect of 6-hydroxydopamine (6-OHDA)- on amantadine (AMANT)-induced stereotyped behaviour in chicks. Chicks were pretreated with 6-OHDA 16 hr. prior to administration of amantadine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviation. * $P < 0.001$, t - test. n = 12 pairs per histogram.

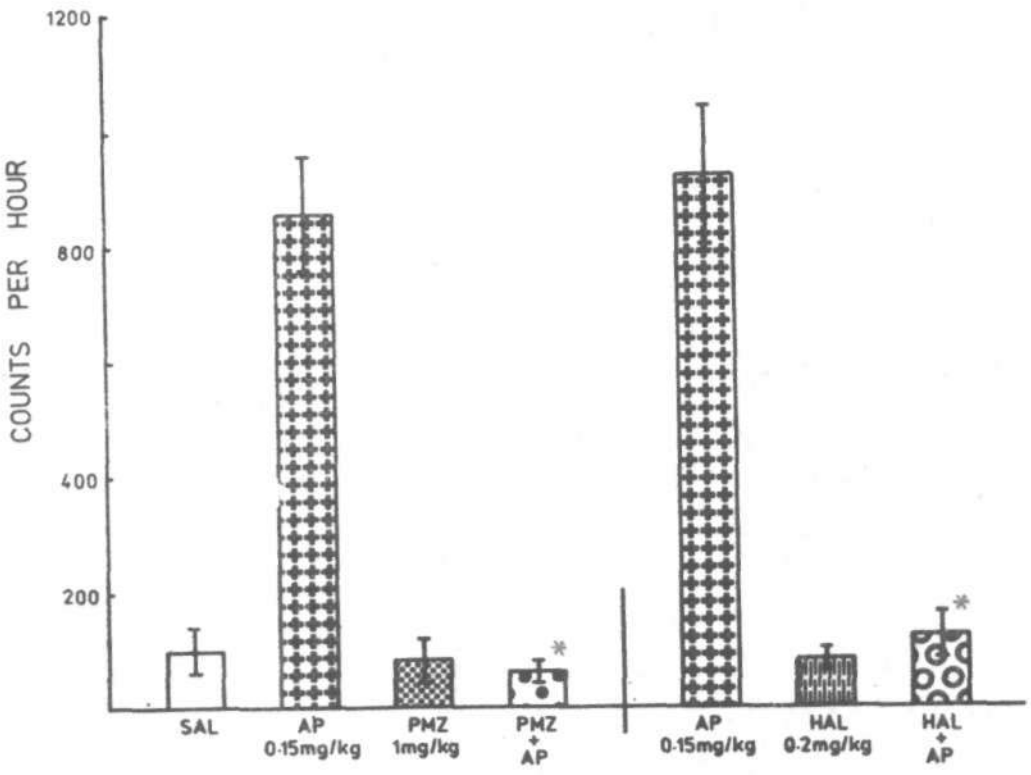


Fig. 4.15. Effects of pimozide (PMZ) and haloperidol (HA) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with pimozide and haloperidol 1 hr. prior to administration of apomorphine in each case. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, t - test. n = 12 pairs per histogram.

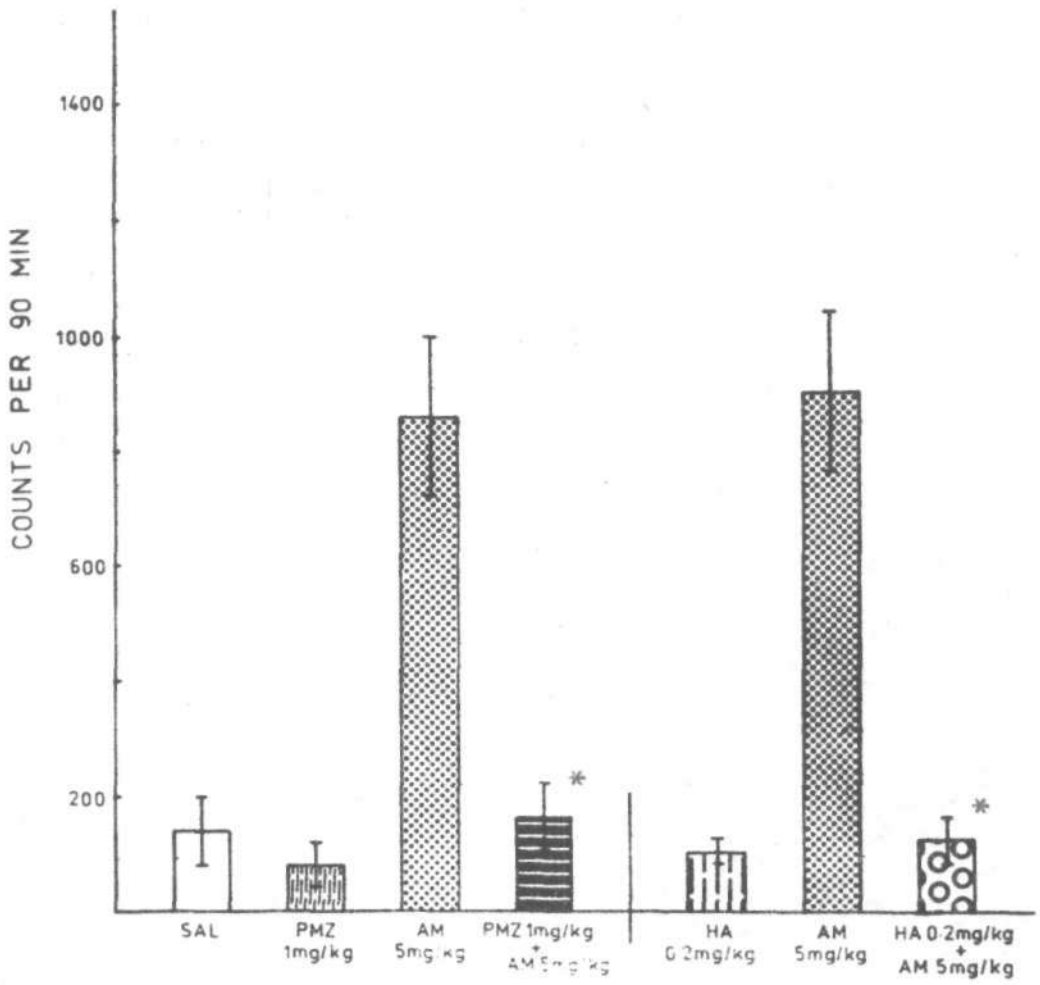


Fig. 4.16. Effect of pimozide (PMZ) and haloperidol (HA) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with pimozide and haloperidol 1 hr. prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, t - test. n = 12 pairs per histogram.

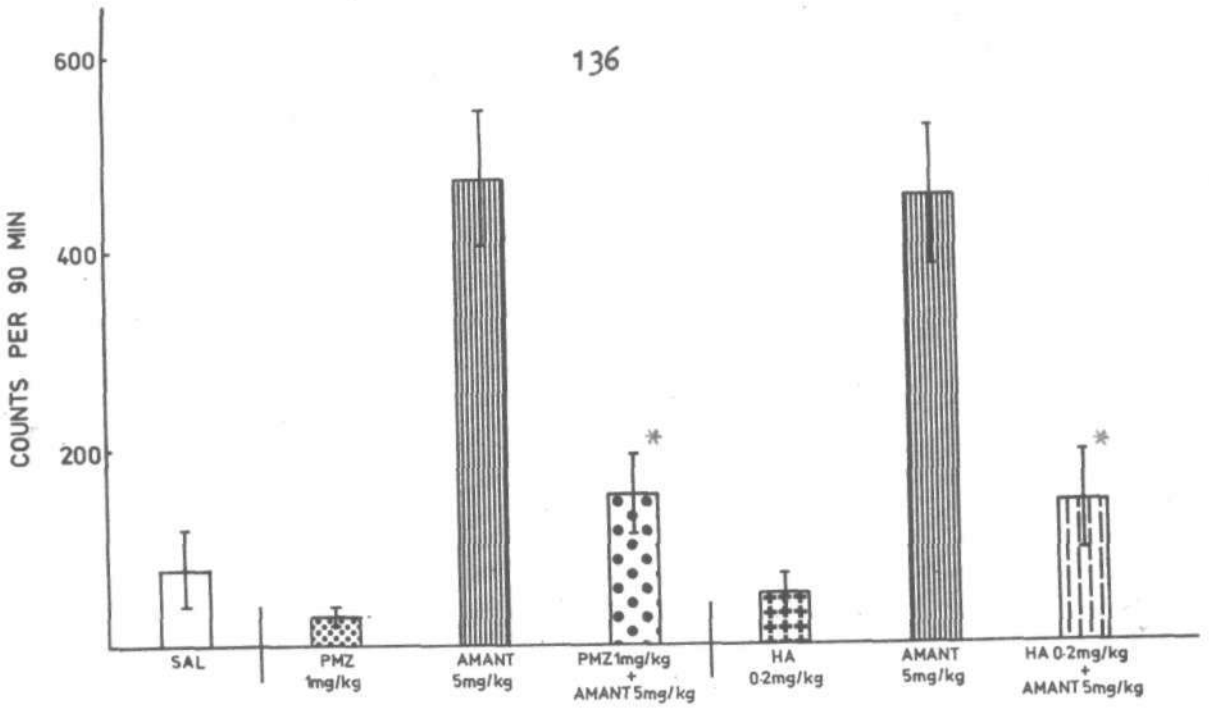


Fig. 4.17. Effects of pimozide (PMZ) and haloperidol (HA) on amantadine (AMANT)-induced stereotyped behaviour in chicks. Chicks were pretreated with pimozide and haloperidol 1 hr. prior to administration of amantadine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.001$, t - test. n = 12 pairs per histogram.

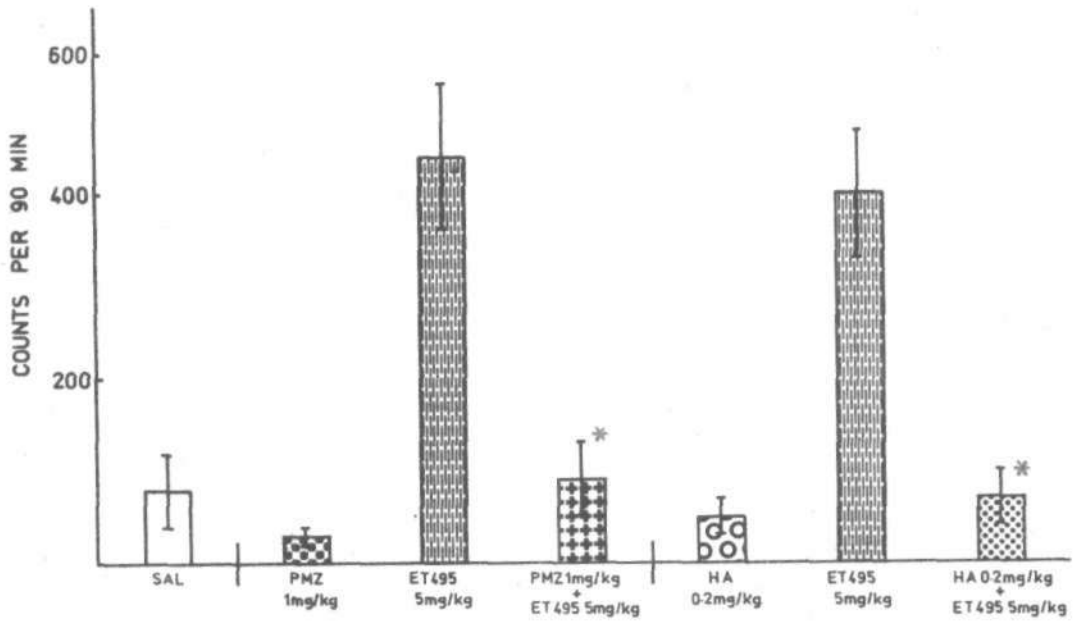


Fig. 4.18. Effects of pimozide (PMZ) and haloperidol (HA) on piribedil (ET495)-induced stereotyped behaviour in chicks. Chicks were pretreated with pimozide and haloperidol 1 hr. prior to administration of piribedil. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations * $p < 0.001$, t - test.
n = 12 pairs per histogram.

Janssen et al, 1965). Both drugs produced behavioural depression in the chicks when administered alone. Pimozide (1 mg/kg) and haloperidol (0.2 mg/kg) administered 1 h. previously, antagonized d-amphetamine, and apomorphine-induced stereotyped behaviour. The postural changes induced by d-amphetamine were not antagonized by either pimozide (1-4 mg/kg) or haloperidol (0.2 - 1 mg/kg). The behavioural effects of amantadine and piribedil were also antagonized by both pimozide and haloperidol (figs. 4.15 - 4.18).

4.3.6. Propranolol (5 - 20 mg/kg).

Propranolol (a beta adrenergic receptor blocking agent) when administered alone, depressed the chicks in a dose-dependent manner. There was usually a decrease in locomotor activity of the chicks. When the chicks were pretreated with propranolol (5 - 20 mg/kg) 30 min previously, the behavioural effects of apomorphine (0.15 mg/kg), d-amphetamine (5 mg/kg), piribedil (5 mg/kg) and

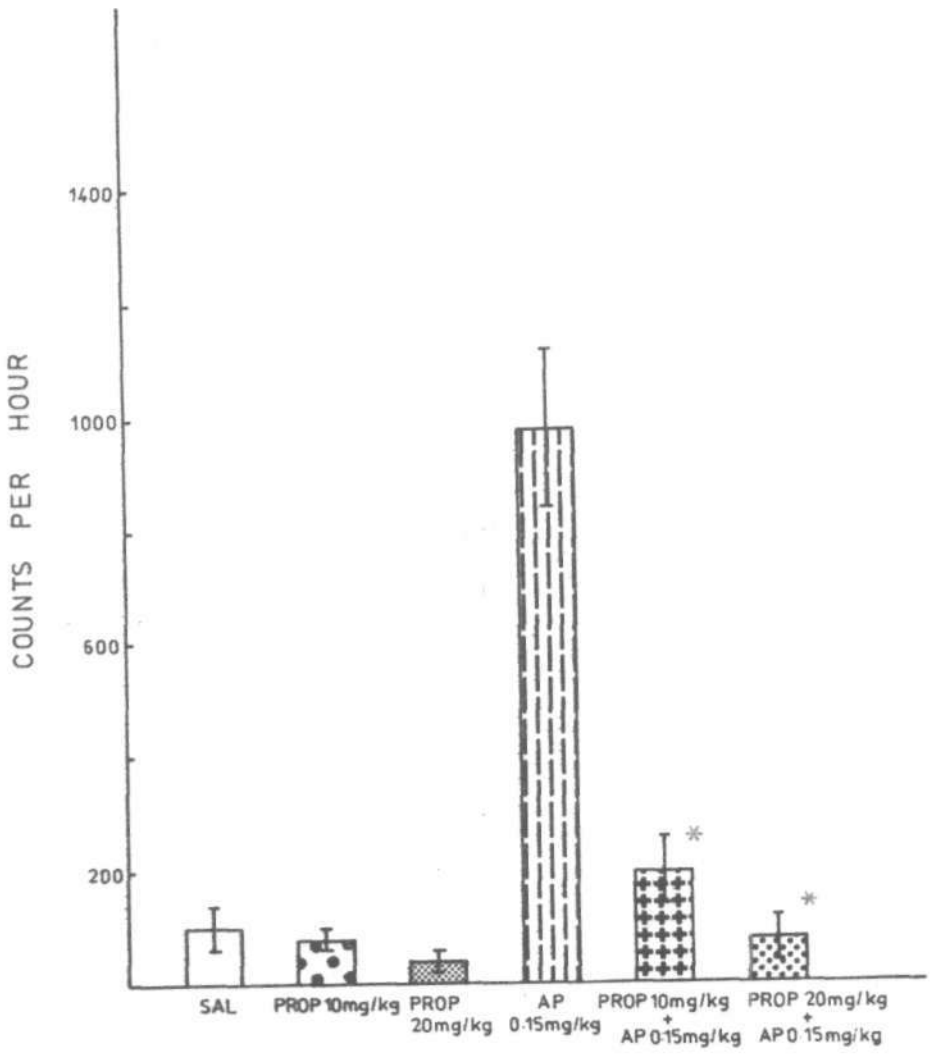


Fig. 4.19. Effect of propranolol (PROP) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with propranolol 30 min. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, t - test. n = 12 pairs per histogram.

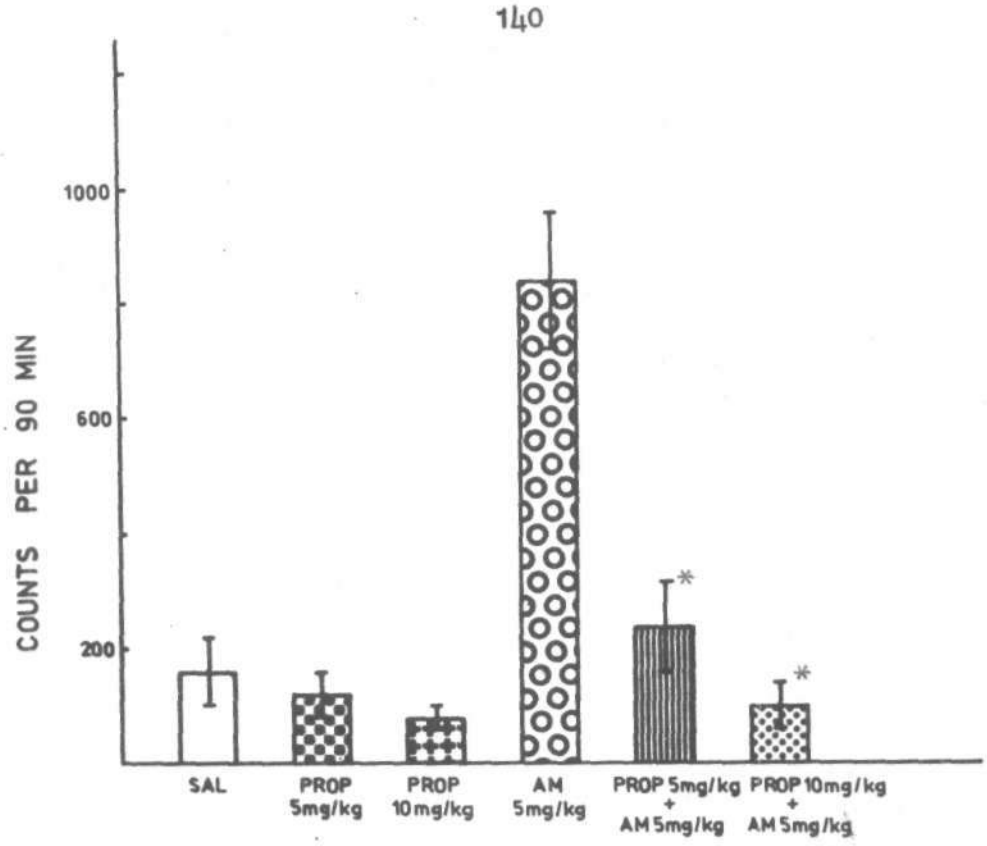


Fig. 4.20. Effect of propranolol (PROP) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with propranolol 30 min. prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.001$, t - test (n = 12 pairs per histogram).

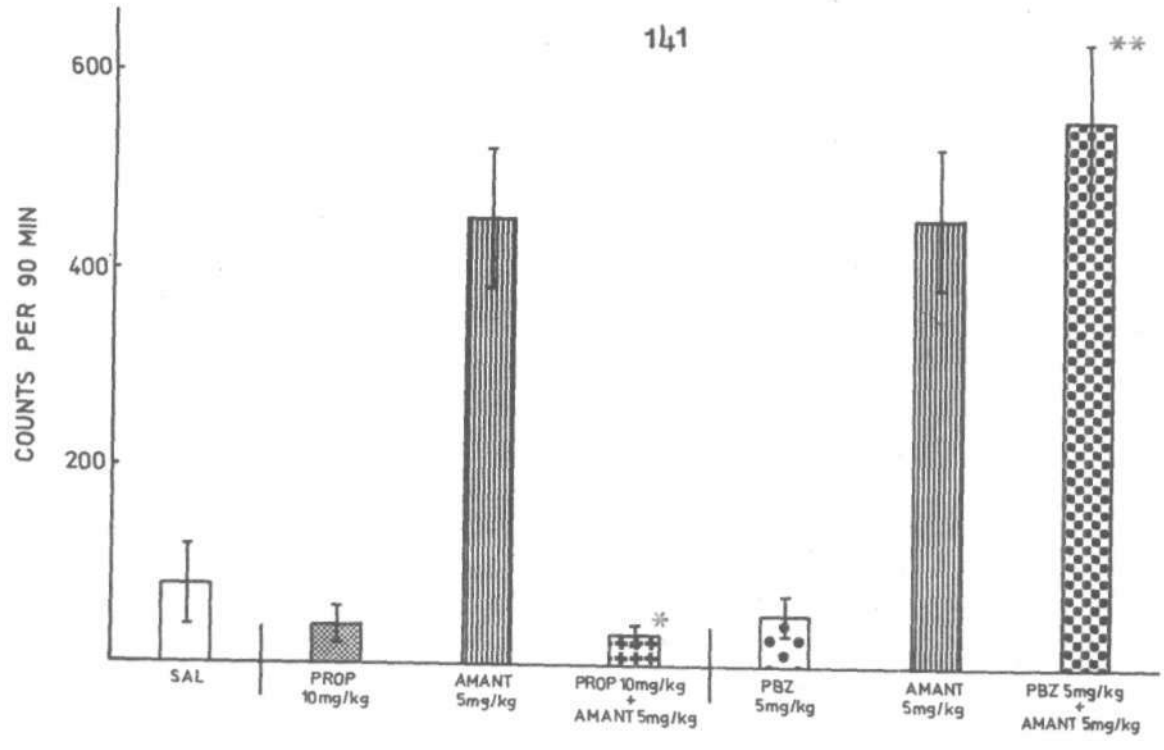


Fig. 4.21. Effects of propranolol (PROP) and phenoxybenzamine on amantadine (AMANT)-induced stereotyped behaviour in chicks. Chicks were pretreated with propranolol and phenoxybenzamine 30 min. in each case prior to administration of amantadine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, ** $p < 0.05$, t - test (n = 12 pairs per histogram).

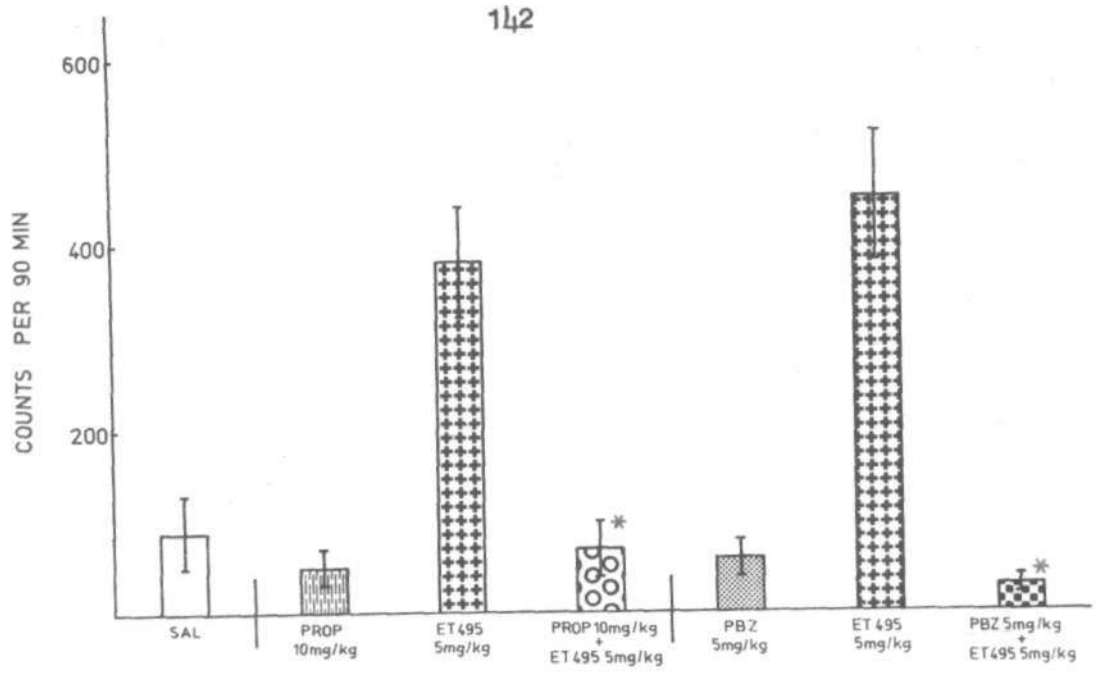


Fig. 4.22. Effects of propranolol (PROP) and phenoxybenzamine (PBZ) on piribedil (ET495)-induced stereotyped behaviour in chicks. Chicks were pretreated propranolol and phenoxybenzamine 30 min. in each case prior to administration of piribedil. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, t - test, (n = 12 pairs per histogram).

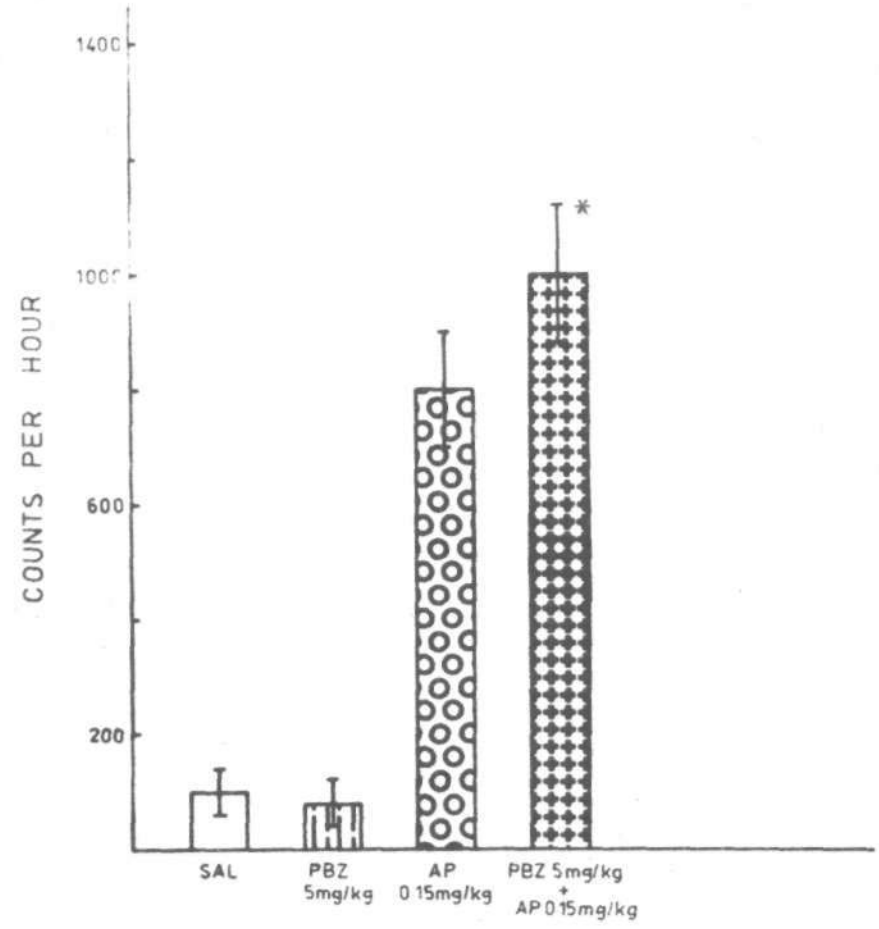


Fig. 4.23. Effect of phenoxybenzamine (PBZ) on apomorphine-induced stereotyped behaviour in chicks. Chicks were pretreated with phenoxybenzamine 30 min. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations.
* $p < 0.05$, t - test. n = 12 pairs per histogram.

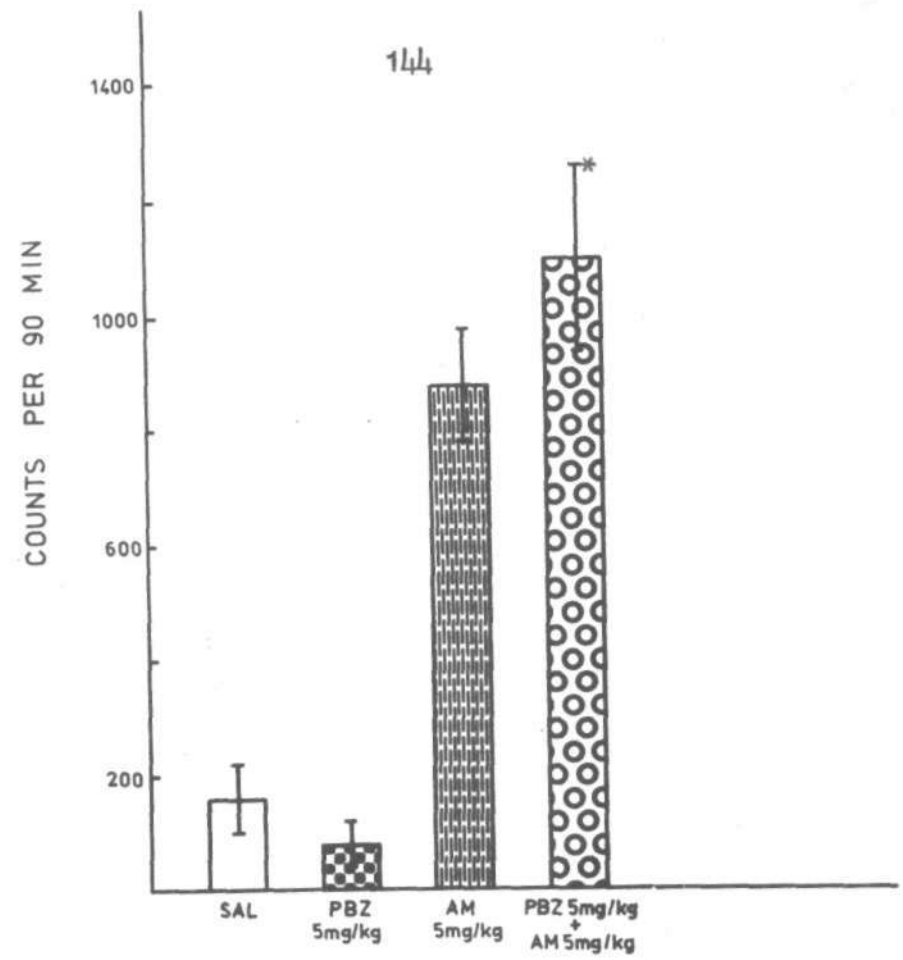


Fig. 4.24. Effect of phenoxybenzamine (PBZ) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with phenoxybenzamine 30 min. prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars indicate standard deviations. * $p < 0.05$, t - test. n = 12 pairs per histogram.

amantadine (5 mg/kg) were antagonized. Propranolol potentiated ergometrine-induced sedation in the chicks (figs. 4.19 - 4.22).

4.3.7. Phenoxybenzamine (5 - 20 mg/kg).

Phenoxybenzamine (an alpha adrenergic receptor antagonist) sedated chicks dose-dependently. Phenoxybenzamine (5 mg/kg) administered to chicks 30 min. previously, slightly potentiated apomorphine (0.15 mg/kg) d-amphetamine (5 mg/kg) and amantadine (5 mg/kg) induced stereotyped behaviour (figs. 4.21 - 4.24). Inconsistent results were obtained when higher doses of Phenoxybenzamine (10 - 20 mg/kg) were used. The depressant effects of piribedil (5 mg/kg) and ergometrine (5 mg/kg) were enhanced in chicks pretreated with phenoxybenzamine (5 - 10 mg/kg).

4.4. Discussion.

The observations made in this study show that drugs which influence catecholamine levels and receptor activity affect drug-induced

stereotyped behaviour in various ways. Thus, these results suggest a role of catecholamines in stereotyped behaviour induced by these drugs in chicks.

Reserpine has been shown to deplete brain catecholamines and serotonin (Bertler et al, 1956). It has been recently shown that catecholamine receptors become more sensitive to stimulation after either denervation (Ungerstedt, 1971b), and long-term, or short-term treatments with agents interrupting catecholaminergic transmission (Tarsy and Baldessarini, 1973; Costentin et al, 1975). Thus, the observed potentiation of apomorphine and piribedil-induced effects after reserpine pretreatment could be due to increased sensitivity of the receptors on which these drugs act. Since serotonin has been reported to exert inhibitory influence on drug-induced stereotyped behaviour (Weinner et al, 1973; Cheng and Long, 1974), the depletion of serotonin by reserpine would lead to a reduction of this

inhibitory influence which would further potentiate apomorphine- and piribedil-induced effects.

The antagonism of d-amphetamine and amantadine-induced effects shows that these drugs may not act directly on DA receptors in the chick to produce their characteristic behavioural effects. Both d-amphetamine and amantadine are believed to act indirectly by releasing catecholamines and inhibiting their reuptake (Weissman et al, 1966; Farnebo et al, 1971). It has however been reported that in the rat, amphetamine-induced stereotyped behaviour is not abolished after reserpine pretreatment (Van Rossum et al, 1962; Smith, 1963) and this led to the suggestion that amphetamine has a direct effect on catecholamine receptors (Van Rossum et al, 1962; Smith , 1963). However, the present data suggest that d-amphetamine acted mainly via an indirect mechanism. Neurochemical measurement of DA or homovanillic acid (major DA metabolite) turnover in the presence of

d-amphetamine may throw more light on the biochemical basis of these results.

The fact that the action of ergometrine was not significantly altered after reserpine pretreatment shows that this drug probably has no agonistic actions on DA receptors in the chick.

FLA-63 inhibits dopamine beta hydroxylase enzyme and thereby increases the level of DA (Corrodi et al, 1970). Hence, the potentiation of amphetamine-induced stereotyped behaviour by FLA-63 was probably due to the increase in the amount of transmitter available for release by d-amphetamine. The inhibitory effect of FLA-63 on apomorphine and piribedil induced stereotyped behaviour is probably due to the high level of DA available at the receptor sites, under the influence of FLA-63. This would prevent apomorphine and piribedil from having access to the receptors and thereby inhibiting their action.

The potentiation of apomorphine and d-amphetamine-induced effects by phenoxybenzamine suggests that activation of central alpha adrenergic receptors has an inhibitory influence on drug-induced stereotyped behaviour in chicks. Mogilnicka and Braestrup (1976), made a similar observation in rats. The observation made with DOPS, however, contradicts this suggestion, since the behavioural effects of apomorphine were enhanced when the chicks were pretreated with DOPS. This enhancement could probably be due to increase sensitivity of DA receptors to apomorphine after pretreatment with DOPS. This supersensitivity could be brought about by DOPS - induced reduction of brain DA levels. It has been shown that DA receptors become more sensitive to stimulation under such conditions (Ungerstedt, 1971b; Tarsy and Baldessarini, 1973).

Conflicting data have been reported on the role of beta adrenergic receptor blockers in drug-induced stereotyped behaviour. While some workers have not observed any effect on stereotyped behaviour (Randrup et al, 1963; Herman, 1967),

other workers have reported potentiation of stereotyped behaviour by beta adrenergic receptor blockers (Simon et al, 1972). In this study, piribedil, amantadine, apomorphine and d-amphetamine-induced stereotyped behaviour was antagonized by propranolol. It is possible that beta adrenergic receptors play a modulatory role in drug - induced stereotyped behaviour in chicks. On the other hand, propranolol might have acted on DA receptors to produce the observed antagonism.

The fact that both pimozide and haloperidol (antagonists of DA receptors, Anden et al, 1970; Janssen et al, 1965) antagonized the effects of apomorphine, piribedil, d-amphetamine and amantadine suggests that DA mechanisms might be involved in the behavioural effects of these drugs in chicks as in other species. The fact that ergometrine and d-amphetamine - induced postural effects were not antagonized by pimozide

and haloperidol suggest that these effects were probably not mediated via DA mechanisms.

6-OHDA selectively destroys catecholamine nerves (Ungerstedt, 1971b). The young chick has an underdeveloped blood-brain barrier (Waelsch, 1956; Key and Marley, 1962), and it is used to advantage to study the effects of drugs which do not cross the blood-brain barrier in the adult. Hence 6-OHDA when administered parenterally to young chicks is capable of destroying central catecholamine nerves and this leads to the development of supersensitive postsynaptic catecholamine receptors (Ungerstedt, 1971b). The observed potentiation of apomorphine and piribedil - induced stereotyped behaviour is therefore probably due to the action of these drugs on supersensitive DA receptors. This observation further shows that piribedil has a direct effect on DA receptors just like apomorphine. On the other hand, the antagonism of

d-amphetamine and amantadine effects after 6-OHDA suggests an indirect mode of action for these drugs.

The observations made in this study indicate a primary role for the dopaminergic system in the behavioural effects of these drugs. The observations also suggest that in the chick, piribedil has a direct effect on central DA receptors while d-amphetamine and amantadine act mainly via an indirect mechanism. However, neurochemical studies might further elucidate the mechanisms of the observed actions.

CHAPTER V

5. INFLUENCE OF SEROTONERGIC (5-HT) AGENTS ON APOMORPHINE AND d-AMPHETAMINE-INDUCED STEREOTYPED BEHAVIOUR IN CHICKS.

5.1. Summary.

5.1.1. The influence of serotonergic agonists and antagonists on apomorphine and d-amphetamine-induced stereotyped behaviour was studied in chicks.

5.1.2. Serotonin and quipazine antagonized apomorphine and d-amphetamine-induced stereotyped behaviour.

5.1.3. Cyproheptadine and parachlorophenylalanine potentiated apomorphine and d-amphetamine-induced stereotyped behaviour.

5.1.4. The results suggest that serotonergic system plays an inhibitory role in apomorphine and d-amphetamine-induced stereotyped behaviour in chicks.

5.2. Introduction.

Serotonergic mechanisms have been

associated with the ability of dopaminergic agonists to produce stereotyped behaviour (Costal and Naylor, 1974a) and thereby suggesting a possible relationship between DA and 5-HT in parkinsonism. However, conflicting results have been reported on the role of 5-HT in drug-induced stereotyped behaviour. Several authors have reported data consistent with an inhibitory action of 5-HT on drug-induced stereotyped behaviour (Cheng and Long, 1974; Winner et al, 1973; Saxena et al, 1977), while others have not observed any effect (Randrup and Munkvad, 1964; Rotrosen et al, 1972). In addition, it has been reported that drug-induced stereotypy is not significantly affected by lesions placed in the midbrain raphe (Samanin et al, 1972) or by PCPA pretreatment, experimental situations in which there is marked decrease in brain serotonin. In view of these conflicting observations, this study was designed to investigate the role of serotonergic mechanisms

in apomorphine and d-amphetamine-induced stereotyped behaviour in chicks.

5.3. Results:

5.3.1. 5-HT (1-20 mg/kg).

Moderate doses (1-5 mg/kg) produced slight behavioural sedation and caused the chicks to chirp intermittently with eyes closed, while higher doses (10 - 20 mg/kg) produced greater sedation. The stereotyped behaviour induced by apomorphine (0.15 mg/kg) and d-amphetamine (5 mg/kg) was antagonized in chicks pretreated 30 min previously with 5-HT (5 mg/kg, figs. 5.1 & 5.2). d-Amphetamine-induced postural changes were not antagonized by 5-HT.

5.3.2. Quipazine (1-20 mg/kg).

Quipazine, a direct acting 5-HT receptor agonist (Hong, Sancilio, Vargas and Pardo, 1969), in moderate doses (1-5 mg/kg), induced immobility, intermittent chirping, prominent head shaking and

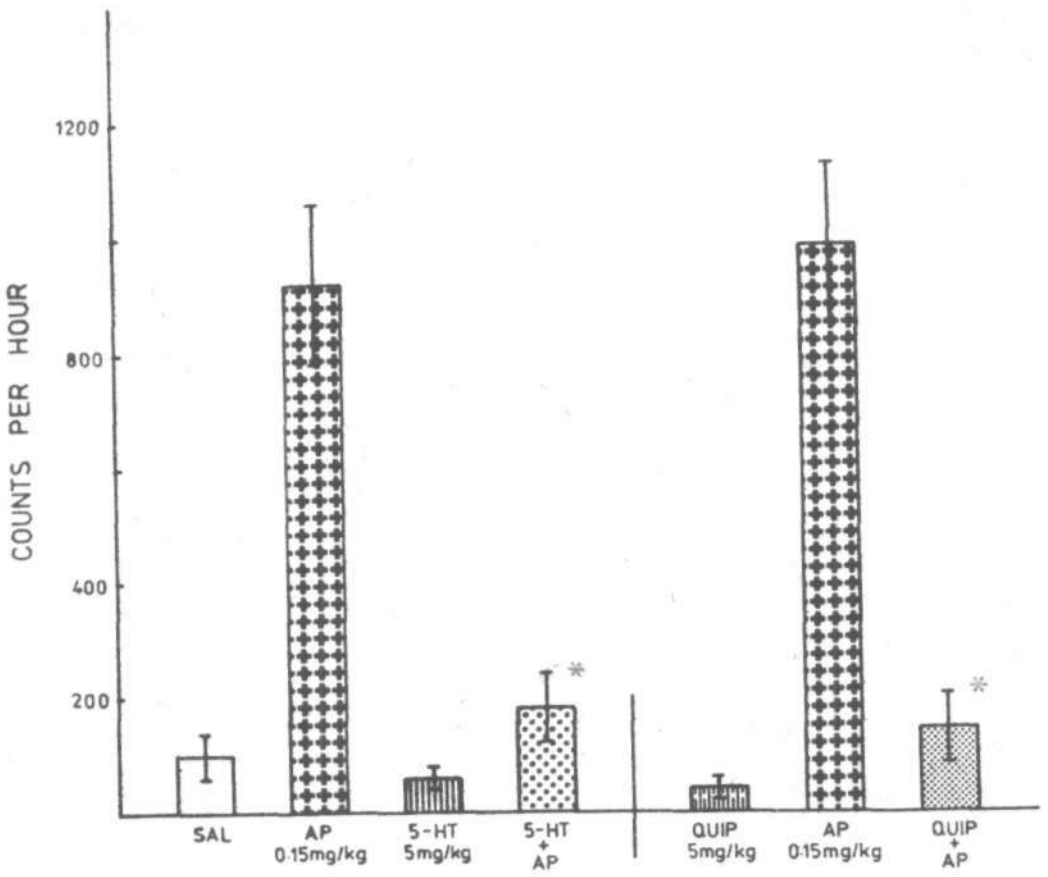


Fig. 5.1. Effect of 5-HT and quipazine (QUIP) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with 5-HT and quipazine 30 min. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$ (n = 12 pairs per histogram).

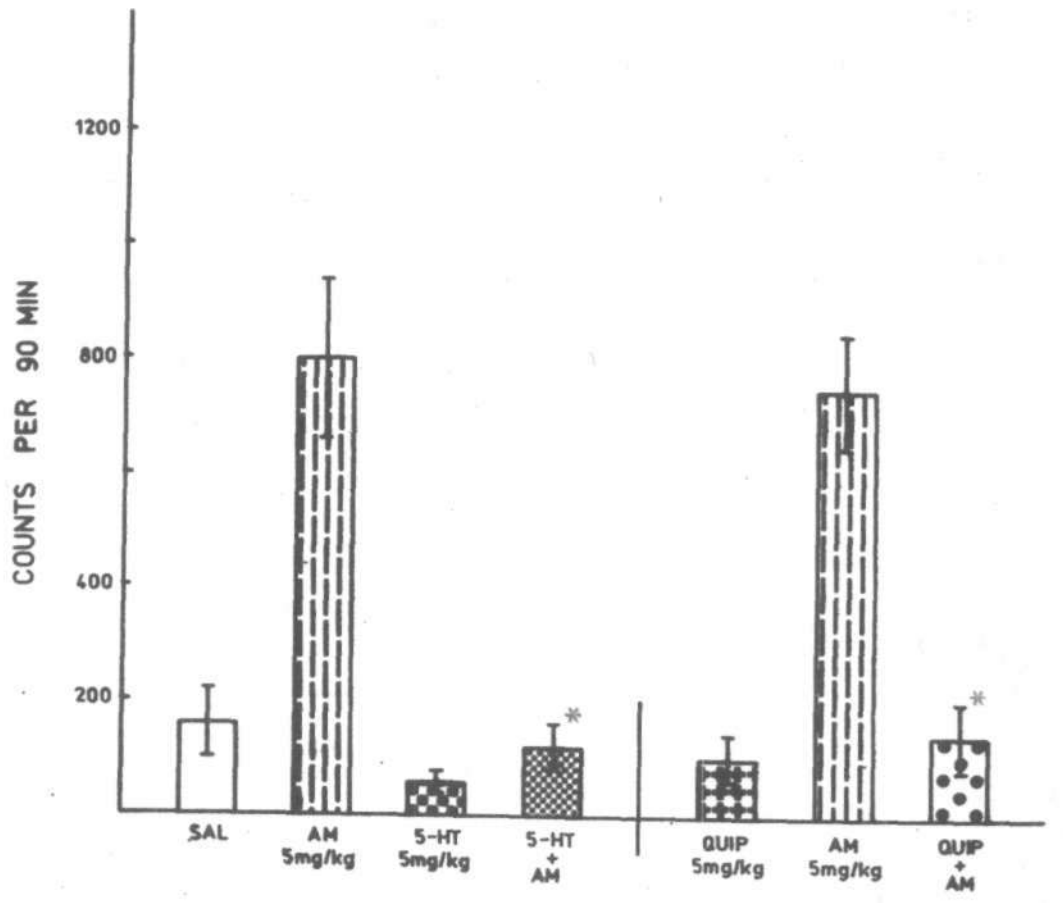


Fig. 5.2. Effects of 5-HT and quipazine (QUIP) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with 5-HT and quipazine 30 min. prior to administration with amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. * $p < 0.001$, (n = 12 pairs per histogram).

frequent cloacal evacuation. Higher doses (10 - 20 mg/kg) produced postural changes and weak compulsive pecking at non-food objects. Quipazine (5 mg/kg, 30 min pretreatment) antagonized the behavioural effects of both apomorphine (0.15 mg/kg) and d-amphetamine (5 mg/kg, figs. 5.1 & 5.2).

5.3.3. Methysergide (0.5 - 10 mg/kg). Low doses (0.5 - 2.5 mg/kg) of methysergide (a 5-HT receptor antagonist) produced a mixture of excitation and depression. The chicks usually went down on the metatarsus with wings spread out and chirped intermittently. Higher doses (5 - 10 mg/kg) produced greater depression. Methysergide (0.5 mg/kg) administered 1 h previously, potentiated apomorphine (0.15 mg/kg)-induced stereotyped behaviour, while higher doses (1 - 5 mg/kg) antagonized the effects of apomorphine. On the other hand, d-amphetamine effects were antagonized by all the doses of methysergide used in this study (figs. 5.3 & 5.4).

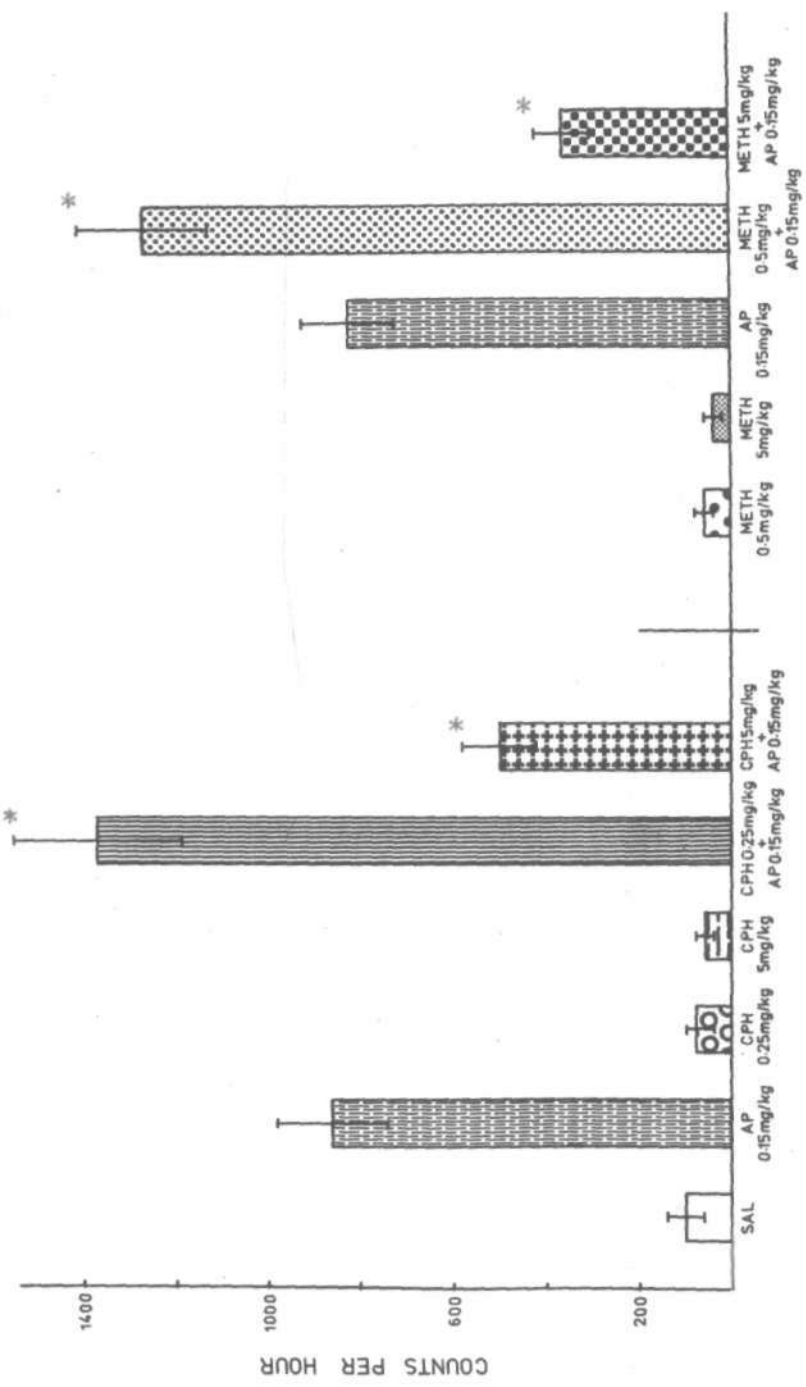


Fig. 5.3. Effects of cyproheptadine (CPH) and methysergide (METH) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with cyproheptadine and methysergide 1 hr. prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. *p $\angle$ 0.01, t - test, (n = 12 pairs).

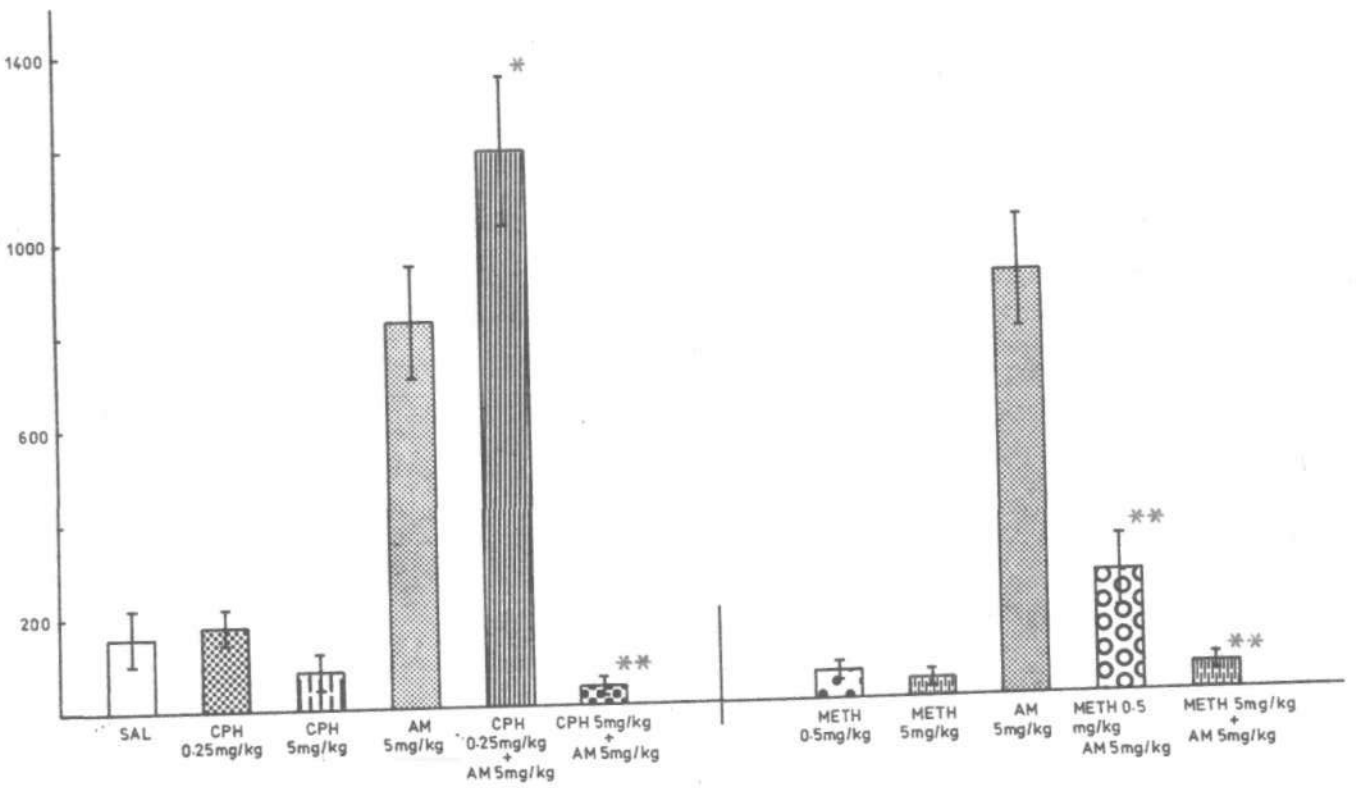


Fig. 5.4. Effect of cyproheptadine (CPH) and methysergide (METH) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with cyproheptadine and methysergide 1 hr. prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviation. * $p < 0.01$, ** $p < 0.001$, t - test, (n = 12 pairs per histogram).

5.3.4. Cyproheptadine (0.25 - 10 mg/kg).
With low doses (0.25-2.5 mg/kg) of cyproheptadine (a specific 5-HT receptor antagonist) the chicks chirped and closed their eyes intermittently. 5-10 mg/kg produced slight behavioural sedation, and chirping was also intermittent. When the chicks were pretreated with cyproheptadine, 0.25 mg/kg 1 h previously, the behavioural effects of apomorphine (0.15 mg/kg) and d-amphetamine (5 mg/kg) were enhanced. Higher doses (5-10 mg/kg of cyproheptadine antagonized apomorphine and d-amphetamine effects (figs. 5.3 & 5.4). The postural effects induced by d-amphetamine were antagonized by cyproheptadine (5 mg/kg).

5.3.5. Parachlorophenylalanine (PCPA, 100 mg/kg for 3 days). PCPA inhibits the synthesis of 5-HT (Koe and Weissman, 1966). PCPA pretreated chicks appeared slightly sedated and inactive. The behavioural effects of apomorphine (0.15 mg/kg) and d-amphetamine (5 mg/kg) were enhanced in chicks

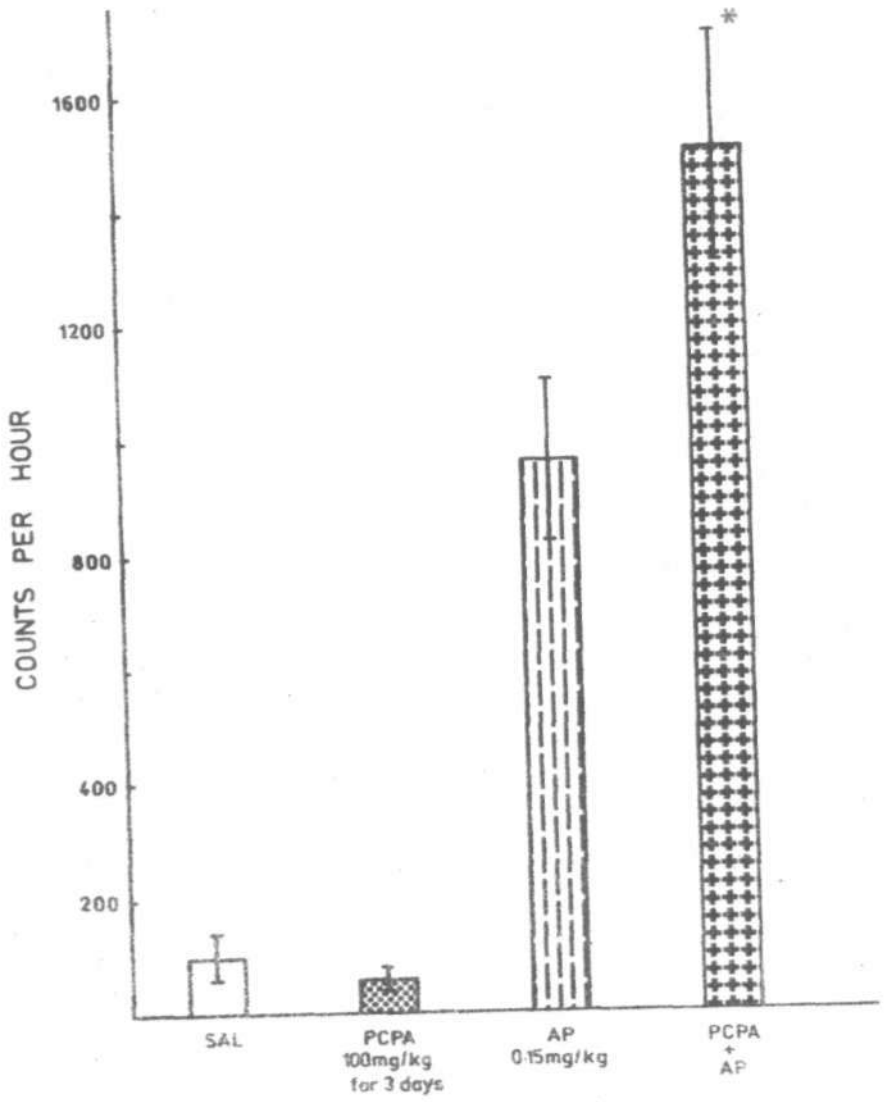


Fig. 5.5. Effect of parachlorophenylalanine (PCPA) on apomorphine (AP)-induced stereotyped behaviour in chicks. Chicks were pretreated with PCPA for 3 consecutive days prior to administration of apomorphine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviations. *$p < 0.005$, t - test, ($n = 12$ pairs per histogram).

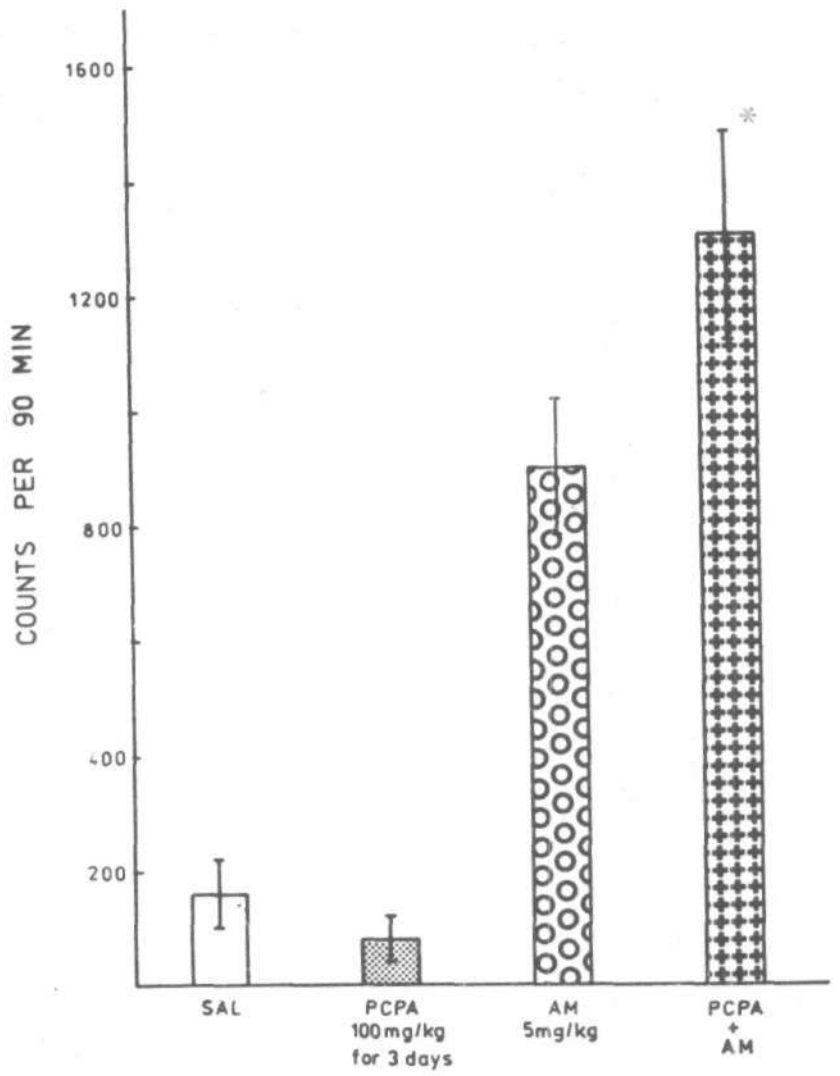


Fig. 5.6. Effect of parachlorophenylalanine (PCPA) on amphetamine (AM)-induced stereotyped behaviour in chicks. Chicks were pretreated with parachlorophenylalanine for 3 consecutive days prior to administration of amphetamine. Each histogram is the mean of the total activity recorded. Vertical bars represent standard deviation. * $p < 0.01$, (t - test, n = 12 pairs per histogram).

pretreated with PCPA (100 mg/kg i.p. daily for 3 days) while the postural changes induced by d-amphetamine (5 mg/kg) were antagonized (Figs. 5.5 & 5.6).

5.4. Discussion:

The results obtained in this study suggest a modulatory role for 5-HT in apomorphine and d-amphetamine induced stereotyped behaviour. Quipazine has been reported to have serotonin-like actions in the central nervous system (Hong et al, 1969). Quipazine also induces stereotyped sniffing and gnawing in rats and these actions are antagonized by haloperidol and pimozide (Grabowska et al, 1974a). The stereotyped pecking activity observed with higher doses of quipazine is probably due to an indirect interaction of the drug with central DA mechanisms through serotonin system.

The antagonism of apomorphine and d-amphetamine induced behavioural effects by

5-HT and quipazine shows that activation of central serotonergic receptors may inhibit drug-induced stereotyped behaviour in chicks. Several authors have reported similar observations in other species (Cheng and Long 1974; Weiner et al, 1973; Maj 1977). The potentiation of d-amphetamine and apomorphine effects by cyproheptadine is a further evidence in support of the suggestion that serotonin has an inhibitory influence on apomorphine and d amphetamine induced stereotyped behaviour. Cyproheptadine has also been shown to enhance apomorphine-induced pecking in pigeons (Saxena et al, 1977). The potentiation of apomorphine effect by methysergide agrees with the observation of Weiner et al,(1975). The reason for the inability of methysergide to potentiate d-amphetamine's effects is not obvious from this study. It is probable that d-amphetamine effects are more susceptible to blockade by methysergide than apomorphine effects,since the effects of

the later were also blocked by higher doses of methysergide. In view of the similarity between the postural changes induced by methysergide and d-amphetamine, it is tempting to suggest that both drugs produce these effects via a similar mechanism. According to Schrold and Squires (1971), d-amphetamine produces its postural effects in newly hatched chicks via a serotonergic mechanism. Methysergide could be producing the postural effects via an initial release of serotonin prior to receptor blockade. It is also probable that in the chick, methysergide exerts both agonistic and antagonistic effects on 5-HT mechanism. Further studies are required to elucidate the mechanism by which methysergide produces its effects in young chicks.

The fact that PCPA pretreatment potentiated both apomorphine and d-amphetamine effects, further suggests an inhibitory role of 5-HT on apomorphine and d-amphetamine-induced

stereotyped behaviour. PCPA inhibits hyptophan hydroxylase enzyme (Koe and Weissman, 1966) and thereby lowers the level of 5-HT in the brain. Thus, the observed potentiation is probably due to a reduction of the inhibitory influence of 5-HT as less 5-HT would be available under the influence of PCPA. The observed antagonism of d-amphetamine-induced postural effects by cyproheptadine and PCPA supports the previous suggestion by Schrold and Squires (1971) that serotonin is probably involved in these actions of d-amphetamine.

The data reported in this study indicate that 5-HT exerts antagonistic effects on apomorphine and d-amphetamine induced stereotyped behaviour in chicks. However, neurochemical studies are required to elucidate the mechanisms of the observed antagonism and potentiation of the behavioural effects.

CHAPTER VI

6. EFFECTS OF TRICYCLIC ANTIDEPRESSANTS ON APOMORPHINE-INDUCED LOCOMOTOR ACTIVITY IN CHICKS.

6.1. Summary.

6.1.1. The influence of some tricyclic antidepressants on apomorphine-induced locomotor activity was studied in chicks.

6.1.2. Desipramine, imipramine and amitriptyline induced a biphasic effect in chicks when administered alone.

6.1.3. Desipramine, imipramine and amitriptyline when administered 1 h previously inhibited apomorphine-induced locomotor activity.

6.1.4. Imipramine and amitriptyline when administered 30 min previously, potentiated apomorphine-induced locomotor while the effect was inhibited by desipramine.

6.1.5. The results suggest that tricyclic antidepressants produce a time - dependent effect on apomorphine-

induced locomotor activity in chicks.

6.2. Introduction:

There has been much debate about the possible role of central catecholamines in motor activity. The results of pharmacological manipulation of the amine system has led some authors to claim that NA enhances certain aspects of motor activity (Svensson, 1971; Maj et al, 1972). There are however some workers with a contrary view (Thormbung and Moore, 1973). According to the hypothesis of Van Rossum and Hurkmans (1964), stimulation of central DA receptors causes an increase in motility. It seems therefore that apomorphine could be a good tool for studying the role of dopaminergic system in motility since it possesses agonistic activity on DA receptors.

The behavioural effects of imipramine have been studied in chicks (Mandell et al, 1968; Schrold, 1970). These workers observed a biphasic action of imipramine in young chicks with

an initial decrease in locomotor activity followed by excitation and aggressive behaviour. In addition, Schrold (1970) observed that other tricyclic antidepressants produced the same characteristic behavioural changes in young chicks. Tricyclic antidepressants are generally believed to act by inhibiting monoamine neuronal reuptake systems (Schanberg, Schildkraut and Kopin; 1967; Carlsson et al, 1969).

This work was designed to study the influence of tricyclic antidepressants on apomorphine-induced locomotor activity in young chicks, with the aim of establishing whether this interaction would produce a consistent locomotor pattern and the possibility of using this as a behavioural model for screening compounds for antidepressant properties.

6.3. Results:

6.3.1. Apomorphine (0.01 - 0.05 mg/kg).

With a dose of 0.01 mg/kg of apomorphine

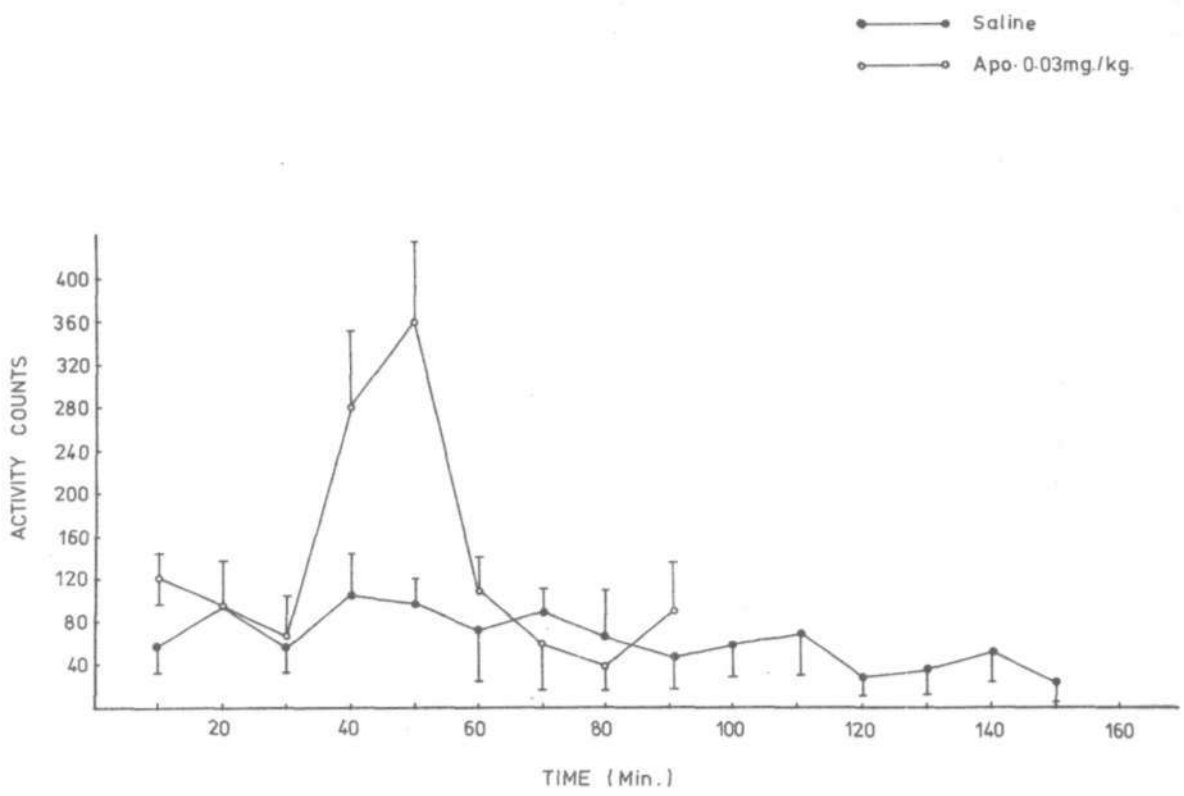


Fig. 6.1. Effect of apomorphine (0.03 mg/kg) on locomotor activity in 3-day old chicks. The values plotted are arithmetic mean ± s.d. Vertical bars represent standard deviations.

the chicks appeared sedated, inactive and chirped intermittently, while 0.02 mg/kg apparently increased locomotor activity. When 0.03 mg/kg was administered, the chicks became excited and actively moved around the cage with loud chirping. Higher doses (0.05 - 0.15 mg/kg) induced greater excitation and stereotyped pecking activity. 0.03 mg/kg was chosen as the dose which produced optimum increase in locomotor activity without stereotyped pecking and aggressive behaviour, and this dose was used in the drug interaction studies (Fig. 6.1,.

6.3.2. Imipramine (2.5 - 15 mg/kg).

Imipramine (2.5 & 5 mg/kg) when administered alone initially decreased the activity of the chicks. This period of decreased locomotor activity (lasting about 30 min) was followed by a prolonged period of increased locomotor activity With these doses, no stereotyped pecking activity or aggressive behaviour was observed. Higher

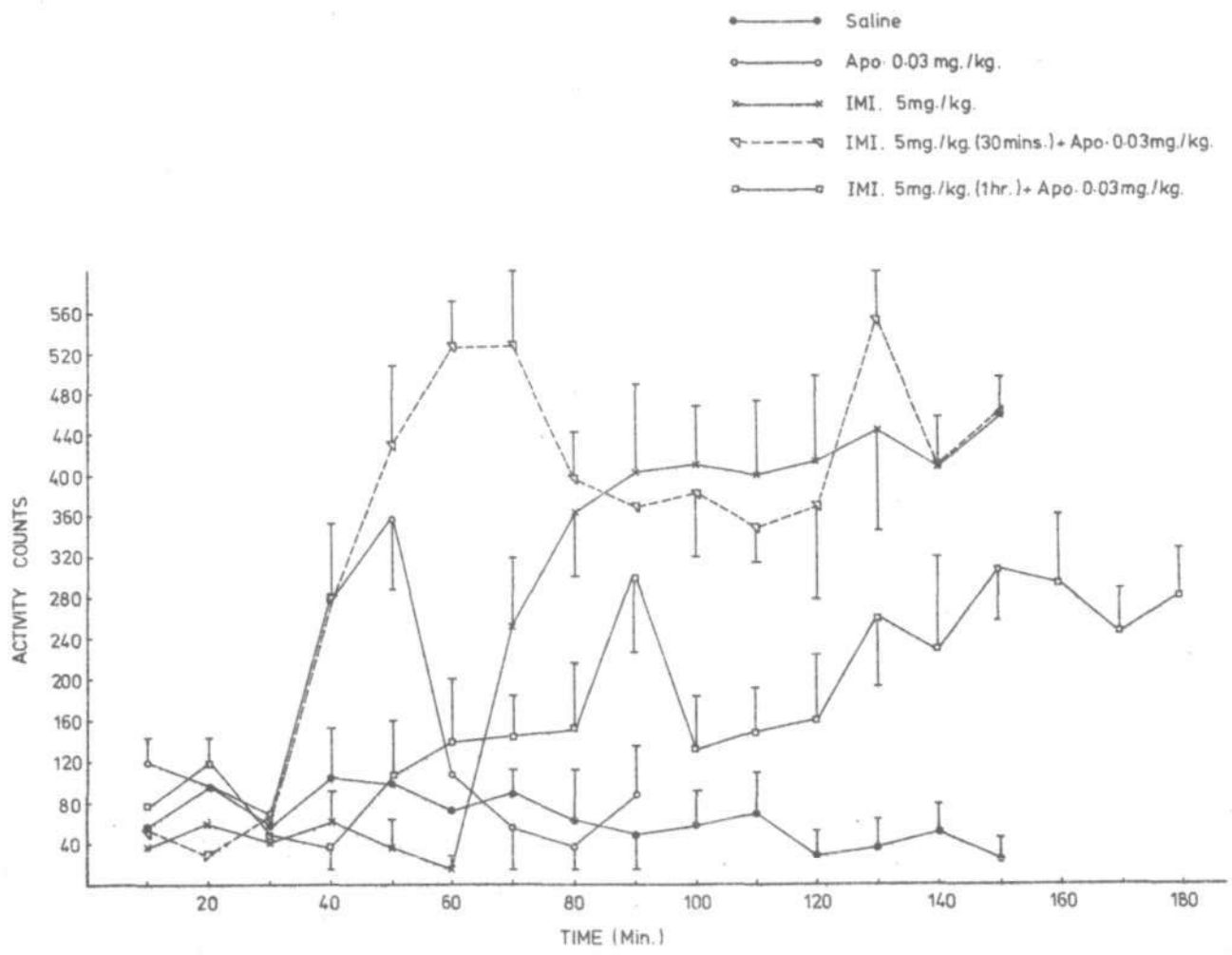


Fig. 6.2. Effect of imipramine (IMI) on apomorphine-induced locomotor activity in 3-day old chicks. The values plotted are arithmetic mean ± s.d. Vertical bars represent standard deviations.

doses (10 - 15 mg/kg) induced intense aggressive behaviour in chicks. This was characterized by pecking at each other in the region of the beak.

When the chicks were pretreated with imipramine (5 mg/kg) 30 min previously, apomorphine-induced locomotor activity was enhanced. On the other hand, administration of imipramine (5 mg/kg) 1 h previously, imhibited apomorphine-induced locomotor activity (Fig. 6.2).

6.3.3. Desipramine (2.5 - 15 mg/kg)

The pattern of behaviour induced by desipramine was similar to that reported for imipramine. When the chicks were pretreated with desipramine (5 mg/kg) 30 min and 1 h previously, apomorphine-induced locomotor activity was inhibited. (Fig. 6.3).

6.3.4. Amitriptyline (2.5 - 15 mg/kg)

Amitriptyline also induced a behavioural pattern similar to that of imipramine and desipramine. Pretreatment of chicks with

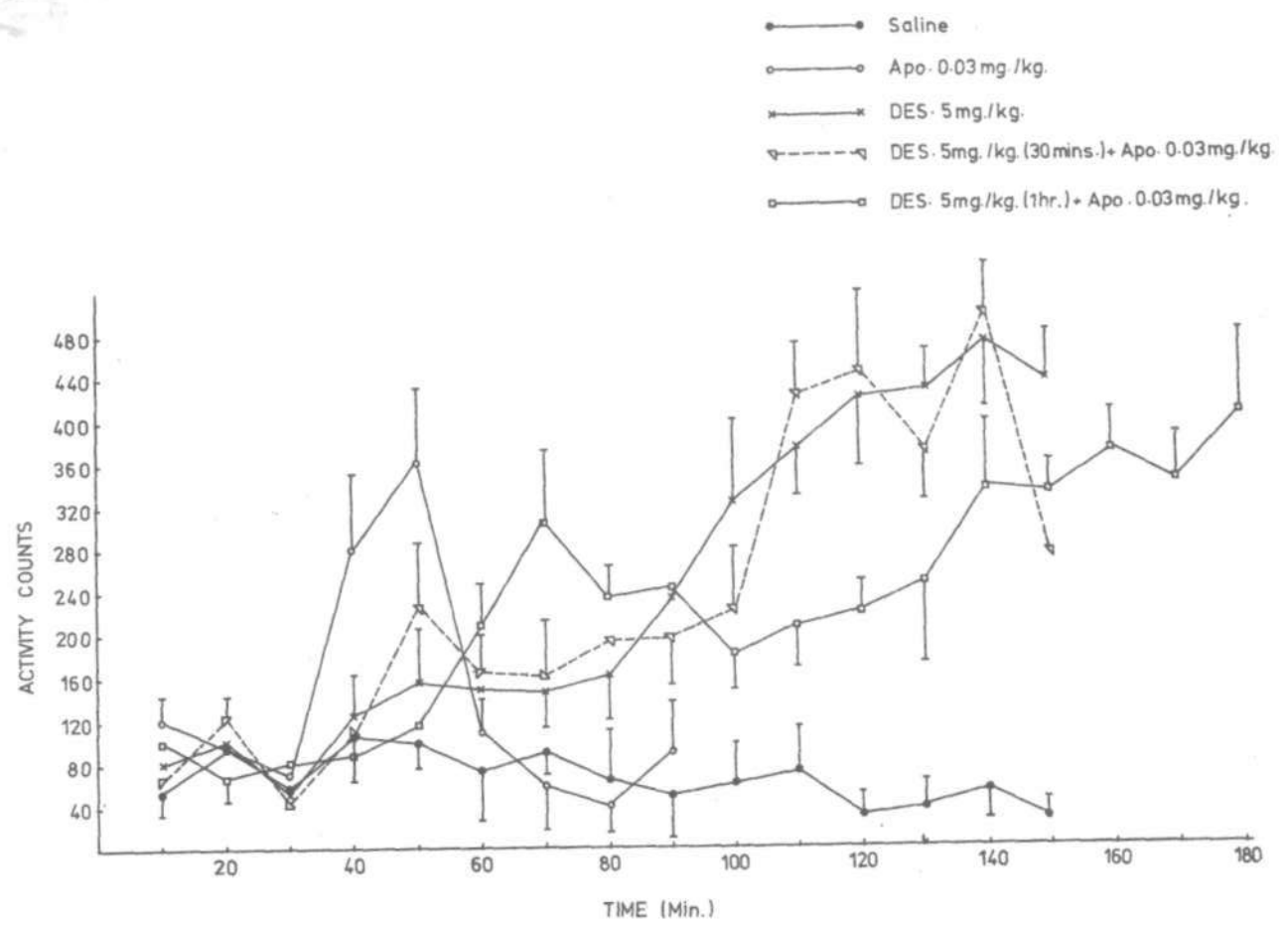


Fig. 6.3. Effect of desipramine (DES) on apomorphine-induced locomotor activity in 3-day old chicks. The values plotted are arithmetic mean ± s.d. Vertical bars represent standard deviations.

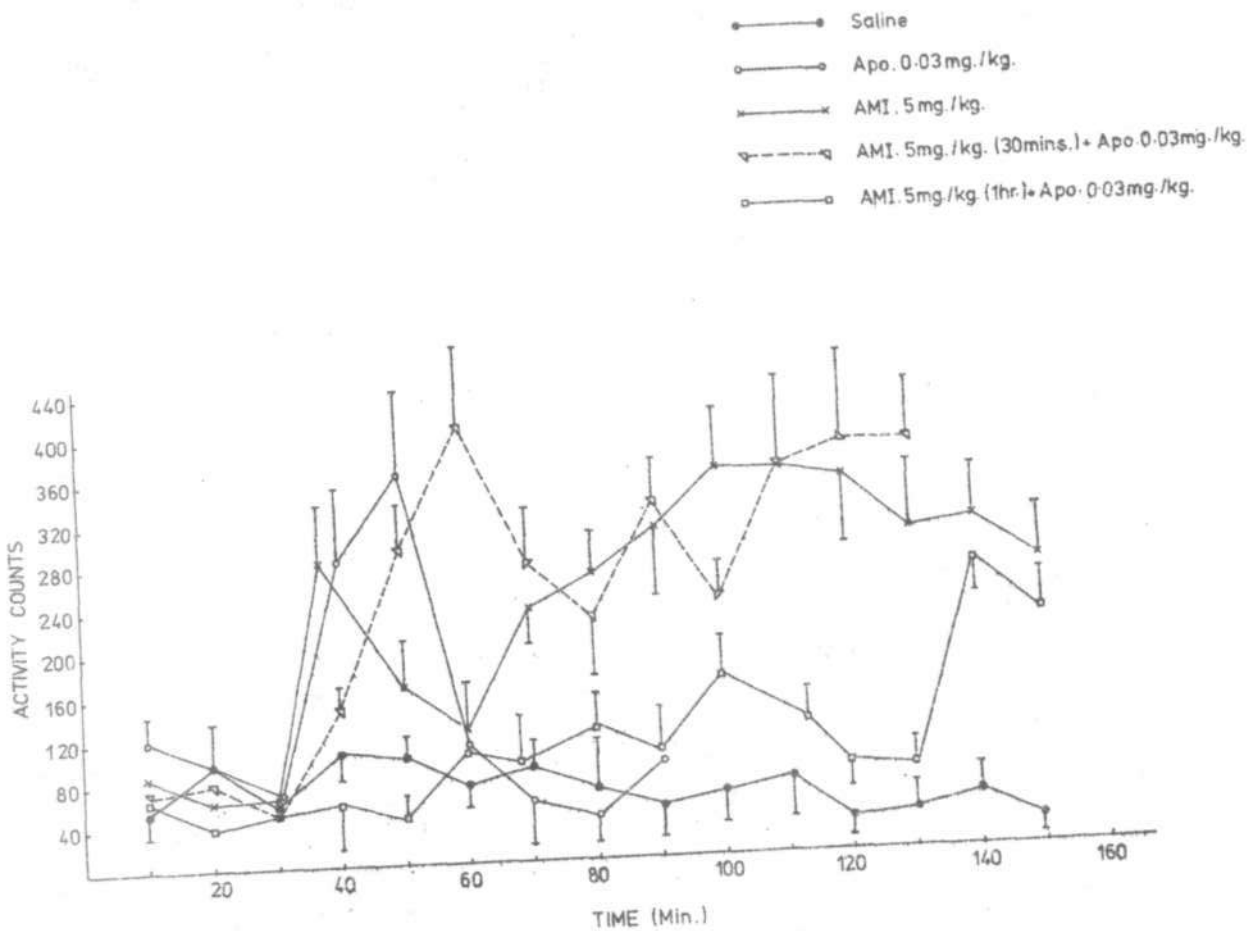


Fig. 6.4. Effect of amitriptyline (AMI) on apomorphine-induced locomotor activity in 3-day old chicks. The values plotted are arithmetic mean ± s.d.

amitriptyline (5 mg/kg) 30 min previously, potentiated apomorphine effect while pretreatment for 1 h inhibited the effect of apomorphine (fig.6.4).

6.4. Discussion:

Apomorphine is believed to produce its behavioural effects through direct stimulation of central DA receptors (Ernst, 1967; Andén et al, 1967). The observations made in this study show that apomorphine produces a dose-dependent increase in locomotor activity in young chicks, as previously reported in rats (Maj et al, 1972; Grabowska et al, 1974b). The depressant effect observed with 0.01 mg/kg of apomorphine could probably be due to an action of the drug on inhibitory presynaptic receptors located on DA neurons, which have been termed autoreceptors by Carlsson, Kehr, and Linqvist (1975). It has been shown that these receptors are preferentially activated by low doses of a DA agonist, presumably because of greater affinity for these agonists or a greater number relative to postsynaptic receptors.

Autoreceptor activation causes inhibition of DA synthesis and release and thereby secondarily reduces motor activity (Carlsson et al, 1975, Strombom, 1975). The increase in locomotor activity observed with higher doses is probably due to a greater activation of postsynaptic DA receptors.

The behavioural effects of imipramine and other tricyclic antidepressants have been attributed to inhibition of 5-HT and NA reuptake processes. Hence, the initial decrease in locomotor activity observed with these drugs is probably due to the increase level of 5-HT and NA at the receptor sites. These drugs probably caused subsequent increase in locomotor activity by inhibiting both 5-HT and NA from having access to their respective receptor sites, as previously suggested by Mandell et al (1968).

The potentiation of apomorphine-induced locomotor activity by both imipramine and

amitriptyline probably suggests that these drugs enhance the action of apomorphine at DA receptors. Imipramine and other tricyclic antidepressants have been shown to inhibit the reuptake of DA Pscheidt and Himwich, 1968) and hence, the observed potentiation could be due to increase level of DA at the receptor sites. It may also be possible that a supposed anticholinergic effect of imipramine and amitriptyline significantly reduced cholinergic inhibitory influence on DA neurotransmission in the striatum and thereby potentiating the action of apomorphine.

The observed inhibition of apomorphine effect by the three antidepressants administered 1 h previously could probably be due to an indirect inhibitory influence of NA and 5-HT on dopaminergic mechanisms. A similar inhibitory effect of tricyclic antidepressants on apomorphine-induced locomotor activity has been reported in rats (Grabowska et al, 1974b). These workers attributed

this action to a predominant inhibitory influence of 5-HT. A functional relationship between serotonergic and dopaminergic neurons has been proposed to explain various behavioural responses. Antagonism between serotonin and DA has been suggested in the striatum on the basis of the finding that amphetamine stereotypy is inhibited by tryptophan and enhanced by methysergide, a 5-HT receptor blocker (Weiner et al, 1973).

The observations made in this study suggest that tricyclic antidepressants exert a biphasic action on apomorphine-induced locomotor activity in chicks. A similar biphasic action of trimipramine (a tricyclic antidepressant) on apomorphine-induced stereotyped behaviour has been reported in chicks (Osuide and Adejoh, 1973). However, the observed potentiation and inhibition cannot be precisely explained in the light of the present data and further work is required to elucidate the mechanism of these effects.

CHAPTER VII

7. BEHAVIOURAL EFFECTS OF B, B-IMINODIPROPIONITRILE (IDPN) IN CHICKS.

7.1. Summary:

7.1.1. The behavioural effects of IDPN and the influence of various drugs were studied in chicks.

7.1.2. Repeated administration of IDPN (75 mg/kg) for five days induced distinct behavioural changes in chicks which were characterized by excitation, choreiform head and neck movements and circling (i.e. ECC - syndrome).

7.1.3. d-Amphetamine enhanced the ECC-syndrome while apomorphine and piribedil enhanced the choreiform head and neck movements with minimal effect on the circling behaviour.

7.1.4. Pimozide antagonized the ECC-syndrome and also inhibited amphetamine-induced potentiation of the syndrome.

7.1.5. Atropine and hyoscine enhanced the ECC-syndrome and also potentiated the effects of amphetamine.

7.1.6. Quipazine in low doses abolished and in high doses enhanced the ECC-syndrome. Pretreatment with either cyproheptadine or pimozide prevented quipazine-induced potentiation of the ECC-syndrome in chicks.

7.1.7. Fluorimetric determinations of biogenic amines showed significant reductions in NA, DA and 5-HT levels in the hyperstriatum, optic tectum, cerebellum and pons-medulla of syndromized chicks.

7.1.8. The results suggest that DA and serotonergic mechanisms may be involved in IDPN-induced effects in chicks. A further suggestion is that the cholinergic system may play an inhibitory influence on the ECC-syndrome.

7.2. Introduction.

The behavioural and neurotoxic effects of IDPN have been studied in rats and mice (Selye, 1957, Delay et al, 1952). The most prominent behavioural features of the syndrome induced by IDPN include excitation, choreiform head and neck movements and circling and this has led to the syndrome being called the

ECC-syndrome. Histological lesions occurring after the drug have been described in many regions of the central nervous system of IDPN-treated animals (Chou and Hartmann, 1964). Neurochemical and pharmacological studies have indicated a role for 5-HT and DA in IDPN's neurotoxic and behavioural effects (Langlai, Huang and Gabay, 1975, Gabay, Langlai, and Huang, 1974).

However, inspite of all these studies, the primary anatomical and neurochemical basis for the ECC-syndrome has not yet been clearly elucidated. The present work was designed to study the effects of IDPN in chicks with the aim of comparing such effects with those reported in rodents.

7.3. Results.

7.3.1. Syndromized chicks:

After five days of repeated administration of IDPN (75 mg base/kg) to chicks, the ECC-syndrome

appeared on the sixth day. The hyperactive behaviour was characterized by continuous loud chirping, jerky and involuntary movements of the head and neck, and circling. The chicks turned their heads, from side to side and also up and down (fig. 7.1.). In some chicks the neck and head were usually curved under the trunk of the body and this posture was usually maintained for prolonged periods (fig. 7.2) while in others the neck and head were usually thrusted backwards with the beak pointing upwards while the chicks performed backward movements (fig. 7.3). Circling behaviour was both clockwise and anticlockwise. During feeding, the beak was usually aimlessly thrusted outwards to pick food particles, and when drinking water, the neck and head were often curved backwards to facilitate swallowing. A high mortality rate was recorded when the chicks were injected with high doses (200 - 300 mg/kg) of IDPN.

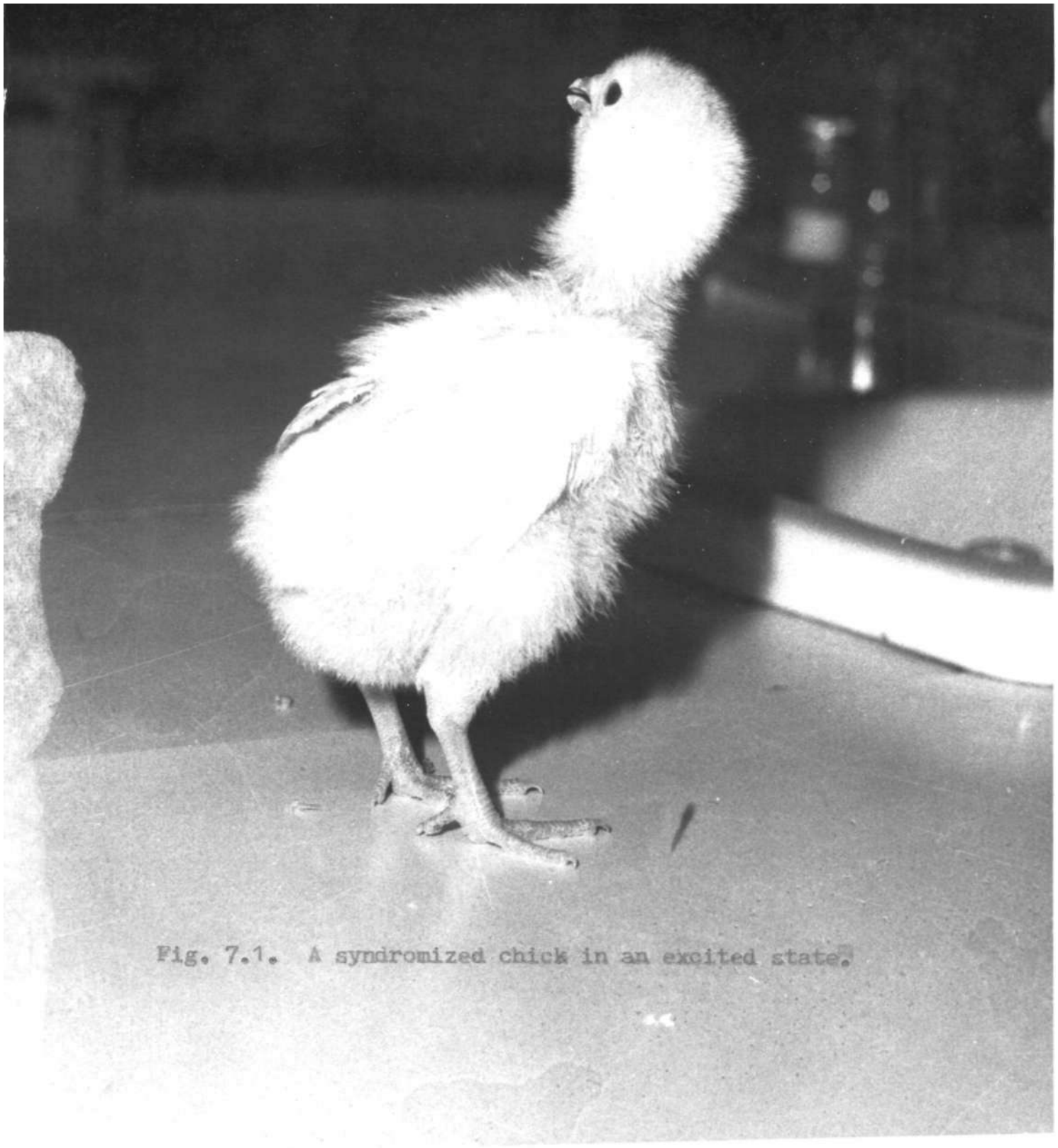


Fig. 7.1. A syndromized chick in an excited state.



Fig. 7.2. A syndromized chick with its head and neck curved under the trunk of the body. The chick usually remained in this posture for prolonged periods and also performed backward locomotion when in this posture.

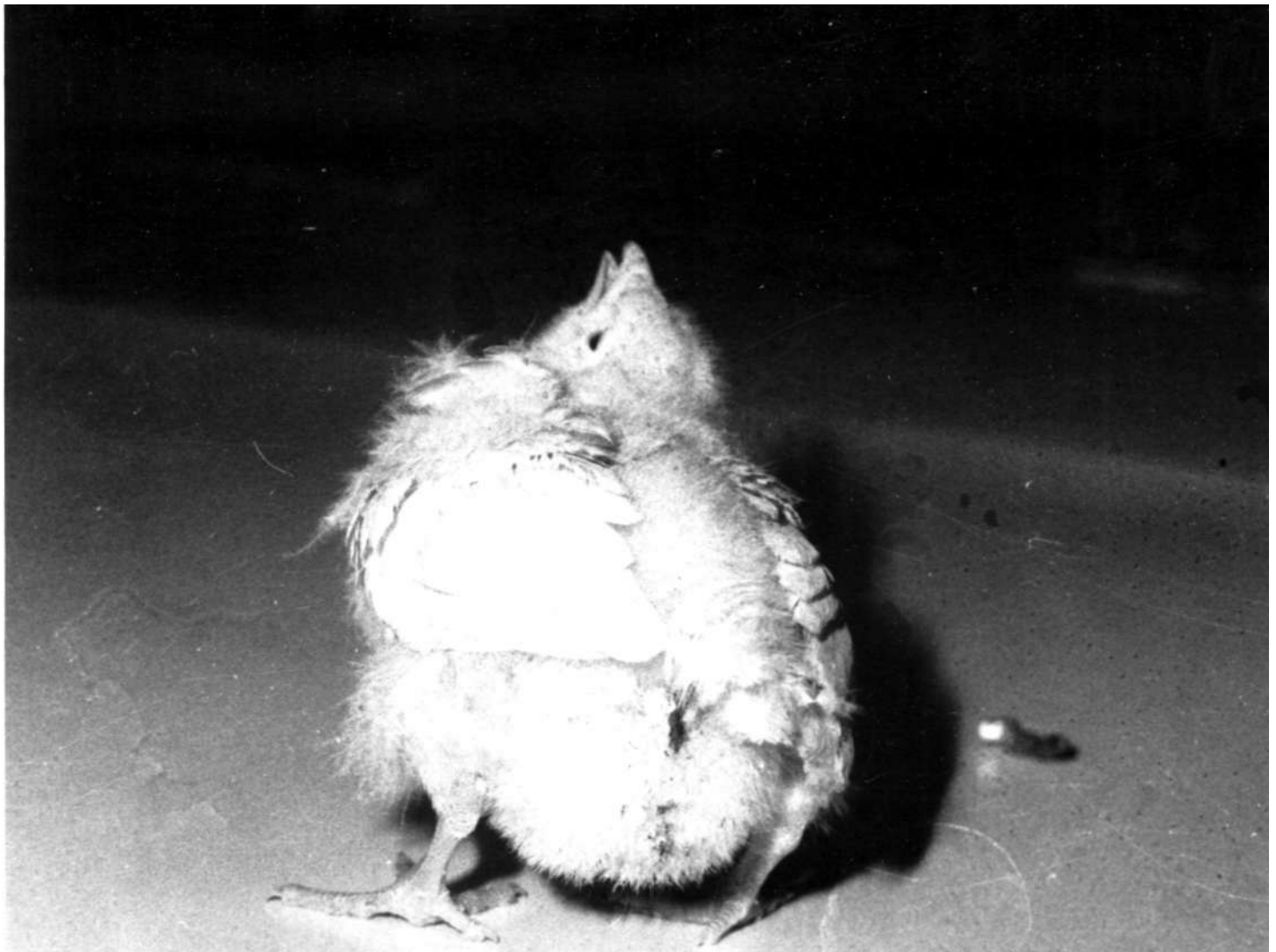


Fig. 7.3. A syndromized chick with its beak pointing upwards. The chick usually performed backward locomotion when in this posture.

During IDPN administration no loss in body weight was observed, instead the chicks gained weight. The chicks however, lost weight after the appearance of the ECC-syndrome probaly due to the increase in activity and change in eating habit. The chicks usually regained their weight after the syndrome had stabilized.

The intensity of most of the neurotoxic effects mentioned above usually decreased with time. Infact, the chicks usually appeared behaviourally normal after a period of three to four months.

7.3.2. Drug Studies.

(a) Amphetamine (2.5 & 5 mg/kg).

Amphetamine (5 mg/kg) potentiated both the circling and the choreiform head and neck movements in the chicks, as has been reported in rats (Gabay et al, 1974). The chicks spread their wings and chirped aloud while circling (fig. 7.4, table 7.1).

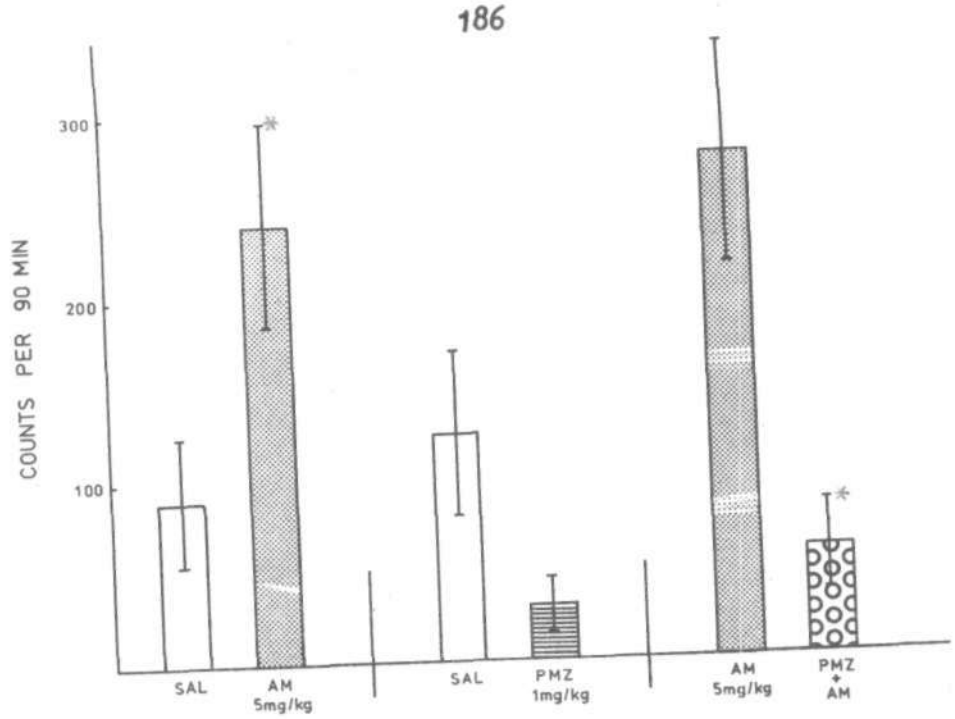


Fig. 7.4. Effect of amphetamine (AM) and its interaction with pimozide (PMZ) on IDPN-induced circling behaviour in chicks. The ECC-syndrome was allowed to stabilize for 1 week before drug studies were carried out. Chicks were pretreated with pimozide 1 hr. prior to administration of amphetamine. Vertical bars represent standard deviations. *p $\lesssim$ 0.001, t - test, (n = 10 per histogram).

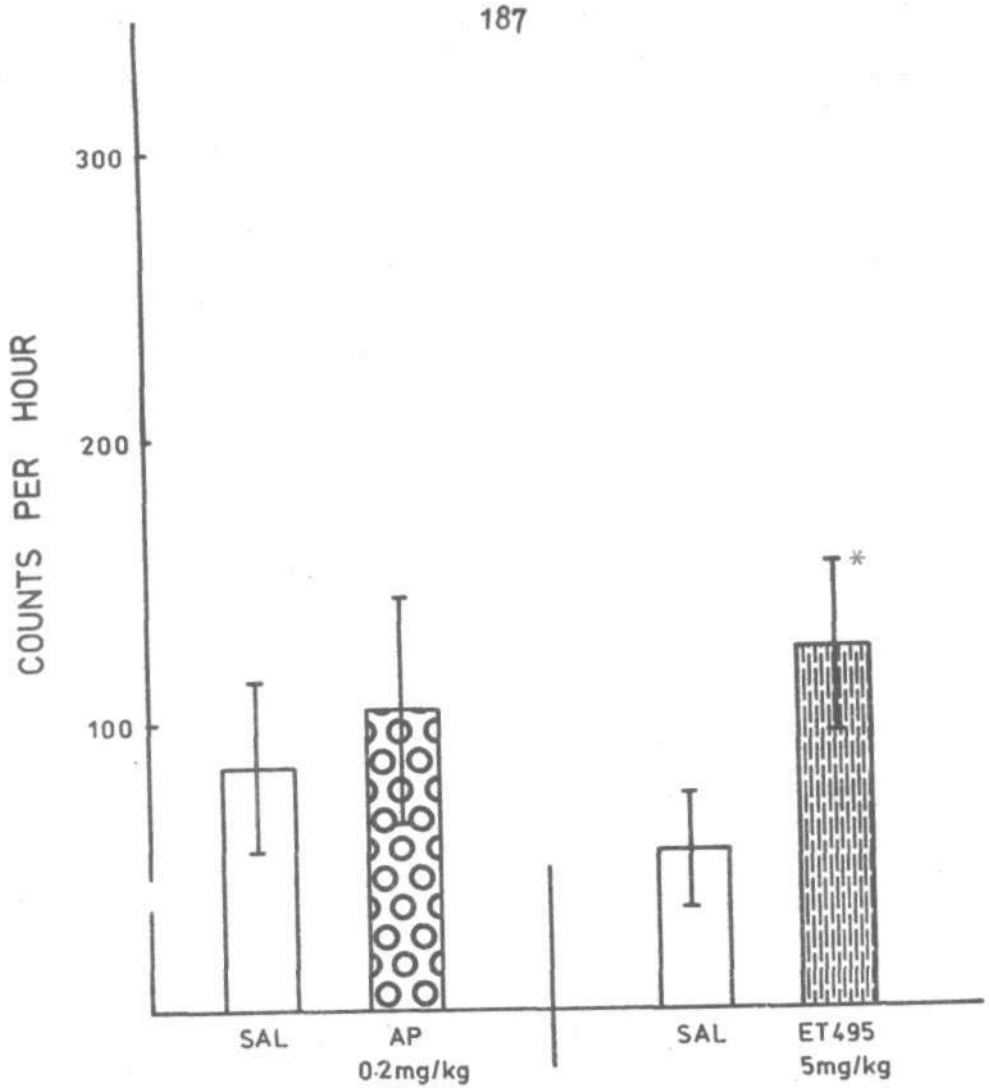


Fig. 7.5. Effect of apomorphine (AP) and ET 495 on IDPN-induced circling behaviour in chicks. The ECC-syndrome was allowed to stabilize 1 week before drug studies were carried out. Vertical bars represent standard deviations. * $p < 0.05$, t - test. n = 10 per histogram.

(b) Apomorphine (0.1 & 0.2 mg/kg).

Apomorphine (0.1 & 0.2 mg/kg) did not produce any marked effect on the circling behaviour. The choreiform head and neck movements were, however, potentiated by apomorphine (fig. 7.5, table 7.1). The effect of apomorphine was antagonized when the chicks were pretreated 1 h. previously with pimozide (1 mg/kg).

(c) Piribedil (2.5 & 5 mg/kg).

Piribedil (5 mg/kg) potentiated the circling and choreiform head and neck movements (fig. 7.5, table 7.1).

(d) Pimozide (1 mg/kg).

Pimozide (1 mg/kg) when administered alone, inhibited the ECC-syndrome. Pimozide (1 mg/kg 1 h. previously) also antagonized amphetamine-induced potentiation of the ECC-syndrome (fig. 7.4, table 7.1).

(e) Atropine (2.5 & 5 mg/kg).

Atropine (2.5 mg/kg) when administered alone, potentiated the ECC-syndrome, while a higher dose (5 mg/kg) produced antagonistic

Drugs	Dose(mg/kg)	Mean counts + s.d.
Saline		18.14 ± 3.67
Amphetamine	5	64.33 ± 7.1+
Pimozide	1	6.12 ± 1.02+
Pimozide	1	
Amphetamine	5	14.31 ± 2.73*
Apomorphine	0.2	73.76 ±12.15+
Pimozide	1	
Apomorphine	0.2	24.05 ± 4.01*
Piribedil	5	56.36 ± 6.21+
Pimozide	1	
Piribedil	5	16.81 ± 3.37*
Atropine	2.5	45.01 ± 5.62+
Atropine	2.5	
Amphetamine	5	136.14 ± 10.03*
Hyoscine	5	30.16 ± 5.19+
Hyoscine	5	
Amphetamine	5	115.92 ± 16.34*
Quipazine	20	55.83 ± 6.57+
Pimozide	1	
Quipazine	20	19.75 ± 3.11*

Table 7.1. Effect of various drugs on IDPN-induced choreiform head and neck movements in chicks. The ECC-syndrome was allowed to stabilize for 1 week before drug studies were carried out. Observation was carried out for 90 min in each case.

+ Statistical analysis was performed using saline result.

* Statistical analysis was performed using respective single drug controls.

In this study, all the drug treatments showed significant differences when compared to the respective controls; $p < 0.001$ t-test, N = 7 per drug treatment.

effects. Atropine also potentiated the effects of amphetamine on the ECC-syndrome (fig. 7.6)

(f) Hyoscine (2.5 & 5 mg/kg).

Hyoscine (2.5 & 5 mg/kg) potentiated the ECC-syndrome. These doses of hyoscine also potentiated the effects of amphetamine on the ECC-syndrome (fig. 7.7, table 7.1).

(g) Phystostigmine (0.5 mg/kg).

Physostigmine (0.5 mg/kg) inhibited both the circling and choreiform head and neck movements. Physostigmine (0.5 mg/kg) when administered 15 min previously, antagonized amphetamine-induced potentiation of the ECC-syndrome (fig. 7.8).

(h) Quipazine (5 - 20 mg/kg)

Quipazine (5 mg/kg) inhibited the circling and choreiform head and neck movements in IDPN - treated chicks. The chicks usually remained at one corner of the cage and chirped

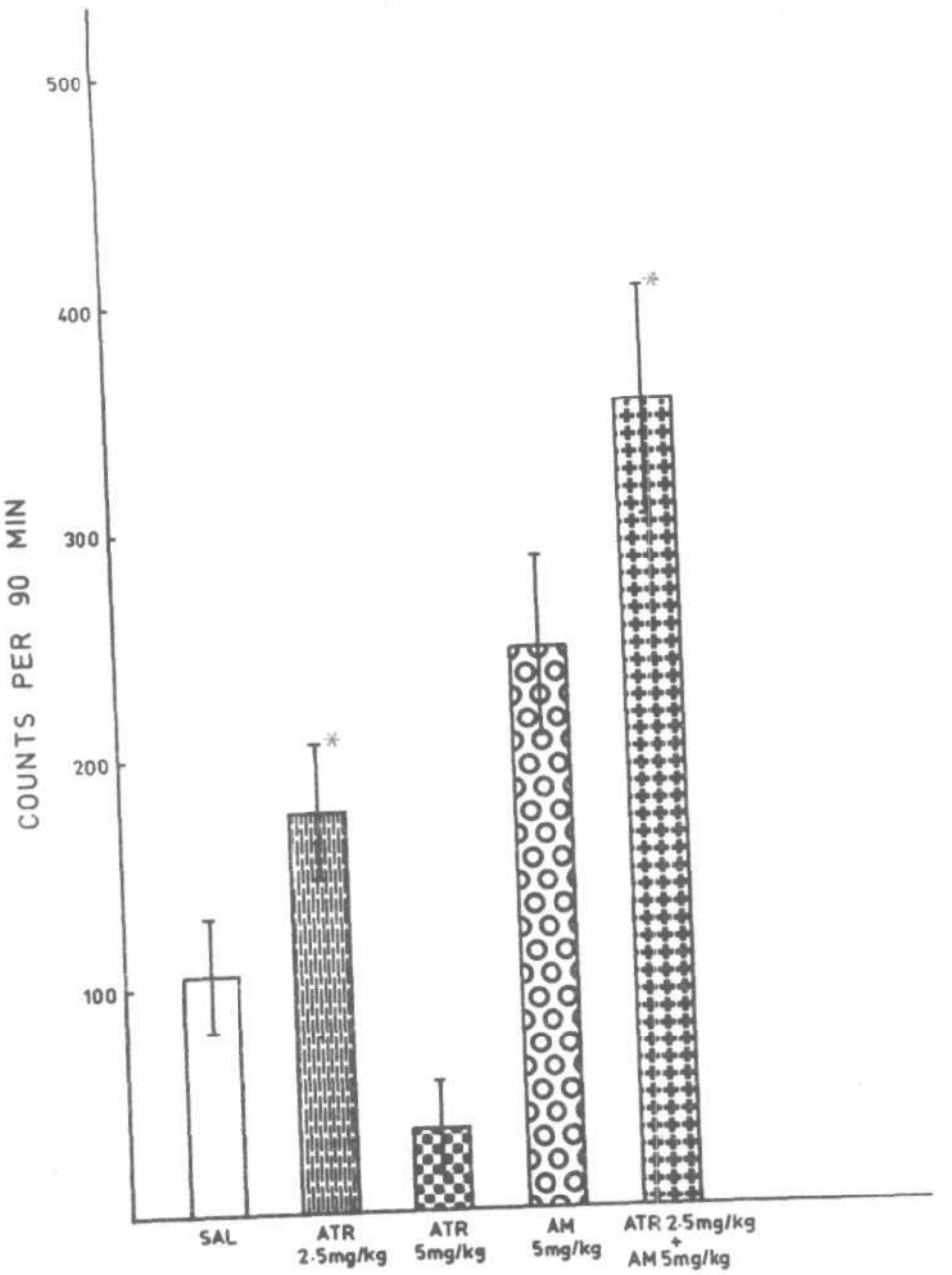


Fig. 7.6. Effect of atropine (ATR) and its interaction with amphetamine (AM) on IDPN-induced circling behaviour in chicks. The ECC-syndrome was allowed to stabilize for 1 week before drug studies were carried out. Chicks were pretreated with atropine 15 min. prior to administration of amphetamine. Vertical bars represent standard deviations. *$p < 0.05$, **$p < 0.01$, t - test, (n = 10 per histogram).

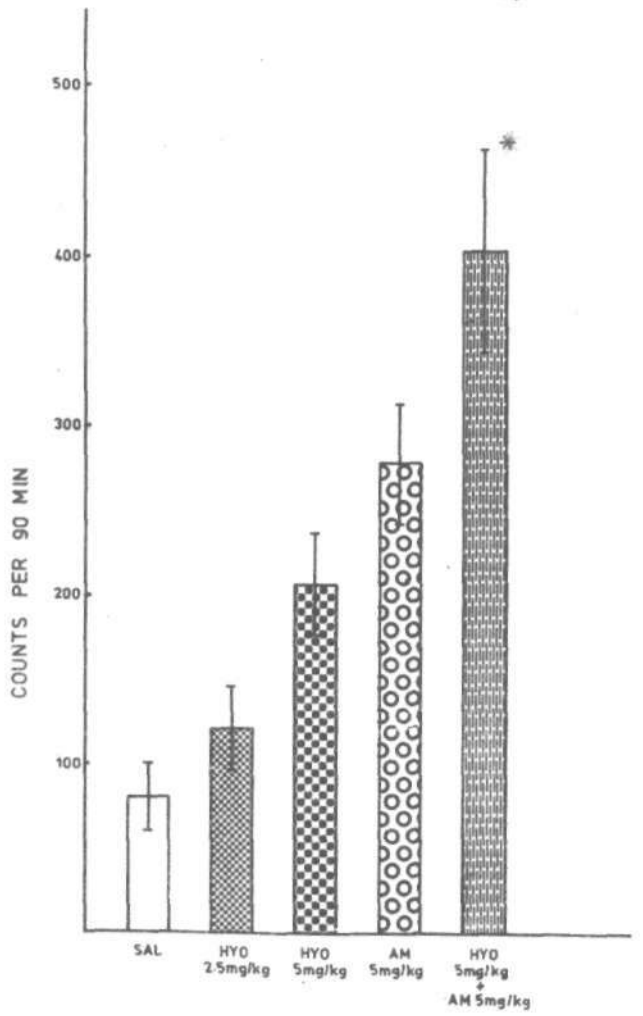


Fig. 7.7. Effect of hyosine (HYO) and its interaction with amphetamine (AM) on IDPN-induced circling behaviour in chicks. The ECC-syndrome was allowed to stabilize for 1 week before drug studies were carried out. Chicks were pretreated with hyosine 15 min. prior to administration of amphetamine. Vertical bars indicate standard deviations. * $p < .01$ (n = 10 per histogram).

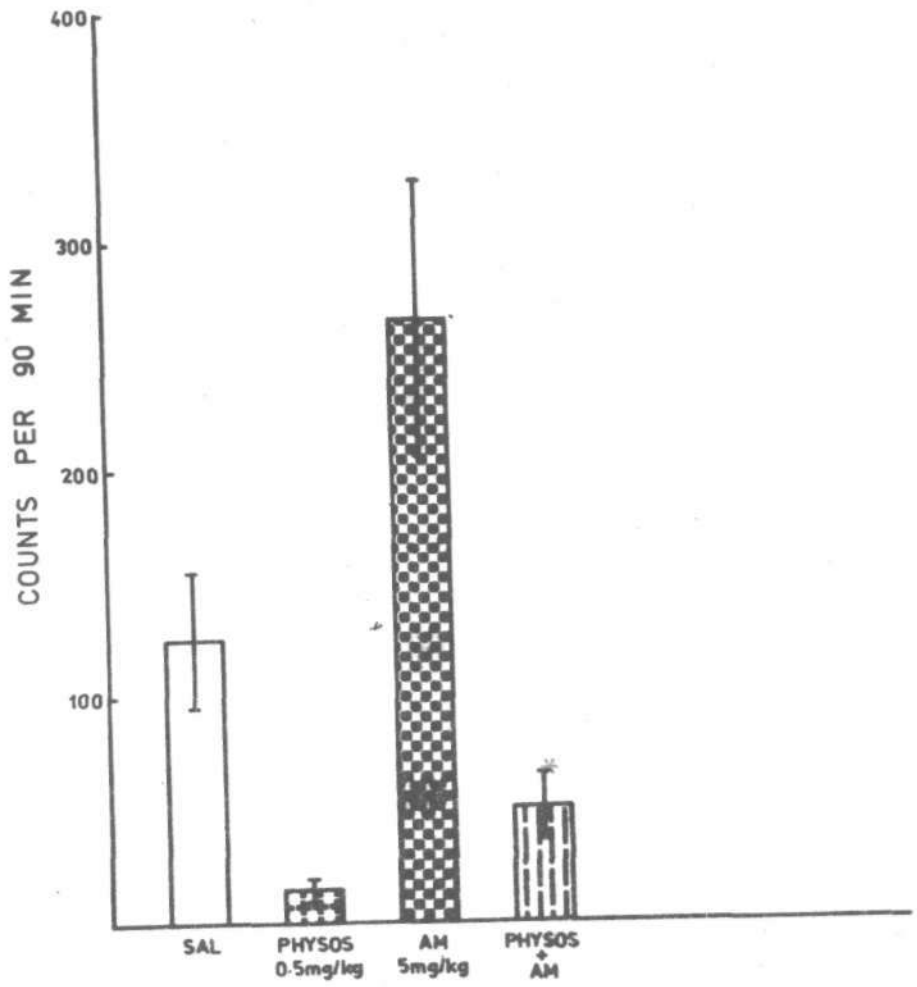


Fig. 7.8. Effect of physostigmine (PHYSOS) and its interaction with amphetamine (AM) on IDPN-induced circling behaviour in chicks. The ECC-syndrome was allowed to stabilize for 1 week before drug studies were carried out. Chicks were pretreated with physostigmine 15 min. prior to administration of amphetamine. Vertical bars indicate standard deviations. *$p < 0.001$ (n = 10 per histogram).

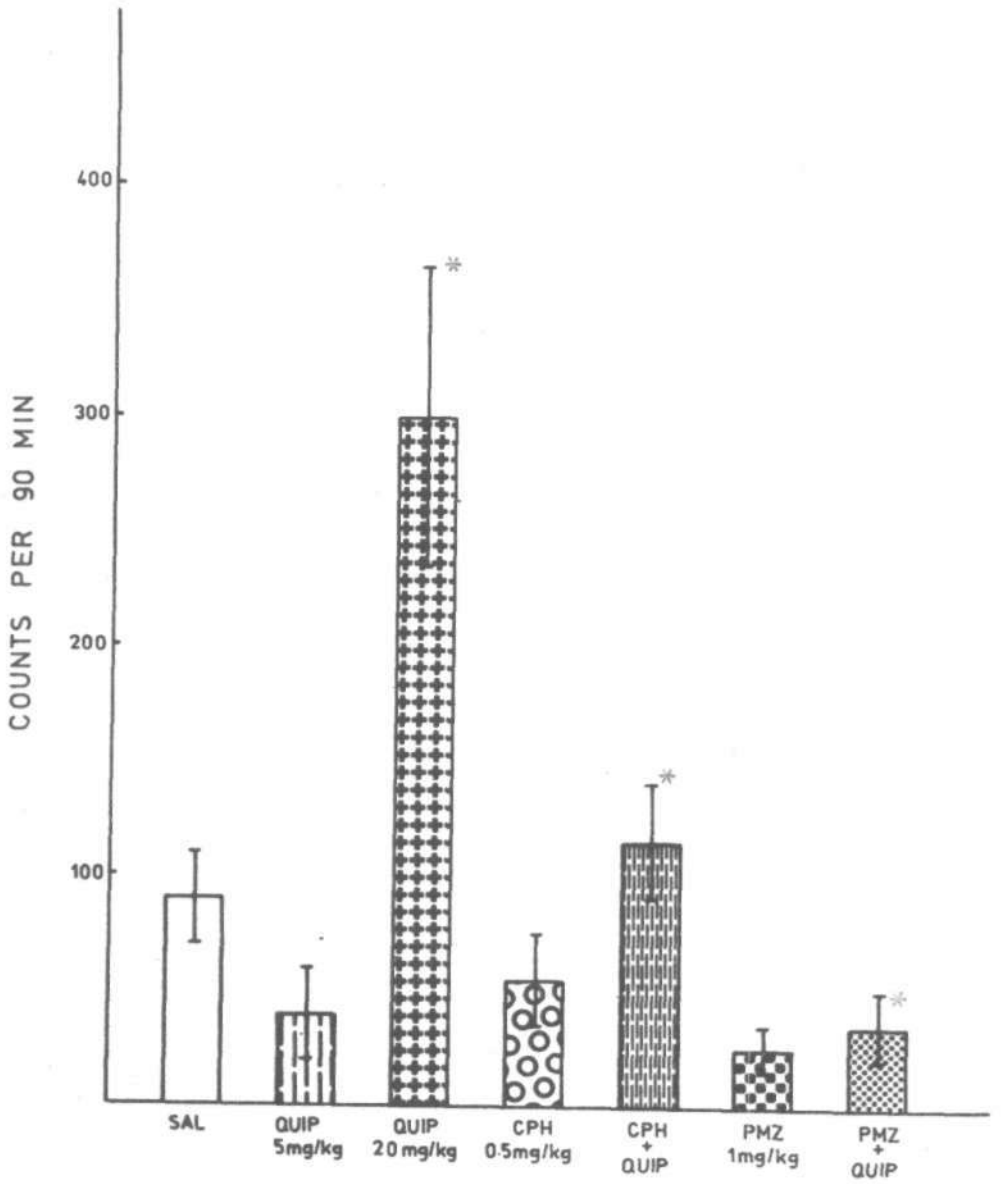


Fig. 7.9. Effect of quipazine and its interaction with cyproheptadine (CPH) and pimozide (PMZ) on IDPN-induced circling behaviour in chicks. Chicks were pretreated with cyproheptadine and pimozide 1 hr. prior to administration of quipazine. Vertical bars represent standard deviations. * $p < 0.001$, t - test, (n = 10 per histogram).

intermittently. With higher doses (15 - 20 mg/kg) the chicks became more active and chirped more frequently with wings spread out. The intensity of the ECC-syndrome was enhanced with these higher doses of quipazine. The potentiation of the ECC-syndrome by quipazine was inhibited when the chicks were pretreated with cyproheptadine (0.5 mg/kg) and pimozide (1 mg/kg). The inhibitory effect of low doses of quipazine was not altered by pimozide, while cyproheptadine slightly antagonized this effect. Administration of cyproheptadine (0.5 mg/kg) alone, slightly inhibited the ECC-syndrome in chicks. (fig. 7.9, table 7.1).

7.3.3. Fluorimetric determination of NA, DA, and 5-HT in IDPN-treated chicks.

The biogenic amine levels in the hyperstriatum, optic tectum, cerebellum and pons-medulla were determined in control and IDPN-treated chicks when the ECC-syndrome appeared (table 7.2.).

(a) Hyperstriatum.

There was a significant reduction ($P < 0.001$) in the levels of DA and 5-HT.

(b) Optic tectum.

NA and DA levels significantly decreased ($P < 0.001$) while 5-HT level slightly increased.

(c) Cerebellum.

There was a significant reduction ($P < 0.001$) in the levels of the three biogenic amines.

(d) Pons-medulla.

The level of NA and 5-HT significantly decreased while a slight (but significant, $P < 0.005$) reduction was observed in the level of DA.

7.4. Discussion:

The observations made in this study show that IDPN induces similar behavioural changes in chicks as has been reported in rats (Gabay et al, 1974). This probably suggests that the same mechanisms are involved in IDPN-induced behavioural effects in both rats and chicks. However, contrary to the observation in rats, the chicks showed behavioural recovery after a

period of time. The basis for this recovery is not obvious from this study. It has been observed that pretreatment with thyroxine inhibits the appearance of the ECC-syndrome in rats (Gabay, personal communication). In the light of this observation, it is probable that IDPN interferes with the synthesis of thyroxine to produce the ECC-syndrome. The permanent character of the syndrome in rats could be due to an irreversible suppression of thyroxine synthesis, while in chicks, synthesis of thyroxine is probably restored leading to a subsequent recovery when administration of IDPN is stopped.

Gabay et al , (1974) observed potentiation of the ECC-syndrome in rats by d-amphetamine. The observation made in the present study shows that amphetamine similarly potentiates IDPN effects in chicks. Amphetamine is believed to act indirectly by releasing catecholamines and inhibiting their reuptake (Weissman et al, 1966;

Glowinski and Axelrod, 1966). The major catecholamine that has been implicated in the behavioural effects of amphetamine is dopamine. The observed potentiation of the ECC syndrome by amphetamine is therefore probably due to increase in the brain level of dopamine at the receptor sites. The fact that pimozide (a specific dopamine receptor antagonist) inhibited the amphetamine effect further supports this suggestion.

The inability of the directly acting dopamine agonist (apomorphine) to significantly potentiate IDPN-induced circling while potentiating the choreiform head movements is rather paradoxical. It is probable that circling and choreiform head movements are mediated via different dopamine mechanisms. Cools and Van Rossum (1976) postulated the existence of two types of dopamine receptors i.e. excitation - mediating and inhibition mediating dopamine receptors. According to

workers, amphetamine is an agonist of both receptor types while apomorphine acts on one type i.e. excitation mediating type, with little or no action on the other. It is therefore probable that apomorphine failed to significantly potentiate the circling movement because the receptors mediating this response are not sensitive to its actions.

The potentiation of the ECC-syndrome by atropine and hyoscine which are anticholinergic drugs suggest that cholinergic mechanisms exert an inhibitory influence on this syndrome. This is further supported by the observed antagonism of the syndrome after the administration of physostigmine. Thus the observed potentiation of the amphetamine effects by atropine and hyoscine shown in this study is probably due to blockade of central cholinergic sites by these drugs. Anticholinergic drugs have been shown to similarly potentiate

amphetamine and apomorphine-induced stereotyped behaviour in experimental animals and this has led to the conclusion that cholinergic system exert a modulatory influence on stereptyped behaviour (Arnfred and Randrup, 1968; Osuide and Adejoh, 1973).

Several studies have shown that quipazine directly stimulates 5-HT receptors, (Hong et al, 1969; Costall and Naylor, 1975) and also inhibits neuronal reuptake of 5-HT (Median, Leeling and Phillips, 1973). In this study, quipazine produced a dose-dependent effect on the ECC-syndrome. The observed inhibition of the ECC-syndrome by quipazine (5 mg/kg) probably indicates that activation of serotonergic mechanisms exert an inhibitory influence on IDPN-induced effects. The potentiation of the ECC-syndrome by a higher dose (20 mg/kg) of quipazine and the antagonism of this effect by pimozide suggests an involvement of dopaminergic mechanisms in this action of quipazine. The observation is similar to that reported by Langlai and Gabay (1977) in rats. Grabowska et al (1974a) have reported evidence in support of the involvement of dopaminergic

mechanisms in the behavioural actions of quipazine. However, the antagonism of quipazine-induced potentiation of the ECC-syndrome by cyproheptadine also probably suggests that quipazine is producing this effect indirectly, via serotonergic mechanisms, as previously suggested by Langlai and Gabay (1977). These observations further suggest that low doses of quipazine act predominantly on serotonergic receptors while higher doses may influence both serotonergic and dopaminergic mechanisms in chicks.

The data obtained from the fluorimetric determinations of NA, DA and 5-HT, show that IDPN administration influences the levels of these brain biogenic amines. The results show significant reductions in the levels of NA, DA and 5-HT in the brain regions studied. Such reductions in transmitter level have been shown to induce supersensitivity in postsynaptic receptors (Tarsy and Baldessarini, 1973; Costentin et al, 1975). Thus, the observed

potentiation of the ECC-syndrome by DA agonists may be due to the influence of these drugs on supersensitive DA receptors. Though NA was not markedly altered in the striatum highly significant reductions in DA and 5-HT levels were observed, and this probably reflects the extent of involvement of these two biogenic amines in IDPN effects. A functional relationship between serotonergic and dopaminergic neurons has been used to explain various behavioural responses. Antagonism between serotonin and DA has been suggested in the striatum on the basis of the finding that amphetamine stereotypy is inhibited by tryptophan and enhanced by methysergide (Weiner et al, 1973). Based on this suggestion, a reduction in the level of serotonin in the hyperstriatum would lead to an enhancement of dopaminergic activity. Thus, the observed hyperactive behaviour in IDPN-treated chicks could probably be partly due to the reduced inhibitory influence of serotonergic system on DA mechanisms in the hyperstriatum. Apart from the hyperstriatum, the observed alterations in the levels of these biogenic

amines in the other brain areas studied, may probably contribute to the overall picture of the ECC-syndrome in chicks. Langlai et al, (1975), in a similar study in rats, observed significant reductions in 5-HT and 5-HIAA levels, one week after the appearance of the ECC-syndrome, and these workers suggested that serotonergic systems may be involved in the toxic actions of IDPN. However, these workers did not observe any significant alterations in DA level and this probably suggests that IDPN exerts differential effects, on dopaminergic mechanisms in rats and chicks.

The observations made in the present study show that IDPN, induces behavioural effects in chicks similar to those reported in rats. However, the basis for the recovery of the chicks from the effects of IDPN is not apparent from this study, and this requires further elucidation. The data obtained in this study indicate a role for both DA and 5-HT in IDPN-induced effects while an inhibitory role is indicated for the

cholinergic system. Further neuro-chemical studies may throw more light on the possible involvement of these neurotransmitters in the behavioural effects of IDPN in chicks.

CHAPTER VIII

8. PHOTIC-EVOKED RESPONSE RECORDING AND THE INFLUENCE OF VARIOUS DRUGS IN CONSCIOUS YOUNG CHICKS.

8.1. Summary.

8.1.1 Evoked responses to photic stimulation recorded under artificial light and dark adaptation from three different brain areas in conscious young chicks were compared.

8.1.2 The magnitude of the evoked responses varied from one experiment to another, but for a particular chick consistent responses were obtained under the same behavioural state, stimulus intensity, and recording conditions.

8.1.3 The responses recorded under dark adaptation were larger in magnitude and more reproducible when compared with those recorded under artificial light.

8.1.4. The photic-evoked responses under dark adaptation were very sensitive to drug effects.

8.1.5. These observations suggest that photic-evoked response could probably be utilized in elucidating actions of psychoactive drugs.

8.2. Introduction.

The young chick demonstrates adult-like electroencephalographic characteristics (Key and Marley, 1962). It also responds to environmental stimuli and to most neuropharmacological agents in a manner comparable to adult mammalian species (Spooner and Winters, 1966). The blood-brain barrier is under-developed in the young chick during the first four weeks of life (Waelsch, 1955; Key and Marley 1962) and this species is used to advantage to study the effects of systemically administered compounds which do not cross the blood-brain barrier in the adult. These observations make the young chick suitable for

neuropharmacological studies.

Various techniques have been used to record evoked responses to sensory stimulation in chicks (Scholes and Roberts, 1964; Scholes and Gutnick, 1970; Spooner and Winters, 1967; Kramer, Sherman and Seifter, 1967). Some of these workers used curarized or anaesthetized chicks, and under such conditions it would not be possible to appreciate the behavioural correlates of the evoked responses. Furthermore, the anaesthetic conditions, the necessity for artificial respiration and the central effects of curare in the young chick (Scholes, 1965) place limitations on the interpretations of results obtained under these conditions. Several studies have reported the effects of various centrally-acting drugs on sensory evoked responses from different brain regions of various species. Such drugs include anaesthetics (Pradhan and Galambos, 1963) gamma aminobutyric acid and gamma hydroxybutarate

Scholes and Roberts, 1964; Kramer et al, 1967; Winters and Spooner, 1965), hallucinogens (Purpura 1956a).

However, there has been a reluctance to use photic-evoked response in conscious unrestrained animal studies due to inconsistent results (Winters, 1968).

The first part of this study was designed to investigate the pattern and utility of photic-evoked response in conscious young chicks. The second part of this work was designed to study the influence of various centrally acting drugs on the photic-evoked response.

8.3. Materials and Methods.

8.3.1. Implantation of electrodes.

Chicks were used for this study. Each chick was anaesthetized with halothane and the head was held firmly in a stereotaxic apparatus. The scalp was exposed and the head was properly leveled using a spirit level. Co-ordinates were

taken using the bregma as the zero point. Electrodes made from the pointed ends of stainless steel insect pins (No. 00) were implanted onto the hyperstriatum (HS) and optic tectum (OT) using surface markings and secured chronically to the skull with dental cement according to the method of Spooner (1965) as modified by Osuide (1972). Bipolar electrodes made from stainless steel insect pins (No. 000) were implanted into the pontine reticular formation (RF) using the following coordinates: Anterior - posterior, -2 mm; lateral, + 1.5 mm; Vertical, 8.1 mm. Electromyograph (EMG) electrodes were made with stainless steel insect pins bent at the ends to make hooks. The electrodes were positioned in the proximity of the head and insertion of semispinalis capitis muscle of one side of dorsolateral aspect of the neck. A reference electrode similar to those used for the EMG was positioned in the comb of each chick. The

chicks were allowed 24 h. for recovery.

8.3.2. Recording procedure.

During recording the chick was placed in a screened cage in a quiet dark room. The electrodes from the chicks were connected to the terminals of a grass polygraph (model 79D) using amphenol connectors (Part No. 222 - 11 No. 7). The recordings from the hyperstriatum and optic tectum were performed using the comb as the reference. The polygraph was connected to an averaging computer (Nicolet model 1072) and the averaged evoked responses were displayed on a Tetronix Oscilloscope (model R5 103N), and recorded on an x - Y recorder (Hewlett Packard, model 7044A). The computer was triggered 100 ms prior to the flash by using a Grass stimulator (model 588) while the lamp of the photic stimulator (Grass P522A) was placed 50 cm. in front of the chick's cage. The chick usually settled down in the cage after a period of

5 - 25 min. The EEG and EMG were recorded before, during and after the time the chick was exposed to photic stimulation at a frequency of one flash in 2 sec. A series of 32 flashes were presented to the chick. Before drug administration photic-evoked responses were recorded approximately every 30 min in the quiet awake state. After drug administration photic-evoked responses were recorded at appropriate intervals to illustrate changes in the evoked responses with various drug-induced states. The evoked responses were added and averaged by the computer, displayed on the oscilloscope and written out with the recorder.

8.3.3. Electrode verification.

At the end of each experiment, the electrodes in reticular formation were located by a lesion made at the tip of the electrodes by passing a direct current of 0.5 mA using a Grass D.C. constant - current lesion maker

(model D.C. LM 5A). The chick was subsequently opened up and perfused with 10 ml of 1% w/v sodium chloride solution through the cardiac ventricle, followed by 20 ml of 1% potassium ferrocyanide in 10% formalin. The potassium ferrocyanide reacted with the iron deposit to leave a blue spot at the location of the electrode tip which was verified histologically.

8.4. Results.

8.4.1. Characteristics of the photic-evoked response.

Computer averaged electrical responses to flashes were obtained from all the recording sites studied, and these indicated a wide distribution of visual responses throughout the brain (fig. 8.1). In the hyperstriatum, the evoked responses were not visually distinguishable from the back-ground spontaneous activity. The latter was characterized by high amplitude waves of 8 - 13 c/s frequency. The averaged evoked responses consist of an initial small negative component

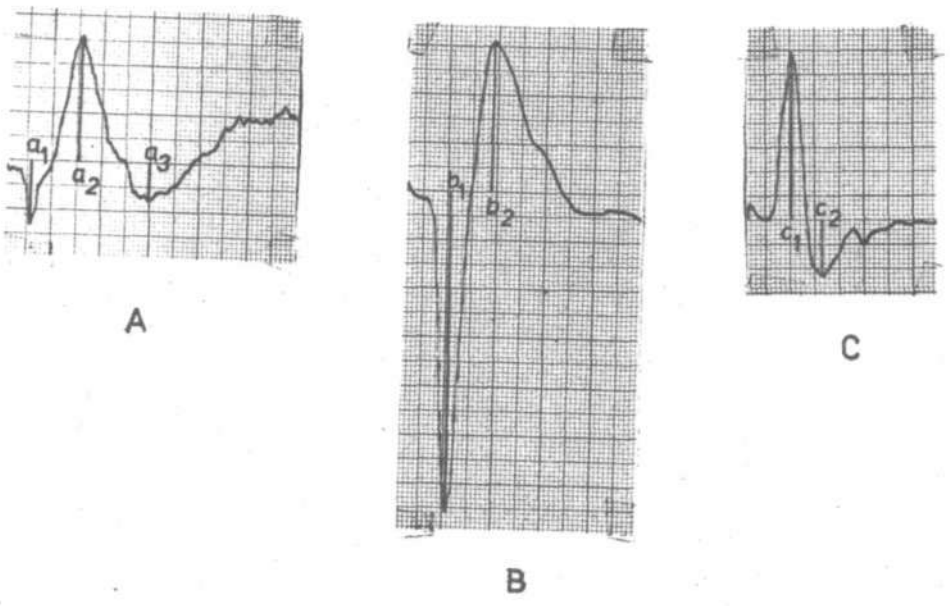


Fig. 8.1. Pattern of photic-evoked response in the young chick, showing the main component parts of each evoked response. The evoked responses recorded from the hyperstriatum, optic tectum, and reticular formation are shown as A,B and C respectively, with their primary components represented as a_1,a_2,a_3; b_1,b_2; and c_1,c_2.

TABLE Averaged Photic-Evoked Response Values Recorded Under Dark Adaptation

HYPERSTRIATUM (A_2 WAVE)				OPTIC TECTUM(OT) (B_1 WAVE)				PONTINE RETICULAR FORMATION(RF)(C_1WAVE)			
RECORDING 1	RECORDING 2	RECORDING 3	MEAN ± SE (uV)	RECORDING 1	RECORDING 2	RECORDING 3	MEAN ± SE (uV)	RECORDING 1	RECORDING 2	RECORDING 3	MEAN ± SE (uV)
39.3	33.7	40.2	37.73 ± 3.52	-344.3	-369.8	-369.7	-361.27 ± 14.69	64.6	68.2	70.0	67.60 ± 2.75
93.2	96.5	111.9	100.53 ± 9.98	-314.1	-316.2	-321.9	-317.40 ± 4.04	28.2	28.8	32.0	29.67 ± 2.04
40.37	56.25	45.54	47.39 ± 8.10	-239.50	-232.33	-229.2	-233.68 ± 5.28	33.47	31.19	32.48	32.38 ± 1.14
87.6	75.57	76.38	79.85 ± 6.72	-431.89	-426.32	-413.68	-423.96 ± 9.33	33.15	38.50	31.67	34.44 ± 3.59

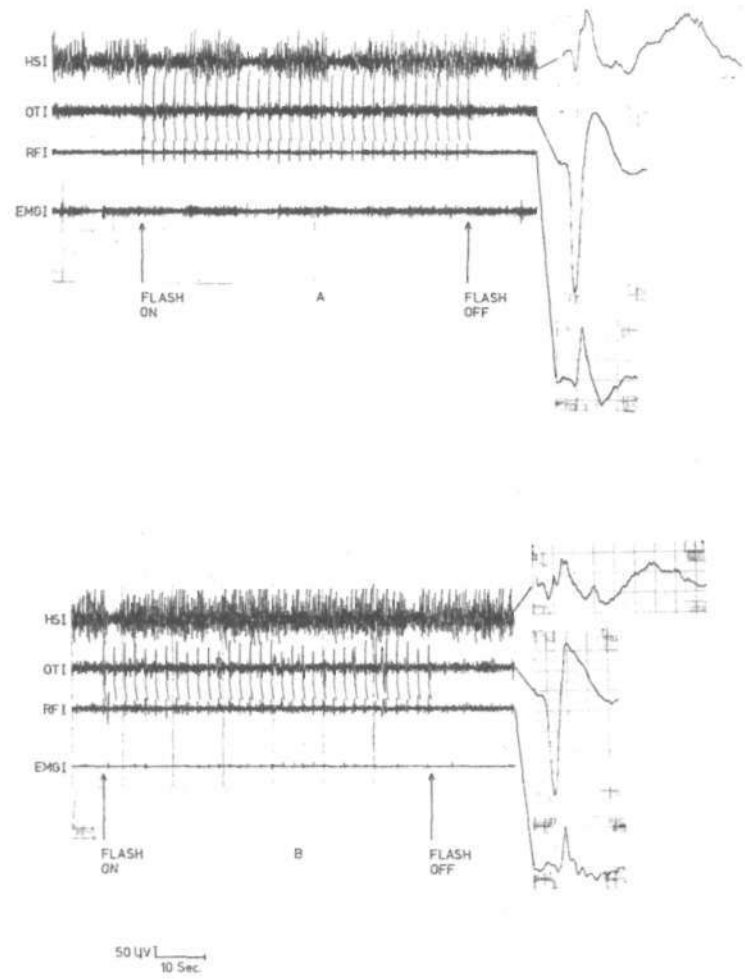


Fig. 8.2. EEG and photic-evoked response taken during the awake (A) and sleep (B) behavioural states in a 5-day old chick.

followed by a positive component of higher amplitude. In most of the animals, but not invariably, this positive wave is usually followed by a negative component. In the optic tectum, the EEG recordings show marked evoked responses to photic stimulation consisting of an initial high amplitude negative wave (229 - 431 mV) followed by a positive component of smaller amplitude (100 - 200 mV). The spontaneous EEG was characterized by low - voltage fast activity of 20 - 30 c/s frequency. In the pontine reticular formation, the evoked responses consisted of an initial positive wave (28 - 70 mV) followed by a negative component of smaller amplitude. The spontaneous EEG was characterized by low-voltage fast activity.

In all the brain areas studied, the amplitude of the evoked responses was greater and more consistent under dark adaptation than under artificial light. The amplitude of the

TABLE Averaged Photic-Evoked Response Values Recorded under Light Adaptation

HYPERSTRIATUM (A_2 WAVE)				OPTIC TECTUM (B_1 WAVE)				PONTINE RETICULAR FORMATION (C_1 WAVE)			
RECORDING 1	RECORDING 2	RECORDING 3	MEAN ± SE (uv)	RECORDING 1	RECORDING 2	RECORDING 3	MEAN ± SE (uv)	RECORDING 1	RECORDING 2	RECORDING 3	MEAN ± SE (uv)
14.14	30.30	20.87	21.77 ± 8.12	- 70.68	- 101.14	-130.33	-100.72± 29.83	30.23	18.10	26.51	24.95 ± 6.21
28.9	41.0	36.59	35.50 ± 6.12	-163.0	- 65.71	-150.98	-126.56± 53.04	20.14	11.45	8.36	13.32 ± 6.11
10.13	23.40	16.11	16.55 ± 6.65	-200.92	- 146.30	-100.28	-149.17± 50.38	23.06	13.23	17.0	17.76 ± 4,96
30.11	40.0	18,26	29.46 ± 10.88	-184.13	- 260.54	-173.0	-205.89± 47.65	17.66	9.34	11.21	12,74 ± 4,37

evoked responses varied with the behavioural state of the animals. The amplitude was larger when the chicks were awake and smaller when they appeared sedated (fig. 8.2). For a particular chick, the averaged evoked responses recorded under the same behavioural state, stimulus intensity, and recording conditions, were found to be consistent (tables 8.1a & b).

8.4.2. Drug studies.

a) Amphetamine: (1 - 5 mg/kg).

Amphetamine (1 mg/kg) produced a slight behavioural excitation, EEG desynchronization and a reduction in the amplitude of the evoked response . After 5 mg/kg there was a greater behavioural excitation and an increase in the amplitude of the averaged evoked response. Behavioural depression and a decrease in the amplitude of the evoked responses usually followed the excitatory phase (fig. 8.3, table 8.2).

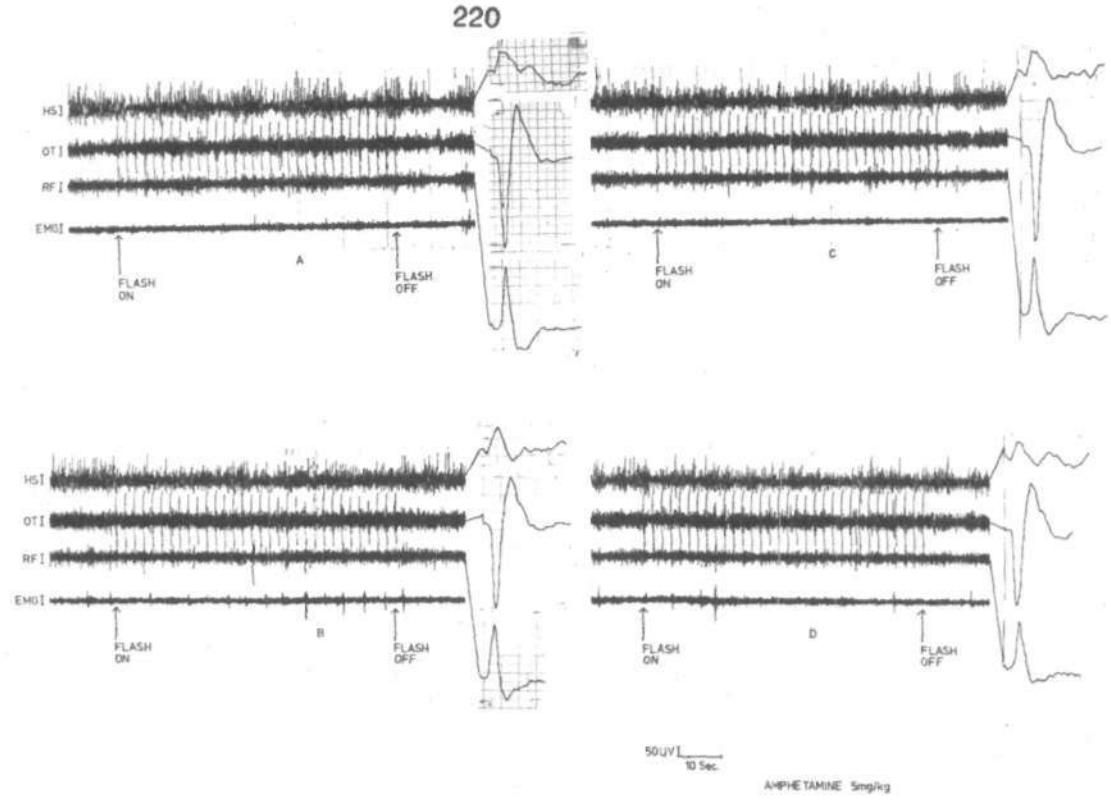


Fig. 8.3. Effect of amphetamine (5 mg/kg) on EEG, EMG and photic-evoked response in a 4-day old chick. A, is control, while B,C, and D are responses recorded at 15 min, 30 min and 1 hr. respectively, after drug administration.

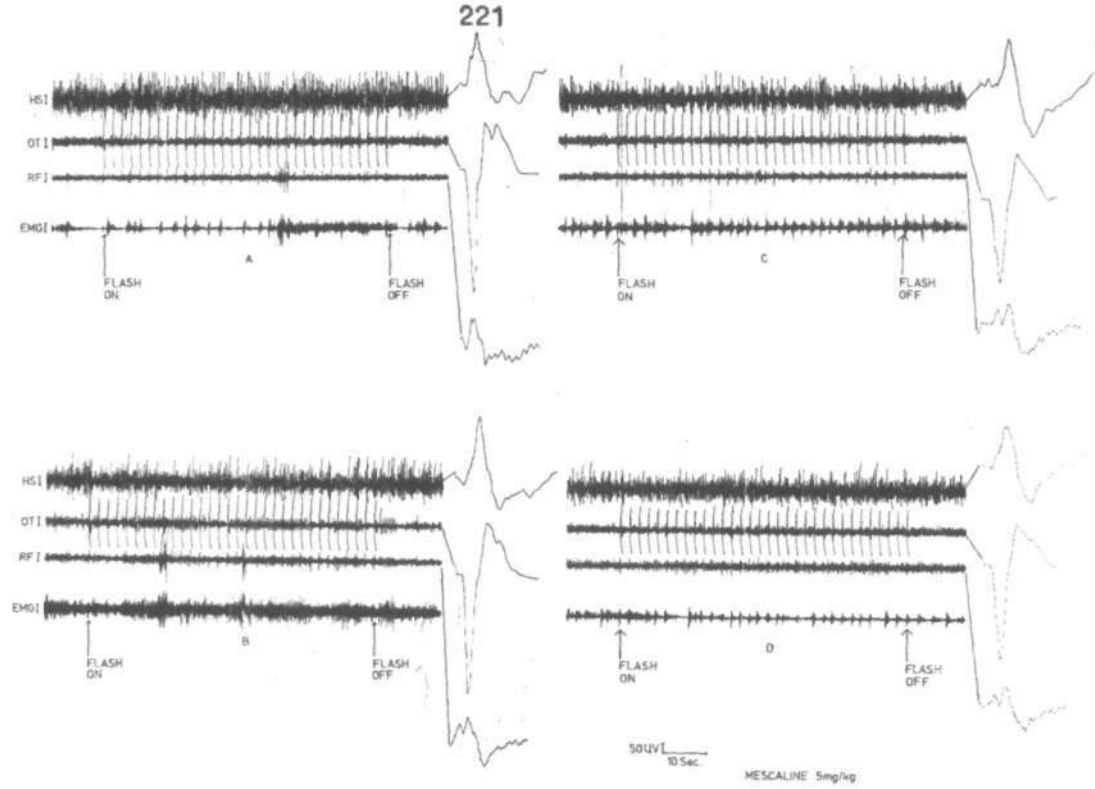


Fig. 8.4. Effect of mescaline (5 mg/kg) on EEG, EMG and PER in a 4-day old chick. A, is control, while B,C and D are responses recorded at 15 min. 1 hr. and 2 hr. respectively after drug administration.

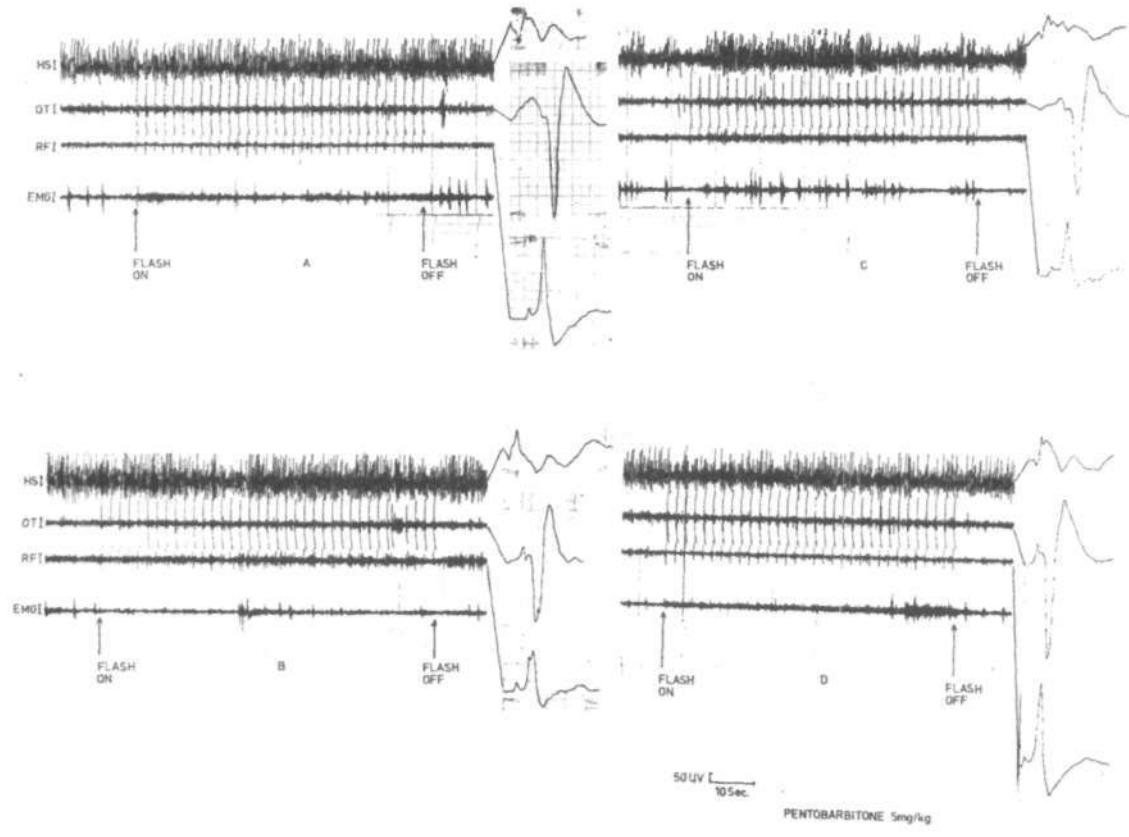


Fig. 8.5a. Effect of pentobarbitone (5 mg/kg) on EEG and photic-evoked response in a 7-day old chick. (A), control recording, while the chick was quiet with eyes closed; (B), 5 min. after pentobarbitone; (C), 2½ hr. after pentobarbitone when the EEG and behaviour looked normal; and (D), 5 hr. after pentobarbitone when the photic-evoked response, behaviour, and EEG had fully recovered from the pentobarbitone effects.

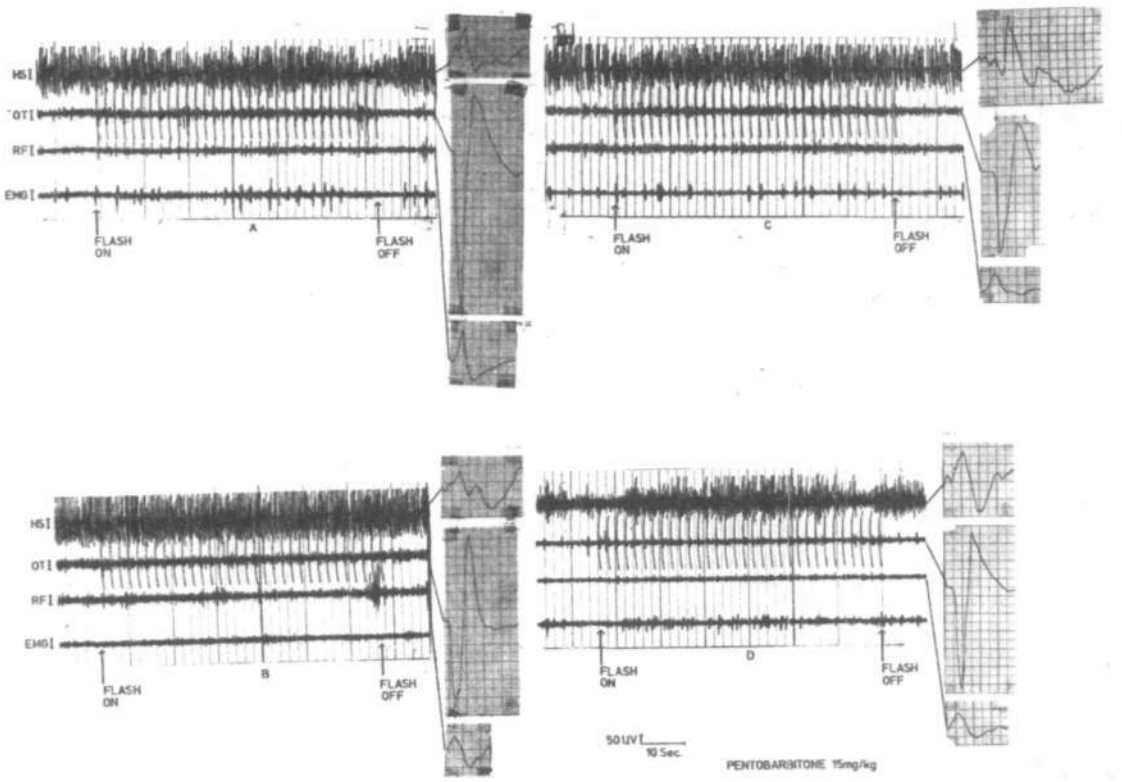


Fig. 8.5b. Effect of pentobarbitone (15 mg/kg) on EEG, EMG and PER in a 7-day old chick. A, is control, while B,C and D are responses recorded at 5 min, 1 hr and 5 hr. respectively, after drug administration.

b) Mescaline (1 and 5 mg/kg).

Mescaline (1 mg/kg) produced behavioural sedation and slightly reduced the amplitude of the evoked responses. In the hyperstriatum, the initial decrease in amplitude of the evoked response was followed by an increase. After 5 mg/kg, there was behavioural arousal and a slight decrease in the amplitude of the evoked responses from the optic tectum while an increase was observed in the hyperstriatum and reticular formation (fig. 8.4, table 8.2).

c) Pentobarbitone (5 - 15 mg/kg).

Pentobarbitone (5 mg/kg) produced a dose-dependent behavioural depression in chicks. The EEG was syndronized while the amplitude of the evoked response was reduced in the optic tectum and reticular formation. In the hyperstriatum, the initial decrease in amplitude was followed by an increase. The effect of pentobarbitone on the evoked response was still

TABLE Effect of some drugs on PER in the Hyperstriatum (a_2), Optic Tectum (b_1), and Reticular Formation (c_1) of the Young Chick

	MEAN ± SE CONTROL RESPONSE (uv)	TIME				
		5 MIN	30 MIN	1 HR	2 HR	3 HR
Amphetamine 5 mg/kg i.p. HS (a_2)	116.67 ± 6.59	149.41	157.10	82.47		
OT (b_1)	-145.01 ± 10.44	-160.46	-210.9	-87.99		
RF (c_1)	39.05 ± 5.13	30.66	52. 3	22.58		
Mescaline 5 mg/kg i.p. HS(a_2)	100.37 ± 4.10	130.90	131.90	101.60	73.7	70.4
OT (b_1)	-317.40 ± 4.04	-314.30	-266.30	-217.3	-236.20	-253.0
RF (c_1)	29.67 ± 2.04	36. 0	41.0	32.4	35.8	29.9
GHB 1g/kg						
HS (a_2)	48.01 ± 0.09	50. 43	258.86	214.71	198.95	54.13
OT (b_1)	-205.21 ± 6.13	-186.72	-160.14	-141.30	-129.23	-152.93
RF (c_1)	58,62 ± 2.53	38.31	35.56	29.23	20.46	42.04
GABA 2 g/kg						
HS (a_2)	86.43 ± 3.61	154.46	12.01	63.03	96.38	189.71
OT (b_1)	-261.38 ± 4.0	-242.31	- 10.23	-15.64	-101.83	-200.55
RF (c_1)	49.82 ± 1.24	68.14	2.50	3.85	5.61	31.46

obvious even when the drug-induced behavioural depression and EEG synchronization had returned to normal (figs. 8.5a & b).

d) Lysergic acid diethylamide (LSD, 10 & 20 ug/mg).

LSD (10 & 20 ug/kg) produced initial behavioural excitation characterized by loud chirping and violent locomotor movements. There was an increase in the amplitude of the averaged evoked response from all the recording sites. This excitatory phase was subsequently followed by behavioural depression and a decrease in the amplitude of the averaged evoked response (fig. 8.6).

e) Gamma hydroxybutyrate (GHB, 0.5 - 1 g/kg).

GHB produced behavioural depression with an accompanying EEG synchrony. There was a progressive increase in the amplitude of the positive and negative components of the averaged photic evoked response in the hyperstriatum, while a decrease in amplitude was observed in the optic tectum and reticular formation. The highest

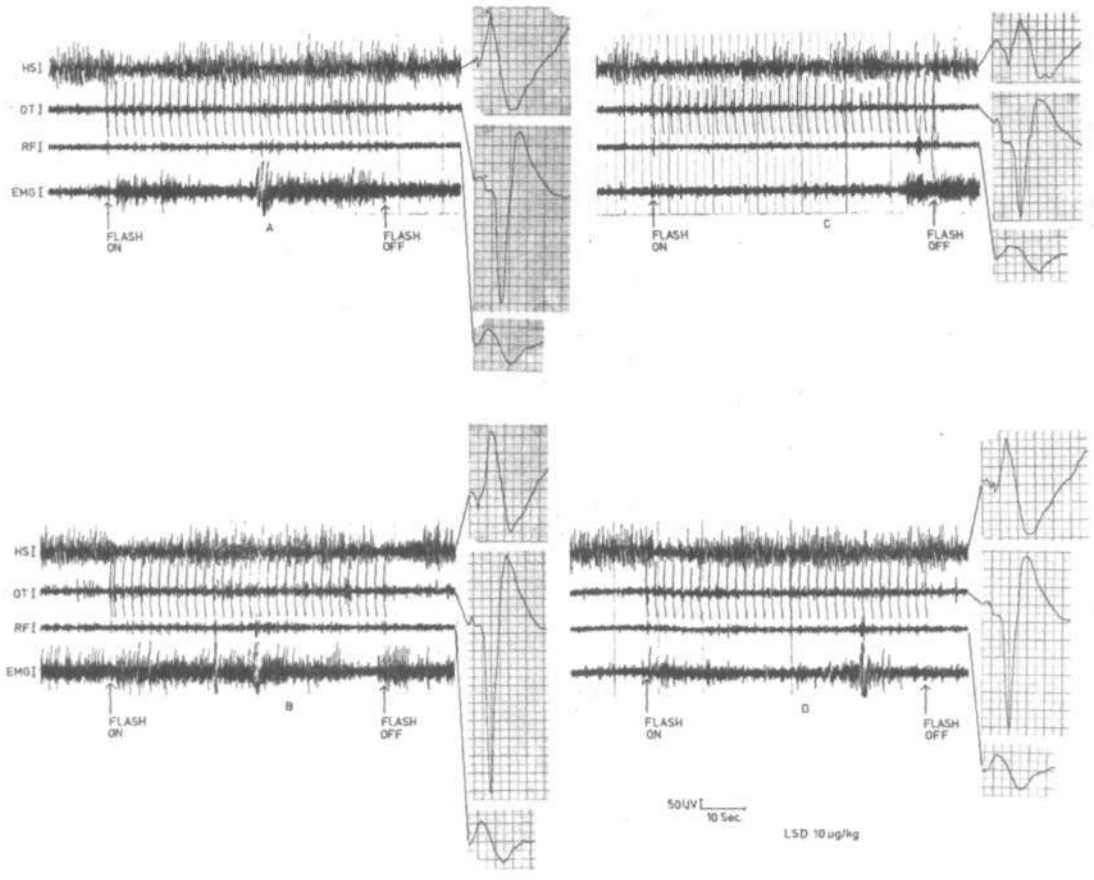


Fig. 8.6. Effect of LSD (10 ug/kg) on EEG, EMG and PER in a 5-day old chick. A, is control, while B,C, and D are responses recorded at 5 min, 1 hr. and 5 hr. respectively after drug administration.

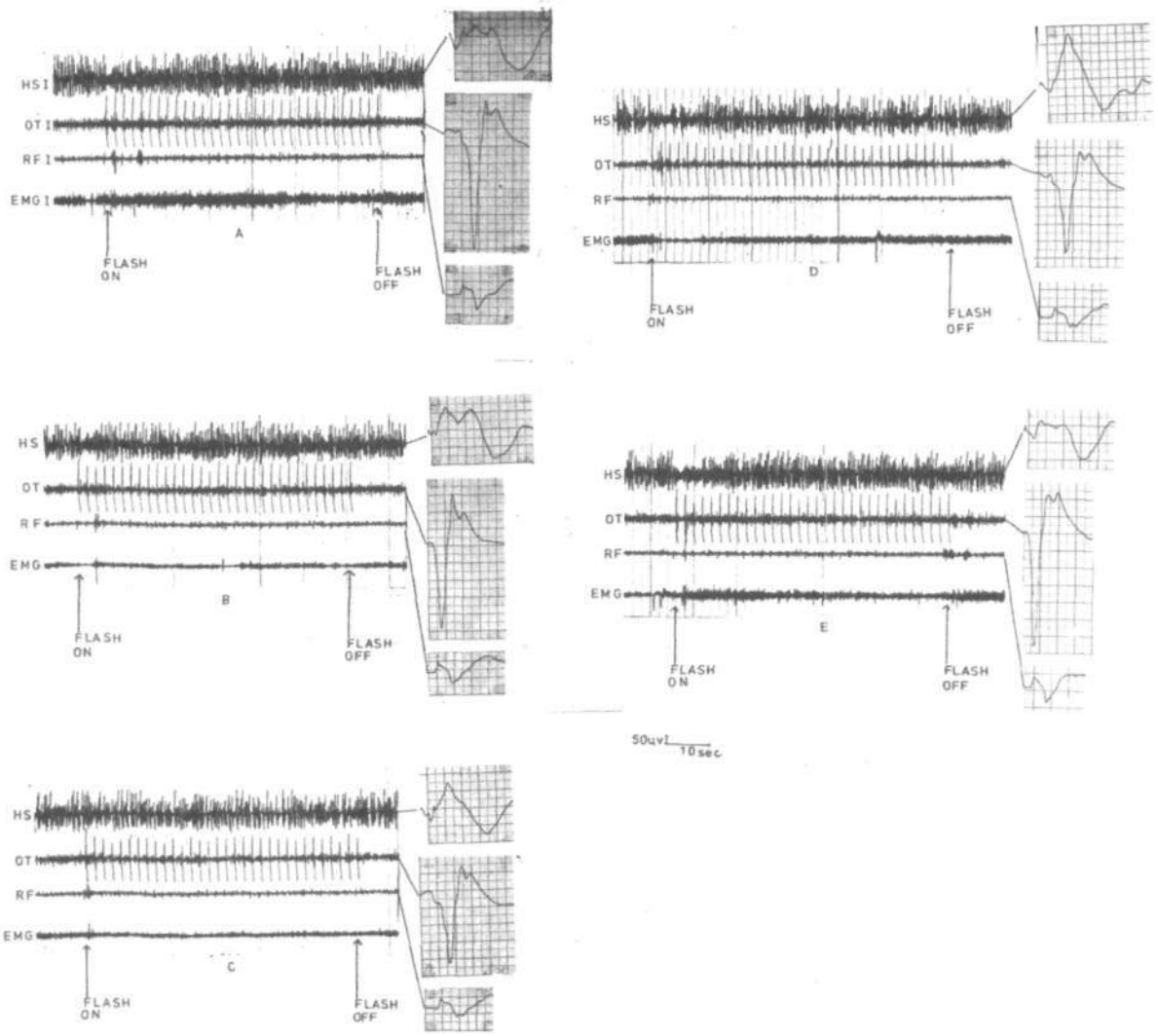


Fig. 8.7a. Effect of GHB (500mg/kg) on EEG, EMG and photic-evoked response in a 5-day old chick, A, is control, while B,C,D and E are responses recorded at 5 min, 30 min, 1 hr. and 3 hr. respectively, after drug administration.

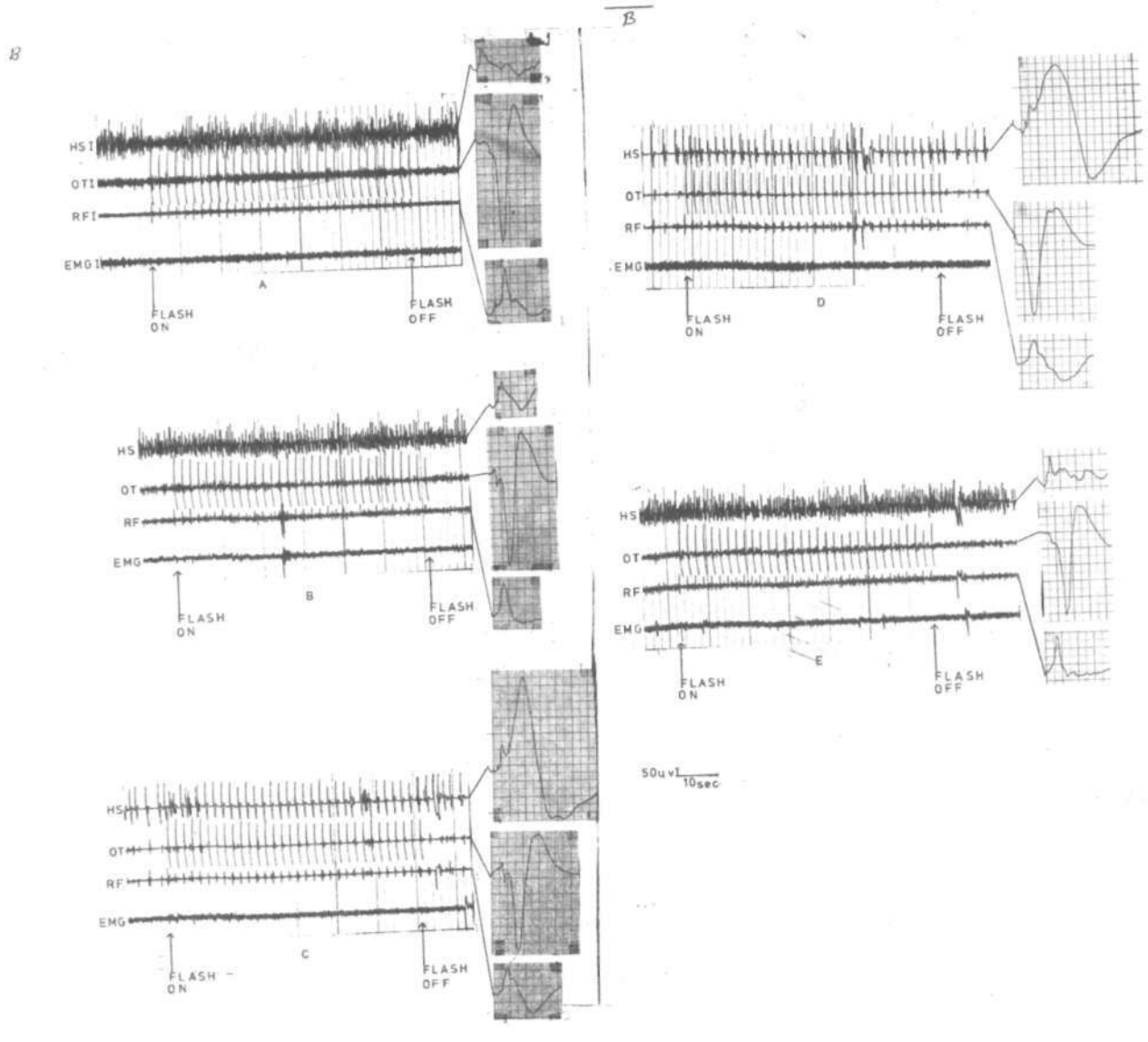


Fig. 8.7b. Effect of gamma hydroxybutyrate (GHB; 1 g/kg) on EEG, EMG and photic-evoked response in a 5-day old chick. A, is control recording, while B,C,D and E are responses recorded at 5 min. 30 min. 2 hr. and 3 hr. respectively, after drug administration.

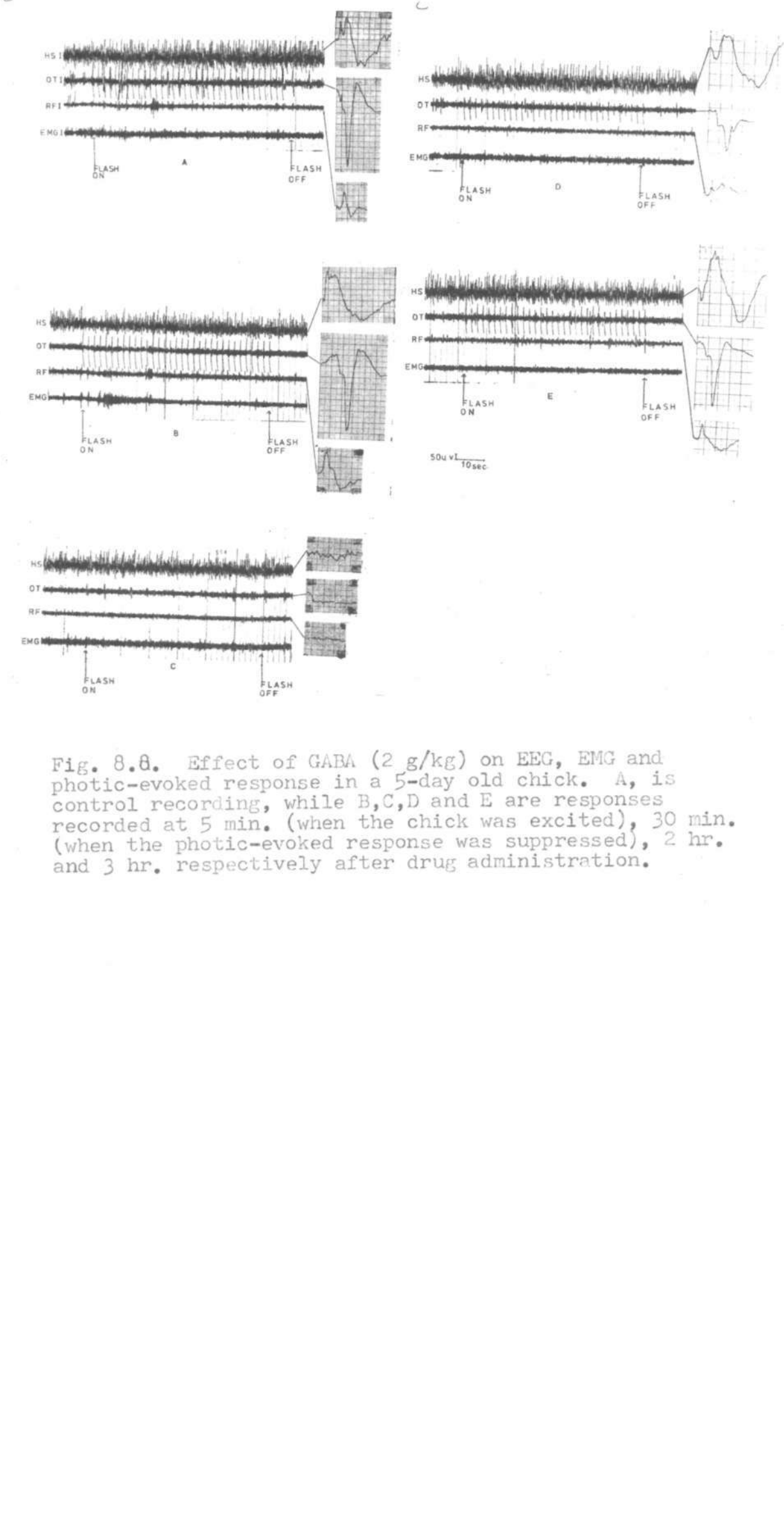


Fig. 8.8. Effect of GABA (2 g/kg) on EEG, EMG and photic-evoked response in a 5-day old chick. A, is control recording, while B,C,D and E are responses recorded at 5 min. (when the chick was excited), 30 min. (when the photic-evoked response was suppressed), 2 hr. and 3 hr. respectively after drug administration.

amplitudes were recorded during periods of spikes with electrical silence. The EMG was usually suppressed. During this period, there was synchronization of spike activity and photic induced spikes after each flash. In some chicks a series of myoclonic jerks were recorded after photic stimulation (figs. 8.7a & b).

f) Gamma aminobutyric acid (GABA, 0.5 - 2 g/kg).

With GABA (0.5 - 2 g/kg) there was an initial behavioural excitation characterized by loud chirping and violent movements, EEG desynchronization and an increase in the amplitude of the PER in the hyperstriatum and reticular formation. This excitatory phase lasted for a brief period and was subsequently followed by behavioural depression accompanied by suppresion of the PER in all the brain areas studied. The EEG was synchronized in the hyperstriatum and reticular formation while in the optic tectum, there was no observable change in the EEG. The

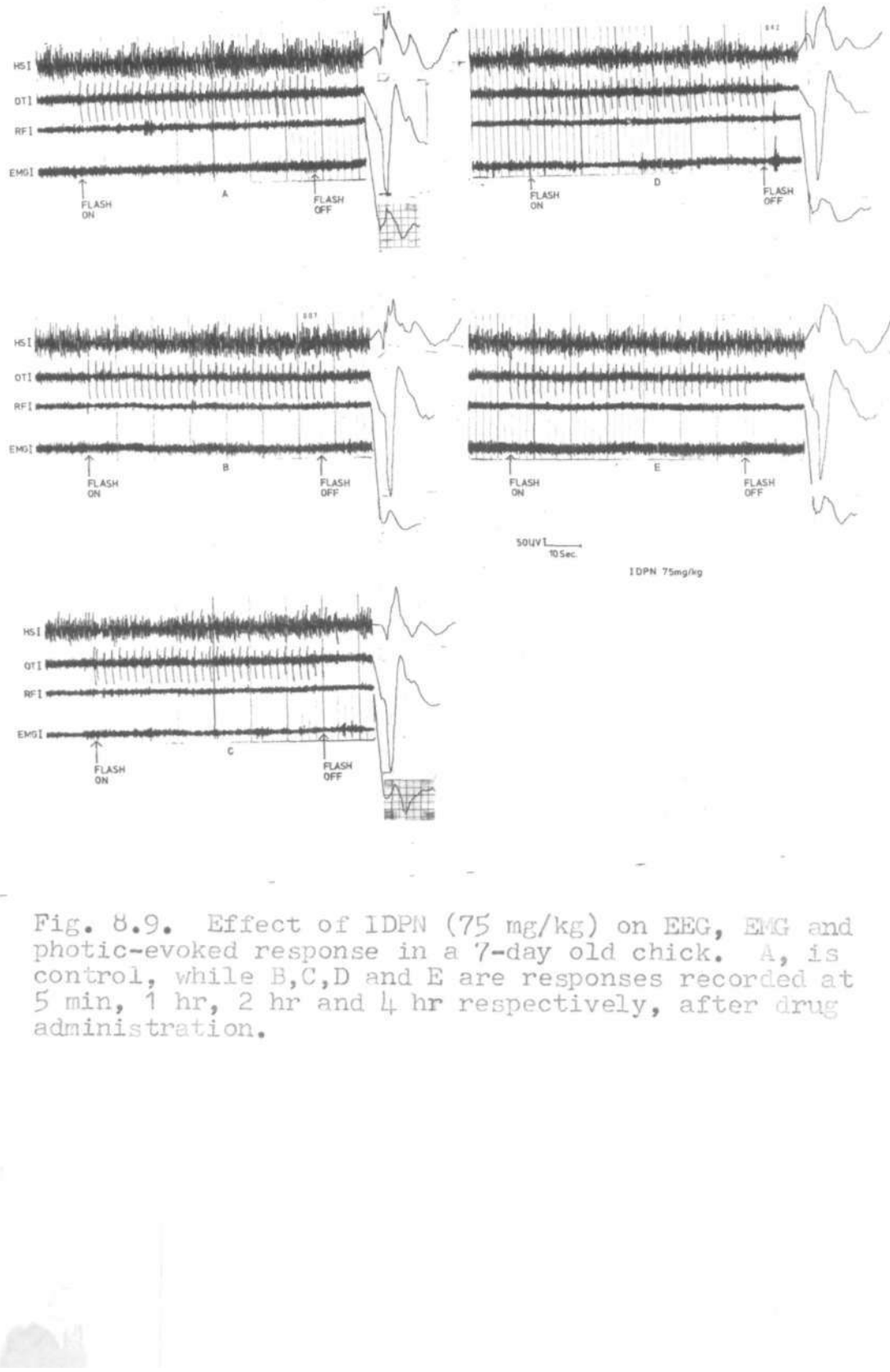


Fig. 8.9. Effect of IDPN (75 mg/kg) on EEG, EMG and photic-evoked response in a 7-day old chick. A, is control, while B,C,D and E are responses recorded at 5 min, 1 hr, 2 hr and 4 hr respectively, after drug administration.

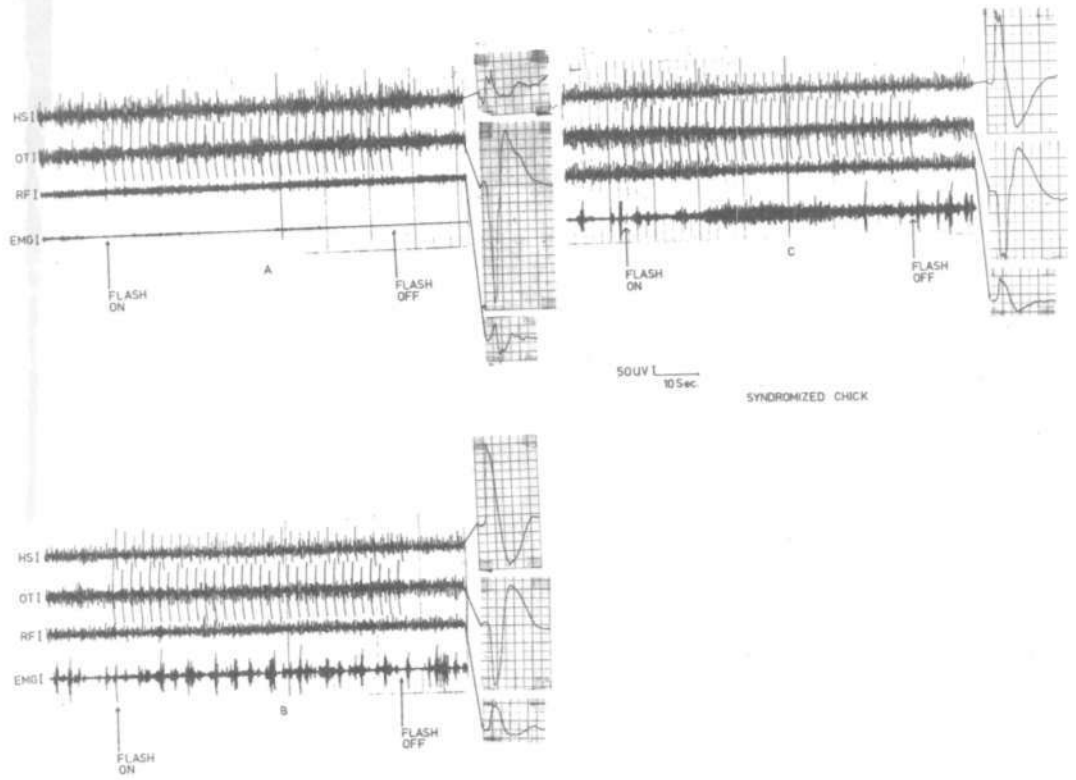


Fig. 8.10. Effect of chronic administration of IDPN (75 mg/kg for 5 days) on EEG, EMG and PER in a 2-week old chick. A, is control, while B and C are responses recorded on the 6th and 10th day respectively, after the appearance of the ECC-syndrome.

EMG activity was sometimes increased with GABA (figs. 8.8)

g) IDPN (75 mg/kg).

With a single dose of IDPN (75 mg/kg) the chicks were slightly sedated with intermittent closure of the eyes. There was a progressive desynchronization of the EEG and an increase in amplitude of the evoked response in the HS, while a decrease in amplitude was observed in the OT and RF (fig. 8.9).

h) Syndromized chicks.

The ECC-syndrome appeared in the implanted chicks after 5 days of repeated administration with IDPN (75 mg/kg). The EEG was synchronized and there was a marked increase in the amplitude of the photic evoked response in the hyperstriatum while in the optic tectum a decrease in amplitude was observed. In the reticular formation, a slight increase in amplitude was observed (fig. 8.10).

8 .5. Discussion.

The method described in this study is

sensitive, gives distinct evoked response which are clearly visible in the ongoing EEG (particularly in the optic tectum and reticular formation) and can be used for chronic studies. In addition, the method does not involve interaction with sedatives, anaesthetics and muscle relaxants which can influence respiration with consequent effect on the central nervous system. Under dark adaptation, photic stimulation exerted a more intense and consistent effect on the retina, so that the direction of the chick's eyes produced less significant influence on the PER in any behavioural state, and hence the amplitude of the evoked response was larger and more consistent than under artificial light. The axons from the retina terminate in the optic tectum. Since this is the primary visual pathway, the responses from the optic tectum were greater in amplitude than those recorded from the other brain ares. The variability of the photic-evoked response with behavioural state of the animal is a reverse of what has been observed by previous workers

using auditory stimulation (Spooner and Winters, 1967; Winters, 1964). Thus, the evoked responses were larger when the animals were awake and smaller when they were asleep while the reverse is true for auditory-evoked response (AER). A possible explanation for the differences between the AER and the PER during different behavioural states might be that the animals focused on visual information when awake and on auditory stimulus when asleep (Winters, 1968).

The observations made in the drug studies show that the PER is sensitive to the effects of various centrally-acting drugs. The increase in amplitude observed with amphetamine is probably due to a direct excitatory effect at the various sites.

The effects of mescaline and LSD on the young chick have been described by Zaimis (1960). Key and Marley (1962) have observed behavioural and EEG signs of sleep in three week old chicken following 1 or 2 mg/kg of mescaline. In this study mescaline

(1 mg/kg) produced behavioural sedation in young chicks. Hence the observed decrease in amplitude could be attributed to the depressant effect of the drug at the various recording sites. The increase in amplitude of the evoked response observed after the initial decrease in the hyperstriatum might be due to a subsequent facilitating effect of the drug on the hyperstriatum. The effect of 5 mg/kg mescaline shows that at this dose, the drug has an inhibitory effect on the PER from the optic tectum while the responses from the other brain areas were increased. This differential effect is probably due to a direct depressant effect of the drug on the optic tectum.

The effects observed with LSD show that this drug has both stimulant and depressant effects in chicks central nervous system. The marked increase in amplitude of the evoked responses is probably due to the excitatory

effects of the drug. Scholes (1970) made a similar observation with LSD in chicks. However, this observation is in contrast to that of Winters (1963) who reported suppression of auditory evoked response (AER) in cats by LSD. The subsequent progressive behavioural depression and decrease in amplitude of PER suggest a biphasic action of this drug on behaviour and PER in the chicks.

The effect of pentobarbitone on the PER is similar to its effect on AER (Winters, 1964) and it is probably due to a direct effect of the drug at the various sites. The subsequent increase in amplitude observed in the hyperstriatum may be due to a faster recovery of this site from the drug effect coupled with loss of modulatory influence of sites which normally control sensory input to the hyperstriatum. The fact that the effect of pentobarbitone on the evoked responses lasted longer than its effect on the EEG shows

that the evoked response is more sensitive to the effect of pentobarbitone than the gross EEG.

The observations made in this study show that GHB and GABA have differential effects on PER in conscious chicks. The effect of GHB on the PER is similar to its effect on AER as observed by previous workers (Winters and Spooner, 1965). However, while these workers observed a general increase in the amplitude of the AER from all the sites they studied, the results of the present study show an increase in amplitude only in the hyperstriatum and a decrease in the optic tectum and reticular formation. Several studies have suggested that the reticular formation plays an important role in the modulation of sensory input (Hernandez - peon, 1961; Killam, 1962). The observed increase in the amplitude of the PER in the hyperstriatum is therefore probaly due to the depression of the reticular formation

resulting in its inability to modulate the amplitude of sensory input. It is also probable that the hyperstriatum becomes more sensitive to sensory stimulation under the influence of GHB either due to increased level of neurotransmitter substance or due to involvement of certain facillitatory neuronal pathways which are brought in to play in the presence of GHB. The suppression of the PER in the optic tectum and reticular formation is probably due to a direct depressant effect of GHB at the two sites or due to an effect on the primary visual pathways. The initiation of myoclonic jerks in GHB treated chicks by photic stimulation show that photic stimulation enhances the seizure activity by GHB.

The observations made in this study show that GABA has an initial excitatory effect in young chicks. This excitatory effect could be due to activation of excitatory GABA receptors.

The suppression of the PER by GABA in this study is similar to that reported by Scholes and Roberts (1964) and Kramer et al (1967). This suppression of PER is probably due to depression of the primary visual pathways or due to activation of inhibitory pathways, resulting in loss of response to photic stimulation. The fact that the doses of GABA used in this study did not produce any obvious effect on tectal spontaneous activity even when the PER was markedly suppressed supports the view that GABA probably has no effect at the tectal level, as previously suggested by Scholes and Roberts (1964), and Kramer et al (1967). These observations suggest that both GHB and GABA have facilitatory and inhibitory influences on PER in young chicks.

The observations made in synchromized chicks after repeated administration of IDPN

shows that this compound produced a differential effect on PER recorded from different areas of the chick brain and this might probably reflect the extent of involvement of these areas in IDPN-induced behavioural dnd neurotoxic effects. However, the observations cannot be clearly explained in the light of the present data and further work is required to elucidate the mechanism of the observed effects.

In conclusion, the observations made in this study show that PER in young chicks under dark adaptation is sensitive to changes in behavioural state and to drug effects, and could probably be utilized in elucidation of the actions of drugs on the central nervous system.

CHAPTER IX

9. PHOTIC-EVOKED RESPONSE STUDIES IN PHENOBARBITONE AND ETHANOL-DEPENDENT RATS.

9.1. Summary.

9.1.1. The influence of acute and chronic administration of phenobarbitone and ethanol on photic evoked response was studied in rats.

9.1.2. Acute administration of phenobarbitone (5-50 mg/kg i.p.) and ethanol (1-3 g/kg i.p.) produced behavioural depression, EEG synchronization, a biphasic effect on the amplitude of the PER recorded from the frontal cortex (FC) and optic cortex (OC), while a reduction in amplitude was observed in the midbrain reticular formation (MBRF).

9.1.3. The amplitude of the averaged PER in the FC and OC was enhanced in phenobarbitone and ethanol-dependent rats, while in the MBRF, a reduction in amplitude was observed.

9.1.4. The withdrawal phase was characterized by behavioural excitation and an enhancement of the averaged PER

recorded from the brain areas studied in both phenobarbitone and ethanol-dependent rats.

9.1.5. The results suggest that both phenobarbitone and ethanol produce a similar pattern of effects on PER in rats and that the effects are probably characteristic of the sedative-hypnotic group of drugs.

9.2. Introduction.

Several studies indicate that ethanol and barbiturate withdrawal syndrome is characterized by the release of a latent state of neural hyperexcitability (Kalant, LeBlane and Gibbins, 1971). This notion has been largely inferred from the observed spontaneous and sound-induced seizures after ethanol and barbiturate withdrawal in animals (Freund and Walker, 1971). The EEG correlates of ethanol and barbiturate dependence and withdrawal states have been studied by some workers (Guerrero-Figueroa et al, 1970; Walker and Zarnetzer, 1974). Such studies have reported

seizure activity in the EEG after ethanol and barbiturate withdrawal in animals (Hunter et al, 1973; Walker and Zarnetzer, 1974). In the light of these EEG observations, it was thought necessary to study the sensory evoked response correlates in ethanol and barbiturate dependent rats, with the aim of investigating any characteristic effects these drugs might have on the evoked response. Such a study may also determine the possibility of using evoked response technique for testing drugs for dependence liability.

9.3. Materials and Methods.

Male albino rats weighing 250-300 g were used in this study. They were housed one rat to a cage and provided with food and water ad libitum. The rats were divided into two groups of six for chronic administration of phenobarbitone sodium (British Drug Houses Ltd, and ethanol. Control rats were kept under the same conditions except that they did not receive any drug treatment.

9.3.1. Chronic Phenobarbitone.

Six rats were injected (i.p.) daily with phenobarbitone sodium. The initial dose of 20 mg/kg was increased by 20 mg/kg every third day until a dose of 150 mg/kg/day was reached. The rats were maintained on this dose of phenobarbitone for three weeks before withdrawal. Control rats were injected with distilled water.

9.3.2. Chronic ethanol.

Ethanol was administered to six rats through drinking water into which was added saccharin sodium (0.2 mg/ml) to mask the taste, according to the method of Puri and Lal (1974). The starting concentration of 5% v/v was increased by 5% v/v every other day until a concentration of 20% v/v was reached. The rats were maintained on this concentration of ethanol for four weeks before withdrawal. Control rats were provided with food and drinking water containing saccharin sodium (0.2 mg/ml).

In both groups, when dependence was established, each rat was implanted with cortical, subcortical and electromyogram electrodes for EEG, EMG and photic-evoked response recordings. The influence of acute administration of phenobarbitone and ethanol on averaged PER was also studied.

9.3.3 Implantation of electrodes:

Each rat was anaesthetized with pentobarbitone (30 mg/kg) and the head was held firmly in a stereotaxic apparatus. The procedure for implantation of electrodes was similar to that used for chicks (Chapter VIII), except that in the present study, stainless steel screws were used as cortical electrodes. The brain sites implanted include the frontal cortex (FC) optic cortex (OC) and the midbrain reticular formation (MBRF). These brain areas were implanted using the following coordinates:- FC: anterior - posterior, + 1.9 mm, Lateral, + 1.5 mm; OC: anterior - posterior, - 9.2 mm, lateral,- 2.8 mm; MBRF: anterior - posterior,- 3.8 mm, Lateral, + 2.0 mm, Vertical,- 8.2 mm. The procedure for recording the EEG, EMG and averaged photic-evoked response

in both control and treated rats was similar to that used for chicks as stated in chapter VIII.

9.4. RESULTS

9.4.1. Characteristics of PER Pattern in rats.

Distinct averaged evoked responses to photic stimulation were recorded from the brain areas studied. In the FC, the averaged evoked response was characterized by an initial negative wave followed by a positive component of higher amplitude (40-70 μV). The averaged PER recorded from the OC was characterized by an initial positive wave (150-200 μV), followed by a negative component (120-190 μV). The EEG was characterized by low voltage fast activity of 25-30 c/sec frequency. In the MBRF, the averaged PER consisted of a positive component (25-60 μV) followed by a negative component of smaller amplitude, while the spontaneous activity was characterized by low voltage fast activity of 20-35 c/sec frequency. In the FC and MBRF, the evoked spikes were not visually distinguishable from the ongoing spontaneous activity, while in the OC, the

evoked spikes were slightly distinct from the EEG (fig. 9.1 A). The averaged PER from different animals were similar when recorded under the same conditions.

9.4.2. Acute Phenobarbitone:

Acute Administration of low doses (5-10 mg/kg) of phenobarbitone produced slight behavioural sedation and EEG synchronization. The amplitude of the averaged evoked response recorded from the FC and MBRF was slightly decreased while that recorded from the OC was not significantly affected. Higher doses (30-50 mg/kg) produced marked behavioural depression and EEG synchronization. In the FC, there was an initial decrease in the amplitude of the averaged evoked response followed by an increase. In the OC, there was an increase in the amplitude of the evoked response while in the MBRF a decrease in amplitude was observed (fig. 9.1).

9.4.3. Chronic injection of Phenobarbitone in rats.

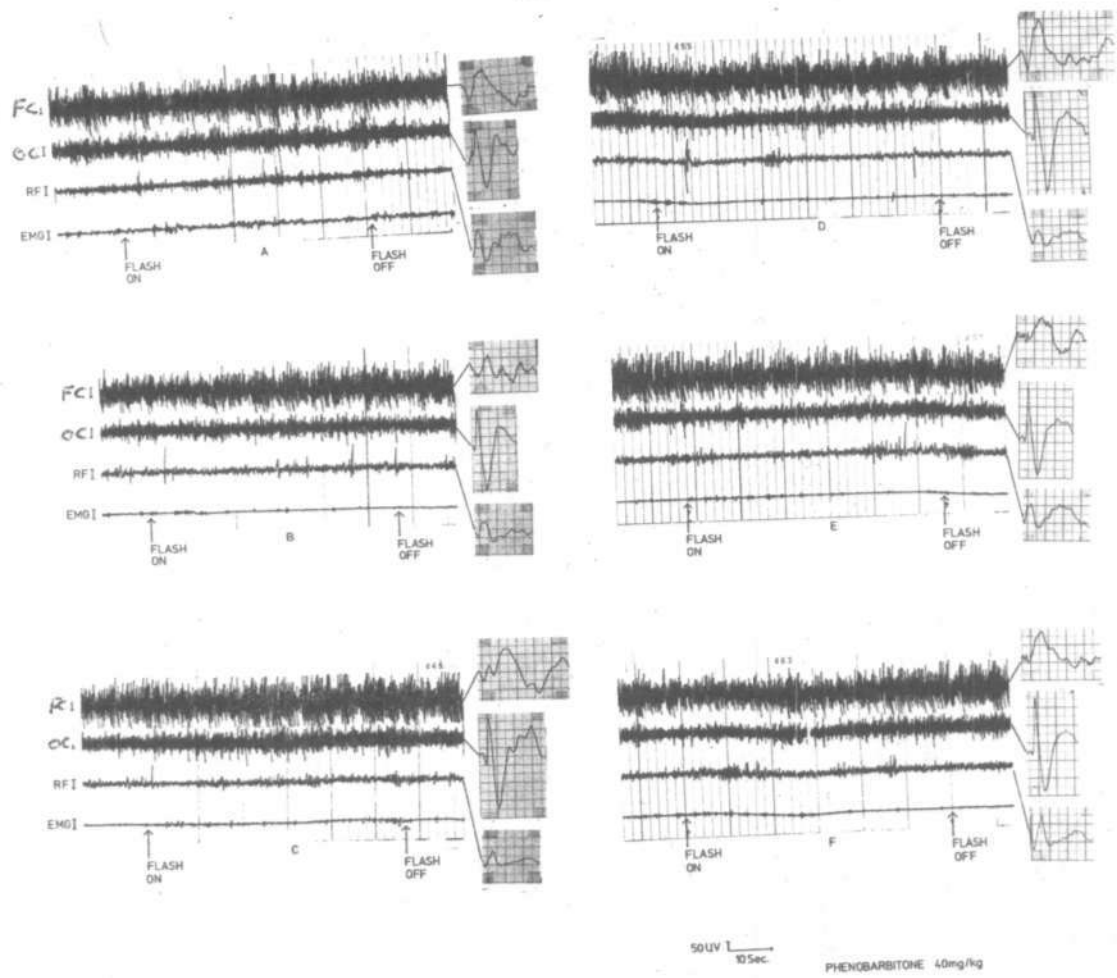


Fig. 9.1. Effect of phenobarbitone (40 mg/kg) on EEG, and PER recorded from the frontal cortex (FC), optic cortex (OC) and midbrain reticular formation (RF) of a rat. A, is control, while B,C,D,E, and F are responses recorded at 5 min, 30 min, 1hr, 2 hr and 4½ hr. respectively, after drug administration. The effect on the EMG is also shown.

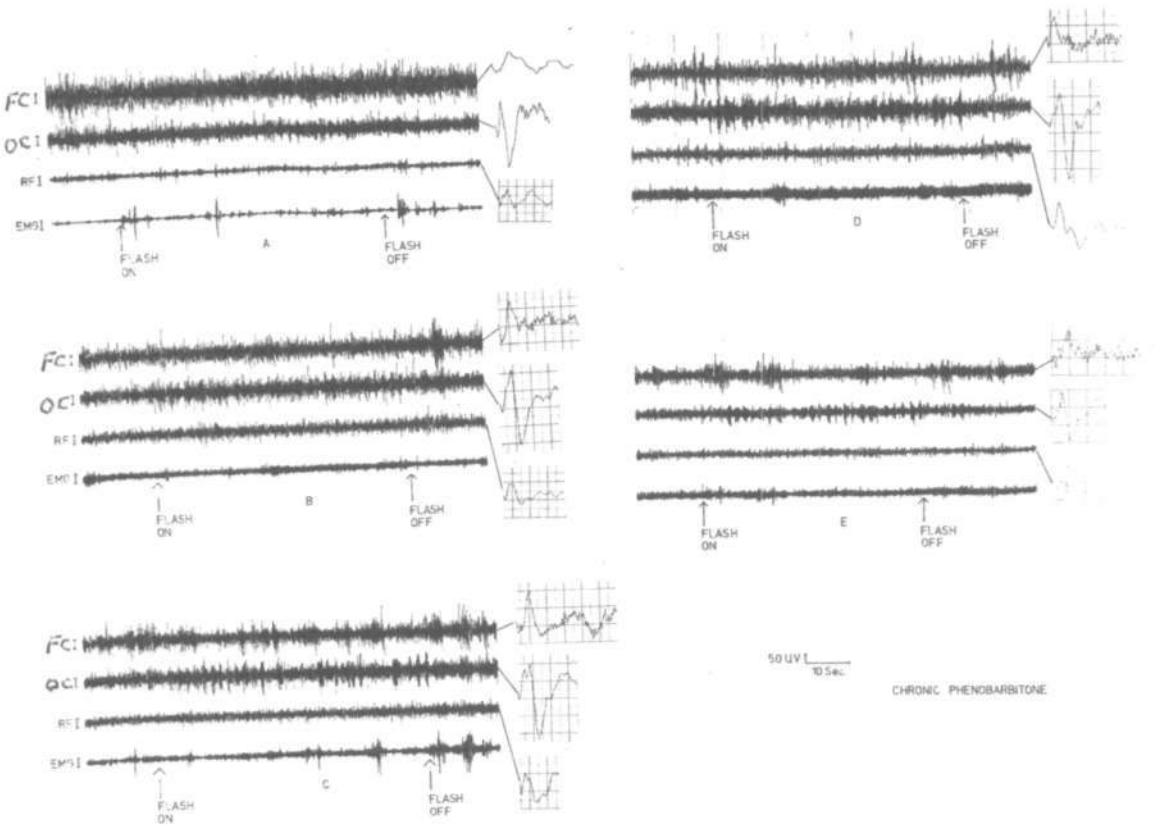


Fig. 9.2. Effect of chronic administration of phenobarbitone and its withdrawal on EEG, EMG and PER in a rat. A, is control, B, is recording taken when the rat was addicted to phenobarbitone, while C,D and E are responses recorded 24 hr. 48 hr. and 1 week respectively, after withdrawal of the drug.

Moderate doses (20 mg/kg) of phenobarbitone produced behavioural sedation. With higher doses (50 mg/kg) the rats were more depressed and usually remained calmly at one corner of the cage for a period of about 2 h before normal activity was resumed. The barbiturate dependence in these rats was ascertained by the sound-induced convulsions in one of the rats 24 and 48 h after withdrawal. Other signs observed during withdrawal included muscle fasciculations, vocalization and increased irritability. In the FC and OC, the amplitude of the averaged PER was enhanced in phenobarbitone-dependent rats, while a decrease in amplitude was observed in the MBRF. The ongoing EEG was synchronized in all the recording sites. During withdrawal (24 and 48 h) the behavioural excitation was characterized by EEG desynchronization and an increase in the amplitudes of the evoked responses recorded from the three brain areas studied. There was also an increase in the EMG activity. One week after withdrawal,

	FC (μV)	OC (μV)		MBRF (μV)
Control	+50.53 ± 14.49	+172.36 ± 22.6	-136.57 ± 10.22	+30.2 ± 3.66
Phenobarb.(addicted)	+68.0 ± 10.03	+197.0 ± 29.1	-173.14 ± 27.0	+17.24± 3.02
Phenobarb.(withdrawal, 24 h.)	+86.47 ± 15.21	+221.40 ± 30.04	-170.16 ± 22.0	+44.32± 8.51
Phenobarb.(withdrawal, 48 h.)	+58.21 ± 11.3	+180.8 ± 34.6	-207.0 ± 29.4	+46.15+ 6.2
Phenobarb.(withdrawal, 1 wk.)	+35.0 ± 5.4	+126.05 ± 21.0	-114.6 ± 15.1	+20.0 + 3.30

Table 9.1. Effect of chronic administration of phenobarbitone and its withdrawal on photic-evoked response in rats. The values given in the table are the means of 5 separate (control and treated) recordings from the frontal cortex (FC), optic cortex (OC) and midbrain reticular formation (MBRF).

there was a reduction in the amplitudes of the evoked responses recorded from the three brain sites (fig. 9.2, table 9.1).

9.4.4. Acute ethanol.

Administration of ethanol (1-3 g/kg) produced behavioural depression. The EEG was synchronized while there was a decrease in the EMG activity. With these doses of ethanol, the amplitude of the averaged PER was initially decreased and subsequently increased in the FC while in the OC, there was an initial increase in amplitude followed by a decrease. In the MBRF, there was a decrease in amplitude (fig. 9.3).

9.4.5. Chronic ethanol treated rats.

The rats given ethanol were usually weak and showed significant loss in weight. With concentration higher than 20% v/v, the daily intake of ethanol decreased markedly. During withdrawal, the rats showed increased motor activity and were found irritable to touch but

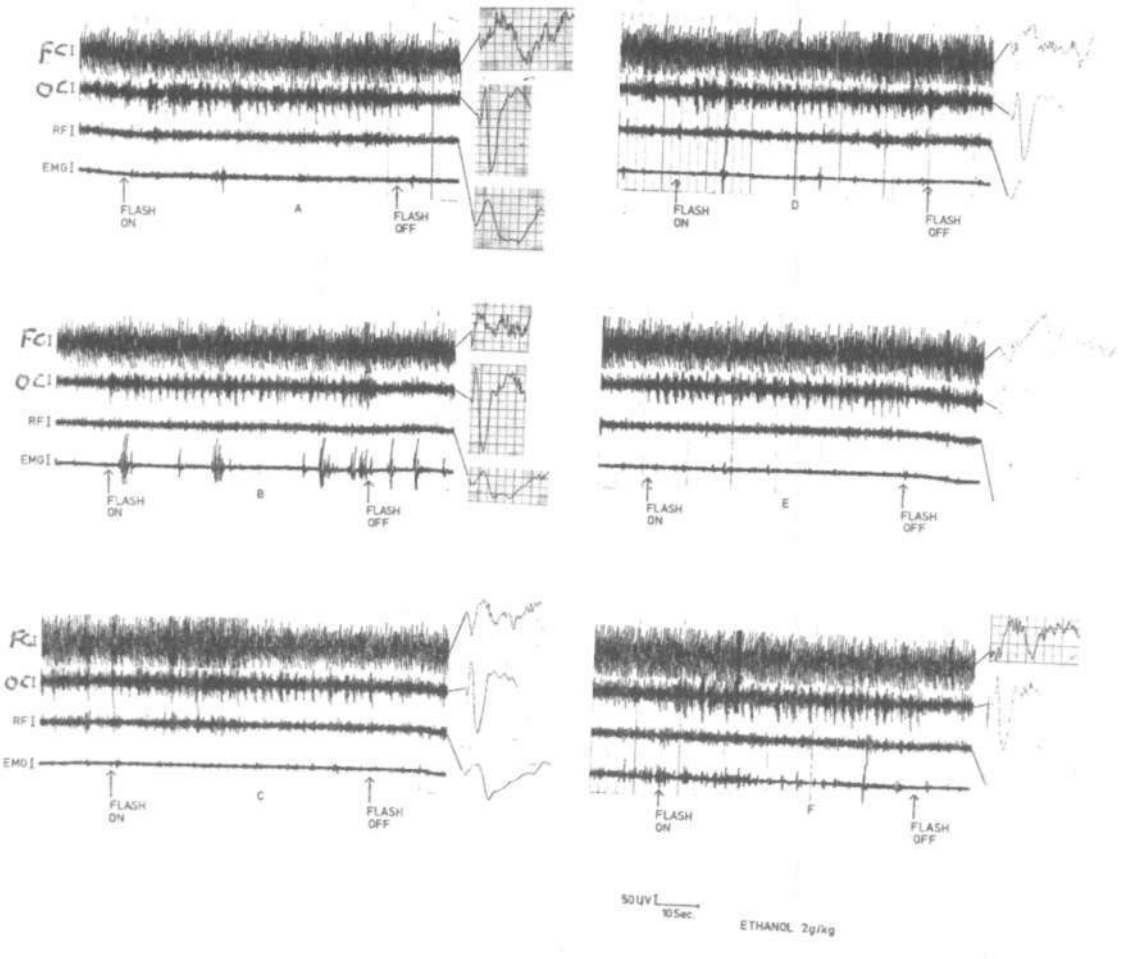


Fig. 9.3. Effect of ethanol (2 g/kg) on EEG, EMG and PER of a rat. A, is control, while B,C,D,E and F are responses recorded at 5 min, 30 min, 1hr, 2 hr and 3 hr. respectively, after ethanol administration.

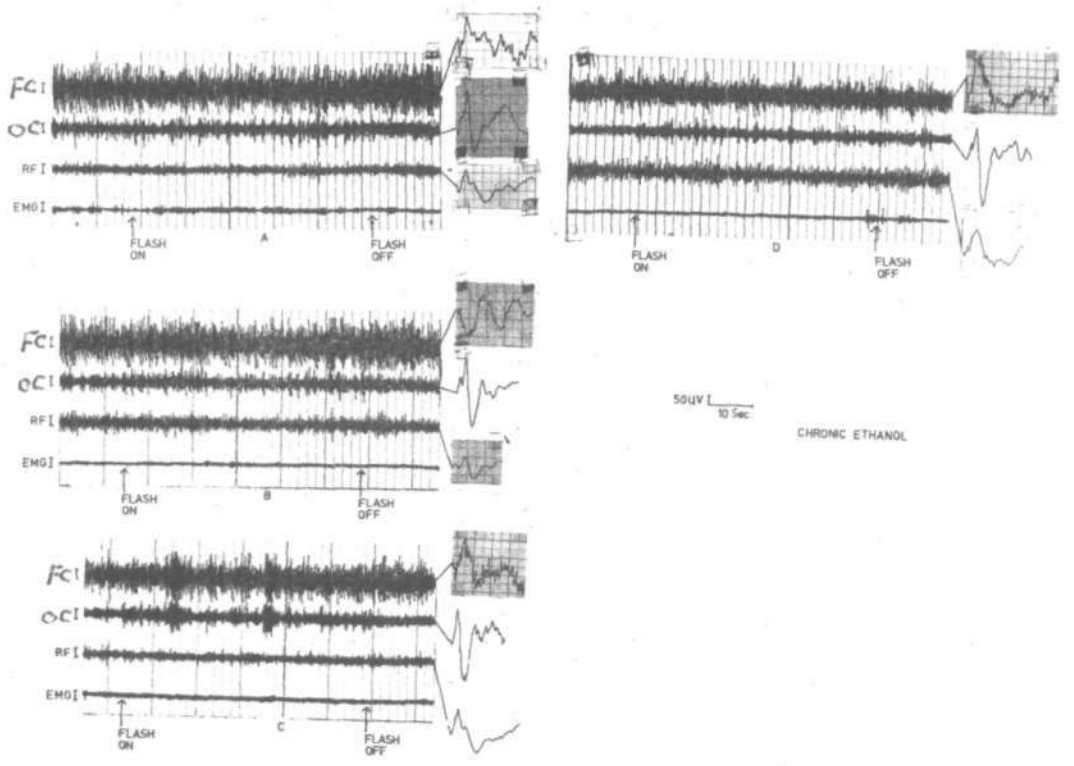


Fig. 9.4. Effect of chronic administration of ethanol and its withdrawal on EEG, EMG and PER in a rat. A, is control, B, is recording taken when the rat was addicted to ethanol, while C and D are responses recorded 24 hr and 48 hr. respectively, after ethanol withdrawal.

	FC (μV)	OC (μV)		MBRF (μV)
Control	+59.1 ± 12.3	+160.0 ± 22	-121.41 ± 17.0	+36.1 ± 4.8
Ethanol (addicted)	+88.09 ± 16.43	+181.20 ± 26.34	-155.52 ± 23.0	+22.01 ± 4.11
Ethanol(withdrawal, 24 h.)	+100.71± 18.6	+201.0 ± 31.22	-189.3 ± 24.0	+51.82 ± 9.0
Ethanol (withdrawal, 48 h.)	+70.93 ± 13.1	+179.53 ± 22.0	-198.0 ± 17.74	+38.20 ± 5.4
Ethanol (withdrawal, 1 wk.)	+38.0 ± 5.0	+131.1 ± 15.13	-119.0 ± 20.03	+25.31 ± 4.09

Table 9.2. Effect of chronic administration of ethanol and its withdrawal on photic-evoked response in rats. The values given in the table are the means of 5 separate (control and treated) recordings from the frontal cortex (FC), optic cortex (OC) and midbrain reticular formation (MBRF).

did not show spontaneous or sound induced convulsions. The EEG of the ethanol addicted rats was synchronized in all the brain areas studied, while the EMG activity was decreased. The amplitude of the averaged PER during this state was increased in the FC and OC while in the MBRF the amplitude was reduced. The withdrawal phase was characterized by desynchronized EEG and increased EMG activity. The amplitude of the averaged PER was increased in the FC, OC, and the MBRF when recorded 24 and 48 h after withdrawal. A decrease in amplitude was observed in all the three brain areas when recording was done one week after withdrawal (fig. 9.4, table 9.2). By this time the rats appeared behaviourally calm and inactive.

9.5. Discussion.

The observations made in this study show that photic stimulation induces distinct evoked responses in rats as observed in chicks, and could be used for elucidating the action of drugs on the central nervous system. While the

patterns of the responses recorded from the FC and MBRF were similar to those recorded from corresponsing areas in the chick brain, the responses from the OC consisted of an initial positive component followed by a negative one, in contrast to the reverse pattern observed in the optic tectum of chicks. This probably reflects differences in the process of sensory transmission through the primary pathways in the two species.

The effects of acute administration of phenobarbitone and ethanol on the evoked responses recorded from the FC were similar to the observed effect of pentobarbitone on the PER recorded from the corresponding region of the chick brain.

The withdrawal signs observed after chronic administration of phenobarbitone and ethanol are similar to those reported by previous workers (Crossland and Leonard, 1963; Maxson and Sze, 1976). However, in the present study, ethanol

withdrawal failed to induce spontaneous or audiogenic seizures in the rats as observed by Freund and Walker (1971). This was probably due to the low daily alcohol intake by the rats used in this study. Other workers have reported higher alcohol intake (20 mls/day), using the same concentration (20% v/v) and method of administration (Puri and Lal, 1974). It is probable that the rats used in the present study showed more aversion to the taste of alcohol than those used by Puri and Lal (1974). It has been generally observed that the taste of alcohol appears to be aversive to rats, and this has led some studies to employ intragastric and intravenous routes to induce dependence on alcohol (Mello, 1973).

The EEG synchronization which was observed when the rats became dependent on both phenobarbitone and ethanol correlate with the behavioural depression which characterized that state. The observed increase in

the amplitude of the averaged PER in the FC and OC in phenobarbitone and ethanol dependent rats was probably due to a depression of the reticular formation which normally modulates the amplitude of sensory input to these brain areas. This suggestion is supported by the observed reduction in the amplitude of the evoked response recorded from the MBRF during this state. The increase in amplitude of the evoked responses during the withdrawal phase in both phenobarbitone and ethanol dependent rats probably suggests an enhanced sensitivity of those sites to sensory stimulation when drug administration was stopped. Such a suggestion is consistent with the notion that a release of a latent state of neural hyperexcitability takes place after ethanol or barbiturate withdrawals (Kalant et al., 1971).

The observations made in this study show that both phenobarbitone and ethanol produced similar pattern of effects on the averaged PER recorded from the brain areas studied. Since

both drugs belong to the sedative hypnotic group, the observed effects are probably characteristic of this group of drugs. However, the significance of the increases and decreases in amplitude of the PER is not evident from the present study and this requires further elucidation.

CHAPTER X

GENERAL DISCUSSION AND CONCLUSION.

The observations made with the various DA agonists used in this study show that these drugs elicit a similar pattern of stereotyped behavioural effects in chicks. The ability to induce stereotyped behaviour in experimental animals is widely used as an index of central dopaminergic stimulation and as an indicator of antiparkinsonian potentiality (Costall, Naylor and Pinder, 1974). Thus, stereotyped behaviour in chicks, could be used as a behavioural model for screening drugs for antipsychotic or antiparkinsonian properties. The differences in the intensity of the activity induced by these DA agonists may reflect differences in their mechanisms of action and to what extent their actions are influenced by other modulatory mechanisms within the central nervous system.

Among the DA agonists used, apomorphine was found to be the most potent in producing

stereotyped pecking and aggressive behaviour. The inability of injected DA to produce distinct behavioural effects in the chicks cannot be precisely explained in the light of the available data. It could be due to rapid conversion of DA to NA or due to differences in affinity for DA receptors involved in the induction of stereotyped behaviour. Such a proposal is consistent with the hypothesis of Cools and Van Rossum (1976) that there may be two types of DA receptors. Both ergometrine and piribedil, have been reported to exert a direct agonistic effect on central DA receptors (Woodruff et al, 1974; Costall and Naylor, 1973b). However,the observations made in this study suggest that piribedil most probably has a weak agonistic effect while ergometrine has a predominant inhibitory effect on central DA receptors in the chick. In addition to the weak agonistic effect, the observed antagonism of apomorphine and

d-amphetamine-induced stereotyped behaviour by piribedil also suggest that this drug may exert antagonistic effects on DA receptors. However, several studies also suggest that piribedil affect brain NA. Garattini and his co-workers (1974) have shown that piribedil causes depletion of brain NA and accumulation of MOPEG-SO4 and that these effects are dose-dependent. Hence, some of the actions observed with piribedil in the present study could probably be due to the influence of the drug on other neurotransmitter systems. Further neurochemical studies may throw more light on the mechanism of the observed effects.

The effects observed with the various catecholaminergic agents used in this study suggests a primary role for DA in stereotyped behaviour. The observed potentiation of apomorphine-induced stereotyped behaviour by reserpine is in contrast to the observervation of Fakete, Kurti and Pribuz (1970) in mice.

These workers observed inhibition of apomorphine action by reserpine in mice. This apparent differential effect of reserpine on apomorphine action could probably be due to species variation. According to Horn and Synder (1971), the therapeutic efficacy of neuroleptics in schizophrenic patients generally parallels their potency in antagonizing animal stereotypies and their tendency to produce extrapyramidal side effects. These relationships suggest that similar mechanisms may underly stereotyped behaviour in animals, psychosis induced by central nervous system stimulants and certain forms of naturally occurring psychotic disorders (Rotrosen et al, 1972). Thus the observed antagonism of the behavioural effects of apomorphine and other DA agonists by pimozide and haloperidol suggest that stereotyped behaviour in chicks could be used for screening drugs for antipsychotic properties.

Though several conflicting data have been reported on the role of 5-HT in drug-induced stereotyped behaviour, the observations made in the present study indicate that 5-HT plays a predominant inhibitory role in stereotyped behaviour in chicks. These observations agree with those of Weiner et al (1973), Cheng and Long (1974), and Saxena et al (1977). A number of studies have shown that apomorphine increases the level of 5-HT and 5-hydroxyindoleacetic acid in the whole brain and accelerates 5-HT turnover, and it has further been shown that these biochemical changes are susceptible to blockade by DA receptor blocking agents (Grabowska et al, 1973). These biochemical data indicate that apomorphine activates 5-HT neurons indirectly via stimulation of DA receptors. Thus the observations made in the present study show that activation of 5-HT neurons appears to be antagonistic towards its causative factor i.e. dopaminergic stimulation.

The results obtained with methysergide suggest that this drug may have both 5-HT agonistic and antagonistic actions in young chicks. The inability of methysergide to potentiate the actions of d-amphetamine cannot be explained in the light of the present data and the actions of methysergide in young chicks requires further study.

The observations made with the tricyclic antidepressants show that this group of drugs exert a biphasic effect on locomotor activity in young chicks. Many pharmacological and neurochemical studies have implicated the level of synaptically available NA and 5-HT in the mechanism of action of drugs modulating mood and motility (Schildkraut and Kety, 1967). Thus, the observed potentiation and antagonism of apomorphine-induced locomotor activity by imipramine, amitriptyline and desipramine could probably be due to the action of these drugs on

catecholaminergic and serotonergic mechanisms. DA-5-HT interaction has been implicated in locomotor activity. For example, apomorphine-induced locomotor stimulation is enhanced by lesions of raphe nuclei, 5-HT synthesis inhibitors and 5-HT receptor blockers (Grabowska et al, 1974a). This therefore suggests that serotonergic mechanisms may be involved in the inhibitory effects of these tricyclic antidepressants on apomorphine-induced locomotor activity in young chicks. Though the precise mechanisms of these actions are not obvious from this study, it is tempting to suggest that the observed antagonism of apomorphine-induced locomotor activity is probably common to this group of drugs and could probably be useful for screening purposes. Further studies with other types of antidepressants might help to verify this suggestion.

The fact that IDPN induced behavioural

changes in chicks as has been reported in rats suggest that similar mechanisms may be involved in the induction of the ECC-syndrome in both species. The results of pharmacological studies indicate a role for DA and 5-HT in the ECC-syndrome. On the other hand, cholinergic influences appear to play an inhibitory role in this syndrome. The apparent recovery of the chicks from the ECC-syndrome is in contrast to the permanent nature of the syndrome in rats. Though, the factors responsible for the behavioural recovery in chicks are not obvious from this study, the observations suggest that further neurochemical studies on the behavioural effects of IDPN in chicks may elucidate the mechanisms underlying the ECC-syndrome.

The technique of photic evoked response recording described in this study is sensitive, gives distinct evoked responses clearly visible in the ongoing EEG, particularly in the optic

tectum and reticular formationaand can be used for chronic studies. Furthermore, the method does not involve interaction with sedatives, anaesthetics and muscle relaxants which can influence respiration with consequent effect on the central nervous system. Though Winters (1968) indicated that the position of an animal's head with respect to the light source is critical in visual evoked response studies, the observations made in the present studies show that under dark adaptation, the position of the chicks' eyes has no significant influence on the photic-evoked response in any behavioural state. The results of pharmacological studies also show that various centrally-acting drugs modify the evoked responses in various ways. The observations made with LSD appear to be quite interesting in view of the fact that LSD induces visual hallucination. If one can speculate that a visual hallucination results from a reduction in modulation of the visual input system, then an abnormally large number of visual stimuli would enter the central nervous system and overload the

visual system resulting in bizzarre sensory manifestations. Some evidence for this speculation has been presented by Purpura (1956) in which he demonstrated an increase in the visual evoked response in the lateral geniculate and cortex following LSD. Apter and Pfeiffer (1957) reported that the visual system activity induced by LSD starts in the retina and travels through visual pathways up to the cortex. The same workers have also reported evidence indicating that in patients with interruption of the optic nerve, LSD does not induce hallucinations. This shows that an intact visual system is necessary for the behavioural effects of LSD. These observations suggest that photic evoked response could probably be used alone or in combination with other methods, in elucidating the action of some drugs on the central nervous system.

The observations made in the drug dependence studies show that both phenobarbitone

and ethanol produced a similar pattern of effects on PER recorded from brain areas studied. Since both drugs belong to the sedative-hypnotic group, the observed effects may probably be characteristic of the group. This suggests that the evoked response technique could probably be useful for studying the dependence liability of this class of drugs. However, further electrophysiological studies are required to elucidate the significance of the observed enhancements and reductions in amplitudes of the evoked responses.

Finally, the work was quite exciting and challenging. The basic objectives were achieved and suggestions for further work in the aspects studied have been made.

REFERENCES

ADRIAN, E.D. and Matthews, B.H.C. (1934). The interpretation of potential waves in the cortex. J. Physiol. 88: 127.

AJURIAGUERRA, J., Constantinidis, J., Eisering J.I., Yanniotis, G. and Tissot, R. (1972). Behaviour disorders and L-Dopa therapy in Parkinson's syndrome. In "Parkinson's Disease", Vol. II, J. Seigfried (ed.) pp. 201-217. Hans Huber Bern.

AMASSIAN, V.E., Waller, H.J. and Macey, J., J.R. (1964): Neural mechanisms of the Primary Somatosensory evoked potential. Ann. N.Y. Acad. Sci. 112: 5-32.

AMSLER, C. (1923). Beitrage Zur Pharmacologie des Gehirns. Arch. Exptl. Pathol. Pharmacol. 97: 1.- 14.

ANDEN, N.E., Butcher, S.G., Corrodi, H., Fuxe, K. and Ungerstedt, U. (1970). Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. Eur. J. Pharmacol. 11: 303 - 314.

ANDEN, N.E., Carlsson, A., Dahlstrom, A., Fuxe, K. Hillarp, N.A. and Larsson, K. (1964). Demonstration and mapping out of nigro - neostriatal dopamine neuron. Life Sci. 3: 523-530.

ANDEN, N.E., Rubenson, A., Fuxe, K. and Hokfelt, T., (1967). Evidence for dopamine receptor stimulation by apomorphine. J. Pharm. Pharmacol. 19: 627-629.

ANGRIST, B. (1974). Amphetamine Psychosis. In catecholamines and their enzymes in the Neuropathology of Schizophrenia (S. Kety and S. Mattysse. eds). J. Psychiat. Res. 11: 1.

APTER, I.T. and Pfeifer, C.C. (1957). The effect of the hallucinogenic drugs LSD-25 and mescaline on the electro-retinogram. Ann. N.Y. Acad. Sci. 66: 508.

ARNFRED, T. and Randrup, A. (1968). Cholinergic mechanism in brain inhibiting amphetamine induced stereotyped behaviour. Acta. Pharmacol. Toxicol. 26: 384 - 394.

BAROLIN, G.S., Bernheimer, H., and Hornykiewicz, O. (1964). Seitenverschiedenes ver halten des dopamines. (3,hydroxytyramin) in gehirn eines falles von hemiparkinson - nismus. Schweij. Arch. Neurol. Psychiat. 94: 241 - 248.

BARTHOLINI, G. and Pletscher, A. (1968). Cerebral accumulation and metabolism of ^{14}C-dopa after selective inhibition of peripheral decarboxylase. J. Pharmacol. exp. Ther. 161, 14-20.

BERNHEIMER, H. and Hornykiewicz, O. (1973). Brain amines in Huntington's chorea. In Advances in Neurology", Vol. 1, A. Barbeau, T.N. Chase and G.W. Paulson (Eds.) Raven Press, New York pp. 525 - 531.

BERNHEIMER, H., Birkmayer,W., Hornykiewicz, O., Jellinger, K., and Seitelberger, F. (1973). Brain dopamine and the syndromes of parkinson and Huntington, J. Neurol. Sci. 20: 415.

BERTLER, A., Carlsson, A., Rosengren, E. (1956). Release by reserpine of catecholamines from rabbits hearts. Naturwissenschaften 22: 521.

BERTLER, A. and Rosengren, E. (1959). On the distribution in brain of monoamines and of enzymes responsible for their formation. Experientia 15: 382 - 384.

BLASCHKO, H. (1957). Metabolism and storage of biogenic amines. Experientia, 13: 9-12.

BREESE, G.R., Cooper, B.R. and Mueller, R.A. (1974). Evidence for involvement of 5-hydroxytryptamine in the actions of amphetamines. Br. J. Pharmacol. 52. 307-314.

BROWN, B., (1960). CNS drug actions and interactions in mice. Arch. Int. Pharmacodyn 128: 391-413.

BROWNLOW, B.K. and Heath, H. (1969). Biochemical change in B, B-iminodipropionitrile-treated rat brain and prevention of toxicity by ethionine. J. Neuro-Chem. 16: 567-575.

CANNON, W.B. (1915). Bodily changes in pain and hunger, fear and rage. D. Appleton and Company, New York.

CARLSSON, A. (1959). The Occurrence, distribution and physiological role of catecholamines in the nervous system. Pharmacol. Rev. 11: 490-493.

CARLSSON, K.R., and Almasi (1978). Sensitivity to apomorphine in the guinea pig as a function of age and body weight. Psychopharmacology 57: 279-282.

CARLSSON, A., Corrodi, H., Fuxe, K. and Hokfelt, T. (1969). Effects of antidepressant drugs on the depletion of intra-neuronal brain 5-hydroxytryptamine stores caused by 4-methyl-α-α-ethyl-m-tyramine. Eur. J. Pharmacol. 5 35[illegible] - 366.

CARLSSON, A., Kehr, W., Lindqvist, M. (1975). Agonist-Antagonist interaction on dopamine receptors in brain as reflected in the rate of tyrosine and tryptophan hydroxylation. In "proceedings fifth international symposium on Parkinson's Disease".

CARLSSON, A. and Lindqvist, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta. Pharmacol. Toxicol. 20: 140-144.

CARLSSON, A., Lindqvist, N. and Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature (Lond.) 180: 1200.

CARLSSON, A., Persson, T., Roos, B.E. and Walinder, J. (1972). Potentiation of Phenothiazines by -methytryosine in treatment of chronic schizophrenia. J. Neural transmission. 33: 88.

CHASE, T.N., and Murphy, D.L. (1973). Serotonin and Central nervous system function. Ann. Rev. Pharmac. 13: 181 - 197.

CHENG, H.C. Long, J.R. (1974). Dopaminergic nature of apomorphine-induced pecking in pigeons. European J. Pharmacol. 26: 313 - 320.

CHOU, S.M. and Hartmann, H.A. (1964). Axonal lesions and Waltzing Syndrome after B,B-iminodipropionitrile administration in rats with a concept of 'Axostasis', Acta Neurophath. 3: 428-450.

CLYMER, N.V. and J. Seifter, (1947). A method of screening sympathomimetic amines for stimulant action on the cerebrum, J. Pharmac. Exp. Ther. 89: 149 - 152.

COLE, J.O. and Davis, J.M. (1968). Clinical efficienty of the phenothiazines as antipsychotic drugs. In "psychopharmacology" D.H. Efron (ed). pp. 1057 - 1063.

COOLS, A.R. & Van Rossum, J.M. (1976). Excitation-Mediating and Inhibition-Mediating Dopamine-receptors: A new Concept towards a better understanding of electrophysiological, Biochemical Pharmacological, Functional and clinical Data. Psychopharmacologia (Berl.) 45: 243-254.

CORRODI, H., Farnebo, L.O., Fuxe, K. and Hamberger, B. (1975). Effect of ergot drugs on central 5-hydroxytryptamine neurons: evidence for 5-hydroxytryptamine receptor stimulation. European J. Pharmacol. 30, 172-181.

CORRODI, H., Fuxe, K., Hokfelt, T., Lidbrink, P. and Ungerstedt, U. (1973). Effect of ergot drugs on central catecholamine neurons: evidence for stimulation of central dopamine neurons. J. Pharm. Pharmacol. 25, 409-412.

CORRODI, H., Fuxe, K., Ljungdahl, A. and Orgren S.O (1970). Studies on the action of some psychoactive drugs on central noradrenaline neuron after inhibition of dopamine-β -hydroxylase. Brain Res. 24: 451-470.

CORRODI, H., Fuxe, K. and Ungerstedt, U. (1971). Evidence for a new type of dopamine receptor stimulating agent. J. Pharm. Pharmacol. 23: 989-991.

COSTALL, B. and Naylor, R.J. (1973a). The role of telencephalic dopaminergic systems in the mediation of apomorphine-stereotyped behaviour. Eur. J. Pharmacol. 24: 8-24.

COSTALL, B. and Naylor, R.J. (1973b). The site and mode of action of ET. 495 for the mediation of stereotyped behaviour in the rat. Naunyn-Schmiedebergs Arch. Pharmacol. 278: 117-133.

COSTALL, B. and Naylor, R.J. (1974b). Extrapyramidal and mesolimbic involvement with the stereotypic activity of d-and l-amphetamine. European J. Pharmacol. 25: 121-129.

COSTALL, B., and Naylor, R.J. (1974a). Stereotyped and circling behaviour induced by dopaminergic agonists after lesions of the midbrain raphe nucleu, Eur. J. Pharmacol. 29: 206-222.

COSTALL, B. and Naylor, R.J. (1975). The role of the raphe and extrapyramidal nuclei in the stereotyped and circling responses to quipazine. J. Pharm. Pharmacol. 27: 368-371.

COSTALL, B., Naylor, R.J. & Olley, D.E. (1972). The substantia Nigra and stereotyped behaviour. Eur. J. Pharmacol. 18: 95-

COSTALL, B., Naylor, R.J. and Pinder, R.M. (1974). Design of agents for stimulation of neostriatal dopaminergic mechanisms. J. Pharm. Pharmac. 26: 753-762.

COSTENTIN, J., Protais, P., Schwartz, J.C. (1975). Rapid and dissociated changes in sensitivities of different dopamine receptors in the mouse brain. Nature (Lond) 257: 405-407.

CREESE, I. and Iversen, S.D. (1972). Amphetamine response after dopamine neurone destruction. Nature New Biol. 238: 247 - 248.

CREESE, I., and Iversen, S.D. (1974). The role of forebrain dopamine systems in amphetamine induced stereotyped behaviour in the rat. Psychopharmacologia. 39: 345-357.

CREUTZFELDT, O.D., Watanebe, S. and Lux, H.D. (1966). Relations between E.E.G. Phenomena and potentials of single Cortical Cells. Evoked responses after thalamic and epicortical stimulation. Electroenceph. Clin. Neurophysiol., 20: 1-13.

CROSSLAND, J., Leonard, B.E. (1963). Barbiturate withdrawal convulsions in the rat. Biochem. Pharmacol. Suppl. 12: 103.

CROW, T.J., and Gillbem D. (1973): Dopamine antagonism and antischizophrenic potency of neuroleptic drugs. Nature New. Biol. 245: 27-28.

CUTTING, W., Furst, A., Read, D., Read, G., and Parkman, H. (1960). Circling syndrome produced in mice by dimethylaminohexose reductone. Proc. Soc. Exp. Bio. Med. 104. 381 - 335.

DAVIDSON, L., Lloyd, K.G., Dan Kova, J., and Hornykiewicz, O. (1971). L-dopa treatment in parkinsons' disease: Effect on dopamine and related substances in discrete brain regions. Experientia. 27: 1048.

DAWSON, G.D. (1947). Cerebral responses to electrical stimulation of peripheral nerve in man. J. Neurol. Neurosurg. Psychiat 10. 134-140.

DAWSON, G.D. (1951). A summation technmique for detecting small signals in a large irregular background. J. Physiol. 115: 2-3.

DELAY, J., Pichot, P., Thuillier, J., and Marquiset, J.P. (1952). Action de l'amino-dipropionitile sur le compartment moteur de la so lins, blanche. C.R. Soc. Biol. (Paris) 146: 533-534.

DENEAU, G., Yanagita, T., and Seevers, M.H. (1969). Self administration of Psychoactive substances by the monkey. A measure of psychological dependence. Psychopharmacologia 16: 30-48.

DEWHURST, W.G. Marley, E. (1965). The effects of -methyl derivatives of noradrenaline, phenylethylamine and tryptamine on the central nervous system of the chicken. Brit. J. Pharmacol. 25: 682-704.

DHAWAN, B.N., and Saxena, P.N. (1960). Apomorphine-induced pecking in pigeons, Brit. J. Pharmacol. 15: 285-289.

DIEZEL, P.B., and Ule, G. (1963). Histochemische Untersuchun qen an den 'Ghost Cells' beim experimetelle neurolathyrismus. Acta Neuropath. 3: 150-163.

DIVAC, I. (1972). Drug-induced syndromes in rat with large, chronic lesions in the Corpus Striatum. Psychopharmacologia 27: 171-178.

DUFFY, E. (1962). Action and Behaviour. New York. Wiley.

EHRINGER, H., and Hornykiewicz, O. (1960). Verteilung Von. Noradrenalin and Dopamin (3-hydroxytyramin) im Gehirn des menschen und ihr verhalten bein Erkrankungen des extra pyramidalen systems. Klin. Wscher. 38: 1236-1239.

ELLINWOOD, E.H., Sudilovsky and Nelson, L.M. (1973). Evolving behaviour in the Clinical and experimental amphetamine (model) Psychosis. Am. J. Psychiat. 730: 1088.

EMELE, J., Shanaman, J. and Warren, M. (1961). Chlorphentermine hydrochloride, P-chlor-α-α-dimethylphenethylamine hydrochloride, a new anorexigenic agent. II. Central Nervous System activity Fed. Proc. 20 (Part I), 328.

ERNST, A.M. (1967). Mode of action of apomorphine and dex-amphetamine in gnawing compulsion in rats. Psychopharmacologia (Berlin) 10: 316-323.

EVERETT, G.M. (1966). The dopa response potentiation test and its use in screening for antidepressant drugs. In anti-depressant Drugs (Garattini, S., and Drikes, M.N.G. Eds.) pp. 164-167. Excerpta Medica Foundation. Amsterdam.

EVERETT, G.M. and Wiegand, R.G. (1962). Central amines and behavioural states. A critique and new data. In 'proceedings of the first international pharmacological meeting'. Borje Uvnas. (Ed). Pergamon Press. New York. pp. 85-95.

FAKETE, M., Kurti., A.M. and Pribusz, J. 1970). On the dopaminergic nature of gnawing compulsion induced by apomorphine in mice. J. Pharm. Pharmacol. 22: 377-379.

FARNEBO, L.O. FUXE, K., Goldstein, M., Hamberger, B., Ungerstedt, U. (1971). Dopamine and noradrenaline releasing action of amantadine in the central and peripheral nervous sustem. a possible mode of action in Parkinson's disease, Eur. J. Pharmacol. 16: 27-38.

FELDBERG, W. and Sherwood, S.L. (1954,. Behaviour of cats after intraventicular injections of eserine and DFP. J. Physiol. 125: 488-500.

FELTZ, P. and Dechamplain, J. 1973: The postsynaptic effect of amphetamine on striatal dopamine-sensitive neurones. In "Frontiers in Catecholamine Research", (E. Usdin.and S. Snyder eds.) Pergamon Press, New York.

FIBIGER, H.C., Lytle, L.D. and Campbell, B.A. (1970). Cholinergic modulation of adrenergic arousal in the developing rat. J, Comp. Physiol. 72: 384-389.

FOG, R. (1972). On Stereotype and Catalepsy Studies on the effect of amphetamine and neuroleptics in rats. Acta Neurol Scand. Suppl. 48: 50.

FOG, R., Randrup, A., and Pakkenberg, H., (1968). Neuroleptic action of quaternary chlorpromazine and related drugs injected into various brain areas in rats, psychopharmacologia 12: 423-432.

FORGACS, J. (1959). Les alterations oculaires dan le lathyrisme experimental dues a l'iminodipropionionitrile (IDPN). Arch. Ophthalmol (Paris) 20: 275-284.

FREUND, G. & Walker, D.W. (1971). Sound-induced seizures during ethanol withdrawal in mice. Psychopharmacology. (Berl.) 22: 45-49.

FUXE, K. (1973). Tools in the treatment of Parkinson's disease: Studies on new types of dopamine receptor stimulating agents. Advance Neurol. 3. 273.

FUXE, K., and Ungerstedt, U. (1970). Histochemical. biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration. In "Amphetamines and related compounds", E. Costa and S. Garattini (ed) New York. Raven Press, pp. 257-288.

GABAY, S. (1966). Brain transminases in a chemically induced behaviour abnormality. Recent Ad. Biol. Psychiat. 8: 73-85.

GABAY, S., and Huang, K.H. (1969). Studies of brain aminotransferases and psychotropic agents. I. Interaction of β,β'-iminodipropionitrile and rat brain aromatic aminotransferases. Biochem. Pharmac. 18: 767-775.

GABAY, S., Langlais, P.J. and Huang, P.C. (1974). Evidence for the involvement of dopaminergic mechanism in the ECC. Syndrome. The Pharmacologist. 16. 308.

GABAY, S., Vivanco, F., Ramos, F., and Jimenez Diaz, C. (1961). Influence of D. L-thryroxine and cortisone on femurs of rats with Odoratism. Arch. Biochem. Biophys. 92: 87-93.

GARATTINI, S., Bareggi, S.R., Marc, V., Galderini, G., Moselli, P.L. (1974). Effects of piribedil on noradrenaline and MOPEG-504 levels in the rat brain. Eur. J. Pharmacol. 28: 214-216.

GERSTENBRAND, F., Pateisky, K., and Prosenz. (1963). Erfahrungen mit. L.dopa in der therapie des parkinsonismus psychiat. Neurol. 146: 246.

GLOWINSKI, J., Axelrod, J. and Iversen, L.L. (1966). Regional Studies of Catecholamines in the rat brain IV. Effects of drugs on the disposition and metabolism of H^3-nor-epinephrine and H^3-dopamine J. Pharmacol. Exper. Therap. 153: 30-41.

GODWIN, S.K., Murphy, D.L., Brody, H.K.H., and Bunney, W.E., (1970). L-dopa, catecholamines and behaviour. A clinical and biochemical study in depressed patients. Biol. Psychiat. 2: 341-366.

GOLDIN, A., Noe, H.A., Landing, B.H., Shapiro, D.M. and Goldberger, B. (1948). A neurobiological syndrome induced by administration of some chlorinated tertiary amines. J. Pharm. Exp. Ther. 94: 249-261.

GOLDSTEIN, M.H. (1961). Averaging techniques applied to evoked responses. Electroenceph. Clin. Neurophysiol. Suppl. 20: 59-63.

GOLDSTEIN, M., Anagnoste, B., Owen, W.S. and Battista, A.F. (1967). Effects of ventromedial tegmental lesions on the disposition of dopamine in the caudate nucleus. Brain Res. 4: 298-300.

GRABOWSKA, M. (1974). Influence of midbrain raphe lesions on some pharmacological and biochemical effects of apomorphine in rats. Psychopharmacologia 39: 315-322.

GRABOWSKA, M., Antkiewicz, L., Maj, J., and Michaluk, J. (1973). Apomorphine and central serotonin neurons. Pol. J. Pharmac. Pharm. 25: 29-39.

GRABOWSKA, M., Antkiewicz, L. and Michaluk, J. (1974a). A possible interaction of quipazine with central dopamine structures. J. Pharm. Pharmac. 26: 74-76.

GRABOWSKA, M., Antkiewicz, L., and Michaluk, J. (1974b). The influence of the tricyclic antidepressants on the apomorphine induced hypermotility in rats. Pol. J. Pharmac. Pharmac. 26: 411-417.

GUERRERO-FIGUEROA, R., Rye, M.M., Gallant, D.M. and Bishop, M.P. (1970). Electrographic and behavioural effects of diazepam during alcohol withdrawal state in cats. Neuropharmacology 9: 143-150.

GUMULKA, W., Ramirez, D.A., Samanin, R. and Valzeli, L. (1970). Lesion of substantia nigra: biochemical and behavioural effects in rats. Eur. J. Pharmacol. 10: 79-82.

HARTMANN, H.A., Lin, J., and Shively, M.C. (1968). RNA of Nerve Cell bodies and axons after β,β'-iminodipropionitrile. Acta. Neuropath. 11: 275-281.

HEIKKILA, R.E. & Cohen, G. (1972). Evaluation of Amantadine as a releasing agent or uptake blocker for H^3-dopamine in rat brain slices. Eur. J. Pharmacol. 20: 156-160.

HEIMANS, R.L.H., Rand, M.J., and Fennessy, M.R. (1972). Effects of amantadine on uptake and release of dopamine by a particulate fraction of rat basal ganglia, J. Pharm. Pharmacol. 24: 875-879.

HERMAN, Z.S. (1967). Influence of some psychotropic and adrenergic blocking agents upon amphetamine stereotyped behaviour in white rat. Psychopharmacologia 11: 136-142.

HERNANDEZ-PEION, R., (1961). Reticular mechanisms of sensory control. In "Sensory communications". W. Rosenblith (ed.) MIT, Wiley, New York, pp. 497-520.

HOLLISTER, A.S., Brese, G.R. and Cooper, B.R. (1974). Comparison of tyrosine hydroxylase and dopamine-hydroxylase inhibition with the effects of various 6-hydroxydopamine treatments on d-amphetamine-induced motor activity. Psychopharmacologia 36: 1-16.

HONG, E., Sancilio, L.K. Vargas, R., and Pardo, E.G. (1969). Similarities between the pharmacological actions of quipazine and serotonin. Eur. J. Pharmacol. 6: 274.

HORN, H.S., and Snyder, S.H. (1971). Chloropromazine and dopamine: conformational similarities that correlate with antischizophrenic activity of phenothizine drugs. Proc. nat. Acad. Sci. (Wash.) 68: 2325-2328.

HORNYKIEWICZ, O. (1966). Dopamine (3,hydroxytrytamine) and brain function. Pharmacol. Rev. 18: 925-964.

HUNTER, B.E. Boast, C.A., Walker, D.W., and Zornetzer, S.F. (1973). Alcohol withdrawal syndrome in rats: neural and behavioural correlates. Pharmacol. Biochem. Behav. 1: 719-925.

JACOBOWITZ, D.M. and Richardson, J.S. (1978). Method for the rapid determination of norepinephrine, dopamine and serotonin in the same brain region. Pharmacol. Biochem. & Behav. 8: 515-519.

JANSSEN, P.A.J., Niemegeers, C.J.E., Schellekens, K.H.L. (1965). Is it possible to predict the Clinical effects of neuroleptic drugs (major tranquilizer) from animal data?. "Neuroleptic activity spectra" for rats. Arzneim, Forsch. 15. 104-110.

KALANT, H., LeBlanc, A.E., Gibbins, R.J. (1971). Tolerance to, and dependence on ethanol. In: Biological basis of alcoholism. Y. Israel and J. Mardones (eds.), pp. 235-269. New York: John Wiley and Sons.

KEBABIAN, J.W., Petzold, G.L. and Greengard, P. (1972). Dopamine sensitive adenylate cyclase in caudate nucleus of rat brain and its similarity to the "dopamine receptor". Proc. Nat. Acad. Sci. U.S.A. 69: 2145-2149.

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

KEY, B.J. & Marley, E. (1962). The effect of the sympathomimetic amines on behaviour and electrocortical activity of the chicken. Electroenceph. Clin. Neurophysiol. 14: 90-105.

KHAZAN, N., and Brown, P. (1970). Differential effects of three tricyclic antidepressants on sleep and REM sleep in the rat. Life Sci. 9: 279-284.

KILLAM, E.K. (1962). Drug action on the brain-stem reticular formation. Pharmacological Rev. 14: 175-223.

KOE, B.K., and Weissman, A. (1966). P-chlorophenylalanine: a specific depletor of brain serotonin. J. Pharmacol. Exp. Ther. 154: 499-516.

KOSTOWSKI, W., Giacolone, E., Garattini, S., and Valzelli, L. (1968). Studies on behaviour and biochemical changes in rats after lesions of the midbrain raphe. Eur. J. Pharmacol. 4: 371-376.

KRAMER, S.Z., Sherman, P.A., Seifter, J. (1967). Effects of gamma-aminobutyric acid (GABA) and Sodium L-glutamate (glutamate) on the visual system and EEG of chicks. Int. J. Neuropharmacol. 6: 463-472.

LANGLAI, P.J. and Gabay, (1977). Quipazine excerbation of a hyperkinetic syndrome: Involvement of brain dopamine and Serotonin. Journal of Neuroscience Research 3: 135-141.

LANGLAI, P.J., Huang, P.C., and Gabay, S. (1975). Regional Neurochemical Studies on the effect of B,B'-iminodipropionitrile (IDPN) in the rat. Journal of Neuroscience Research 1: 419-435.

LEIMDOFER, A. (1948). A pulmonary cardiovasular reflex in man. Federation Proc. 6: 151.

LIDBRINK, P., Johnson, G., and Fuxe, K. (1971). The effect of imipramine-like drugs and antihistaminic drugs on uptake mechanism in the central noradrenaline and 5-hydroxytryptamine neurons. Neuropharmacology. 10: 521-536.

MAJ, J. (1977). Dopamine agonists and interaction with other neurotransmitter systems. Naunyn-Schmiedeberg's Arch. Pharmacol. 297: 553-554.

MAJ, J., Grabowsky, M., and Gajda, L. (1972). Effect of apomorphine on motility in rats. Eur. J. Pharmacol. 17: 208-214.

MANDELL, A.J. and Spooner, C.E. (1968). Psychochemical research studies in man. Science 162: 1442.

MANDELL, A.J., Spooner, C.E. Winters, W.D., Cruikshank, M., and Sabbot, I.M. (1968). Neurochemical correlates of the Imipramine antagonism of the behavioural effects of norepinephrine. Life Sci. 7: 1317-1324.

MAXSON, S.C. and Sze (1976). Electroencephalographic correlates of audiogenic seizures during ethanol withdrawal in mice. Psychopharmacology 47: 17-20.

McCLEAN, J.R., McCartney, M. (1961). Effect of d-amphetamine on the rat brain noradrenaline and Serotonin. Proc. Soc. Exp. Biol. (N.Y.) 107: 77.

McKENZIE, G.M., and Szerb, J.C. (1968). The effect of dihydroxyphenylalanine, pheniprazine and dextramphetamine on the in vivo release of dopamine from the caudate nucleus. J. Pharmacol. Exp. Ther. 162: 302-308.

MEDON, P.J., Leeling, J.L., and Phillips, B.M. (1973). Influence of quipazine, a potential antiparkinsonian agent on the uptake of 3H-dopamine and 3H-serotonin into rat striatal tissue in vitro. Life Sci. 13: 685-691.

MELLO, N.K. (1973). A review of methods to induce alcohol addiction in animals. Pharmacol. Biochem. Behav. 1: 89-101.

MENON, M.K., Clark, W.G., and Fleming, R.M. (1973). Blockade of the central effects of d-amphetamine sulfate by amantadine hydrochloride. Eur. J. Pharmacol. 21: 311-317.

MILLER, R.J., Horn, A.S., and Iversen, L.L. (1974). The action of neuroleptic drugs on dopamine stimulated adenosine-3'-5'-monophosphate production in rat neostriatum and limbic fore brain. Mol. Pharmacol. 10: 759-766.

MOGILNICKA, E., and Braestrup, C. (1976). Noradrenergic influences on the stereotyped behaviour induced by amphetamine, phenylethylamine and apomorphine. J. Pharm. Pharmacol. 28: 253-255.

MORGAN, C.T. (1965). Physiological Pscychology, New York: McGraw-Hill.

MORGANE, P. (1964). Limbic-hypothalamic-midbrain interaction in thirst and thirst-motivated behaviour. In "Thirst". M.J. Wayner (ed.), Pergamon: London, pp. 94-126.

MUNKVAD, I. and Randrup, A. (1966). The persistence of amphetamine stereotyped of rats in spite of strong sedation. Acta. Psychiat. Scand. Suppl. 191, 42: 178-187.

MUNKVAD, I. and Randrup, A. (1970). Evidence indicating the role of brain dopamine in the psychopharmacology of schizophrenic psychosis in psikofarmakologija 2. (N. Bolacek, ed) pp. 45-47. Medicinska Naklada, Zagreb.

NAYLOR, R.J. and Olley, J.E. (1972). Modification of behavioural changes induced by amphetamine in the rat by lesions in the caudate nucleus. The caudate putamen and globus pallidus. Neuropharmacology 11: 91-99.

NICHOLS, J.R., Headlee, C.P., and Coppock, H.W. (1956). Drug addiction 1. Addiction by escape training, J. Amer. Pharm. Assoc. 45: 788-791.

OSUIDE, G. (1972). Pharmacological properties of amino-oxyacetic acid in chicken. Br. J. Pharmacol. 44: 31-44.

OSUIDE, G. and P.O. Adejoh, (1973). Effects of apomorphine and its interaction with other drugs in the domestic fowl. Eur. J. Pharmacol. 23: 56-66.

OSWALD, I., Brezinova, V., and Dunleavy, D.L.F. (1972). On the slowness of action of tricyclic antidepressant drugs. Br. J. Psychiat. 120: 673-677.

PAASOMEN, M.K., and Vogt, M. (1956). The effect of drugs on the amounts of substance P and 5-hydroxytryptamine in mammalian brain. J. Physiol. (Lond) 131: 617-626.

PALMER, G.C. (1976). Influence of tricyclic antidepressants on the adenylate cyclase phosphodiesterase system in the rat cortex. Neuropharmacology 15: 1-7.

PAYNE, R. and Anderson, D.C. (1967). Scopolamine produced changes in activity and in the startle response. Implication of behavioural activation. Psychopharmacologia. 12: 83-90.

PERSSON, I., and Waldeck, B. (1969). The interaction between different metabolic pathways of catecholamines in the brain studied by means of 3H-dopa. Acta. Pharmacol. Toxicol 27: 225-236.

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

PIJNENBURG, A.J.J., Woodruff, G.N. and Van Rossum J.M. (1973). Ergometrine-induced locomotor activity following intracellular injection into the nucleus accumbens. Bromn Res. 59: 289-302.

PRADHAN, S.N. and Galambos, R., Brain (1963). Some effects of anaesthetics on the evoked responses in the auditory cortex of cats. J. of Pharmac. and Exp. Ther. 139: 97-106.

PRICE, M.T.C., and Fiberger, H.C. (1974). Apomorphine and amphetamine stereotypy after 6-hydroxydopamine lesions of the substantia nigra. Eur. J. Pharmacol. 29: 249-252.

PSCHEIDT, G.R., Himwich, H.E. (1968). Imipramine affects dopamine uptake by rat brain. J. Pharm. Pharmac. 20: 809-810.

PURI, S.K., and Lal, H. (1974). Reduced threshold to pain-induced aggression specifically related to morphine dependence. psychopharmacologia (Berl.) 35: 237-241.

PURPURA, D.P. (1956). Electrophysiological analysis of psychotogenic drug action. Effect of LSD on specific afferent systems in the cat. Arch. Neurol. Psychiat, 75: 122-131.

RANDRUP, A. and Jonas, W.J. (1967). Brain dopamine and the amphetamine-reserpine interaction. J. Pharm. Pharmacol. 19: 483-484.

RANDRUP, A., and Munkvad, I., (1964). On the relation of tryptaminergic and serotonergic mechanisms to amphetamine-induced abnormal behaviour. Acta Pharmacol. Toxicol. 21: 272-282.

RANDRUP, A., and Munkvad, (1966). Dopa and other naturally occuring substances as causes of stereotypy and rage in rats. Act. Psychiat. Scand. Suppl. 191, 42: 193.

RANDRUP, A., and Munkvad, I. (1967). Stereotyped activities produced by amphetamine in several animal species and man. Psychopharmacologia 11: 300-310.

RANDRUP, A., and Munkvad, I. (1968). Behavioural stereotypies induced by the pharmacological agents. Pharmakopsychiat. Neuropsychopharmakol. 1: 18-26.

RANDRUP, A., and Munkvad, I. (1972). Evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain. Orthomol. Psychiat. 1(1):2.

RANDRUP, A., Munkvad, I., and Usden, P. (1963). Adrenergic mechanism and amphetamine-induced abnormal behaviour. Act. Pharmacol. Toxical 20: 145-157.

RANDRUP, A.J., Shreelkruger, (1966). Diethyldithiocarbamate and amphetamine stereotype behaviour. J. Pharm. Pharmacol. 18: 152.

RENSHAW, B., Forbes, A., and Morison, B.R. (1940). Activity of isocortex and hippocampus: Electrical studies with microelectrodes. J. of Neurophysiol. 3: 74-105.

ROTROSEN, J., Angrist, B.M., Wallach, M.B. and Gershon, S. (1972). Absence of serotonergic influence on apomorphine induced stereotypy. Eur. J. Pharmacol. 20: 133-135.

RYLANDER, G. (1969). Clinical and medico-criminological aspects of addiction to central stimulating drugs. In: Abuse of central stimulants, F. Sjoquist and M. Tottie (eds.) pp. 251-273, Almqvist and Wiksell, Stockholm.

SAMANIN, R., Ghezzi, D., Volzelli, L. and Garattini, S. (1972). The effects of selective lesioning of brain serotonin or catecholamine containing neurones on the anorectic activity of fenfluramine and amphetamine. Eur. J. Pharmacol. 19: 318-322.

SAXENA, P.N., Narsesh Chawla, M.B.L. Johri and Shama Iqbal. (1977). Nature of receptors involved in apomorphine responses in pigeons. Psychopharmacology 53: 89-95.

SCHAMBERG, S.M., Schildkraut, J.J. and Kopin, I.J. (1967). The effects of psychoactive drugs on norepinephrine-^{3}H metabolism in brain. Biochem. Pharmacol. 16: 393-399.

SCHEEL-KRUGER, J., Randrup, A. (1967). Stereotype hyperactive behaviour produced by dopamine in the absence of noradrenaline. Life Sci. 6, 1389-1398.

SCHELKUNOV, E.L. (1964). The technique of 'Phenamine' stereotypy for evaluating the effect produced by remedial agents on the central adrenergic processes. Pharmacol. Toxical (russ) 27. 628.

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

SCHILDKRAUT, J.J., Winokur, A., and Applegate, C.W. (1970). Norepinephrine turnover and metabolism in rat brain after long term administration of imipramine. Science, N.Y. 168: 867-868.

SCHLAG, J., (1973). Generation of brain evoked potentials. In: Bioelectric Recording Techniques, Vol. 1-A, R.F. Thompson and M.M. Patterson (eds.), pp. 273-316. Academic Press, New York.

SCHNIEDEN, H., and Cox, B. (1976). A comparison between amantadine and bromocriptine using stereotyped behavioural response test (SBR) in the Rat. Eur. J. Pharmac. 39: 133-141.

SCHOLES, N.W. (1965). Central action of intravenous curare. Nature 207: 303-305.

SCHOLES, N.W., and Gutnick, M.J. (1970). Relative activity of psychotoxic drugs on the avian optic lobe, Eur. J. Pharmacol. 12: 289-296.

SCHOLES, N.W., and Roberts, E. (1964). Pharmacological studies of the optic system of the chick. Effect of gamma-aminobutyric acid and pentobarbitone Biochem. Pharmacol. 13: 1319-1329.

SCHROLD, J., (1970). Aggressive behaviour in chicks induced by tricyclic antidepressants. Psychopharmacologia (Berlin). 19: 225.

SCHROLD, J., (1972). Behavioural Effect of d-Amphetamine alone and in combination with antidepressants, antihistamines or other psychotropic Drugs in young chicks. Psychopharmacologia (Berl.) 23: 115-124.

SCHROLD, J., and R.F. Squires, (1971). Behavioural effects of d-amphetamine in young chicks treated with p-cl-phenylalanine. Psychopharmacologia (Berl.) 20: 85.

SCHUSTER, C.R., and Thompson, (1969). Self administration and behavioural dependence on drugs. Annu. Rev. Pharmacol. 9: 483-502.

SELLE, R.M. (1940). An effect of benzedrine sulfate on chicks. Science 91: 95.

SELYE, H. (1957). Lathyrism. Rev. Canad. Biol. 16: 1-82.

SELYE, H., Kourounakis, P., Szabo, S. (1972). Protection against the Neurolathyrism produced by arsanilic acid in the rat. Exp. Neurol. 35: 513-518.

SENAULT, B. (1970). Comportement d'aggresivite intraspecifique induit par l'apomorphine chez le rat. Psychopharmacologia (Berl.) 18: 271.

SIEGG, E.B., Soffer, L., and Gyermek, L. (1963). Influence of imipramine and related psychoactive agents on the effects of 5-hydroxytryptamine and catecholamines on the cat. nictitating memberance. J. Pharmac. exp. Ther. 142: 13-20.

SIMON, P., Chermat, R., Fosset, M.TH. et Boissier, J.R. (1972). Inhibiteurs B-adrenergiques et stereotypies provoquees par l'amphetamine ou l'ampomorphine chez le Rat. Psychopharmacologia (Berl.) 23: 357-364.

SINCLAIR, J.D. (1974). Rats learning to work for alcohol. Nature London 249: 590-592.

SMITH, C.B., (1963). Enhancement by reserpine and -methyl DOPA of the effects of d-amphetamine upon the locomotor activity of mice. J. Pharmacol. Exp. Ther. 142: 343-349.

SNYDER, S.H., Banerjee, S.P., Yamamura, H.I., and Greenberg, D. (1974). Drugs, negrotransmitters and Schizophrenia. Science. 184: 1243-1253.

SNYDER, S.H., Taylor, K.M., Coyle, J.T. & Meyerhoff, J.L. (1970). Role of brain dopamine in behavioural regulation and the actions of psychotropic drugs. Amer. J. Psychiat. 127: 199-207.

SPOONER, C.E. (1965). Preparation and implantation of brain electrodes in the young chick. Electroenceph. Clin. Neurophysiol 18: 419-421.

SPOONER, C.E., Winters, W.D. (1966). Neuropharmacological profile of the young chick. Int. J. Neuropharmacol. 5: 217-236.

SPOONER, C.E. Winters, W.D. (1967). Evoked responses during spontaneous and monoamine-induced states of wakefulness and sleep. Brain. Res. 4: 189-205.

STELLAR, E. (1954). The Physiology of Motivation. Psychol. Rev. 61: 5-22.

STOLERMAN, I.P. and Kumar, R. (1970). Preferences for morphine in rats: Validation of an experimental model of dependence, psychopharmacologia 17: 137-150.

STROMBOM, U. (1975). Effects of low doses of catecholamine receptor agonists on exploration in mice. J. Neurol. Transm. 37: 229-235.

SVENSSON, T.H.,(1971). On the role of central noradrenaline in the regulation of motor activity and body temperature in the mouse. Naunyn-Schmiedebergs Arch. Pharmakol. 271: 111-120.

TARSY, D., Baldessarini, R. (1973). Pharmacologically induced behavioural supersensitivy to apomorphine. Nature New Biol. 245: 262 263.

TAYLOR, M., and Randall, P.K. (1975). Depletion of S-adenosyl-L-methionine in mouse brain by antidepressant drugs. J. Pharmac. Exp. Ther. 194: 303-310.

THOMPSON, T., and Schuster, C.R. (1964). Morphine self administration, food-reinforced and avoidance behaviours in rhesus monkeys, Psychopharmacologia 5: 87-94.

THORNBURG, J.E., and Moore, K.E. (1973). The relative importance of dopaminergic and noradrenergic neuronal systems for the locomotor stimulation induced by amphetamine and other drugs. Neuropharmacology 12: 363-366.

TSENG, L.S., Wei, E., and Loh, H. (1973). Brain areas associated with Bulbocapine catalepsy. Eur. HJ. Pharmacol. 5: 107-110.

UNGERSTEDT, U. (1971a). Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behaviour. Acta. Physiol. Scand. 83 (Suppl. 367) 49-68.

UNGERSTEDT, U. (1971 b). Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system, Acta Physiol. Scand. Suppl. 367: 69-93.

VAN ROSSUM, J.M. (1968). Significance of dopamine-receptor blockade for the action of neuroleptics. Excerpta. Med. Internat. Congr. 129.

VAN ROSSUM, J.M., and Hurkmans, J.A. (1964). Mechanism of action of psychomotor stimulant drugs. Significance of dopamine in locomotor stimulant action. Int. J. Neuropharmacol. 3: 227-239.

VAN ROSSUM, J.M. Van der Schoot, J.B., and Hurkmans, J.A. (1962). Mechanism of action of cocaine and amphetamine in the brain. Experientia 18: 229-235.

WAELSCH, H. (1955). Blood-brain barrier and gas exchange. In: Biochemistry of the developing nervous system., H. Waelsch (ed) New York Academic Press, pp. 187-207.

WALKER, D.W. and Zornetzer, S.F. (1974). Alcohol Withdrawal in mice: electroencephalographic and behavioural correlates. Electroenceph. Clin. Neurophysiol. 36: 233-243.

WEINER, W.J., Goetz, C. and Klawans, H.L. (1975). Serotonergic and antiserotonergic influences on apomorphine-induced stereotyped behaviour. Acta. Pharmacol. Toxicol. 36. 155 - 160.

WEINER, W.J., Goetz, C., Westheimer, R., and Klawans, H.L. (1973). Serotonergic influences on amphetamine-induced behaviour, J. Neurol. Sci. 20: 373-379.

WEISSMAN, A., Koe, B.K., and Tenen, S.S. (1966). Antiamphetamine effects following inhibition of tyrosine hydroxylase. J. Pharmacol. Exp. Ther. 151: 339-352.

WILKER, A., Prescor, F.T., Fraser, H.F., and Isbell, H. (1956). Electroencephalographic changes associated with chronic alcohol intoxication and the alcohol abstinence syndrome. Amer. J. Psychiat. 113: 106-114.

WILLIAMS, S., Paterson, R.A. and Heath, H. (1968). The effects of iminodipronitrile and anaesthesia on some adenine nucleotides of rat brain and retina. J. Neurochem. 15: 227-233.

WINTERS, W.D. (1964). Comparison of the average cortical and subcortical evoked response to chicks during various stages of wakefulness slow wave sleep and rhombencephalic sleep. Electroenceph Clin. Neurophysiol. 17: 234-245.

WINTERS, W.D. (1968). Neuropharmacological studies utilizing evoked response techniques in animals. Psychopharmacology: A Review of Progress, 1957-1967. D.H. Eiron, ed. P.H.S. Publication No. 1836, Washington D.C. pp. 453-477.

WINTERS, W.D. and Spooner, C.E. (1965). A neurophysiological comparison of gamma-hydroxybutyrate with pentobarbital in cats. Electroenceph. Clin. Neurophysiol., 18: 287-297.

WOODRUFF, G.N., Elkhawad, A.O., Crossman, A.R. (1974). Further evidence for the stimulation of rat brain dopamine receptors by ergometrine J. Pharm. Pharmacol. 26: 455-456.

WOODRUFF, G.N., Kelly, P.H., and Elkhawad, A.O. (1976). Effects of dopamine receptor stimulant on locomotor activity of rats with electrocytic or 6-hydroxydopamine-induced lesions of the nucleus accumbens psychopharmacology 47: 195-198.

YANAGITA, T., Deneau, G.A., Seevers, M.H. (1965). Evaluation of pharmacologic agents in the monkey by longer-term intravenous self or programmed administration. Excerpta Med. Int. Congr. Ser. 87. 453-457.

ZAIMIS, E. (1960). Discussion. In Vane, J.R. Wolstenholme, G.E.W. and O'Connor, M., Ciba Foundation Symposium on Adrenergic Mechanisms Churchill, London. pp. 562.

APPENDIX

A. STATISTICS.

A.1. HP 25 calculator was used for the statistical calculations using standard programmes for student - t - test.

A.2. Theory for student - t - test.

Assuming that

X_{1i} X_{2i} .....X_1n_1 and

X_{21} X_{22} .....X_2n_2

are random samples from two normal populations with the same varience -

$$\bar{X}_1 = \frac{1}{n_1} \sum_{i=1}^{n_1} X1i$$

$$\bar{X}_2 = \frac{1}{n_2} \sum_{i=1}^{n_2} X_{2i}$$

The combined estimator of the population is given by

$$S^2 = \frac{\sum_{i=1}^{n_1} (X_{1i} - \bar{X}_1)^2 + \sum_{i=1}^{n_2} (X_{2i} - \bar{X}_2)^2}{n_1 + n_2 - 2}$$

In evaluating S^2, it is more conveniently written as $S^2 = \sum_{i=1}^{n_1} X_{1i}^2 + \bar{X}_1 \sum_{i=1}^{n_1} X_{1i} + \sum_{i=1}^{n_2} X_{2i}^2 + \bar{X}_2 \sum_{i=1}^{n_2} X_{2i}$

Then the standard error of $\bar{X}_1 - \bar{X}_2$ is $S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$

The t statistic to test the equality of the two population means is given by:- $t = \bar{X}_1 - \bar{X}_2 \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$