THE EFFECT OF GONADAL STEROIDS AND ALTERED TRYPTOPHAN METABOLISM ON BEHAVIOUR

BY

Stephen William Bond

Ph.D. Thesis

Department of Pharmacy

University of Aston in Birmingham

April 1979

S U M M A R Y

GONADAL STEROIDS - TRYPTOPHAN METABOLISM - BEHAVIOUR

Gonadal steroids have been shown to affect tryptophan metabolism in both brain and body, and are thus thought capable of altering the rate of 5-hydroxyptamine synthesis, and consequently, serotoninergic neuronal activity. Subsequent behavioural changes should result, and a primary aim of the research was to elucidate such changes within specific behavioural parameters.

In order to discern more exactly the effect of gonadal steroids on behaviour, other, more specific, alterations in tryptophan metabolism were undertaken using various pharmacological manipulations. Alterations in behaviour after such manipulations were then observed, within the previous behavioural parameters.

A clinical study was carried out in order to ascertain the effect of gonadal steroids, in the form of the oral contraceptive, on plasma tryptophan, mood and behaviour in the human female.

The results suggest that gonadal steroids influence behaviour by increasing 5-hydroxytryptamine synthesis. In general, such an effect led to an exhibition of behavioural depression, manifested as reduced locomotor activity and startle response magnitude.

A paradoxical effect of tryptophan on behaviour was discovered. Increasing doses of l-tryptophan were found to induce heightened startle response magnitude while reducing spontaneous locomotor activity.

It was concluded that both the contraceptive effect and changes in behaviour, brought above after the administration of synthetic gonadal steroids, were caused by increased serotoninergic neuronal activity. It was suggested that these gonadal steroids induce changes in the equilibrium between the catecholaminergic and serotoninergic neuronal systems, causing the latter to predominate.

The hyperactivity syndrome, reported after the concurrent administration of a monoamine oxidase inhibitor and either l-tryptophan or 5-hydroxytryptophan, was thought to be due to increased brain tryptamine levels, rather than the associated increase in brain 5-hydroxytryptamine levels.

ACKNOWLEDGEMENTS

This thesis is an account of original work carried out in the Department of Pharmacy, University of Aston in Birmingham. The study was carried out during the tenure of a research scholarship from the Science Research Council, to whom I am grateful.

I should like to thank Professor C.B. Ferry for providing the facilities for this research. I am extremely grateful to Dr. Sheila L. Handley for her considerable help, encouragement and friendship during the supervision of this study. I should also like to thank Dr. Judith M. Baker for her assistance with the tryptophan assays.

Finally, I should like to thank my wife Mary, for her encouragement and tolerance during the course of this work, and for her help in typing the manuscript.

THE AIM OF THE THESIS

One of the most widely discussed current ideas concerning a possible biological basis of the affective disorders, is that the metabolism of biogenic amines may be altered in such conditions. A series of similar hypotheses have been derived from the theory that biologically active monoamines, known to affect smooth muscle and other peripheral tissues, function in the central nervous system as neurotransmitters at chemically mediated synapses, modulating the activity of central neurones involved in the regulation of mood and behaviour. The amines usually discussed in this context are the catecholamines, noradrenaline and dopamine, the indoleamine, 5-hydroxytryptamine (serotonin) and the quaternary amine, acetylcholine. These hypotheses suggest that depression is associated with altered availability of one or other of these amines at functionally important sites (termed "receptors"), and that mania is associated with a converse alteration in availability to that in depression.

The aim of this thesis is to study further the hypothesis concerning 5-hydroxytryptamine, with particular reference to biochemical and behavioural changes associated with the administration of synthetic contraceptive steroids in animals. Such substances have been shown to increase the incidence of depression in women taking them.

CONTENTS

INTRODUCTION

THE ANATOMICAL DISTRIBUTION AND METABOLISM OF 5-HYDROXYTRYPTAMINE (5-HT) IN THE CENTRAL NERVOUS SYSTEM

(i) Anatomical Distribution

Significant amounts of 5-HT have been found in mammalian brain (Twarog and Page 1953, Pletscher et al 1956), especially in the mesencephalon (mid-brain) and diencephalon (hypothalamus and thalamus), the highest concentration being found in the hypothalamus and the caudate nucleus, the lowest in the cortex, and none in the cerebellum (Amin et al 1954, Bertler and Rosengren 1959).

The advent of fluorescence histochemistry (Falck et al 1962) enabled 5-HT to be more precisely located in the CNS, 5-HT giving a characteristic yellow fluorescence after treatment with formaldehyde. Work following the introduction of this technique categorised levels of 5-HT, noradrenaline and dopamine in the various areas and nuclei of the brain.

High concentrations of 5-HT have been found in the limbic system and basal ganglia (Garrattini and Valzelli 1965), the lower brain stem and spinal cord (Carlsson et al 1962, Fuxe 1965) and only in the suprachiasmatic nucleus and anterior part of the median eminence of the hypothalamus (Hamon et al 1970, Fuxe et al 1970).

It has been possible to map out pathways of 5-HT noradranaline and dopamine in the brain, by cutting nerve fibres or making lesions in precisely located areas and noting the fall in fluorescence in other areas, which must, therefore, be innervated by axons coming from cell bodies in the lesioned area (Ungerstedt 1971). For example, lesions in the median forebrain bundle in the lateral hypothalamus cause a fall in 5-HT in all the more rostral areas of the brain (Harvey et al 1963, Heller and Moore 1965, Parent et al 1969). Confirmation of the pathways can be obtained by stimulating the areas of their origin (the cell bodies) and noting the decrease in fluorescence at the nerve terminals, in animals pretreated with amine synthesis inhibitors (Fuxe and Gunne 1964, Arbuthnott et al 1970).

Serotoninergic fibres originate caudally in the cell bodies of the median raphe of the pons and descend into the spinal cord. They ascend from the more rostral raphe in the median forebrain bundle, pass to the inderpeduncular nucleus where they divide, the dorsal branch going to the cortex and the ventral branch travelling anteriorly through the lateral hypothalamus. The 5-HT neurones then separate out to innervate the septum, the cingulum, the amygdala and particularly, the suprachiasmatic nucleus, the retrochiasmatic area and the median eminence of the hypothalamus (Fuxe et al 1968, Parent et al 1969, Ungerstedt 1971).

(ii) Synthesis and Metabolism

5-HT is synthesised from the exogenous aminoacid tryptophan within the neurones, under the control of the enzymes tryptophan hydroxylase and l-aromatic amino acid decarboxylase.

Tryptophan → 5-Hydroxytryptophan → 5-Hydroxytryptamine

Tryptophan Hydroxylase; l-aromatic amino acid decarboxylase

The distribution of tryptophan hydroxylase correlates with the distribution of neurones liberating 5-HT at their synapses (Ichiyama et al 1970). It is synthesised in the cell bodies of the raphe nuclei and slowly transported by axonal flow to the nerve terminals (Meek and Neff 1972). It has been shown to exist in two forms, molecular and soluble. The molecular form exists in parts of the brain containing many 5-HT nerve endings, such as the frontal cortex, septum, sacral and lumbar spinal cord (Graham-Smith 1964), whereas the soluble form is found in parts of the brain containing many 5-HT nerve bodies, such as the mesencephalon and the brain stem (Dahlström and Fuxe 1964, Knapp and Mandell 1973).

The hydroxylation of tryptophan to 5-hydroxytryptophan was initially considered as the rate limiting step in the synthesis of 5-HT (Green and Sawyer 1966, Moir and Eccleston 1968). However, tryptophan hydroxylase has a Michaelis constant for its substrate much higher than the concentration of tryptophan normally present in the mammalian brain (Jequier et al 1967, Peters et al 1968). Thus it was suggested that the rate of synthesis of 5-HT in the brain should depend on the availability of tryptophan (Gessa and Tagliamonte 1974), because intraneuronal concentrations of tryptophan may not be sufficient to saturate the enzyme and small variations in the availability of the amino acid could rapidly effect the rate of 5-HT synthesis (Glowinski et al 1963). Knott and Curzon (1974), indicated that the overall rate of 5-HT synthesis is at least partially dependent on brain tryptophan levels.

Brain tryptophan levels appear to be controlled by two major factors, the efficiency of the uptake process (Hamon et al 1974) and the availability of plasma tryptophan. Tryptophan is unusual in that it is the only amino acid that is bound to a plasma protein, being approximately 90% bound to plasma albumin (McMenamy and Oncley 1958). As only the unbound fraction of tryptophan can be taken up by the brain, plasma free tryptophan has been suggested as being another important factor in controlling the transport of tryptophan to the sites of 5-HT synthesis (Gessa and Tagliamonte 1974).

Tryptophan is taken up by two transport systems, one of high affinity, the other of low affinity (Parfitt and Graham-Smith 1974), and can be influenced by a number of factors. From experiments involving studies of the passage of amino acid from blood to brain (Guroff and Udenfriend 1962, Olendorff 1971), and the uptake of amino acids by brain slices (Blasberg and Lajtha 1965), and synaptosomes (Graham-Smith and Parfitt 1970), it has been suggested that aromatic

amino acids share the same transport system from blood to brain in such a way that a high concentration of one can lower the uptake of the others. Thus tryptophan concentration in the brain might depend not only on the concentration of plasma free tryptophan but on the plasma concentration of other amino acids competing for the same transport system (Fernström and Wurtman 1971).

Fernstrom and Wurtman have suggested that dietary factors may influence tryptophan levels in both blood and brain, and thus alter 5-HT synthesis. Carbohydrate ingestion elicits insulin secretion, thus raising plasma tryptophan and lowering the concentrations of competing neutral amino acids (Fernström and Wurtman 1972), hence the ratio of plasma tryptophan to competing amino acids increases, leading to elevations in brain tryptophan and 5-HT. In contrast consumption of protein provides the plasma with an exogenous source of all amino acids. However, the ratio of tryptophan to its competitor amino acids is almost always lower in dietary proteins than it is in the plasma. For this reason, protein ingestion increases the plasma concentrations of tryptophan less than it does the concentrations of competing amino acids, thereby decreasing the ratio of tryptophan to competitor amino acids. The insulin secretion elicited by protein consumption will, by itself, produce an opposite change in this ratio. Thus the amount of tryptophan and 5-HT in the brain can decrease, increase or remain unchanged after eating, according to the proportion of protein to carbohydrate in the diet, and the amino acid composition of particular proteins. It has been suggested that diets containing high concentrations of protein actually decrease the synthesis of 5-HT in rat brains (Fernström and Wurtman 1974).

Glowinski (1973), proposed a hypothetical model for the assimilation of tryptophan into the brain. This suggests that cyclic-AMP, present in the glial cells of the brain, is responsible

for the accumulation of tryptophan into brain neuronal fibres. Further studies (Hamon and Glowinski 1974), suggested that tryptophan newly taken up into the serotoninergic neurones is preferentially used in 5-HT synthesis. This suggests that tryptophan is not localised in a homogenous pool in tissues but compartmentalised. Thus the size and turnover of the tryptophan pool, contributing mainly to the synthesis of 5-HT, would be an extremely important influence on the rate of 5-HT synthesis. If this is so, "global" estimation of the specific activity of tryptophan in tissues, made in order to calculate the rate of 5-HT synthesis, would be of limited value.

Hÿppa (1974), has also suggested the importance of the existence of multiple pools of brain 5-HT.

In contrast to the catecholamines, which inhibit the activity of tyrosine hydroxylase within the neurone, 5-HT has no effect on the activity of tryptophan hydroxylase (Jequier et al 1969). This is why the hypothesis of an end-product regulation in vivo was not retained for many years. However, 5-HT synthesis is reduced when intraneuronal levels of the amine reach 2.5 times normal level in vivo (Macon et al 1971), following M.A.O. inhibition. This effect can also be observed in brain slices of animals pretreated with M.A.O. inhibitors (Hamon et al 1973). Inhibition of 5-HT synthesis is also observed in slices when intraneuronal stores of 5-HT in serotoninergic terminals are increased with exogenous 5-HT. This inhibitory process occurs during the first step of amine synthesis (Carlsson and Lindqvist 1972, Hamon and Glowinski 1974).

The existence of an end-product regulation of 5-HT synthesis in pharmacological circumstances is now well established. Whether or not this mechanism plays a part in controlling the rate of tryptophan hydroxylation under physiological stimulation or inhibition of serotoninergic neurone activity remains undemonstrated.

Kleinrock (1975), has suggested that both mechanisms, that is, the ability of neurones to take up tryptophan, and to a lesser degree, the level of 5-HT in the brain, are essential in the regulation of 5-HT synthesis.

Only a very small percentage of plasma tryptophan is actually utilised by the brain, the major metabolic route being the kynurenine pathway of the liver (Figure I).

5-HT is stored similarly to the other amines within the brain, that is, either in a "bound" form or a "functional" store (Iversen 1967, Aprison and Hingtgen 1972). As the most newly synthesised transmitter is the most easily released (Hamon et al 1970, Farnebo et al 1971), it seems that the amines must fill the "functional" store before filling the "bound" store. Electron microscope studies have shown that the amines are present in small granulated vesicles within the nerve terminals, which presumably are the storage sites (Kobayashi and Matsui 1969), although the anatomical distribution of the "bound" and "functional" stores are not known. The storage granules are thought to be formed in the cell bodies of the neurones and pass down the axon to the nerve terminals. This has been shown using histofluorescent studies with noradrenergic axons (Dahlstrom 1967).

5-HT is released from its nerve terminals after an action potential, and is dependent on the presence of chloride ions (Goodwin et al 1969, Katz and Kopin 1969). Release of 5-HT after electrical stimulation has been shown in vivo. Stimulation of the mid-brain raphé causing a fall in 5-HT in the forebrain with a concomitant rise in its degradation product 5-HIAA . (Aghajanian et al 1967, Sheard and Aghajanian 1968). Electron microscopy has shown that the amines are released from their storage granules by exocytosis, in which the contents are released into the synaptic cleft. At noradrenergic

FIGURE 1

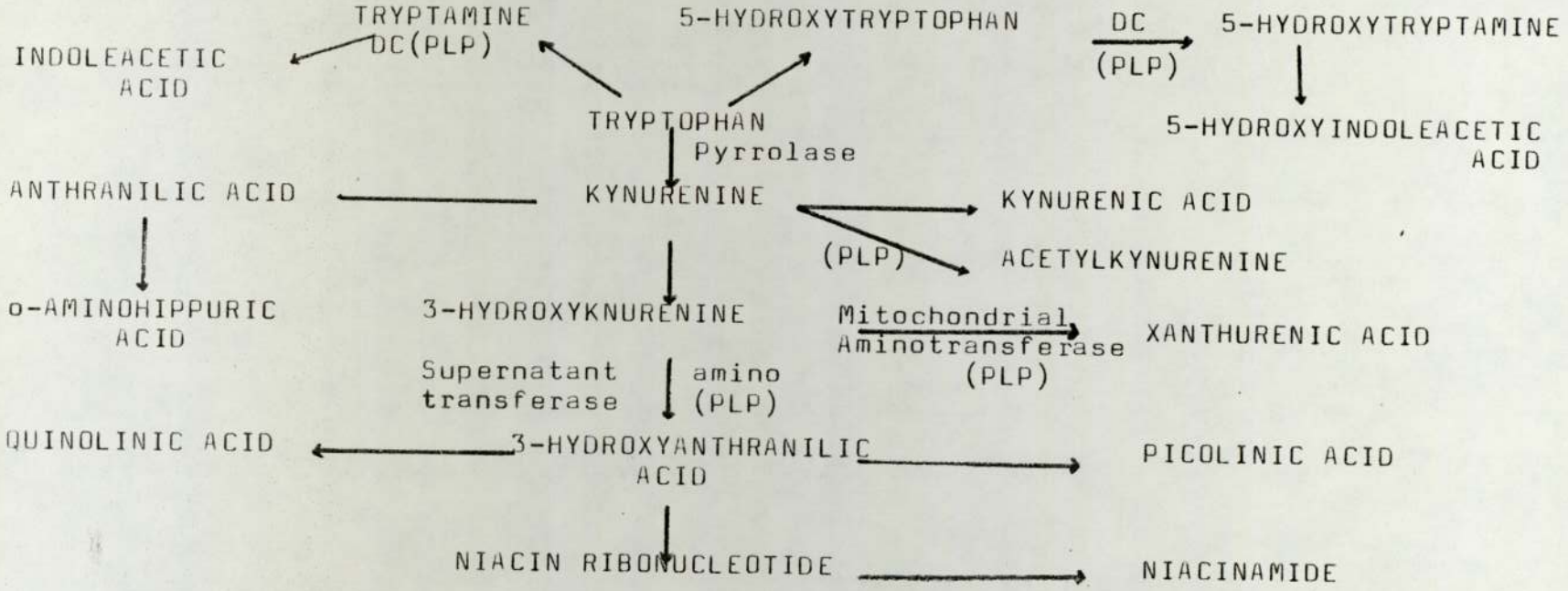


FIG. 1 : Tryptophan Metabolism. A simplified diagramatic pathway.
PLP: Pyridoxal Phosphate
DC: Aromatic Amino Acid Decarboxylase

nerve terminals, noradrenaline, dopamine B-hydroxylase and specific proteins have been shown to be discharged after stimulation (Malamad et al 1968).

5-HT is removed from its site of action by a specific re-uptake system back into the nerve terminals, where it is either restored or metabolised by the intraneuronal enzyme, monoamine oxidase, which is found associated with the mitochondria (Schnaitman et al 1967). Monoamine oxidase has no regional specificity, but has its highest concentrations in the hypothalamus (La Motte et al 1969). M.A.O. converts 5-HT to 5-HIAA. Unlike the catecholamines, tryptophan derivatives are not degraded by catechol-o-methyltransferase, but enzymes have been found in the exclusivity of the pineal gland, for converting 5-HT to melatonin i.e. N-acetylase and hydroxyindole-o-methyltransferase (HIOMT) (Wurtman et al 1968). Two further enzymes capable of metabolising 5-HT have also been found. One is an N-methyltransferase which has been identified particularly in the pituitary and pineal (Mandell and Morgan 1971), and converts 5-HT to its methyl derivatives, which may be psychogenic (Himwich 1971, Brimblecombe 1974). The other enzyme is 5-hydroxytryptamine sulphotransferase, which has been found in the soluble fraction of neuronal tissue and this enzyme converts 5-HT to 5-hydroxytryptamine-o-sulphate (Hidaka et al 1969).

Figure 2 shows the metabolic pathways for 5-HT.

FIGURE 2

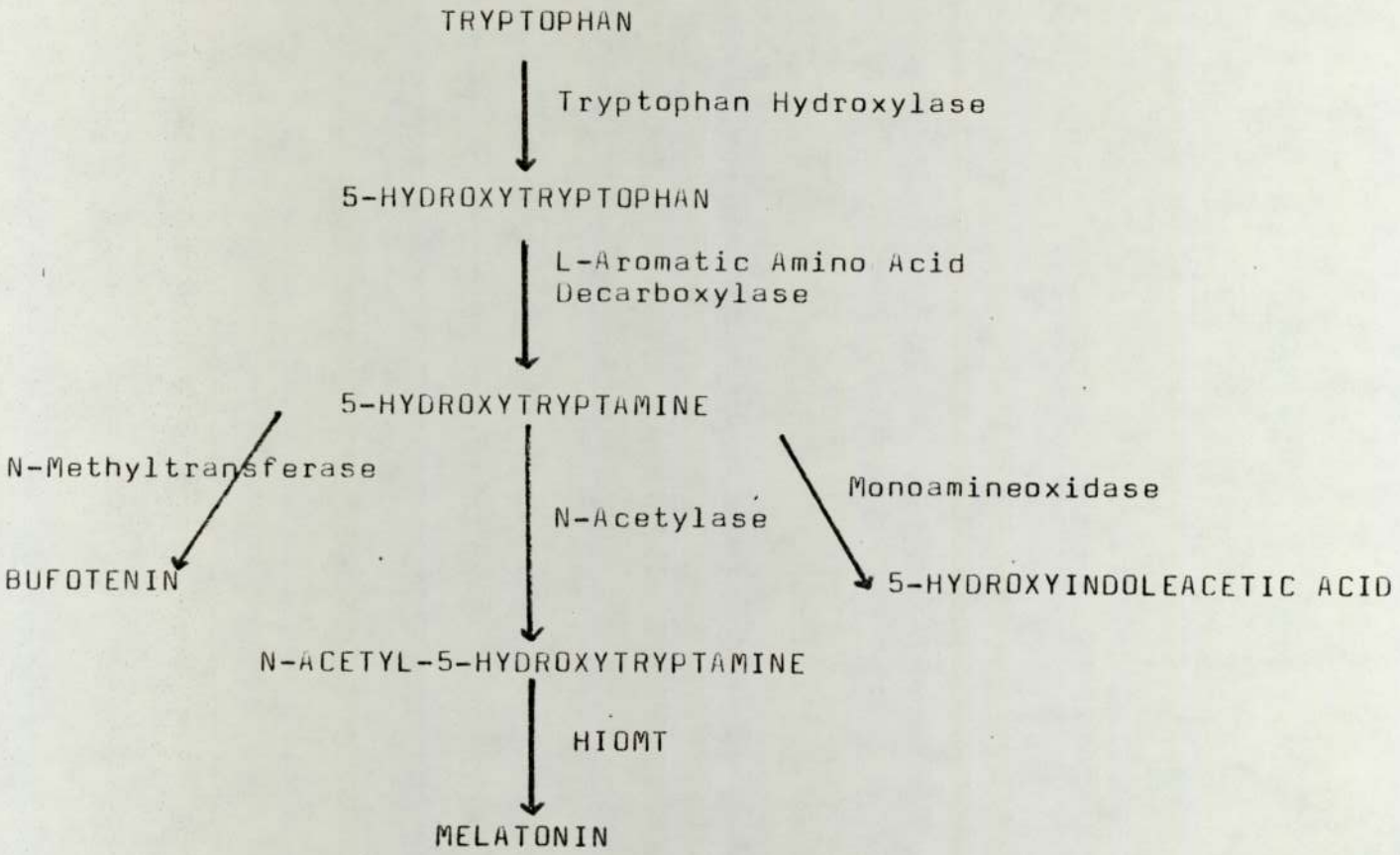


FIG. 2 : Biosynthesis and Metabolism of 5-HT.
A simplified diagramatic pathway.

BIOGENIC AMINES AND DEPRESSION

There is now a considerable amount of evidence suggesting that the biogenic amines are involved in the aetiology of the affective disorders. Initial hypotheses suggested that clinical depression was associated with a functional deficiency of noradrenaline or 5-HT at certain receptor sites in the brain (Schidkraut 1965, Prange 1964). They were based on the investigation of the pharmacological action of antidepressant drugs plus those drugs thought to induce a depression like syndrome.

Monoamine oxidase (MAO) inhibitors inactivate the monoamine degrading enzyme MAO, thus inhibiting the degradation of the monoamine in the neurone. This results in a "leakage" of unbound monoamine (i.e. monoamine not taken up into the synaptic vesicles) to the synaptic cleft. Tricyclic antidepressants block the monoamine "uptake-pump", that is, the active transport system by which a monoamine, having transmitted the impulse from one neurone to the next, is pumped back from the synaptic cleft to the synaptic vesicles. The two types of antidepressants, therefore, influence central monoamine metabolism in different ways, while producing the same net effect, an increased concentration of physiologically active monoamine at postsynaptic receptors, and thus an increased activity of the monoaminergic neuronal systems. It would seem that almost all clinically useful antidepressant drugs, will on acute administration, increase the amount of monoamines at postsynaptic receptor sites (Lapin and Oxenkrug 1969).

However, most of these studies involved the acute administration of such drugs, and certain investigations report a reduction in brain 5-HT concentrations with chronic tricyclic administration (Alpers and Himwich 1972). Post and Goodwin (1975), suggested that chronic tricyclic administration may lead to a decrease in 5-HT turnover, and

Bowers (1974), also discovered a reduction in serotonin (5-HT) turnover in depressed patients undergoing chronic amitriptyline administration. This paradox might be resolved if measurement of amine turnover reflects presynaptic events. Thus if postsynaptic 5-HT receptor activity were actually increased in some depressed patients, one might expect to find decreased 5-HT turnover presynaptically, as a result of feedback regulatory mechanisms (Post and Goodwin 1974). If this were the situation, the antidepressants might work by a direct action on the presynaptic neurone (Bruinvels1972), resulting in a further reduction om 5-HT turnover, a reduction sufficient to overcome the excessive postsynaptic activity.

EVIDENCE FOR THE INVOLVEMENT OF 5-HT IN DEPRESSION

In the exploration of the indoleamine hypothesis of depression several distinct experimental stratagies have been adopted to elucidate whether a central deficiency of 5-HT exists in depressive patients.

a) Post-mortem Studies

Several independent investigations have determined the concentrations of 5-HT and its principal metabolite 5-HIAA in the brainstem of depressive suicide victims. The findings, although not identical, presented the same trend, a lower concentration of 5-HT and 5-HIAA than in a comparable control group (Shaw et al 1967, Bourne et al 1969, Pare et al 1969, Lloyd et al 1974). This would seem to suggest a decreased synthesis of 5-HT. Birkmayer and Riederer (1975), found decreased 5-HT levels in all brain areas of depressed patients who had died from causes other than suicide.

b) Measurement of Amine Metabolites in the Lumbar Spinal Fluid

Another source of information on whether a central 5-HT deficiency occurs in depressed patients, is the concentration of 5-HT metabolites in the cerebrospinal fluid (CSF). Studies of this type are based on the

argument that the concentration of the metabolite in the CNS is related to the amount of corresponding amine which is locally metabolised. There is also a relationship between the concentration of the metabolite in the brain and spinal cord, and that in the CSF. Thus the CSF concentration of an amine metabolite reflects the amount of corresponding amine metabolised in the CNS (Van Praag 1974).

It must be emphasised that any differences found in lumbar CSF must be interpreted with caution, as many factors are known to influence the CSF concentration of 5-HIAA, the main metabolite of 5-HT. CSF concentrations are known to vary with age, and ventricular and cisternal fluid have higher concentrations than lumbar fluid, so that the test must be standardised, and the same amount of fluid taken off by the lumbar tap. It has also been suggested that, as 5-HIAA is transported out of the CSF by an active process, any change in the concentration of lumbar 5-HIAA may reflect changes in the transport of 5-HIAA out of the CSF, rather than any change in central 5-HT turnover (Coppen 1972). A further significant point, is the suggestion that amine metabolities in the CSF reflect spinal cord metabolism rather than brain metabolism.

Early reports found decreased concentrations of 5-HIAA in the CSF of depressive patients (Ashcroft et al 1966, Dencker et al 1966), but this was not confirmed by Bowers et al (1969). Later reports (Coppen 1972), would seem to be in agreement with the finding of reduced concentrations of 5-HIAA. However, both Nordin (1971), and Coppen (1972), report that there is no change in the concentration of 5-HIAA in lumbar CSF after clinical recovery. Thus if these low levels of CSF 5-HIAA are an index of abnormal 5-HT activity in the CNS, it would seem that it persists even after apparently full recovery.

In an attempt to gain more meaningful conclusions about the functional state of dynamic pools of biogenic amines a technique which might reflect the "turnover" of such amines in the CNS was sought. To

this end the probenecid test was used. The rationale for the use of probenacid in the study of 5-HT turnover in the CNS is based on the findings that probenecid inhibits the transport of 5-HIAA out of the CSF and that the rate of elevation of these metabolites gives turnover values which are comparable to those obtained by other methods (Neff et al 1967). Slight accumulation of 5-HIAA suggests a low production and, therefore, a low degree of degradation of 5-HT and thus low turnover of 5-HT. Marked accumulation suggests the reverse: high production of 5-HIAA, a high degree of degradation of 5-HT, and, therefore, a high turnover of 5-HT (Van Praag 1974). The findings of a number of investigators indicate that in depressive patients, 5-HIAA accumulation is significantly smaller than in the control group (Roos et al 1969, Goodwin et al 1973, Van Praag et al 1973, Post and Goodwin 1974). This is interpreted as suggesting that the turnover of 5-HT is lower in depressive patients.

The finding that 5-HIAA does not rise on clinical recovery, unlike the levels of noradrenaline metabolites, has lead to the suggestion that a 5-HT dysfunction may underly the tendency to repeated attacks of depression, while noradrenaline dysfunction may be responsible for the actual attack (Kety 1971).

c) Administration of Tryptophan

With the realisation that the rate of cerebral 5-HT synthesis is largely determined by the amount of tryptophan which is locally available, and that brain tryptophan concentration is closely related to the plasma concentration of free tryptophan (i.e. the tryptophan fraction not bound to serum albumins), (Knott and Curzon 1972), recent studies have concentrated on tryptophan metabolism in depressive patients.

Both the CSF concentration of tryptophan and plasma free tryptophan concentration have been found to be lower in depressive

patients than in a matched control group (Coppen et al 1972a, 1972b). Should the CSF concentration of tryptophan be representative of the situation in the brain, then this finding could explain the decreased 5-HT turnover suspected on the basis of the probenecid test (Van Praag 1974).

There are also indications suggestive of a different explanation - a reduced capacity to convert tryptophan to 5-HT. Van Praag (1974), showed that in depressive patients given an oral load of tryptophan, tryptophan increased markedly in the CSF, whereas 5-HIAA in the CSF showed a less marked increase compared to the control group. This suggests that the tryptophan administered congests in the CNS of depressive patients, and that it is less readily converted to 5-HT. This could also explain a diminished 5-HT turnover. However, Ashcroft et al (1972), found no change in 5-HIAA levels in the CSF of depressed patients compared to the controls.

Conflicting reports abound on the use of tryptophan as an effective antidepressant. Coppen et al (1967, 1972), has reported it to be as effective an antidepressant as imipramine or electroconvulsive therapy (ECT), whereas Murphy et al (1972), and Mendels et al (1975), have found it to have no significant antidepressant effects. The concurrent administration of tryptophan and MAO inhibitors will enhance the antidepressant effects of the latter (Coppen et al 1963, Pare 1963, Glassman et al 1969), but the pharmacological basis of this remains uncertain.

Studies of plasma tryptophan have shown that while total tryptophan levels remain unchanged, the unbound fraction appears to be markedly reduced in depressed patients (Coppen et al 1972a, 1974). Aylward and Maddock (1973), demonstrated the existence of a correlation between plasma tryptophan, and the exhibition of symptoms of mental depression. Prior to this, tryptophan metabolism had been reported to be abnormal

in patients with rheumatoid arthritis (McMillan 1960, Finn et al 1964), and that most of the clinically effective anti-rheumatic drugs would displace tryptophan from human plasma proteins in vitro (McArthur et al 1969). Aylward and Maddock (1973), investigated the relationship between clinically active anti-inflammatory drugs and their ability to displace tryptophan from plasma proteins in vivo. They found that the amelioration in depression scores was greatest in those patients with the highest plasma free tryptophan.

However, more recent reports have cast doubts on the simplistic suggestion that plasma free tryptophan is inevitably lowered in depression. Peet et al (1976), found it to be unchanged and Niskanen et al (1976), have found it to be raised. Stein et al (1976), recently found that on day six, post partum, plasma free tryptophan concentrations were significantly reduced in patients with the most severe depression.

EVIDENCE FOR THE INVOLVEMENT OF CATECHOLAMINES IN DEPRESSION

Considerable evidence has been put forward relating altered catecholamine synthesis to affective disorders. In the early and mid-1960's, it was hypothesised that clinical depression was associated with a functional deficiency of noradrenaline at crucial receptor sites in the brain, whereas mania would be associated with a functional excess (Prange 1964, Bunney and Davis 1965, Schildkraut 1965). The evidence for such a relationship results mainly from the mechanism of action of mood-altering drugs. Thus a depletion of noradrenaline effected by reserpine is related to its behaviourally depressant properties, and an elevation by MAO inhibitors is associated with the antidepressant effects of these drugs. Similarly, the antidepressant effect of the tricyclic drugs is thought to be due to a potentiation of the response of released noradrenaline by their inactivation of the neuronal re-uptake mechanism.

Until recently, the available data were consistent with the above hypothesis. Now, however, it has been suggested that depression may not be explained solely by a functional deficiency of noradrenaline (Shopsin et al 1974, Maas 1975).

Although it has been suggested that reserpine could induce clinical depression by a reduction in noradrenergic activity, reserpine induced behavioural depression in animals is reversed by l-Dopa, a compound found to be ineffective in most cases of clinical depression (Goodwin et al 1970).

Initial reports suggested that depressed patients excrete significantly less 3-methoxy-4-hydroxyphenethylene glycol (MHPG) into the urine than non-depressed controls (Maas et al 1968), MHPG being the principal metabolite of brain noradrenaline. Other reports have shown that patients excreted less MHPG during periods of depression than they did during period of euthymia or hypomania (Bond et al 1973, Jones et al 1973).

It has also been shown that recovered unipolar depressed patients excreted greater amounts of MHPG relative to the period of depression (Shaw et al 1973).

Maas (1975), has suggested that while the studies indicate that depressed patients, as a group, excrete considerably less MHPG than do healthy subjects, close inspection of individual patients shows that many with depression excrete normal, or greater than normal amounts of MHPG. This led to the conclusion that patients who excrete less than normal amounts of MHPG are of a particular sub-group, that has special biochemical or pharmacological characteristics.

Citing other biochemical studies on such patients,(Maas 1975) has suggested that there are two identifiable sub-groups of depressed patients. The first group is characterised by low pre-treatment MHPG level, favourable response to imipramine treatment, brightening of mood following dexamphetamine, modest increases or no change in urinary MHPG after imipramine, and failure to respond to amitriptyline. The second group of patients is characterised by normal or high urinary MHPG level, favourable response to amitriptyline treatment, lack of mood change after dexamphetamine, reduction in urinary MHPG after imipramine and a failure to respond to imipramine.

In a summary of the amine hypotheses in affective illness, Baldessarini (1975), states: "On balance, the behavioural and pharmacological data support a catecholamine hypothesis much more consistently than an indoleamine hypothesis. An alternative position would be that an indoleamine deficiency might help to explain some features of depressive illness, such as insomnia and possibly some aspects of agitation, while a deficiency of catecholamines would perhaps better explain decreased drive, pleasure, enthusiasm, and appetite for food and sex, particularly in retarded depressions. Since both amines undergo importance diurnal variations, a deficiency in either one might underline the diurnal pattern of depressive symptoms."

NEUROCHEMICAL CORRELATES OF BEHAVIOUR

1. LOCOMOTOR ACTIVITY

The administration of the 5-HT precursor tryptophan has been found to have little effect on locomotor activity. Both Graham-Smith (1971), and Jacobs et al (1974), found tryptophan to have no effect on locomotor activity when given systemically, and the latter investigators found no effect after dietary variations in tryptophan. It has been hypothesised that tryptophan does not affect behaviour because, despite increasing 5-HT synthesis, little or none of the newly formed 5-HT can be stored and thus it is metabolised by intra-neuronal MAO (Moir and Eccleson 1968, Graham-Smith 1971, Marsden and Curzon 1976). Very high doses of tryptophan(800mg/µg IP) have been found to cause inhibition of locomotor activity (Modigh 1973), and in attempting to analyse the role of different pathways for the effect on locomotion, Modigh administered tryptophan to mice pre-treated with inhibitors of different serotonergic synthesis pathways. Tryptophan was found to induce locomotor activity after blocking 5-HT and tryptamine formation, which was prolonged after pyrrolase inhibition. Both Modigh and Carlsson (1973), have suggested that the inhibitory action of tryptophan on locomotor activity may be due to be action of the aminoacid itself rather than any action on 5-HT synthesis. They have suggested that the inhibitory effect may be caused by tryptophan blocking the transport of other essential aminoacids into the neurones of the CNS.

Motor activity has been reported both to decrease after treatment with 5-HTP (Brown 1960, Jacobs and Eubank 1974), and to undergo no change (Smith and Davis 1962). In contrast, after treatment with a peripheral decarboxylase inhibitor, animals given 5-HTP underwent marked behavioural excitation (Harita and Hamilton 1970), motor activity being stimulated in mice. Previous depletion of catecholamine stores

using α-methylparatyrosine (AMPT), did not modify this action of 5-HTP, suggesting 5-HT receptor activation may be responsible for this stimulation in activity.

It would seem that the sedative effect of 5-HTP, when given alone, may be at least partially due to the accumulation of 5-HT in the peripheral tissues. In support of this contention, systemically administered 5-HT has been shown to inhibit locomotor activity in mice (Brown 1957, Kobinger 1971, Jacobs and Eubanks 1974), although a central effect might account for this is, as some investigators have reported, 5-HT does cross the blood-brain barrier in significant quantities (Costa and Aprison 1958, Bulat and Supek 1967). 5-HTP may also act indirectly by displacing brain catecholamines (Fuxe et al 1971, Butcher et al 1972), besides increasing brain 5-HT synthesis.

Manipulations of forebrain 5-HT levels by selective lesions of the raphe nuclei, have been shown to reduce locomotor activity significantly. Geyer et al (1976), investigated differential lesions of the raphe nuclei. Lesions of the lateral raphe nuclei had no effect on tryptophan hydroxylase activity or behaviour, although lesions of the dorsal nuclei did produce significant reductions in tryptophan hydroxylase activity without any significant alterations in behaviour. However, lesions of the median raphe nuclei produced significant reductions in tryptophan hydroxylase in the hippocampus, septal nuclei, hypothalamus and cortex with significant behavioural changes reflecting a general increase in responsivity. These lesions produced similar increases in locomotor activity to those reported after combined raphe lesions (Kostowski et al 1968, Lorens et al 1971, Neill et al 1971), with a general increase in locomotor activity during the dark phase of the light cycle.

Jacobs and Cohen (1976), have also reported increases in locomotor activity after median raphe lesions, and have attributed this

specifically to almost total depletion of hippocampal 5-HT.

Increased locomotor activity has also been reported after lesions to Guddens tegmental nuclei. Such lesions significantly reduced 5-HT levels in the diencephalon, hippocampus and the telencephalon, producing similar activity increases to those reported after lesions of the midbrain raphe (Lorens et al 1975). The GTN are known to be linked to the midbrain raphe nuclei by a neuronal pathway.

Several reports have shown that reductions in brain 5-HT synthesis in the brain after p-chlorophenylalamine (p-CPA) caused an increase in locomotor activity (Pirch 1969, Kulkarni et al 1974, Jacobs et al 1975). This effect has been shown to be reversed by the immediate 5-HT precursor, 5-HTP (Geller and Blum 1970, Fibiger and Campbell 1971), but not by the more distant precursor tryptophan. It has been suggested that this has been due to the dose of p-CPA being sufficient to inhibit tryptophan hydroxylase almost completely, so that tryptophan was no longer an effective precursor of 5-HT (Marsden and Curzon 1976). However, tryptophan has been shown to increase 5-HT in rats given p-CPA at a dosage which decreased brain 5-HT by about 50% (Curzon and Marsden 1975), and has been shown to reverse the increase in activity brought about by such a depletion in 5-HT (Marsden and Curzon 1976).

5-HT has also been shown to inhibit locomotor activity previously stimulated by various pharmacological agents. Neuberg and Thut (1974), reported that 5-HT will antagonise the locomotor activity caused by amphetamine, a finding recently confirmed by Warbritten et al (1978), who found a dose dependent decrease in locomotor activity when 5-HT was administered intraventricularly. Grabowska and Michaluk (1974), found that the increase in locomotor activity caused by apomorphine, a central dopaminergic stimulating agent, was stronger in rats pre-treated with Bol or methysergide, both 5-HT antagonists. Earlier experiments by Grabowska et al (1973), reported that apomorphine - stimulated locomotor activity was greater in rats depleted of brain

5-HT either by inhibition of tryptophan hydroxylase activity, or by destruction of the mid-brain raphe nuclei, while it was weaker in 5-HTP pre-treated animals.

Other investigations have described the potentiation of amphetamine induced locomotor activity after lesions of the mid-brain raphe and 5-HT synthesis inhibition (Mabry and Campbell 1973, Neill et al 1972).

Evidence also exists for the stimulatory role of catecholamines in locomotor activity. Central noradrenergic lesions produced by 6-hydroxydopamine reduced activity considerably (Sorensen and Ellison 1973), while intraventricular infusion of catecholamines (primarily noradrenaline, but also dopamine to a lesser degree) significantly increased locomotor activity in hypothermic rats (Stone and Medlinger 1974). In the same experiment 5-HT was seen to reduce the already low activity level of such animals. Gordon and Shellenberger (1974), have found significant positive correlations between locomotor activity and noradrenaline and dopamine levels in specific brain regions.

The Hyperactivity Syndrome after l-Tryptophan and MAO Inhibition

The administration of a MAO inhibitor in conjuction with tryptophan has been found to produce a characteristic syndrome of hyperactivity including tremor, rigidity, hyperreactivity, stereotyped head response and large increases in activity (GrahameSmith 1971). Pre-treatment with spiroperidol, a presumed dopamine receptor blocker, abolished all signs of the syndrome except for rigidity and hyperactivity, suggesting that 5-HT receptor activity was involved in the mediation of the hyperactivity syndrome (Jacobs et al 1974). Pugsley and Lippmann (1977), report that the antagonistic effect of spiroperidol on the tryptophan/MAOI hyperactivity syndrome is entirely due to dopaminergic receptor blockage, rather than any action on 5-HT neuronal mechanisms. Later work by GrahameSmith and co-workers, led to the suggestion that at some point between the post-synaptic 5-HT

receptor sites initiating the production of the hyperactivity syndrome and the mechanisms responsible for the expression of the syndrome, lies a system of dopaminergic neurones. The activity of these neurones depends on adequate dopamine concentrations, depletions of which break the neuronal sequences necessary for the behavioural expression of 5-HT receptor site stimulation (Green and Graham-Smith 1974).

Further evidence for increased 5-HT levels producing hyperactivity is demonstrated by the use of p-chloroamphetamine (PCA). This compound is known to release 5-HT from its storage sites within the neurone, inhibit 5-HT re-uptake and inhibit MAO activity. Green and Kelly (1976), suggested that PCA produced the hyperactivity syndrome by releasing 5-HT, which cannot be metabolised intraneuronally by MAO, thus causing it to spill into the synaptic cleft and stimulating post-synaptic 5-HT receptors. However, Messing et al (1976), suggest that since PCA exerts a maximal effect on 5-HT depletion after two days, which corresponds to the period of maximum locomotor activity, this is further evidence for the existence of a serotonergic system which inhibits behavioural arousal.

In two reports by Green and co-workers, it is stated that both 5-HT and dopamine are normally metabolised by type-A MAO in vivo (Green and Youdin 1976, Green et al 1977). However, when type-A is inhibited, they can both be metabolised by type-B MAO. Only when both forms are inhibited almost totally is the largest rise in both 5-HT and dopamine seen, and this increase in functional activity is manifested in the hyperactivity syndrome.

It should be noted that the MAOI used almost exclusively in the production of this syndrome was tranylcypromine. Recent reports suggest that this compound alone increased brain tryptophan which led to an accumulation of tryptamine in the brain, three times greater than when the MAOI pargyline was used (Tabakoff et al 1977). Marsden and Curzon (1978), have stated that the increase in tryptamine, which occurs in

rats pre-treated with tranylcypromine and given l-tryptophan, is responsible for at least part of the resultant hyperactivity syndrome.

Fluctuations in Locomotor Activity over the Oestrous Cycle

Experimental analysis of motivation began in the 1920's with Richter (1927), who viewed motivated behaviour as a homeostatic mechanism "driven" by various biological needs, and such "physiological drives" manifested themselves in the amount of general activity.

It has been accepted for many years that the 4 or 5 day oestrous cycle of the female rat is paralleled by variations in the general activity level. The peak of activity is normally reached during the stage of cornified vaginal epithelium, which is also marked by sexual receptivity. The original work performed by Wang (1923), and confirmed by Slonaker (1924), and Richter, made use of the running wheel. Since then much doubt has been cast on results using such a method.

Finger (1961), compared the scores of mature female rats using both a running wheel and a photocell recording stationary cage and reported that the amplitude of fluctuation between successive peaks and troughs averaged significantly greater with the wheel. Bolles (1963), observed adult female rats in the home cage environment, and while in some cases there appeared to be an indication of heightened activity at approximately 5 day intervals, no regular relationship was discerned with the results of subsequent receptivity tests. He concluded that the apparent activity cycles were merely coincidental, and speculated that true oestrous cyclicity "is a phenomenon specific to activity wheels".

Further work (reviewed by Bolles 1967, Gress 1968), indicated that the use of running wheels may stimulate extra running and may also act as a reward (Liversey et al 1972).

Richards (1966), also described a discrepancy between wheel running

and home cage activity with golden hamsters. He suggested that this was hardly surprising, since wheel running measured a single activity, and activity in the home cage, while producing a single score (using both stabilimeter cages or activity boxes), was equivalent to the sum of all behavioural categories involving movement.

Finger (1969), using a running wheel and a photocell stationary cage, found 70% agreement between the two methods when testing for increased activity during the oestrus period, but again reported increased amplitude of fluctuation using the running wheel.

Such observations leave in doubt the nature of the behavioural changes of the reproductive cycle. In particular it is not clear whether the large fluctuations reported accurately reflect behaviour in an environment in which locomotor activity is not mechanically stimulated. To this end, Barnett and McEwan (1973), designed an apparatus which allowed some distinction to be made between "(i) movements provided by changing need for food and water, (ii) movement apparently uninfluenced by any internal deficit." No increase in activity was observed at oestrus.

2. STARTLE RESPONSE

Attention to the functional role of brain 5-HT has begun to focus on the involvement of serotonergic neuronal systems in behavioural reactivity, and manipulations of central 5-HT neurones have consistently produced alterations in the responsivity to sensory stimuli.

A number of reports, commencing with Koe and Weissmann (1966), have described a gross hyperreactivity of rats to environmental stimuli following reduction of brain 5-HT levels. More discrete measures of altered reactivity indicate that rats depleted of 5-HT by p-CPA treatment (Tenen 1967), or by brain lesions (Lints and Harvey 1969), exhibit lowered shock thresholds. Noting the effects of p-CPA on shock induced fighting behaviour (Connor et al 1970a), suggested that

5-HT may "play a role in modulating overall levels of responsivity to dynamic changes in the level of environmental stimulation."

Some support for this hypothesis has been reported by Aghajanian and Sheard (1968). These investigators found that electrical stimulation of the mid-brain raphe area, the region containing most of the 5-HT nerve cell bodies in the brain, causes dishabituation of a habituated skeletal-motor startle response. Prior treatment with p-CPA abolished the effects of raphe stimulation, but normal responding was restored by the administration of 5-HTP. This suggests that brain 5-HT is related to the dis-habituating effects of raphe stimulation.

Connor et al (1970 b), reported that depletion of brain 5-HT by p-CPA slowed down but did not prevent habituation of the skeletal - motor startle response. When given to habituated rats, p-CPA caused a transitory increase in startle response magnitude.

Davis and Sheard, in a series of experiments, showed that a reduction in brain 5-HT levels could be associated with an increase in sensitivity to various forms of sensory stimulation. Raphe lesioned rats were shown to exhibit higher startle response magnitudes, caused by heightened tone elicited sensitization (Davis and Sheard 1974a). LSD (lysergic acid diethylamide) is known specifically to inhibit cells within the mid-brain raphe nuclei (Aghajanian et al 1972), and low doses augmented the startle response magnitude. High doses were shown initially to potentiate the response and then reduce it. It has been suggested that this is due to a dual action of LSD, initially inhibiting the raphe thus enhancing the startle response, followed by an inhibition of cells post-synaptic to the raphe neurones thus depressing the startle (Davis and Sheard 1974b). Another known hallucinogenic drug NN-dimethyltryptamine (DMT), with similar effects on the raphe neuronal system, was shown to elicit similar responses in relation to startle magnitude (Davis and Sheard

1974c). From these results it was concluded that raphe neurones do not seem to modulate the startle response directly (e.g. by exerting a tonic level of inhibition on the startle circuit), rather they appear to inhibit other systems which themselves are involved in the facilitation of the startle response (Davis and Sheard 1976). In an experiment involving the specific depletion of 5-HT by PCA, it was suggested that startle sensitization enhancement is associated with 5-HT depletion, and startle sensitization inhibition with enhanced release of 5-HT (Davis and Sheard 1976).

Fechter (1974a), unlike other investigators, reported that p-CPA had no effect on the magnitude of the response, but that a large amine-depleting dose of reserpine enhanced the startle amplitude. Selective replacement with 5-HTP and a MAO inhibitor further enchanced the response. When given to non-reserpinised animals, 5-HTP and the MAO inhibitor again enhanced the response. In further work, Fechter (1974b), reports that activation of central noradrenergic terminals produced an inhibitory influence on the startle response, whereas stimulation of dopaminergic receptors failed to alter the startle reaction. This has led to the suggestion, by Fechter, that 5-HT exerts a facilitatory influence on motor output when measured behaviourally, and since p-CPA had no effect on the startle response, the action of reserpine reflects interference with catecholamine neurotransmission. Thus a catecholamine system normally exerts an inhibitory effect upon 5-HT neurones which are themselves excitatory to the test behaviour.

Harlington (1970), found that the startle response in rats exhibited an age related circadian rhythm, beginning at sexual maturity, rising to a peak beteen 70-100 days, followed by a fall. At night, when levels of 5-HT are known to be low and levels of noradrenaline high (Hery et al 1972, Morgan et al 1973, DiRaddo and

Kellog 1975), the startle response magnitude was found to be 90% greater than during the light period, when roughly opposite levels of the neurotransmitters were known to exist. Davis and Sollberger (1971), produced comparable results using a similar age range.

Geyer et al (1975), infused 5-HT intraventricularly into rats and their results would seem to act as confirmation of the hypothesis that behavioural responsivity, as reflected in the rat startle response, is reduced by increased brain 5-HT levels. 5-HT was found to produce a dose dependent decrease to the startle stimulus. Further investigations by Geyer et al (1976), reinforce this hypothesis. Lesions of the median raphe nuclei produced significant reductions in tryptophan hydroxylase activity in various structure in the forebrain resulting in, amongst other behaviour changes, an increase in magnitude of the startle response.

Throughout these experiments similar results have been obtained from stimuli elicited from both auditory and air-puff sources.

Recent work has suggested that 5-HT may be an inhibitory transmitter in the auditory pathway (Bhargava and McKeown 1977). This may be another factor to be taken into consideration when studying the startle response after auditory stimuli.

3. OPEN-FIELD BEHAVIOUR

The "open-field" developed by Hall (1934), has most commonly been used to measure "emotionality". It is a distinctly different environment from that which the test animals have previously encountered, its dimensions being vastly greater than the boundaries of usual living quarters. This ensures that the "open-field" is both strange and mildly noxious (Denenberg 1969).

The rationale behind the open-field test is that many animals, when exposed to strange or noxious stimuli, will typically freeze. This appears to have an adaptive significance in that it is more

difficult for a predator to observe a non-moving animal. It is suggested that such a situation will trigger activity in the autonomic nervous system, resulting in defaecation. "Thus an emotional animal may be defined as one which, when exposed to noxious or novel stimuli, does not move about, and will defaecate" (Denenberg 1969). Broadhurst (1957), has suggested that "defaecation is the emotionality of choice". Ambulation is also usually taken as a measure of emotionality (Broadhurst 1957), and such an activity score can be taken as a measure of both emotional reactivity and exploratory behaviour (Denenberg 1969).

Ellison and Bresler (1974), compared the behaviour syndromes of rats with lowered noradrenaline levels (produced by 6-hydroxydopamine) to those with lowered 5HT levels (after p-CPA). Animals with reductions in 5HT were seen to exhibit less locomotor activity than control animals and huddle motionlessly ("freeze"). Marsden and Curzon (1976), observed animals also given p-CPA to locomote less, rear less and groom less, while tending to huddle motionlessly upon being exposed to the novel environment. The number of faecal pellets produced was also less than the controls, although this is suggested as being a reflection of reduction in food intake seen after treatment with p-CPA. However, Kulkarni et al (1974), noted that while p-CPA reduced grooming and rearing responses, ambulation was seen to increase.

Rats given 6-hydroxydopamine to reduce noradrenaline levels were seen to locomote more than the controls, but initially reared less, although as the test progressed, continued to rear, unlike the p-CPA treated animals.

This has led Ellison to propose a hypothesis for anxiety and depression, based on such animal models (Ellison 1975, 1977). In it, he observes that noradrenaline-deficient rats have drive deficits and are lethargic in their home environment but are less fearful than

controls in a novel environment. This he suggests as a model of depression. Conversely, the 5HT depleted rat is active and exploratory in familiar environments but frightened in novel environments, a model of anxiety.

Doybanski (1975), using intraventricular injections of 5-HT found them to prolong immobility and decrease rearing significantly in the open-field. When given to rats of high activity, a reduction in locomotor activity and exploration was noted.

Over the oestrous cycle, Burke and Broadhurst (1966), found a slight but insignificant reduction in defaecation in the rat at oestrus, a result reproduced by Guttman et al (1975), in the mouse. The latter investigators also found a significant increase in motor activity at oestrus, while grooming episodes were found to increase, but not with significance. They also measured "peeking", which they suggest is a complex variable thought to contain elements of anxiety, exploration and activity. They found a slight but insignificant decrease at oestrus.

Gray (1971), reports that differences in "emotional" behaviour are not usually found between the sexes in mice, which Bruell (1969), showed to be a product of the predominant use of inbred strains within the species. In rats, however, Gray noted that females defaecate less and ambulate more than males, in the novel environment of the "open-field".

4. SEXUAL BEHAVIOUR

(i) Female Sexual Behaviour

Evidence from a number of studies suggested that hormones may control sexual receptivity in female mammals by altering monoamine neurotransmitter activity (Mayerson 1964, Ahlenius et al 1972, Zemlan et al 1973, Everitt et al 1974). Mayerson (1964), showed that increased levels of 5-HT in the brain reduced the lordosis response,

and that reductions in brain 5-HT levels, after p-CPA or reserpine, increase sexual behaviour, suggesting that these drugs could be given in place of progesterone in order to induce lordosis (Mayerson 1964, Mayerson and Lewander 1970). Zemlan et al (1973), confirmed the action of p-CPA and also showed the same effect after methysergide, a 5-HT receptor blocker.

Mayerson suggested that there is an inhibitory serotonergic control on female sexual behaviour which is overcome by progesterone. It has been further suggested that the mid-brain raphe may be the centre for the inhibitory control because it contains cell bodies with high concentrations of 5-HT, and whose axons run via the median forebrain bundle to the hypothalamus. Implants of progesterone in the mid-brain raphe are more effective in stimulating lordosis than those placed in the hypothalamus (Ross et al 1971).

Mayerson also looked at the effect of altering brain amines on sexual motivation, and found that MAO inhibitors decreased it, while p-CPA increased it, but only in oestrogen primed animals.

Disagreement about the relative importance of indoleamines and catecholamines in this context has occured principally out of the lack of precision in the effects of many agents used. p-CPA, while depletes 5-HT, has been shown to deplete catecholamines to varying extents, and specific depletion of catecholamines by AMPT has produced increased sexual activity similar to that produced by p-CPA (Ahlenius et al 1972).

Results from Everitt et al (1974), supported the hypothesis of 5-HT being inhibitory in such behaviour. They report that the action of p-CPA in stimulating the intensity and duration of lordosis could be specifically attributed to depletion of 5-HT. Dopamine was found to have a role not dissimilar to 5-HT. In a later report Everitt (1977a), submits that depression of serotonergic and dopaminergic activity in the CNS can each induce sexual behaviour.

It has been suggested that 5-HT agonists potentiate sexual receptivity by stimulating pre-synaptic 5-HT receptors and also inhibit receptivity by the inhibition of post-synaptic receptors (Everitt and Fuxe 1977). Clomipramine, an inhibitor of 5-HT uptake, has been shown to depress sexual receptivity in monkeys to a low level (Everitt 1977b).

(ii) Male Sexual Behaviour

It has been shown that by raising brain amine levels with MAO inhibitors, sexual activity in both intact and castrated rats treated with testosterone was reduced (Malmnas and Mayerson 1970). By selectively increasing amine concentrations, the inhibition was shown to be due to raised 5-HT levels, since increasing catecholamine levels had no effect (Malmnas and Mayerson 1970).

When brain 5-HT levels are reduced, or its action inhibited, an increase in both homo and heterosexual activity has been reported. Tagliamonte et al (1972), found that p-CPA induced hypersexuality, which the administration of 5-HTP was found to suppress. Both p-CPA and p-bromoethylamphetamine (depletors of 5-HT) have been shown to stimulate sexual activity in sexually sluggish males, although only p-CPA was found to be effective in sexually inactive males (Dallo 1977).

A significant correlation between altered sexual behaviour and changes in 5-HT uptake has been reported recently, suggestion that 5-HT does indeed serve as an inhibitory transmitter in the neural processes of male sexual behaviour (Larsson et al 1978).

In humans, increased levels of 5-HT and 5-HIAA have been found in the urine of sub-fertile men (Segal et al 1975), while tryptophan has been reported to have no effect on sexual stimulation in male patients (Hyppa and Falck 1977).

It has been suggested that in males elevating brain dopamine levels will induce sexual behaviour, a contrary state to that which

exists in the female rat (Everitt 1977a).

5. AGGRESSION

Decrements in 5-HT function have been corrleated with increases in both irritable aggression (e.g. pain-elicited) and predatory aggression (e.g. muricide).

Lagerspetz et al (1967), showed that in mice specially bred for their aggressive tendency, 5-HT levels were reduced in the forebrain, while showing little difference in the brain stem, whereas brain stem levels of noradrenaline were increased.

Koe and Weissmann (1966), observed that rats injected with p-CPA, an inhibitor of 5-HT biosynthesis, displayed increased aggression when handled. Everitt et al (1975), observed females lesioned with 5,7-dihydroxytryptamine (5,7,-DHT), with reduced brain 5-HT content by about 75%. The females were seen to initiate biting attacks on males in a non-aggressive situation. Aggression did not reflect sexual unresponsivity since the females displayed high responsivity and the attacks were not prompted by mounting attempts. When 5,7-DHT was used to initiate relatively specific destruction of ascending 5-HT pathways, muricidal behaviour was induced in the rat (Marks et al 1977). Hole et al (1977), also found that lesions to such pathways, whether they were electrolytically or chemically induced or involved pharmacological alteration of neuronal function, resulting in reduced 5-HT activity, produced an increase in muricide. Copenhauer et al (1978), reported that the latency and intensity of filicidal attacks by female rats, after p-CPA, varied inversely to 5-HT content in the brain. p-CPA stimulated aggression generally, but when the brain content of 5-HT fell below 0.1ng/g, filicidal behaviour was induced. 5-HTP was shown to reverse this behaviour.

Jacobs and Cohen (1975), observed that electrolytic lesions of the dorsal raphe nuclei produced an 83% increase in pain-elicited

aggression relative to the post-operative level. Kostowski et al (1975), found that 5-HT depleting lesions of the mid-brain raphe nuclei (extensively to the dorsal raphe, partially to the median raphe) induce intraspecific aggression in grouped male rats, but did not increase the incidence of muricide. Lesions to the lateral raphe failed to alter forebrain 5-HT levels and had no effect on aggressive behaviour.

Kilbey et al (1977), found that delta-9-tetrahydrocannabinol (Δ-9-THC) inhibited predatory aggression while producing an increase in 5-HT levels in the mid-brain and medulla oblongata. However, Segawa et al (1977), reported that Δ-9-THC reduced both the rate constant and turnover rate of 5-HT synthesis and stimulated muricide. Conflicting evidence in support of the hypothesis that increased brain 5-HT synthesis reduces aggressive behaviour.

6. SLEEP

Biochemical rhythms have been observed in many aspects of behaviour and biochemical functioning, and one of the most clearly observed is the onset of sleep in rodents, induced by the onset of light (Hanselmann and Barbely 1976). These observers found that the onset of light induced slow wave sleep (SWS) and correlated with a rapid rise in brain 5-HT, particularly in the cortex, during the light period. Noting that 5-HT rose when sleep occured, and fell at the onset of darkness, with a corresponding rise in motor activity (Morgan et al 1973, Hery et al 1972, Diraddo and Kellog 1975, Philo et al 1977), it has been suggested that a link may exist between brain 5-HT rise and the onset of sleep.

Jouvet (1967, 1969), postulated that 5-HT was primarily involved in initiating and sustaining non-rapid eye movement (REM) sleep. It was suggested that 5-HIAA, a 5-HT metabolite, was also necessary to trigger REM sleep, as MAO inhibitors were shown to prevent the

occurence of REM sleep, by inhibiting the metabolism of 5-HT to 5-HIAA.

5-HT has been generally thought to be related to the initiation and duration of SWS (Kupfer 1977). Depletion of 5-HT, with lesions of the rostral raphe system or p-CPA, has been shown to produce insomnia, loss of SWS and a lesser reduction in REM (Pujol et al 1971). More recent work has indicated that, despite a reduction in forebrain 5-HT and 5-HIAA of about 60-70% after raphe lesions, test and control rats could not be distinguished in parameters such as SWS and REM (Bouhuys and An Den Hoofdakker 1977).

The effect of 5-HTP on sleep seems to be associated with REM. Mandell et al (1965), reported an increased percentage time spent in REM sleep, and increased REM efficiency, a finding confirmed by Wyatt et al (1971). However, 5-HTP has been shown to produce a significant improvement in moderately insomniac patients, with the most important changes occuring during the first three hours of sleep and between 6-7:00a.m., which would indicate a selective action on SWS (Soulairac and Lambinet 1977).

The effect of tryptophan on sleep remains unclear. Both tryptophan free and enriched diets failed to produce any significant effects on total sleep time (TST) or percentage REM, although tryptophan free diets were associated with increased length of the waking cycle (Hartmann 1967). When administed orally, tryptophan again failed to produce any significant change in TST, SWS or REM. With high doses a reduced latency to sleep was noted (Hartmann (1972). Hartmann and Elion (1977), also reported a lowering of sleep latency with oral tryptophan administration, when investigating insomnia of sleeping in a strange place. Conflicting reports exist on the influence of tryptophan on REM. Hartmann et al (1974), reported that tryptophan induced a reduction in REM, while Griffiths et al (1972), reported an increase.

The results of recent findings support the suggestion that 5-HT appears to be primarily associated with the modification of REM parameters (Hill and Reyes 1978). However, the authors of another recent report, having found that dietary deficiency of tryptophan produced no significant alteration in time spent awake, in SWS or in paradoxical sleep, cast doubt on the suggested relationship between reduced 5-HT levels and the occurence of insomnia (Clancy et al 1978).

HYPOTHALAMIC AMINES AND GONADOTROPHIN RELEASE

1. OVULATION AND FEEDBACK EFFECTS

The secretion of gonadotrophins from the anterior pituitary is controlled by hormones from both the hypothalamus and the ovary (Wilson 1974). The hypothalamus is connected to the pituitary by a blood supply passing from a series of capillary loops in the median eminence, down the pituitary stalk in sinusoidal vessels into the anterior pituitary. This has been termed the hypophyseal portal system (Harris 1948), and it had been suggested that substances synthesised in the CNS might pass via this system to the pituitary, where they could stimulate pituitary hormone release.

Recently, such substances have been isolated. Basers et al (1970), isolated a releasing factor for thyroid-stimulating hormone (TSH), and Metsuo et al (1971), have both isolated and synthesised a releasing factor for luteinizing hormone (LH). The latter was found to be a decapeptide which could stimulate both LH and FSH (follicle stimulating hormone) release. Initially it was thought that there was only one releasing factor for both gonadotrophins, but a predominantly FSH releasing factor has since been isolated (Johansson et al 1973).

On stimulation, the releasing factors are secreted from their storage sites and pass into the hypophyseal portal system and on to the pituitary. Here they stimulate the release of the pituitary gonadotrophins LH and FSH, which pass into the blood stream. On reaching the ovaries LH and FSH stimulate the secretion of the gonadal steroids, oestradiol and progesterone (Wilson 1974).

The gonadal steroids have been found to exert a negative feedback on the secretion of LH and FSH, thus effectively controlling their own secretion. The principal sites for such feedback appears to be at the hypothalamic level (Ajilla et al 1972), although it has been suggested that oestrogen can both stimulate and inhibit LH release by

actions at the hypothalamic and pituitary levels. Labhsetwar (1975), has suggested that progesterone inhibits LH release by interfering with the release of LH-RF from the hypothalamus.

In certain circumstances, it has been suggested that the gonadal steroids may also exerta positive feedback effect. This is illustrated by the effect of oestradiol just prior to ovulation. Ovulation will only occur after the sudden release of LH, and this only occurs when the plasma levels of oestradiol rise to a certain critical concentration. This surge can be inhibited by the administration of antibodies to oestradiol (Ferin et al 1969), antioestrogen compounds (Labhsetwar 1970, 1972), or by critically timed ovariectomy (Schwartz 1964).

2. THE PHYSIOLOGY OF OVULATION

The study of the physiology of ovulation has been most detailed in the rat. Under the influence of FSH, and a low basal level of LH, the ovarian follicles mature and at a certain stage, start to secrete oestradiol (Schwartz 1969, Ely and Schwartz 1971). A peak level of plasma oestradiol has been found to occur at about mid-day on the day before ovulation - proestrus (Shaikh 1971), following which there is a sudden surge of LH, FSH and prolactin from the pituitary. This occurs in the late afternoon on the day of proestrus, and lasts between two and four hours (Mahesh and Goldman 1971, Naftolin et al 1972, Freeman et al 1972). The surge of LH, but probably not FSH or prolactin, appears to be necessary for ovulation. It initiates the secretion of progesterone from the follicles and/or interstitial cells (Leavitt et al 1972), and initiates changes in the follicle walls, so that the follicles rupture about 12 hours later, each follicle releasing an ovum. Luteal tissue then fills the follicles, forming the corpora lutea. These secrete progesterone at low levels unless fertilisation takes place (Wilson 1974).

In the rat, stimulation of LH release by the rise in plasma

oestrodiol can only take place during a "critical period" of two hours during the afternoon of proestrus. The exact time is controlled by the start of the photo-period for that day (Everett et al 1949). The release of LH into the blood begins about an hour after the stimulation by raised oestradiol levels has been completed, and lasts for about three hours (Miyake 1968).

Raised progesterone levels occur simultaneously with, or just after the LH surge (Miyake 1968), and have been shown to be due to stimulation by the LH surge (Barraclough et al 1971, Piacsek et al 1971). They decline slowly over the following dark period, and are thought to be necessary for inducing sexual receptivity (Boling and Blandeau 1939), and in controlling the duration of LH surge (Kobayaski et al 1970). In the rat, these changes occur over a period of 4 to 5 days, known as the oestrus cycle.

In the human female, an analagous series of changes occur over a period of about 28 days, called the menstrual cycle. During the first half of the cycle (the follicular phase), low levels of FSH and LH stimulate the ovarian cycles to secrete oestrogen, which reaches a peak concentration at mid-cycle. Then, on either the same or the following day, there is a dramatic increase in LH secretion, and to a lesser extent FSH, which lasts up to two days. This is equivalent to the LH surge in the rat, and ovulation takes place towards the end of this mid-cycle period. After ovulation, the corpus luteum secretes progesterone and some oestrogen, in the second half of the cycle known as the luteal phase. If fertilization does not take place, the corpus luteum regresses about two weeks after its formation, and gonadal steroid secretion falls to zero (Ross et al 1970, Van deWiele et al 1970).

Wilson (1974), has suggested three main difference between the human menstrual cycle and the oestrous cycle. In general, only one

follicle ruptures during the menstrual cycle, while an average of twelve may occur in the rat. The second difference involves progesterone secretion from the interstitial cells at the time of the LH surge in the rat. This does not occur in the human at all. In the rat, the corpora lutea produce very little progesterone and regress after 2 or 3 days unless stimulated by coitus (or vaginal irritation) when they become functional and secrete progesterone for 11 to 14 days. In the human, the corpus luteum is always functional.

The sites of steroidal feedback effects in the hypothalamus, have been the objects of much work. It is now considered that there is a tonic centre in the arcuate nucleus and median eminence area, which controls low tonic secretion of gonadotrophin necessary for maintaining gonadal function. In the female, there is an additional centre in the preoptic area, which controls the cyclic release of LH just before ovulation (Gorski 1966). It is thought that the cyclic centre sends pulses at a regular time in the afternoon to stimulate the tonic centre in the median eminence (Tejasen and Everett 1967, Gorski 1966), but the latter only responds on the day of proestrus, when oestradiol levels are high. It has been suggested that oestradiol lowers the threshold of stimulation of the median eminence (McDonald and Gilmore 1971, Sawyer and Hilliard 1971). Everett (1964), has shown that there is a neural pathway connecting the two centres. The site of negative feedback by the gonadal steroids appears to be at the median eminence level (Chowers and McCann 1967, Smith and Davidson, Taleisnik et al 1970), and it is thought that they have a biphasic effect on the threshold of stimulation of the median eminence, first lowering and then raising it.

3. THE EFFECT OF BRAIN AMINES ON GONADOTROPHIN RELEASE

(a) Noradrenaline

Noradrenaline levels have been seen to change during the oestrous cycle (Stefano and Donoso 1967). Initial experiments proved contradictory. Donoso et al (1966), Stefano and Donoso (1967), reported noradrenaline levels to be highest on the day of proestrus, coinciding with the gonadotrophin surge, this fluctuation only being observed in the anterior hypothalamus. Kurachi and Hirota (1969), however, found hypothalamic noradrenaline levels to be at a minimum at proestrus, rising to a maximum in late dioestrus, with cortical levels remaining unchanged throughout the complete cycle. Recently, Negro-Vilar et al (1977), reported that there is a significant increase in the levels of noradrenaline in the median eminence near the time of the "critical period" on the day of proestrus, which precedes the afternoon preovulatory surge of LH and prolactin.

Noradrenaline turnover, judged to be a superior estimation of noradrenergic activity, has also been shown to rise on the day of proestrus, preceeding the LH surge (Coppola 1969, Donoso and Moyano 1970).

Two recent reports indicate that noradrenaline has the facility to release LH. When given intraventricularly it has been shown to release LH (Vijayan and McCann 1978), by the activation of LH-RF (Sawyer and Radford 1978).

As both levels and turnover rate of noradrenaline increase when gonadotrophin release in enhanced, it would seem that increased noradrenaline systhesis is responsible. Support for this hypothesis is strengthened by the existence of a negative correlation between oestradiol and hypothalamic MAO (an enzyme involved in the catabolism of noradrenaline). A time-lag has been shown between increased oestradiol levels and its effect. At proestrus both oestradiol and

MAO levels are high (Kamberi and Danhof 1968), but a small transient trough in hypothalamic MAO levels has been observed just after the "critical period" of proestrus (Kamberi and Kobayashi 1970), with levels rising in early oestrus followed by a dramatic fall in late oestrus as the raised oestradiol levels become effective (Holzbauer and Youdim 1973).

(b) Dopamine

Dopamine levels have been shown to increase steadily from the first day of dioestrus to a maximum at oestrus (Lichtensteiger 1969). Lichtensteiger (1969), indicated that increased dopamine levels can be correlated with a rise in plasma LH levels, a fact supported by the observation of Negro-Vilar et al (1977), who reported an increase in the level of dopamine in the median eminence near to the time of the "critical period". Hery et al (1978), have suggested that dopamine contributes to the regulation of LH by modulating the amplitude of its circadian pattern rather than by the generation of this pattern. Keller and Lichtensteiger (1971), indicated that the increase in dopamine levels correspond to an increase in dopamine synthesis. Vijayan and McCann (1978), reported that when dopamine is given intraventricularly, LH release in stimulated.

There has been a suggestion that dopamine inhibits gonadotrophin release, and that the gonadal steroids exert their negative feedback effect by increasing dopaminergic activity (Fuxe et al 1974). This was supported by evidence from Hackmann et al (1973), who reported a negative correlation between dopamine and LH-RF levels. However, more recent evidence points to the fact that dopamine only inhibits prolactin secretion. Vijayan and McCann (1978), reported that dopamine clearly acts to suppress prolactin release, which they suggest is due to dopamine either stimulating the release of a prolactin inhibiting factor (PIF) or by acting as a PIF itself after

being secreted into the hypophyseal portal vessels. Enjalbert et al (1977) have shown that the mediobasal hypothalamus does contain dopamine-free PIF in the nerve endings. Further support for the inhibition of prolactin secretion by dopamine comes from Szabo et al (1977) and Wiggins and Fernstrom (1977), who both reported that central but not peripheral decarboxylase inhibition will prevent l-dopa from inhibiting the proestrus surge of prolactin, and Langer and Sachar (1977), who found that dopamine inhibited neuroleptic induced prolactin release.

It would seem most likely that the gonadal steroids initiate their negative feedback effect by their influence over noradrenaline metabolism rather than that of dopamine. It has been shown that oestradiol and progesterone inhibit the formation of noradrenaline by reducing tyrosine hydroxylase activity (Beattie et al 1972, Beattie and Sojka 1973), and that oestradiol can both inhibit the release (Donoso et al 1969), and increase the uptake of noradrenaline (Endersby and Wilson 1973, 1974). Thus in circumstances when the gonadal steroids inhibit gonadotrophin release, they also reduce the general metabolic activity of noradrenaline. At times of enhanced gonadotrophin release, there is an increase in noradrenaline metabolism.

(c) 5-Hydroxytryptamine

Since the discovery that 5-HT caused atrophy of the reproductive organs and delayed puberty in immature mice (Robson and Botros 1961), it has been considered likely that 5-HT is an inhibitor of gonadotrophin release.

Several reports indicate that electrochemical stimulation of both the ventral tegmentum and the mid-brain raphe will inhibit spontaneous ovulation and exert an inhibitory influence on the episodic release of LH (Carrer and Taleisnik 1970, 1972; Arendash and Gallo 1978).

The latter report suggests that this is due to the activation of an ascending serotonergic pathway originating from this region of the mid-brain.

5-HT levels have been seen to fall significantly in the median eminence prior to the LH surge (Wheaton et al 1972). When gonadal steroids were administed to castrated rats, thus initiating a negative feedback on gonadotrophin release, a rise in hypothalamic tryptophan levels was reported (Bapna et al 1971), as were rises in 5-HT levels in the mid-brain (Tonge and Greengrass 1971).

5-HT has been shown to act peripherally on ovulation. If given intravenously or subcutaneously after the "critical period" before the LH surge, ovulation was shown to be inhibited (Currie et al 1969; Labhsetwar 1971a), which has led to the suggestion that this may be due to peripheral vasoconstriction, preventing the passage of the gonadal steroids from the ovary to the hypothalamus (Wilson and McDonald 1974, 1973).

Intraventricular administration of 5-HT has been shown to inhibit LH and FSH release in intact female rats (Kamberi et al 1970, 1971; Kamberi 1973). The effects of such injections on spontaneous ovulation have been varied. Several workers have reported that high doses of 5-HT (50-200µg per rat) injected at various times during the day of proestrus, had no effect on ovulation (Rubenstein and Sawyer 1970; Schneider and McCann 1970; Wilson and McDonald 1974). However, Kamberi (1973), found that much lower doses (1-5µg per rat) would inhibit ovulation when given just before the critical period. Intraventricular 5-HT has also been found to inhibit the facilitatory effect of progesterone induced ovulation (Zolovik and Labhsetwar 1973).

It has been reported that 5-HT inhibits the release of LH at the level of the mediobasal hypothalamus (Domarski et al 1975), by inhibiting the release of LH-RF (Leonardelli et al 1974).

Lippmann (1968), suggested the hypothesis that gonadotrophin release was controlled not by individual amine levels, but by the relative proportions of noradrenaline and 5-HT. When 5-HT levels are in the ascendent, gonadotrophin release is inhibited. When both amines are depleted to the same extent, no effect on gonadotrophin release has been reported (Labhsetwar 1971b). Dopamine has been shown to reverse 5-HT induced inhibition of ovulation, induced by pregnant mares serum (PMS) and progesterone (Zolovik and Labhsetwar 1973).

It has been suggested that one action of progestertone is the suppression of the 5-HT mediated inhibitory pathway from the mid-brain to the hypothalamus. By interfering in the conversion of tryptophan to 5-HT, and thus reducing 5-HT synthesis (Kordon and Glowinski 1972), progesterone may facilitate ovulation (Mayerson 1964).

Although the majority of results indicate that 5-HT has an inhibitory effect on gonadotrophin release, and thus ovulation, some evidence exists to show that 5-HT can stimulate both tonic and cyclic gonadotrophin release (Kordon et al 1972; Parker et al 1972; Takehashi 1973). It has been suggested that the circadian rhythm of gonadotrophin release may be controlled by the pineal principals 5-HT, melatonin, 5-hydroxytryptophol and 5-methoxytryptophol (Fraschini et al 1971), which travel to the basal hypothalamus via the cerebrospinal fluid, thereby altering releasing hormone activity. Quay (1963), has shown that pineal 5-HT levels are at a maximum daily at mid-day, in the rat, and higher on the day of proestrus than any other day. Hypothalamic 5-HT levels have also been shown to exhibit a circadian rhythm, with a peak each day in the late afternoon, at the expected time of the "critical period", and when the levels of pituitary gonadotrophins were at a maximum (Quay 1969). It would seem possible that 5-HT is involved in the control of the release of the ovulatory surge of LH. This may be due to an enhancement of the

oestradiol positive feedback effect, with 5-HT facilitating the uptake of oestradiol into the hypothalamus (Kordon and Glowinski 1972).

Much recent work has centered on the effect of 5-HT on prolactin release. All evidence points to 5-HT having a facilitatory effect on prolactin secretion. Ferrari et al (1978), have shown 5-HT antagonists, such as methysergide, to inhibit prolactin release. They suggest that this action is not, or only partly related to any action of methysergide on dopamine receptors. Both Larson et al (1977), and Lancranjan et al (1977), report 5-HTP to have a stimulatory effect on prolactin release, while Woolf and Lee (1977), have shown that plasma prolactin levels rose slightly after the administration of tryptophan, but markedly when tryptophan administration is followed by water, taken orally.

STEROID HORMONES AND DEPRESSION

Initial research descovered that patients suffering from clinical depression had high cortisol (and its derivatives) levels (Hullin et al 1967; Bridges and Jones 1966; Doig et al 1966). Refinements in various experimental designs and techniques have resulted in considerable clarification of diverse findings once apparently contradictory. Sachar et al (1976), summarizes such findings thus:

(i) A large minority of depressed patients hypersecrete cortisol.

(ii) Factors of psychological distress and emotional arousal account for some of the excessive cortisol secretion, especially in patients suffering from neurotic, reactive depressions.

(iii) Many depressed patients, however, manifest a pervasive hypersecretion of cortisol, apparently unrelated to measures of psychological stress, suggesting an underlying neuroendocrine abnormality. Such patients are most commonly of the kind with severe illness of the endogenomorphic and psychotic types - that is, with syndromes characterised by reduction of interest and pleasure, depressed mood, reduced emotional responsivity to the environment, psychomotor retardation or agitation, pessimism and often, suicidal preoccupations of delusional proportions.

(iv) The cortisol hypersecretion ceases after clinical recovery.

Sachar et al (1976), have suggested that cortisol hypersecretion reflects ACTH hypersecretion which in turn reflects hyperactivity of the neuroendocrine cells secreting corticotropin releasing hormone (CRH).

In an attempt to relate this neuroendocrine abnormality to current theories of brain neurotransmitter disturbances in depressive illness, they speculate that cortisol hypersection in depressed patients reflects a disinhibition of CRH secretion

secondary to brain noradrenaline depletion. It is suggested that such a hypothesis would gain more credance if a noradrenergic system were found to be responsible for the normal circadian inhibition of CRH-ACTH-cortisol secretion in late evening and early morning.

Sachar et al (1976), have also implicated a noradrenergic deficiency in the discovery of reduced LH levels in depressed menopausal women.

Depression is also one of the recognised side effects in women taken oral contraceptives, occuring in between 5-7% of such women (Herzberg and Coppen 1970; Herzberg et al 1970; Adams et al 1973). Attempts have been made to link peripheral changes in tryptophan metabolism to the pathogenesis of this mood change.

Tryptophan is metabolised along two main pathways, the quantitatived major route being the kynurenine-niacin pathway, while the other results in the formation of 5-HT. The first enzyme of the kynurenine-niacin pathway is liver tryptophan pyrrolase.

This enzyme has been found to be induced by hydrocortisone, corticosterone (Knox and Auenbach 1955) and betamethasone, with an associated fall in brain 5-HT levels (Green and Gurzon 1968; Scapagnini et al 1969). In animals, large doses of cortisol have been found to reduce brain 5-HT levels markedly (Curzon and Green 1968; Green and Curzon 1968; Yuweiler et al 1971; Fuxe et al 1973; Yuweiler and Geller 1974), although chronic administration of cortisol appeared to produce different effects to those of acute administration, in that brain 5-HT levels returned to normal in animals given repeated doses. It has been suggested that the induction of liver tryptophan pyrrolase decreased the availability of tryptophan for 5-HT synthesis (Curzon 1971; Green et al 1975; Green et al 1975).

Oestrogens appear to induce tryptophan pyrrolase by their known action of increasing plasma cortisol (Keller et al 1969). It has

long been known that oestrogens increase total plasma cortisol without increasing the urinary excretion of cortisol metabolites (Petesson et al 1960). This is explained by the fact that oestrogen-treated subjects show increased cortisol binding to plasma proteins (Sanberg and Slaunwhite 1959). The increase occurs in the corticosteroid-binding globulin (CBG) and depends on the oestrogen level (Doe et al 1967), being similar to that of other liver enzymes induced by oestrogen (Musa et al 1967).

Another induced enzyme is alanine aminotransferase, which is elevated in both the liver and pancreas by cortisol. Keller et al (1969), have shown that, in the rat, when corticosteroids are raised by oestrogens, there is an increase in the activity of the liver enzyme, but not that of the pancreas. Their explanation was that CBG can gain access to the enzyme-producing systems of the liver by pinocytosis, followed by the liberation of the free steroid, whereas the thick basement membrane of the pancreatic cell prevents entrance of the protein-bound steroid into that organ. Toseland (1974), suggested that oestrogens increase tryptophan pyrrolase in the same way, the effect being dose related.

Thus various investigators have suggested that oestrogens increase pyrrolase activity, which in turn would decrease 5-HT synthesis, thereby producing the mood change (Rose 1966; Rose and Braidman 1971; Rose 1972; Winston 1973; Adams et al 1973; Grant et al 1975; Wynn 1975; Malik-Ahmed and Berkmann 1976). A recent report by Green et al (1978) has, however, failed to find increased pyrrolase activity in women taking oral contraceptives.

The evidence for increased pyrrolase activity is that the urinary excretion of several kynurenine-niacin pathway metabolites, such as 3-hydroxykynurenine, xanthurenic acid and 3-hydroxyanthranilic acid, is higher in women taking oral contraceptives, following the

administration of an oral tryptophan load (Rose 1966; Adam et al 1973; Leklem et al 1975; Luhby et al 1971; Green et al 1978). The agreement of the latter investigators in this indicates that their failure to demonstrate increased pyrrolase activity while on oral contraceptives, is not due to current oral contraceptives having a lower oestrogen content than those of a few years ago. This is supported by their observation that there was no difference in the metabolic response of the subjects on low oestrogen dose contraceptives to those on higher oestrogen dose contraceptives.

Unlike previous reports (Adams et al 1973; Leklem et al 1975; Rose and Adams 1972; Price et al 1967), increased excretion of kynurenine by subjects on oral contraceptives was not observed, either before or during the tryptophan load, by Green and his co-workers (1978). Indeed the absolute excretion of kynurenine by this group was lower. They have suggested that since the production of kynurenine from tryptophan appears to be the same in both groups, the catabolism of kynurenine may be different in the oral contraceptive group, in that it is broken down more rapidly to other metabolites, such as acetylkynurenine, excretion of which is raised in oral contraceptive users (Leklem et al 1975).

One possible reason for the changed excretion of tryptophan metabolites by women on oral contraceptives is suggested by the work of Mason and Gullekson (1960). They demonstrated that sulphate esters of oestrogens interfere, in vitro, with the activity of some pyridoxal phosphate-dependent enzymes by competing for sites on the apoenzyme. Rose et al (1972), suggested that esters formed from the oestrogenic component of oral contraceptives may reach sufficient concentrations in the liver to inhibit supernatant kynureninase, but that kynurenine aminotransferase, protected with the mitochondria, retains its activity and metabolises the accumulating 3-hydroxykynurenine to xanthurenic

acid. The observation of Leklem et al (1975) is consistent with this theory, since it showed that when Vitamin B_6 deficiency is sufficiently severe, the build-up of 3-hydroxykynurenine exceeds that of xanthurenic acid, suggesting that B_6 deficiency impaired mitochondrial aminotransferase activity. Also consistent with this hypothesis are the observations that abnormal excretion of tryptophan metabolites seen in subjects taking oral contraceptives returns to normal after B_6 administration (Rose 1966; Luhby et al 1971; Adams et al 1973), and that B_6 administration induced a clinical improvement in those oral contraceptive subjects suffering from endogenous depression (Adams et al 1973).

EXPERIMENTAL METHODS

1. ANIMALS, ANIMAL HUSBANDRY AND LABORATORY CONDITIONS

Experiments reported in this thesis were carried out on Aston University-bred male and female albino mice of the TO strain. Subsequent to weaning, mice were kept in groups of twelve in polypropylene cages in the experimental laboratory. This room was maintained at 21 ± 1°C, with relative humidity between 50–60%, the animals being exposed to a 12 hour light/12 hour dark cycle. They were fed a conventional 41B cube diet (Pilsbury's Ltd., Birmingham) and received tap water "ad libitum".

At between 10–12 weeks old, the mice were divided into experimental groups of 3 or 4, and kept in cages (42x28x15cm). This ensured a synchronised oestrus cycle in each experimental group of female mice.

2. INJECTION TECHNIQUES

(a) Subcutaneous (sc) Injection

Injection was made into the loose skin at the back of the neck. The injection volume was 10ml/kg.

(b) Intraperitoneal (ip) Injection

Injection was made by inserting the hypodermic needle obliquely and upwards through the abdominal wall. Care was taken not to penetrate too deeply. Again the injection volume was 10ml/kg.

3. OESTROUS CYCLE EVALUATION

Daily vaginal smears were obtained from each female mouse using a thin glass rod dipped in 0.9% sodium chloride solution. Staining and fixing of the smears was found to be unnecessary.

The stages of the oestrous cycle were elucidated using the description of Allen (1922). For convenience, the cycle was divided into four stages, each histologically distinct.

1. Dioestrus: The smears were characterised by few epithelial cells, in various stages of nuclear degeneration and cytoplasmic shrinkage plus polymorphonuclear leucocytes.

2. Proestrus: In this stage of the cycle, the smear only showed nucleated epithelial cells.

3. Oestrus: The smear showed only cornified, non-nucleated cells.

4. Metoestrus: This period may be subdivided into two sections. The smears of early metoestrus were characterised by numerous cornified non-nucleated cells bunched or cakes together. In late metoestrus, there was a decrease in the number of cornified cells plus a heavy leucocytic infiltration.

All experiments took place on mature, regularly cycling mice, sexual maturity being attained at nine weeks (Parkes 1925).

4. BEHAVIOURAL TESTS

(a) Measurement of Locomotor Activity

The locomotor activity of mice was measured by means of an Animex activity meter type SE, and Animex counter type 1-X (both supplied by LKB Farad). A thorough investigation of the Animex system has been made by Svensson and Thieme (1969).

The Animex activity meter consists of an inductance coil together with a variable capacitance, forming a resonant circuit. This circuit is fed from a high frequency oscillator. As an animal approaches or moves away from one of the six sensitive coils, a change in current of the resonant circuit occurs, resulting in "offtuning". Each change within any of the coils is amplified and registered.

The Animex activity meter was placed in the experimental laboratory with the counter in an adjoining room to prevent noise from it interfering in the experiments.

Experiments were performed on groups of three mice, which were

placed in polypropylene cages (42x28x15cm) each containing a very thin layer of sawdust. A cage of such dimensions just fitted over the six coils of the Animex. In all cases, the experiments were carried out with mice in their "home cage", thus preventing any increase in activity due to exploration of a novel environment.

The activity meter was tuned to 40μA, and the sensitivity at 25μA. Preliminary experiments showed this sensitivity to be sufficient to detect gross locomotor activity, but too slight to detect small movements such as grooming and tremor.

Two types of experiment were carried out using the activity meter. In the first, counting began five minutes after the last injection treatment had been performed. Counts were made at fifteen minute intervals, and the experiment lasted for two hours from the time of the initial count. In the second, counts were made at one hour intervals and the experiments lasted for one complete light/dark cycle (24 hours).

Data obtained were statistically evaluated by means of the t-test (Student 1908).

(b) Measurement of Startle Response

Evaluation of the startle response of mice was made possible by the development of a startle box similar in design to that of Kirkby et al (1972). A puff of air replaced the sound stimulus.

The apparatus consisted of a perspex box 15x15cm, standing 12cm high, with a perforated lid. A small perspex box (8x8x8cm), in which the mouse was housed, was attached to the lid. A sheet of flexible metal, supported at each end, formed the floor, and was positioned 8cm from the top of this box. This unit fitted inside a wooden box (40x30x32cm), which was ventilated by a fan. The fan also provided a standard background noise. The box was illuminated by a 20 volt bulb, and fitted with a one-way glass lid, such that a mouse

could be viewed without disturbance.

Compressed air was delivered at 15lb/sq.inch from a cylinder, by means of a British Oxygen head, and maintained by a valve (Festo Pneumatic). A timer and stimulator (Scientific and Research Instruments) allowed standardised puffs of air to be displaced at intervals of five seconds. These puffs of air were directed at the mouse by a piece of tubing secured to the lid of the box.

The metal floor was attached to a strain guage. Sudden jerks produced by the mouse activated a transducer and impulses were amplified and recorded on a Devices instrument.

Mice were caged in groups of four. At a suitable time after injection, a mouse was placed in the startle box for fifteen minutes before application of the stimulus. This was found to be sufficient time for the mouse to habituate to its new environment inside the box. Twenty stimuli at five second intervals, were then directed at the mouse. In the oestrous cycle experiments, all mice were smeared immediately after removal from the box, on completion of each individual experiment.

Comparisons between drug treatments or oestrous cycle stages were made using the t-test.

(c) Open Field Assessment

The four parameters of "emotionality" in rodents, as defined by Burke and Broadhurst (1966), were evaluated in the open field. These were ambulation, defaecation, rearing and grooming episodes.

The observation area consisted of an open polypropylene box, measuring 50x30cm and standing 15cm high. The floor was divided into 5cm squares. This area was centrally illuminated by a 100w bulb. Counting the various behavioural parameters started immediately the mouse had been placed centrally in the observation area and lasted for five minutes. In order to standardise conditions, all

experiments were carried out between 12-14:00hrs.

5. BIOCHEMICAL METHODS

Whole brain noradrenaline and 5-Hydroxytryptamine levels were determined spectrofluorimetrically.

Mice were sacrificed by cervical dislocation and decapitated. Their brains were quickly dissected out and weighed.

(a) Estimation of 5-Hydroxytryptamine

The method of Maickel et al (1968), with modification by Curzon and Green (1970), was followed. Each mouse brain was homogenised in 3ml of ice-cold acidified n-butanol and centrifuged at 200g for five minutes. 2.5ml of the supernatant from the acidified n-butanol homogenate was added to 5ml of heptane and 0.4ml 0.1N hydrochloric acid containing 0.2% of cysteine to prevent oxidation. This mixture was shaken in a glass stoppered tube for one minute, using a Whirlimixer (Fisons Scieftific Apparatus) and then centrifuged at 2000g for five minutes. The organic phase was aspirated off and an 0.2ml aliquot of the acid phase added to 0.6ml of O-phthaldehyde solution. The tube contents were shaken and then heated on a water bath at 70°C for twenty minutes. The fluorescence intensity of 5-HT was read at the activation and emission wavelength 360/470 μm respectively in an Aminco Bowman Spectrophotofluorimeter. The activation and emission slits were 3.0mm. The value of the recovery was 95%.

(b) Estimation of Noradrenaline

Essentially the method of Chang (1964), was used. After weighing, each brain was homogenised in 3ml of ice-cold acidified n-butanol containing 0.01% EDTA. This was centrifuged at 2000g for five minutes. A 2.5ml portion of the supernatant fluid was then taken and transferred to a glass stopped tube containing 5ml of heptane plus 2.5ml 0.01N HCl. The tubes were shaken for one minute

and centrifuged at 2000g for five minutes. 2ml of the acid phase was transferred to a glass stoppered tube containing 200g of alumina and 1ml of 2.0M sodium acetate with 0.1% EDTA (pH 7.0). The tube was shaken in a horizontal fashion for one minute and centrifuged at 2000g for five minutes. The sodium acetate was aspirated off and the alumina washed by shaking with 2.0ml of double distilled water for one minute and centrifuging. After aspiration, the water soluble catecholamines were eluted by shaking with 2.0ml of 0.1N acetic acid for one minute and centrifuging at 2000g for five minutes. A 1ml sample of eluate was added to 0.2ml 1M sodium acetate containing 0.1M EDTA to give a pH of 6.5. 0.1ml of 0.1N iodine in absolute alcohol was added to oxidise the amines. After precisely two minutes the oxidation was stopped by the addition of 0.2ml alkaline sulphite. Exactly two minutes later, the solution was adjusted to a pH of 5.4 by the addition of 0.2ml 5N acetic acid.

In order to assay noradrenaline, the mixture was heated in a boiling water bath for exactly two minutes, then cooled in ice. The fluorescence of noradrenaline was read at activation and emission wavelengths 385/485 μm, the slit widths being 3.0mm. The value of the recovery was 65%.

(c) Estimation of Tryptophan

The method used was that of Denckla and Dewey (1967), with modification by Bloxam and Warren (1974).

(i) Plasma Tryptophan

The mice were sacrificed by cervical dislocation and decapitated, the blood being collected in heparinised tubes. These were then centrifuged at 11,000rpm for ten minutes. 10ml of plasma was than deproteinised by the addition of 2.0ml of 10% trichloracetic acid solution (TCA), and centrifuged for ten minutes at 20,000g. The supernatant was then decanted to a glass stopped centrifuge tube

(calibrated to 2.0ml) to which 0.2ml of 2% (w/v) formaldehyde solution and 0.1ml of $6x10^{-3}$M $FeCl_3$ in 10% TCA were added. The tubes were then placed in a water bath at 99-101°C for one hour. (Temperatures below 99°C have been found to seriously affect the yield). Following the reaction the tubes were cooled to room temperature and 10% TCA solution was used to replenish to the 2ml mark, the fluid lost during incubation. The product was read at excitation and emission wavelengths of 368μm and 448μm respectively, using an Aminco Bowman Spectrophotofluorimeter. The excitation and emission slits were 3.0mm.

(ii) Brain Tryptophan

The brain was weighed and immediately transferred to a cold glass homogenising tube to which 5.0ml of 0.9% saline was added. 0.04ml of the homogenate was pipetted into a plastic centrifuge tube to which was added 1.8ml of $3x10^{-4}$M $FeCl_3$ in 10^{-4}M HCl - this dilution being necessary for a quantitative recovery. After thorough mixing, 0.2ml of 75% TCA solution was added, the solution thoroughly mixed again and then centrifuged at 20,000g for ten minutes. All further steps were carried out with the supernatant as for plasma.

(d) Estimation of Cortisol

0.5ml of plasma was added to 5.0ml of dichloromethane in stoppered tubes. These were then shaken for ten minutes - consistent shaking being ensured. A 2.0ml sample was then added to 1.0ml of fluorescent reagent (30% absolute alcohol and 70% sulphuric acid). This was then shaken for thirty seconds.

All of the lower acid/alcohol phase was then placed in a cuvette and read in an Aminco Bowman Spectrophotofluorimeter, the excitation wavelength being 460μm and the emission wavelength 520μm. The excitation and emission slits were 3.0mm.

(e) Estimation of Tryptophan Binding to Serum Albumin using ^{14}C Tryptophan

The method used was essentially that of Bender et al (1975). Using human plasma, 300µl was pipetted into a plastic cup of 250µl capacity, thereby forming a convex meniscus. The cup was then covered with a square of cellophane dialysis membrane, previously soaked in distilled water for several hours and blotted dry immediately prior to use. Such an operation expelled excess plasma leaving 250µl inside the cup. The cellophane membrane was then secured in place using an open ended plastic tube, thereby creating a second chamber above the membrane. 100µl of a solution of ^{14}C tryptophan (220,000 DPM/100µl) in 0·15M NaCl was then pipetted into the upper chamber, above the membrane. The upper chamber was then capped, and the complete dialysis apparatus placed in an airtight container and left overnight at $4^{o}C$, to achieve equilibrium (Bender et al have suggested that equilibrium should be obtained within 8 hours without need for agitation).

After equilibrium, replicate samples of 20µl were withdrawn from the upper chamber and, after the removal of the tube and membrane, the lower chamber, in order to count radioactivity. The 20µl samples were placed in counting tubes with 10ml of scintillation fluid. The water-miscible scintillation fluid was composed of 3g 2,5-diphenylphenyloxazole+0·3g 1,4,-bis-(5-phenyloxazole -2gl) benzene in 400ml of ethoxyethanol+600ml toluene. Each sample was counted for 10 minutes in the scintillation counter, 0·15M NaCl being used in the blanks.

With experiments using mice, a smaller plastic cup was not used as suggested by Bender et al. The blood from four animals was combined in order to obtain 300µl plasma samples for use in the procedure described above.

6. REAGENTS, DRUGS AND CHEMICALS

All reagents were Analar grade unless specified.

(a) Reagents

Acidified n-butanol - 0·85ml concentrated HCl dissolved in one litre of n-butyl alcohol.

0·1M EDTA - 1·86g disodium ethylenediamine tetraacetate dihydrate (EDTA) was dissolved in 500ml 1M sodium acetate. The pH was adjusted to 6·7 - 7·0 by the addition of 10N sodium hydroxide solution.

o-phthalaldehyde - A solution was prepared of o-phthalaldehyde 40ug/ml in 10N HCl. o-phthalaldehyde was obtained from Regis Chemical Co., Chicago, U.S.A.

Alkaline sulphite - 1ml of sodium sulphite solution (2·5g of anhydrous salt in water in 10ml water) was diluted with 9ml 5N sodium hydroxide before use.

(b) Drugs and Chemicals

A stock solution of 5-HT 1mg/ml was prepared in 0·1N hydrochloric acid containing 0·2% cysteine.

A stock solution of noradrenaline 1mg/ml was prepared in 0·1N hydrochloric acid.

L-tryptophan was prepared as a suspension in 0·9% saline containing 0·5% Tween 80 (BDH).

MK 486 was dissolved in a few drops of concentrated HCl, made up to volume with 0·9% saline, the pH being adjusted to 6·7 - 7·0 using 10N sodium

hydroxide. A similar procedure was used in the preparation of a solution of 5-HTP.

The synthetic sex steroids, norethisterone acetate and ethinyloestradiol were dissolved in a few drops of 95% ethanol, and made up to volume with 0·9% saline.

p-CPA was prepared as a suspension in 0·9% saline, an equal weight of powdered acacia being used as the suspending agent.

The drugs used and their sources were as follows:

Drug	Source
5-hydroxytryptamine creatinine sulphate	Sigma Ltd.
noradrenaline hydrochloride	Sigma Ltd.
l-tryptophan	Sigma Ltd.
DL-5-hydroxytryptophan	Sigma Ltd.
reserpine	British Drug Houses Ltd.
MK 486	Gift from M.S.D. Ltd.
chlorgyline	Gift from M.S.D. Ltd.
p-chlorophenylalanine	Sigma Ltd.
norethisterone acetate	Sigma Ltd.
ethinyloestradiol	Sigma Ltd.

7. CLEANSING OF ALUMINA

Neutral chromatographic grade alumina was acid washed by the method of Anton and Sayre (1962). 100g of alumina was added to 500ml 2N HCl and stirred for 45 minutes at 90-100°C. After cessation of stirring the particles were allowed to settle for 90 seconds

and then the supernatant discarded along with the finer particles of aluminium oxide. This procedure was repeated with 250ml 2N HCl, stirred for 10 minutes at 70°C, and 250ml 2N HCl, stirred for 10 minutes at 50°C. The precipitate of alumina was then repeatedly washed with double distilled water until a pH of 3 was achieved. The alumina was finally heated in a dry oven for one hour at 120°C, and for 2 hours at 200°C.

8. GENERAL CLEANING OF GLASSWARE

Special attention was paid to the cleaning of glassware to ensure accurate results in the spectrofluorimetric assays. Glassware was soaked in Decon solution (prepared from Decon 90 Concentrate) for 24 hours. Several rinses were made with tap water and finally double distilled water. Glassware was then dried in a hot air oven.

9. CALCULATION OF RESULTS

Concentrations of 5-HT, noradrenaline, cortisol and tryptophan were calculated by running known standards, internal standards and reagent blanks through the assays. Data were assessed for significance by the Students t-test. Open-field results were assessed initially by analysis of variance (ANOVA) followed by the students t-test where necessary.

RESULTS

CHAPTER ONE

LOCOMOTOR ACTIVITY IN THE MOUSE OESTROUS CYCLE, IN MALE AND AGED FEMALE MICE, AND IN FEMALE MICE RECEIVING SYNTHETIC GONADAL HORMONES.

The following work is an attempt to quantify locomotor activity in both female and male mice. Studies were undertaken to examine changes in locomotor activity thought to occur during the oestrous cycle of sexually mature female mice, and also in "senile" female mice, and sexually mature male mice. The results are expressed in the activity units as recorded by the Animex activity meter, these units being arbitrary units of activity.

1. LOCOMOTOR ACTIVITY CHANGES DURING THE OESTROUS CYCLE OF SEXUALLY MATURE VIRGIN FEMALE MICE UNDER A 12 HOUR LIGHT/12 HOUR DARK LIGHTING SCHEDULE

The age of the mice used was 3 months, to ensure sexual maturity and regular oestrous cycles. The three mice of each set were housed together for 30 days prior to the test, to ensure synchrony of oestrous cycles within the group. Vaginal smears were taken daily between 12:00 - 1:00p.m. for three complete oestrous cycles, prior to the test, to ascertain oestrous status. If any memeber of such a group showed irregularity of cycle, the group as a whole was rejected from the experiment.

Each experimental group was housed on the Animex activity meter for six days to ensure the completion of one oestrous cycle. Daily vaginal smears continued to be taken between 12:00 - 1:00p.m. throughout the six day experimental period.

(a) Total Activity Changes

Two distinct phases of activity were exhibited by the test animals. During the light period, the mice were

seen to spend much of their time asleep, thus recording a negligible activity count. In the dark phase, however, the mice showed considerable activity. The table below shows the effect of the lighting schedule on activity during the oestrus and dioestrus stages of the cycle:

Stage of Cycle	Total Dark Phase Activity	Total Light Phase Activity	Total Light Phase Activity as a % of Total Activity
Oestrus	8868 ± 639	2525 ± 289	22·18
Dioestrus	5824 ± 522	1728 ± 229	22·88

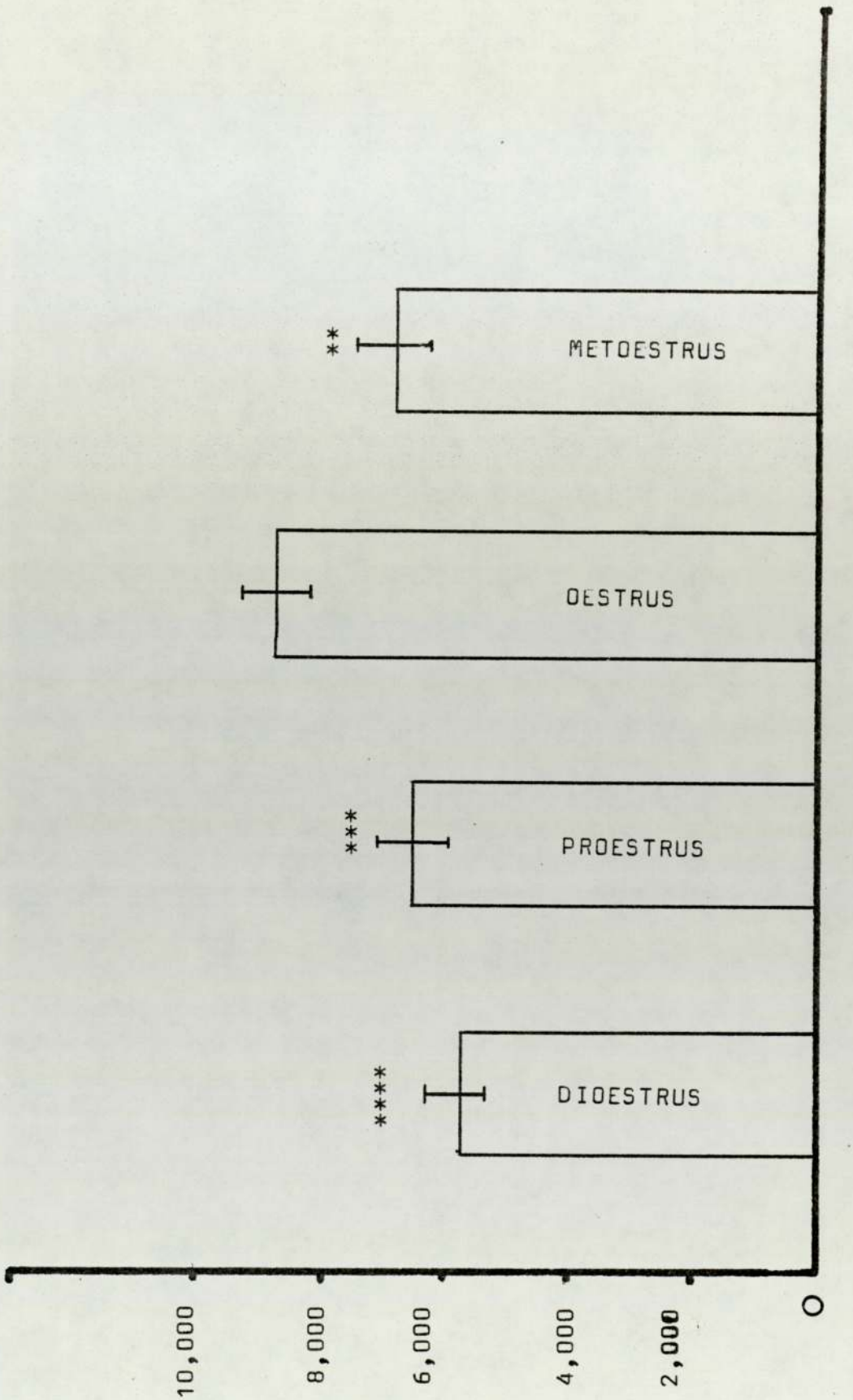
(b) Total Dark Phase Activity

As shown above, nearly 80% of the total activity was exhibited during this phase of the lighting schedule, throughout all phases of the oestrous cycle. A distinct increase in activity was seen during the oestrus phase, which was found to be significant when compared to three other phases (Fig 3). Dioestrus was found to be the phase of least activity (5824 ± 522, n=16, $P < 0{\cdot}001$). Activity then increased with the advent of the proestrus stage (6565 ± 597, n=19, $P<0.01$), and continued to increase, reaching a maximum at oestrus (8868 ± 638, n=15). During the metoestrus phase activity started to decline (6930 ± 587, n=17, $P<0{\cdot}02$).

(c) Hourly Changes in Activity Throughout the Oestrous Cycle

The lighting schedule in this experiment was 07·00 hours to 19·00 hours - light; 19·00 hours to 07·00 hours the following day - dark.

Figure 4 shows the patterns of activity which occured in the oestrus and dioestrus phases, Fig 5, that



TOTAL DARK TIME ACTIVITY

FIG. 3 : Total Dark Time Activity over the Mouse Oestrous Cycle.

** P<0.02; *** P<0·01; **** P<0·001 (Oestrus-control, t-test).

FIG. 4 : Hourly Dark Time Activity for the Oestrus and Dioestrus phases of the Oestrous Cycle.

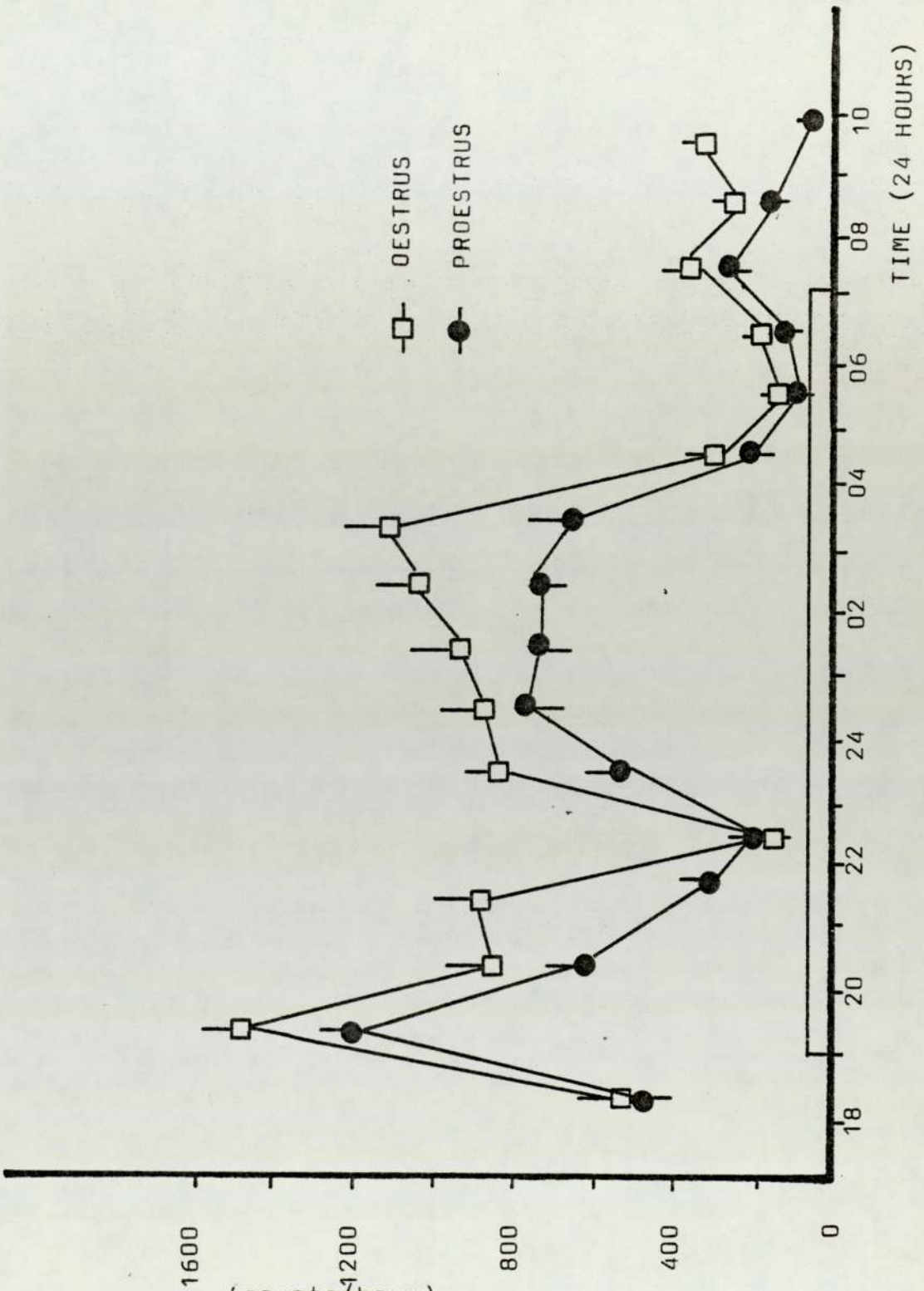


FIG.5: Hourly Dark Time Activity for the oestrus and proestrus phases of the oestrous cycle.

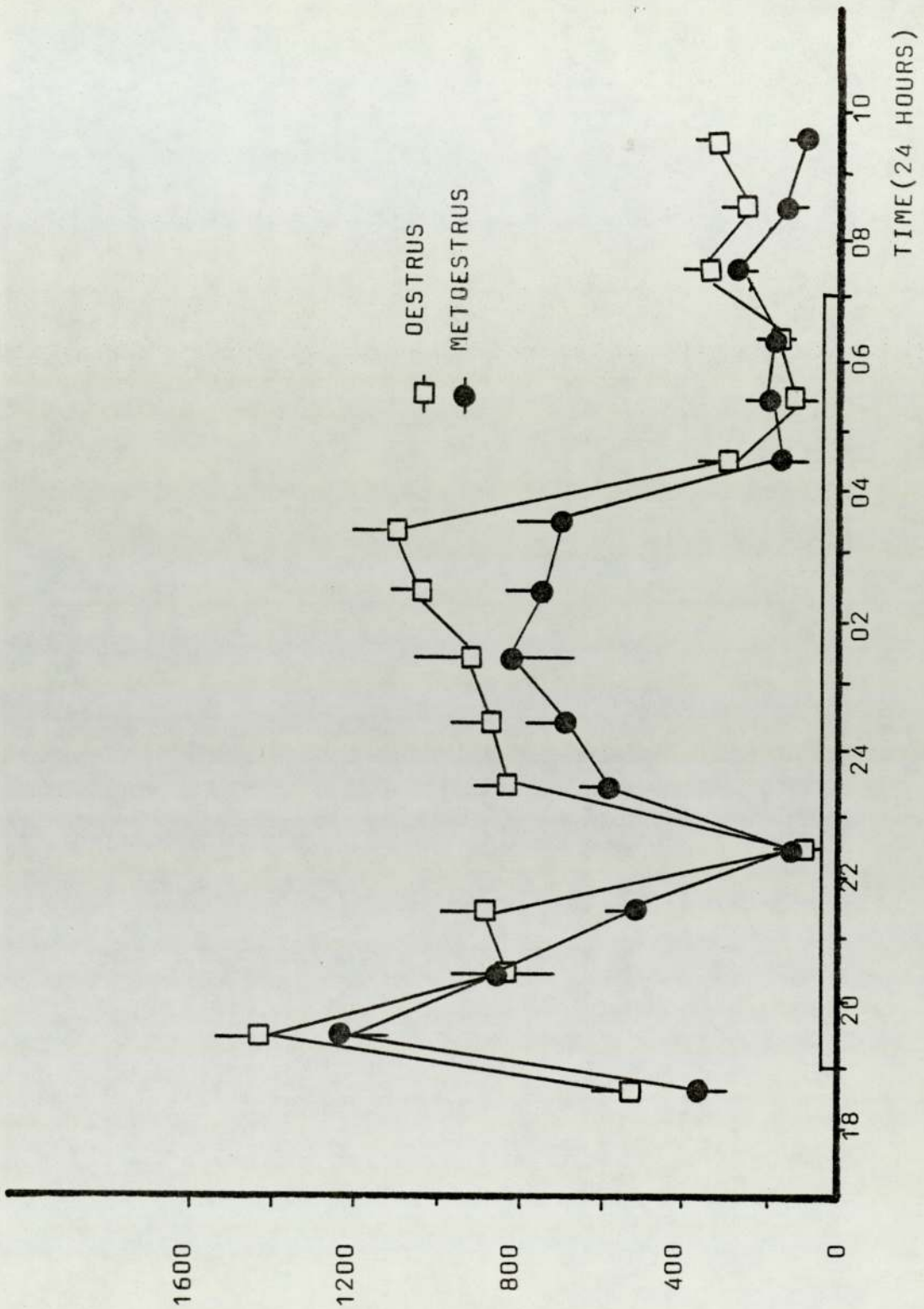


FIG.6 Hourly dark time locomotor activity for the oestrus and metoestrus phases of the oestrous cycle.

occuring in proestrus, and Fig 6, that occuring in metoestrus. In Figures 5 and 6, the oestrus activity pattern is included for means of comparison.

Table 1, summarises the hourly scores in all four phases of the cycle.

Hourly activity counts between 10·00 and 18·00 hours (during the period of illumination) where negligible in all four phases of the cycle, and thus are not included in Figures 4,5,6 or Table 1.

The first hour of darkness (19·00 - 20·00 hours) produced the most intense activity count in all four phases, the count at oestrus (1504 ± 123, n=15) being significantly greater than at dioestrus (1150 ± 116, n=16, $P<0.05$), or proestrus (1181 ± 92, n=19, $P<0.05$), but not that at metoestrus (1269 ± 96, n=17). Activity then declined between 20·00 - 21·00 hours, with no significant variations between the four phases. Between 21·00 and 22·00 hours a distinct change occured in the activity pattern. The oestrus count rose slightly above that of the previous hour, whereas the counts at dioestrus, proestrus and metoestrus continued to decline. Between 22·00 and 23·00 hours, the count for all four phases was at a minimum for the dark period, after which a prolonged period of activity occured between 23·00 - 04·00 hours, the counts at oestrus being consistently, and in most cases significantly, above those of the other phases (see Table 1). Between 03·00 and 04·00 hours the count at oestrus continued to rise, whereas the counts of the other phases had started to decline. The last 3 hours of darkness showed a fall-off in activity, without any significant variations. In the

TIME (24 Hours)	OESTRUS (n=15)	DIOESTRUS (n=16)	METOESTRUS (n=17)	PROESTRUS (n=19)
18-19	526±84	426±59	372±53	485±69
19-20	1504±123	1150±116*	1269±96	1181±92
20-21	844±127	589±104	847±125	650±94
21-22	908±134	486±73***	523±111*	316±60****
22-23	144±22	143±23	158±30	179±46
23-24	838±93	519±112*	533±76**	511±86**
24-1	892±108	443±77***	639±96	804±90
1-2	923±142	539±93*	809±168	743±70
2-3	1029±96	733±89*	759±88*	735±49***
3-4	1078±114	606±45*****	683±106**	648±109****
4-5	274±68	211±40	179±58	224±42
5-6	169±50	203±46	207±55	160±46
6-7	196±44	186±65	202±60	184±50
7-8	345±121	206±58	303±91	278±77
8-9	235±82	140±44	151±57	208±51
9-10	295±75	153±59	128±29*	85±22****

TABLE 1

Hourly variation in activity throughout the oestrous cycle. The degree of significance for each set of results is expressed as follows:

* = < 0·05; ** = <0.02; *** = < 0·01
**** = < 0·001

All results are compared with the corresponding oestrus value. Where no asterisks appear with the results, there was no significant difference in the results. The number of observations (n) appears in paranthesis below the phase of the cycle.

first three hours after the onset of light all four groups showed a slight increase in activity in comparison to the previous three hours. Only at oestrus between 09·00 and 10·00 hours, was the count significantly above that of metoestrus and proestrus.

It is clear, therefore, that the activity counts at oestrus are not only consistently greater than those of the other three phases, but also more sustained.

A later, subjective observation was carried out to ascertain the behaviour associated with the peaks in activity. In this experiment, a reversed light/dark illumination schedule was adopted, with a dim red light, rather than total darkness, for ease of observation. The most noteworthy result was that the first peak of activity between 19·00 and 22·00 hours had associated with it high levels of general locomotor activity and the consumption of food and water, whereas the later peak between 23·00 and 04·00 hours had little associated food and water intake.

2. LOCOMOTOR ACTIVITY CHANGES IN FEMALE MICE NO LONGER EXHIBITING OESTROUS CYCLES DUE TO AGE, AND MAINTAINED UNDER A 12 HOUR LIGHT/12 HOUR DARK LIGHTING SCHEDULE

The lifespan for virgin female mice has been found to be between 500 - 750 days, depending upon the strain of mouse involved (Green 1966). Several workers have examined the relationship between advanced age and occurence of cycles. It would appear that such a relationship is quite variable, and is again dependent upon the particular strain under study. Caschera (1959), found increasing irregularity in cycles with advanced age, including some prolonged periods of dioestrus. Thung et al

(1956), also found decreasing numbers of cycles per unit of time in advanced age, cycles being characterised by somewhat lengthened metoestrus and dioestrus.

It was, therefore, decided to examine the daily locomotor activity pattern in female mice with an age in excess of 400 days.

The mice were housed in groups of 3 for 30 days prior to the experiment. Daily vaginal smears were taken and in all cases persistent dioestrus was observed.

(a) Total Activity Changes

The table below shows the effect of the lighting schedule on both light and dark phase activity of the aged females. The results for oestrus females are included as a means of comparison:

Status	Total Dark Phase Activity	Total Light Phase Activity	Total Light Phase Activity as a % of Total Activity
Aged ♀	4240 ± 227	2374 ± 129	35·89
Oestrus ♀	8868 ± 639	2525 ± 289	22·18

No evidence of cyclicity was observed with either locomotor activity or vaginal smearing in the case of the aged females.

In the dark phase, total activity was significantly below that of oestrus ($P<0.001$) and also below that of dioestrus. Total light phase activity was similar in intensity to that of the oestrus females.

(b) Hourly Activity Changes

Figure 7, shows the 24 hour pattern of activity counts exhibited by the aged females.

Between 10·00 - 16·00 hours (during the period of

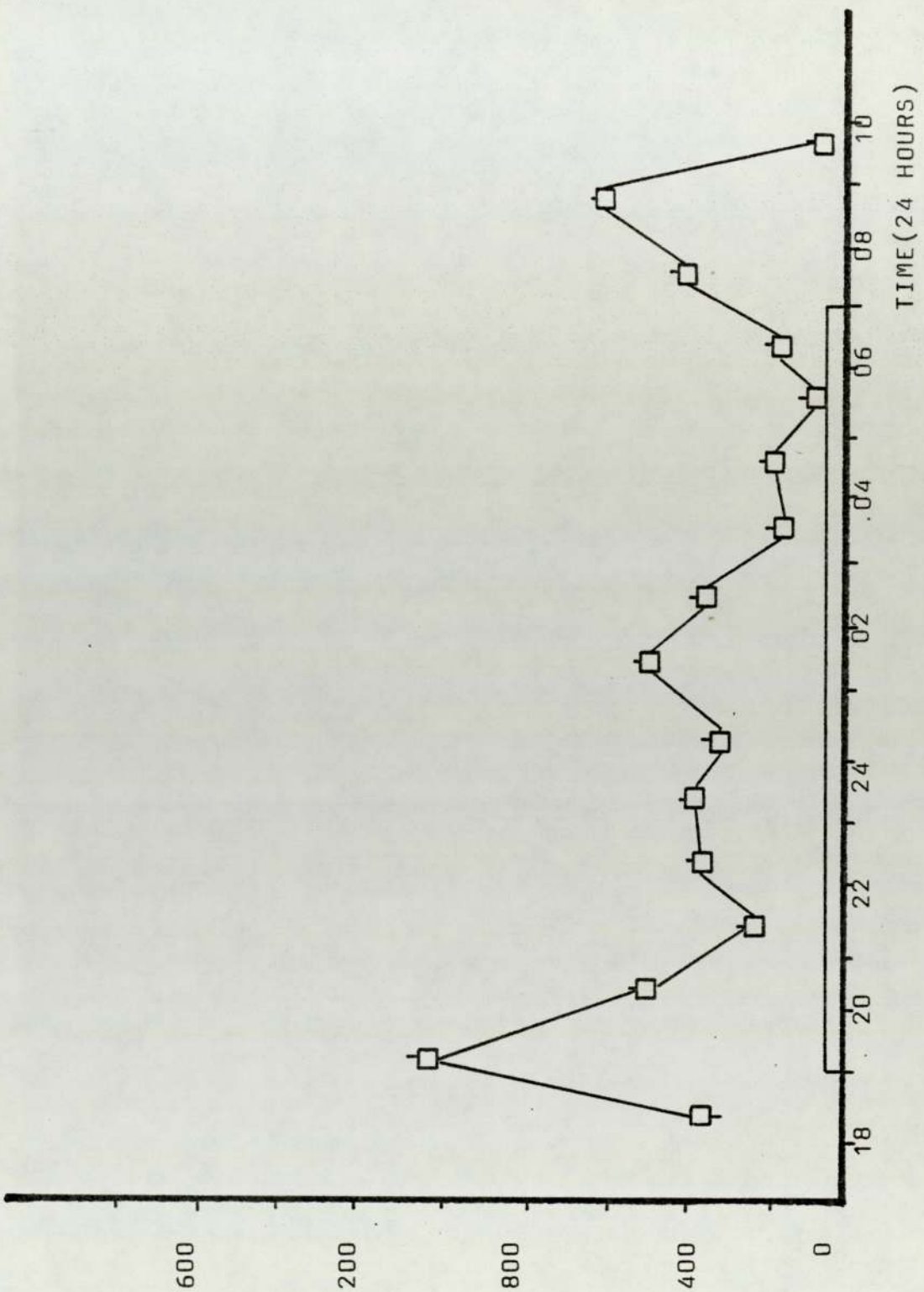


FIG.7 Hourly dark time locomotor activity in aged female mice.

illumination) activity was negligible. From 16·00 to 19·00 hours activity was seen to rise slowly, reaching a maximum at 19·00 - 20·00 hours, the hour immediately following the onset of darkness. Activity then fell, remaining reasonably stable between 300 - 400 counts per hour (c.f. oestrus cycle) for the following six hours. From 03·00 - 07·00 hours, the activity count then fell to around 200 counts per hour, the minimum for the dark period. Following the onset of the light phase, activity again reached a peak between 08·00 - 09·00 hours. At this point, the activity score for the aged females (651 ± 60, n=8) was significantly higher than the corresponding score for the oestrus females (236 ± 82, n=16, $P<0;001$) - the only time this occurs. After this peak, activity fell to the constant light-time low.

3. LOCOMOTOR ACTIVITY CHANGES IN SEXUALLY MATURE MALE MICE MAINTAINED UNDER A 12 HOUR LIGHT/12 HOUR DARK LIGHTING SCHEDULE

Five groups of three sexually mature male mice (aged 3 months) underwent the same experimental schedule as the two previous groups of females, above. Each male mouse was handled daily in order to mimick the handling associated with the taking of vaginal smears in the females.

(a) Total Activity Changes

The table below shows the effect of the lighting schedule on locomotor activity in male mice, with that for oestrus females included for comparison:

Status	Total Dark Phase Activity	Total Light Phase Activity	Light Phase Activity as a % of Total Activity
Male	11236 ± 471	7411 ± 310	39·74
Oestrus	8868 ± 639	2525 ± 289	22·18

A significant feature of these results is the high total activity exhibited by male mice in a 24 hour period, and in particular, the light phase activity. The total light phase activity of the males was greater than the dark phase activity of females in the dioestrus, proestrus and metoestrus phases of the oestrus cycles, and that of the aged females. Total dark phase activity in the males was significantly greater than that of the oestrus females ($P<0·02$).

(b) Hourly Activity Changes

Figure 8, shows the 24 hour pattern of activity counts for male mice.

During the last two hours of light, prior to the onset of darkness, considerable activity was exhibited, which reached a maximum in the first hour of darkness (19·00 - 20·00 hrs), falling to relatively less activity between 21·00 - 23·00 hours. A further burst of activity was sustained for the following four hours between 23·00 and 03·00 hours. The last four hours of darkness between 03·00 - 07·00 hours was the period of minimum activity for the dark phase. The first three hours after the onset of the light phase (07·00 - 10·00 hours) produced a peak in activity equivalent in intensity and duration to that occuring after the onset of darkness. The activity count recorded in the first three hours, and the last two hours,

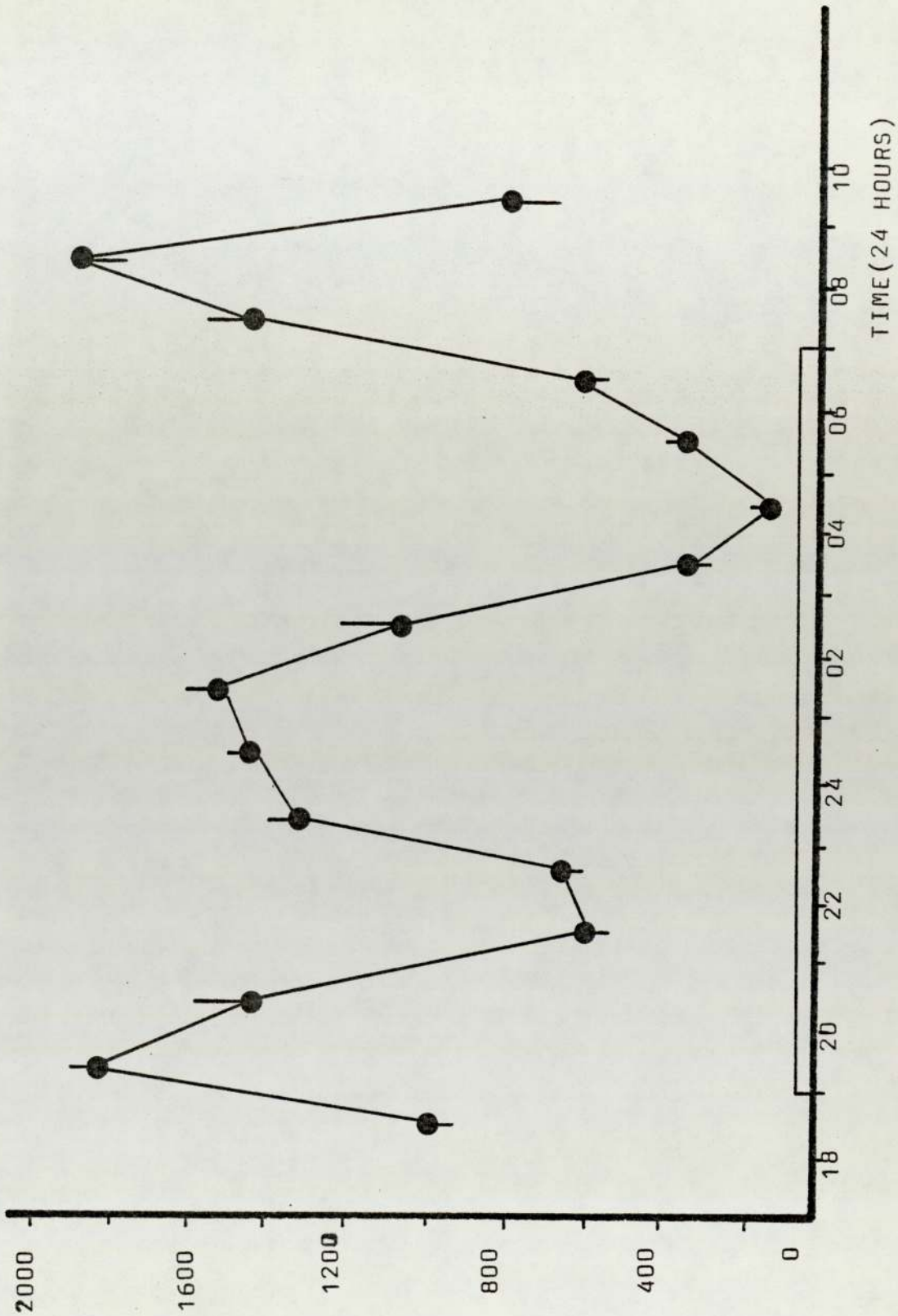


FIG.8: Hourly Dark Time Activity in Male Mice.

of the light phase, represent 81·5% of the total light time activity.

4. THE EFFECT OF THE SYNTHETIC SEX HORMONES ETHINYL-OESTRADIOL AND NORETHISTERONE ACETATE ON LOCOMOTOR ACTIVITY IN SEXUALLY MATURE FEMALE MICE MAINTAINED UNDER A 12 HOUR LIGHT/12 HOUR DARK LIGHTING SCHEDULE

Experiment 1

This was a preliminary experiment to ascertain the effect of continuous daily does of ethinyloestradiol (1µg/kg), and norethisterone acetate (20µg/kg), when given by subcutaneous injection on the cyclicity of the oestrous cycle.

The doses chosen represent the equivalent dose of such steroid hormones that are present in oral contraceptives such as "Minovlar", (which contains 1mg norethisterone acetate and 50µg ethinyloestradiol per tablet).

The experiment was performed on a single group of three sexually mature female mice aged 12 weeks. Subcutaneous injections of normal saline were given daily for four complete oestrous cycles prior to the start of the treatment. Daily vaginal smears were taken to ensure that regular cycling occured.

Table 2, summarises the results obtained. The hormone treatment was started on the afternoon after ovulation had occured, the vaginal smear still indicating the oestrus state. The following day the mice were found still to be in oestrus, but from that day onwards a normal oestrous cyclicity was resumed. On days 6 and 7, after the start of treatment, the metoestrus phase was seen to last

Days after Treatment	Total Dark Phase Activity	Stage of Cycle	Days after Treatment	Total Dark Phase Activity	Stage of Cycle
	5491	Met	16	5958	Di
	5454	Di	17	3827	
	6055	Pro	18	4834	
	8832	Oe	19	3446	
	4083	Met	20	4561	
	1308	Di	21	2669	
	5062	Pro	22	1201	
	5363	Oe	23	2031	
Start of Treatment			24	2347	
1	8019	Oe	25	1920	
2	4129	Met	26	2025	
3	6307	Di	27	2021	
4	6235	Pro	28	1824	
5	8609	Oe	29	2973	
6	8315	Met	30	2319	
7	6054	Met	31	4441	
8	2692	Di	32	3296	
9	2809		33	4000	
10	3119		34	2305	
11	4970		35	2131	
12	6236		36	2341	
13	5186		37	2200	
14	4047		38	2038	
15	4063		39	1784	

TABLE 2

The effect of continuous daily administration of 1μg/kg ethinyloestradiol and 20 μg/kg norethisterone acetate on locomotor activity during the dark period and on the vaginal oestrous cycle.

for two days, and from day 8 onwards, the mice exhibited a vaginal smear indicating a state of constant dioestrus, which persisted to the end of the experiment. After day 8, no regular activity peaks associated with oestrus behaviour could be associated with changes in dark time activity.

Experiment 2

Five groups of three mice were used in this experiment, experimental conditions being as in experiment 1, above. Each set of mice were placed on the Animex for one day in every seven, the other two days being taken up by mice receiving vehicle injections only, which acted as an "internal control".

(a) Total Dark Phase Activity

See Figure 9

Oestrous cycle readings were obtained from each group for two cycles prior to the start of hormone treatment. The results were similar to those obtained in experiment 1. The first day of hormone treatment produced activity readings (6803 ± 807), similar to those obtained at oestrus (6919 ± 523). Only a slight fall was seen by Day 8 (6196 ± 705), which was not found to be statistically significant. However, by Day 15, the activity count for the dark period had fallen significantly to 4161 ± 611 ($P<0.05$) when compared to the reading obtained on Day 1 of treatment. A similar result was obtained on Day 22, (4229 ± 477, $P< 0.05$). Another sharp fall in activity had occured by Day 29 (2825 ± 552, $P<0.01$), but this level of activity remained reasonably constant throughout t e rest of the experiment, being 2601 ± 282 ($P<0.001$) on Day 36, and

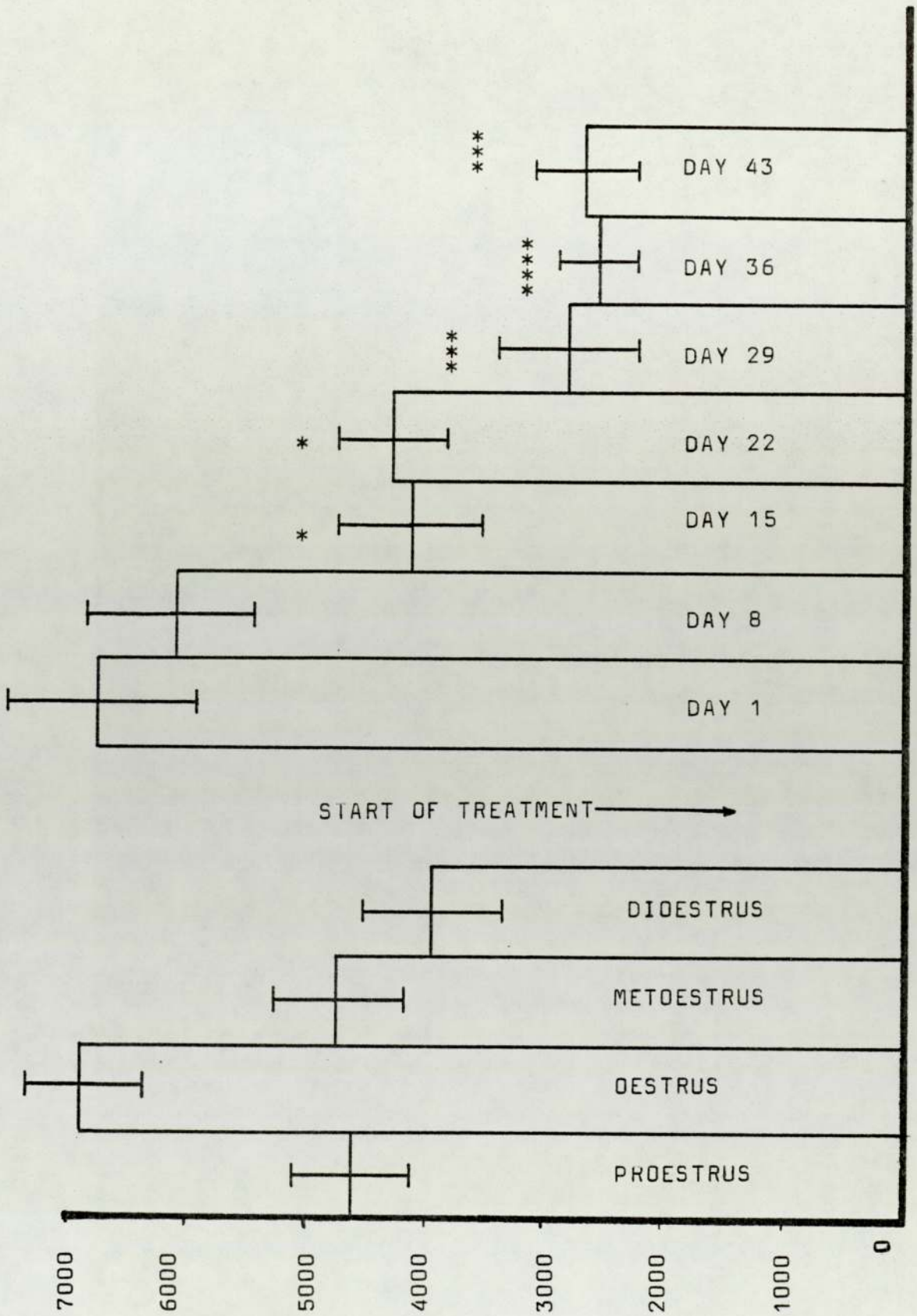


FIG. 9 : Total dark time locomotor activity in female mice receiving norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg.
* P<0·05; *** P<0·01; **** P<0·001.
(Day 1 - control, t-test).

2787 ± 394 ($P<0.01$) on Day 43, the final day of the experiment.

(b) 24 Hour Activity Patterns

Days 15 and 36 were chosen from the above result, as a comparison to the oestrus pattern, as on both of these days the level of activity during the dark phase was similar to that obtained seven days previously. Each of these days respresents an activity plateau, Day 15 = 4000 counts/12 hour dark phase, Day 33 = 2500 counts/ 12 hour dark phase.

Figure 10 illustrates such changes in the activity recorded during the 24 hour period.

Quite distinct changes in the activity pattern can be seen by Day 15. The initial peak in activity which occured at oestrus after the onset of darkness was markedly reduced by Day 15, with a further slight reduction on Day 36. The mid-dark phase activity peak of oestrus was considerably reduced on Days 15 and 36 of the treatment. However, on Day 15, a sustained activity peak, occuring between 03·00 - 07·00 hours, was produced, which was not found to correspond with any activity peak in the oestrus females. This peak was again visible on Day 36, but with reduced intensity.

Little difference in activity was noticed during the light phase between the treated and non-treated mice.

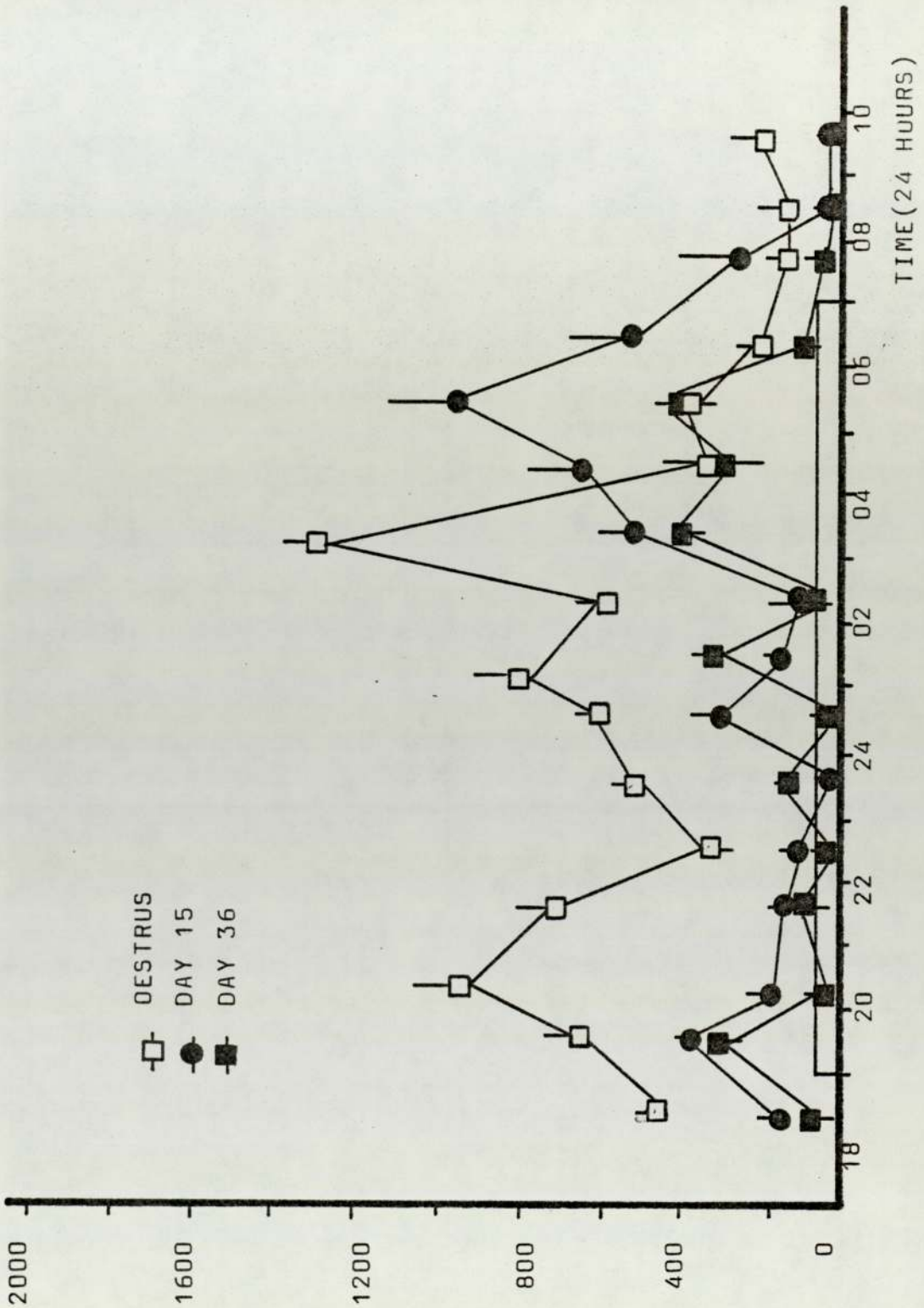


FIG.10: Hourly dark time locomotor activity in female oestrus controls & in female mice on days 15 & 36 of treatment with norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg.

5. THE EFFECT OF THE SYNTHETIC SEX HORMONE NORETHISTERONE ACETATE ON LOCOMOTOR ACTIVITY IN SEXUALLY MATURE FEMALE MICE MAINTAINED UNDER A 12 HOUR LIGHT/12 HOUR DARK LIGHTING SCHEDULE

The experimental conditions for this experiment were identical to those instituted in the previous experiment. Five groups of three mice received 20µg/kg norethisterone acetate by subcutaneous injection, daily.

(a) Total Dark Phase Activity

See Figure 11

The first day of treatment again produced a total dark phase activity of similar magnitude (8939 ± 1453) to that of the corresponding oestrus value (8880 ± 823). By Day 8 activity had fallen significantly below that of oestrus to 5623 ± 1010 ($P<0.05$), and observations of the vaginal smears indicated that the period of constant dioestrus had begun. These smears showed that constant dioestrus began after only one cycle, Day 1 of treatment exhibiting an oestrus smear, Days 2 and 3 metoestrus smears and Day 4 dioestrus. From Day 4 onwards, the smears were all found to be dioestrus. This differs from the previous experiment involving the concurrent administration of norethisterone acetate and ethinyl-oestradiol, when constant dioestrus began after two oestrous cycles had been completed - on Day 8 of treatment.

By Day 15, activity again fallen to 5098 ± 887 ($P<0.02$), and on Day 22 the activity reading fell again to 4032 ± 319 ($P<0.001$). From this day onwards, activity remained at a constant level being 4254 ± 785 ($P<0.01$) on Day 29, 4797 ± 557 ($P<0.001$) on Day 36, and

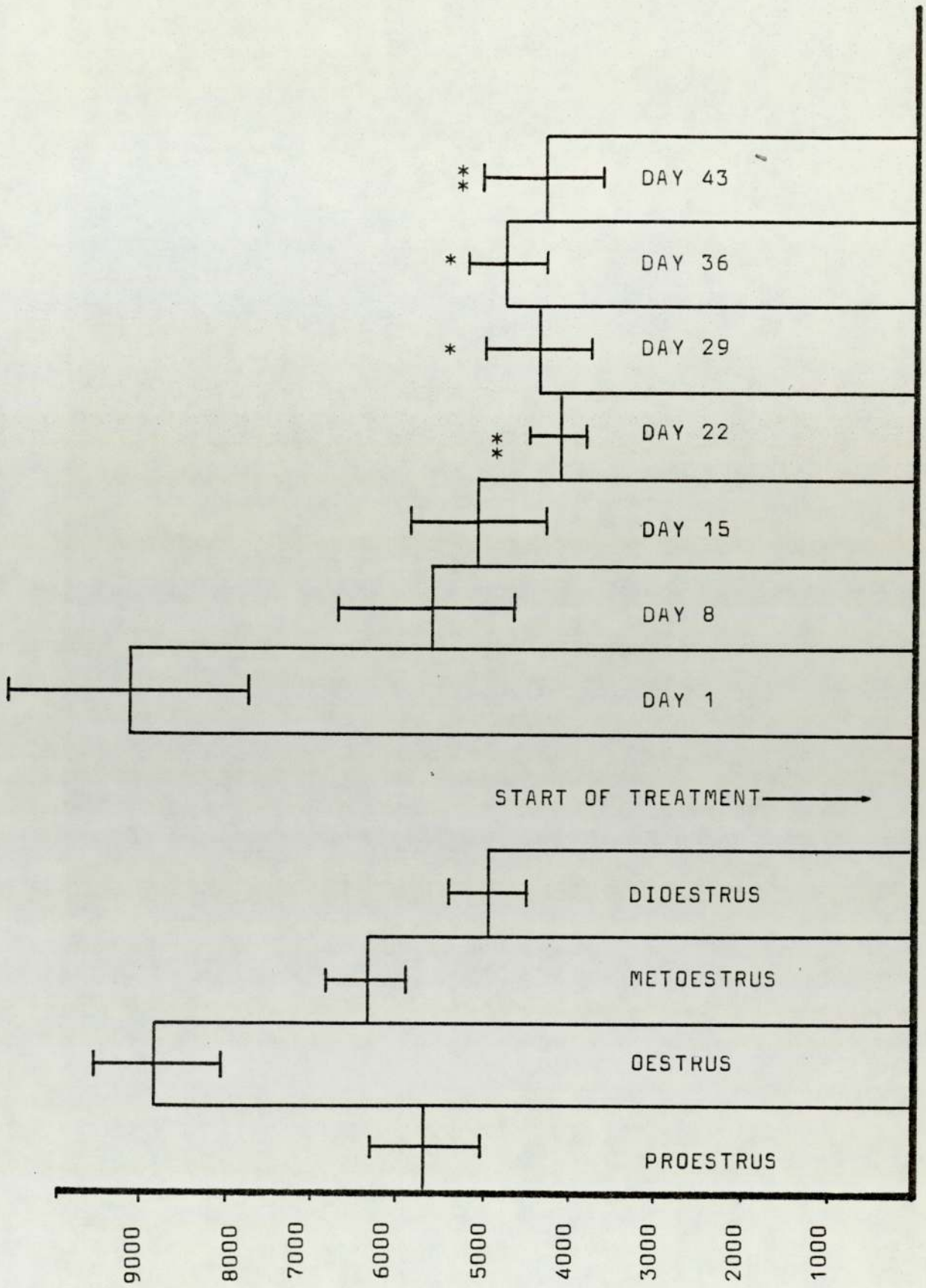


FIG.11: Total dark time locomotor activity in female mice receiving norethisterone acetate 20mcg/kg. * P 0·05; ** P 0·02. (Day 1 - control, t-test).

4130 ± 769 ($P<0.001$) on Day 43, the final day of the experiment.

(b) 24 Hour Activity Patterns

Days 15 and 43 were used as a comparison with the oestrus pattern, and were choosen using the same criteria as in the preceding experiment.

Figure 12 represents the activity changes over the 24 hour period.

The 24 hour pattern in activity was altered much less by the administration of 20µg of norethisterone acetate alone, than when given in combination with 1µg/kg of ethinyloestradiol. While the peak in activity seen in the hour after the onset of darkness was reduced from 1116 at oestrus to 661 by Day 15, the reduction was not so great as on Day 15 of the combined treatment. This peak value is sustained at a similar level on Day 43, a feature not seen with the combined treatment where the peak was further reduced. The sustained activity peak which occured in the last four hours of darkness during the combined treatment is again present on Day 15 of norethisterone acetate alone, the intensity of activity being reduced only slightly. This peak was the most noticeable difference between both forms of sex hormone treatment and oestrous cycle activity patterns. On Day 43 of norethisterone acetate alone, this activity peak was much reduced, and the total dark phase activity pattern produced was much more akin to that of the dioestrus pattern of Figure 2. Once again, little difference in activity was observed during the light phase between treated and non-treated animals.

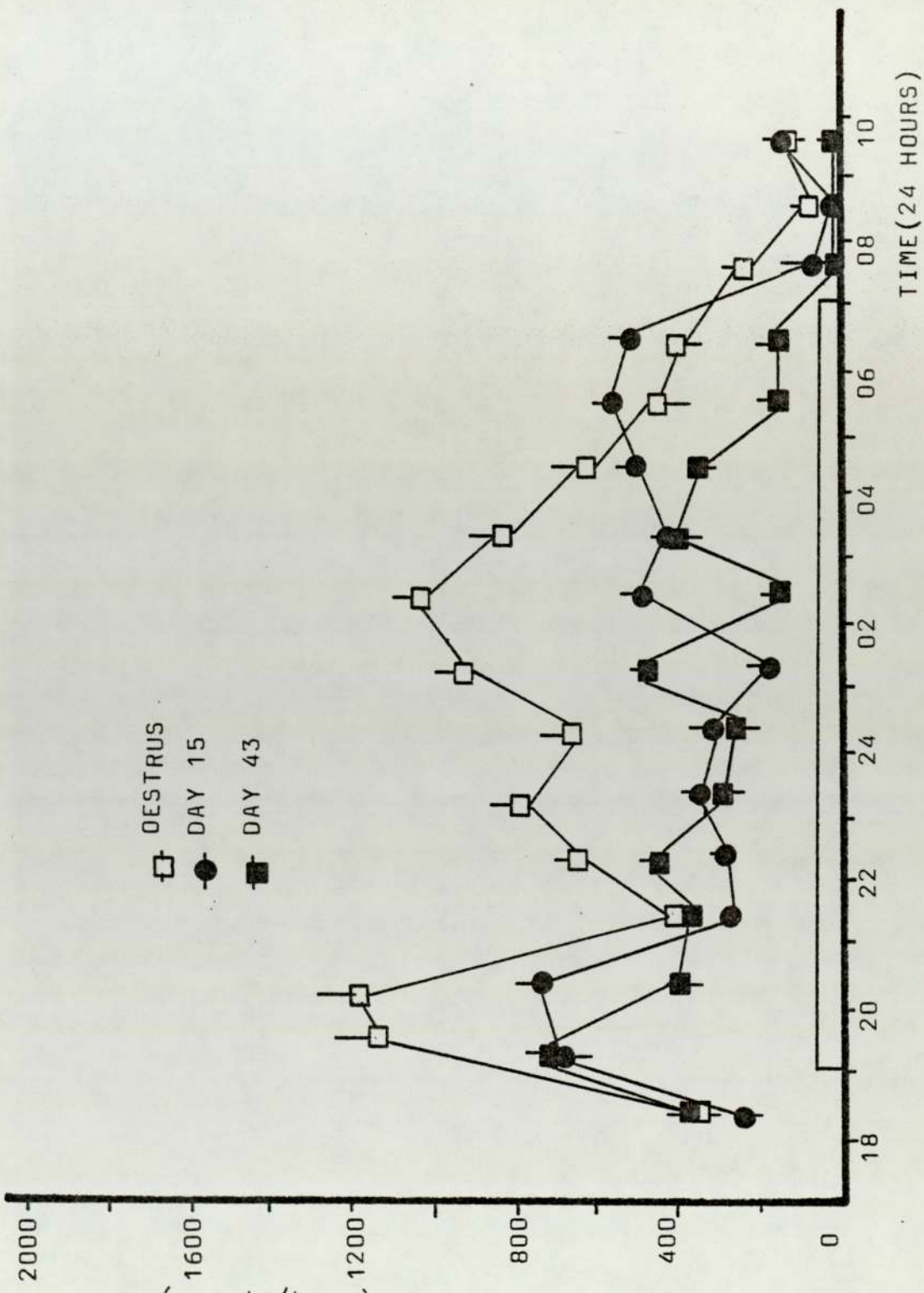


FIG.12: Hourly dark time locomotor activity in female oestrus controls & in female mice on days 15 & 43 of treatment with norethisterone acetate 20mcg/kg.

6. THE EFFECT OF SALINE/VEHICLE INJECTIONS ON LOCOMOTOR ACTIVITY IN SEXUALLY MATURE FEMALE MICE MAINTAINED UNDER A 12 HOUR LIGHT/12 HOUR DARK LIGHTING SCHEDULE

This experiment was carried out in order to ascertain the effects of prolonged subcutaneous injections on both locomotor activity and the oestrous cycle.

(a) Effect on Oestrous Cycle

The most significant effect of such a continued series of injections was not to disrupt the cycle completely, but only to extend the period of dioestrus. Normally, all the female mice studied had regular four day oestrous cycles, which rarely extended above five days. However, the effect of the injections was to increase the length of the dioestrus period to at least two days, thus producing a total cycle length of around five days. During the experiment, eight cycles were completed after the injections had been started and in only two was the length in excess of five days. Both cycles had periods of dioestrus of three days, lengthening the cycles to a total of six days. It must be stressed, however, that the three other phases of the oestrous cycle all occured at regular intervals throughout the time the experiment was carried out.

(b) Effect on Total Dark Phase Activity

See Figure 13

The effect of this series of injections was to produce a slight reduction in total dark phase activity after treatment had started. On Days 1 and 43, the vaginal smears showed that the mice were in the metoestrus phase of the cycle. The total dark phase activity counts registered were 6422 $\pm$ 542 on Day 1

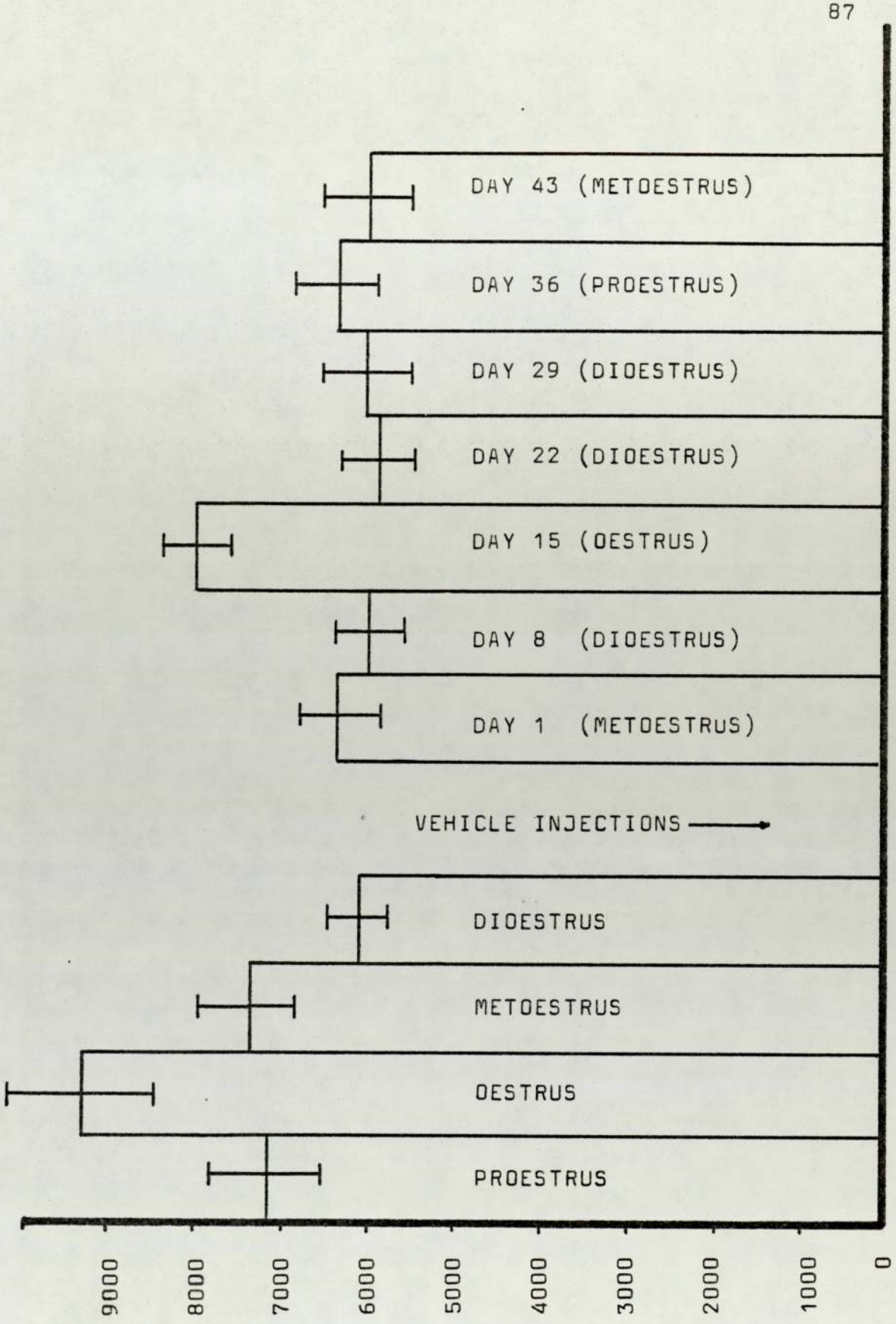


FIG. 13: Total dark time locomotor activity in female mice receiving daily vehicle injections (s.c.).

and 6134 ± 574 on Day 43. This compares with a pre - injection total dark phase activity count of 7409 ± 570 at metoestrus. The differences were not found to be significant. Similar situations occured in the oestrus, proestrus and dioestrus phases of the cycle.

Despite this slight reduction in total dark phase activity after the injections, the normal oestrous cycle activity rhythm was exhibited, with peaks occuring at oestrus and troughs at dioestrus.

The 24 hour activity patterns produced by such vehicle injections were found to be similar to those of the female mice exhibiting natural oestrous cycles. It would seem unlikely that injections alone are solely responsible for the alteration in the hourly dark time activity pattern, in particular, in those mice which received steroid hormone treatment.

It should be noted that ethinyloestradiol alone was not administered to the mice because it is known to produce a state of constant oestrus in animals receiving it. Other oestrogenic compounds are known to possess similar properties, eg oestradiol benzoate.

CHAPTER TWO

OPEN-FIELD BEHAVIOUR DURING THE MOUSE OESTROUS CYCLE, IN MALE AND AGED MICE AND IN FEMALE MICE RECEIVING SYNTHETIC GONADAL HORMONES.

In two reports, Ellison (1975,1977), suggested that changes in both noradrenaline and 5-HT brain levels can induce altered behaviour when animals are placed in the situation of the open field compared to that exhibited in the home cage environment. Thus, it was decided to test this hypothesis to see whether it could be applied to animals with a natural variation in such biogenic amine brain levels, as is suspected to occur during the oestrous cycle. Further experiments were carried out to determine the effect of variations in hormone levels and sex differences, on behaviour in the open field.

All experiments were carried out between the hours of 12·00 - 14·00, the mice being maintained under a 12 hour light/12 hour dark lighting schedule, with the period of illumination between 07·00 - 19·00 hours.

1. THE EFFECT OF THE OESTROUS CYCLE ON OPEN FIELD BEHAVIOUR

Three month old virgin female mice were caged in groups of three for thirty days prior to the experiment to ensure a regular synchronised oestrous cycle. Animals were chosen at random, tested in the open field situation, vaginal smears being taken on completion of the test, to ascertain oestrous status.

(a) Locomotor Activity (Ambulation)

This activity was measured by counting the number of squares entered by all four paws of each animal in a period of five minutes.

A significant increase in open field locomotor activity was found at oestrus, compared to that at both metoestrus and dioestrus ($P<0.05$). Locomotor activity was

also greater at oestrus than at proestrus, but the difference was found not to be significant. (Fig 14)

(b) Rearing

The count for rearing included both the number of free-standing and wall-supported rearing episodes occuring in five minutes.

No significant differences were found between the phases of the oestrous cycle, although less rearing was seen to occur at oestrus (Fig 15).

(c) Defaecation

The defaecation score was the number of faecal boli produced by each animal during the five minutes of the test.

No significant difference were found in the defaecation counts during the oestrous cycle, although the number of faecal boli produced at oestrus was reduced (Fig 16).

(d) Grooming

No significant differences were found in the number of grooming episodes occuring in the various phases of the oestrous cycle, although a slight increase in such episodes was found at oestrus (Fig 17).

2. THE EFFECT OF INCREASED AGE IN FEMALE MICE ON OPEN FIELD BEHAVIOUR

Virgin female mice with an age of 400 days were used in this experiment. They were caged in groups of three for thirty days prior to the test. Daily vaginal smears indicated that these females no longer exhibited regular oestrous cycles, being in a state of constant dioestrus (anoestrus).

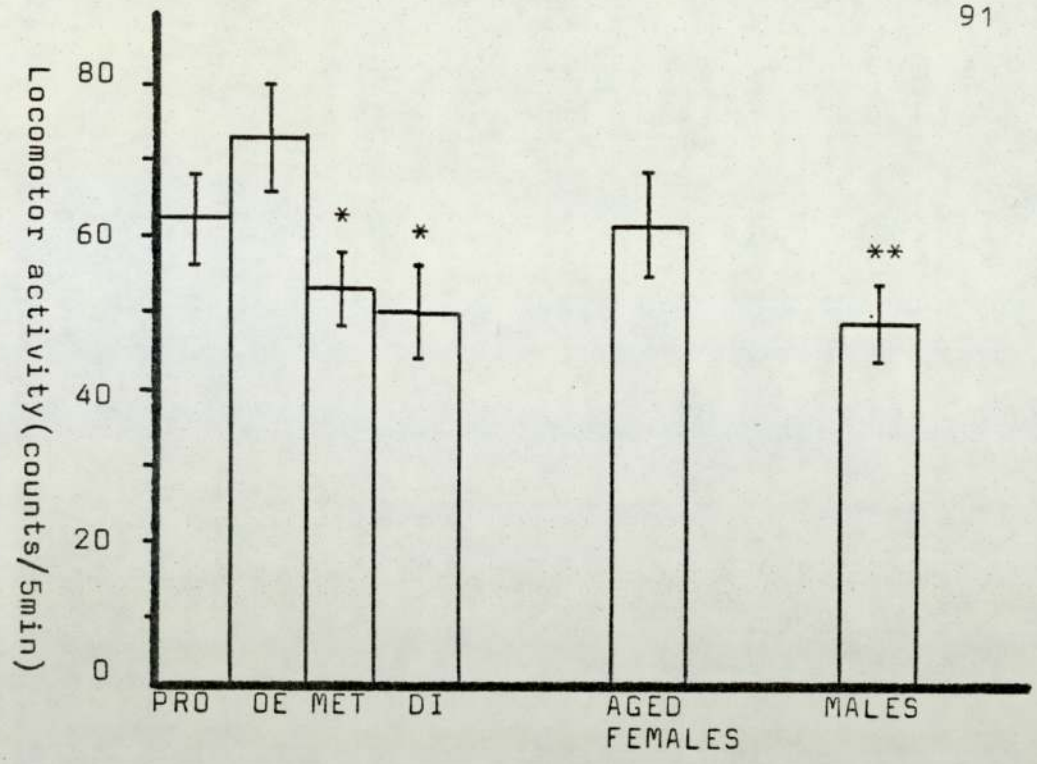


FIG.14:Locomotor activity in open-field; Mouse oestrous cycle,aged female & male mice.
* P<0·05; ** P<0·02:(oestrus-control,t-test).

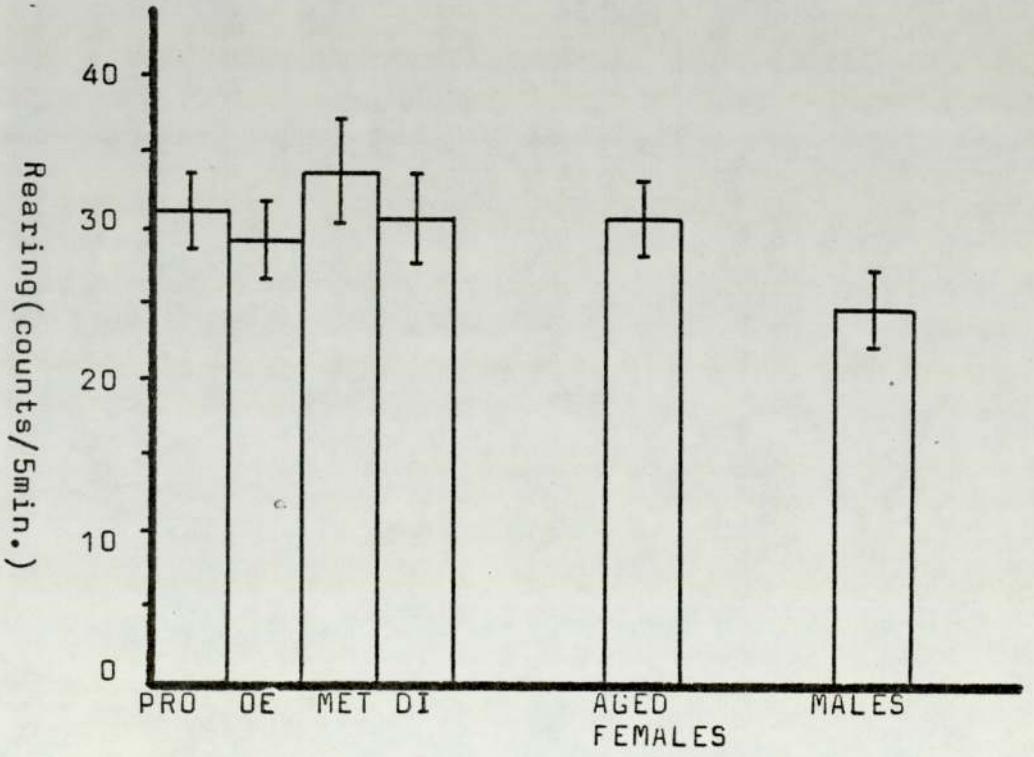


FIG. 15:Rearing in open-field;Mouse oestrous cycle,aged female and male mice.

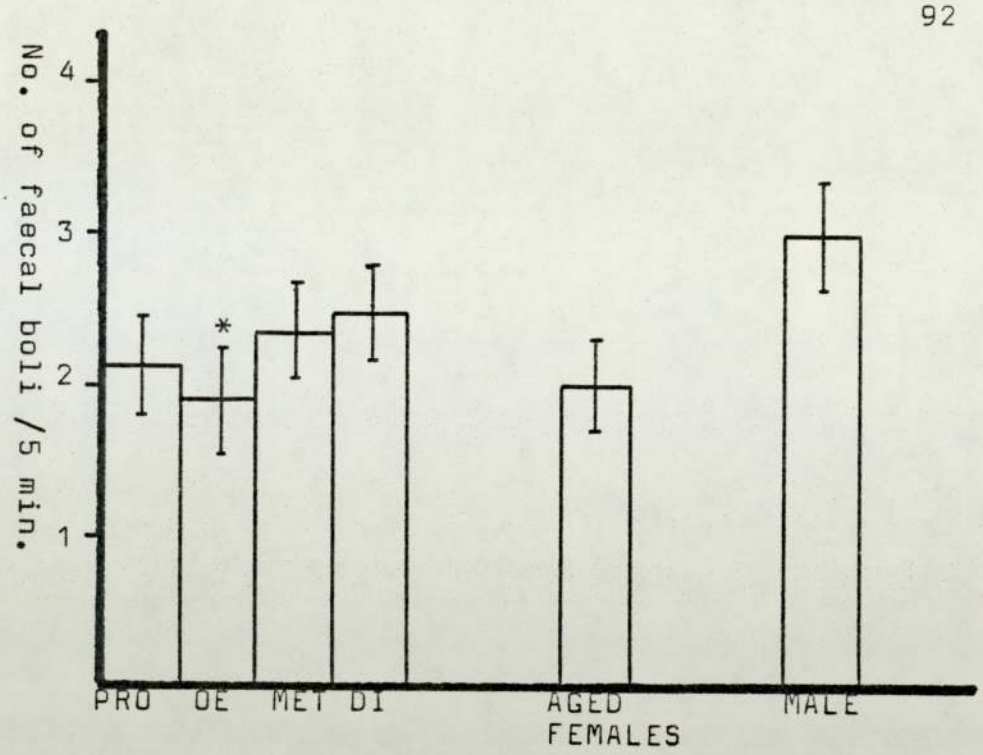


FIG.16:Defaecation in open-field;Mouse oestrous cycle,aged female and male mice. * $p<0.05$: (male-control,t-test).

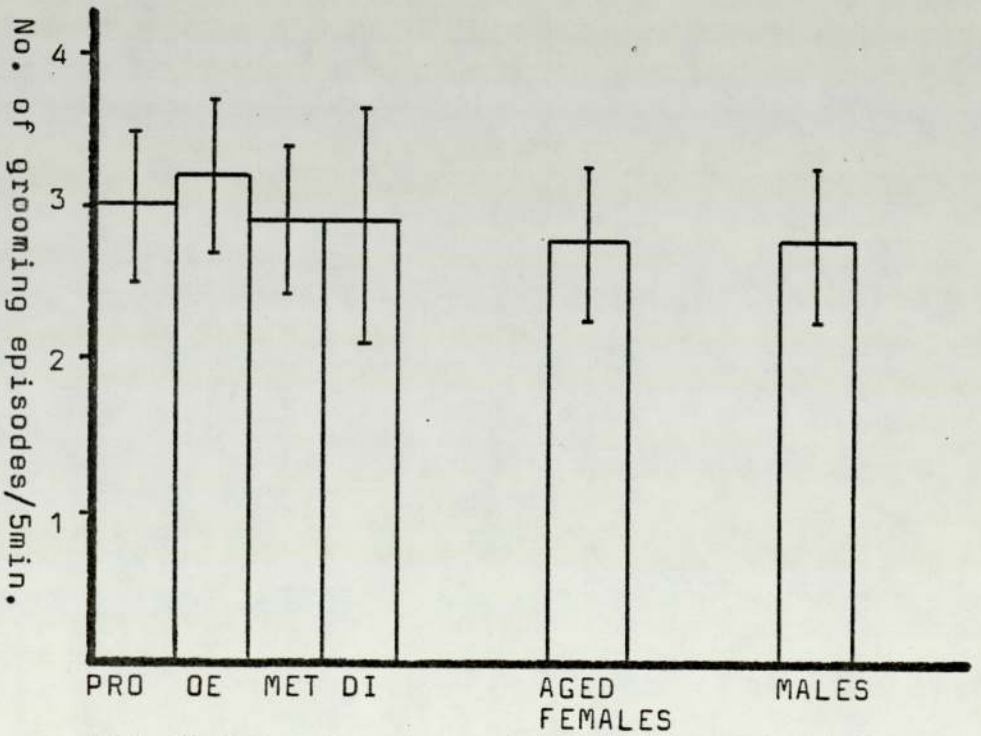


FIG.17:Grooming episodes in open-field;Mouse oestrous cycle,aged female and male mice.

The aged females exhibited only slightly less locomotor activity than the oestrus females and more than the females of the three other phases of the cycle (Fig 14). Their rearing count was found to be almost the same as that of the dioestrus females (Fig 15), while they produced slightly more faecal boli than oestrus females but less than those of the dioestrus, proestrus and metoestrus females (Fig 16). They were seen to exhibit fewer grooming episodes than any of the females in any of the four phases of the oestrous cycle (Fig 17).

3. THE PERFORMANCE OF MALE MICE IN THE OPEN FIELD SITUATION

The male mice used in this experiment were three months old, of similar age to the females of the oestrous cycle experiment. They were caged in groups of three for thirty days prior to the experiment and handled daily in order to compensate for the taking of daily vaginal smears in the experiments involving female mice.

The male mice exhibited significantly less locomotor activity in the open field than the oestrus females, $P<0.02$ (Fig14), and also reared less than the female mice in any of the stages of the oestrous cycle and the aged female mice (Fig 15). The males were seen to defaecate significantly more than the oestrus females, $P<0.05$ (Fig 16), and exhibit fewer grooming episodes than any of the female mice tested (Fig 17).

4. THE EFFECT OF THE CHRONIC ADMINISTRATION OF NORETHISTERONE ACETATE (20µg/Kg) AND ETHINYL-OESTRADIOL (1µg/Kg) TO FEMALE MICE ON OPEN FIELD BEHAVIOUR

The mice in this experiment underwent similar

experimental procedures to those from the locomotor activity experiments of Chapter 1. The open field experiments took place after approximately six weeks of continuous daily injections of 1µg/kg ethinyloestradiol and 20µg/kg norethisterone acetate. The control group for this experiment received vehicle injections over the same period, and exhibited regular 5-6 day oestrous cycles. The hormone treated animals were again seen to exhibit c ontinuous dioestrus as detailed in Chapter 1.

(a) Locomotor Activity

The hormone treated animals exhibited slightly less locomotor activity than the dioestrus controls. Both of these groups exhibited significantly less locomotor activity than the oestrus controls, $P<0.05$ (Fig 18).

(b) Rearing

The hormone treated group reared slightly more than the oestrus controls, but less than the dioestrus controls. The differences were not significant (Fig 19).

(c) Defaecation

The hormone treated group produced a significantly greater number of faecal boli than the oestrus controls ($P<0.05$) and also showed a non-significant increase over the dioestrus group (Fig 20).

(d) Grooming

Although the hormone treated mice exhibited only 60% of the grooming episodes of the oestrus controls and 67% of the dioestrus controls, the differences were found not to be significant (Fig 21).

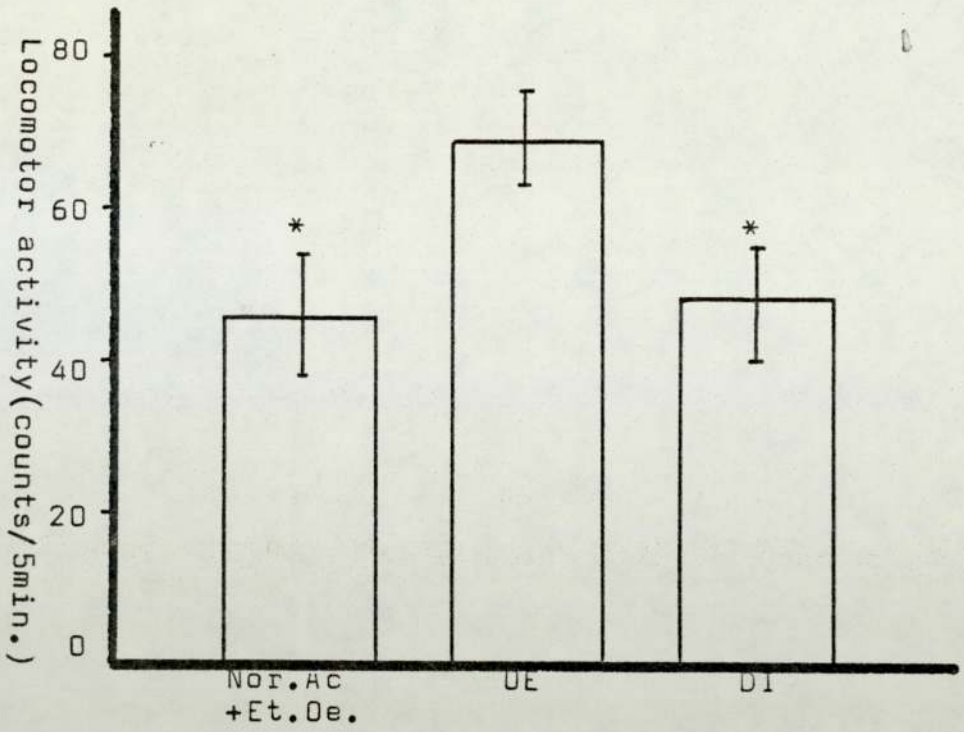


FIG.18:Locomotor activity in open-field; female mice-oestrus,dioestrus and norethisterone acetate 20mcg/kg+ ethinyloestradiol 1mcg/kg.
* $p<0.05$.(oestrus-control,t-test.)

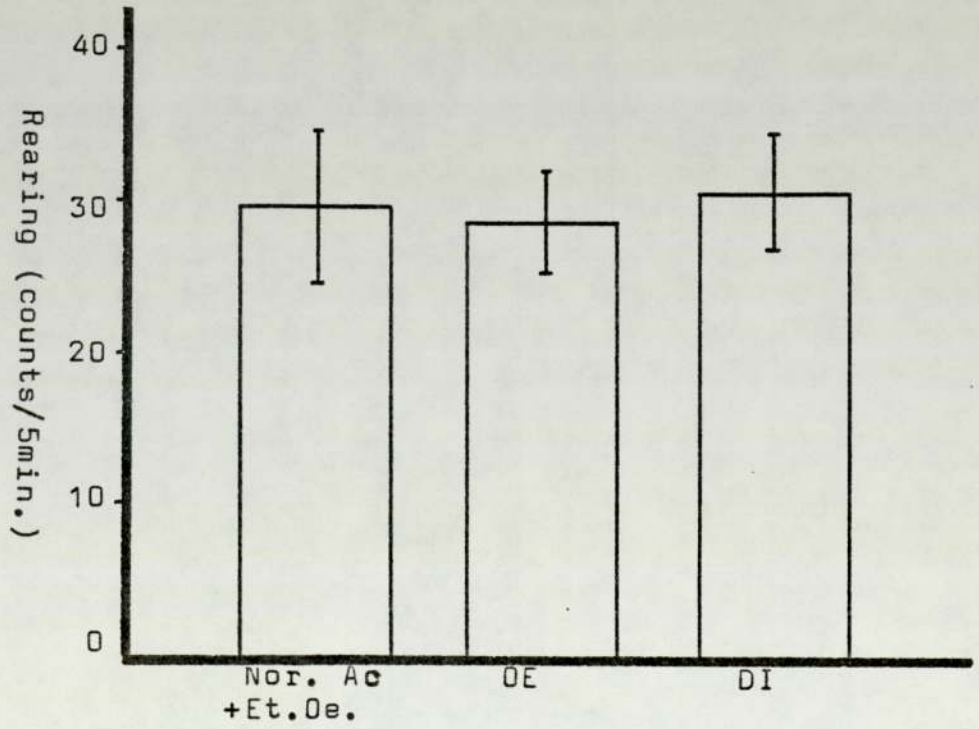


FIG.19:Rearing in open-field;female mice-oestrus,dioestrus and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg.

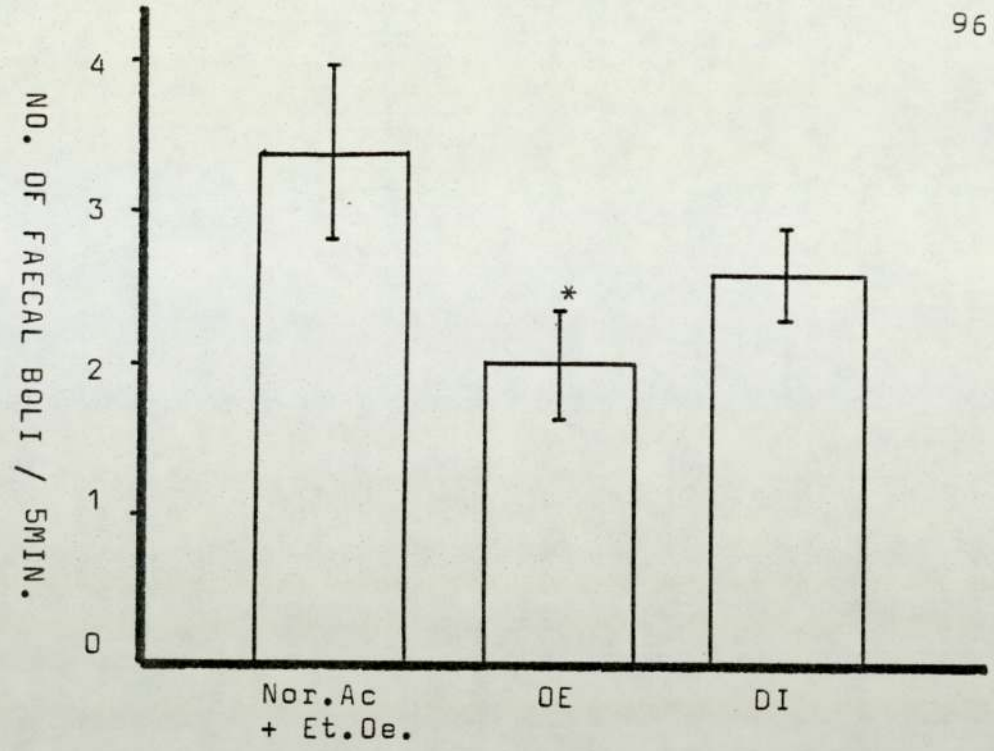


FIG.20:Defaecation in open-field;female mice -oestrus,dioestrus and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg.
* P 0·05.(nor.ac + et.oe-control,t-test).

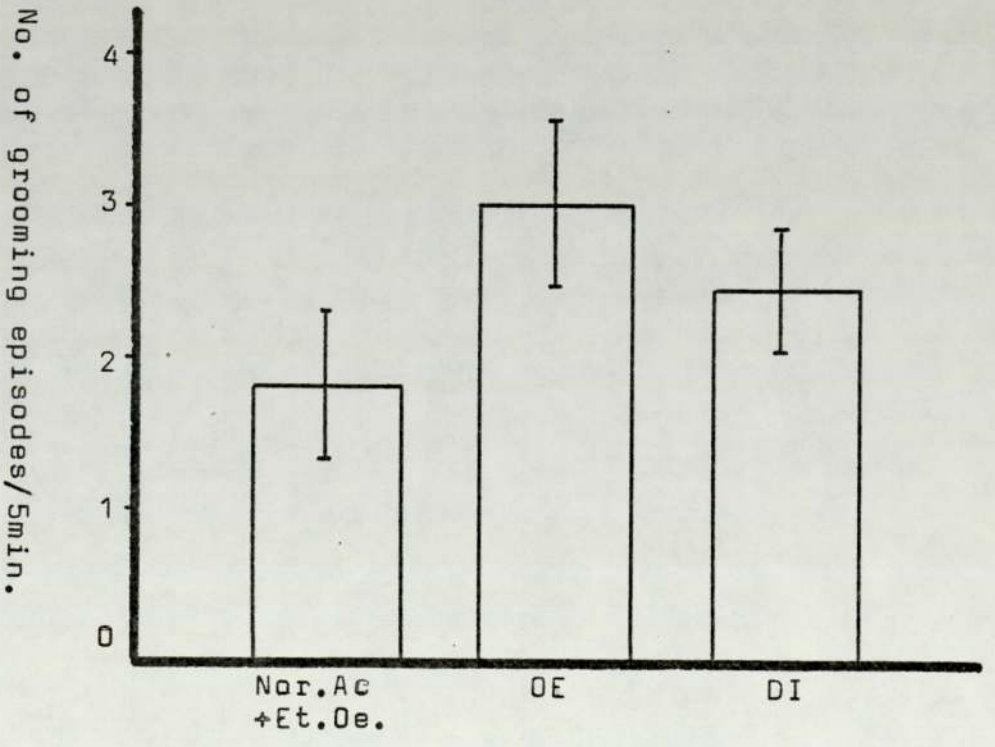


FIG.21:Grooming in open-field;female mice -oestrus,dioestrus and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg.

5. THE EFFECT OF THE CHRONIC ADMINISTRATION OF NORETHISTERONE ACETATE (20µg/Kg) TO FEMALE MICE ON OPEN FIELD BEHAVIOUR

The same experimental conditions existed as in the previous experiment for the combined administration of ethinyloestradiol and norethisterone acetate, except that only 20ug/kg of norethisterone acetate was given.

(a) Locomotor Activity

Both the hormone treated group and the dioestrus controls exhibited significantly less locomotor activity than the oestrus controls, ($P<0.05$). A slightly greater reduction in activity was seen in this experiment, where norethisterone acetate was given alone, than in the previous experiment where norethisterone acetate was combined with ethinyloestradiol (Fig 20).

(b) Rearing

Unlike the mice which received combined hormone treatment, the mice receiving norathisterone acetate alone were seen to rear less than both the oestrus and dioestrus controls, although the reduction was not found to be significant (Fig 23).

(c) Defaecation

The group receiving norethisterone acetate produced a significantly greater number of faecal boli than the oestrus controls ($P<0.05$) and also produced a greater number than the dioestrus controls, which was found not to be significant (Fig 24).

(d) Grooming

The group receiving norethisterone acetate alone exhibited the greatest number of grooming episodes of the three groups tested. There was a 5·4% increase over the oestrus controls and a 17·7% increase over the dioestrus controls, neither of which were found to be significant (Fig 25).

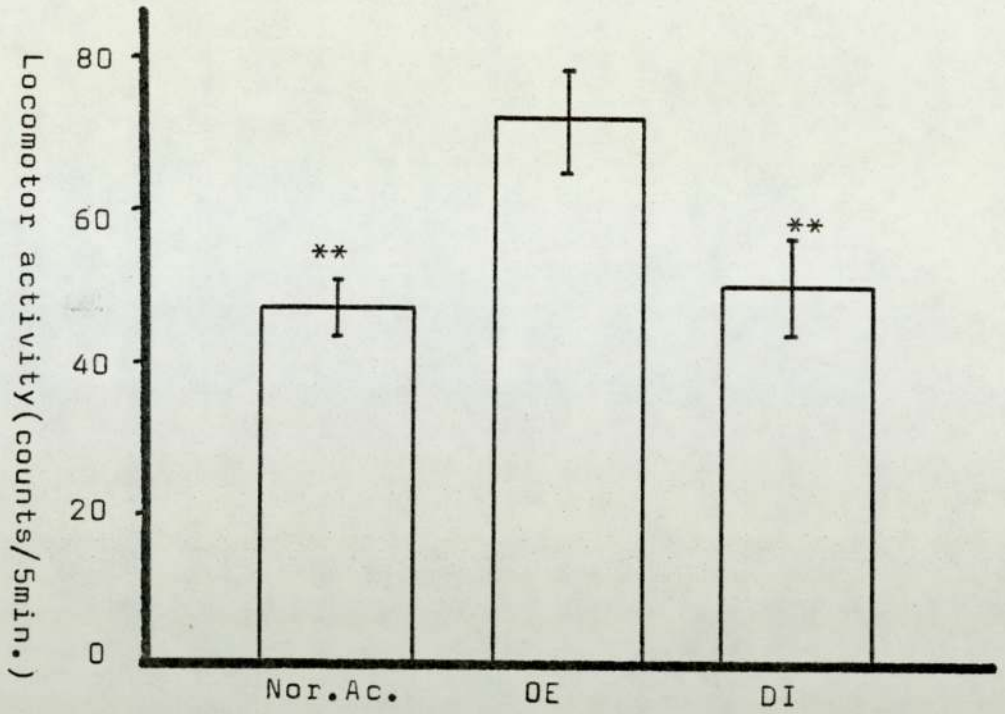


FIG.22:Locomotor activity in open-field; female mice-oestrus,dioestrus and norethisterone acetate 20mcg/kg. ** P<0·02.(oestrus-control, t-test.)

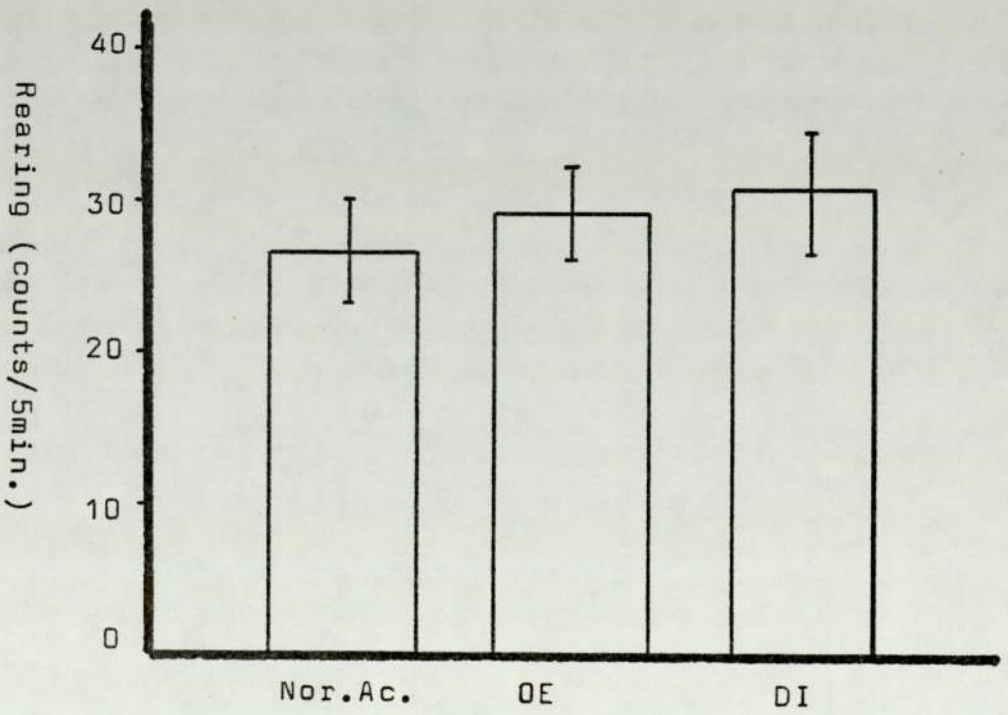


FIG.23:Rearing in open-field;female mice-oestrus,dioestrus and norethisterone acetate 20mcg/kg.

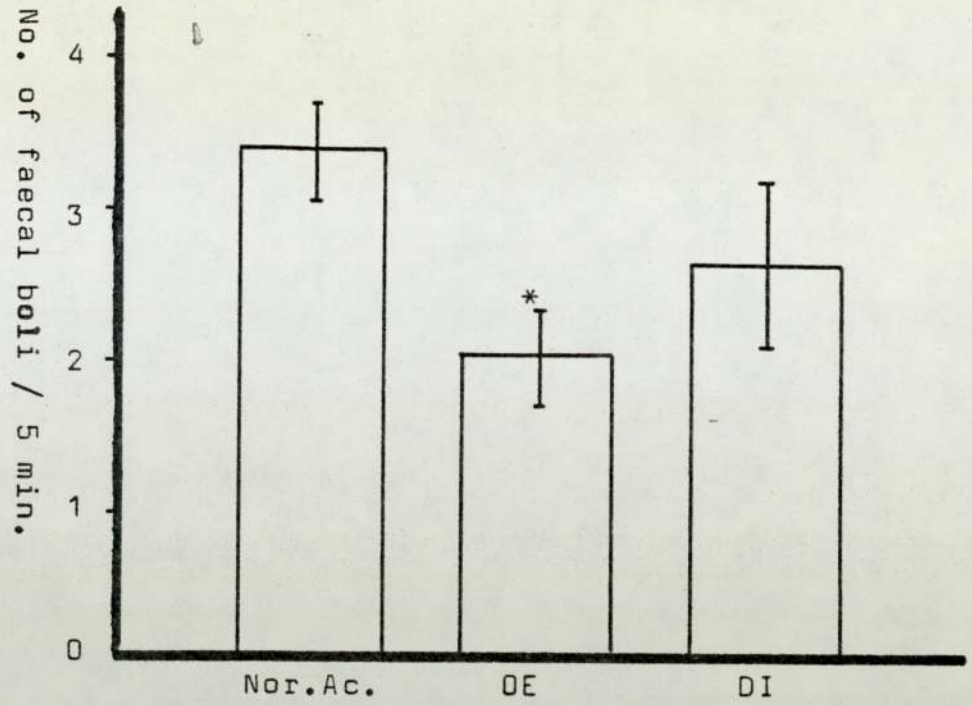


FIG.24:Defaecation in open-field;female mice -oestrus,dioestrus and norethisterone acetate 20mcg/kg. * P<0·05.(nor.ac-control,t-test.)

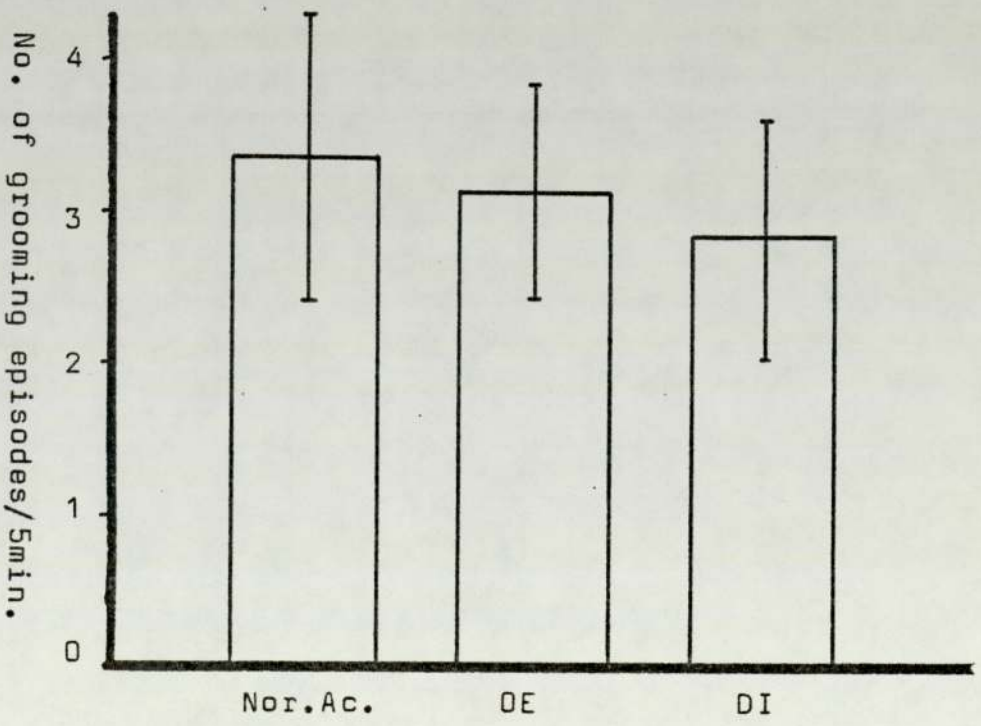


FIG.25:Grooming episodes in open-field;female mice-oestrus,dioestrus and norethisterone acetate 20mcg/kg.

CHAPTER THREE

VARIATION IN THE STARTLE RESPONSE DURING THE MOUSE OESTROUS CYCLE AND IN FEMALE MICE RECEIVING SYNTHETIC GONADAL HORMONES.

All mice tested for the startle response were caged in groups of three for thirty days prior to the start of the experiment, except in the case of those mice which received norethisterone acetate alone and in conjunction with ethinyloestradiol, where the experimental procedure described in previous chapters was followed. The lighting schedule was 12 hours light/ 12 hours dark, with illumination occuring between 07·00 and 19·00 hours. All experiments were carried out between 11·00 - 15·00 hours.

Previous experience with the apparatus had shown that a minimum adaption time of 15 minutes was necessary for each mouse. The startle response was found to be almost absent in mice which had been placed in the box only five minutes before the initiation of the startle stimulus. Adaption times of 15, 30 and 60 minutes produced similar startle responses and habituation occured over a similar time course (Thomas, personal communication). Thus an adaption time of 15 minutes was selected and used throughout the course of experiments.

In all cases, 20 puffs of air, one every five seconds, were directed at each mouse, the mean result being considered.

1. THE EFFECT OF FOUR CONSECUTIVE DAYS TESTING ON THE HABITUATION OF THE STARTLE RESPONSE

In the experiment for the measurement of the startle response over the oestrous cycle, it was thought to be more satisfactory to follow the progress of an individual mouse throughout the length of a complete cycle than select mice at random to undergo a single test.

Thus it was necessary to discover whether habituation to the startle stimulus might occur when such mice were tested on four consecutive days.

In this experiment three month old male mice were used. The results were as follows:-

	DAY 1	DAY 2	DAY 3	DAY 4
n=11	9·41±1·16	9·59±0·68	9·7±0·67	9·56±0·66

Thus, it can be seen that no habituation occured in the startle response when 20 puffs of air were directed at a mouse at five second intervals on four consecutive days of testing.

2. THE EFFECT OF TIME DIFFERENCES ON THE STARTLE RESPONSE

Two groups of male mice were tested between 11·00 - 15·00 hours. The first group were housed under lighting conditions where illumination commenced at 07·00 hours and finished at 19·00 hours. The second group were housed under reversed lighting conditions from weaning. The results obtained were:-

	LIGHT	DARK
n=16	11·05±0·8	13·65±0·91

It was found that the group tested when in the middle of their dark phase exhibited a significantly greater response to the startle stimulus than the group tested in the middle of their light phase ($P<0·05$).

3. VARIATION OF THE STARTLE RESPONSE OVER THE OESTROUS CYCLE

Virgin female mice aged three months were used for this experiment, daily vaginal smears being taken to assess the oestrous status. The mice were tested on four

consecutive days, only mice with regular four day cycles being used, without a regular stage of the oestrous cycle for the starting point. This was carried out in order to counteract any habituation to the startle stimulus which might occur in female mice - despite the suggestion from the previous experiment that habituation does not occur under such conditions.

The mice at oestrus were seen to exhibit a significantly greater startle response than mice at metoestrus ($P<0.05$), proestrus ($P<0.05$) and dioestrus ($P<0.001$). The startle response was found to reach a minimum during the dioestrus phase of the cycle (Fig26).

4. EFFECT OF SEX DIFFERENCES ON THE STARTLE RESPONSE

Within the experiment to discover any variation in the startle response that might occur over the oestrous cycle, an experiment comparing the response of male mice to female mice was carried out. This involved testing male mice at random times during the oestrous cycle experiment, thus reproducing similar test conditions for both sexes.

Although the male mice exhibited a greater response to the startle stimulus, this was found not to be significantly greater than that obtained for females in the oestrus phase of the cycle (Fig 26).

5. THE EFFECT OF THE CHRONIC ADMINISTRATION OF NORETHISTERONE ACETATE (20µg/Kg) AND NORETHISTERONE ACETATE (20µg/Kg) & ETHINYLOESTRADIOL (1µg/Kg) ON STARTLE RESPONSE OF FEMALE MICE

In this experiment both groups of mice had received treatment for 43 days. The first group received 20µg/kg

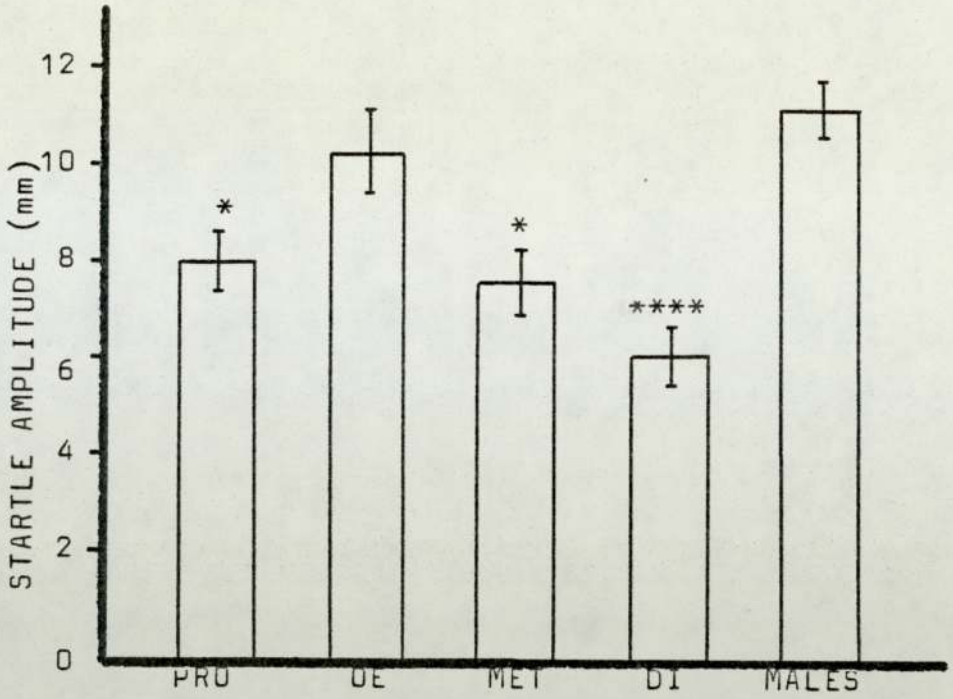


FIG.26: Variation of startle response over the mouse oestrous cycle, + startle response of male mice. * P<0·05; **** P<0·001: (oestrus-control, t-test.)

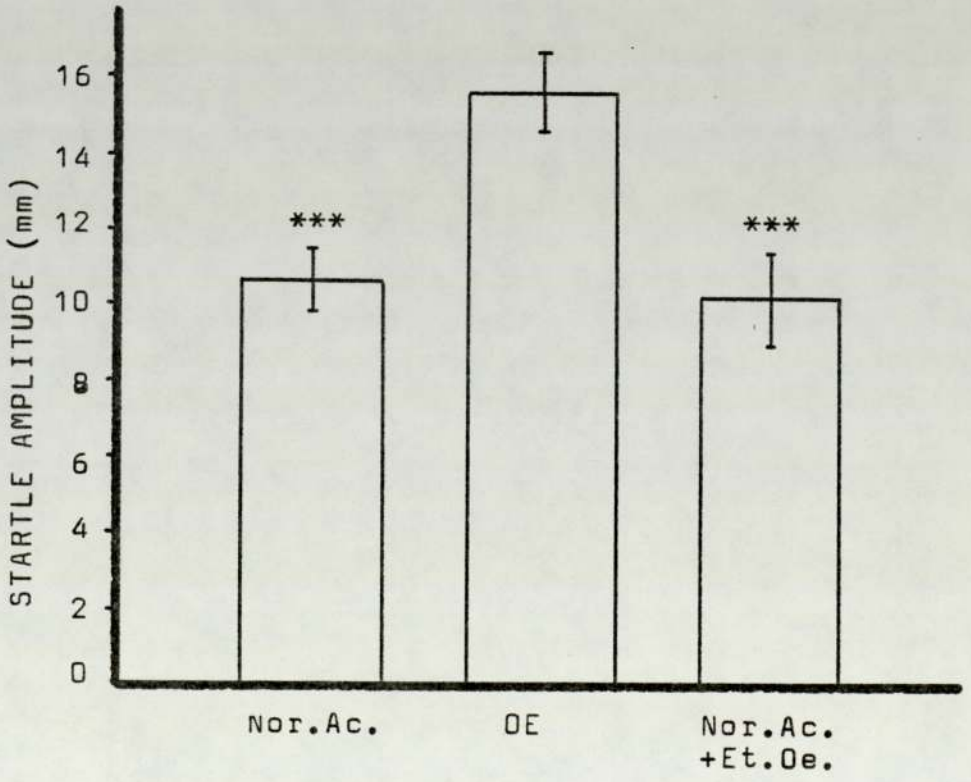


FIG.27: The effect of norethisterone acetate 20mcg/kg and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg on the startle response of female mice. *** P 0·001. (oestrus-control, t-test.)

norethisterone acetate daily, the second group 20µg/kg norethisterone acetate plus 1µg/kg ethinyloestradiol. A control group received vehicle injections daily. As in previous ecperiments both groups of hormone treated mice exhibited constant dioestrus, while the control group exhibited regular 5-6 day oestrous cycles.

For the purpose of comparison the oestrus stage of the control group was used (Fig 27).

Both groups of hormone treated mice exhibited a similar startle response magnitude, both of which were significantly below the values obtained from the oestrus controls ($P<0.01$).

The group receiving norethisterone acetate alone had a startle response magnitude of 66·7% of that of the oestrus control, whereas for the group receiving norethisterone acetate and ethinyloestradiol, this declined only slightly to 65·45%. These values compare with the 58·2% obtained by female mice in dioestrus from experiment three.

CHAPTER FOUR

BIOCHEMICAL CHANGES DURING THE MOUSE OESTROUS CYCLE, AND IN MALE AND AGED FEMALE MICE, AND IN FEMALE MICE RECEIVING SYNTHETIC GONADAL HORMONES.

The following work was an attempt to relate changes in certain aspects of brain biochemistry to changes in behaviour as examined previously.

1. VARIATION IN BRAIN 5-HT LEVELS OVER THE OESTROUS CYCLES AT TWO POINTS IN THE LIGHTING SCHEDULE

Three month old virgin female mice were maintained under a 12 hour light/12 hour dark lighting schedule. They were caged in groups of three for thirty days prior to the start of the experiment to ensure synchronous oestrous cycling withing each group. All groups registered a regular four day oestrous cycle, vaginal smears being taken daily. The mice were sacrificed at the mid-point times of the light and dark periods.

Figure 28 shows the variation in brain 5-HT levels at the mid-point of the dark phase for the four stages of the oestrous cycle. A significant increase above the value at oestrus was found at the metoestrus stage ($P<0.05$) and dioestrus ($P<0.05$). No significant difference was found at proestrus. The mid-point in the dark period at oestrus is roughly the time at which ovulation is thought to occur.

Figure 29 shows the variation in brain 5-HT levels at the mid-point in the light phase for the four stages of the cycle. The levels at metoestrus ($P<0.05$) and dioestrus ($P<0.05$) were again significantly above those found at oestrus.

It can be seen from Figures 28 and 29, that a diurnal variation exists in 5-HT brain levels between the light and dark photo-periods. In all stages of the oestrous cycle the levels during the period of illumination were

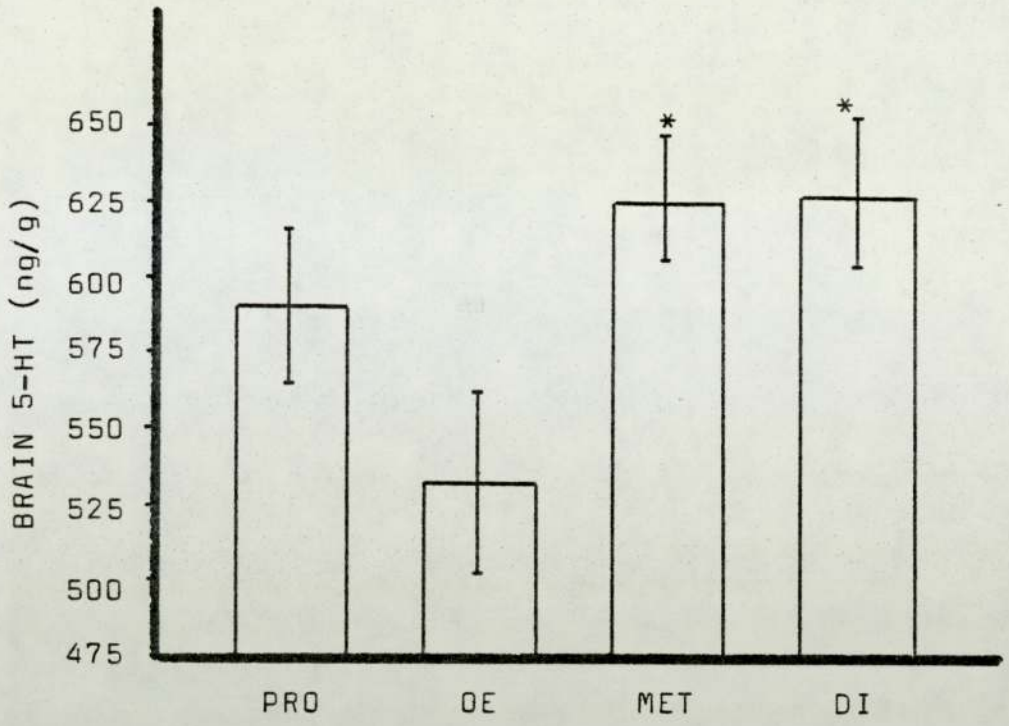


FIG.28:Brain 5-HT over the mouse oestrous cycle at the mid-point in the period of darkness. * P<0·05;(oestrus-control,t-test.)

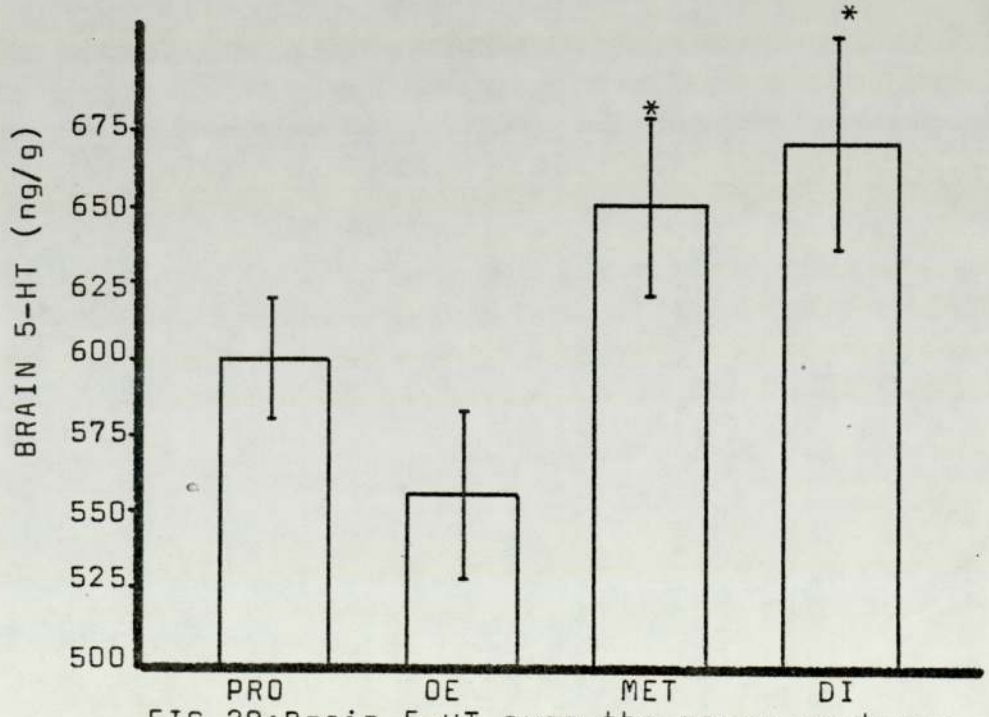


FIG.29:Brain 5-HT over the mouse oestrous cycle at the mid-point in the period of illumination. * P<0·05;(oestrus-control,t-test.)

found to be greater than those of the corresponding stage in the period of darkness. These difference were found not to be statistically significant.

2. DIURNAL VARIATIONS IN BRAIN 5-HT LEVELS IN MALE MICE

Three month old male mice were caged in groups of three for thirty days prior to the beginning of the experiment. They were sacrificed at either the mid-point in the light phase (L+6) or the dark phase (D+6).

	L+6	D+6
n=15	690·06±31·83	617·26±30·28

Although the levels were found to be lower at the mid-point in the dark phase, the difference was found not to be statistically significant.

3. THE EFFECT OF THE CHRONIC ADMINISTRATION OF NORETHISTERONE ACETATE (20µg/Kg) OR NORETHISTERONE ACETATE (20µg/Kg) & ETHINYLOESTRADIOL (1µg/Kg) ON BRAIN 5-HT LEVELS

The procedure carried out in this experiment was the same as that detailed in previous chapters when the chronic administration of either norethisterone acetate (20µg/kg) or norethisterone acetate (20µg/kg) plus ethinyloestradiol (1µg/kg) was performed. The control animals received vehicle injections over the same 43 day period. The mice were sacrificed at the mid-point of the light phase on the day of the last injection (Day 43).

The combined administration of norethisterone acetate (20µg/kg) and ethinyloestradiol (1µg/kg) raised the 5-HT levels in the brain to a significantly greater extent than the administration of norethisterone acetate (20µg/kg) alone (Fig 30). The combined "therapy"

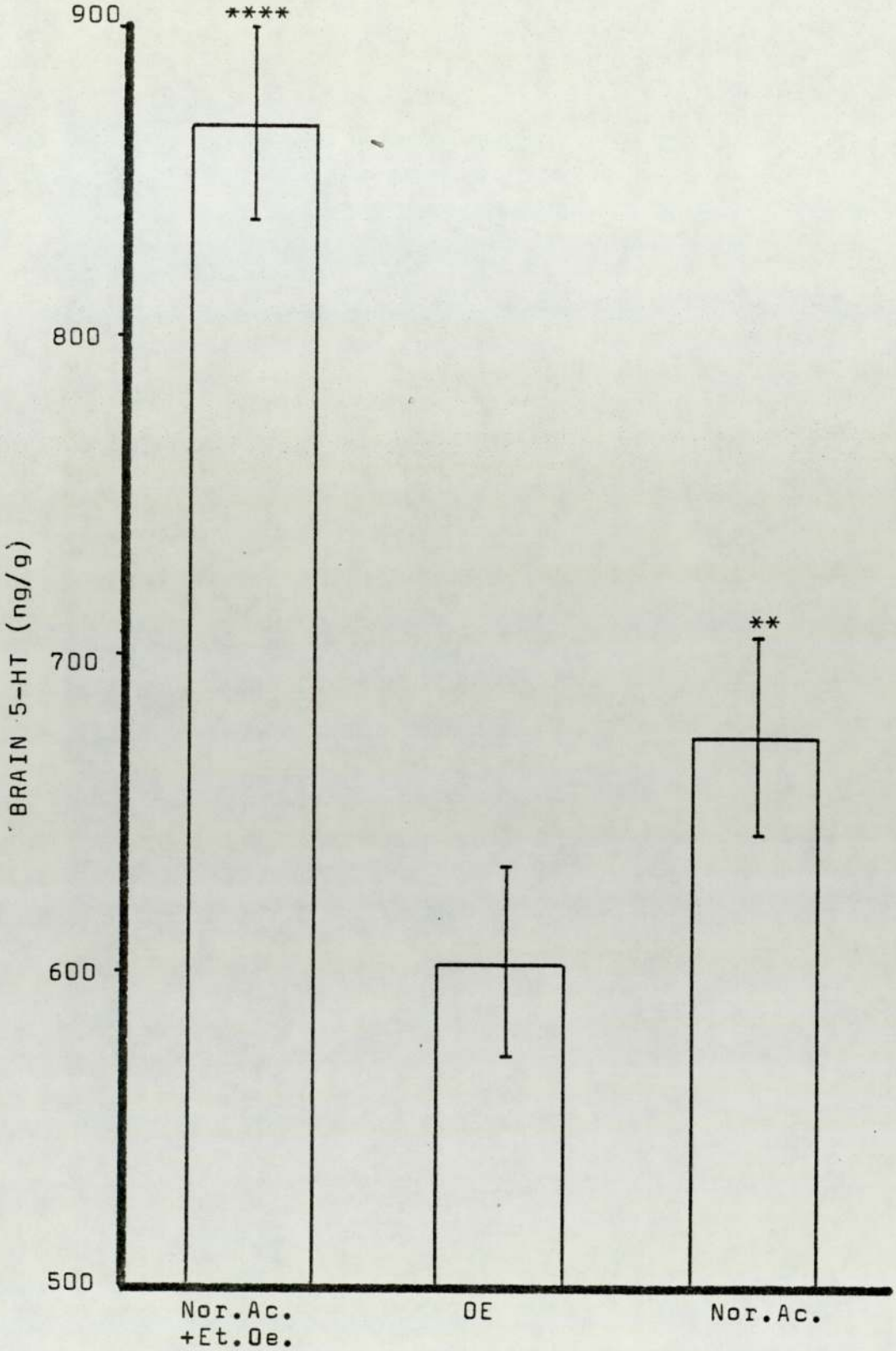


FIG.30: The effect of norethisterone acetate 20mcg/kg and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg on brain 5-HT levels in female mice at the mid-point in the period of illumination.
** P<0·02; **** P<0·001: (oestrus-control, t-test).

produced an increase of 43% over the oestrus control ($P<0.001$), while norethisterone acetate produced a corresponding increase of 12% ($P<0.02$).

4. DIURNAL VARIATIONS IN BRAIN 5-HT LEVELS IN AGED FEMALE MICE

The aged females were unmated and at least 400 days old. They were caged in groups of three for thirty days prior to the experiment, during which period no signs of any oestrous cycle were present. The mice were sacrificed at either the mid-point in the light or dark phase.

	L+6	D+6
n=15	672·06±30·4	623·82±31·92

The levels were found to be lower at the mid-point of the dark period, but the difference was found not to be statistically significant.

5. VARIATION IN TRYPTOPHAN LEVELS OVER THE OESTROUS CYCLE

The experiments were carried out as described in METHODS. The mice were sacrificed at the mid-point in the light period.

(a) Plasma Tryptophan (Fig 31)

Plasma tryptophan levels were found to decline from proestrus through oestrus to reach a minimum at metoestrus. Maximum plasma tryptophan levels were found at dioestrus. The value at metoestrus was found to be significantly less than those at proestrus ($P<0.02$) and dioestrus ($P<0;02$).

(b) % Free Tryptophan (Fig 32)

A minimum value for % free tryptophan was seen at oestrus rising through metoestrus to reach a maximum at dioestrus. The value for proestrus was between that of metoestrus and dioestrus. The value at oestrus was

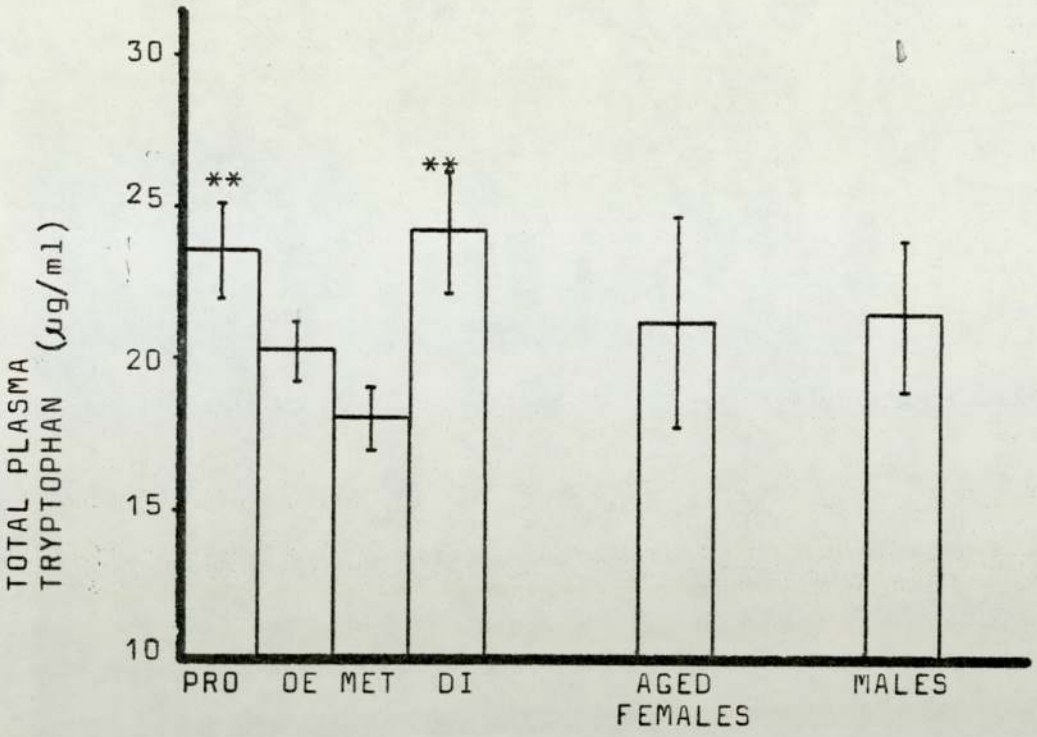


FIG.31: Total plasma tryptophan over the mouse oestrous cycle and in aged female and male mice. ** P<0·02;(oestrus-control, t-test.)

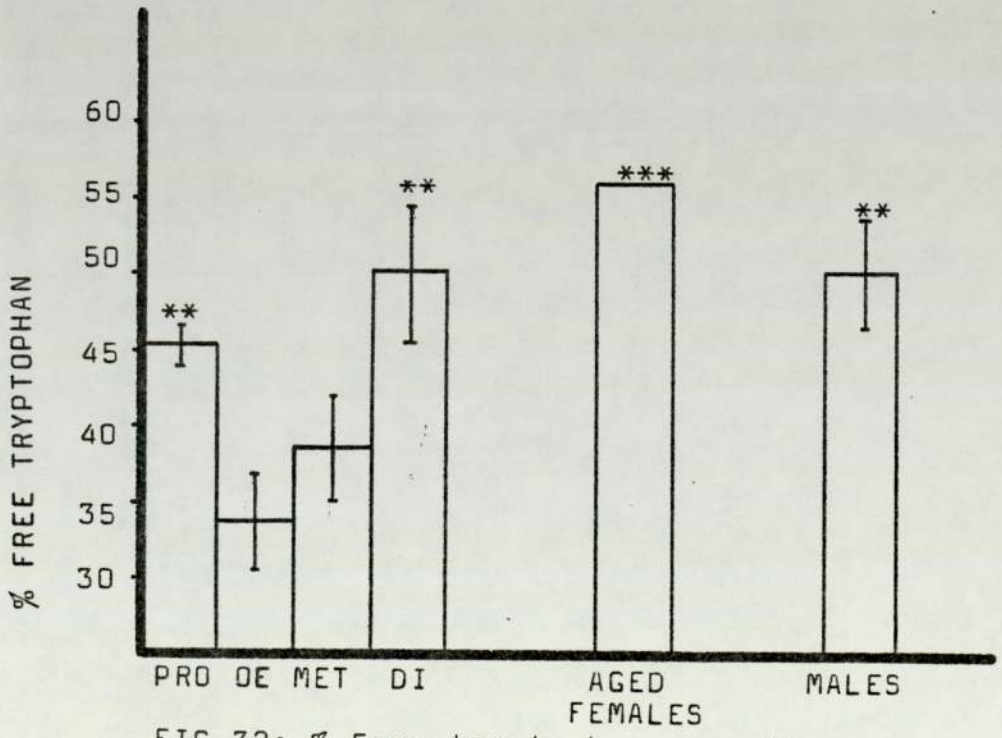


FIG.32: % Free tryptophan over the mouse oestrous cycle and in aged female and male mice. ** P<0·02; *** P<0.01:(oestrus-control, t-test).

significantly less than those at proestrus ($P<0.02$) and dioestrus ($P<0.03$).

(c) Concentration of Free Tryptophan (Fig 33)

A similar pattern to that of % free tryptophan was observed. Little difference was found to exist between the values obtained at metoestrus and oestrus, or between those at proestrus and dioestrus. The concentrations of free tryptophan for the former levels were significantly below those of the latter ($P<0.001$).

(d) Brain Tryptophan (Fig 34)

Brain tryptophan levels were found to be at a minimum at metoestrus. They rose through the dioestrus and proestrus stages of the cycle to reach a maximum at oestrus. The value at oestrus was significantly above the value at metoestrus ($P<0.01$).

6. TRYPTOPHAN LEVELS IN AGED FEMALES

The aged females were found to have a plasma tryptophan level (21.06 ± 3.48) between that of oestrus and proestrus, from the experiment above (Fig 31). Both the % free tryptophan (56.0 ± 2.3) and the concentration of free tryptophan (12.46 ± 1.31) in the aged females were found to be above the dioestrus values which were the maximum values observed in the oestrous cycle (Figs 32, 33). Brain tryptophan levels (3.52 ± 0.62) in aged females also exceeded the maximum value obtained during the oestrous cycle (Fig 34).

7. TRYPTOPHAN LEVELS IN MALE MICE

The male mice had a plasma tryptophan level (21.36 ± 2.48) similar to that of the aged females (Fig 31). They were found to have a % free tryptophan level

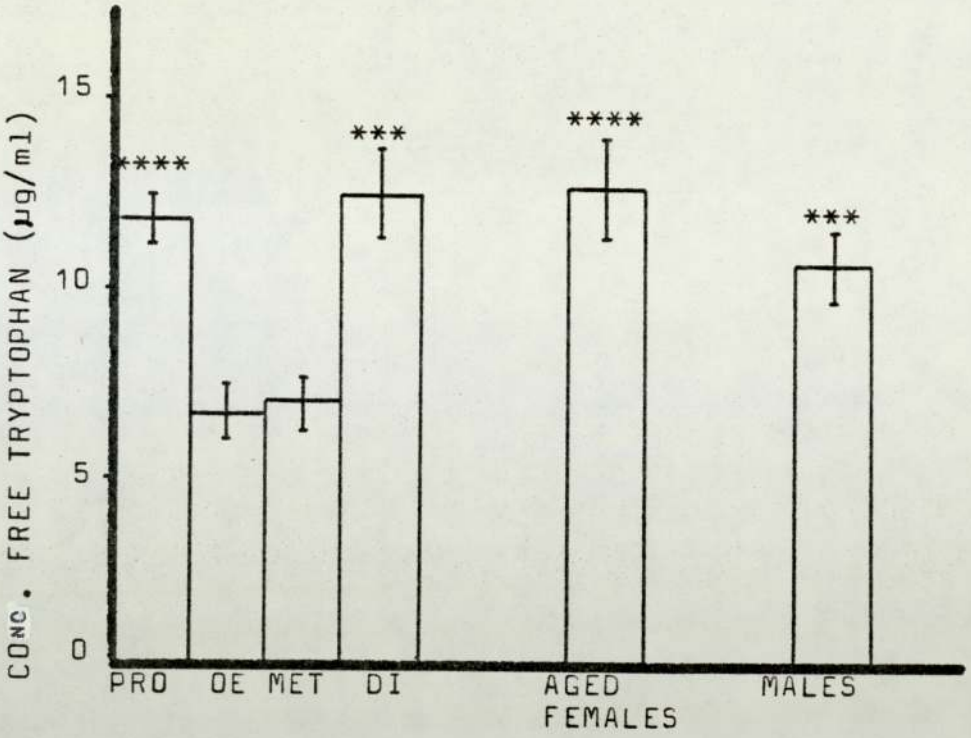


FIG.33:Concentration of free tryptophan over the mouse oestrous cycle and in male and aged female mice. *** P<0·01;**** P<0·001: (oestrus-control,t-test).

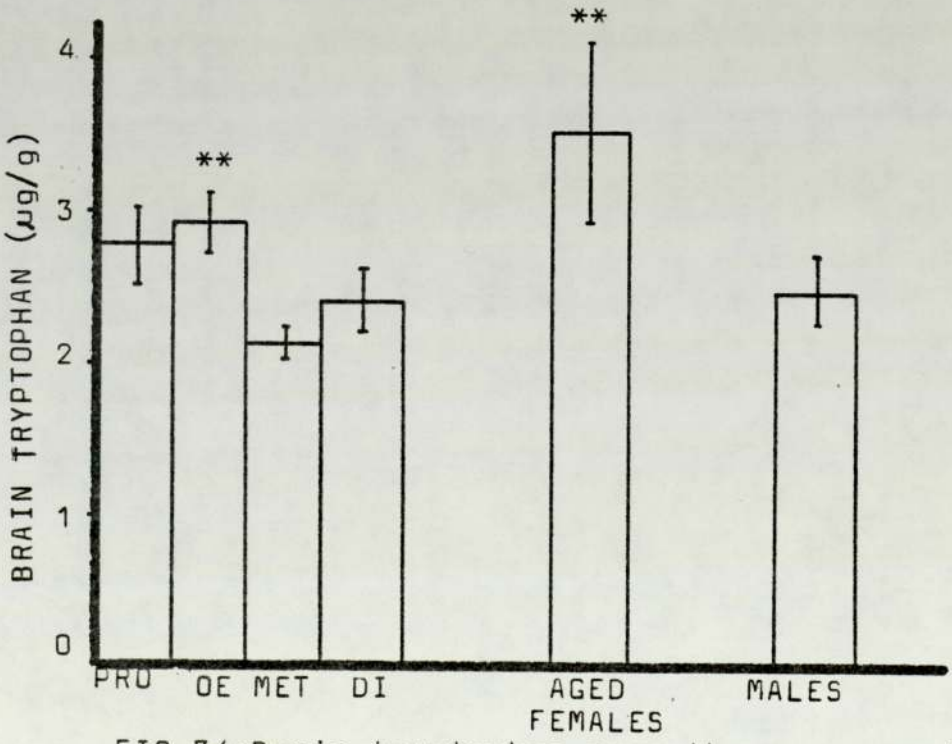


FIG.34:Brain tryptophan over the mouse oestrous cycle and male and aged female mice. ** P<0·02: (metoestrus-control,t-test).

(50·0±3·72) similar to that of dioestrus females (Fig 32), and a concentration of free tryptophan (10·49±0·85) above those of both metoestrus and oestrus but below those of proestrus, dioestrus and the aged females (Fig 33). The brain tryptophan levels in male mice (2·43±0·22) were found to be above that if metoestrus and dioestrus but below that of proestrus, oestrus and aged females (Fig 34).

8. THE EFFECT OF THE CHRONIC ADMINISTRATION OF NORETHISTERONE ACETATE (20µg/Kg) AND NORETHISTERONE ACETATE (20µg/Kg) PLUS ETHINYLOESTRADIOL (1µg/Kg) ON TRYPTOPHAN LEVELS

Control animals were given vehicle injections over the same period as the test animals, as in similar experiments described previously. The values for all tryptophan levels in the metoestrus and oestrus control animals were found to be only slightly above those of the metoestrus and oestrus animals from experiment five, above, where the animals received no injections (the differences not being statistically significant).

(a) Plasma Tryptophan (Fig 35)

In both cases, theplasma tryptophan levels were found to be significantly above those of the metoestrus controls (norethisterone acetate 20µg/kg, $P<0·02$: norethisterone acetate 20µg/kg plus ethinyloestradiol 1µg/kg, $P<0·01$). Little difference was found between the plasma tryptophan levels of the two groups receiving the steroid treatment.

(b) % Free Tryptophan (Fig 36)

The % free tryptophan levels for the group receiving norethisterone acetate 20µg/kg were found to be significantly higher than those of the oestrus control

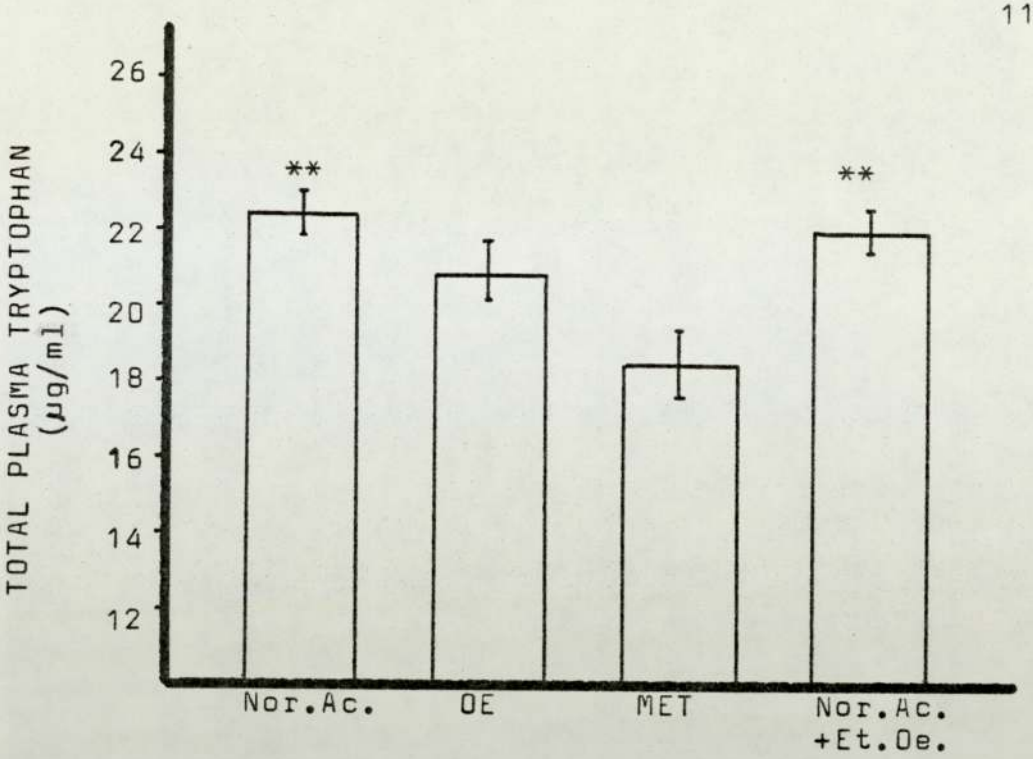


FIG.35: The effect of norethisterone acetate 20mcg/kg and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg on total plasma tryptophan of female mice. ** p<0.02:(metoestrous -control,t-test).

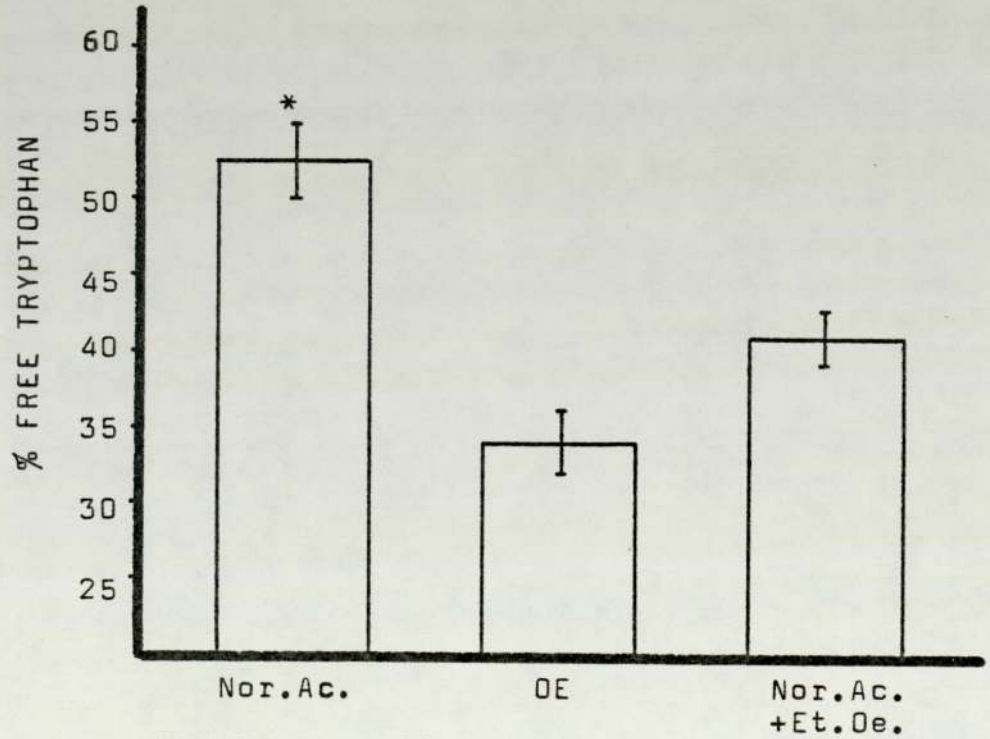


FIG.36: The effect of norethisterone acetate 20mcg/kg and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg on % free tryptophan of female mice. * P<0·05:(oestrus-control,t-test).

group ($P<0.05$). Although the % free tryptophan value for the group receiving the dual steroid injections was found to be greater than the oestrus controls, the increase was not found to be statistically significant.

(c) Free Tryptophan Concentration (Fig 37)

The free tryptophan concentration was significantly greater in both of the groups receiving the synthetic hormone treatment than the oestrus controls. The group receiving norethisterone acetate 20µg/kg had a free tryptophan concentration almost twice that of the oestrus controls ($P<0.001$), while the group receiving both norethisterone acetate 20µg/kg and ethinyloestradiol 1µg/kg showed a 33% increase ($P<0.01$).

(d) Brain Tryptophan (Fig 38)

The brain tryptophan levels for the two groups receiving the hormone treatment were found to be similar, that of the group receiving norethisterone acetate 20ug/kg being slightly higher. Both this group ($P<0.02$) and the group receiving the combined treatment ($P<0.05$) had brain tryptophan levels significantly above those of the metoestrus controls.

9. VARIATION IN BRAIN NORADRENALINE LEVELS OVER THE OESTROUS CYCLE

The procedure in this experiment was similar to that instituted in the measurement of 5-HT levels over the oestrous cycle. The assay was performed immediately after the mice had been sacrificed at the mid-point in either the light or dark period.

Figure 39 shows the variation in brain noradrenaline levels over the oestrous cycle at the mid-point in the

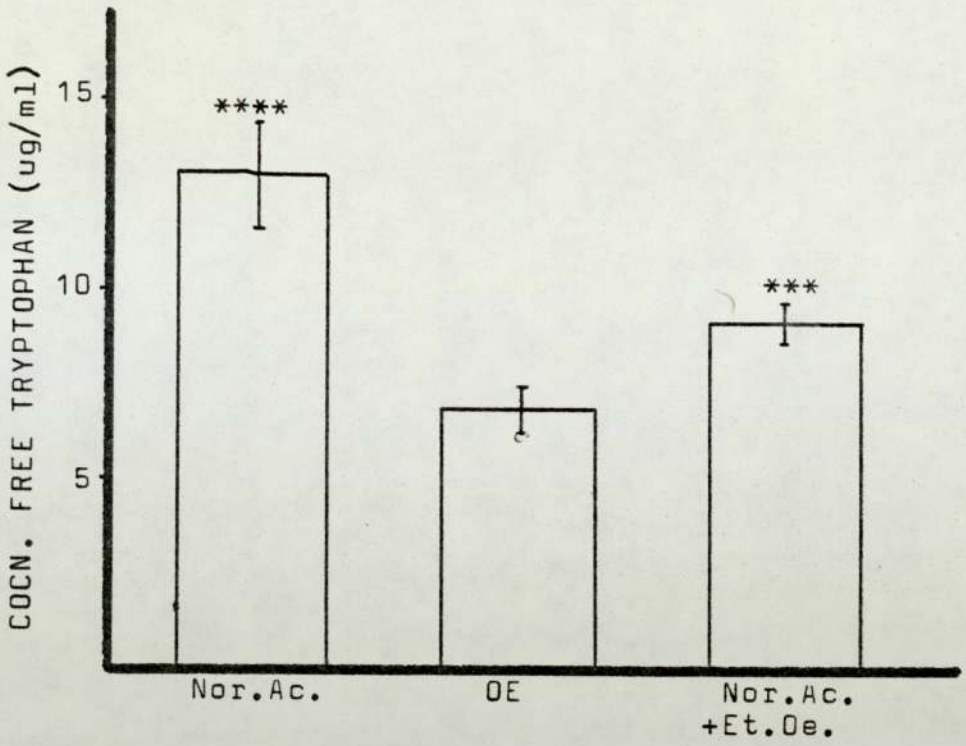


FIG.37:The effect of norethisterone acetate 20mcg/kg and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg on the concentration of free tryptophan in the plasma of female mice. *** P<0·01; **** p<0.001:(oestrus-control, t-test).

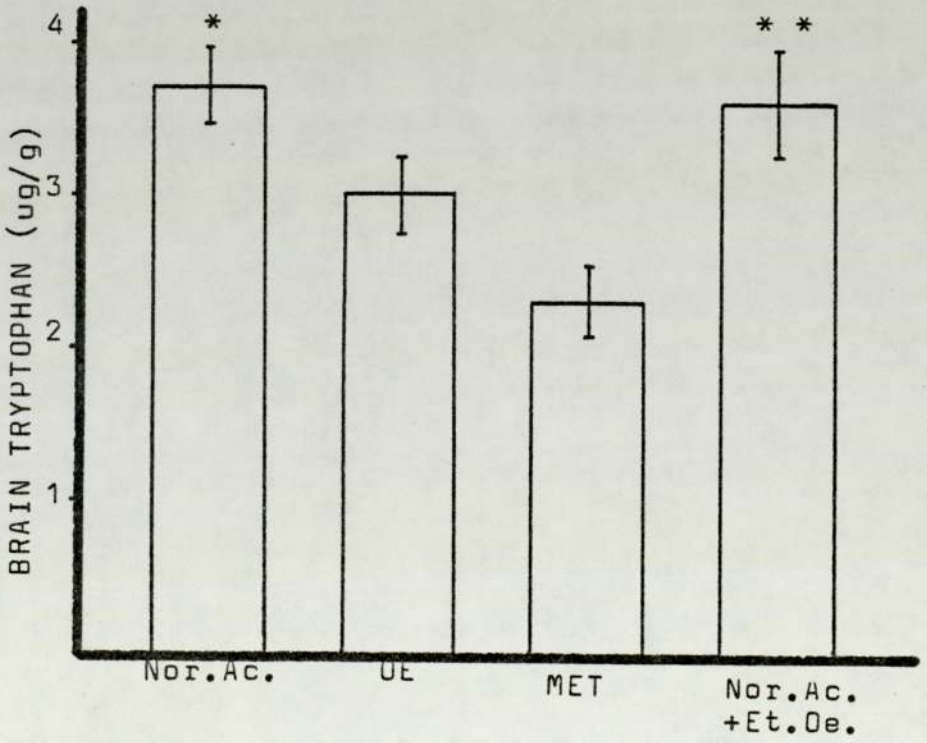


FIG.38: The effect of norethisterone acetate 20mcg/kg and norethisterone acetate 20mcg/kg + ethinyloestradiol 1mcg/kg on brain tryptophan of female mice. * P<0·05; ** P<0·02:(metoestrus-control,t-test).

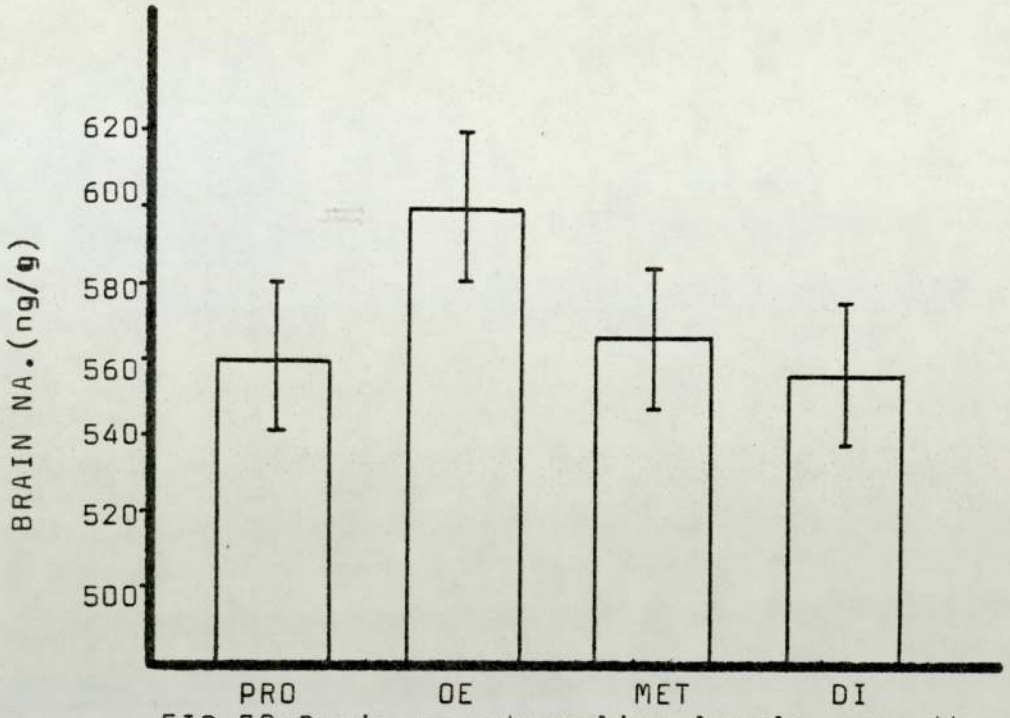


FIG.39:Brain noradrenaline levels over the mouse oestrous cycle at the mid-point in the period of darkness.

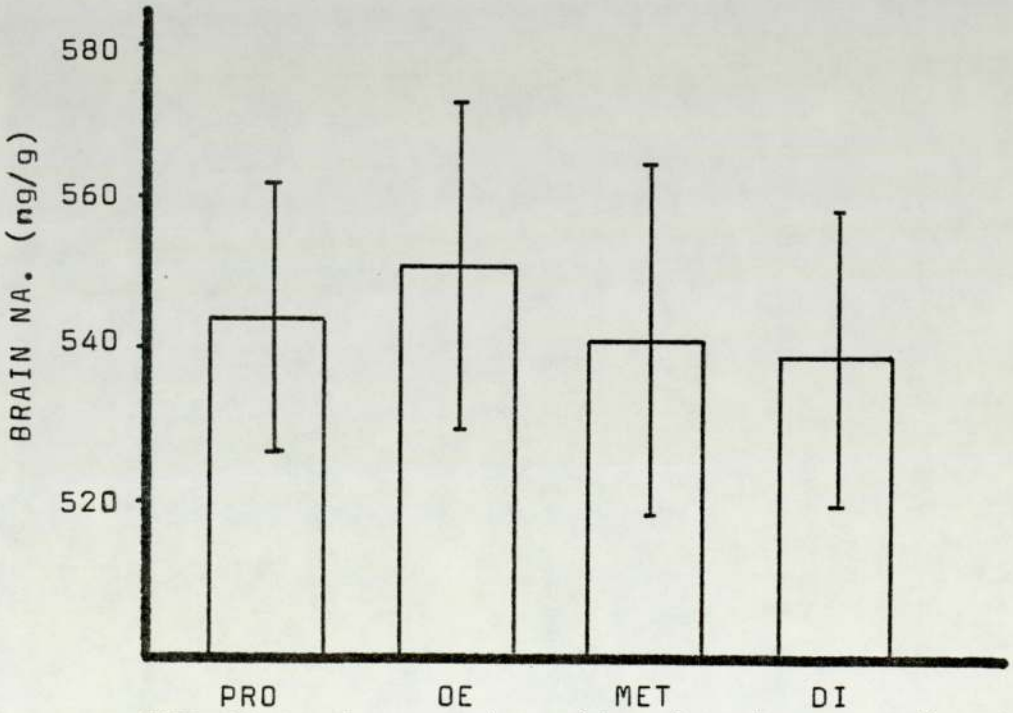


FIG.40:Brain noradrenaline levels over the mouse oestrous cycle at the mid-piont in the period of illumination.

dark period. The peak value was found to be at oestrus, the lowest value at dioestrus.

Figure 40 represents the variation in brain noradrenaline levels over the oestrous cycle at the mid point in the light period. The maximum was again found to occur at oestrus with the minimum at dioestrus. The differences in brain noradrenaline levels over the oestrous cycle were not significant in either the light or dark periods.

10. DIURNAL VARIATION IN BRAIN NORADRENALINE LEVELS OF MALE MICE

Two groups of male mice were used for this experiment, one being sacrificed at the mid-point in the light phase (L+6), the other at the mid-point in the dark phase (D+6).

L+6	D+6
534·2±23·65 (n=15)	603±27.52 (n=11)

Although the noradrenaline levels were found to be higher in the dark period, the difference was found not to be statistically significant.

CHAPTER FIVE

DISCUSSION OF RESULTS FROM CHAPTERS ONE - FOUR.

DISCUSSION: CHAPTERS 1 - 4

(a) Locomotor Activity

The results obtained lend support to the hypothesis that a variation in locomotor activity occurs during the oestrous cycle, the peak of which coincides with a peak in sexual receptivity. This is the oestrus phase of the cycle, ovulation occuring during this period.

This hypothesis was originally put forward by Wang (1923), and supported by the work of Slonaker (1924), and Richter (1927). More recently, doubt has been cast on the value of these results because of the use made of running wheels to measure such activity. It was suggested that running wheels may both stimulate extra running (Bolles 1967, Gress 1968), and act as a reward (Livesey et al 1972).

The Animex Motility Meter used in this series of experiments produces a single score which, as was suggested by Richards (1966), is the sum of all behavioural categories involving movement. Using such equipment, a significant increase in total dark phase activity was found to occur during the oestrus phase of the cycle. The nature of the equipment was such that there was little liklihood of it either stimulating or rewarding activity. Thus it would seem unlikely that the relationship between peak activity at oestrus and sexual receptivity is purely coincidental as had been suggested by Bolles (1963).

Much of the locomotor activity exhibited by mice was found to occur during the dark phase - minimal activity

was recorded during the period of illumination. It was also found that during the dark phase, when activity was high, brain noradrenaline levels were raised and brain 5-HT levels reduced. The converse was found to occur in the period of illumination. Such results correspond with previous reports by Hery et al (1972), Morgan et al (1973) and DiRaddo and Kellog (1975).

Brain noradrenaline and 5-HT levels were also seen to vary over the oestrous cycle. At dioestrus, when activity was reduced, brain 5-HT levels were found to be increased and brain noradrenaline to be reduced. During the oestrus phase of the cycle, 5-HT levels were found to have declined and noradrenaline levels risen.

This latter finding supports more detailed evidence already obtained that noradrenaline and 5-HT levels in the brain may control ovulation. It has been reported that brain noradrenaline levels are raised several hours prior to ovulation, coinciding with the gonadotrophin surge (Donoso et al 1966, Stefano and Donoso 1967), and that raised noradrenergic activity preceeds the high surge (Coppola 1969, Donoso and Moyano 1970). More recent findings have indicated that noradrenaline has the facility to release LH (Vijayan and McCann 1978). It has been suggested that LH is necessary for ovulation (Wilson 1974). 5-HT levels have been seen to fall significantly in the median eminence prior to the LH surge (Wheaton et al 1972). When given by intraventricular injection into the brain, 5-HT has been shown to inhibit both LH and FSH release in intact female rats (Kamberi et al 1970, 1971: Kamberi 1973), and the facilitatory

effect of progesterone induced ovulation (Zolovik and Labhsetwar 1973). Evidence suggests that 5-HT inhibits the release of LH at the level of the mediobasal hypothalamus (Domarski et al 1975), by inhibiting the release of LH - RF (Leonardelli et al 1974).

Both the aged females and the female mice receiving the synthetic sex hormones had reduced levels of activity. At the end of the 43 day treatment period, the mice that received both the norethisterone acetate and ethinyloestradiol had a reduction in total dark period activity of 60% when compared to the oestrus control value. Brain 5-HT levels showed a 43% increase at the end of the same period for this group. For the mice receiving norethisterone acetate, the reduction in total dark phase activity after 43 days was 53·5%, while the increase in brain 5-HT levels was found to be 11·6%.

The aged females showed an increase of 21% in brain 5-HT levels and a reduction of 52% in total dark phase activity, when compared to the oestrus females.

Although the evidence is circumstantial, it would seem possible that locomotor activity is increased when brain 5-HT levels are lowered, and reduced when brain 5-HT levels are raised. These results lend support to the hypothesis that gonadotrophin is not controlled by individual amine levels, but by the relative proportions of noradrenaline and 5-HT (Lippmann 1968). Immediately prior to ovulation, noradrenaline levels are raised and 5-HT levels reduced. This circumstance facilitates ovulation and may also lead to the increase in activity during the oestrus phase of the cycle.

It should be noted, however, that raised levels of noradrenaline do not necessarily indicate an increase in neuronal activity: it has been suggested that raised levels may indicate reduced neuronal activity. However, noradrenaline turnover, judged to be a superior estimation of noradrenergic activity, has been found to increase as noradrenaline levels rise (see introduction), and in this case, it would seem likely that raised noradrenaline levels do reflect raised noradrenergic activity. A converse situation would seem to exist in the case of 5-HT, where lowered levels have been shown to coincide with reduced turnover (see introduction).

When the oestrus cycle was abolished, due to either the administration of synthetic sex hormones or increased age, the hourly activity patterns were seen to change.

Male mice were seen to produce 3 peaks in activity in their 24 hour activity cycle. The first two coincided with those found in the female oestrous cycle - although the level of activity was greater in the males. However, a further peak not found in females of the oestrous cycle was present. This peak was initiated in the last hour of darkness and sustained through the first three hours of illumination, after which activity fell to the minimal level characteristic of the light period.

In aged females, no longer exhibiting any sexual activity or experiencing regular oestrous cycle, total dark phase activity was much reduced in comparison to sexually active female mice. The initial activity peak was found to be present, as was the second peak of more sustained activity - if much reduced in intensity.

However, a third peak also occured in the first two hours of illumination mid-way in intensity between the previous two peaks.

This third peak was also visible in female mice receiving synthetic sex hormones in order to inhibit sexual activity and suppress the oestrous cycle. When norethisterone acetate was given alone, it seemed to abolish the second peak which had occured mid-way in the dark period in the oestrous cycle mice, and produce one which occured in the last three hours of darkness. This latter activity peak was more in evidence on Day 15 of the treatment than Day 43, the final day, when the peak was reduced in intensity. Those female mice receiving both norethisterone acetate and ethinyloestradiol had both the characteristic activity peaks of the oestrous cycle considerably reduced, while a relatively intense activity peak was seen to occur during the last four hours of darkness. No previous reports exist detailing this observation and its significance would seem unclear.

(b) Startle Response

The startle response magnitude was found to reach a maximum during the oestrus phase of the cycle and fall to a minimum at dioestrus. It would seem that the startle response can also be correlated to changes in brain 5-HT and noradrenaline levels. In a series of experiments, Davis and Sheard (1974a,b,c; 1976), proposed a hypothesis suggesting that a reduction in brain 5-HT levels could also be associated with an increase in sensitivity to various forms of sensory stimulation. At oestrus brain 5-HT levels were seen to be reduced and the startle

response magnitude to increase.

A diurnal variation in the startle response was also found. Using oestrus female mice, it was seen that the magnitude of the startle response was significantly greater in the dark period than in the light, the increase being 23·5%. This contrasts with the results of Horlington (1970), and Davis and Sollberger (1971), who found the startle response magnitude to be 90% greater during the dark period. It should be stressed, however, that in both of these reports male rats were used in conjunction with an auditory startle stimulus. As 5-HT has been suggested as an inhibitory transmitter in the auditory pathway (Bhargava and McKean 1977), it may be that changes in brain 5-HT levels magnify variations in startle response magnitude when an auditory stimulus is used.

Little difference could be found in startle response magnitude between oestrus female and male mice.

Both the administration of norethisterone acetate and norethisterone acetate plus ethinyloestradiol produced a significant reduction in startle response magnitude when compared to the oestrus controls. In both cases, brain 5-HT levels were raised significantly after 43 days treatment (see above), thus lending further support to Davis and Sheard's hypothesis. It should be noted that despite a considerable increase in brain 5-HT levels (43%) in the group receiving the combined treatment, the startle response magnitude was only marginally below that of the group receiving norethisterone acetate alone, where the brain 5-HT levels were found to be increased by only11·6%.

(c) Open Field Behaviour

The results obtained for changes in open field behaviour in female mice over the oestrous cycle were similar to those reported by Guttman et al (1975). A significant increase in locomotor activity was seen at oestrus ($P<0.05$) along with slight reduction in both rearing and defaecation scores. A slight increase in the number of grooming episodes was also noted at oestrus.

Male mice were found to exhibit less locomotor activity than females in any stage of the oestrus cycle, and significantly less than oestrus females ($P<0.02$). Male mice were also found to rear less than oestrus females, to exhibit slightly fewer grooming episodes and to produce a significantly greater number of faecal boli ($P<0.05$).

Despite the suggestion that differences in "emotional" behaviour are not usually found between the sexes in mice (Gray 1971), due to the predominant use of inbred strains within the species (Bruell 1969), it would seem that male mice of the T.O. strain are more "emotional" than the females of the strain, by the definition of Dannenberg (see introduction).

Using this definition of "emotionality", females in dioestrus can be said to be more "emotional" than their counterparts in oestrus, as they were seen to exhibit less locomotor activity and produce more faecal boli when placed in this novel environment.

The female mice that received either norethisterone acetate or norethisterone acetate and ethinyloestradiol were also found to be more "emotional" than oestrus controls.

Both groups exhibited significantly less locomotor activity (norethisterone acetate $P<0.01$; norethisterone acetate and ethinyloestradiol $P<0.05$), and produced significantly more faecal boli (norethisterone acetate $P<0.05$; norethisterone acetate and ethinyloestradiol $P<0.05$), than oestrus controls.

The oestrus females were active and exploratory in their home cage, and less fearful than the females in other stages of the cycle when in the "open-field". At this time brain 5-HT levels were found to be reduced. This situation does not conform to Ellisons hypothesis of the 5-HT depleted animal as a model of anxiety (Ellison 1975, 1977). Using this hypothesis, the oestrus female should be more fearful in the open field situation, while remaining active and exploratory in the home cage environment. It may be that when brain 5-HT levels fluctuate within normal physiological levels, as in the oestrous cycle, the hypothesis is not valid.

(d) Biochemical Changes

Brain 5-HT levels were found to undergo a diurnal variation, being greater in the period of illumination than in the period of darkness. The converse was found to be true in relation to brain noradrenaline levels. These results are in agreement with those of Hery et al 1972, Morgan et al 1973, and DiRaddo and Kellog 1975.

As stated previously, brain 5-HT levels were found to be at a minimum around the time of ovulation, when brain noradrenaline levels reached a maximum. It would seem likely that raised noradrenaline levels reflect increased noradrenergic neuronal activity and lowered

5-HT levels reflect reduced serotoninergic neuronal activity in order to facilitate the occurence of ovulation, as suggested by Lippmann (1968).

Although tryptophan is a known metabolic precursor to 5-HT, the various tryptophan levels were not found to parallel the variation of brain 5-HT throughout the oestrous cycle. Total plasma tryptophan was found to reach a minimum at metoestrus with a maximum at dioestrus. The concentration of free (unbound) tryptophan in the plasma was found to be at a minimum at oestrus, rising only very slightly at metoestrus, to reach a maximum at dioestrus. Brain tryptophan levels were found to be at a maximum at oestrus, with a minimum at metoestrus.

It should be rememb ered that tryptophan in the human plasma is distributed between two pools: about 10-20% circulating as the free amino acid, while the remainder is bound to serum albumin (McMenamy and Oncly 1958). No other amino acid has been found to bind appreciably to plasma proteins. It was found, however, that in the T.O, strain of mouse, the percentage of free tryptophan circulating in the plasma varied between 30-50%.

Because binding in general implies storage, several investigators have suggested that the plasma free tryptophan is biologically important in determining the availability of circulating tryptophan to the brain and other tissues (Knott and Curson 1972). It was shown that brain tryptophan levels and 5-HT turnover were time related to changes in plasma free tryptophan but not total plasma tryptophan. However, Fernstrom et al (1974), have

suggested that a paradoxical situation exists, where total plasma tryptophan and albumin-bound tryptophan, but not plasma free tryptophan, change in parallel with brain tryptophan, in response to various diets. This is explained by the assumption that plasma free tryptophan is in equilibrium with circulating albumin-bound tryptophan, and with the tryptophan in such tissues as brain, heart and skeletal muscle. For albumin-bound tryptophan to enter any of these tissues, it must pass through a free tryptophan phase, however transiently. If the affinity of albumin for the tryptophan molecules is greater than that of muscle or heart, but less than that of brain, the phenonenon of albumin binding will thenfavour tryptophan molecules entering the brain, even though the size of the pool within heart and muscle may be greater than in brain. It is presumed that such affinity constants can be varied by other circulating factors, e.g. insulin may affect tryptophan uptake into muscle, while competing neutral amino acids will affect its uptake into heart. In any case, all the tryptopan in the plasma would be available to the brain even though only the free part actually entered brain tissue.

It was found that changes in total plasma tryptophan and the concentration of free tryptophan in the plasma were similar, with minimum values being obtained during either the metoestrus or oestrus phases, with values rising to a maximum at dioestrus and persisting through proestrus.

The variation in the concentration of free tryptophan in the plasma may be explained by an apparent

positive correlation between total plasma oestrogen content and the concentration of free tryptophan in the plasma (Thomson et al 1977). Oestradiol is known to reach a peak between 11a.m. and 3p.m. on the day of proestrus (Shaikh 1971), with ovulation occuring some 12-14 hours later. This would account for the relatively hi h levels of free tryptophan in the plasma that were found on the day of proestrus. After ovulation has occured, oestrogen levels decline with a corresponding reduction in the concentration of plasma free tryptophan visible on the days of oestrus and metoestrus.

The question then arises of why concentrations of plasma free tryptophan appear to be at a maximum on the day of dioestrus. In the human female plasma oestradiol levels reach a maximum prior to ovulation, decline rapidly, then rise again, although less markedly than before, in the post ovulatory phase. This situation does not, however, occur in the rodent oestrous cycle. It may be that the maximum concentration of plasma free tryptophan seen on the day of dioestrus are an illusion of the timing of the experiment. The oestrogen secretion rate is known to start to rise from virtual zero at the beginning of the period of illumination on the day of dioestrus. This rise continues gradually until reaching a maximum at around mid-day on the day of proestrus. After reaching this peak the decline is rapid, falling to virtual zero within 12 hours (Shirley et al 1968). The experiments were undertaken at about 2p.m. each day. It may be that at 2p.m. on the day of dioestrus, oestradiol levels had risen to a point higher than that existing at

the same time some 24 hours later, when the rapid decline in oestradiol levels had commenced. The exact time of the peak in oestradiol plasma levels is closely linked to the photoperiodicity existing during the particular oestrous cycle.

Neither total plasma tryptophan levels nor the concentration of free tryptophan in the plasma were found to parallel the changes in brain tryptophan. Brain tryptophan levels were found to be at a maximum at oestrus, falling to a minimum at metoestrus, rising again through the dioestrus and proestrus stages of the cycle, Christensen (1975), reported that oestrogens promote uptake of amino acids into cellular tissue, while reducing renal transport. Thus brain tryptophan would be expected to be high on the day of proestrus, and rise to a maximum on the day of oestrus, assuming that this facilitation of tryptophan uptake is a gradual rather than instantaneous process. Coinciding with this situation 5-HT synthesis has been shown to be reduced at oestrus in order to facilitate ovulation (see introduction), and these two factors either in combination, or alone, may account for the increase in brain tryptophan during proestrus/oestrus period.

Similarly, brain tryptophan levels may reach a minimum at metoestrus due to the changes in oestradiol secretion and 5-HT synthesis known to follow ovulation. Oestradiol levels in the plasma reach relatively low levels during metoestrus, while 5-HT synthesis increases rapidly in order to halt the ovulatory process. In this situation, uptake of tryptophan into brain neurones will

be reduced, and the metabolism of brain tryptophan increased, in comparison to the oestrus and proestrus phases of the cycle, thereby producing a relative decline in brain tryptophan levels at metoestrus.

It would seem that in the case of the mouse oestrous cycle, there is a stronger relationship between the amount of circulating oestrogens in the plasma and brain tryptophan levels, than between the plasma oestrogen levels and the concentration of free tryptophan in the plasma. This may be due to the large percentage of free tryptophan present in mouse plasma (40-50%), masking the effect of plasma oestrogens, an effect not seen in humans where the percentage of free tryptophan is much smaller (10%) and, therefore, more liable to modification by the plasma oestrogens.

Where brain 5-HT levels were raised due to the administration of synthetic sex hormones, brain tryptophan levels were found to be significantly above those of the metoestrus controls (norethisterone acetate $P<0.02$: norethisterone acetate and ethinyloestradiol $P<0.05$). A similar result was found to occur in aged females, which were found to have significantly increased brain tryptophan levels ($P<0.02$) coinciding with raised brain 5-HT levels.

The administration of the synthetic sex steroids produced a definite pattern in relation to tryptophan and 5-HT levels. Significant increases in total plasma tryptophan and the concentration of free tryptophan in the plasma were found to coincide with significant increases in brain tryptophan and 5-HT levels. This was

not unexpected as far as the combined therapy was concerned, as oestrogens have been suggested to increase free tryptophan in the plasma by the possible displacement of tryptophan from albumin binding sites (Aylward 1976). The oestrogenic component might also be expected to increase brain tryptophan levels. Little difference was found in any of the tryptophan levels when norethisterone acetate was given alone in comparison to when it was administered in conjunction with the oestrogenic compound, ethinyloestradiol.

More difficult to understand was why similar brain tryptophan levels produced by norethisterone acetate alone, or in conjunction with ethinyloestradiol, produced an 11·6% increase in brain 5-HT levels in the former and 43% increase in the latter, when compared to oestrus controls. It may be that the oestrogenic component ethinyloestradiol can be implicated in some way, although no significant behavioural differences were discovered between the two groups of animals despite this considerable variation in brain 5-HT levels. It may be that two such dissimilar brain 5-HT levels reflect similar levels of serotoninergic neuronal activity, which, if it were true, would further underline the inaccuracy of whole brain 5-HT levels as a reflection of serotoninergic neuronal activity within the brain.

CHAPTER SIX

THE EFFECTS OF l-TRYPTOPHAN ON LOCOMOTOR ACTIVITY

Male mice weighing between 20-25g were used. The animals were housed, from weaning, under a 12 hour light/ 12 hour dark lighting schedule, the period of illumination beginning at 7·00a.m. and finishing at 7·00p.m. The experiments were performed between mid-day and 2·00p.m. Locomotor activity was measured in groups of three animals by means of an Animex Motility Meter. Recordings were made over a period of two hours, counts being taken at 15 minute intervals. The initial count started five minutes after injection (i.p.).

When Modigh (1973), studied the effect of tryptophan on the locomotor activity of mice, the experiments were carried out for 20 minutes, beginning one hour after the injection of tryptophan. Thus it was decided to record locomotor activity from the time of injection until two hours after the time of injection in order to discover whether the effect of tryptophan on locomotor activity was time dependent as well as dose dependent.

Figure 41 shows the effect of increased doses of tryptophan on total activity in the two hour period after injection, while Figure 42 illustrates the effect of increased doses of tryptophan on locomotor activity measured at 15 minute intervals during the 2 hours after injection.

(i) <u>l-Tryptophan 100mg/kg (i.p.)</u>

No significant reduction in total activity was seen during the two hour period, although total activity was reduced by 37% when compared to the control value.

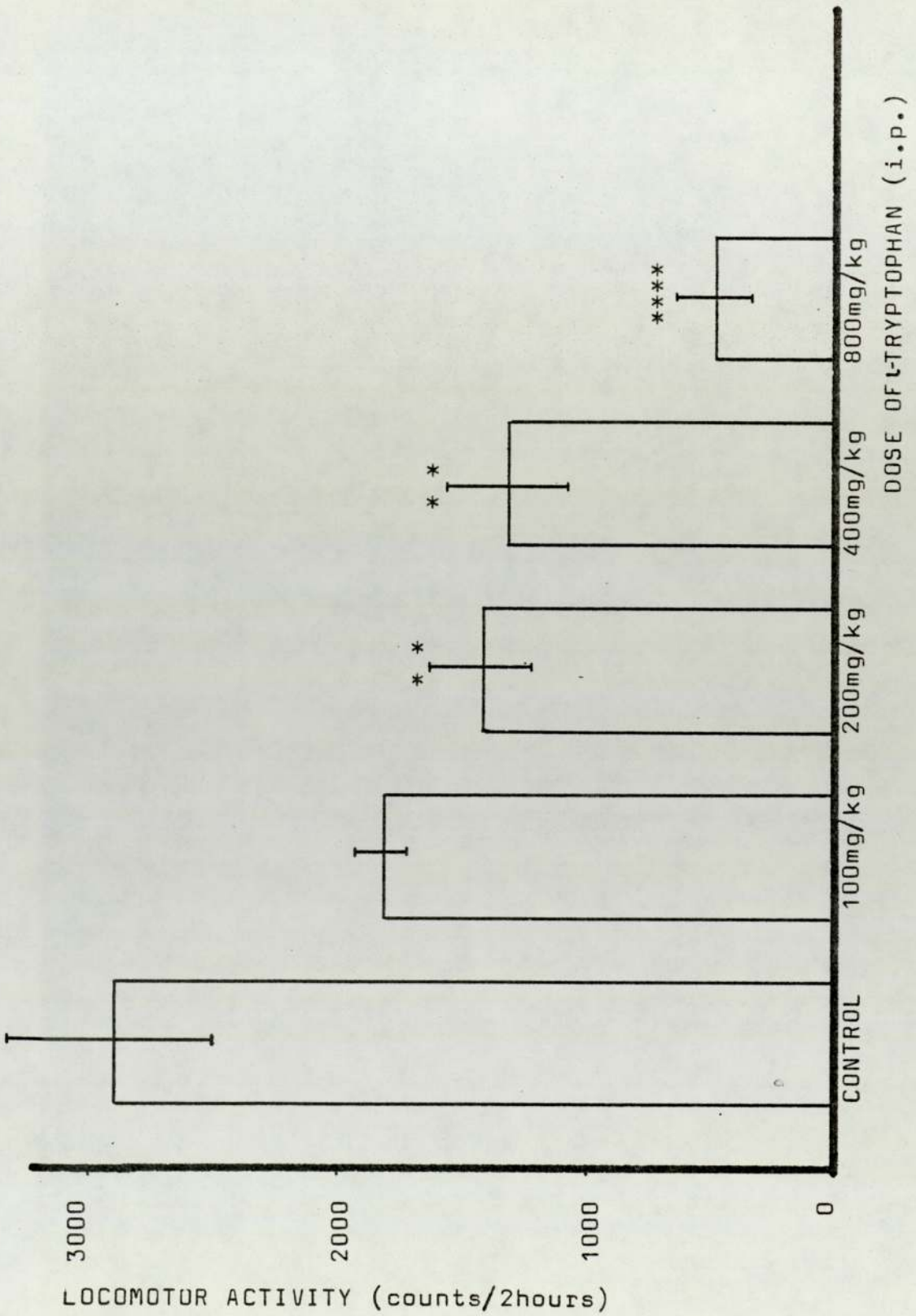


FIG.41: The effects of various doses of l-tryptophan (i.p.) on locomotor activity for a two hour period after injection.** P<0·02;**** P<0·001.

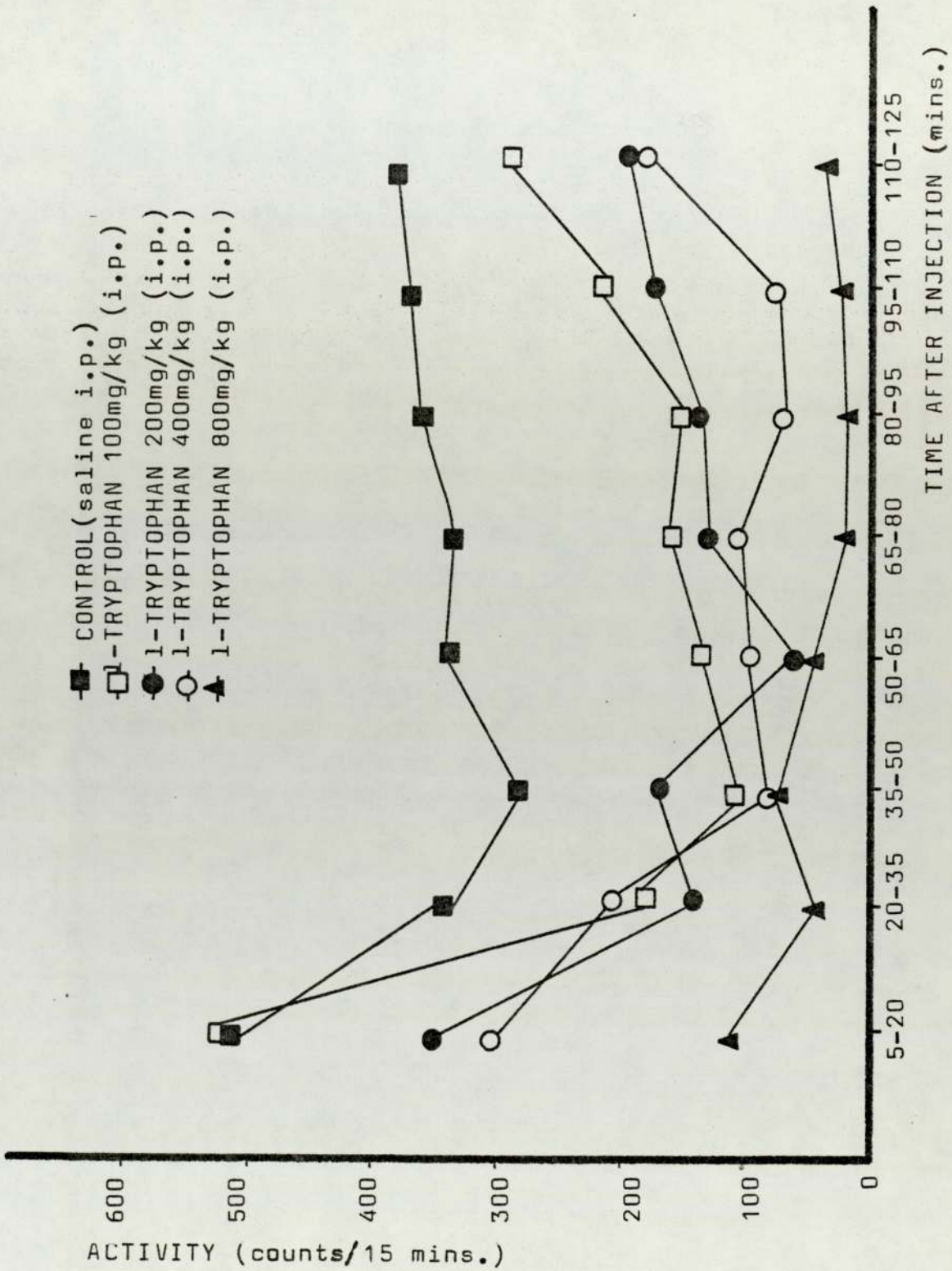


FIG.42: The effects of various doses of l-tryptophan on locomotor activity for a 2 hour period after injection.

Little difference was seen between control and test values up to 20 minutes after injection, but between 20-35 minutes locomotor activity in the test group declined by 50% when compared to the control value. The difference was found not to be statistically significant. Between 35-50 minutes the locomotor activity was reduced in the test animals to only 37% of that of the controls, but again this was not found to be statistically significant. Between 50-65 minutes a significant reduction in locomotor activity was found in the test animals ($P<0.05$), the test value being 45% of that of the control. From 65 minutes onwards, locomotor activity in the test mice began to rise slowly, and between 110-125 minutes it had reached a value of 74% of that of the control.

(ii) <u>l-Tryptophan 200mg/kg (i.p.)</u>

A significant reduction of 52·5% ($P<0.02$) was found to occur in total locomotor activity during the 2 hour period after the injection of a dose of 200mg/kg (i.p.) of l-tryptophan.

Between 5-20 minutes locomotor activity in the test animals was found to be 69% of that of the controls, and between 20-35 minutes this had declined to 37%, the difference being statistically significant ($P<0.05$). Between 35-50 minutes, locomotor activity in the test animals rose to 57% of that of the controls but between 50-65 minutes it had fallen to the minimum value recorded with this dose of tryptophan, being only 19% of that of the control value, the difference being statistically significant ($P<0.01$). Locomotor activity in the test

mice then rose until the end of the experiment, being 39% of the control value between 65-80 minutes, 41% between 80-95 minutes and 50% between 95-110 minutes. This latter difference was found to be statistically significant ($P<0.05$). In the last 15 minutes of the experiment, locomotor activity attained a value of 54% of the control value, in the test animals.

(iii) <u>l-Tryptophan 400mg/kg (i.p.)</u>

A significant reduction of 55% ($P<0.02$) was found to occur in total locomotor activity in the 2 hour period after the injection of a dose of 400mg/kg (i.p.) of l-tryptophan.

Between 5-20 minutes the locomotor activity of the test animals was found to be 60% of that of the controls and between 20-35 minutes this had risen very slightly to 64%. Between 35-50 minutes the locomotor activity of the test animals fell to a minimum value being only 27% ofthat of the controls, the difference being statistically significant ($P<0.02$). Between 50-65 minutes the difference in locomotor activity between the test and control groups remained statistically significant ($P<0.02$), the locomotor activity of the test animals being 30% of that of the controls. Between 65-80 minutes, this had risen to 32% but between 80-95 minutes had fallen again to 26% of the control value, this latter difference being statistically significant ($P<0.05$). Between 95-110 minutes the locomotor activity of the test animals was again significantly below that of the controls ($P<0.01$), the test values remaining at 26% of that of the controls. Between 110-125 minutes, the locomotor activity of the test animals had risen to 53%

of that of the control values.

(iv) 1-Tryptophan 800mg/kg (i.p.)

With a dose of l-tryptophan of 800mg/kg (i.p.) an 83% reduction in locomotor activity occured in comparison with the control animals during the 2 hour period after the injection. This difference was found to be statistically significant ($P<0.001$). The total activity in this 2 hour period was also found to be significantly below that recorded for the dose of tryptophan of 400mg/kg (i.p.), ($P<0.05$).

At each of the eight 15 minute recordings, the reductions in locomotor activity for the test animals were significantly below the corresponding control values, as shown in the following table:

Time after Injection (minutes)	% of Control Value	P Value
5-20	23	$P<0.001$
20-35	13	$P<0.01$
35-50	25	$P<0.05$
50-65	14	$P<0.01$
65-80	7	$P<0.02$
80-95	6	$P<0.02$
95-110	7	$P<0.001$
110-125	13	$P<0.05$

Thus, the maximum effect exerted by a dose of 800mg/kg of l-tryptophan would appear to occur between 65-110 minutes after injection. This contrasts with the situation produced by the previous doses of tryptophan where the maximum reduction in locomotor activity occurs between 35-50 minutes for a dose of 100mg/kg, between 50-65 minutes for a dose of 200mg/kg and between 35-50

minutes for a dose of 400mg/kg.

(v) Discussion

l-Tryptophan has been reported to reduce spontaneous locomotor activity in laboratory animals of different species (Brown 1960; Ashcroft et al 1964; Modigh 1973), and to cause sedation and changes in the sleep pattern of man (Wyatt et al 1970; Hartman et al 1971).

Brown (1960), reported a marked depression of motor activity in mice after the administration of tryptophan 0·07mg/kg, and this depression was not increased further when the dose was increased to 150mg/kg. These results were not evaluated statistically. Modigh (1973), however, found that only a large dose of l-tryptophan (800mg/kg) reduced spontaneous locomotor activity with lower doses being ineffective. It was suggested that the discrepancy between such results may be due to the differing constructions of the activity meters used in the two experiments, which may have measured differing aspects of motor activity. It would seem unlikely, however, that such an explanation would account completely for such extreme variations in the two sets of results.

The discrepancy between the results presented above and those of Modigh may be partially attributable to the difference in the timing of the experiments. Modigh's results were based on a count of locomotor activity lasting 20 minutes, starting one hour after the time of injection. From the results above, it can be seen that between 65-80 minutes after injection (the corresponding period

to that in the experiments of Modigh), only a dose of 800mg/kg produced a significant reduction in locomotor activity. This result corresponds to that of Modigh. However, for doses between 50mg/kg and 400mg/kg, his results show a maximum decline in locomotor activity of 20%, in comparison to the control values, which was not seen to be dose dependent. This does not correspond with the above results, where it was found that a dose of 100mg/kg of tryptophan produced a 47% decline in locomotor activity in comparison to the control values, 200mg/kg, a 61% reduction and 400mg/kg, a 68% reduction, over the equivalent time period. It would hardly seem likely that the discrepancy between such results could be attributed to any variation on motility meter construction.

It is known that in mice, the l-tryptophan induced increase of 5-HT synthesis in the brain reaches a maximum after the i.p. administration of 300mg/kg (Carlsson and Lindquist 1972). This would seem to be confirmed when it was found that a dose of 800mg/kg i.p. of l-tryptophan only raised brain 5-HT levels 2% above that produced by a dose of 400mg/kg i.p. (See Chapter 6). It can be seen that a dose of 100mg/kg i.p. of l-tryptophan produced a maximum reduction in locomotor activity of 63% in comparison with the control value 100mg/kg = 81%; 400mg/kg = 75% and 800mg/kg = 94%. It would seem likely that, up to a dose of 300mg/kg, l-tryptophan will increase 5-HT synthesis such that it causes a type of behavioural depression, reflected as reduced levels of spontaneous locomotor activity. The negligible difference in reduced locomotor activity levels by doses of 200mg/kg and 400mg/kg would

seem to support this contention.

Clearly, in the case of a dose of 800mg/kg, the situation would seem to be altered somewhat. The results presented above seem to support the contention of Modigh "that the inhibitory effect of l-tryptophan on motor activity is not mainly mediated via any of its known neurotrophic metabolites" - at least in doses in excess of 400mg/kg. It would seem that in doses above such a figure, the inhibitory effect is more likely to be potentiated by the amino acid itself. It is known that l-tryptophan is taken up into the brain (Guroff and Udenfriend 1962), and into synaptasomes (Graham-Smith and Parfitt 1970), by means of active transport, and other amino acids compete for these transport mechanisms (Peters 1972). It may be that in high doses, the observed reduction in motor activity could be due to both maximum 5-HT synthesis and the induced deficiency of other amino acids necessary for the maintenance of spontaneous locomotor activity by the saturation of transport mechanisms with l-tryptophan.

It is unlikely that such a reduction in locomotor activity can be associated with increased tryptamine formation as Curzon and Marsden (1977, 1978) have shown tryptamine to have a facilitatory effect on locomotor activity.

CHAPTER SEVEN

THE EFFECT OF ALTERED TRYPTOPHAN METABOLISM ON THE STARTLE RESPONSE

Three month old male mice, weighing between 20-25g were used. The animals were housed, from weaning, under a 12 hour light/12 hour dark lighting schedule, illumination lasting from 7·00a.m. until 7·00p.m. This series of experiments was performed between mid-day and 2;00p.m., as previous experience had shown that the timing of such experiments was critical (see Chapter 3).

Startle response magnitude was measured using the startle box and associated equipment as described in "Methods". The animals were pre-housed individually in the apparatus for fifteen minutes and then twenty startle stimuli, in the form of air puffs, were directed at each animal at five second intervals. The mean of these twenty responses was taken as the startle response magnitude for each mouse.

(i) The Effect of l-tryptophan on the Startle Response

The startle response was tested at two differing times after the i.p. injection of l-tryptophan. The times were thirty minutes and sixty minutes after injection, the doses of l-trypotphan administered ranging between 50mg/kg and 800mg/kg.

The table below illustrates the results obtained at each dose level of l-tryptophan at the two differing times. The results are expressed as a percentage of the control value.

Dose of l-tryptophan Mg/Kg	S.R. Magnitude % Control 30 min after injection(n=10)	S.R. Magnitude % Control 60 min after injection(n=10)
50	85·18±7·04	86·64±9·2
100	92·79±17·18	93·96±5·82
200	96·68±4·41	94·57±7·83
300	107·63±5·15	98·60±8·87
400	122·65±8·03*	102·78±3·85
800	126·96±6·13*	129·12±10·22*

Control startle response magnitude = 100·00±6·5 (n=30)

*Significant difference $P<0·05$

It can be seen that tryptophan produces a dose dependent effect on startle response magnitude, for, as the dose of l-tryptophan increased so did the startle response magnitude. At lower doses the effect of time would seem to be negligible, but above a dose of 300mg/kg the startle response magnitude was reduced at 60 minutes after injection, in comparision to that at 30 minutes. This was particularly noticeable at the dose of 400mg/kg, where after 30 minutes the startle response magnitude was significantly above that of the controls ($P<0·05$). No significant difference was seen to occur in the startle response magnitude between the test animals receiving l-tryptophan 400mg/kg, and the controls, 60 minutes after injection. A dose of 800mg/kg increased the startle response magnitude significantly ($P<0·05$) at both 30 minutes and 60 minutes after injection, the level of increase being similar.

Doses of l-tryptophan from 50mg/kg to 200mg/kg were seen to reduce the startle response magnitude,

which became closer to the control value as the dose approached 200mg/kg.

(ii) The Effect of l-tryptophan on the Startle Response after pre-treatment with the Peripheral Decarboxylase Inhibitor, MK 486

It is known that the aromatic amino acid decarboxylase enzyme is that which catalyses the formation of 5-HT from its immediate precursor 5-HTP (Udenfriend et al 1956), and also the formation of tryptamine from tryptophan. This conversion was inhibited preipherally using L-α-hydrazino-α-methyl-β-(3,4,dihydroxyphenyl) propionic acid-MK 486 (Parker et al 1962; Bartholini and Pletscher 1969).

A dose of 100mg/kg MK 486 i.p. was given 30 minutes prior to the various i.p. doses of l-tryptophan. The startle response was assessed 30 minutes after the injection of l-tryptophan. The results in the table below are again expressed as a percentage of the control value.

Dose of l-tryptophan Mg/kg	Startle Response Magnitude % Control (n=8)
50	62·14±4·82*
100	98·07±18·01
200	91·34±6·37
300	92·29±9·42
400	107·96±19·36
800	168·68±24·04*

Control startle response magnitude = 100·00±12·46

*Statistically significant $P<0·05$

It was found that pre-treatment with MK 486 potentiated the inhibitory effect of tryptophan significantly at a dose of 50mg/kg while significantly

increasing the startle response at a dose of 800mg/kg.

Biochemistry

Brain 5-HT levels were measured 30 minutes after doses of l-trypotphan had been administered by i.p. injection. The doses of l-tryptophan used were 50mg/kg, 400mg/kg and 800mg/kg. This process was repeated after pre-treatment with 100mg/kg MK 486 i.p., administered 30 minutes prior to the tryptophan injections.

Dose of l-tryptophan mg/kg	Brain 5-HT Levels ng/g (n=6)	
	l-tryptophan	l-tryptophan + MK 486
50	690±26	737±24***
400	789±27***	810±23****
800	806±22****	813±26****

Control Brain 5-HT levels: 651±25

Statistically significant * $P<0.05$ ** $P<0.02$
*** $P<0.01$ **** $P<0.001$

MK 486 was found to increase brain 5-HT levels at all three doses of l-tryptophan, the most noticeable increase being at 50mg/kg. The 5-HT levels produced after the administration of doses of l-tryptophan of 400mg/kg and 800mg/kg were found not to be significantly different, either with MK 486 pre-treatment or without it.

(iii) The Effect of 5-HTPon the Startle Response

A dose of 50mg/kg of 5-HTP was administered fifteen minutes prior to the assessment of the startle response. 5-HTP is the immediate metabolic precursor of 5-HT.

	Startle Response Magnitude (mm)
Control (n=10)	10·59±0·61
5-HTP 50mg/kg (n=12)	10·82±0·81

At this dose level 5-HTP was found to have little effect on startle response magnitude, despite inducing a threefold increase in brain 5-HT levels. The 5-HT levels were found to be 1727±70 ng/g in 5-HTP treated animals compared to 631±16 in the controls. The difference was found to be statistically significant.

(iv) Discussion

Recent reports suggest that behavioural excitation, expressed as increased locomotor activity and produced by a combination of l-tryptophan and a MAO inhibitor, may be partially due to increased brain tryptamine levels (Marsden and Curzon 1978).

In the previous chapter, a high dose of l-tryptophan (800mg/kg) was seen to produce a depressant effect on locomotor activity, whereas a similar dose induced behavioural excitation, illustrated by increased startle response magnitude. An explanation of such an apparent paradox may be the effect of increased brain tryptamine on the two different modes of behaviour.

It would seem likely that at a dose of 50mg/kg, l-tryptophan acts only to increase brain 5-HT levels. This would seem to be confirmed by the action of the peripheral decarboxylase inhibitor in producing increased brain 5-HT levels after 50mg/kg dose of l-tryptophan, which further reduced the startle response magnitude significantly.

However, as the dose of l-tryptophan increased towards 300mg/kg, (the dose of l-tryptophan suggested as producing maximum 5-HT synthesis)behavioural depression, illustrated by changes in startle response magnitude, decreased. It may be that as the dose of l-tryptophan is increased, more tryptamine is synthesised. Thus the excitatory effect of tryptamine on such behaviour gradually overcomes the depressant effect of 5-HT, as the latter nears the maximum synthesis level, produced by the 300mg/kg dose of l-tryptophan. This results in the neglibible difference in startle response magnitude between the test animals, at doses of 100mg/kg, 200mg/kg and 300mg/kg of l-tryptophan, and the controls. Above 300mg/kg, behavioural excitation, in the form of increased startle response magnitude, began to manifest itself. This could be due to the excitatory effect of tryptamine on behaviour exceeding the now static inhibitory effect of 5-HT. Thus in doses of 400mg/kg and above, much of the l-tryptophan in the brain will be converted to tryptamine.

Pre-treatment with MK 486 produced differing effects after doses of l-tryptophan exceeding 400mg/kg. After a dose of 400mg/kg the startle response magnitude was reduced by 15% in comparison to the effect produced by a similar dose of tryptophan alone, whereas a dose of 800mg/kg produced a 42% increase. The reduced startle response magnitude, after MK 486, is probably due to the increase in 5-HT levels after a dose of l-tryptophan of 400mg/kg.

At 800mg/kg, the levels of brain 5-HT remained virtually unchanged after pre-treatment with MK 486. This would seem to suggest that a considerable increase in brain tryptamine may be responsible for the 42% increase in startle response magnitude, especially as it has been suggested that MK 486 may have a central effect in restricting 5-HT accumulations to brain regions containing endogenous indole stores (Bedard et al 1971).

Support for this hypothesis is gained from the fact that 5-HTP, which can only be decarboxylated to 5-HT, produced a threefold rise in brain 5-HT levels, while producing little effect on startle response magnitude.

This does not, however, explain why increasing doses of l-tryptophan increase startle response magnitude while decreasing spontaneous locomotor activity. It can only be surmised that the central and peripheral neural pathways controlling locomotor activity are less responsive, in some way, to the excitatory effect of tryptamine than those responsible for controlling the startle response. It may be that such a neural pathway only becomes responsive after the considerable increase in brain tryptamine levels brought about by the concurrent administration of l-tryptophan and an MAO inhibitor.

CHAPTER EIGHT

THE EFFECT OF SEROTONINERGIC METABOLIC PRECURSORS ON THE STARTLE RESPONSE OF ANIMALS DEPLETED OF 5-HT.

(A) Depletion of 5-HT by Synthesis Inhibition

p-CPA has been shown to be a relatively specific inhibitor of 5-HT synthesis (Koe and Weissmann, 1966), although some interference with catecholamine synthesis has been suggested at higher doses. Conflicting reports exist on the ability ofp-CPA to affect locomotor activity and the startle response, while producing reductions in brain 5-HT levels.

(i) p-CPA 150mg/kg i.p.

A single dose ofp-CPA 150mg/kg i.p. was administered 24 hours prior to the start of the startle response experiment. Such a dose has been reported to reduce brain 5-HT levels by about 50% due to partial inhibition of 5-HT synthesis (Curzon and Marsden, 1975).

	Startle Response Magnitude (% control)
Control (n=12)	100·0±7·7
p-CPA 150mg/kg (n=8)	102·5±3·1

This dose of p-CPA reduced brain 5-HT by 68%, which was found to be statistically significant ($P<0·001$), while producing only a slight, non-significant increase in startle response magnitude.

(ii) p-CPA 400mg/kg i.p.

This dose was administered on two occasions, the first 48 hours before the start of the experiment, the second 24 hours before. Both injections were given i.p. Such a dose has been shown to be capable of reducing brain 5-HT levels to a minimum (Curzon and Marsden 1975).

	Startle Response Magnitude (% control) (n=12)
Control	100·0±5·65
p-CPA 400mg/kg	128·2±6·55

Such a dose of p-CPA was seen to increase the startle response magnitude significantly ($P<0·02$) while also reducing brain 5-HT levels by 89%, a level significantly below that produced by a single dose of p-CPA 150mg/kg ($P<0·01$).

(B) Selective Replacement of Brain 5-HT after Synthesis inhibition with p-CPA

The increase in locomotor activity reported after the administration of p-CPA has been shown to be reversed by the immediate precursor of 5-HT, 5-HTP (Geller and Blum, 1970; Fibiger and Campbell, 1971).

(i) 5-HTP 50mg/kg i.p. + p-CPA 150mg/kg i.p.

A single dose of p-CPA 150mg/kg i.p. was given 24 hours prior to the start of the experiment. 5-HTP 50mg/kg was administered 15 minutes before the start of the experiment. Control animals received saline rather than 5-HTP.

	Startle Response Magnitude (% control) (n=16)
Control	100·0±8·5
p-CPA + 5-HTP	72·9±4·92

A significant reduction in startle response magnitude ($P<0·01$) was found to correspond with six-fold increase in brain 5-HT levels, which was also found to be statistically significant ($P<0·001$).

(ii) 5-HTP 50mg/kg i.p. + p-CPA 400mg/kg i.p.

Two doses of p-CPA 400mg/kg were administered, one 48 hours before the start of the experiment, the other 24 hours beforehand. A dose of 5-HTP 50mg/kg was administered 15 minutes before the start. Control animals received saline rather than 5-HTP.

	Startle Response Magnitude (% control) (n=10)
Control	100·0±8·7
p-CPA + 5-HTP	76·3±8·4

A significant reduction in startle response magnitude ($P<0·05$) was found to correspond with a nine-fold increase in brain 5-HT levels, which was also found to be statistically significant ($P<0·001$).

Such results support previous reports of the ability of 5-HTP to reverse behavioural hyperreactivity induced by p-CPA. The fact that p-CPA induced considerable reductions in brain 5-HT levles, while only producing a significant increase in startle response magnitude with the higher dose level, may be partially due to interference with catecholamine synthesis. The results also support the observation of Connor et al (1970b), who reported that p-CPA was responsible for a transitory rise in startle response magnitude.

(C) Depletion of 5-HT by Reserpine

Further effects of amine depletion on the startle response were investigated by the administration of a large, single dose of reserpine 18 hours prior to the start of the experiment. Although reserpine does not

produce specific 5-HT depletion, it does offer two important advantages in the context of such an investigation. The first is, that it provides a means of studying the effects of amine depletion on the startle response, accomplished by a method other than synthesis inhibition, and, secondly, it provides a basic pharmacological model for subsequent experiments in which 5-HT levels can be selectively replaced by the administration of the metabolic precursors 5-HTP and l-tryptophan.

A dose of 5mg/kg reserpine was administered by i.p. injection 18 hours prior to the start of the experiment.

	Startle Response Magnitude (% control) (n=12)
Control	100·0±5·02
Reserpine	39·0±3·01

A significant reduction of 78% in brain 5-HT levels ($P<0·001$) was found to correspond with a significant reduction in startle response magnitude ($P<0·001$).

(D) Selective Replacement of 5-HT in Reserpinised Animals

(i) 5-HTP 50mg/kg i.p.

The previous experiment demonstrated that general monoamine depletion, which included depletion of 5-HT, resulted in the reduction of startle response magnitude. In this experiment, 5-HT was selectively increased in reserpine-treated animals by the administration of its immediate metabolic precursor 5-HTP.

The animals were given 5mg/kg reserpine i.p., 18 hours prior to the start of the experiment, followed by 5-HTP 50mg/kg i.p. 15 minutes prior to the start. Control animals received saline rather than 5-HTP.

	Startle Response Magnitude (% control) (n=20)
Control	100·0±9·94
Reserpine + 5-HTP	68·66±10·65

The administration of 5-HTP to reserpinised animals produced a significant four-fold rise in brain 5-HT levels ($P<0·001$) which corresponded with a significant reduction in startle response magnitude ($P<0·001$).

(ii) 1-Tryptophan

In this experiment 5-HT was again selectively increased in reserpine treated animals by the administration of its more distant metabolic precursor, the amino acid l-tryptophan.

The animals were again pre-treated with reserpine 18 hours before the start of the experiment. One group of test animals received l-tryptophan 100mg/kg i.p. 30 minutes prior to the start of the experiment, while the second group received 400mg/kg. The control animals received saline rather than l-tryptophan.

	Startle Response Magnitude (% control) (n=12)
Control	100·0±12·74
Reserpine + l-tryptophan 100mg/kg	69·1±8·62
Reserpine + l-tryptophan 400mg/kg	65·7±7·83

The dose of l-tryptophan 100mg/kg was found to increase brain 5-HT to a level twice that of the controls, the increase being statistically significant ($P<0.001$). The 400mg/kg dose increased brain 5-HT levels by a further 24% ($P<0.001$) while reducing the startle response magnitude by 3·4%, in comparison to the 100mg/kg dose. Both doses reduced the startle response magnitude significantly (100mg/kg $P<0.05$; 400mg/kg $P<0.02$).

(E) Selective Replacement of 5-HT in Reserpinised Animals Pre-treated with a MAO Inhibitor

(i) 5-HTP 50mg/kg i.p.

In an attempt to increase brain 5-HT levels further than in previous experiments, the MAO inhibitor chlorgyline (N-methyl-N-propargyl-3-(2,4,dichlorphenoxy) propylamine)-M and B 9302, was administered. Chlorgyline has been shown to be an irreversible substrate selective inhibitor of MAO type A, thought to be specific to indoleamines (Johnston 1968; Hall et al 1969). A dose of 50mg/kg i.p. was administered 30 minutes prior to the dose of 5-HTP. The rest of the experimental details were as in experiment d (i) of this chapter. Controls received saline rather than 5-HTP.

	Startle Response Magnitude (% control) (n=12)
Control	100·0±10·17
Reserpine + Chlorgyline + 5-HTP	143·7±9·8

The startle response magnitude was found to be increased significantly ($P<0.02$) after 5-HTP. The effect of chlorgyline on brain 5-HT levels was such that

animals receiving reserpine + 5-HTP had brain 5-HT levels of 596±25ng/g, whereas the additional administration of chlorgyline produced brain 5-HT levels of 1382±24ng/g. This increase was found to be significant ($P<0.001$).

Reserpinised animals receiving both chlorgyline + 5-HTP exhibited some of the characteristics of the hyperactivity syndrome reported after the concurrent administration of a MAOI and 5-HTP or l-tryptophan (Grahame-Smith 1971). Those characteristics present were tremor, rigidity, hyperreactivity and stereotyped head response. In addition, hyperextensions and athetoid movements of the hind legs were seen. These latter behavioural manifestations have been partly explained by an increased excitability of the α-motoneurones. It has been suggested that the effects are due to the formation of 5-HT from 5-HTP in the caudal part of the spinal cord, 5-HT diffusing from the cytoplasm of the monoamine nerve terminals to receptors on the effector cells, without being released by nerve inpulses (Anden 1975).

A similar increase in startle response magnitude was found in non-reserpinised animals after similar doses of chlorgyline and 5-HTP. In this experiment the controls received saline rather than 5-HTP.

	Startle Response Magnitude (% control) (n=10)
Control	100·0±5·2
Chlorgyline + 5-HTP	122·74±6·6

The increase in startle response magnitude after 5-HTP was found to be significant ($P<0.05$). The

characteristics of the hyperactivity syndrome were again present, with associated hyperextensions and athetoid movements of the hind legs.

(ii) l-Tryptophan

In this experiment, l-tryptophan was administered in place of 5-HTP. Chlorgyline 50mg/kg i.p. was given 30 minutes prior to the i.p. administration of l-tryptophan which was itself given 30 minutes prior to the start of the experiment. Doses of 100mg/kg and 400mg/kg l-tryptophan were used. Reserpine 5mg/kg i.p. had been administered 18 hours before the start of the experiment. The control animals received saline rather than l-tryptophan.

	Startle Response Magnitude (% control) (n=10)
Control	100·0±2·3
l-tryptophan 100mg/kg	105·84±7·4
l-tryptophan 400mg/kg	113·2±4·42

At a dose of 100mg/kg, l-tryptophan produced a negligible increase in startle response magnitude. The increase was found to be significant at a dose of 400mg/kg ($P<0·05$). The7·5% difference in brain 5-HT levels produced by the two doses of tryptophan was reflected as a 7·5% difference in startle response magnitude.

(F) Biochemistry

Table 3 shows brain 5-HT levels (ng/g) after the various pharmacological manipulations undertaken in this series of experiments. Statistical analysis of these results was given in the preceeding text of this chapter.

TABLE 3

(i) SYNTHESIS INHIBITION

Drugs Administered	Brain 5-HT Levels (ng/g)
Saline Control	651±25
p-CPA 150mg/kg	209±18
p-CPA 400mg/kg	136±10
p-CPA 150mg/kg + 5-HTP 50mg/kg	1365±72
p-CPA 400mg/kg + 5-HTP 50mg/kg	1286±60

(ii) AMINE DEPLETION

Drugs Administered	Brain 5-HT Levels (ng/g)
Saline Control	630±25
Reserpine 5mg/kg	136±12
Reserpine 5mg/kg + 5-HTP 50mg/kg	596±25
Reserpine 5mg/kg + l-tryptophan 100mg/kg	273±12
Reserpine 5mg/kg + l-tryptophan 400mg/kg	305±14
Reserpine + Chlorgyline + 5-HTP 50mg/kg	1382±74
Reserpine + Chlorgyline + l-tryptophan 100mg/kg	538±12
Reserpine + Chlorgyline + l-tryptophan 400mg/kg	575±20

The dose of reserpine was 5mg/kg and chlorgyline 50mg/kg in the last three entries of the table. All drugs were administered by i.p. injection. The number of animals in each group was six.

The animals that underwent biochemical analysis for brain 5-HT were from the same weaning groups as those that underwent the startle response experiment, and thus from weaning all animals in this series of experiments were housed under similar conditions. All the animals experienced the same experimental conditions except that the animals for biochemical analysis were removed from the startle box after experiencing 15 minutes adaptation time, and sacrificed by cervical dislocation immediately. These animals did not receive any startle stimuli.

(G) Discussion

Conflicting reports exist on the effect of p-CPA on behaviour, in particular, its effect on the startle response and locomotor activity. p-CPA has been shown to increase locomotory activity (Pirch 1969; Kulkarni et al 1974; Jacobs et al 1975), to have no effect on locomotory activity (Modigh and Svensson 1972), and even to reduce locomotor activity (Estler 1973). It may be that difference dose levels are responsible for such a divergence in results, as p-CPA has also been found to interfere with catecholamine synthesis at higher dose levels (Aprison and Mintgen 1972). This explanation would appear to gain credence by the fact that 5-HTP, the immediate metabolic precursor of 5-HT, has been shown to reverse p-CPA induced increases in locomotor activity (Geller and Blum 1970; Fibiger and Campbell 1971). Marsden and Curzon (1975, 1976), have shown that reductions in brain 5-HT levels, brought about by doses of p-CPA which partially inhibit tryptophan hydroxylase, increase locomotor activity, and that the administration of the more distant metabolic precursor to 5-HT, l-tryptophan, can reverse this increase in activity.

p-CPA has also been shown to produce conflicting effects on the startle response and its rate of habituation, which may reflect differences in the measurement, the intensity of the stimulus and the species of animal used. Fechter (1974a), reported p-CPA to cause no alteration of the prepulse inhibition, amplitude of startle reaction or rate of habituation. The ineffectiveness of p-CPA on the startle response

and habituation had previously been reported by Aghajanian and Sheard (1968). Connor et al (1970b), reported a transitory increase in startle response magnitude and retardation of the course of habituation after p-CPA, the latter finding being confirmed by Carlton and Advokat (1973).

The present investigation found that doses of p-CPA which partially inhibited 5-HT synthesis, increased startle response magnitude to a lesser extent than those which were found to inhibit totally 5-HT synthesis. 5-HTP was found to reverse the action of p-CPA on the startle response. Its effectiveness in increasing 5-HT levels and reducing startle response magnitude was greater after the partially inhibiting doses of p-CPA on 5-HT synthesis. This was presumably due to the fact that 5-HT was still able to be synthesised from endogenous sources of tryptophan, a situation unable to occur with increased doses of p-CPA, which totally inhibit tryptophan hydroxylase. At least part of the inhibitory effect of 5-HTP on the startle response may be due to its decarboxylation in catecholaminergic neurones (Fuxe et al 1971; Sutcher et al 1972).

Despite reporting considerably reduced brain 5-HT, noradrenaline and dopamine levels after reserpine, Fechter (1974a), found an increase in startle response magnitude. Such a result does not concur with the results of the present study, which found reserpine to effect a significant reduction in startle response magnitude. This may be explained partially by the fact that Fechter administered reserpine some six or seven

hours before the start of the startle response experiments, whereas the time period in the present investigation was 18 hours. Reserpine is thought to deplete biogenic amines by disrupting the storage of the amines from their various storage granules (Glowinski et al 1966). Maximum depletion of brain 5-HT from storage has been shown to occur after six hours with reserpine levels only rising slightly after 18 hours. Sedation, a characteristic feature of reserpine treatment, has been shown to be due entirely to this release of 5-HT from storage sites (Brodie et al 1966). The increase in startle response reported by Fechter (1974a), after reserpine may be due to the disruption of noradrenaline storage. Glowinski et al (1966), showed maximum noradrenaline release from storage to occur up to eight hours after the time of injection. By 18 hours the release had fallen to a minimum. Thus the facilitatory effect of noradrenaline on the startle response (see introduction) would be at a minimum 18 hours after reserpine injection, whereas a converse situation would exist 6 hours after injection.

In reserpinised animals, a dose level of 50mg/kg 5-HTP was found to increase brain 5-HT levels considerably more than doses of 100mg/kg and 400mg/kg of l-tryptophan, although each increased brain 5-HT levels significantly. Such differences in brain 5-HT levels were not, however, reflected in changes in startle response magnitude, where 5-HTP (50mg/kg) produced a 32% reduction, l-tryptophan (100mg/kg) a 31% reduction and l-tryptophan (400mg/kg) a 34% reduction, in comparison to the control values.

It is interesting to compare the effect of l-tryptophan on startle response magnitude in reserpinised and non-reserpinised animals. In the previous chapter, 100mg/kg l-tryptophan was seen to have little effect on the startle response, whereas 400mg/kg produced a significant increase in startle response magnitude. In the reserpinised animal, both doses were found to decrease the startle response magnitude significantly. Thus, if the tryptamine hypothesis of behavioural arousal is correct, such increases in startle response megnitude will only occur when brain 5-HT levels reach a maximum. When intraneuronal 5-HT levels become so high, end-product inhibition occurs (Macon et al 1971; Hamon et al 1973) thereby increasing the tryptophan to be metabolised to tryptamine. This situation does not apply in the reserpinised animal because 5-HT synthesis is still not at a maximum even after a dose of 400mg/kg l-tryptophan, and as such, reductions in startle response magnitude are recorded.

However, when l-tryptophan was administered to reserpinised animals after pre-treatment with the MAO inhibitor chlorgyline, startle response magnitude was found to be increased significantly, although only at a dose of 400mg/kg. 5-HTP was also found to produce a significant increase in startle response magnitude under similar circumstances, at a dose of 50mg/kg. In both cases, the characteristic hyperactivity syndrome was present.

It has been suggested that raised 5-HT levels associated with increased post-synaptic 5-HT receptor

activity are the cause of such a syndrome, despite considerable evidence to the contrary which suggests that 5-HT may have a depressant effect on behaviour. Although increased accumulation seems an essential feature of this syndrome, it is possible that another metabolite of tryptophan or 5-HT could be involved acting either directly on 5-HT receptors or indirectly by increasing the amount of 5-HT available to the receptors. Squires (1975), suggested that the syndrome could depend upon the synthesis of an N-substituted derivative of 5-HT, although there is no direct evidence for the synthesis of such derivatives when l-tryptophan is given to MAOI pre-treated rats (Marsden 1978).

In the rat, brain tryptamine levels have been found to be markedly increased when MAO was inhibited with a more striking increase occuring when l-tryptophan was given to MAOI pre-treated animals. In the latter case, brain tryptamine levels were reported to have undergone a proportionally greater increase than brain 5-HT levels, (Marsden and Curzon 1974; Tabakoff et al 1977). Administered alone, l-tryptophan has been shown to produce only a slight increase in brain tryptamine levels (Saavedra and Axelrod 1973; Marsden and Curzon 1974). However, the administration of tryptamine to rats pre-treated with an MAOI was reported to produce hyperactivity similar to that seen on giving l-tryptophan to similarly pre-treated rats (Foldes and Costa 1975). Marsden and Curzon (1977, 1978), have suggested that peripherally synthesised tryptamine may also enhance the syndrome, as tryptamine is synthesised both inside and

outside the brain, and unlike 5-HT, is able to cross the blood/brain barrier (Green and Sawyer 1960).

From this data, Marsden (1978), has surmised that tryptamine may act either through the release of 5-HT or by direct action on 5-HT receptors. As tryptophan uptake is not specific to 5-HT neurones and aromatic amino acid decarboxylase is also present in catecholaminergic neurones, Marsden has further suggested that the effects of tryptamine may not be solely mediated by its effect on 5-HT neuronal systems, but may also involve dopaminergic neurones, through the release of dopamine.

In the present study, when l-tryptophan was administered to a reserpinised animal pre-treated with the MAOI chlorgyline, the startle response magnitude was seen to be increased. In these circumstances, intraneuronal levels of 5-HT will build up more rapidly than in the animals not given the MAOI. End-product inhibition of 5-HT synthesis thus occurs more rapidly, resulting in more of the l-tryptophan being converted to tryptamine. As the MAOI inhibited tryptamine catabolism, the effect on the hyperactivity syndrome will be enhanced.

More difficult to explain is the ability of 5-HTP to increase the startle response magnitude to a greater extent than l-tryptophan, in reserpinised animals pre-treated with an MAOI. As 5-HTP can only be decarboxylated to 5-HT, the only tryptamine produced would be from endogenous stores of tryptophan. Thus in this case much less of the substrate from tryptamine formation will be present. It may be that the suggestion of Squires (1975), of an N-methylated metabolite of 5-HT being responsible

for the induction of the syndrome is true in this case, although there is little evidence in support of such a hypothesis.

CHAPTER NINE

THE EFFECT OF ORAL CONTRACEPTIVES ON TRYPTOPHAN METABOLISM, CORTISOL LEVELS, MOOD AND BEHAVIOUR.

(A) Introduction

This study was an attempt to compare quantitative changes in tryptophan metabolism with alterations in mood and behaviour in women taking oral contraceptives.

The women studies numbered fifteen in total, all of whom were second year Pharmacy undergraduates at the University of Aston in Birmingham. Approval of the Human Sciences Ethical Committee of the University was obtained before commencing the study. The voluntary nature of participation was emphasised to the students, and they were informed that no advantage or disadvantage, in academic terms, would occur as a result of their volunteering. The volunteers were then informed, in writing, of the procedures involved and of the conifdentiality of the study, and their written consent obtained.

The subjects were aged between 19 - 21 years. Seven of the women were taking a variety of oral contraceptives (see Table 6), and, although the duration of use was not established, it is unlikely in view of age that the duration of use could have exceeded five years.

The most commonly used oral contraceptive pill is a combination of an oestrogen and a progestogen. The two oestrogens most widely used are ethinyloestradiol and its 3-methyl ether, mestranol. The synthetic progestogens are related to either 19-nortestosterone or 17-α-hydroxyprogesterone, although modifications have been made to each of these compounds. The 19-norsteroids (including norethisterone and norgestrel) have been shown

to be partially metabolised to oestrogenic compounds (Brown and Blair 1960; Paulsen 1965), although only norethynodrel and ethynodiol diacetate demonstrate oestrogenic activity of clinical importance. The 17-hydroxyprogestogens have been found to be devoid of oestrogenic and androgenic effects, but like the nortestosterone group, they oppose the effect of oestrogen at a cellular level.

The combined contraceptive is usually taken as a daily tablet for 21 consecutive days, starting, where possible, on the fifth day of the menstrual cycle. On completion of the twenty-one day course of tablets, medication is stopped for seven days, and, whether or not menstruation has occured, is started again after this interval. Normally menstruation occurs two or three days after the last "pill".

In those women not undertaking oral contraceptive medication, the length of the menstrual cycles varied between 26-36 days. Generally, the menstrual cycle can be divided into five arbitrary phases. The first is the period of menstrual flow, following which is the follicular phase; ovulation; the post-ovulatory phase; and, finally, the pre-menstrual phase. In the standard 28 day menstrual cycle, the menses last from days 1-5, the follicular phase from days 6-13, ovulation then occurs between days 14-16, followed by the post-ovulatory stage from days 17-21, and finally the pre-menstrual stage from days 22-28. The period following ovulation is generally termed the luteal phase of the cycle. Any alteration in menstrual cycle length is reflected in alterations of the

length of the follicular phase (Van de Wiele et al 1970). In this study the stage of cycle was verified by the measurement of basal body temperature, which established the time of ovulation. The expected date of onset of the next menses had veen verified at the initial interview along with the age of the subject and the usual length of cycle. Having established the date of ovulation, by the method of basal body temperature measurement, the arbitrary divisions of the menstrual cycle were reassessed, in the knowledge that any increase in cycle length is reflected as an increase in the length of the follicular phase. Thus, in the instance of a 33 day menstrual cycle, menstruation would occur from day 1-5, the follicular phase from day 6-18 (an increase of 5 days over the follicular phase of a 28 day cycle), with ovulation occuring between days 18-20.

Although the menstrual cycle is suppressed by the administration of the gonadal hormones in the oral contraceptive, the terminology from the various stages of the cycle was retained in the figures as a point of reference between the two sets of results.

(i) Basal Body Temperature

In order to assess whether ovulation occured in the subjects under study, each was asked to record basal body temperature daily. It was suggested that the reading was taken immediately prior to rising in the mornings in order to obtain such readings under similar daily conditions, and for it to be truly basal.

Basal body temperature is thought to fall on the

day of ovulation from its level in the follicular phase, and then rise to a maximum in the luteal phase.

(ii) Biochemistry

Blood samples were taken from each subject on either four or five occasions during a single menstrual or contraceptive cycle between 9:00 - 9:30a.m. It was hoped to have one sample from each subject for each stage of the cycle, but this proved impossible in a few cases, due to their personal and educational commitments. A minimum of four samples was taken from each subject.

From the samples obtained, total plasma tryptophan, concentration of the free plasma tryptophan and plasma cortisol levels were estimated using the biochemical procedures described in Methods.

(iii) Behaviour

Farris (1958), reported a rise in locomotor activity in women at the time of ovulation. Similar results have been obtained with animals, both in this research and others. Thus, it was decided to monitor the effect of gonadal steroids, in the form of the oral contraceptive, on locomotor activity in human females. To this end, each subject was fitted with a pedometer, to measure distance travelled daily, during working hours. The group not receiving oral contraceptive medication acted as a control group, and from this group it was possible to assess whether peak activity coincided with the time of ovulation.

(iv) Mood

(a) Multiple Affect Adjective Check List

Affect has been defined as the "psychological aspects of emotion, or the emotional response which is assessed by means of verbal reports" (Zuckerman and Lubin 1965). There has been considerable study of emotional change as reflected by physiological measures. However, most psychological measures have been constructed to assess affect as a "trait rather than a state", trait being an inbuilt facet of character, and state, a time-variable change in mood. Zuckerman and Lubin (1965), have suggested "that such questionnaires phrase their items in terms which ask the subject how he "generally" or "occasionally" feels, rather than specifying the time referent e.g. "today" or "now". These tests have often shown decreases in mean scores as the subjects have become adapted to the test items. Furthermore, many of these tests have become so time consuming that the subject's mood may have changed before the completion of the test. Because of the lack of adequate instruments for measuring changes in affect, it has been suggested that psychologists have frequently resorted to "poorly standardised and ad hoc self-rating scales" (Zuckerman and Lubin 1965).

The Multiple Affect Adjective Check List (MAACL), was designed by Zuckerman and Lubin to fill the need for a self-administered test which would provide valid measures of the three clinically relevant negative affects: anxiety, depression and hostility. They also state that

the MAACL was not designed in an attempt to measure positive affects, but that some evidence indicates that the scales are bipolar, and that low scores on the full scale indicate states if positive affect. Two other advantages of the MAACL are that it is brief, and seldom requires more than five minutes to administer.

Table 4 shows the form in which the MAACL was presented to the subjects. It consisted of three columns of adjectives. The "response alternatives" were to make a check by ringing a particular adjective, or to make no check, by not ringing the adjective. The adjective list was preceeded by the instruction "... how you feel today". Factor analysis of the MAACL (Zuckerman and Lubin 1965), has shown three main factors: depression, hostility and anxiety, and the adjectives to be classified, according to their maximum loading on the scale, as positive or negative. To obtain a score from the completed MAACL's, a system of keyed words (Zuckerman and Lubin 1965), was used, as shown in Table 5.

To obtain the anxiety score, the anxiety key was used. The total anxiety score was the number of positive items checked plus the number of negative items not checked. The hostility and depression scores were obtained similarly using the relevant hostility and depression keys. Having obtained the score, the greater the score, the greater the incidence of the particular negative affect.

(b) Visual Analogue Scale

On finishing the MAACL, the subjects were then asked to complete a visual analogue self-rating scale.

TABLE 4

Form in which MAACL was presented to Subjects:

unhappy
sympathetic
fearful
willful
loving
contented
low
cheerful
good
terrified
agreeable
lost
understanding
calm
rejected
amiable
upset
glad
bitter
mad
inspired
discontented
tame
active
kindly
whole
desperate
cooperative
safe
tense
strong
merry
secure
devoted

outraged
lucky
sad
disagreeable
discouraged
blue
warm
angry
lonely
gay
steady
stormy
joyful
miserable
hopeless
tormented
happy
cruel
alive
awful
fit
offended
irritated
healthy
furious
clean
destroyed
panicky
thoughtful
enthusiastic
sunk
fine
worrying
polite
disgusted
peaceful
forlorn
nervous
friendly
alone

good-natured
mean
young
pleasant
suffering
wilted
unsociable
terrible
frightened
gloomy
tender
shaky
afraid
vexed
interested
free
enraged

TABLE 5

Keyed Words for MAACL

Anxiety		Depression		Hostility	
Plus	Minus	Plus	Minus	Plus	Minus
afraid	calm	alone	active	angry	agreeable
desperate	cheerful	awful	alive	bitter	amiable
fearful	contented	blue	clean	cruel	cooperative
frightened	happy	destroyed	enthusiastic	disagreeable	friendly
nervous	joyful	discouraged	fine	discontented	good-natured
panicky	loving	forlorn	fit	disgusted	kindly
shaky	pleasant	gloomy	free	enraged	polite
tense	secure	hopeless	gay	furious	sympathetic
terrified	steady	lonely	glad	irritated	tame
upset	thoughtful	lost	good	mad	tender
worrying		low	healthy	mean	understanding
		miserable	inspired	offended	willful
		rejected	interested	outraged	(devoted)
		sad	lucky	stormy	(warm)
		suffering	merry	unsociable	
		sunk	peaceful	vexed	
		terrible	safe		
		tormented	strong		
		unhappy	whole		
		wilted	young		

TABLE 6

Product Name	Manufacturers	Constituents		No of Volunteers
Eugynon 30	Schering	Ethinyloestradiol 30mcg	Levonorgestrel 250mcg	3
Microgynon 30	Schering	Ethinyloestradiol 30mcg	Levonorgestrel 150mcg	2
Ovranette	Wyeth	Ethinyloestradiol 30mcg	Levonorgestrel 150mcg	1
Norinyl-1	Syntex	Mestranol 50mcg	Norethisterone 1mg	1

TABLE 6: The constituents of the oral contraceptives used in Chapter 9.

This consisted of a straight horizontal line, 10cm in length, with the words "I feel as low as I ever have" to the left of the line, and "I feel on top of the world" at the opposite end. The subjects were asked to place a cross somewhere along the line to correspond with the mood they felt at that particular time, in relation to the two statements. Scores were obtained by measuring the distance from the left hand end of the line.

The two scales were completed by each subject on the days of blood sampling, and on at least one other day for each stage of the cycles.

(B) Results

(i) Basal Body Temperature

The pattern in the women not taking oral contraceptive medication was found to adhere to the classical temperature fluctuation of the menstrual cycle. The minimum temperature was found to coincide with the expected time of ovulation, after which the temperature rose to maximum levels during the luteal phase. Such a pattern was obtained from each of the menstrual cycle group, suggesting that ovulation occured in each of the women in this group.

In the group taking the oral contraceptives, little temperature fluctuation was seen to occur. There was, however, a slight reduction in temperature at the mid point of the cycle which was recorded in every case. As no subsequent rise in temperature occured after mid-cycle, it would seem that ovulation did not occur in any of the subjects in this group.

(ii) Biochemistry

(a) Cortisol

The women taking oral contraceptives were found to have higher plasma cortisol levels throughout the cycle, than those women with an unaltered menstrual cycle (Fig 43). The difference was found to be at a maximum during the premenstrual stage, when cortisol plasma levels were found to be significantly greater ($P<0.02$) in the women undergoing oral contraceptive medication.

Cortisol levels altered only slightly over the menstrual cycle, being at a minimum (11.6 ± 1.16 µg/100ml) during the menses, rising to a maximum in the follicular phase (13.6 ± 2.1) and during ovulation (13.6 ± 1.6), then falling through the post-ovulatory phase (12.1 ± 2.6) and premenstrual phase (12.0 ± 2.6), to the minimum at menstruation.

In the women taking the oral contraceptive, cortisol levels were found to remain steady during menstruation (18.3 ± 5.5 µg/100ml) and the first week of medication (18.3 ± 3.1), rising slightly at the mid point of the cycle (19.1 ± 2.4), then falling to a minimum during the second week of medication (17.6 ± 2.4). Maximum cortisol levels in the plasma were reached in the third, pre-menstrual week of medication (24.4 ± 2.3).

(b) Total Plasma Tryptophan

See Figure 44.

Total plasma tryptophan was found to vary considerably when the menstrual cycle pattern was compared to that produced by oral contraceptives.

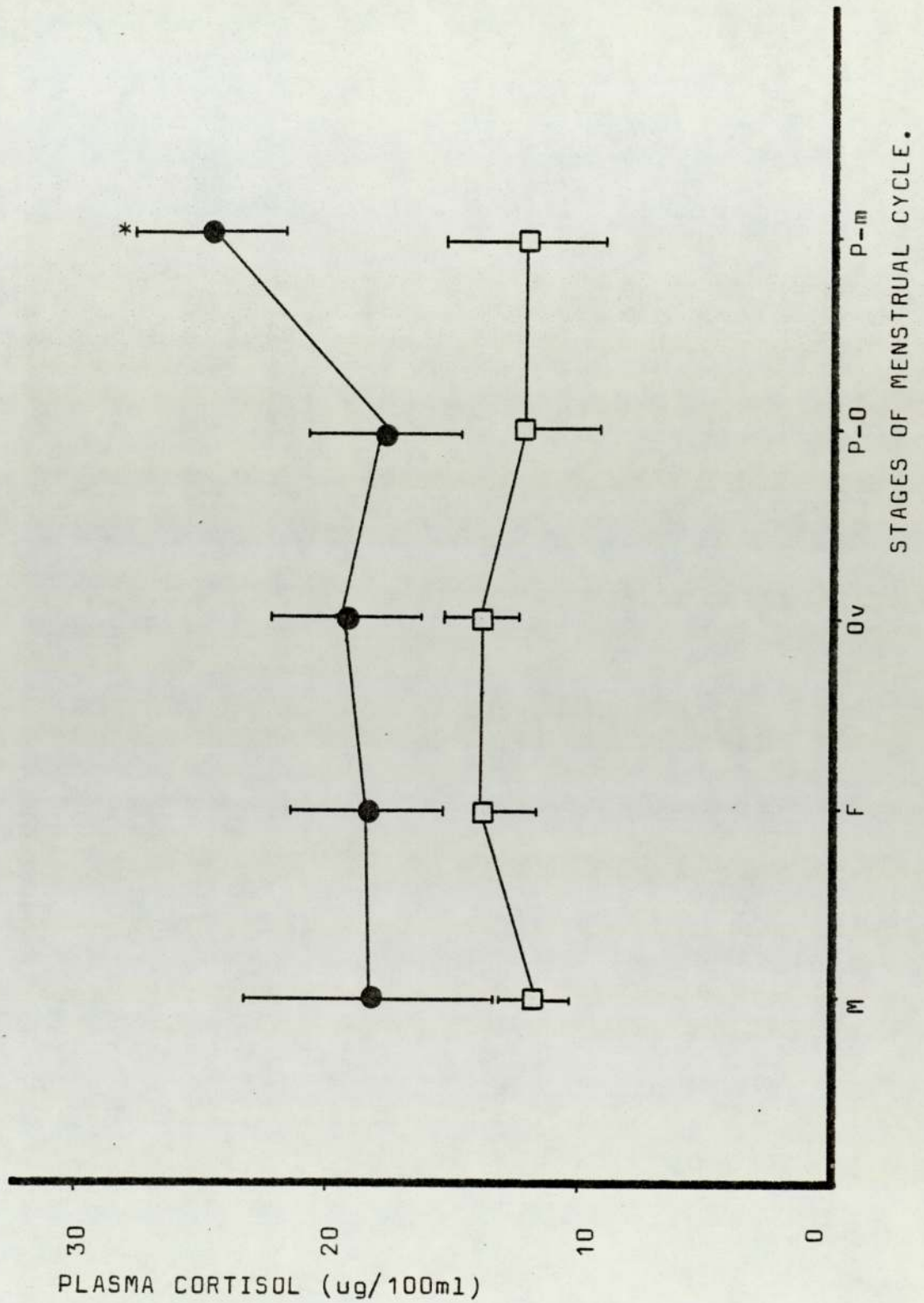


FIG.43: Plasma cortisol in women taking oral contraceptives and in women with normal menstrual cycles.
Stages of cycles: M-menses; F-follicular; Ov-ovulation; P-O -post ovulatory; P-M -premenstrual.

Menstrual cycle.

Oral contraception.

* $P<0.05$.

During the mestrual cycle, total plasma tryptophan was found to be at a minimum during menstruation (9·48±0·81 µg/ml), rising during the follicular phase (10·7±0·8) to reach a maximum during the ovulatory (13·5±1·6) and post-ovulatory stages (13·5±1·2). It was then seen to decline during the premenstrual stage to 11·8±0·6µg/ml.

The pattern of total plasma tryptophan in the oral contraceptive group was almost the reverse of that found in the menstrual cycle group. During the menses and for the first few days of medication, total tryptophan levels were similar (menses 11·5±1·2; initial medication 11·7±1·1), both being greater than those of the menstrual and follicular phases of the menstrual cycle, the difference not being statistically significant. In mid-cycle, levels were seen to fall (10·5±2·3), and continued to fall into the second week of medication (10·3±1·0). The total plasma tryptophan level in this second week of contraceptive medication was found to be significantly less than the level in the corresponding, post-ovulatory phase of the menstrual cycle ($P < 0·05$) after analysis using a 1-tail t-test. Doubt might be cast on the validity of such a statistical analysis in these circumstances, as no previous reports exist suggesting that total plasma tryptophan should rise in this period of the menstrual cycle and fail to rise in the equivalent period of the contraceptive cycle. Analysis of the data by paired t-test, showed no significant differences. Maximum levels were reached in the third, pre-menstrual week of medication (12·4±2·1). Although total

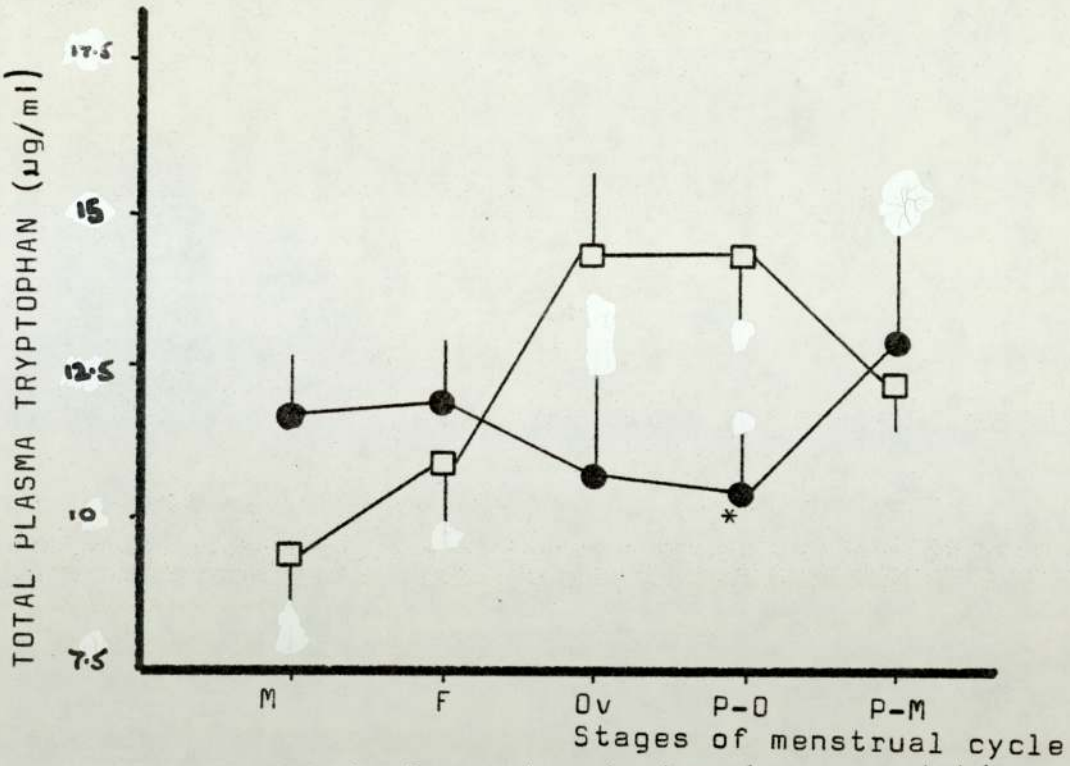


FIG.44: Total plasma tryptophan in women taking oral contraceptives & in women with normal menstrual cycles. (Stages of cycles-see Fig 43)

* P<0·05

Menstrual cycle.

Oral contraception.

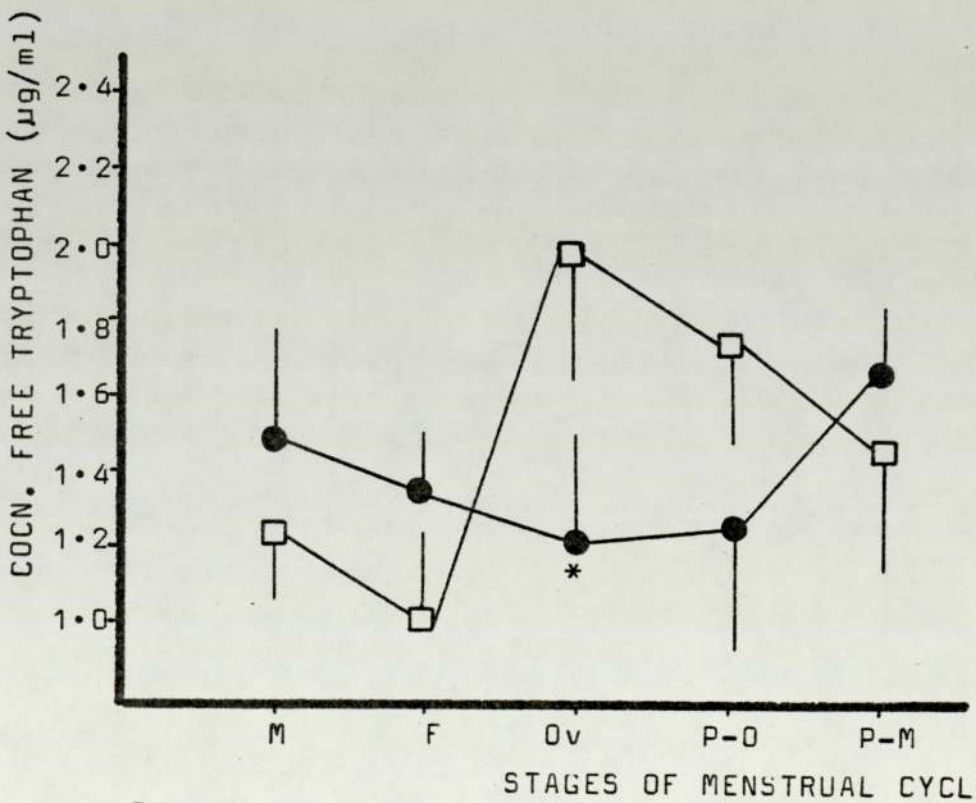


FIG.45: Concentration of free tryptophan in the plasma of women taking oral contraceptives & in women with normal menstrual cycles.

(Stages of cycles: see Fig 43) * P<0·05

Menstrual cycle

Oral contraception

tryptophan was found to be a maximum during this pre-menstrual stage of the contraceptive cycle, numerically the level was only 5% above the levels obtained during the premenstrual stage of the menstrual cycle.

(c) Absolute Free Plasma Tryptophan

See Figure 45.

As in the case of total plasma tryptophan, free plasma tryptophan levels were found to vary considerably, widely differing patterns being exhibited by the two cycles.

In the menstrual cycle group, levels were found to be low during the menses (1·24±0·27 μg/ml), falling slightly during the follicular phase (1·04±0·15), then rising to a maximum at ovulation. At ovulation the level of 2·01±0·34 ug/ml was found to be significantly greater ($P<0·05$) than the level during the follicular phase. Levels were seen to decline during both the post-ovulatory (1·79±0·26) and premenstrual (1·46±0·17) stages.

In the group taking oral contraceptives, free plasma tryptophan was found to be 1·51±0·18 μg/ml during menstruation - a level higher, although not significantly so, than that obtained during menstruation in the menstrual cycle group. As medication was resumed, levels fell to 1·38±0·24 μg/ml, reaching a miminum at mid-cycle (1·22±0·29). At the mid-cycle the free tryptophan concentration in the plasma was seen to be significantly below that obtained at ovulation in menstrual cycle, after a 1-tailed t-test. In this case it would seem valid to use such analysis as the concentration of free

tryptophan has been shown to be positively correlated with plasma oestrogen concentrations (Thomson et al 1977). At ovulation plasma oestrogens have been shown to be raised (Collins and Newton 1974), and during combined oral contraceptive medication plasma oestradiol levels have been shown to be reduced (Mishell et al 1972). During the second week of medication plasma free tryptophan was seen to rise (1·28±0·32), and to reach a maximum during the third, premenstrual week of medication at 1·69±0·32 μg/ml.

Percent free tryptophan in the plasma varied from 10·4 to 14·9% over the menstrual cycle, being at a minimum during menstruation and a maximum at ovulation. In the oral contraceptive group, the variation was between 11·4 and 13·6%, being at a maximum during the third week of medication and menstruation, and varying between 11·4 and 11·8% during the first two weeks of medication.

(iii) Behaviour

Figure 46, shows the fluctuation in locomotor activity in both the menstrual and contraceptive cycle groups.

In the menstrual cycle group, the results obtained using pedometers, were somewhat inconclusive. Although the peak value of 5·095±0·71 miles per day was recorded at the time of ovulation, the minimum value recorded during the post-ovulatory phase of the cycle was only 13·5% below this. During menstruation, locomotor activity was found to be 4·815±0·43 miles per day.

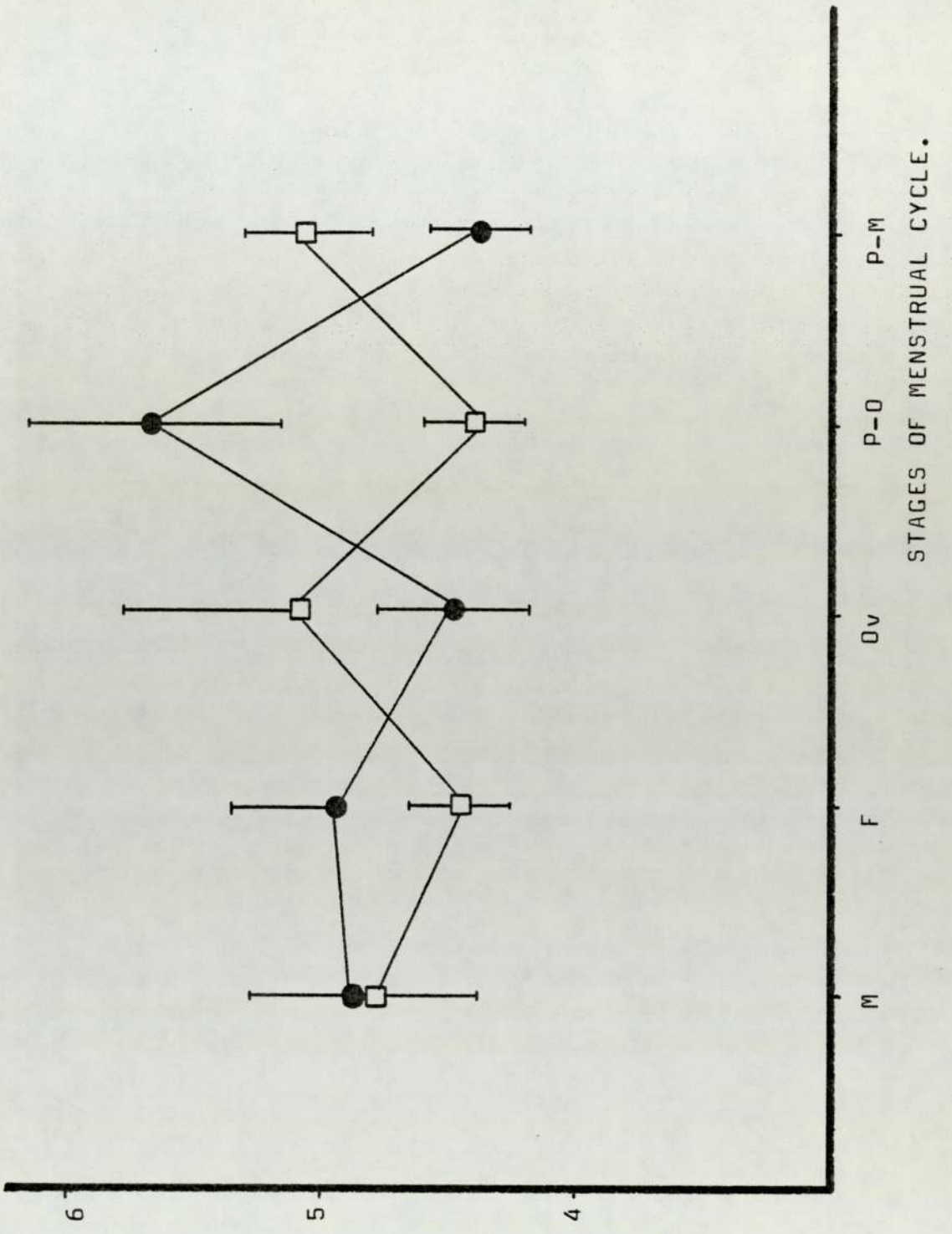


FIG.46: Pedometer readings (daily activity) in women taking oral contraceptives & in women with normal menstrual cycles. (Stages of cycles: see Fig 43)

- Menstrual cycle.
- Oral contraception.

Activity then fell slightly during the follicular phase (4·61±0·24) rising to a maximum at ovulation. The minimum locomotor activity was recorded during the post-ovulatory phase (4·457±0·29), activity then rising during the premenstrual phase to 5·044±0·34 miles per day.

In the oral contraceptive group, no peak in locomotor activity was seen at mid-cycle. Levels of activity were much the same as in the menstrual cycle group, ranging from 4·374 to 5·65 miles daily. During menstruation locomotor activity was recorded as 4·853±0·44 miles per day. Little change was noted after the first few days of medication (4·91±0·44). By the mid-cycle activity had declined to 4·63±0·42 miles per day. Maximum activity levels were recorded during the second week of medication (5·65±0·49) while minimum activity levels were recorded during the third, premenstrual week of medication (4·374±0·26).

No statistically significant differences were found between any of the recorded values.

(iv) Mood

The principal aim of this study was to associate biochemical changes produced by the oral contraceptive with the possible onset of depression in those subjects on such medication. Thus, in this section, emphasis has been placed on changes in mood measured using the MAACL depression 'D' Score and the V.A.S.

With the MAACL 'D' score, increased numerical values indicated increased incidence of depression. From Figure 47, it can be seen that the scores in the

two groups varied considerably.

In the menstrual cycle group the 'D' score was found to be 18±1 during the premenstrual phase, during menstruation and during the follicular phase. Mood was seen to improve at ovulation when the 'D' score was found to reach a minimum of 16±2, increasing to 17±1 in the post-ovulatory phase.

In the oral contraceptive group, the 'D' score was generally higher throughout the test period. It can be seen that the mean 'D' score for the menstrual cycle group varied between 16-18, whereas the variation in the oral contraceptive group's scores was between 18-22. In the initial week of contraceptive medication, the 'D' Score was 18±1, which remained the same at the mid point of the cycle. By the second week of medication the 'D' Score was found to have increased to 19±1. A further increase to reach a maximum of 22±1 was found to have occured by the third, premenstrual week of medication. This value was significantly above the corresponding premenstrual value from the menstrual cycle group (P 0·001). A 'D' score of 22 and over has been suggested as being equivalent to a clinically significant depression of mood, as assessed in a psychiatric interview (Handley - personal communication). During menstruation, the 'D' score of the contraceptive group was seen to fall to 20±1.

The maximum 'D' score in the contraceptive group, found to occur in the third, premenstrual week of medication, coincided with maximum 'H' and 'A' scores. It should be noted that these scores were only slightly

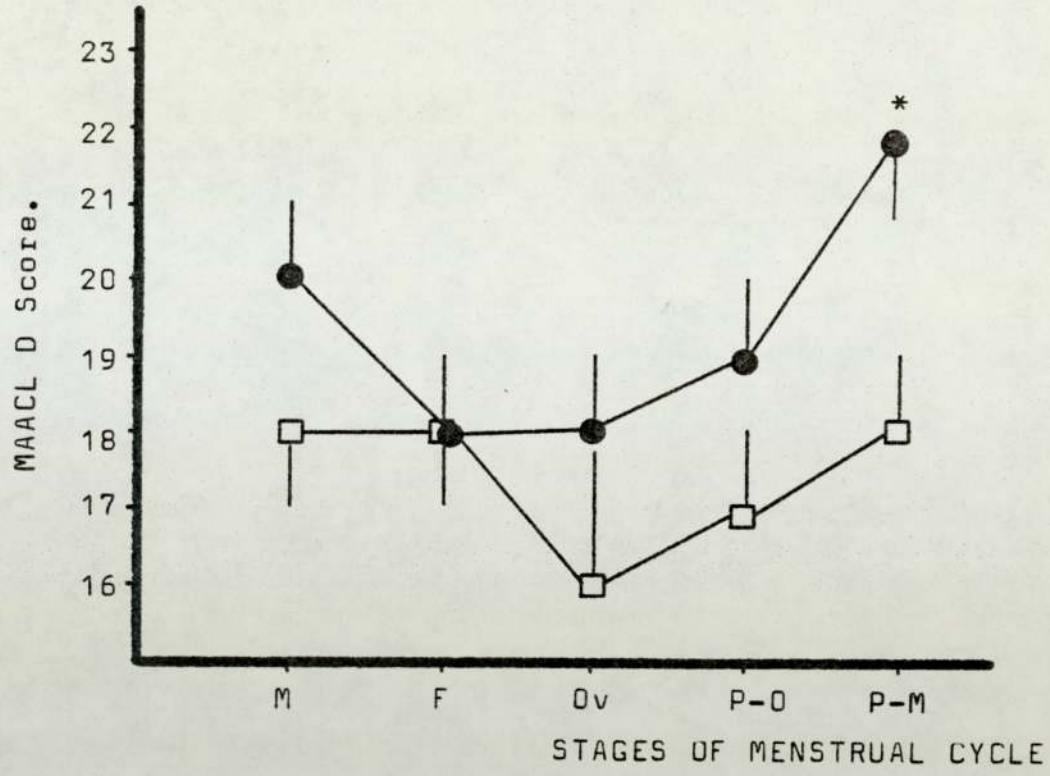


FIG.47: MAACL D score in women taking oral contraceptives & in women with normal menstrual cycles. (Stages of cycles: see Fig 43) * $P<0.05$.

Menstrual cycle.
Oral contraception.

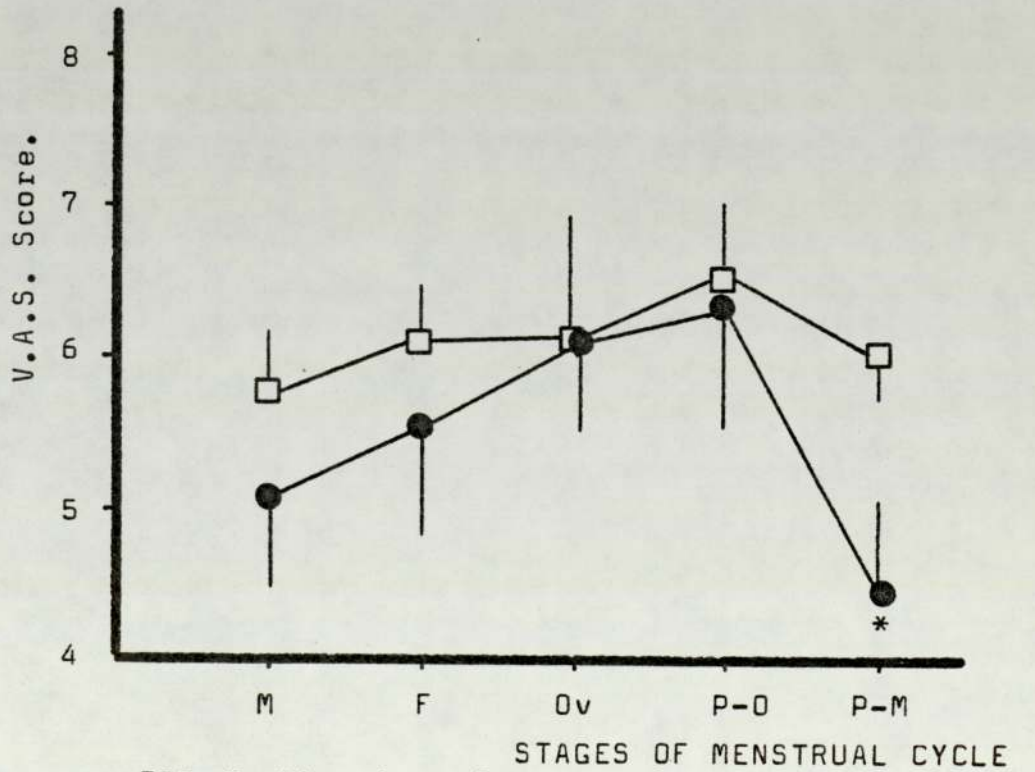


FIG.48: Visual analogue scale score in women taking oral contraceptives &in women with normal menstrual cycles. (Stages of cycles: see Fig 43) *$P<0.05$.

Menstrual cycle.
Oral contraception.

above the scores obtained for the other phases of the contraceptive cycle. In the menstrual cycle group, the differences in scores were again found to be only slight with the maximum 'A' score occuring during menstruation, and minimum 'A' score at ovulation. The 'H' score remained static throughout the cycle except for a minimal rise at ovulation.

With the visual analogue scale (V.A.S.) reduced numerical values represented a decline in mood. For the menstrual cycle group the V.A.S. score varied between 5·7-6·5, whereas the variation was found to be between 4·5-6·4 in the group undergoing contraceptive medication. Figure 48 shows the changes in V.A.S. scores over the two cycles.

In the menstrual cycle group, the V.A.S. score was found to be 5·7±0·4 during menstruation; mood increased as the cycle progressed, the score increasing to 6·1±0·4 in the follicular phase, at which it remained at ovulation (6·1±0·8). The maximum was reached in the post-ovulatory phase (6·5±0·5), after which it was found to decline in the premenstrual phase to 5·9±0·3.

In the group undergoing contraceptive medication the V.A.S. score was found to be 5·1±0·7 during menstruation which increased to 5·5±0·8 during the first week of medication. At the mid-point of the cycle, the score was found to have increased to 6·1±0·6, reaching a maximum of 6·4±0·8 during the second week of medication. In the third, premenstrual week, the V.A.S. score was found to have reached a minimum of 4·5±0·6 which was significantly below the premenstrual V.A.S.

score from the menstrual cycle group ($P<0.05$).

In summary, the oral contraceptive group was found tohave generally higher 'D' scores throughout the four week cycle, with a significantly higher score being recorded during the premenstrual phase. With the V.A.S. the oral contraceptive group had generally lower values over the same period, with a significantly lower value being recorded during the premenstrual phase.

(C) Discussion

The subjects undergoing oral contraceptive medication were seen to have consistently higher plasma cortisol levels throughout the complete cycle, which in the premenstrual phase were found to be significantly greater than plasma cortisol levels in the equivalent phase of the menstrual cycle. The effects produced on cortisol metabolism by the administration of oral contraceptives relate largely to oestrogen component, for the administration of progestogens alone has not been shown to have any consistent effect. The oestrogen component appears to increase total plasma cortisol levels without increasing the urinary excretion of cortisol metabolites (Robertson et al 1959; Peterson et al 1960). This is explained by the increase in cortisol binding to plasma proteins which occurs following oestrogen administration (Sandberg and Slaunwhite 1959; Sandberg et al 1960). A concomitant increase in the plasma concentration of corticosteroid-binding globulin also occurs (Doughaday et al 1962 ; Doe et al 1964; DeMoor et al 1966), which is apparently due to an increased hepatic

synthesis of CBG in parallel with the increase in other specific carrier proteins induced by oestrogens (Musa et al 1967). It seems likely that non-protein bound as well as protein bound and total cortisol levels are all increased (Burke 1970).

The subjects undergoing oral contraceptive medication were also seen to be consistently more depressed than their menstrual cycle counterparts, and depression was found to be significantly greater during the premenstrual phase of the contraceptive cycle - the time of significantly increased cortisol levels.

Sachar et al (1976), reported increased cortisol levels in a large minority of depressed patients (returning to normal on clinical recovery) which was suggested as being due to reduced brain noradrenaline levels causing a disinhibition of corticotrophin releasing hormone. In depressed menopausal women, Sachar and his co-workers implicated reduced L.H. levels with a noradrenaline deficiency. It should be noted that the primary mode of action of the combined oral contraceptive is the suppression of ovulation by the inhibition of FSH and LH releasing factors at the hypothalamic level, and the consequent reduction in LH and FSH. Recent work has shown 5-HT to have an inhibitory effect on LH release, by direct action on the neurosecretory nerve endings releasing LH-RF (Charli et al 1978), whereas noradrenaline has been shown to mediate a stimulatory feedback effect releasing LH-RF from the mediobasal hypothalamus (Kalra et al 1978).

The oestrogen component has also been suggested as

being implicated in the alteration of tryptophan metabolism. It would seem that by increasing plasma cortisol levels, oestrogens induce liver tryptophan pyrrolase (Braidman and Rose 1971). Such an induction of hepatic tryptophan pyrrolase would produce increased tryptophan metabolism via the kynurenine-niacin pathway, thereby reducing 5-HT synthesis. This is turn may lead to changes in mood, as reduced brain 5-HT levels have been suggested as a possible cause of depression (see Introduction).

This study found no significant differences in either total plasma tryptophan or free plasma tryptophan between the group receiving oral contraceptive medication and the menstrual cycle group, although the patterns of both tryptophan levels throughout the four week cycle were seen to vary.

Thomson et al (1977), suggested that a positive correlation between total plasma oestrogen and the concentration of free tryptophan in the plasma might exist. A similar pattern was seen in this study in the way that such levels varied over the menstrual cycle. Low levels of oestradiol are known to occur during menstruation, and throughout the follicular phase they rise gradually. Immediately prior to ovulation oestradiol levels have been shown to rise dramatically, falling slightly as ovulation occurs. In the post-ovulatory phase, there is another less intense, more gradual rise in plasma oestradiol which then falls premenstrually to reach a minimum during the menses (Collins and Newton 1974). In this study the concentration of free

tryptophan was seen to vary similarly.

Although total tryptophan levels in the plasma did not show such a dramatic variation over the menstrual cycle as the concentration of free trypotphan, the pattern of variation was also found to parallel the expected changes in plasma oestradiol.

In the oral contraceptive group no such pattern in tryptophan levels were seen to exist. The hormonal changes occuring in such a contraceptive cycle have been reported as an absence of the normal mid-cycle peaks of LH and FSH and the loss of the biphasic oestrogen excretion pattern together with an absent progesterone elevation in the luteal phase of the cycle (Pennington and Naik 1974).

In the oral contraceptive group, cortisol levels were found to be at a maximum during the third, pre-menstrual week of medication. However, during this phase of the contraceptive cycle, total plasma tryptophan and the concentration of free plasma tryptophan were also found to be at a maximum. This would seem to be the converse of previous reports, which suggest that raised cortisol levels induce hepatic tryptophan pyrrolase, thereby reducing tryptophan availability (Green et al 1975a; Green et al 1975b), thus affecting mood (Grant 1975; Wynn 1975).

In this study such biochemical maxima coincided with "maximum depression" as scored on the MAACL 'D' score and the V.A.S. A more recent report has suggested that although oral contraceptives may alter tryptophan metabolism, this is not caused by an increase in hepatic

tryptophan pyrrolase activity.

Handley et al (1977), found a significant positive correlation between the concentration of free plasma tryptophan and mood. However, this finding was unable to be reproduced in a larger sample. In this latter study, it was found that in women the failure of total plasma tryptophan levels to rise immediately after parturition corresponded with depressed mood in the first post-partum week. Such an occurrence may explain the elevation in mood seen in women in this study at ovulation, when free tryptophan levels in the plasma were found to be at a maximum, the 'D' score at a minimum, and the V.A.S. score within 7% of the maximum.

It has also been shown that oestradiol promotes tryptophan uptake in cells (Christensen 1975). Thus at ovulation, brain tryptophan levels would also be expected to be raised. However, high concentrations of free tryptophan in the plasma and brain tryptophan are unlikely to reflect the stage of brain 5-HT synthesis, as it has been suggested that for ovulation to occur, the inhibitory action of 5-HT must be at a minimum, relative to noradrenergic activity, although this may only apply to specific regions of the brain.

It may be that the relationship between raised plasma cortisol, reduced noradrenergic activity and depression, proposed by Sachar and his co-workers, could be thought to be a more credible explanation for the premenstrual depression seen in the oral contraceptive group.

Farris (1958), studied variations in activity in

fifteen women and six men. Men, were found to exhibit relatively constant activity, whereas women were shown to exhibit three peaks of activity which occured during menstruation, at the mid-cycle, around the expected day of ovulation and during the premenstrual period. Maximum activity was found to occur at mid-cycle which coincided with "elation and happiness" in the subjects.

Similar results were found in the menstrual cycle group in this study although the differences in activity were less marked. Farris recorded a basal activity of 6·5 miles per day with 9·6 recorded on day 4 (during menstruation), 10·1 miles on day 15 (ovulation) and 9·8 miles on day 24 (premenstrual). In this study, the difference between the maximum activity (at ovulation) and the minimum activity (during the post-ovulatory phase) was only 13% compared to 36% between the maximum and basal activities recorded by Farris.

In the oral contraceptive group, activity was found to vary little throughout the cycle, with the exception of the second week of medication when the maximum was recorded. This value proved to be the maximum recorded in either group during the study and coincided with maximum V.A.S. score and minimum levels of plasma cortisol, total plasma tryptophan, and the concentration of free tryptophan in the plasma.

While the study did not provide sufficient evidence for the involvement of altered tryptophan metabolism as a cause of depression in women undergoing oral contraceptive therapy, it may be said to lend support to the following contentions:-

A. In the menstrual cycle:

(i) The possibility of a positive correlation between plasma oestradiol levels and the concentration of free tryptophan in the plasma.

(ii) Locomotor activity reaches a peak during the time of ovulation and coincides with elevated mood.

B. In women taking oral contraceptives:

(i) Plasma cortisol levels are raised during such therapy.

(ii) There is a definite basis for the suggestion that such women suffer increased incidence of depression as a result of oral contraceptive therapy (Herzberg and Coppen 1970; Herzberg et al 1970; Adams et al 1973).

GENERAL DISCUSSION

1. The Effect of Altered 5-HT Synthesis on some Aspects of Behaviour

(a) Locomotor Activity

The administration of tryptophan, the metabolic precursor to 5-HT has previously been shown to have little effect on locomotor activity. Both Graham-Smith (1971), and Jacobs et al (1974), found it to have no effect when given systemically. From this it was hypothesised that tryptophan does not affect behaviour because, despite increasing 5-HT synthesis, little or none of the newly formed 5-HT can be stored, and thus is metabolised by intraneuronal MAO (Moir and Eccleston, 1968, Graham-Smith, 1971, Marsden and Curzon, 1976). Modigh (1973), did find very high doees of l-tryptophan (800mg/hg) to inhibit locomotor activity in mice, and as a result concluded that the inhibitory action of tryptophan on locomotor activity may be due to the amino acid itself rather than any action on 5-HT synthesis. Carlsson (1975), suggested that the inhibitory action may be caused by tryptophan blocking the transport of other essential amino acids into the neurones of the C.N.S.

The results of the present study would seem to be at variance with those of such previous investigations. Doses of 100mg/kg l-tryptophan, and above, were seen to reduce locomotor activity in a dose dependent manner. In doses of 200mg/kg and above, the reduction in total locomotor in the 2 hours following injection was found to be significant. In general, the time at which locomotor

activity was reduced to a minimum was between 35-50 minutes after injection. As Modigh carried out his experiments for 20 minutes, starting one hour after injection, it would seem that the variation in the two results may be a function of experimental timing.

A dose of 800mg/kg was found to reduce locomotor activity significantly at all times during the 2 hour period following injection, when compared with the controls, and the total activity in this 2 hour period after such a dose was found to be significantly below that of animals given l-tryptophan 400mg/kg. As 5-HT synthesis has been suggested to reach a maximum after l-tryptophan 300mg/kg (Carlsson and Lindqvist 1972), it may be that the increase in the inhibition of locomotor activity seen after l-tryptophan 800mg/kg is due to tryptophan blocking the uptake of other essential amino acids, as was suggested by Carlsson (vide supra).

The fact that tryptophan has been found to increase brain 5-HT synthesis (Carlsson and Lindqvist 1972, Gessa and Tagliamonte 1974), and, in this study, to reduce locomotor activity may lend further support for the finding that 5-HT has an inhibitory effect on locomotor activity. Both Neuburg and Thut (1974), and Warbritten et al (1978), found 5-HT to antagonise amphetamine-induced increases in locomotor activity, and Grabowska et al (1973), and Grabowska and Michaluk (1974), found that the increase in locomotor activity after apomorphine was greater in rats treated with 5-HT antagonists and in those depleted of 5-HT by the

inhibition of tryptophan hydroxylase or by mid-brain raphe lesions. Such manipulations of 5-HT synthesis have also been shown to potentiate amphetamine-induced locomotor activity (Neill et al 1972, Mabry and Campbell 1973).

(b) Startle Response

Considerable evidence exists to show that 5-HT has an inhibitory effect on the startle response. In a series of experiments, Davis and Sheard (1974a,b,c), showed that lesions of the mid-brain raphe, the area of the brain containing most of the 5-HT cell bodies, were found to increase the magnitude of the startle response. They also suggested that the enhancement of the startle response could be associated with 5-HT depletion and inhibition of the startle response with enhanced release of 5-HT (Davis and Sheard 1976).

This latter finding may explain the effect that reserpine was seen to have on the startle response. Brodie et al (1966), suggested that the sedative action of reserpine was due to the continual release of 5-HT into the synapse, thereby enhancing 5-HT release. From the suggestion of Davis and Sheard (1976), it would be expected that reserpine should reduce the startle response magnitude, a finding confirmed in the present study. The fact that the administration of tryptophan was seen to potentiate the reduction in startle response magnitude after resperine may be due to selective increases in brain 5-HT synthesis, and increased 5-HT release. 5-HT was found to have a similar effect on

startle response magnitude to tryptophan, and it may be presumed that the mechanism of effect is also similar.

Other investigators have shown that brain lesions and synthesis inhibition which produce reduced serotonin-ergic neuronal activity, will increase the magnitude of the startle response. Geyer et al (1976), found that lesions of the median raphe nuclei produced significant reductions in tryptophan hydroxylase activity in the forebrain, and increased the startle response magnitude. p-CPA, and inhibitor of tryptophan hydroxylase (Koe and Weisman 1966), has also been found to increase the startle response magnitude (Connor et al 1970b), although this has been disputed by Fechter (1974a), who found p-CPA to have no effect.

In this study p-CPA was seen to enhance the startle response, presumably by its inhibitory action on tryptophan hydroxylation and consequent reduction of 5-HT synthesis. 5-HT, being already hydroxylated, would bypass this initial hydroxylation step, and was found to reverse the action of p-CPA on the startle response and increase brain 5-HT very considerably in animals treated with p-CPA.

In both reserpinised and p-CPA treated animals the metabolic precursors of 5-HT, tryptophan and 5-HTP, were found to increase brain 5-HT levels. They were also found to exert an inhibitory effect on the startle response reflex, which was seen in the potentiation of the inhibitory effect of reserpine, and in the inhibition of the facilitatory effect of p-CPA.

2. The Possible Role of Tryptamine in the Mediation of some of the Behavioural Effects of Tryptophan

In contrast to the findings of the present study, where the administration of l-tryptophan was found to reduce locomotor activity in a dose dependent manner while putatatively increasing the rate of 5-HT synthesis, Graham-Smith (1971), had found that a low dose of l-tryptophan (100mg/kg) administered in conjunction with a MAO inhibitor induced a characteristic hyperactivity syndrome. Features of this syndrome included tremor, rigidity, hyperreactivity, stereotyped head response and large increased in locomotor activity, all of which were found to correspond with a considerable increase in the rate of 5-HT synthesis.

Jacobs et al (1974), suggested that increased 5-HT receptor activity was involved in the mediation of this syndrome, a contention which gained further support from the work of Green and Kelly (1976). Pugsley and Lippmann (1977), reported that dopamine receptor blockade could antagonise the syndrome, which lent support to a previous hypothesis proposed by Green and Graham-Smith (1974), which suggested that at some point between the post-synaptic 5-HT receptor sites initiating the production of the syndrome and the mechanism responsible for its expression lay a system of dopaminergic neurones. Depletion of dopamine in this neuronal system would thus break the neuronal sequence for the behavioural expression of 5-HT receptor site stimulation. Work by Green and co-workers

showed that both 5-HT and dopamine are normally metabolised in vivo by MAO type A (Green and Youdim 1976, Green et al 1977). However, when type A is inhibited, both can be metabolised by type B. When both forms are almost totally inhibited, the largest rise in brain 5-HT and dopamine levels are seen, and this increase in functional activity is manifested in the hyperactivity syndrome.

Recently the suggestion has been made that tryptamine, another metabolite of trypotphan, may be responsible for at least part of the syndrome. Raised brain tryptamine levels have been shown to induce certain characteristics of the hyperactivity syndrome (Marsden and Curzon 1978). Tabakoff et al (1977), also showed that tranylcypromine, the MAO inhibitor used almost exclusively in the production of this syndrome, produced an accumulation of tryptamine three times greater than when pargyline was used.

It may be that the putative stimulatory action of tryptamine can explain the biphasic effect that tryptophan was seen to exert on the startle response, during the present study. A low dose of l-tryptophan (50mg/kg) was seen to reduce the startle response magnitude significantly from the control value, but as the doses were increased towards 300mg/kg, the dose of l-tryptophan suggested as producing maximum 5-HT synthesis (Carlsson and Lindqvist 1972), the startle response was seen to approach the control value. In doses above 300mg/kg, the startle response magnitude was found to be increased above the control value, such an increase

being found to be statistically significant at a dose of 800mg/kg.

It is possible that as the dose of l-tryptophan is increased towards 300mg/kg and both 5-HT and tryptamine synthesis in the brain is increased, the putative stimulatory effect of tryptamine gradually counteracts the inhibitory effect of 5-HT, the net effect being to return the startle response magnitude to the control value. Above a dose of 300mg/kg, the rate of 5-HT synthesis should have reached a maximum, but as tryptamine synthesis may increase, this would explain the manifestation of behavioural excitation in the form of increased startle response magnitude.

Thus, in doses of 300mg/kg l-tryptophan and above, it might be expected that the excitatory effects of tryptamine overcome the inhibitory effects of 5-HT thus producing the resultant increase in startle response magnitude. It should be noted that the present study also found tryptophan to have an inhibitory effect on locomotor activity after such doses. It can only be concluded that the neurel pathways responsible for the mediation of the two types of behaviour vary in sensitivity to raised 5-HT and tryptamine levels, thus accounting for the variation in behaviour seen after increasing doses of tryptophan.

In the reserpinised animal, doses of 100mg/kg and 400mg/kg l-tryptophan both decreased the magnitude of the startle response significantly. This can be explained using the tryptamine hypothesis, since increased startle response magnitude would only occur when brain

5-HT synthesis has reached a maximum. When 5-HT synthesis reaches a maximum, intraneuronal levels are such to initiate end-product inhibition (Macon et al 1971, Hamon et al 1973), thereby increasing the amount of tryptophan available for metabolism to tryptamine. In the reserpinised animal this is unlikely to occur, since the principal action of reserpine is to disrupt amine storage, thereby increasing release and metabolism. Since end-product inhibition would not occur in the reserpinised animal doses of tryptophan would serve only to increase 5-HT synthesis and release, and thereby enhance the inhibitory effect of reserpine on the startle response reflex.

When doses of 100mg/kg and 400mg/kg of l-tryptophan were administered to reserpinised animals pre-treated with the MAO inhibitor, chlorgyline, the magnitude of the startle response was found to be increased. In these circumstances intraneuronal levels of 5-HT should build up more rapidly, under the influence of MAO inhibition, and thus end product inhibition should occur more rapidly. This will result in more tryptophan being metabolised to tryptamine, the effect of tryptamine being enhanced due to the inhibition of MAO.

The characteristic hyperactivity syndrome normally associated with the concurrent administration of a MAO inhibitor and tryptophan was not found to be present. This may be due to the disruptive effect of reserpine on the dopaminergic system, which has been suggested by Green and Graham-Smith (1974), as being

responsible for the mediation of the hyperactivity syndrome (vide supra).

The "tryptamine hypothesis", however, fails to explain the effect of 5-HTP in conjunction with a MAO inhibitor in increasing the startle response magnitude to a greater extent than l-tryptophan under similar circumstances. As 5-HTP can only be decarboxylated to 5-HT, the only tryptamine formed would be from endogenous tryptophan, in which case, much less substrate for tryptamine formation would be present. It may be that the suggestion of Squires (1975), of an N-methylated metabolite of 5-HT being responsible for the induction of the syndrome is true, despite little other evidence to support it.

3. The Influence of 5-HT on the Oestrous Cycle

Previous investigations have suggested that 5-HT may have a considerable influence on the oestrous cycle.

At the beginning of the oestrous cycle under the influence of FSH and a low basal level of LH, the ovarian follicles mature, and at a certain stage, begin to secrete oestradiol (Ely and Schwartz 1971). The peak level of plasma oestradiol has been found to occur about 12 hours prior to ovulation (Sharkh 1971), and following this peak in oestradiol levels, there is a sudden surge of LH, FSH and prolactin from the pituitary. It has been suggested that the LH surge is vital for ovulation to occur (Wilson 1974).

Both Carrer and Taleisnik (1970, 1972), and Arendash and Gallo (1978), report that electrochemical

stimulation of both the ventral tegmentum and the mid brain raphe will inhibit spontaneous ovulation via the inhibition of the episodic release of LH. The report by Arendash and Gallo (1978), has suggested that this is caused by the activation of an ascending serotoninergic pathway originating from the region of the mid-brain. Recently, Charli et al (1978), have shown 5-HT to act directly by inhibiting the release of LH-RF at the level of the secretory nerve endings in the mediobasal hypothalamus. This confirmed the previous findings of Domarski et al (1975), and Leonardelli et al (1974). Wheaten et al (1972), have shown 5-HT levels to fall significantly in the median eminence prior to the LH surge.

In the present study, brain 5-HT levels were found to be at a maximum at dioestrus and a minimum at oestrus. Brain tryptophan and total and free tryptophan levels were not found to correspond with brain 5-HT levels over the oestrous cycle, a finding which would seem contrary to that of Gessa and Tagliamonte (1974), who suggested that the concentration of free tryptophan in the plasma has a direct influence on brain tryptophan levels and 5-HT synthesis. However, in the case of the oestrous cycle, the influence of plasma oestradiol levels may be of some importance, since Thomson et al (1977), have shown a positive correlation between plasma oestradiol and free tryptophan concentrations in human subjects, and Christensen (1975), has reported oestradiol to possess the facility to promote plasma amino acid uptake into certain tissues, while reducing renal

clearance. Such findings may account for brain tryptophan levels to be at a maximum when plasma oestradiol levels would be expected to be at a maximum - at oestrus, and to be at a minimum when plasma oestradiol levels have been shown to reach their basal level - at dioestrus.

Although brain amine levels are not, in themselves, a satisfactory measure of neuronal activity, a point which has been stressed in previous sections of this thesis, it would seem unlikely that the low levels of brain 5-HT found at oestrus, reflect anything other than reduced serotoninergic neuronal activity, particularly in view of the evidence that raised serotoninergic neuronal activity has been found to inhibit ovulation (Arendash and Gallo 1978, Charli et al 1978). As oestrus is the point in the cycle at which sexual receptivity is facilitated (Slonaker 1924), it would be expected that serotoninergic neuronal activity should be reduced at this time since Mayerson (1964), found increased 5-HT levels to inhibit sexual receptivity and Everitt et al (1974), and Zemlan et al (1973), have found reduced 5-HT levels to promote sexual receptivity.

4. <u>The Influence of 5-HT on certain Behavioural Parameters during the Oestrous Cycle</u>

In view of the fact that 5-HT may have an inhibitory influence on some aspects of behaviour, and that serotoninergic neuronal activity may vary over the oestrous cycle (wide supra), it was expected that startle response magnitude, home-cage locomotor activity and open-field behaviour would also vary depending upon the

phase of the cycle. This was seen to be the case.

Both startle response magnitude and home-cage locomotor activity were found to be increased at oestrus, when compared to the dioestrus values. This was the expected result, as, previous investigators have suggested that serotoninergic neuronal activity in the brain is reduced at oestrus in order to facilitate ovulation (Everitt et al 1974).

A further illustration of the inhibitory nature of 5-HT on such behaviour, was the changes in startle response magnitude and home-cage locomotor activity during the 24 hour photo-period. In darkness, when brain 5-HT levels were seen to fall, sexually mature female mice were seen to exhibit about 80% of their total 24 hour activity, irrespective of the stage of the cycle. The dark period lasted for 50% of the total 24 hour photo-period.

In aged females, activity during the period of darkness was seen to be 65% of the total, and it would seem likely that the influence of circulating gonadal steroids, the levels of which are reduced in anoestrus, aged females, may be responsible for such a difference in activity. Greengrass and Tonge (1974), have suggested that progesterone can increase 5-HT turnover, and oestrogen can increase noradrenaline turnover. Reduced levels of these gonadal steroids may influence the synthesis of 5-HT and noradrenaline enough to produce the alteration in the activity pattern exhibited by the aged females.

The definition of "emotion" as applied to a rodent has been suggested by Denenberg (1969) as "an animal that when exposed to noxious or novel stimuli, will not move about, and will defaecate." In the novel environment of the open-field, oestrus females were seen to be less "emotional" than those females in the other phases of the cycle. Such a decrease in emotionality was found to correspond with reduced brain 5-HT levels.

Ellison (1975, 1977), proposed a hypothesis in which noradrenaline-deficient rodents represent an animal model of depression, and 5-HT-deficient animals represent a model of anxiety. The latter suggestion was proposed after the observation that 5-HT depleted animals were seen to be exploratory and active in the home-cage, but "fearful" (i.e. reduced locomotor activity, rearing and grooming in combination with a tendency to huddle motionless) in the open-field.

However, in this study, the oestrus females were found to correspond more nearly to 5-HT depleted animals since this group were found to have lower 5-HT levels than the females in other phases of the cycle, and although they were found to be more active in the home cage, they were not found to be "fearful" in the open field. Such a finding may cast doubt on the validity of such a hypothesis.

An interesting paradox was revealed by the behaviour of the aged females. It has been suggested that such animals have reduced biogenic amine levels due to the increased efficiency of MAO or a considerable

reduction in the number of cortical neurones (Davis and Himwich 1975). However, biochemicalanalysis found the aged females to have brain 5-HT levels slightly in excess of those of the dioestrus females, with free tryptophan concentrations in the plasma and brain tryptophan levels also above those of the dioestrus females. This would normally result in increased serotoninergic neuronal activity (Gessa and Tagliamonte 1974). The aged females were also found to exhibit less home-cage locomotor activity in a 24 hour period than the dioestrus females, which would also support the contention that these animals had increased serotoninergic neuronal activity, since it has been suggested that 5-HT has an inhibitory effect on locomotor activity. However, if this were so, it would be expected that the aged females should be more "emotional" in the open-field than the dioestrus females. This, however, was found not to be so, as they were found to produce only slightly more faecal boli, and exhibit only slightly less locomotor activity than the oestrus females, the least "emotional" phase of the cycle. This lack of activity in the home-cage and lack of "fearfulness" in the open field would see them as models of depression according to the Ellison hypothesis. Although brain noradrenaline levels in the aged females were not measured in this study, it may be that a functional noradrenaline deficiency does exist if the increased levels of free tryptophan in the plasma, brain tryptophan and brain 5-HT do reflect increased serotoninergic activity.

5. The Influence of 5-HT in the Modification of the Oestrous Cycle by the Administration of Synthetic Sex Steroids

The action of the synthetic progestogenic steroid, norethisterone acetate, administered alone and in combination with the synthetic oestrogenic steroid, ethinyloestradiol, was to induce a depressent effect on home-cage locomotor activity and reduce the magnitude of the startle response.

The combined therapy was found to produce a 32% increase in brain 5-HT levels in comparison to when norethisterone acetate was administered alone. Christensen (1975), has implied that oestrogens may possess the facility to increase tryptophan uptake into tissues. However, little difference was found in brain tryptophan levels between the two groups. The concentration of free tryptophan in the plasma was found to be 30% lower in the combined therapy group than in the group receiving norethisterone acetate alone. It may be that the presence of ethinyloestradiol stimulates the negative feedback effect of oestrogens, thereby reducing plasma oestradiol - a finding shown by Mishell et al (1970), to occur in women taking oral contraceptives. Since Thomson et al (1977), have shown the existence of a positive correlation between oestradiol and the concentration of free tryptophan, it might be expected that free tryptophan levels in the plasma may be reduced under such circumstances.

If the increase in brain 5-HT levels in the group receiving the combined therapy represents increased 5-HT

turnover, difference in behaviour between the two groups might be expected. After 43 days of continuous treatment, home-cage locomotor activity was found to have declined to a greater extent in the combined therapy group, than in the group receiving norethisterone acetate, when compared to their respective pre-treatment oestrus and dioestrus values. This may be explained by increases in serotoninergic neuronal activity which would be greater in the combined therapy group.

It should be noted that the startle response magnitude in the two groups receiving steroid treatment was reduced by a similar degree, and from other experiments conducted in this study, it would seem that any increase in serotoninergic neuronal activity should inhibit the startle response reflex (vide supra). It may be that the apparently conflicting results concerning the effect of the combined therapy on locomotor activity and the startle response reflex can be explained by the neural mechanisms controlling the startle response mechanism being less sensitive to changes in serotoninergic neuronal activity than those controlling locomotor activity. It must be stressed that such an explanation is not based on any direct evidence but only on the circumstantial evidence presented above.

In the open-field, both groups receiving hormone treatment were found to be considerably more "emotional" than oestrus females, exhibiting significantly less locomotor activity and producing significantly more faecal boli. As in the caseof the oestrous cycle, the increase in emotionality was found to correspond with

raised brain 5-HT levels.

6. Modifications in the Human Menstrual Cycle and Mouse Oestrous Cycle after the administration of Synthetic Sex Steroids

The administration of synthetic sex steroids was found to inhibit ovulation in both humans and mice. This was illustrated by the failure of basal body temperature to rise in the luteal phase of the cycle in women undergoing oral contraception. In the mouse, a state of constant dioestrus was found to occur after the administration of synthetic sex steroids, and the failure of the cycle to progress into oestrus would seem to preclude the possibility of ovulation having occurred.

After such treatment, considerable variation in certain biochemical parameters was seen between the two species, and it would seem likely thatsuch variations were a function of the fundamental differences between the menstrual and oestrous cycles. The most significant of these difference is the lack of a functional corpus luteum in the rodent oestrous cycle.

In the human menstrual cycle, two peaks in oestradiol secretion have been noted (Van de Wiele et al 1970). The first occurs immediately prior to ovulation, and the rise and decline in plasma levels have been shown to be rapid. This is followed by a more gradual, less intense rise, which is initiated by the corpus luteum. Thomson et al (1977), have shown the existence of a positive correlation between plasma oestradiol levels and the concentration of free tryptophan in the plasma, in human subjects. Such a

correlation may explain the findings of the present study in which the concentration of free tryptophan in the plasma was found to correspond with expected levels of plasma oestradiol in the human menstrual cycle.

In the mouse oestrous cycle, no such relationship between oestradiol and free tryptophan in the plasma was evident. It has been shown that plasma oestradiol in the rodent rises gradually from dioestrus to reach a maximum several hours prior to ovulation (Schwartz 1969). During the same period both total tryptophan and the concentration of free tryptophan in the plasma were seen to decline. There did, however, seem to be a connection between putative plasma oestradiol levels and brain tryptophan . At oestrus, when plasma oestradiol levels are know to be high, brain tryptophan levels were found to be at a maximum, despite low levels of total and free tryptophan in the plasma. This would suggest a lack of dependence of brain tryptophan on plasma tryptophan levels, which would seem to oppose the finding of Gessa and Tagliamonte (1974), who suggested that brain tryptophan levels were dependent on the amount of free rather than total tryptophan in the plasma. It may be that the raised brain tryptophan levels found at oestrus can be explained by the facility that oestrogens are said to possess in promoting amino acid uptake into tissue (for review see Christensen 1975).

The effect of contraceptive medication on the menstrual cycle was to lessen the variation in plasma tryptophan concentrations. This was not particularly

surprising since the principal action of combined steroid contraceptives has been shown to be the suppression of release of FSH-RF and LH-RF (Pennington and Naik 1974), thereby inhibiting ovulation. Because of this, little variation in plasma oestradiol levels has been found to occur during combined steroid contraception (Mishell et al 1972), and thus little variation in plasma tryptophan levels would be expected to occur. Experimental results confirmed this expectation, particularly in respect to the concentration of free tryptophan in the plasma. The overall mean value of free plasma tryptophan was only slightly reduced in the contraceptive cycle, which may be a function of reduced plasma oestradiol levels during steroid contraception. It would also seem to indicate that the daily dose of synthetic oestrogen included in the combined oral contraceptive "pill", may not replace the missing natural oestradiol in this respect.

In the mouse, the administration of the synthetic progestogen, norethisterone acetate, was generally seen to increase all plasma tryptophan levels above the pre-treatment dioestrus values, the phase of the cycle at which maximum values were seen to occur. When the synthetic oestrogen, ethinyloestradiol, was administered with norethisterone acetate, a reduction in the concentration of free tryptophan in the plasma was seen to occur. The reduction was such that the level of free tryptophan fell to only a little above the minimum value seen during the oestrus phase of the cycle. It may be that the presence of a synthetic oestrogen induces

increased inhibitory feedback on the mechanism controlling oestradiol secretion. This would effectively reduce oestradiol secretion, and possibly reduce the concentration of free tryptophan in the plasma. This would, of course, depend upon there being a similar positive correlation between plasma oestradiol and free tryptophan in the mouse, as Thomson et al (1977), have shown to exist in the human. It should be noted that previous experiments in this study, relating to the mouse oestrous cycle, found little evidence of such a connection (vide supra).

It would seem possible that the inhibition of ovulation and the depression of certain aspects of behaviour following the administration of contraceptive sex steroids may have been mediated by a functional increase in serotoninergic neuronal activity. Evidence exists to show that 5-HT has an inhibitory effect on ovulation, and the results of this study would seem to suggest that behavioural parameters such as startle response magnitude and home-cage locomotor activity were decreased when 5-HT turnover was increased. As these behavioural parameters were also decreased after the administration of synthetic sex steroids (locomotor activity being reduced in human females taking oral contraceptives as well as in mice undergoing synthetic sex steroid administration), this factor and the accompanying biochemical results would seem to indicate that such a situation may have been a result of increased serotoninergic neuronal activity.

7. Depression as a Side-Effect of Oral Contraception

Depression has been recognised as one of the side-effects in women taking oral contraceptives, and has been suggested to occur in 5-7% of such women (Herzberg and Loppen 1970, Herzberg et al 1970, Adams et al 1973). It was suggested that since oestrogens are known to raise plasma cortisol (Keller et al 1969), they possess the facility to induce hepatic tryptophan pyrrolase. This would have the effect of diverting tryptophan metabolism via the kynurenine-niacin pathway, thereby effectively reducing brain 5-HT synthesis, due to decreased tryptophan availability (Curzon 1971, Green et al 1975a,b).

Such a possibility would lend further support to the hypothesis that a functional deficiency in brain 5-HT may be responsible for the generation of depressive illness, and in particular, depressive side-effects associated with oral contraceptive medication. The present study also found an increased incidence of depressive symptoms in women taking oral contraceptives, and these symptoms were particularly pronounced in the third premenstrual week of medication, the time when plasma cortisol levels were seen to be significantly increased and total plasma tryptophan and free tryptophan in the plasma were reduced.

While there would seem to be little doubt that raised oestradiol levels do raise plasma cortisol, doubt does exist on the effectiveness of the oestrogenic component of the combined oral contraceptive to induce

hepatic tryptophan pyrrolase. Recent work by Green et al (1978), found little difference in kynurenine production,after an oral load of tryptophan,between women undergoing oral contraceptive medication and menstrual cycle controls. It should be noted, however, that Curzon and Green (1968), reported that the chronic effects of cortisol appear to differ from the acute effects, in that brain 5-HT levels tend to return to normal within 5 days of repeated dosing.

In the present study, plasma cortisol was seen to parallel expected oestradiol secretion over the menstrual cycle, yet both total tryptophan and free tryptophan in the plasma were also seen to exhibit such patterns. This situation would seem unlikely, if, under normal physiological conditions a primary function of raised oestradiol levels on plasma tryptophan was via the induction of hepatic tryptophan pyrrolase. It may be, however, that such reductions in plasma tryptophan levels produced by the induction of tryptophan pyrrolase are offset by the general homeostatic role of the liver in controlling amino acid levels (for review see Christensen 1975).

Greengrass and Tonge (1974), have suggested that oestrogen may improve mood and motivation by the promotion of a functional excess of brain noradrenaline. Such a connection would explain the improvement in mood seen at ovulation, when oestradiol levels are at a maximum, and the subsequent decline in mood as oestradiol levels fall in the premenstrual and menstrual phase of the reproductive cycle. After the occurrence of

ovulation, progesterone levels are known to reach a maximum in the luteal phase of the cycle. Greengrass and Tonge have suggested an opposing role for progesterone in comparison to the effects of oestrogen, in that it may induce a relative increase in brain 5-HT concentrations.

In the third, premenstrual week of oral contraceptive medication, plasma cortisol levels were seen to be more than double the corresponding menstrual cycle level, and about 80% above the maximum menstrual cycle plasma cortisol levels, seen at ovulation. Sachar et al (1976), have suggested reduced noradrenergic activity to be associated with reduced LH levels, which are known to occur in women taking the oral contraceptive (Goldzieher et al 1970). This, they suggest, may be responsible for such elevated plasma cortisol levels, the disinhibition of CRH secretion being secondary to a reduction in noradrenergic activity. The reduction in noradrenergic activity may also be responsible for the depressive episodes associated with raised cortisol levels.

It has been suggested that such "psychiatric" side-effects could be avoided by the proper choice of oral contraceptives. Briggs and Briggs (1972), have shown raised plasma progesterone and total plasma corticosteroid levels in oral contraceptives containing levonorgestrel in comparison to other preparations with differeing progestational agents. As has been suggested above, raised plasma progesterone may be associated with raised brain 5-HT activity, and raised corticosteroid

levels with reduced noradrenergic activity. This situation may precipitate depressive episodes because it is interesting to note that all but one of the women taking oral ceontraceptive medication in the present study were prescribed a brand containing levonorgestrel.

The "hormone shock" hypothesis has been proposed as an explanation for the variability in all side-effects seen in women taking oral contraceptives (Kurner and Duffy 1970). It suggests that women have various levels of natural oestrogen and progesterone, which predisposes them to react in different ways to the same exogenous hormones. Specifically, women with less endogenous hormone will respond more acutely to the sudden large supply of exogenous hormone. Because of the relatively fixed doses of hormones, the dose administered will be either too high or too low for a similar proportion of women.

Motivation for the use of such preparation has also been suggested as being behind the incidence of reported side-effects. Cullberg et al (1969), suggest that if premenstual psychiatric symptoms and dysmenorrhoea are reduced by oral contraceptive medication this, and the feeling of great contraceptive security, will lessen the incidence of adverse side-effects - particularly those of a psychiatric nature. Lidz (1969), concludes that depression, as a side effect of oral contraceptive medication, occurs as a result of frustration over the interference with fertility.

In conclusion, it would seem likely that many of the side-effects associated with oral contraceptive

medication could be reduced by a more accurate assessment of the relative oestrogen/progestogen balance of the combined pill in relation to the oestradiol/progesterone balance of the menstrual cycle. However, it would seem unlikely that the depressive side-effects could be eliminated totally, since the intrinsic contraceptive effect of the combined pill is known to be mediated via the amines, noradrenaline (Kalra et al 1978), and 5-HT (Charli et al 1978), and any alteration in their metabolism may precipitate depressive side-effects.

B I B L I O G R A P H Y

Adams, P.W., Rose, D.P., Folkard, J., Wynn, V., Seed, M., Strong, R. (1973): Effect of pyridoxine hydrochloride (Vitamin B6) upon depression associated with oral contraception. Lancet i, 897-904.

Aghajanian, G.K., Sheard, M.H. (1968): Behavioural effects on mid-brain raphe stimulation-dependence on serotonin. Communs. Behav. Biol. 1, 37-41.

Aghajanian, G.K., Foote, W.E., Sheard, M.H. (1970): Action of psychogenic drugs on single mid-brain raphe neurones. J. Pharmac. Exp. Ther., 171, 178-187.

Aghajanian, G.K., Haigher, H.J., Bloom, F.E. (1972): Lysergic acid diethylamide and serotonin: Direct actions on serotinin-containing neurones. Life Sci. 11, 615-622.

Ahlenius, S., Engel, J., Eriksson, H., Modigh, K., Sodersten, P. (1972): Importance of central catecholamines in the mediation of lordosis behaviour in ovariectomised rats treated with oestrogen and inhibitors of monoamine synthesis. J. Neural. Trans 33, 247-254.

Ajika, K., Krulich, L., Fawcett, C.P., McCann, S.M. (1972): Effects of oestrogen on plasma and pituitary gonadotrophins and prolactin and on hypothalamic releasing and inhibiting factors. Neuroendocrinology 9. 304-309.

Allen, E. (1922): The oestrous cycle in the mouse. Am. J. Anat. 30, 297-371.

Alpers, H.S., Himwich, H.E. (1972): The effects of chronic imipramine administration on rat brain levels of serotonin, 5-hydroxyindoleacetic acid, norepinephrine and dopamine. J. Pharmac. Exp. Ther. 180, 531-538.

Amin, A.H., Crawford, T.B.B., Gaddum, J.H. (1954): The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. J. Physiol. 126, 596-618.

Anden, N.E. (1975): Lesions of the nigro-neostriatal dopamine neurones on the bulbospinal norepinephrine and 5-hydroxytryptamine neurones in rats: action of drugs. Pharmac. Therap. B.1, 371-380.

Anton, A.H., Sayre, D.F. (1962): A study of factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmac. Exp. Ther. 138, 360-375.

Aprison, M.H., Hingtgen, J.N. (1972): Serotonin and behaviour: a brief summary. Fedn. Proc. 31, 121-129.

Arbuthnott, G.W., Crow, T.J., Fuxe, K., Olson, K., Ungerstedt, U. (1970): Depletion of catecholamines in vivo induced by electrical stimulation of-central monoamine pathways. Brain Res. 24, 471-483.

Arendash, G.W., Gallo, R.V. (1978): Serotonin involvement in the inhibition of episodic luteinizing hormone release during electrical stimulation of the mid-brain dorsal raphe nuclei in ovariectomised rats. Endocrinology 102, 1199-1206.

Ashcroft, G.W., Crawford, T.B.B., Eccleston, D., Sharman, D.F., McDougall, E.J., Stantan, J.B., Binns, J.F. (1966): 5-hydroxyindole compounds. Lancet ii, 1049.

Ashcroft, G.W., Eccleston, D., Murray, L.G., Glen, A.M., Crawford, T.B.B., Pullar, I.A., Schilds, P.J., Walter, O.S., Blackburn, I.M., Connechan, J., Lanergan, M. (1972): Modified amine hypothesis for the aetiology of effective illness. Lancet ii, 573-577.

Aylward, M. (1976): Estrogens, plasma tryptophan levels in perimenopausal patients. In: The management of the menopause and post-menopausal years, pp 135-147. Ed. S. Campbell MTP Press.

Aylward, M., Maddock, J. (1973): Plasma tryptophan levels in depression. Lancet i, 936.

Baldessarini, R.J. (1975): Basis for the amine hypothesis of affective disorders. Arch. Gen. Psychiat. 32, 1087-1093.

Bapna, J., Neff, N.H., Costa, E. (1971): A method for studying norepinephrine and serotonin metabolism in small regions of rat brain: effect of ovariectomy on amine metabolism in anterior and posterior hypothalamus. Endocrinology 89, 1345-1349.

Barnett, S.A., McEwan, I.M. (1973): Movements of virgin, pregnant and lactating mice in a residential maze. Physiol. Behav. 10, 741-746.

Barraclough, C.A., Collu, R., Massa, R., Marfinch, L. (1971): Temparal interrelationships between plasma L.H., ovarian secretion rates and peripheral plasma progestin concentrations in the rat: effects of nembutal and exogenous gonadotrophins. Endocrinology 88, 1437-1447.

Bartholini, G., Pletscher, A. (1968): Cerebral accumulation and metabolism of C^{14}-Dopa after selective inhibition of peripheral decarboxylase. J. Pharmac. Exp. Ther. 161, 14-20.

Beattie, C.W., Rogers, C.H., Soyka, L.F. (1972): Influence of ovariectomy and ovarian steroids on hypothalamic tyrosine hydroxylase activity in the rat. Endocrinology 91, 276-279.

Beattie, C.W., Soyka, L.F. (1973): Influence of progestational steroids on hypothalamic tyrosine hydroxylase activity in vitro. Endocrinology 93, 1453-1455.

Bedard, P., Carlsson, A., Fuxe, K., Lindqvist, M. (1971): Origin of 5-hydroxytryptophan and l-dopa accumulating in brain following decarboxylase inhibition. Naunyn-Schmiedebergs Arch. Pharmac. 269, 1-6.

Bender, D.A., Boulton, A.P., Coulson, W.F. (1975): A simple method for the study of tryptophan binding to serum albumin by small-scale equilibrium dialysis: Application to animal and human studies. Biochem. Soc. Trans. 3, 193-194.

Bertler, A., Rosengren, E. (1959): Occurence and distribution of catecholamines in brain. Acta. Physiol. Scand. 47, 350-361.

Bhargava, V.K., McKean, C.M. (1977): Role of 5-hydroxytryptamine in the modulation of acoutic brain stem (far-field) potentials. Neuropharmacology 16, 447-449.

Blasberg, R., Lajtha, A. (1966): Heterogenicity of the mediated transport systems of amino acid uptake in brain. Brain Res. 1, 86-104.

Bloxam, D.L., Warren, W.H. (1974): Error in the determination of tryptophan by the Method of Denkla and Dewey. A revised procedure. Analytical Biochem. 60, 621-625.

Birkmayer, W., Riederer, P. (1975): Biochemical post-mortem findings in depressed patients. J. Neural. Trans. 37, 95-109.

Boling, J.L., Blandau, R.J. (1939): The estrogen-progest4rone induction of mating responses in the spayed female rat. Endocrinology 25, 359-364.

Bolles, R.C. (1963): A failure to find evidence of the estrus cycle in the rat's activity level. Psychol. Rep. 12, 530.

Bolles, R.C. (1969): Theory of motivation. Harper, New York.

Bond, P.A., Jenner, F.A., Sampson, G.A. (1972): Daily variations of the urine content of MHPG in two manic depressive patients. Psychol. Med. 2, 81-85.

Bouhuys, A.L., AnDen Hoofdakker, R.H.(1977): Effects of mid-brain raphe destruction on sleep and locomotor activity in rats. Physiol. Behav. 19, 1143-1153.

Bowers, C.Y., Schally, A.V., Enzmann, F., Boler, J., Folkers, K. (1970): Porcine Thyrotropin-releasing hormone is (pyro) glu-his-Pro (NH_2). Endocrinology 86, 1143-1153.

Bowers, M.B. (1974): Amitriptyline in man: decreased formation of central 5-hydroxyindoleacetic acid. Clin. Pharmac. Therap. 15, 167-170.

Bowers, M.B., Heninger, G.R., Gerbode, F. (1969): Cerebrospinal fluid 5-hydroxyindoleacetic acid and homovanillic acid in psychiatric patients. Int. J. Neuropharmac. 8, 255-262.

Bridges, P.K., Jones, M.T. (1966): The diurnal rhythm of plasma cortisol concentration in depression. Br. J. Psychiat. 112, 1157-1162.

Briggs, M., Briggs M. (1972): Plasma hormone concentrations in women receiving steroid contaceptives. J. Obstet. Gynaec. Br. Commonwealth. 79, 946.

Brimbelcombe, R.W. (1973): In: Advances in drug research, Vol 7. Ed. Simmonds, A.B., Academic Press, New York.

Broadhurst, P.L. (1957): Determinants of emotionality in the rat. 1-situational factors. Br. J. Psychol. 48, 1-12.

Brodie, B.B., Comer, M.S., Costa, E., Dlabac, A. (1966): The role of brain serotonin in the mechanism of the central action of reserpine. J. Pharmac. Exp. Ther. 152, 340-349.

Brown, B.B. (1957): Lysergic acid diethylamide antagonism of certain drugs. Ann. N.Y. Acad. Sci. 66, 677-685.

Brown, B.B. (1960): C.N.S. drug action and interaction in mice. Archs. Int. Pharmacodyn Ther. 128, 391-414.

Brown, J.B., Blair, H.A.F. (1960): Urinary estrogen metabolites of 19-horethisterone and its esters. Proc. R. Soc. Med. 53, 433.

Bruell, J.H. (1969): Genetics and the adaptive significance of emotional defaecation in mice. Ann. N.Y. Acad. Sci. 159, 825-830.

Bruinvals J. (1972): Inhibition of the biosynthesis of 5-hydroxytryptamine in rat brain by imipramine. Eu. J. Pharmac. 20, 123-127.

Bulat, M., Supek, Z. (1967): The penetration of 5-hydroxytryptamine through the blood-brain barrier. J. Neurochem. 14, 265-271.

Bunney, W.E., Davis, J.N.: Norepinephrine in depressive reactions. A review. Arch. Gen. Psychiat. 13, 483-494.

Burke, A.W., Broadhurst, P.L. (1966): Behavioural correlates of the oestrous cycle of the rat. Nature 209, 223-224.

Burke, C.W. (1970): The effect of oral contraceptives on cortisol metabolism. J. Clin. Pathol. 23, Suppl. 3, 11.

Butcher, L.L., Engel, J., Fuxe, K. (1972): Behavioural, biochemical and histochemical analyses of the central effects of monoamine precursors after peripheral decarboxylase inhibition. Brain Res. 41, 387-411.

Carlsson, A. (1975): Monoamine precursors and analogues. Pharmac. Therap. B. 1, 381-392.

Carlsson, A., Falck, B., Hipparp, N.A. (1962): Cellular localisation of brain monoamines. Acta. Physiol. Scand. 56, Suppl. 196.

Carlsson, A., Lindqvist, M. (1972): The effect of l-tryptophan and some psychotropic drugs on the formation of 5-hydroxytryptophan in the mouse brain in vivo. J. Neural. Trans. 33, 23-43.

Carlton, P.L., Advocat, C. (1973): Attenuated habituation due to parachlorophenylalamine. Pharmac. Biochem. Behav. 1, 657-663.

Carrer, H.F., Taleisnik, S. (1970): Effect of mesencephalic stimulation on the release of gonadotrophins. J. Endocr. 48, 527-540.

Carrer, H.F., Taleisnik, S. (1972): Neural pathways associated with the mesencephalic inhibitory influence on gonadotropin secretion. Brain Res. 38, 299-313.

Caschera, F. (1959): La "menopause" nei topi femmine vergini (R 111/DmSe, C3Hb/Se, A/He/Se substrains). Lav. Anat. Patol. Perugia. 19, 13-20.

Chang, C.C. (1964): A sensitive method for spectro-photofluorimetric assay of catecholamines. Int. J. Neuropharmac. 3, 643-649.

Charli, J.L., Rotztejn, W.H., Pattou, E., Kordon, C. (1978): Effect of neurotransmitters on in vivo release of LH releasing hormone from the mediobasal hypothalamus of male rats. Neurosci. Lett. 10, 159-163.

Christensen, H.N. (1975): Biological Transport.

W.A. Benjamin Inc. Reading, Mass.

Chowers, I., McCann, S.M. (1967): Comparison of the effect of hypothalamic and pituitary implants of oestrogen and testosterone on reproductive system and adrenal of female rats. Proc. Soc. Exp. Biol. Med. 124, 260-268.

Clancy, J.J., Caldwell, D.F., Oberlas, D., Sangiah S., Villeneuve, M.J. (1978): Effect of chronic tryptophan dietary deficiency on the rats sleep waking cycle. Brain Res. Bull. 3, 83-87.

Collins, W.P., Newton, J.R. (1974): The ovarian cycle. In: Biochemistry of women: clinical concepts. pp 1-22 Eds. Curry, A.S., Hewitt, J.V. CRC Press, Cleveland.

Connor, R.L., Stolk, J.M., Barchas, J.D., Dement, W.C., Levine, S. (1970a): The effect of parachlarophenylalanine on shock induced fighting behaviour in rats. Physiol. Behav. 5, 1215-1219.

Connor, R.L., Stolk, J.M., Barchas, J.D., Levine S. (1970b): Parachlorophenylalanine and habituation to repetetive auditory startle stimuli in rats. Physiol. Behav. 5, 1215-1219.

Copenhaver, J.H., Schalock, R.L., Carver, M.J. (1978): para-chloro-Dh-phenylalanine induced filicidal behaviour in the female rat. Pharmac. Biochem. Behav. 8, 263-270.

Coppen, A. (1972): Biogenic amines and affective disorders. J. Psychiat. Res. 9, 163-175.

Coppen, A., Shaw, D.M., Farrell, J.P. (1963): Potentiation of the antidepressive effect of a monoamine-oxidase inhibitor by tryptophan. Lancet i, 79-81.

Coppen, A., Shaw, D.M., Herzberg, B. (1967): Tryptophan in the treatment of depression. Lancet ii, 1178-1180.

Coppen, A., Eccleston, E.G., Peet, M. (1972a): Total and free tryptophan concentrations in the plasma of depressive patients. Lancet ii, 1415-1416.

Coppen, A., Brooksbank, B.W.L., Peet, M. (1972b): Tryptophan concentration in the cerebrospinal fluid of depressive patients. Lancet ii, 1393.

Coppen, A., Whybrow, P.C., Noguera, R., Maggs, R., Prange, A.J. (1972c): The comparative antidepressant value of l-tryptophan and imipramine with and without potentiation by liothyronine. Arch. Gen. Psychiat. 26, 234-241.

Coppen, A., Eccleston, E.G., Peet, M. (1974): Plasma tryptophan binding and depression. Adv. Biochem. Psychopharmac. 11, 325-332.

Crow, T.J., Deakin, J.F.W. (1977): Role of tryptaminergic mechanism in the elements of the behavioural syndrome evoked by tryptophan and a monoamine oxidase inhibitor. Br. J. Pharmac. 59, 461P.

Cullberg, J., Gelli, M., Jonsson, C.O. (1969): Mental and sexual adjustment before and after six months use of an oral contraceptive. Acta.Psychiat. Scand. 45, 259-276.

Currie, G.N., Black, D.L., Armstrong, D.T., Greep, R.O. (1969): Blockade of ovulation in the rabbit with catecholamines and sympathomimetics. Proc. Soc. Exp. Biol. Med. 130, 598-602.

Curzon, G. (1971): Relationships between stress and brain 5-hydroxytryptamine and their possible significance in affective disorders. Adv. Ment. Sci. 4, 163-176.

Curzon, G., Green, A.R. (1968): Effect of hydrocortisone on brain 5-hydroxytryptamine. Life Sci. 7, 657-663.

Curzon, G., Marsden, C.A. (1975): Metabolism of a tryptophan load in the hypothalamus and other brain regions. J. Neurochem. 25, 251-256.

Coppola, J.A. (1969): Turnover of hypothalamic catecholamines during various states of gonadotrophin secretion. Neuroendocrinology 5, 75-80.

Costa, E., Aprison, M.H. (1958): Distribution of intracarotidly injected serotonin in the brain. Am. J. Physiol. 192, 95-100.

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

Dahlstrom, A. (1967): The intraneuronal distribution of noradrenaline and the transport and life span of amine storage granules in the sympathetic adrenergic neurone. Naunyn-Schmiedeberg's Arch. Pharmac. Exp. Path. 257, 93-115.

Dallo, J. (1977): Effect of two brain serotonin depletors on sexual behaviour of male rats. Pol. J. Pharmac. Pharm. 29, 247-251.

Doughaday, W.H., Adler, R.E., Mariz, I.K., Rasinski, D.C. (1962): Measurement of the binding capacity of corticosteroid-binding globulin in human plasma. J. Clin. Invest. 22, 704-710.

Davis, J.M., Himwich, W.A. (1975): Neurochemistry of the development and aging of the mammalian brain. Adv. Behav. Biol. 16, 329-357.

Davis, M., Sollberger, A. (1971): Twenty-four hour periodicity of the startle response in rats. Psychon. Sci. 25, 37-39.

Davis, M., Sheard, M.H. (1974a): Habituation and sensitisation of the rat startle response: Effects of raphe lesions. Physiol. Behav. 12, 425-431.

Davis, M., Sheard, M.H. (1974b): Effects of lysergic acid diethylamide (LSD) on habituation and sensitisation of the startle response in the rat. Pharmac. Biochem. Behav. 2, 675-683.

Davis, M., Sheard, M.H. (1974c): Diphasic dose-response effects of NN-dimethyltryptamine on the rat startle reflex. Pharmac. Biochem. Behav. 2, 821-829.

Davis, M., Sheard, M.H. (1976): p-Chloroamphetamine (PCA): acute and chronic effects on habituation and sensitisation of the acoustic startle response in rats. Eu. J. Pharmac. 35, 261-273.

Dencker, S.J., Malm, V., Roos, B.E., Werdinius, B. (1966): Acid monoamine metabolites of cerebrospinal fluid in mental depression and mania. J. Neurochem. 13, 1545-1550.

Denckla, W.D., Dewey, H.K. (1967): The determination of tryptophan in plasma, liver and urine. J. Lab. Clin. Med. 69, 160-169.

Denenberg, V. (1969): Open-field behaviour in the rat: what does it mean? Ann. N.Y, Acad. Sci. 159, 852-859.

DeMoor, P., Steeno, O., Brosens, J., Hendrikx, A. (1966): Data on transcortin activity in human plasma as studied by gel filtration. J. Clin. Endocr. 26, 71-78.

DiRaddo, J., Kellog, C. (1975): In vivo rates of tyrosine and tryptophan hydroxylation in regions of rat brain at four times during the light-dark cycle. Naunyn-Schmiedebergs Arch. Pharmac. 286, 389-400.

Doe, R.P., Mellinger, G.T., Swain, W.R., Seal, U.S. (1967): Estrogen dosage effects on serum proteins: a longitudinal study. J. Clin. Endocr. 27, 1463-1469.

Doig. R.J., Mummery, R.V., Wills, M.R., Elkes, A. (1966): Plasma cortisol levels in depression. Br. J. Psychiat. 112, 1157-1162.

Domanski, E., Przekop, F., Skubiszewski, B., Wolinska, A. (1975): The effect and site of action of indoleamines on the hypothalamic centres involved in the control of LH release and ovulation in sheep. Neuroendocrinology 17, 265-273.

Donoso, E., Stefano, F.J.E., Biscardi (1966): Adrenergic mechanisms in hypothalamic regulation of sexual function. Acta. Physiol. Lat. Am. 16, 301-310.

Donoso, A.O., Moyano, M.B., Santolaja, R.C. (1969): Metabolism of noradrenaline in the hypothalamus of castrated rats. Neuroendocrinology 4, 12-19.

Donoso, A.O., Moyano, M.B.G. (1970): Adrenergic activity in the hypothalamus at ovulation. Proc. Soc. Exp. Biol. Med. 135, 633-635.

Drybanski, A. (1975): Effects of 5-hydroxytryptamine given intraventricularly on rat behaviour. Pol. J. Pharmac. Pharm. 27, Suppl. 83-85.

Ellison, G.D. (1975): Behaviour and the balance between norepinephrine and serotonin. Acta. Neurobiol. Exp. 35, 499-515.

Ellison, G.D. (1977): Animal models of psychopathology: the low-norepinephrine and low-serotonin rat. Am. Psychol. 32, 1036-1045.

Ellison, G.D., Bresler, D.E. (1974): Tests of emotional behaviour in rats following depletion of norepinephrine, of serotonin, or both. Psychopharmacologia 34, 275-288.

Ely, C.A., Schwartz, N.B. (1971): Elucidation of the role of luteinising hormone in estrogen secretion and ovulation by use of antigonadotropic sera. Endocrinology 89, 1103-1108.

Endersby, C.A., Wilson, C.A. (1973): The effect of ovarian steroids on the uptake ^{3}H-noradrenaline, ^{3}H-dopamine and ^{3}H-5-hydroxytryptamine by hypothalamic tissue in vitro. Br. J. Pharmac. 47, 647-648P.

Endersby, C.A., Wilson, C.A. (1974): Effect of ovarian steroids on the accumulation of ^{3}H labelled monoamines by hypothalamic tissue in vitro. Brain Res. 73, 321-331.

Enjalbert, A., Moos, F., Carbonell, L., Priam, M., Kordon, C. (1977): Prolactin inhibiting activity of dopamine-free subcellular fractions from rat mediobasal hypothalamus. Neuroendocrinology 24, 147-161.

Everett, J.W., Sawyer, C.H., Markee, J.E. (1949): A neurogenic timing factor in control of the ovulatory discharge of luteinizing hormone in the cyclic rat. Endocrinology 44, 234-250.

Everett, J.W. (1964): Central neural control of reproductive functions in the adenohypophysis. Physiol. Rev. 44, 373-431.

Everitt, B.J. (1977a): Cerebral monoamines and sexual behaviour. In: Handbook of Sexology. pp 429-488. Eds. Money, J., Musaph, H. Excerpta Medica, Amsterdam.

Everitt, B.J. (1977b): Effects of clomipramine and other inhibitors of monoamine uptake on sexual behaviour of female rats and rhesus monkeys. Postgrad. Med. J. 53, Suppl. 4, 202-210.

Everitt, B.J., Fuze, K. (1977): Serotonin and sexual brhaviour in female rats. Effects of hallucinogenic indolealkylamines and phenylethylamines. Neurosci. Lett. 4, 215-220.

Everitt, B.J., Fuxe K., Hokfelt, T (1974): Inhibitory role of depamine and 5-hydroxytryptamine in the sexual behaviour of female rats. Eu. J. Pharmac. 29, 187-191.

Everitt, B.J., Fuxe K., Jonsson, G. (1975): The effects of 5, 7-dihydroxytryptamine lesions of ascending 5-hydroxytryptamine pathways on the sexual and aggressive behaviour of female rats. J. Pharmac. (Paris) 6, 25-32.

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

Farris, E.J. (1958): Human ovulation and fertility, Pittman Medical, London.

Fechter, L.D. (1974a): Central serotonin involvement in the elaboration of the startle reaction in rats. Pharmac. Biochem. Behav. 2, 161-171.

Fechter, L.D. (1974b): The effects of l-dopa, clonidine and apomorphine on the acoustic startle reaction in rats. Psychopharmacologia 39, 331-344.

Fernstrom, J.D., Wurtman, R.J. (1971): Brain serotonin content: physiological dependence on plasma tryptophan levels. Science N.Y. 173, 149-152.

Fernstrom, J.D., Wurtman, R.J. (1972): Elevation of plasma tryptophan by insulin in the rat. Metabolism 21, 337-343.

Fernstrom, J.D., Wurtman, R.J. (1974): Control of brain serotonin levels by the diet. Adv. Biochem. Psychopharmac. 11, 133-142.

Fernstrom, J.D., Larin, F., Wurtman, R.J. (1971): Daily variations in the concentrations of individual amino acids in rat plasma. Life Sci. 10, 813-820.

Fernstrom, J.D., Madras, B.K., Munro, H.N., Wurtman, R.J. (1974): Nutritional control of the synthesis of 5-hydroxytryptamine in the brain. In: Amino acids in the brain. pp 153-173. Ciba Foundation Symposium. Elsevier Excerpta Med. Amsterdam.

Ferrari, C., Caldera, R., Romussi, M., Rampini, P., Telbi, P., Zaatar, S., Curtarelli, E. (1978): Prolactin suppression by serotonin antagonists in man: further evidence for serotonergic control of prolactin secretion. Neuroendocrinology 25, 319-328.

Fibiger, H.C., Campbell, B.A. (1971): The effect of para-chlarophenylalanine on spontenous locomotor activity in the rat. Neuropharmacology 10, 25-32.

Finger, F.W. (1961): Estrous activity as a function of measuring device. J. Comp. Physiol. Psychol. 54, 524-526.

Finger, F.W. (1969): Estrous and general activity in the rat. J. Comp. Physiol. Psychol. 68, 461-466.

Finn, J.H., Price, J.M., Yess, N., Brown, R.R. (1964): Excretion of tryptophan metabolites by patients with rheumatoid arthritis. Arch. Rheum. 7, 201-209.

Foldes, A., Costa, E. (1975): Relationship of brain monoamine and locomotor activity in rats. Biochem. Pharmac. 24, 1617-1621.

Fraschini, F., Collu, R., Martini, L. (1971): In: Proc. 3rd Int. Cong. Harm. Ster. Eds. James, V.H.T., Martini, L. Excerpta Medica Foundation, Amsterdam.

Freeman, M.E., Reichert, L.E., Neill, J.D. (1972): Regulation of the proestrus surge of prolactin secretion by gonadotropin and estrogens in the rat. Endocrinology 90, 232-238.

Fuxe, K. (1964): Distribution of monoamine nerve terminals in the central nervous system. Acta. Physiol. Scand. 64, Suppl. 247, 38-85.

Fuxe, K., Gunne, L.M. (1964): Depletion of the amine stores in brain catecholamine terminals on amygdaloid stimulation. Acta. Physiol. Scand. 62, 493-494.

Fuxe, K., Hokfelt, T., Ungerstedt, U. (1968): Localisation of indolealkylamines in CNS. Adv. Pharmac. 6A, 235-251.

Fuxe, K., Corrodi, H., Hokfelt, T., Jonsson, G. (1970): Central monoamine neurones and pituitary adrenal activity. Progr. Brain Res. 32, 42-56.

Fuxe, K., Butcher, L.L., Engel, J. (1971): DL-5-hydroxytryptophan-induced changes in central monoamine neurones after peripheral decarboxylase inhibition. J. Pharm. Pharmac. 23, 420-424.

Fuxe, K., Schubert, J., Hokfelt, T., Jonsson, G. (1973): Some aspects of the inter-relationship between central 5-hydroxytryptamine neurones and hormones. Adv. Biochem. Psychopharmac. 10, 67-73.

Fuxe, K., Hokfelt, T., Jonsson, G., Lofstrom, G. (1974): Aminergic mechanisms in neuroendocrine control. In: Neurosecretion - the final neuroendocrine pathway. pp 269-275. Eds. Knowles, F., Vollrath, L., Springer-Verlag, Berlin.

Garattini, S., Valzelli, L. (1965): Serotonin. Elsevier, Amsterdam.

Geller, J., Blum, K. (1970): The effects of 5-HTP on para-chlorophenylalanine (p-CPA) attenuation of "conflict" behaviour. Eu. J. Pharmac. 9, 319-324.

Gessa, G.L., Tagliamonte, A. (1974): Serum free tryptophan: control of brain concentrations of tryptophan and of synthesis of 5-hydroxytryptamine. In: Amino acids in the brain. pp 207-216. Ciba Foundation Symposium. Elsevier Excerpta Med. Amsterdam.

Geyer, M.A., Warburton, J.D., Menkes, D.B., Zook, J., Mandell, A.J. (1973): Opposite effects of intra-ventricular serotonin and bufotenin on rat startle responses. Pharmac. Biochem. Behav. 3, 687-691.

Geyer, M.A., Puerto, A., Menkes, D.B., Segal, D.S., Mandell, A.J. (1976): Behavioural studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. Bran Res. 106, 257-270.

Glassman, A.H., Platman, S.R. (1969): Potentiation of a monoamine oxidase inhibitor by tryptophan. J. Psychiat. Res. 7, 83-88.

Glowinski, J., Iversen, L.L., Axelrod, J. (1966): Storage and synthesis of norepinephrine in the reserpine treated rat. J. Pharmac. Exp. Ther. 151, 385-399.

Goldzieker, J.W., Kleber, J.W., Moses, L.E., Rathmacher, R.P. (1970): A cross-sectional study of plasma PSH and LH levels in women using sequential, combination or injectable steroid contraceptives over long periods of time. Contraception 2, 225-248.

Goodwin, F.K., Murphy, D.L., Brodie, H.K.H., Bunney, W.E. (1970): l-Dopa, catecholamines and behaviour: a clinical and biochemical study in depressed patients. Lancet ii, 805-808.

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

Goodwin, J.S., Katz, R.I., Kopin, I.J. (1969): Effect of bromide on evoked release of monoamines from brain slices and intact atria. Nature, Lond. 221, 556-557.

Gordon, J.H., Shellenberger, M.K. (1974): Regional catecholamine content in the rat brain: sex differences and correlation with motor activity. -Neuropharmacology 13, 129-139.

Gorski, R.A. (1966): Localisation and sexual differentiation of nervous structures which regulate ovulation. J. Reprod. Fertil. Suppl. 67.

Grabowska, M., Antkiewicz, L., Maj, J., Michaluk, J. (1973): Apomarphine and central serotonin neurones. Pol. J. Pharmac. Pharm. 25, 29-39.

Grabowska, M., Michaluk, J. (1974): On the role of serotonin in apomarphine-induced locomotor stimulation in rats. Pharmac. Biochem. Behav. 2, 263-266.

Grahame-Smith, D.G. (1974): Tryptophan hydroxylation in brain. Biochem. Biophys. Res. Comm. 16, 586-592.

Grahame-Smith, D.G. (1971): Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and l-tryptophan. J. Neurochem. 18, 1053-1066.

Grahame-Smith, D.G., Parfitt, A. (1970): Tryptophan transport across the synaptosomal membrane. J.Neurochem. 17, 1339-1353.

Grant, E.C.G. (1975): The influence of hormones on headache and mood in women. Hemicrania 6, 2-10.

Gray, J.A. (1969): Genetics and the adaptive significance of emotional defaecation in mice. Ann, N.Y. Acad. Sci. 159, 825-830.

Green, A.R., Curzon, G. (1968): Decrease of 5-hydroxytryptamine in the brain provoked by hydrocortisone and its prevention by allopurinol. Nature, Lond. 220, 1095-1097.

Green, A.R., Grahame-Smith, D.G.(1974): The role of brain dopaminine in the hyperactivity syndrome produced by increased 5-hydroxytryptamine synthesis in rats. Neuropharmacology 13, 949-960.

Green, A.R., Kelly, P.H. (1976): Evidence concerning the involvement of 5-hydroxytryptamine in locomotor activity produced by amphetamine or tranylcypromine and l-dopa. Br. J. Pharmac. 56, 141-147.

Green, A.R., Youdin, M.B.H. (1975): Effects of monoamine oxidase inhibition by chlorgyline, deprenil or tranylcypromine on 5-hydroxytryptamine concentrations in rat brain and hyperactivity following subsequent tryptophan administration. Br. J. Pharmac. 55, 415-422.

Green, A.R., Woods, H.F., Knott, P.J., Curzon, G. (1975): Factors influencing the effect of hydrocortistone on rat brain tryptophan metabolism. Nature, Lond. 225, 170.

Green, A.R., Sourkes, T.L., Young, S.N. (1975): Liver and brain tryptophan metabolism following hydrocortisone administration to rats and gerbils. Br. J. Pharmac. 53, 287-292.

Green, A.R., Mitchell, B.D., Tardoff, A.F.C., Youdim, M.B.H. (1977): Evidence for dopamine deamination by both type A and type B MAO in rat brain in vivo and for the degree of inhibition of enzyme necessary for increased functional activity of dopamine and 5-hydroxytryptamine. Br.J. Pharmac. 60, 343-349.

Green, A.R., Bloomfield, M.R., Wood, H.F., Seed, M. (1978): Metabolism of an oral tryptophan load by women and evidence against the induction of tryptophan pyrrolase by oral contraceptives. Br. J. Clin. Pharmac. 5, 233-241.

Green, E.L. (1966): Biology of the Laboratory Mouse. McGraw-Hill, New York.

Green, H., Sawyer, J.L. (1960): Correlation of tryptamine induced convulsions in rats with brain tryptamine concentration. Proc. Soc. Exp. Biol. Med. 104, 153-155.

Green, H., Sawyer, J.L. (1960): Demonstration, characterisation and assay procedure of tryptophan hydroxylase in rat brain. Anal. Biochem. 15, 53-57.

Greengrass, P.M., Tonge, S.R. (1974): Suggestions on the pharmacological actions of ethinyloestradiol and progesterone on the control of monoamine metabolism in three regions from the brains of gonadectomised male and female mice and the possible clinic significance. Arch. int. Pharmacodyn. 211, 291-304.

Griffiths, W.J., Lester, B.K., Coulter, J.D., Willins, H.C. (1972): Tryptophan and sleep in young adults. Psychophysiology, 9, 345-356.

Gross, C.G. (1967): General activity. In: Analysis of Behavioural Change. Ed. L. Weiskrantz, Evanston, New York. Harper and Row, London.

Guroff, G., Udenfriend, S. (1962): Studies on aromatic amino acid uptake by rat brain in vivo. J. Biol. Chem. 237, 803-806.

Guttman, R., Lieblich, I., Gross, R. (1975): Behavioral correlates of estrous cycle stages in laboratory mice. Behav. Biol. 13, 127-132.

Hackman, E., Lichtensteiger, W., Wirz-Justice, A. (1973): Uptake of dopamine and 5-hydroxytryptamine in rat brain during progesterone decline. Psychopharmacologia 32, 183-191.

Hall, C.S. (1934): Emotional behaviour in the rat I-defaecation and urination as measures of individual differences in emotionality. J. Comp. Psychol. 18, 385-403.

Hall, D.W.R., Logan, B.W., Parsons, G.M. (1969): Further studies on the inhibitor of monoamine oxidase by Mand B 9302 (chlorgyline). I-substrate specificity in various mammalian species. Biochem. Pharmac. 18, 1447-1454.

Hamon, M., Glowinski, J. (1974): Regulation of serotonin synthesis. Life Sci. 15, 1533-1548.

Hamon, M., Javoy, F., Kordon, C., Glowinski, J. (1970): Synthesis and release of serotonin in the median eminence of the rat. Life Sci. 9, 167-173.

Hamon, M., Bourgoin, S., Glowinski, J. (1973): Feedback regulation of 5-hydroxytryptamine synthesis in rat striatal slices. J. Neurochem. 20, 1727-1745.

Hamon, M., Bourgoin, S., Morot-Gaudry, Y., Herg, F., Glowinski, J. (1974): Role of active transport of tryptophan in control of 5-hydroxytryptamine biosynthesis. Adv. Biochem. Psychopharmac. 11, 153-162.

Handley, S.L., Dunn, T.L., Baker, J.M., Cockshott, C., Gould, S. (1977): Mood changes in puerperium and plasma tryptophan and cortisol concentrations. Br. Med. J. 2, 18-22.

Hanselmann, G., Barberg, A.A. (1976): Response of rat brain indoles and motor activity to short light-dark cycles. J. Neurochem. 26, 951-955.

Harris, G.W. (1948): Neural control of the pituitary gland. Physiol Rev. 28, 139-179.

Hartmann, E. (1967): The effect of l-tryptophan on the sleep-dream cycle in man. Psychon. Sci. 8, 479-480.

Hartmann, E. (1972): Sleep inducing effects of l-tryptophan. J. Pharm. Pharmac. 24, 252-253.

Hartmann, E., Elion R. (1977): The insomnia of "sleeping in a strange place": effects of l-tryptophan. Psychopharmacology 53, 131-133.

Hartmann, E., Chung, R., Chien C. (1971): l-tryptophan and sleep. Psychopharmacologia 19, 114-127.

Hartmann, E., Cravens, J., List, S. (1974): Hypnotic effects of l-tryptophan. Arch. Gen. Psychiat. 31, 394-397.

Harvey, J.A., Heller, A., Moore, R.Y. (1963): The effect of unilateral and bilateral median forebrain bundle lesions on brain serotonin. J. Pharmac. Exp. Ther. 140, 103-110.

Heller, A., Moore, R.Y. (1965): Effect of central nervous system lesions on brain monoamines in the rat. J. Pharmac. Exp. Ther. 150, 1-9.

Hery, F., Rover, E., Glowinski, J. (1972): Daily variations of serotonin metabolism in the rat brain. Brain Res. 43, 445-465.

Hery, M., Laplanke, E., Kordon, C. (1978): Participation of serotonin in the phasic release of luteinizing hormone. ll Effects of lesions of serotonin containing pathways in the CNS. Endocrinology 102, 1019-1025.

Herzberg, B.N., Coppen, A. (1970): Changes in psychological symptoms in women taking oral contraceptives. Br. J. Psychiat. 116, 161-164.

Herzberg, B.N., Johnson, A.L., Brown,S. (1970): Depressive symptoms and oral contraceptives. Br. Med.J. 4, 142-145.

Hidaka, H., Nagatsu, T., Yagi, K. (1969): Occurrence of serotonin sulphotransferase in the brain. J. Neurochem. 16, 783-785.

Hill, S.Y., Reyes, R.B.(1978): Effects of l-tryptophan and ethanol on sleep parameters in the rat. Psychopharmacology 58, 229-233.

Himwich, H.E. (1971): Biochemistry, Schizophrenia and Affective illnesses. Williams and Wilkins, Baltimore.

Hole, K., Johnson, G.E., Berg, O.G. (1977): 5, 7, dihydroxytryptamine lesions of the ascending 5-hydroxytryptamine pathways: habituation, motor activity and agonistic behaviour. Pharmac. Biochem. Behav. 7, 205-210.

Horlington, M. (1970): Startle response circadian rhythm in rats: lack of correlation with motor activity. Physiol. Behav. 5, 49-53.

Horn, A.S. (1973): Structure activity relations for the inhibition of 5-HT uptake into rat hypothalamic homogenates by serotonin and tryptamine analogues. J. Neurochem. 21, 883-888.

Horita, A., Hamilton, A.E. (1970): Potentiation of the central actions of 5-hydroxytryptophan in rabbits by DL-x-hydrazino-x-methyldopa. J. Pharm. Pharmac. 22, 389-391.

Holzbaver, M., Youdim, M.B.H. (1973): The oestrous cycle and monoamine oxidase activity. Br. J. Pharmac. 48, 600-608.

Hullin, R.P., Bailey, A.D., McDonald, R., Dransfield, G.A., Milne, M.B. (1967): Variations in 11-dydroxy-corticosteroids in depression and manic-depressive psychosis. Br.J. Psychiat. 113, 593-600.

Hyppa, M. (1974): Metabolic rates and multiple functional pools of brain biogenic amines: their significance in neuroendocrine regulation. Med. Biol. 52, 170-175.

Hyppa, M.T., Falck, S.C. (1977): l-tryptophan and neuroendocrine regulation in neurologic patients: gonadotrophin secretion, sexual motivation and responsiveness during l-tryptophan treatment in patients with multiple sclerosis. Psychoneuroendocrinology 2, 359-363.

Ichiyama, A., Nakamura, S., Nishizuka, Y., Hayaishi, O. (1970): Enzymic studies on the biosynthesis of serotonin in mammalian brain. J. Biol. Chem. 245, 1699-1709.

Jacbos, B.L., Cohen, A. (1976): Differential behavioural effects of lesions of the median or dorsal raphe nuclei in rats: open field and pain elicited aggression. J. Comp. Physiol. Psychol. 90, 102-108.

Jacobs, B.L., Eubanks, E.E. (1974): A comparison of the locomotor effects of 5-hydroxytryptamine and 5-hydroxy-tryptophan administered by two systemic routes. Pharmac. Biochem. Behav. 2, 137-139.

Jacobs, B.L., Eubanks, E.E., Wise, W.D. (1974a): Effect of indolealkylamine manipulations on locomotor activity in rats. Neuropharmacology 13, 575-583.

Jacobs, B.L., Wise, W.D., Taylor, K.M. (1946): Differential behavioural and neurochemical effects following lesions of the dorsal median raphe nuclei in rats. Brain Res. 79, 353-361.

Jacobs, B.L., Trimbach, C., Eubanks, E.E., Trulson, M. (1975): Hippocampal mediation of raphe lesions and p-CPA induced hyperactivity in the rat. Brain Res. 94, 253-261.

Jequier,E., Lovenberg, W., Sjoerdsma, A. (1967): Tryptophan hydroxylase inhibition: The mechanism by which p-chlorophenylalanine depletes rat brain serotonin. Mol. Pharmac. 3, 274-278.

Jequier, E., Robinson, D.S., Levenberg, W., Sjoerdsma, A. (1969): Further studies on tryptophan hydroxylase in rat brainstem and beef pineal. Biochem. Pharmac. 18, 1071-1081.

Johansson, K.N.G., Currie, B.L., Folkers, K. (1973): Biosynthesis and evidence for the existence of the follicle stimulating hormone releasing hormone. Biochem. Biophys. Res.Com. 50, 8-13.

Johnston, J.P. (1968): Some observations upon a neu inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmac. 17, 1285-1297.

Jones, F., Maas, J.W., Dekirmenjian, H. (1973): Urinary catecholamine metabolites during behavioural changes in a patients with manic-depressive cycle. Science, 179, 300-302.

Jouvet, M. (1967): Mechanism of the states of sleep: a meuropharmacological approach. In: Sleep and altered states of consciousness. pp 86-126. Eds. S. Kety, M. Evarts, H. Williams. Williams and Wilkins, Baltimore.

Jouvet, M. (1969): Biogenic amines and the state of sleep. Science 163, 32-41.

Kalra, S.P., Kalra, P.S., Chen, C.L., Clemens, J.A. (1978): Effect of norepinephrine synthesis inhibitors and a dopamine agonist on hypothalamic LH-RH, serum gonadotropin and prolactin levels in gonadal steroid treated rats. Acta. Endocr. Copenh. 89, 1-9.

Kamberi, I.A. (1973): The role of brain monoamines and pineal indoles in the secretion of gonadotrophins and gonadotrophin releasing factors. Rec. Progr. Brain Res. 39, 261-280.

Kamberi, I.A., Danhof, I.E. (1968): Monoamine oxidase activity in hypothalamus, amygdala and cerebral cortex during the oestrous cycle. Fedn. Proc. 27, 388.

Kamberi, I.A., Kobayashi, Y. (1970): Monoamine oxidase activity in the hypothalamus and various other brain areas and in some endocrine glands of the rat during oestrous cycle. J. Neurochem. 17, 261-268.

Kamberi, I.A., Mical, R.S., Porter, J.C. (1970): Effect of anterior pituitary perfusion and intraventricular injection of catecholamines and indoleamines on LH release. Endocrinology 87, 1-12.

Kamberi, I.A., Mical, R.S., Porter, J.C. (1971): Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology 88, 1288-1293.

Katz, R.I., Kopin, I.J. (1969): Release of norepinephrine -^{3}H and serotonin -^{3}H evoked from brain slices by electrical-field stimulation - calcium dependency and the effects of lithium, oubain and tetrodotoxin. Biochem. Pharmac. 18, 1935-1939.

Keller, P.J., Lichtensteiger, W. (1971): Stimulation of tubero-infundibular dopamine neurones and gonadotrophin secretion. J. Physiol. 219, 385-401.

Keller, N., Richardson, V.I., Yates, F.E. (1969): Protein binding and the biological activity of corticosteroids: in vivo induction of hepatic and pancreatic alamine amino transferase bycorticosteroids in normal and oestrogen treated rats. Endocrinology 84, 49-62.

Kety, S. (1971): Brain amines and affective disorders. Adv. Behav. Biol. 4, 237-244.

Kilbey, M.M., Johnson, K.M., McLendon, D.M. (1977): Time course of delta-9-tetrahydrocannabinol inhibition of predatory aggression. Pharmac. Biochem. Behav. 7, 117-120.

Kirkby, R.J., Bell, D.S., Preston, A.C. (1972): The effects of methylamphetamine on stereotyped behaviour, activity, startle and orientating responses. Psychopharmacologia 25, 41-48.

Knapp, S., Mandell, A.J. (1972): p-chlorophenylalamine - its three phase sequence of interactions with the two forms of tryptophan hydroxylase. Life Sci. 16, 761-771.

Knott, P.J., Curzon, G. (1972): Free tryptophan in plasma and brain tryptophan metabolism. Nature, Lond. 452-453.

Knott, P.J., Curzon, G. (1974): Effect of increased rat brain tryptophan on 5-HT and 5-HIAA in the hypothalamus and other brain regions. J. Neurochem. 22, 1065-1072.

Knox, W.E., Auerbach, V.H. (1955): The hormonal control of tryoptphan peroxidase in the rat. J. Biol. Chem. 214, 307-313.

Kobayashi, H., Matsui, T. (1969): Fine structures of the median emicence and its functional significance. In: Frontiers in heuroendocrinology, pp 3-46. Eds. Ganong W., Martini L. Oxford Univ. Press.

Kobayashi, F., Hara, K., Miyake, T. (1969): Effect of steroids on the release of LH in the rat. Endocr. Jap. 16, 251-260.

Kobinger, W. (1971): Differentiation between the sedative actions of 5-hydroxytryptophan in mice, by means of two stimulating substances. Acta. Pharmac. Tox. 20, 145-154.

Koe, B.K., Weissman, A. (1966): P-chlorophenylalamine: A specific depletion of brain serotonin. J. Pharmac. Exp. Ther. 154, 499-516.

Kordon, C., Gogan, F., Hery, M., Rotsztejn, W.H. (1971): Interference of serotonin containing neurones with pituitary gonadotropins release-regulation. Gynec. Invest. 2, 116-121.

Kordon, C., Glowinski, J. (1972): Role of hypothalamic monoaminergic neurones in the gonadotrophin release-regulating mechanisms. Neuropharmacology 11, 153-162.

Kostowski, W., Giacolone, W., Garattini, S., Valzelli, L. (1968): Studies on behavioural and biochemical changes in rats after lesions in mid-brain rephe. Eu. J. Pharmac. 4, 371-376.

Kostowski, W., Czlonkowski, A., Markowska, L., Markiewicz, L. (1975): Intraspecific aggressiveness after lesions of mid-brain raphe nuclei. Pharmacology 13, 81-85.

Kulkarni, S.K., Dandiya, P.C., Jain, K.H. (1974): A comparitive study of the influence of 6-hydroxydopamine, x-methyltyrosine, 5,6-dihydroxytryptamine and p-chlorophenylalamine on open field behaviour in rats. Indian J. Physiol. Pharmac. 18, 324-329.

Kupfer, D.J. (1977): EEG sleep correlates of depression in man. In: Animal models in psychiatry and neurology pp. 181-188. Pergamon, Oxford.

Kutner, S.J., Duffy, T.J. (1970): A psychological analysis of oral contraceptives and the IUD. Contraception 2, 289-296.

Kurachi, K., Hirota, K. (1969): Catecholamine metabolism in rat brain related with sexual cycle. Endocr. Jap. Suppl. 69-73.

Labhsetwar, A.P. (1970): The role of oestrogens in spontaneous ovulation: evidence for positive oestrogen feedback in the 4-day oestrous cycle. J. Endocr. 47, 481-494.

Labhsetwar, S.P. (1971a): Effect of 5-hydroxy-tryptamine on spontaneous ovulation in rats. Nature, London, 229, 203-204.

Labhsetwar, A.P. (1971b): Effects of 5-hydroxytryptamine on spontaneous ovulation: a theory for dual hypothalamic control of ovulation. Acta Endocr. Copenh. 68, 334-344.

Labhsetwar, A.P. (1972): Role of monoamines in ovulation: evidence for a serotoninergic pathway for the inhibition of spontaneous ovulation. J. Endocrinol. 54, 269.

Labhsetwar, A.P. (1975): Progesterone: sites of action in inhibiting ovulation in hamsters. J. Reprod. Fertil. 42, 341-350.

Lagerspetz, K.Y.H., Tirri, R., Lagerspetz, K.M.J. (1967): Neurochemical and endocrinological studies in mice selectively bred for aggressiveness. Rep. Inst. Psychol. (Turku) 29, 1-5.

LaMotte, R.H., Schmidt, D.E., Roliffson, W.S. (1969): Multiple substrate determination of monoamine oxidase distribution and iproniazid inhibition in rat brain. J. Neurochem. 16, 725-730.

Lancranjan, I., Wirz-Justice, A., Puehringer, R., Delpozo,E. (1977): Effect of l-5-hydroxytryptophan infusion on growth hormone and prolactin secretion in man. J. Clin. Endocr. Metab. 45, 588-593.

Langer, G., Sachar, E.J. (1977): Dopaminergic factors in human prolacting regulation: effects of neuroleptics and dopamine. Psychoneuroendocrinology 2, 373-378.

Lapin, I.P., Oxenkrug, G.F. (1969): Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. Lancet i, 132-136.

Larson, B.A., Sinha, Y.N., Vanderlaan, W.P. (1977): Effect of 5-hydroxytryptophan on prolactin secretion in the mouse. J. Endocr. 74, 153-154.

Larsson, K., Fuxe, K., Everitt, B.J., Holmgren, M., Sodersten, P. (1978): Sexual behaviour in male rats after intracerebral injection of 5,7-dihydroxy-tryptamine. Brain Res. 141, 293-303.

Leavitt, W.W., Bosley, C.G., Claha, G.C. (1971): Source of ovarian preovulatory progesterone. Nature (New Biol.) 234, 283-284.

Leklem, J.E., Brown, R.R., Rose, D.P., Linkswiler, H., Arend, R.A. (1975): Metabolism of tryptophan and niacin in oral contraceptive users receiving controlled intakes of vitamin B6. Am. J. Clin. Nutr. 28, 146-156.

Leonardelli, J., Dubois, M.P., Poulain, P. (1974): Effect of exogenous serotonin on LH-RH secreting neurones in the guinea pig hypothalamus as revealed by immuno-fluorescence. Neuroendocrinology 15, 69-72.

Lichtensteiger, W. (1969): Cyclic variations of catecholamine content in hypothalamic nerve cells during the estrous cycle of the rat, with a concomitant study of the substantia nigra. J. Pharmac. Exp. Ther. 165, 204-215.

Lidz, R.W. (1969): Emotional factors in the success of contraception. Fertil, Steril. 20, 761-771.

Lints, C.E., Harvey, J.A. (1969): Altered sensitivity to foot shock and decreased brain content of brain serotonin following brain lesions in the rat. J. Comp. Physiol. Psychol. 67, 23-31.

Lippmann, W. (1968): Relationship between hypothalamic norepinephrine and serotonin and gonadotrophin secretion in the hamster. Nature, Lond. 218, 173-174.

Livesey, P.J., Egger, G.J., Meyer, P.N. (1972): Wheel running, a rewarding activity for the rat or a response to increased drive following deprivation. Aust. J. Psychol. 24, 45-53.

Lloyd, K.G., Farley, I.J., Deck, J.N.N., Hornykiewicz, O. (1974): Serotonin and 5-hydroxyindoleacetic acid in discrete areas of the brain stem of suicide victims and control patients. Adv. Biochem. Psychopharmac. 11, 387-397.

Lorens, S.A., Sorensen, J.P., Yunger, L.M. (1971): Behavioural and neurochemical effects of lesions in the raphe system of the rat. J. Comp. Physiol. Psychol. 77, 48-52.

Lorens, S.A., Kohler, C., Goldberg, H.C. (1975): Lesions in Guddens tegmental nuclei produce behavioural and 5-HT effects similar to those after raphe lesions. Pharmac. Biochem. Behav. 3, 653-659.

Luhby, A.L., Brin, M., Gordon, M., Davis, P., Murphy, M., Spiegel, H. (1971): Vitamin B_6 metabolism in users of oral contraceptive agents. 1: Abnormal xanthurenic acid excretion and its correction by pyridoxine. Am. J. Clin. Nutr. 24, 684-693.

Maas, J.W. (1975): Biogenic Amines and depression. Arch. Gen. Psychiat. 32, 1357-1361.

Maas, J.W., Fawcett, J.A., Dekirmenjian, H. (1968): 3-methoxy-4-hydroxyphenyl glycol (MHPG) excretion in depressive states. Arch. Gen. Psychiat. 19, 129-134.

Mabry, P.D., Campbell, B.Y. (1973): Serotonergic inhibition of catecholamine-induced behavioural arousal. Brain Res. 49, 381-391.

Macon, J.B., Sokoloff, L., Glowinski, J. (1971). Feedback control of rat brain 5-hydroxytryptamine synthesis. J. Neurochem. 18, 323-331.

Mahesh, V.B., Goldman, B.D. (1971): In: Proc. 3rd Int. Cong. Harm. Ster. Eds. James, V.H.T., Martini, L. Excerpta Medica Foundation, Amsterdam.

Maickel, R.P., Cox, R.H., Saillant, J., Miller, F.B. (1968): A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. Int. J. Neuropharmac. 7, 275-281.

Malamed, S., Poisner, A.M., Frifaro, J.M., Douglas, W.W. (1968): The fate of the chromaffin granule during catecholamine release from the adrenal medulla. - III Recovery of a purified fraction of electron-translucent structure. Biochem. Pharmac. 17, 241-246.

Malik-Ahmadi, P., Behrmann, P.J. (1976): Depressive syndrome induced by oral contraceptives. Dis. Nerv. System 37, 406-408.

Malmnas, C.O., Mayerson, B.J. (1970): Monoamines and testosterone activated copulatory behaviour in the castrated male rat. Acta. Pharmac. Tox. 28, suppl. 49, 67.

Mandell, M.P., Mandell, A.J., Jacobsen, A. (1965): Biochemical and neurophysiological studies of paradoxical sleep. Rec. Adv. Biol. Psychiatry 7, 115.

Mandell, A.J., Morgan, M. (1971): Indole (ethyl) amine N-methyltransferase in human brain. Nature (New Biol.) 230, 85-87.

Marks, P.C., O'Brien, M., Paxinos, G. (1977): 5, 7-DHT-induced muricide: inhibition as a result of preoperative exposure of rats to mice. Brain Res. 135, 383-388.

Marsden, C.A. (1978): MAOI and l-tryptophan in rats. In: Neurotransmitter systems and their clinical disorders. pp 189-199. Ed. Legg, N.J. Academic Press, London,

Marsden, C.A., Curzon, G. (1974): Effects of lesions and drugs on brain tryptamine. J. Neurochem. 23, 1171-1176.

Marsden, C.A., Curzon, G. (1976): Studies on the behavioural effects of tryptophan and p-chlorophenyl-alanine. Neuropharmacology 15, 165-172.

Marsden, C.A., Curzon, G. (1977): Effects of p-chloro-phenylalanine and x-methyltryptophan on behaviour and brain 5-hydroxyindoles. Neuropharmacology 16, 489-494.

Marsden, C.A., Curzon, G. (1978): The contribution of tryptamine to the behavioural effects of l-tryptophan in tranylcypromine treated rats. Psychopharmacology 57, 71-76.

Mason, M., Gullekson, E.H. (1960): Estrogen enzyme interactions: inhibition and protection of kynurenine aminotransferase by the sulfate esters of diethyl-stilboestrol, oestradiol and estrone. J. Biol. Chem. 235, 1312-1316.

Matsuo, H., Baba, Y., Nair, R.M.G., Arimura, A., Schally, A.V. (1971): Structure of the procine LH and FSH-releasing hormone. 1 The proposed amino acid sequence. Biochem. Biophys. Res. Com. 43, 1334-1339.

Mayerson, B.J. (1964): Central nervous monoamines and hormone-induced oestrus behaviour in the spayed rat. Acta. Physiol. Scand. 63, Suppl. 241

Mayerson, B.J., Lewander, R. (1970): Serotonin synthesis inhibition and oestrous behaviour in female rats. Life Sci. 9, 661-671.

Meek, J.R., Neff, N.H. (1972): Tryptophan-5-hydroxylase: Appriximation of half-life and rate of axonal transport. J. Neurochem. 19, 1519-1525.

Mendels, J., Stinnett, J., Burns,D. (1975): Amine precursors and depression. Arch. Gen. Psychiat. 32, 22-30.

Messing, R.B., Phebus, L., Fisher, L.A., Lyttle, L.D. (1976): Effects of p-chloroamphetamine on locomotor activity and brain 5-hydroxyindoles. Neuropharmacology 15, 157-164.

Mishell, D.R., Thorneycroft, I. H., Nakamura, R.M., Nagata, Y., Stone, S.C. (1972): Serum estradiol in women ingesting combination oral contraceptive steroids. Am. J. Obstet. Cynec. 114, 923-928.

Miyake, T. (1968): In: Integrative mechanisms of neuroendocrine systems. pp 139-149. Ed. Ioh, S. Hokkaido University, Sapparo.

Mödigh, K. (1973): Effects of l-tryptophan on motor activity in mice. Psychopharmacologia 30, 123-134.

Moir, A.T.B., Eccleston, D. (1968): The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. J. Neurochem. 15, 1093-1108.

Morgan, W.H., Yudo, C.A. (1973): Daily rhythms in tryptophan and serotonin content in mouse brain: the apparent independence of these parameters from daily changes in food intake and from plasma tryptophan content. Life Sci. 12, 395-408.

Murphy, D. L., Goodwin, F.K., Bunney, W.E. (1972): A reevaluation of biogenic amines in manic and depressive states. Hospital Practice 7, 85-92.

Musa, B.U., Doe, R.P., Seal, U.S. (1967): Serum protein alterations produced in women by synthetic oestrogens. J. Clin. Endocr. 27, 1463-1469.

McArthur, J.N., Dawkins, P.D. (1969): The effect of sodium salicylate on the binding of l-tryptophan to proteins. J. Pharm. Pharmac. 21, 744-750.

McDonald, P.G., Gilmore, D.F. (1971): The effect of ovarian steroids on hypothalamic thresholds for ovulation in the female rat. J. Endocr. 49, 421-429.

McMenamy, R.H., Oncley, J.C. (1958): The specific binding of l-tryptophan to serum albumin. J. Biol. Chem. 233, 1436-1437.

McMillan, M. (1960): The identification of a fluorescent reducing substance in the urine of patients with rheumatoid arthritis: the excretion of 3-hydroxy-anthranilic acid in this and other conditions. J. Clin. Pathol. 13, 140-148.

Naftolin, F., Brown-Grant, K., Carker, C.S. (1972): Plasma and pituitary luteinizing hormone concentrations and peripheral plasma oestradiol concentration during early pregnancy and after the administration of progestational steroids in the rat. J. Endocr. 53, 31-36.

Neff, N.H., Tozer, T.N., Brodie, B.B. (1967): Application of steady-state kinetics to the studies of the transfer of 5-hydroxyindoleacetic acid from brain to plasma. J. Pharmac. Exp. Ther. 158, 214-218.

Negro-Vilar, A., Chiocchio, S.R., Tramezzani, J.H. (1977): Changes in catecholamine content of the median eminence precede the por-oestrous surges of luteinizing hormone and prolactin. J. Endocr. 75, 339-340.

Neill, D.B., Grant, L.D., Grossmann, S.P. (1972): Selective potentiation of locomotor effects of amphetamine by mid brain raphe lesions. Physiol. Behav. 9, 655-657.

Neuburg, J., Thut, P.D. (1974): Comparison of the locomotor stimulant mechanisms of action of d-amphetamine and d-amphetamine plus l-dopa: possible involvement of serotonin. Biol. Psychiat. 8, 139-150.

Niskanen, P., Huttunen, M., Tamminen, T., Jaaskelainen, J. (1976): Daily rhythm of plasma tryptophan and tyrosine in depression. Br. J. Psychiat. 28, 67-73.

Nordin, G., Ottoson, J.D., Roos, B.E. (1971): Influence of convulsive therapy on 5-hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid in endogenous depression. Psychopharmacologia 20, 315-321.

Oldendorf, W.H. (1971): Brain uptake of radiolabelled amino acids, amines and hexoses after arterial injection. Am. J. Physiol. 1629-1639.

Pare, C.M.D. (1963): Potentiation of monoamine oxidase inhibitors by tryptophan. Lancet ii, 527-528.

Pare, C.M.B., Young, D.P.H., Price, K., Stacey, R.S. (1969): 5-hydroxytryptamine, noradrenaline and dopamine in brainstem, hypothalamus and caudate nucleus of controls and patients committing suicide by coal-gas poisoning. Lancet ii, 133-135.

Parent, A., Saint-Jacques, C., Poirier, L.J. (1969): Effect of interupting hypothalamic nervous connection on the norepinephrine and serotonin content of the hypothalamus. Exp. Neurol. 23, 67-75.

Parkes, A.S. (1925): The age of attainment of sexual maturity of the albino mouse. J. Roy. Microscop. Soc. 315-319.

Parfitt, A., Grahame-Smith, D.G. (1974): The transfer of tryptophan across the synaptosome membrane. In: Amino acids in the brain. pp 175-196. Ciba Foundation Symposium. Elsevier Excerpta Med. Amsterdam.

Paulsen, C.A. (1965): Progestin metabolism: special reference to estrogenic pathways. Metabolism 14, 313-319.

Pennington, G.W., Naik, S. (1974): The biochemistry of contraception. In: Biochemistry of women: clinical concepts. pp 71-84. Eds. Curry, A.S., Hewitt, H.J.V., C.R.C. Press, Cleveland.

Peters, D.A.V. (1972): Inhibition of brain tryptophan 5-hydroxylase by amino acids - The role of l-tryptophan uptake inhibition. Biochem. Pharmac. 21, 1051-1053.

Peters, D.A.V., McGeer, P.L., McGeer, E.G. (1968): The distribution of tryptophan hydroxylase in rat brain. J. Neurochem. 15, 1431-1435.

Peterson, R.E., Nokes, G., Chen, P.S., Black, R.L. (1960): Estrogens and adrenocortical function in man. J. Clin. Endocr. 20, 495-514.

Philo, R., Rudeen, P.K., Reiter, R.J. (1977): A comparison of the circadian rhythms and concentrations of serotonin and norepinephrine in the telencephalon of four rodent species. Comp. Biochem. Physiol. 57, 127-130.

Piacsek, B.E., Schneider, T.C., Gay, V.L. (1971): Sequential study of luteinizing hormone (LH) and "progestin" secretion on the afternoon of proestrus in the rat. Endocrinology 89, 39-45.

Pirch, J.A. (1969): Stimulation of locomotor activity by p-chlorophenylalanine and low dose of reserpine. Arch. Int. Pharmacodyn 181, 434-440.

Pletscher, A., Shore, P.A., Brodie, B.B. (1956): Serotonin as a mediator of reserpine action in brain. J. Pharmac. Exp. Ther. 116, 84-89.

Porter, C.C., Watson, L.S., Titus, D.C., Totaro, J.A., Byer, S.S. (1962): Inhibition of dopa decarboxylase by the hydrazino analog of x-methyldopa. Biochem. Pharmac. 11, 1067-1077.

Porter, J.C., Kamberi, I.A., Ondo, J.G. (1972): Role of biogenic amines and cerebrospinal fluid in the neurovascular transmittal of hypophysiotrophic substances. In: Brain-Endocrine Interaction. Median Eminence Structure and function. pp 243-253. Eds. Knigge, K.M., Scott, D.E., Weindl, A., Karger, Basel.

Post, R.M., Goodwin, F.K. (1974): Effects of amitriptyline and imipramine on amine metabolites in the cerebrospinal fluid of depressed patients. Arch. Gen. Psychiat. 26, 57-63.

Post, R.M., Goodwin, F.K. (1975): Studies of cerebrospinal fluid amine metabolites in depressed patients. Conceptual problems and theoretical implications. In: The psychobiology of depression. pp 47-67. Ed. Mendels, J. Spectrum, New York.

Prange, A.J. (1964): The pharmacology and biochemistry of depression. Dis. Nerv. Syst. 25, 217-221.

Price, J.M., Thornton, M.J., Mueller, L.M. (1967): Tryptophan metabolism in women using steroid hormones for ovulation control. Am. J. Clin. Nutr. 20, 452-456.

Pugsley, T.A., Lippmann, W. (1977): Effect of butaclamol, a new neuroleptic, on serotoningergic mechanisms. J. Pharm. Pharmac. 29, 135-138.

Pujol, J.F., Bobillier, P., Buguet, A., Glowinski, J. (1969): Biosynthese de la serotonine cerebrale: etude neurophysiologique et biochimique apres p-chlorophenylalanine et destruction du system du Raphe. C.R. Acad. Sci. (Paris) 268, 100-102.

Quay, W.B. (1963): Circadian rhythm in rat pineal serotonin and its modification by estrous cycle and photoperiod. Gen. Comp. Endocr. 3, 473-479.

Quay, W.B. (1968): Differences in circadian rhythms in 5-hydroxytryptamine according to brain regions. Am. J. Physiol. 215, 1448-1453.

Richards, M.P.M. (1966): Activity measured by running wheels and observation during the oestrous cycle, pregnancy and pseudopregnancy in the golden hamster. Anim. Behav. 14, 450-458.

Richter, C.P. (1927): Animal behaviour and internal drives. Q. Rev. Biol. 2, 307-343.

Robertson, M.E., Stiefel, M., Laidlaw, J.C. (1959): The influence of oestrogen on the secretion, disposition and biologic activity of cortisol. J. Clin. Endocr. 19, 1381-1398.

Robson, J.M., Botros, M. (1961): The effect of 5-hydroxytryptamine and of monoamine oxidase inhibitors on sexual maturity. J. Endocr. 22, 165-176.

Roos, B.E., Sjostrom, R. (1969): 5-hydroxyindoleacetic acid (and homovanillic acid) levels in the cerebrospinal fluid after probenecid application in patients with manic-depressive psychosis. Pharmac. Clin. 1, 153-155.

Rose, D.P. (1966): The influence of oestrogens on tryptophan metabolism in man. Clin. Sci. 31, 265-272.

Rose, D.P. (1972): Aspects of tryptophan metabolism in health and disease: a review. J. Clin. Path. 25, 17-25.

Rose, D.P., Adams, P.W. (1972): Oral contraceptives and tryptophan metabolism: effects of oestrogen in low dose combined with a progestogen and of a low-dose progestogen (Megestrol acetate) given alone. J. Clin. Path. 25, 252-258.

Rose, D.P., Craidman, I.P. (1971): Excretion of tryptophan metabolites as affected by pregnancy contraceptive steroids and steroid hormones. Am. J. Clin. Nutr. 24, 673-683.

Rose, D.P., Strong, R., Adams, P.W., Harding, P.E. (1972): Experimental vitamin B_6 deficiency and the effect of oestrogen-containing oral contraceptives on tryptophan metabolism and vitamin B_6 requirements. Clin. Sci. 42, 465-477.

Ross, G.T., Cargille, C.M., Lipsen, M.B., Rayford, P.L., Marshall, J.R., Strott, C.A., Rodbard, D. (1970): Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. Rec. Progr. Horm. Res. 26, 1-47.

Ross, J., Claybeugh, C., Clemens, L.G., Gorski, G.(1971): Short latency induction of oestrous behaviour with intracerebral gonadal hormones in ovariectomised rats. Endocrinology 89, 32-38.

Rubinstein, L., Sawyer, C.H.(1970): Role of catecholamines in stimulating the release of pituitary ovulating hormone(s) in rats. Endocrinology 86, 988-995.

Saavedra, J.M., Axelrod, J. (1973): Effect of drugs on tryptamine content of rat tissues. J. Pharmac. Exp. Ther. 185, 523-529.

Sachar, E.J., Roffworg, H.P., Gruen, P.H., Altman, N., Sassin, J. (1976): Neuroendocrine studies of depressive illness. Pharmkopsych. Neurophych. 9, 11-17.

Sanberg, A.A., Slaunwhite, W.R. (1959): Transcortin: a corticosteroid binding protein of plasma. II Levels in various conditions and the effects of oestrogens. J. Clin. Invest. 38, 1290-1297.

Sandberg, A.A., Slaunwhite, W.R., Carter, A.C. (1960): Transcortin: a corticosteroid-binding protein of plasma. III The effects of various steroids. J. Clin. Invest. 39, 1914-1926.

Sawyer, C.H., Hilliard, J. (1971): In: Proc. 3rd Int. Cong. Horm. Ster. Eds. James, V.H.T., Martini, L. Excepta Medica Foundation, Amsterdam.

Sawyer, C.H., Radford, H.M. (1978): Effects of intraventricular injections of norepinephrine on brain-pituitary-ovarian function in the rabbit. Brain Res. 146, 83-93.

Scapagnini, U., DeSchaepdryver, A.F., Preziosi, P. (1969): Influence of restraint stress, corticosterone and betamethasone on brain amine levels. Pharmac. Res. Comm. 1, 63-67.

Schildkraut, J.J. (1965): The catecholamine hypothesis of affective disorders: A review of supporting evidence. Am. J. Psychiat. 122, 509-522.

Schnaitman, C., Erwin, V.G., Greenwalt, J.W. (1967): The submitochondrial localisation of monoamine oxidase. An enzymatic marker for the outer membrane of rat liver mitochondria. J. Cell. Biol. 32, 719-735.

Schneider, H.P.G., McCann,S.M. (1970): Mono-and Indoleamines and control of LH secretion. Endocrinology 86, 1127-1133.

Schwartz, N.B. (1964): Acute effects of ovariectomy on pituitary LH, uterine weight and vaginal cornification. Am. J. Physiol. 207, 1251-1259.

Schwartz, N.B. (1969): A model for the regulation of ovulation in the rat. Rec. Progr. Horm. Res. 25, 1-55.

Segal, S., Sadousky, E., Palti, Z., Pfeifer, Y., Polishuk, W. (1975): Serotonin and 5-hydroxyindole-acetic acid in fertile and subfertile men. Fertil. Steril. 26, 314-316.

Segawa, T., Bando, S., Hosokawa, M. (1977): Brain serotonin metabolism and delta-9-tetrahydrocannabinol-induced muricide behaviour in rats. Jap. J. Pharmc. 27, 581-582.

Shaikh, A.A. (1971): Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. Biol. Reprod. 5, 297-307.

Shaw, D.M., Camps, F.E., Eccleston, E.G. (1967): 5-hydroxytryptamine in the hind brain of depressive suicides. Br. J. Psychiat. 113, 1407-1411.

Shaw, D.M., O'Keefe, J., MacSweeney, D.A. (1973): 3-methoxy-4-hydroxyphenylglycol in depression. Psychol. Med. 3, 333-336.

Sheard, M.H., Aghajanian, G.K. (1968): Stimulation of the mid-brain raphe. Effect on serotonin metabolism. J. Pharmac. Exp. Ther. 163, 425-430.

Shirley, B., Wolinsky, J., Schwartz, N.B. (1968): Effects of a single injection of an estrogen antagonist on the estrous cycle of the rat. Endocrinology 82, 959-968.

Shopsin, B., Wilk, S., Sathanathan, G., Gershon, S., Davis, K. (1974): Catecholamines and affective disorders revised. A critical assessment. J. Nerv. Ment. Dis. 158, 369-383.

Slonaker, J.R. (1924): The effect of pubescence, oestration and menopause on the voluntary activity in the albino rat. Am. J. Physiol. 68, 294-315.

Smith, C.B., Dews, P.B. (1962): Antagonism of locomotor suppressant effects of reserpine in mice. Psychopharmacologia 3, 55-59.

Smith, E.R., Davidson, J.M. (1968): Role of estrogen in the cerebral control of puberty in female rats. Endocrinology 82, 100-108.

Sorensen, C.A., Ellison, G.D. (1973): Nonlinear changes in activity and emotional reactivity scores following central noradrenergic lesions in rats. Psychopharmacologia 32, 313-325.

Soulairac, A., Lambinet, H. (1977): The effects of 5-hydroxytryptophan, a serotonin percursor, on sleep disorders. Ann. Med. Psychol, (Paris) 1, 792-798.

Squires, R.F.(1975): Evidence that 5-methoxy-NN-dimethyltryptamine is a specific substrate for MAO A in rat: implications for the indoleamine dependent behavioural syndrome. J. Neurochem. 24, 47-50.

Stefano, F.J.E., Donoso, A.O. (1967): Norepinephrine levels in rat hypothalamus during the oestrous cycle. Endocrinology 81, 1405-1406.

Stein, G., Milton, F., Bebbington, P., Wood, K., Coppen, A. (1976): Relationship between mood disturbances and free and total plasma tryptophan in postpartum women. Br. Med. J. 2, 457.

Stone, E.A., Mendlinger, S. (1974): Effect of intraventricular amines on motor activity in hypothermic rats. Res. Comm. Chem. Pathol. Pharmac. 7, 549-556.

Student (1908): The probable error of a mean. Biometrica 6, 1-25.

Svensson, T.H., Thieme, G. (1969): An investigation of a new instrument to measure motor activity of small animals. Psychopharmacologia 14, 157-163.

Szabo, M., Nakawatase, C., Kovathana, N., Frohman, L.A. (1977): Effect of the dopa decarboxylase inhibitor MK-486 on L-dopa-induced inhibition of prolactin secretion: evidence for CNS participation in the L-dopa effects. Neuroendocrinology 24, 24-34.

Tabakoff, B., Moses, F., Philips, S.R., Boulton, A.A. (1977): Effects of tranylcypromine and pargyline on brain tryptamine. Experentia 33, 380-381.

Tagliamonte, A., Fratta, W., Mercuro, G., Biggio, G., Camba, R.C., Gessa, G.L. (1972): 5-hydroxytryptophan but not tryptophan inhibits copulatory behaviour in male rats. Riv. Pharmacol. Ter. 111, 405-409.

Taleisnik, S., Velasco, M.E., Astrada, J.J. (1970): Effect of hypothalamic deafferentation on the control of luteinizing hormone secretion. J. Endocr. 46, 1-8.

Tejasen, T., Everett, J.W. (1967): Surgical analysis of the preoptico-tuberal pathway controlling ovulatory release of gonadotropins in the rat. Endocrinology 81, 1387-1396.

Tehen, S.S. (1967): The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behaviour in the rat. Psychopharmacologia 10, 204-219.

Thomson, J., Maddock, J., Aylward, M., Oswald, I. (1977): Relationship between nocturnal plasma oestrogen concentration and free plasma tryptophan in peri-menopausal women. J. Endocr. 72, 395-396.

Thung, P.J., Boot, L.M., Muhlbock, O. (1956): Senile changes in the oestrous cycle and in ovarian structure in some inbred strains of mice. Acta. Endocr. Copenh. 23, 8-32.

Tonge, S.R., Greengrass, P.M. (1971): The acute effects of oestrogen and progesterone on monoamine levels of the brain of ovariectomised rats. Psychopharmacologia 21, 374-381.

Toseland, P.A. (1974): The biochemistry of depression in women. In: Biochemistry of Women: clinical concepts. pp 165-176. Eds. Curry, A.S., Hewitt, J.V. C.R.C. Press, Cleveland.

Twarog, B.M., Page, I.H. (1953): Serotonin content of some mammalian tissues and urine, and a method for its determination. Am. J. Physiol. 175, 157-161.

Udenfriend, S., Titus, E., Weissbach, H., Peterson, R.E. (1956): Biogenesis and metabolism of 5-hydroxyindole compounds. J. Biol. Chem. 219, 335-344.

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

Van Praag, H.M. (1974): Toward a biochemical classification of depression. Adv. Biochem. Psychopharmac. 11, 357-368.

Van Praag, H.M. (1974): Toward a biochemical typology of depression. Pharmacopsychiat. 7, 281-292.

Van Praag, H.M., Korf, J., Schut, D. (1973): Cerebral monoamines and depression. Arch. Gen. Psychiat. 28, 827-833.

Van de Weile, R.L., Bogumil, J., Dyrenfurth, I., Ferin, M., Jewelewicz, R., Wazzen, M., Rizkallah, T., Mikhail, G.F. (1970): Mechanisms regulating the menstrual cycle in women. Rec. Progr. Horm. Res. 26, 63-193.

Vijayan, E., McCann, S.M. (1978): Re-evaluation of the role of catecholamines in control of gonadotropin and prolactin release. Neuroendocrinology 25, 150-165.

Wang, G.H. (1923): The relation between "spontaneous" activity and the oestrous cycle in the white rat. Comp. Psychol. Monogr. ii, No 6, 1-27.

Warbritton, J.D., Stewart, R.M., Bladessarini, R.J. (1978): Decreased locomotor activity and attenuation of amphetamine hyperactivity with intraventricular infusion of serotonin in the rat. Brain Res. 143, 373-382.

Wheaton, J.E., Martin, S.K., Swanson, L.V., Stormshak, F. (1972): Changes in hyppthalamic biogenic amines and serum LH in the ewe during the estrous cycle. J. Anim. Sci. 35, 801-804.

Wiggins, J.F., Fernstrom, J.D. (1977): L-dopa inhibits prolactin secretion in proestrous rats. Endocrinology 101, 469-474.

Wilson, C.A. (1974): Hypothalamic amines and the release of gonadotrophins and other anterior pituitary hormones. Adv. Drug. Res. 8, 119-204.

Wilson, C.A., McDonald, P.G. (1973): Inhibition of ovulation by 5-hydroxytryptamine in the adult rat. Acta. Endocr. (Kbh.) Supp 177, 137.

Wilson, C.A., McDonald, P.G. (1974): Inhibitory effect of serotonin on ovulation in adult rats. J. Endocr. 60, 253-260.

Winston, F. (1973): Oral contraceptives, pyridoxine and depression. Am. J. Psychiat. 130, 1217-1221.

Woolf, P.D., Lee, L. (1977): Effect of the serotonin precursor tryptophan on pituitary hormone secretion. J. Clin. Endocr. Metab. 45, 123-133.

Wurtman, R.J., Axelrod, J., Kelly, D.E. (1968): The pineal. Academic Press, New York.

Wyatt, R.J., Engelman, K., Kupfer, D.J., Fram, D.H., Sjoerdsma, A., Snyder, F. (1970): Effects of l-tryptophan (a natural sedative) on human sleep. Lancet ii, 842-846.

Wyatt, R.J., Zarcone, V., Engelman, K., Denent, W.C., Snyder, F., Sjoerdsma, A. (1971): Effects of 5-hydroxy-tryptophan on sleep in normal human subjects. Electroencephalogr. Clin. Neurophysiol. 30, 505-509.

Wynn, V. (1973): Vitamins and oral contraceptive use. Lancet i, 561-564.

Yuwiler, A., Wetterburg, L, Geller, E. (1971): Relationships between alternate routes of tryptophan metabolism following administration of tryptophan peroxidase inducers or stressors. J. Neurochem. 18, 593-399.

Yuwiler, A., Geller, E. (1974): Rat liver tryptophan oxygenase induced by neonatal corticoid administration and its effects on brain serotonin. Enzyme 15, 161-168.

Zemlan, F.P., Ward, I.L., Crowley, W.R., Margules, D.L. (1973): Activation of lordotic responding in female rats by suppression of serotoninergic activity. Science 179, 1010-1011.

Zolouik, A., Labhsetwar, A.P. (1973): Evidence for the theory of dual hypothalamic control of ovulation. Nature, 245, 158-159.

Zuckerman, M., Lubin, B. (1965): Manual for the multiole affect adjective checklist. Educational and Industrial testing service. San Diego, California.