5-HYDROXYTRYPTAMINE AND SEXUAL BEHAVIOUR IN RHESUS MONKEYS

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To my wife and children

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ABSTRACT

Selective inhibition of 5-hydroxytryptamine by parachlorophenylalanine (PCPA) is able to restore sexual receptivity in female rhesus monkeys made unreceptive by bilateral adrenalectomy. PCPA in the doses used reduces the levels of 5-hydroxyindoleacetic acid (5HIAA) in the cerebrospinal fluid to 40 per cent. of the normal oestradiol-treated condition. Both the increased sexual receptivity and the lowered 5HIAA levels in the CSF are in turn reversed by 5-hydroxytryptophan (5HTP), the immediate precursor of 5HT and the substance whose synthesis is inhibited by PCPA. 5HTP on its own reduces sexual receptivity and increases 5HIAA levels in the CSF of ovariectomised, oestradiol-treated (but otherwise intact) female rhesus monkeys.

A substance other than an adrenal androgen has therefore been shown to restore sexual receptivity in adrenalectomised female monkeys. Testosterone propionate and oestradiol benzoate both lower the turnover rates of 5HT in the brains of ovariectomised female monkeys, as measured by the 2 hour probenecid test. Taken together, these findings suggest that adrenal androgens could act on specific sites in the female monkey brain via 5HT-containing neural systems, to control (or at least influence) sexual receptivity. All the results of administering oestradiol to ovariectomised monkeys in these experiments are consistent with the established roles of this hormone in female sexual attractiveness and in the gonadotrophin-controlling systems of the hypothalamo-hypophyseal axis.

In contrast to these findings on 5HT and sexual receptivity in female monkeys, no clear role for 5HT-containing neural systems could be demonstrated in the grooming, aggressive or social behaviours of female monkeys. No clear role for 5HT could be demonstrated in the

refractory period following ejaculation in male monkeys, or when testosterone replacement is given to castrated male monkeys.

SUMMARY

In contrast to the influential statements of Zuckerman (1932) and others, it is now clear that gonadal hormones do influence and control the sexual behaviour of sub-human primates. To some extent even the human brain is not completely emancipated from such hormonal control. In female rhesus monkeys and baboons, ovarian oestrogens and progesterone control sexual attractiveness while adrenal androgens have a marked effect on sexual receptivity.

Some of the sites of action of such gonadal and adrenal steroids are known. Oestrogens and progesterone have their primary effects on peripheral structures, principally the vaginal tract. Adrenal androgens in physiological doses, on the other hand, have no marked effects on peripheral tissue, but act centrally on the anterior hypothalamus/preoptic area of the diencephalon, and possibly on other brain areas as well.

What is not known is how the adrenal androgens act on specific sites in the female primate's brain to control her receptivity. The suggestion has been made that sex steroids control oestrous behaviour in sub-primate mammals by modifying the activity of monoamine-containing neural systems in the CNS. For example, an inhibitory role for 5-hydroxytryptamine (5HT) in oestrous behaviour in female rats, cats and hamsters has been proposed. It is also known that 5HT is involved in the release of gonadotrophin releasing factors from the basal hypothalamus of the female rhesus monkey. But it is not known whether 5HT is involved in the neuroendocrine control of sexual receptivity in female monkeys. Nor has the possible role of 5HT-containing neural systems in other sexual behaviours such as the post-

ejaculatory refractory period of the male, been properly elucidated.

The role of 5HT in the control of sexual behaviour was therefore studied in 19 adult male and 24 adult female rhesus monkeys. Behavioural observations were made on 14 females paired with males for a series of 30-minute observation periods. Males were intact, but the females were ovariectomised and given oestradiol (15ug or 25ug/day) whenever they were tested with males. Ten were then retested after bilateral adrenalectomy (with cortisol replacement, 3mg/kg/day), and subsequently on parachlorophenylalanine (PCPA, 75mg/kg/every fourth day in phosphate buffer, pH7.4). Five of these were also tested on PCPA as before, but together with 5-hydroxytryptophan (5HTP, 20mg/kg every second day in phosphate buffer, pH7.4), the substance whose synthesis is inhibited by PCPA. The remaining 4 females were not adrenalectomised, but after ovariectomy and oestradiol they were given 5HTP alone and retested.

Parallel biochemical experiments monitored the effects of such treatments on levels of 5-hydroxyindole-3-acetic acid (5HIAA), the principle metabolite of 5HT in the CSF. Cisternal CSF was withdrawn from anaesthetised monkeys early on the day of assay, and 5HIAA was measured spectrophotofluorimetrically. In addition, the effects of small doses of ovarian and adrenal steroids (oestradiol (15ug/day); oestradiol + progesterone (15mg/day); testosterone propionate (250 ug or 400 ug/day) given for 10 days) on both 5HIAA levels and 5HT turn-over rates (2-hour probenecid test; probenecid sodium, 20mg/kg in phosphate buffer) were measured in 10 (different) ovariectomised female monkeys.

The final set of experiments measured the effects of ejaculation and castration plus testosterone replacement on levels of 5HIAA and turn-over rates of 5HT in the brains of 15 male monkeys.

PCPA is able to restore sexual receptivity in female monkeys made unreceptive by adrenalectomy, and this effect is reversed by 5HTP. PCPA in the doses used lowers the levels of 5HIAA in the CSF to about 40 per cent. of the normal levels. Testosterone and oestradiol both lower the turn-over rates of 5HT in the brains of female monkeys. The effects of oestradiol in this respect are antagonised by progesterone.

The findings are important because hitherto only adrenal androgens have been shown to restore sexual receptivity in female monkeys made unreceptive by adrenalectomy or adrenal suppression. Taken together, the findings suggest that adrenal androgens could act on specific sites in the female monkey brain, via 5HT-containing neurons, to control her sexual receptivity.

The effects of oestradiol are compatible with its role in other brain mechanisms, for example, the hypothalamo-hypophyseal system. The effects of progesterone shown here may be important, since the role of progesterone in sexual receptivity and in mood changes following oral contraceptives, for example, is not clear.

In contrast to these findings in the female, no effects of ejaculation or castration on the males' 5HIAA levels or 5HT turn-over rates could be detected. No clear role for 5HT-containing neurons in the aspects of male sexual behaviour studied under these experimental conditions could therefore be shown. In particular, no 5HT effects on the refractory period following ejaculation were demonstrated.

CHAPTER 1

INTRODUCTION

A general problem facing neuroendocrinology is that of specifying how hormones act on a neural substrate to modify the behaviour of an animal. The study of sexual and related behaviours is clearly no exception. There is no doubt that gonadal secretions are one of the important determinants of sexual behaviour in all mammals. But the way in which these hormones act on the brain or body of the animal to influence its sexual behaviour is far from clear.

Recently the suggestion has been made that sex steroids could affect behaviour by modifying the activity of biogenic animes in the brain (see recent reviews by Janowsky, Fann and Davis (1971), and Pfaff (1973)). Evidence is accumulating that this may in fact be the case. The suggestion is that monoamines, particularly 5-hydroxytryptamine (5HT), are implicated in the neural systems underlying oestrous behaviour in sub-primate mammals, such as the female rat (Meyerson 1964, a, b, c; 1968; Zemlan, Ward, Crowley and Margules, 1973), the female cat (Hoyland, Shillito and Vogt, 1970) and the female hamster (Meyerson, 1970).

What is not clear is whether monoamines are also implicated in the processes by which gonadal hormones affect the behaviour of sub-human primates and even man. In the pages that follow, the evidence which suggests that monoamines might also play such a role in primate behaviour will be presented. This will lead on to a statement of the position which pertained before these experiments were started. In the final chapter, the results of these experiments will be discussed.

This will lead on to a statement of the possible role of 5-hydroxytryptamine in the sexual behaviour of female primates.

1.1. The Sexual Behaviour of Non-Human Primates and Man

Considerable insight into the origins of human sexual behaviour could follow from a study of non-human primates (Jensen, 1973). Following Zuckerman (1932), it was held that the primate brain had evolved some measure of emancipation from hormonal control. Certainly the human female will mate at any season, during pregnancy and at nearly all stages of the menstrual cycle. This position contrasts markedly with that found in sub-primate mammals (Young, 1961).

The female sub-primate shows a definite circumscribed period of oestrus with little or no mating behaviour at other times. Sexual receptivity in the female rodent, for example, is limited to a brief period of oestrus when the female shows a special sort of behaviour. At this time, the female will accept the male's mounts without either escape or aggression, and the hollow-backed lordosis pattern is shown in response to a male mount. In the oestrous sow, the farmer is able to induce lordosis simply by pressing with his hands on the pig's back (see also Herbert, 1972). In many species, the oestrous period is associated with increased locomotion, and in some cases, such as cattle, females will mount each other during "heat".

The length of the oestrous period, like the duration of the oestrous cycle, is normally characteristic of each species, although environmental influences can have a marked effect on animals such as the baboon (Rowell, 1970). In the rat this period is a few hours only, during the afternoon or night preceding ovulation. In the sheep, it is one day out of a regular oestrous cycle of sixteen days. In cattle, heat lasts for a few days. But in general, "behavioural oestrus" is not shown at times other than the periods of late follicular growth and ovulation, which are associated with periods of high oestrogen secretion

from the ovaries. The onset of luteal progesterone often signals the end of behavioural oestrus.

Early anthropologists and other scientists were aware that many female monkeys and apes, as well as women, did not fit this pattern (for example, Hartman, 1928; Yerkes and Elder, 1936; Young and Orbison, 1944) because the female was sexually "receptive" throughout the menstrual cycle. However, emphasis on the differences between primate and non-primate mating behaviour could lead to the mistaken idea that sexual activity in primates is not influenced by gonadal hormones. Studies of groups of primates in the wild and of small groups or pairs of animals in the laboratory have shown that sexual behaviour in sub-human primates does vary with the seasons and with the female's menstrual cycle (for example, Lancaster and Lee, 1965; Michael and Herbert, 1963). The exact neuroendocrine basis for sexual behaviour in all living species of primates has not been determined, but an increasing volume of data is available. A brief review of this data follows, since the point to be made here is simply that gonadal hormones do influence the sexual and other related behaviours of sub-human primates and man.

Review of Literature

The central argument in this chapter is developed in three stages:

(1) Evidence is reviewed to establish that gonadal and adrenal steroids are important determinants of sexual and related behaviours in primates, as they are in sub-primate mammals. The literature suggests that ovarian cycles, the seasons, gonadectomy, adrenalectomy and steroid hormone treatments all have marked effects on most primate sexual behaviours, and therefore the point is firmly made.

(2) Evidence is presented on the possible sites of action of these hormones in the female primate's body.

(3) Literature relevant to the suggestion that hormones act via monoamine-containing neural systems at some of these sites of action is reviewed.

1.2. Sexual Behaviour and Ovarian Cycles

Menstruation takes place in the females of Old World monkeys and the great apes, as well as man, although there is little observable blood flow in some guenons (Jolly, 1972) or in the talapoin monkey Miopithecus talapoin (Scruton and Herbert, 1970 a, b). Menstruation is very difficult to detect in New World monkeys and is described by Jolly (1972) as being "microscopically visible". The only exception noted so far appears to be the capuchin Cebus apella, which shows menstrual bleeding for 2 - 7 days (Hamlett, 1939). Such evidence as is available would indicate that the reproductive cycles of prosimians are typified by having no menstruation (Hill, 1953).

Adequate demonstration of the role of ovarian cycles in sexual behaviour requires careful observations of such behaviour at various stages of the oestrous or menstrual cycles. Field studies are usually suggestive in this respect (see also 4.1 below); but they are clearly not conclusive, since a number of variables could be acting on an animal in any large primate group. Controlled laboratory experiments such as those of Michael, Herbert and Welegalla (1967) on Macaca mulatta or of Goldfoot (1971) on Macaca nemestrina study the effects of the ovarian cycle on male / female sexual interaction. Such studies are needed for all species of primate. Then to demonstrate the exact hormonal basis for sexual behaviour, it is necessary to remove the gonads and to test behaviour before and after systematic replacement with physiological doses of those active steroids usually secreted by the ovaries.

Detailed studies have been lacking for the prosimians until very recently (Doyle and Bekker, 1967), and are still not plentiful for either prosimians or New World monkeys. More is known about the ovarian cycle and sexual behaviour in Old World monkeys and apes.

1.3. Prosimians and New World Monkeys

The prosimians are of considerable theoretical interest in that they may provide evolutionary links between higher primates and non-primate mammals. Attention has been drawn to features such as the specialised tactile hairs of the potto (Perodicticus potto) and the highly developed olfactory sense and lack of colour and stereoscopic vision, all of which are found in prosimians and all of which could be viewed as being characteristic of more "primitive" mammals (Doyle, Pelletier and Bekker, 1967). Hill (1953) noted the placenta and the lack of a menstrual cycle as distinguishing features of prosimii. Some Madagascar lemurs live solitary lives (Petter, 1965) quite unlike higher primates which are characteristically social. Most prosimians are nocturnal, again in contrast to Old World monkeys such as the baboon (Stoltz and Saayman, 1970). The young of the lesser bushbaby Galago senegalensis moholi are carried in the mouth of the mother, like a cat (Doyle, Anderson and Bearder, 1969), and the ability to cling to the ventral fur of the mother, which is seen in many Old World monkey infants such as the vervet Cercopithecus aethiops (Struhsaker, 1967 d), is not found.

Although highly "social" prosimii do exist, such as the ringtailed lemur Lemur catta and the great white sifaka Propithecus verrauxi (Jolly, 1966), recent studies of fairly representative species of prosimian have confirmed that the patterns of sexual behaviour in these animals correspond much more closely to those found in the non-primate mammals than they do to those found in the larger Old World monkeys and higher primates (Goy and Resko, 1972).

In the thick-tailed bushbaby Galago crassicaudatus crassicaudatus, mating never occurred outside the circumscribed period of behavioural

oestrus (Eaton, Slob and Resko, 1973). Behavioural oestrus in this species was found only during times of vaginal cornification and maximum plasma oestradiol levels. The period lasted 6 days out of a cycle length of 44 days, although it is possible that this period was affected by the experimentors interrupting coitus by removing the male prior to ejaculation. The luteal phase of the cycle, during which plasma progesterone reached a peak, lasted 24 days, and the female was then not receptive to the male at all. In fact, during dioestrus and outside behavioural oestrus, she would withdraw and either threaten (or even attack) the male, if he persisted in sexual advances.

In the South African lesser bushbaby Galago senegalensis moholi, the male is excited probably by olfactory cues from a white discharge from the vagina (Sauer and Sauer, 1963) which is apparent after the opening of the vulva at oestrus. During the rest of the cycle, which lasts about 40 days in the absence of fertilisation (Butler, 1967; Doyle et al., 1967), the vagina is sealed. Behavioural oestrus, when the female will accept the male's increased sexual attentions, lasts from 1 to 3 days (Doyle, Anderson and Bearder, 1971). At times other than behavioural oestrus, the female will jump away before the male makes physical contact, and she will only rarely permit even a genital inspection or licking. When unreceptive, she sometimes turns to "box" the male, but this is met by retaliation (Doyle et al, 1967).

The lesser bushbaby female comes into oestrus twice a year (Doyle et al., 1967) as does the slender loris Loris tardigradus lydekkerianus (Ramaswami and Anand Kumar, 1962; 1965). Perodicticus potto has an ovarian cycle of about six weeks, with a circumscribed period of oestrus (Ioannou, 1966).

In the ring-tailed lemur Lemur catta, sexual behaviour is restric-

ted to a period of less than one day (probably about 12 hours) out of an ovarian cycle of 5½ weeks (Evans and Goy, 1968). Vaginal oestrus is always seen at this time, suggesting high levels of oestrogen in the blood. At oestrus the vagina opens and turns pink (Jolly, 1972), while Lemur catta breeding seasons appear to be synchronised, and are probably only 2 weeks long (Jolly, 1966).

Conaway and Sorenson (1965) and Sorenson (1970) have shown that Tupaia species have circumscribed periods of oestrus (see also Martin, 1968). This general pattern of a specific period of oestrus and fairly distinct and limited breeding seasons is therefore well established in prosimians.

1.3.1. New World Monkeys

Accounts of communication and social organisation in Platyrrhini are available (for example, Moynihan, 1967 and Mason, 1971, respectively), but sexual behaviour has received specific attention in relatively few studies, perhaps in contrast to the position in the catarrhine monkeys and apes. All the New World primates are arboreal, and observations in the field are therefore difficult (see also 4 below). The tamarins Saguinus spp like the squirrel monkey Saimiri sciureus inhabit the forest fringes and secondary growth (Thorington, 1968; Moynihan, 1967), but nearly all the other New World monkeys live in the forest canopy or various reaches of the mature forest. In general, laboratory studies of the ovarian cycle and sexual behaviour are lacking in the Platyrrhini (see also Dixon, 1973).

The New World primates studied in most detail in the field remain the howlers Alouatta palliata of Barro Colorado island (Carpenter 1934, 1965; Collias and Southwick, 1952; Altmann, 1959; Bernstein, 1964; Chivers, 1969). The female shows a clear oestrous period lasting 2 to

3 days, during which time she will initiate sexual activity with a male. If unsuccessful, she will approach other males in turn, and a consort pair may form. However, this pairing is not exclusive, and during oestrus the female could copulate with all the other males in the group. Characteristic behaviours before copulation include the rhythmic tongue movements seen in both males and females, but there is no distinct genital swelling or colour changes signalling oestrus. The approaching of males during oestrus is remarkable because "normally a female does not approach a male persistently and repeatedly" (Carpenter, 1965, p 279).

Moynihan (1964, 1967) has studied the night monkey Aotus trivirgatus, noting a family group structure of one male, one female and young. Rubbing of the perineum on branches may be sexual, but is more probably related to territorial marking. Detailed observations on sexual behaviour are lacking, and Hill (1960) reports no seasonality of births in captivity. However, captive and zoological garden data on breeding seasonality are probably of extremely limited value (Jolly, 1972, p 200).

Marmosets Callithrix spp tend to be monagamous as well, with a minimum of sexual display. Napier and Napier (1967) regard Callithrix spp as being polyoestrous, and quote early work by Russell and Zuckerman (1935) on sex skin swellings in one species of the genus. The dusky titi Callicebus moloch also forms strong and stable pair relationships, and a definite limited birth period is reported for this species (Mason, 1968).

The tamarins and pinches Saguinus spp show no external evidence of seasonal or menstrual cycling, except that marking may form a part of the female's sexual behaviour (Hampton, Hampton and Landwehr, 1966). Copulation is not often observed, but sometimes rhythmic tongue movements occur during copulation, as in the howler monkey Alouatta palliata.

Thorington (1968) did not observe copulation in Saguinus midas in the wild, but this study was only a week long.

Carpenter (1935) reports that he watched males groom females and manipulate the genitals of females in red spider monkeys Ateles geoffroyi, and quotes "considerable qualitative evidence ... that indicates that the red spider monkey has a rather definite oestrous period" (p 101). Consort pairs were observed, and when females of such pairs were "collected", they were found to have recently-formed vaginal plugs and "greatly enlarged vaginal walls and lumina". An early report by Harms (1956, cited in Napier and Napier, 1967) claims to have found what is described as a "menstrual cycle" of 24 to 27 days in Ateles.

As already mentioned, the capuchin Cebus apella is quoted as having a menstrual cycle, with a variable 15 to 20 days between periods of menstruation of 2 to 7 days (Hamlett, 1939). The same worker found that copulations were restricted to the mid-cycle period around ovulation, as measured by the presence of spermatozoa in the vagina.

In the female squirrel monkey Saimiri sciureus, laboratory investigators have often failed to detect any regular sex cycle (Clewe, 1969; Ploog, Blitz and Ploog, 1963). Du Mond and Hutchinson (1967) have pointed out that the natural synchrony of male/female breeding cycles in squirrel monkeys could be drastically altered by the move from the feral to the laboratory situation. Certainly in the Monkey Jungle in Florida (Northern Hemisphere), the breeding cycles were six months out of phase with the Iquitos home range in Peru, and this change took place gradually over three years. Rosenblum, Nathan, Nelson and Kaufman (1967) put the oestrous cycle for Saimiri at 7 or 8 days, with a period of oestrus of only a few hours. This finding, if substantiated, would make the squirrel monkey cycle the shortest of any living primate. An excellent comparative

study involving Saimiri has been done (Mason, 1971), but unfortunately no data on sexual behaviour was reported (see also Baldwin, 1971).

It is noticeable that very few studies have attempted systematic control of gonadal hormones in New World monkeys. Alvarez (1968) found that gonadectomy changed the normal social contact and structure patterns of Saimiri, and Clewe (1969) found that sexual activity in females of this species declined gradually after ovariectomy, but was restored by oestradiol benzoate replacement. Alvarez was not able to restore his monkeys to the preoperative state with hormone replacement, but Mason (1971) suggests that the doses used may have been too low.

1.4. Old World Monkeys and Apes

1.4.1. Seasonality of Breeding

Lancaster and Lee (1965) distinguished between a mating season, to which fertile matings are restricted; a mating peak, in which most fertile matings occur, and which is indicated by a birth peak later in the year; and a random distribution of matings throughout the year. Jolly (1972) points out that it is relatively easy to detect a distinct birth season in a species, but a great deal of data is required to distinguish species with a birth peak from those that mate and breed randomly throughout the year.

Seasonality of breeding is very much affected by factors in the environment, such as daylight, ambient temperature, precipitation and food availability. For example, Lindburg (1971) detected a distinct mating season from mid-October to mid-January, the autumn and winter months, in wild rhesus monkeys Macaca mulatta in the Asarori forests of North India. This gave a birth season in April and May, when new spring growth of flowering and fruiting trees started. Lactation was coincident with warm temperatures, heavy rainfall and the abundant food supply of the summer monsoon. Breeding seasons in the transplanted groups of rhesus monkeys in Puerto Rican island colonies, such as Cayo Santiago, are distinct (Koford, 1965), and appear to have adapted to climatic conditions on the islands (Conaway and Sade, 1965), possibly involving dietary factors (Vandenbergh and Vessey, 1968).

It is likely that all macaques are seasonal breeders. Abundant evidence exists for the rhesus monkey (Carpenter, 1942; Altmann, 1962; Southwick, Beg and Siddiqui, 1965) and the Japanese macaque Macaca fuscata (for example, Kawai, 1966; Frisch, 1968; Eaton, 1973). The Barbary "ape" Macaca sylvana (Mac Roberts and Mac Roberts, 1966) and the

bonnet macaque Macaca radiata (Simonds, 1965), like the pig-tailed macaque Macaca nemistrina (Bernstein, 1967) are all either seasonal breeders or have a fairly pronounced peak of births. Male rhesus monkeys show seasonal spermatogenisis (Conaway and Sade, 1965), like the squirrel monkey (Du Mond and Hutchinson, 1967). It has also been shown that the presence of oestrous females is enough to cause testicular changes such as sperm and androgen production (the latter affecting the red sex skin) and to induce copulatory behaviour in sexually quiescent male rhesus monkeys in the non-breeding season (Vandenbergh, 1969).

It is also possible that the breeding season is normally terminated by the female becoming pregnant, at least in the Japanese monkey (Eaton, 1973). Eaton implanted interuterine contraceptive devices in some of the Japanese monkey females at Oregon, and noted that the mating period in the I.U.D-carrying females lasted longer than the usual autumn mating season of 4 to 6 months, observed in Japan by Kawai and fellow workers and in the confined troop of Japanese monkeys at Oregon.

Breeding seasons or peaks have also been observed in Hanuman langurs Presbytis entellus (Jay, 1965). In the Nilgiri langur Presbytis johnii, there is a primary birth season in May and June which is coincident with the southwest monsoon, and a secondary birth season in November and extending perhaps into early February, coincident with the northeast monsoon (Poirier, 1970). As far as is known, the guenons of Africa (Cercopithecus spp) all have definite peaks, and may in fact all have true breeding seasons (see Jolly, 1972, p 200). Hall (1965) noted a distinct birth peak in the patas monkey Erythrocebus patas in Uganda.

It is not clear whether there is a breeding season in all baboons. In Kenya, most rain falls between October and May, and the olive baboon

Papio anubis is reported to show a birth peak in October to December, although isolated births were recorded throughout the year (De Vore and Hall, 1965). The same marked trend is recorded for the hamadrayas baboon Papio hamadryas (Kummer, 1968 a, b), only the birth peak is in May to August. However, in the Cape of Good Hope groups of chacma baboons Papio ursinus, there was no evidence of a birth peak, and black infants were seen in all months of the year (Hall, 1962). Nor was there a breeding season, in the strict sense of the term, in olive baboons in Queen Elizabeth National Park, Uganda (Rowell, 1967). From the available evidence, it would seem as if births in chimpanzees are random (for example, Goodall, 1963) and not enough data exist on gorillas for a judgement to be made (Schaller, 1963; 1965; 1967; Fossey, 1972). There is, however, some evidence that human populations may have slight birth peaks. For instance, in Europe most fertile mating is done in the spring, while in America the peak for conceptions is from October through to Christmas (Cowgill, 1966, 1969).

With the possible exception of certain populations of baboon living in specific areas, it seems clear that the Old World monkeys all show marked seasonal breeding peaks. Such seasonality would suggest gonadal hormone influences on sexual behaviour, as is the case in subprimate mammals such as the impala Aepyceros melampus (Fairall, 1972), the red deer Cervus elaphus (Lincoln, Guinness and Short, 1972) and the caribou (Whitehead and Mc Ewan, 1973).

1.4.2. Ovarian Cycles and Sexual Behaviour in Wild Groups

The Old World monkey female is therefore seasonally polyoestrous, although it is unusual for her to cycle and ovulate for any length of time, in the wild, without fertilisation taking place. There is now ample evidence to suggest that the menstrual cycle influences the inci-

dence of sexual behaviour in Old World monkeys in the wild.

In some species such as the baboon, the chimpanzee, the mangabey, the pigtailed macaque, the Barbary "ape" and the talapoin, the female has a prominent sexual swelling of the vulva and perineum during the follicular phase of the menstrual cycle. In the baboon, turgescence of the skin is dependent on oestrogen (Gillman and Gilbert, 1946) from the developing ovarian follicle, and this turgescent phase of the cycle has been divided by Rowell (1967) into two stages, namely,"inflating" (early follicular) and "swollen" (late follicular and mid-cycle). The deturgescent phase is progesterone-dependent, and is divided into "deflating" (early luteal) and "flat" stages. Calculations have been based on an estimate of 7.9.7.9. days for each of the four stages of the cycle (Saayman, 1970).

In the talapoin monkey Miopithecus talapoin, the sexual skin swells for between 15 and 20 days and then subsides during the luteal phase of a 33 day cycle (Scruton and Herbert, 1970). It has been shown that oestrogen controls this swollen sex skin by administering oestradiol to ovariectomised females, and thus reproducing sex skins typical of mid-cycle (Scruton and Herbert, 1972).

In field studies of those monkeys which show marked sex swellings, the relationship between sex behaviour and the stages of the menstrual cycle can therefore be measured with some degree of confidence. In the troops of baboons studied in detail, all observers agree that sexual interactions increase during the late inflating stage and reach a maximum at the swollen mid-cycle stage. There is a rapid decline in sexual interactions during the deflating (luteal) phase of the cycle. This pattern has been found in the chacma baboon (Hall 1962) and confirmed by Saayman (1970); in the olive baboon Papio anubis by Hall and DeVore (1965) in

Kenya and by Rowell (1967, in Papio anubis doguera) in Uganda, and in the hamadryas baboon by Kummer (1968) in Ethiopia. The same pattern is suggested by the remarks made about oestrous and consorting females in the guinea baboon, Papio papio by Dunbar and Nathan (1972).

Full copulatory activity in the chimpanzee Pan troglodytes is maximal at periods of maximum skin turgescence as well, and the oestrous period lasts about 6 days in a 35-day menstrual cycle (Goodall, 1965; Reynolds and Reynolds, 1965; Van Lawick-Goodall, 1968; Sugiyama, 1968). A feature of these matings is a marked "promiscuity" on some occasions, one female copulating with as many as seven successive males while at this stage of the cycle (Goodall, 1965; Van Lawick-Goodall, 1968).

Although sex skin swelling and colour changes are not found to the same extent in macaque species (with exceptions such as Macaca sylvana and Macaca nemistrina), and indeed are absent in some species such as the bonnet of South India (Simonds, 1965), the influence of the menstrual cycle on sexual behaviour is at least as marked. Thus rhesus monkeys show the greatest incidence of full copulatory behaviour in the middle third of the menstrual cycle, particularly during the time of consort pairing (Carpenter, 1942). These early findings have subsequently received considerable support (for example, Altmann, 1962; Conaway and Koford, 1965; Southwick et al 1965; Vandenbergh and Vessey, 1968; Lindburg, 1971). Lindburg notes that in North India the "behavioural oestrous" period was usually about 1 to 3 days in length, during which time copulation reached a peak. Field studies have confirmed similar patterns for the bonnet macaque (Simonds, 1965), the pigtailed monkey (Bernstein, 1967), the Barbary "ape" (Mac Roberts and Mac Roberts, 1966) and the Japanese monkey (Kawai, 1966).

Among the Asian leaf eaters, the Hanuman langur female is sexually

active for about seven days at mid-cycle (Jay 1963,1965), and Bernstein (1968) states that the sexual soliciting and copulations which he observed in the lutong (Presbytis cristatus) were "presumably by oestrous females". However, a puzzling case is that of the Nilgiri langur Presbytis johnii, where Poirier (1970) was not able to observe copulatory behaviour at all during 1250 hours of field study. Poirier suggests tentatively that this could be due to the observer upsetting the study population, and suggests a similar explanation for the paucity of observed copulations in gorilla groups (Schaller, 1963; Fossey, 1972). Certainly Fossey has observed only 8 copulations in more than 2250 hours of field observations of Gorilla gorilla beringei (Fossey 1970, 1972), and she does suggest that play behaviour in young gorillas is inhibited by observer presence. This explanation, if true, raises serious issues, since it would cast doubt on the reliability of all field observations of sub-human primate sexual behaviour. However, it is more likely that the gorilla and the Nilgiri langur are special cases in this respect, and it remains possible that these animals do not copulate very much. There is no suggestion that macaque or baboon groups have been inhibited by observer presence in this way (for example, Lindburg, 1971; Saayman, 1970).

The suggestion that human sexual interaction is influenced by the menstrual cycle in women not taking contraceptive measures has been made, on the basis of studies in the southern states of America (Udry and Morris, 1968). The extent to which these interesting findings can be generalised to other groups is not, however, clear at this stage.

1.4.3. Ovarian Cycles and Sexual Behaviour in Captive Animals

1.4.3.1. Social Groups

In a small captive group of olive baboons in Uganda, Rowell (1967) has confirmed a massive peak of sexual interaction for swollen females.

Inflating females showed more sexual interaction with two virile males in the group than did either deflating or flat females.

In pigtailed macaques, Goldfoot (1971) has drawn attention to the great importance of dominance and other social factors in the group, in determining the extent to which the menstrual cycle is able to influence sexual behaviour. By introducing a male into groups of 3 females in which clear linear dominance hierarchies were already established, he was able to measure the sexual behaviour determined by the interaction of the dominance hierarchy with 3 different stages of the females' menstrual cycle, namely, early follicular, mid-cycle and luteal stages.

The highest score on all measures of sexual behaviour, including ejaculations, was achieved when the alpha female was at mid-cycle. When the beta female was at mid-cycle, her endocrine state was sufficient to "override" her lower dominance status and she scored highest on sexual interaction with the male, at the expense of the alpha female. However, when the gamma female was at mid-cycle, the male copulated (but did not ejaculate) with the alpha female and ignored the gamma female, although the latter was the only mid-cycle female in the group and the former was well into the luteal phase of her cycle. Further attention will be given to this study under 4.2.2. below. For the present, it should be noted that these findings accord well with data from feral groups, where the dominant males form consorts with specific females but not with others (Lindburg, 1971).

1.4.3.2. Paired observations

In the last ten years, intensive laboratory studies, mostly at the Bethlem Hospital in London, have shown that sexual behaviour in oppositely-sexed pairs of rhesus monkeys varies with the female's menstrual cycle

and reaches a peak at mid-cycle (Michael and Herbert, 1963, 1964; Michael, 1965; Herbert and Michael, 1965; Herbert, 1965, 1966; Michael, Herbert and Welegalla, 1967; Michael and Zumpe, 1970). An interesting finding in some cases was that of a secondary, smaller peak of sexual activity just before menstruation. These studies were partly anticipated by Ball and Hartman (1935) who showed that sexual "excitability" increased around ovulation and then fell away (see also Everitt, 1970, p 5).

It should be noted that a contrary finding by Rowell (1963) is available. She claimed that copulations in rhesus monkeys were evenly distributed throughout the cycle, with, if anything, a peak in the infertile phases of the cycle (see also Goy and Resko, 1972, p 709). However, it is clear that this finding reflects the gross behavioural measures used, since each mount by the male was scored as a copulation, and the number of "copulations" per day were then counted. Since the rhesus monkey is a multiple mounter, and since it is relatively common for males to mount even "dioestrous" females (but not to ejaculate with them), it is probable that more sensitive behavioural indices would have produced a different result.

This finding of mid-cycle increases in complete sexual interactions (involving intromitted mounts with thrusts and ejaculation by the male), is supported by studies on other captive species. Yerkes and Elder (1936) and Young and Orbison (1944) report the same pattern for chimpanzees, noting a marked decline in copulations after ovulation. Bullock, Paris, Goy and Resko (1968) found the same in pigtailed macaques.

1.4.4. Gonadectomy and Hormone Replacement

In laboratory sub-primates such as the rat, guinea pig and hamster, all mating behaviour is completely and reliably abolished by ovariectomy

of the female (Beach, 1947; Young, 1961). This pattern is wide-spread in sub-primate mammals such as, for example, the guinea pig (Boling and Blandau, 1939), the mouse (Ring, 1944) and the rat (Ford and Beach, 1951). It is made use of in controlling the behaviour of domestic pets, so that ovariectomised cats become less "attractive" to males in that they are approached less. In addition, they will then not allow copulation, but will meet copulation attempts with escape or aggression. In contrast to this general pattern in the sub-primates, ovariectomy in women has inconsistent and often not very serious effects on sexual behaviour (Filler and Drezner, 1944; evidence reviewed by Money, 1961, 1965).

The sexual behaviour of sub-primate mammals can be restored by replacing ovarian steroids. In the case of the carnivores, oestrogen alone is sufficient to bring the ovariectomised female into full vaginal and behavioural oestrus (cat - Michael and Scott, 1957; ferret - Marshall and Hammond, 1945; ferret - Herbert, personal communication; dog - Leatham, 1938). In rodents and some other mammals, a "synergistic" effect with progesterone is required. In the rat, mouse, hamster and guinea pig, an injection of oestradiol followed several hours later by a larger dose of progesterone will induce behaviour typical of "natural" oestrus (see also Herbert, 1972). By contrast, but consistent with the findings following ovariectomy, sexual behaviour in women is not dependent on oestrogen replacement (Filler and Drezner, 1944; Ford and Beach, 1951). Progestogenic compounds, if anything, have a negative effect on women's "libido" when taken orally for controlling fertility (Grant and Mears, 1967), but this point will be discussed in more detail later.

In rhesus monkeys, ovariectomy abolishes the rhythmical fluctuations in sexual and closely related behaviours (such as grooming) which are seen in pairs of animals which include an intact cycling female (Michael and Herbert, 1963).

Ball (1936) reported that ovariectomy of female rhesus monkeys reduced their "sex interest", but that this was restored again by oestrogen replacement. This effect was in turn antagonised by the addition of progesterone (Ball, 1941). Subsequent research has served to refine Ball's earlier concept of "sex interest", but has not altered his general conclusions. Ovariectomy abolishes the male's mounting rhythms and decreases the number of mounts he makes, while subsequent oestradiol replacement restores these mounting levels to those typical of the mid-cycle condition (Michael and Herbert, 1963, 1964; Herbert 1965, 1966 a, 1967 b, 1968 b; Michael and Saayman, 1967).

The decline in sexual behaviour in oppositely-sexed pairs of rhesus monkeys following ovariectomy could be due to changes either in the male's behaviour or in the female's behaviour, or, indeed, to some interaction of the two. In this connection, two independent hormone-dependent mechanisms have been proposed (Herbert 1965; Michael, Saayman and Zumpe, 1967; Saayman, 1970; Herbert, 1970), namely, (1) female sexual attractiveness and (2) female sexual receptivity.

Female attractiveness affects primarily the sexual behaviour of the male, while a change in female receptivity is detected primarily in the female. However, at least as important are the secondary effects which these changes could have on the other partner of each pair. For example, a female could be made less attractive to a male, and could then respond to his lowered mounting rates by actually "presenting" for sexual interaction more often than she did before. This would not necessarily reflect any increase in her "receptivity", as might perhaps be concluded from her increased presentation rate. Conversely, a female made unreceptive might refuse a male's mounting attempts. This could inhibit his mounting, but is clearly a secondary and not a primary effect on her "attractiveness".

These points have been forcibly put by Herbert (1970 , and elsewhere), and are crucial to an adequate analysis of any changes in sexual behaviour which may result from manipulations of the female's hormonal state in even the simplest laboratory situation, the paired observation test. In laboratory groups of monkeys, these factors assume an even greater significance. However, adequate attention has not always been paid to these factors. For example, Michael (1968) has argued that stilboestrol implanted into the basal diencephalon of ovariectomised female rhesus monkeys makes them more "receptive". But before this interpretation of the results could be justified, it would have to be shown that there was no possibility of enough oestrogen escaping from the brain implant into the general circulation to affect the quality of the female's vagina. It is now clear that cornification of the vagina by oestrogenic substances plays a central part in female sexual attractiveness (see further description below). In this work by Michael, male sexual activity was also stimulated. While this could be an effect secondary to the female's increased "receptivity" (as argued), the possibility that the primary effect was on female "attractiveness" cannot be excluded.

1.4.5. Female Sexual Attractiveness

As noted above, ovariectomy of the female depresses the male's sexual behaviours to low levels. He mounts less frequently and ejaculates less often per test. The time taken to ejaculate lengthens, while mounting rates and the number of thrusts per intromitted mount very often decline as well (see Everitt and Herbert, 1972). A consistent finding is that the proportion of female presentations effective in inducing a male mount declines after ovariectomy (Trimble and Herbert, 1968).

Oestradiol replacement consistently restores male sexual behaviours to high levels, but is not always consistent in its effects on female

behaviour (Herbert, 1970). The male's sexual behaviour with an oestradiol-treated female is effectively the same as that recorded when he is paired with an intact female at mid-cycle (Michael, Herbert and Welegalla, 1967; Herbert, 1967 a). In particular, such female presentations as are made are now much more effective in stimulating male mounting, while in addition the male initiates more sexual behaviour himself.

In view of the arguments presented above, it is possible that the male is now responding to increased female "receptivity" caused by the oestradiol replacement. However, it is clear that this is not the case, for two reasons. First, relatively few of the mounts made by the males are initiated by a presentation from the female. Mounts are mostly male-initiated, AT LEAST IN SOME RHESUS MONKEYS (HERBERT, 1970).

Some females hardly ever initiate sexual behaviour, and so changes in their behaviours are not sufficient to explain the observed changes in the sexual activity of the pair. Secondly, some females actually present less after oestradiol treatment than they did before. Others present more often, but in others presentation rates stay the same (Trimble and Herbert, 1968). Ovariectomised females, like intact female monkeys, refuse male mounting attempts very infrequently, so no changes in this behaviour after oestrogen treatment would be expected (see also Everitt and Herbert, 1972).

Since changes in the postures and behaviour of the females after ovariectomy (and subsequent oestrogen replacement) are not sufficient to account for the marked effects of these treatments on the sex activity of the pair, it is necessary to examine other possible parameters. Rhesus monkeys do not show the marked oestrogen-dependent sex skin swellings found in baboons or talapoins, but the sex skin does show hormone-dependent colour changes, and oedema is seen on occasions (see, for example, Rowell, 1967). The perineum is bright red in the intact female, but

usually fades to a dull pink after ovariectomy (see fig. 1).

However, this colour change per se is not what stimulates male sexual behaviour in the caged situation, although this does not exclude the possibility that it may act in some other role, for instance as a "distance" cue (Wickler, 1967), in the wild. Herbert (1966 b) applied oestrogen cream to the sex skin of ovariectomised females and restored the red colour (see fig. 2), but this had no marked effect on male sex behaviour. The vaginal smears of these females remained uncornified, showing that insufficient oestrogen had been absorbed into the general circulation to cornify the vagina, even though the local application was sufficient to redden the sex skin.

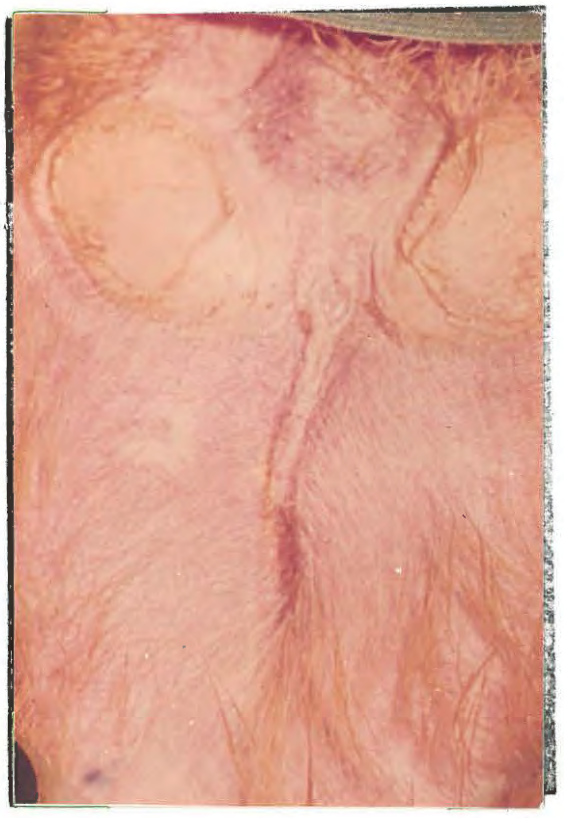
This was in marked contrast to the position when oestrogen cream was applied directly into the vagina as well as to the sex skin. The vaginal smears were then fully cornified, and the males showed marked increases of sexual behaviour; they mounted the females more often, ejaculated regularly with them and accepted their sex invitations (Herbert, 1966 b; see also Everitt and Herbert, 1972; confirmed by Michael and Saayman, 1968).

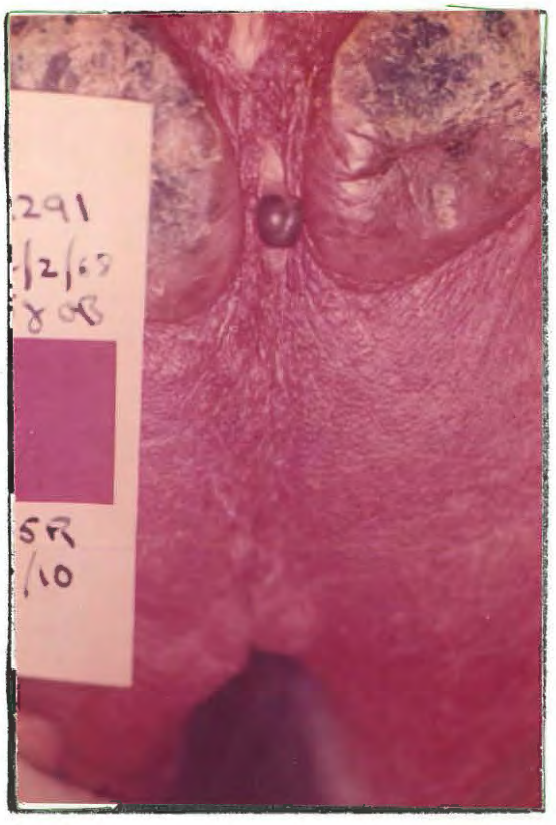
This work showed that "the sex skin is not the origin of the oestrogen dependent stimulus" acting on the male (Herbert, 1970, p 125), but did suggest that olfactory cues (Weiner, 1966), produced by the female's cornified vagina, might be responsible (Herbert, 1966 b). The suggestion was taken up by Michael and Saayman (1968), and a series of elegart experiments followed in which the origin, chemical composition and action of these olfactory cues were detailed.

Males were tested intact and when rendered anosmic in an operant (lever-pressing) laboratory situation (Michael and Keverne 1968 a, b).

Figure 1 Sex skin of female rhesus monkey after ovariectomy. Note pale colour (contrast fig. 2).

Figure 2 Sex skin of female rhesus monkey after oestradiol replacement. Note bright red colour (contrast fig. 1).

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The olfactory cue or pheromone (Gleason and Reynierse, 1969) was found to be of vaginal origin by pipetting vaginal secretions from an oestrogen-treated donor female to the sex-skin of an untreated ovariectomised recipient. Intact males started lever pressing to gain access to the recipient females shortly after the vaginal lavages had been applied, in marked contrast to their earlier behaviours with those females (Michael and Keverne, 1970). Subsequent work showed that the pheromone fractions of the vaginal secretions were soluble in ether (Keverne and Michael, 1971), and were then identified as short-chain aliphatic acids (Michael, Keverne and Bonsall, 1971). Finally, at least five of the particular aliphatic acids were identified, and a "cocktail" of such acids in the correct proportions was synthesised and shown to act on male sexual behaviour in the appropriate way (Curtis, Ballantine, Keverne, Bonsall and Michael, 1971). It seems that this very important aspect of female sexual attractiveness has therefore been demonstrated.

Progesterone antagonises the effects of oestrogen on the vaginal epithelium, and therefore COULD antagonise the production of pheromones. Giving progesterone to oestrogen-treated females also antagonises the effects of oestrogen on the pair's sexual behaviour (Herbert, 1965; 1967 a). Because progesterone COULD HAVE such marked effects on female attractiveness, it is difficult to dissect out the exact effects of progesterone on female sexual behaviour. For example, the suggestion has been made that progesterone causes a loss of sexual receptivity in female monkeys (Michael, Saayman and Zumpe, 1967 a, b, 1968; Michael and Zumpe, 1968; Michael and Welegella, 1968). While this may yet turn out to be the case (see below), FUTURE EXPERIMENTS ON THIS POINT SHOULD always distinguish between a primary effect on the female's receptivity and a secondary effect on the female caused by the male's reduced behaviour because of her loss of attractiveness.

At the same time, Goy and Resko (1972) have drawn attention to a reciprocal relationship existing between the concentrations of progesterone and of testosterone in the systemic plasma of intact cycling rhesus females (p 710). In view of the arguments which will be presented below, which emphasise a central role for adrenal androgens in female sexual receptivity, such a demonstrated reciprocal relationship is important.

For the present, it should be noted that one POSSIBLE effect of luteal progesterone in the intact cycling female is to antagonise pheromone production and hence sexual attractiveness. To the extent that this occurs, it will make isolation of the effects of progesterone on sexual receptivity more difficult.

The findings of Saayman (1972 b) on the effects of slow-absorbing implants of oestrogen and Provera (progesterone) in ovariectomised female baboons released to join free-ranging groups support the above conclusions. It is probable that the males were attracted by olfactory cues from the vaginas of oestrogen-treated females. Indeed, considerable support for all the above findings on sexual attractiveness in the laboratory is given by work on feral groups. The work of Vandenbergh (1969) has already been mentioned, and would argue for some pheromonal influence from the female in inducing spermatogenisis and sexual behaviour in rhesus males out of the breeding season. Saayman (1968, 1969, 1970) has presented valuable evidence to support the notion of olfactory components of female attractiveness in wild groups of chacma baboons in the Northern Transvaal.

1.4.6. Female Sexual Receptivity

Female attractiveness is thus mediated by peripheral cues from the females genital tract, under ovarian hormone control. But ovariectomised monkeys and women remain sexually receptive to males. It is therefore necessary to explain the "baseline" levels of sexual behaviour which

remain after ovariectomy or in the luteal and early follicular phases of the menstrual cycle of higher primates.

The suggestion has been made that androgens mediate sexual receptivity in women (Shorr, Papanicolaou and Stimmel, 1938; Greenblatt, 1943; Salmon and Geist, 1943; Waxenburg, Drellich and Sutherland, 1959; Money 1961; Benjamin, 1967). Unfortunately, however, the evidence is taken from clinical records, and there are difficulties in accepting evidence derived during the course of androgen treatments of, for instance, metastatic tumour conditions. It could be that the effects of androgens in increasing "libido" are clouded by feelings of well-being associated with the alleviation (even if only temporarily) of the cancer (even if this is terminal).

The role of androgens in female receptivity suggested by this clinical material has, however, been investigated in detail in the rhesus monkey. Ovariectomised female monkeys were given testosterone propionate in three different dosages of 1, 5, or 25mg/day (Trimble, 1967; Herbert and Trimble, 1967; Trimble and Herbert, 1968). At the lowest dose of 1mg/day the presentation rates of females were increased significantly. However, these increased presentation rates were not effective in inducing more male mountings, in contrast to the effects of oestradiol (1.4.5 above).

Testosterone does redden the sex skin of the female, but this had no effect on the male, thus supporting the view that in proximal male/female sexual interaction the sex skin colour is not of crucial importance. Significantly, testosterone did not cornify the vaginal epithelium. Hence the conclusion that testosterone affects the female's receptivity but not her attractiveness. Larger doses of testosterone propionate (5mg and 25mg/day) were less effective in stimulating sexual re-

ceptivity, and too large a dose actually induced aggressiveness and sexual unreceptivity in the female (Trimble and Herbert, 1968).

These experiments demonstrated that androgens stimulate sexual receptivity in female monkeys. But it was not clear that this was the mechanism by which receptivity is normally maintained through the menstrual cycle. The main source of androgens in female rhesus monkeys and women is the adrenal cortex (Baird, Horton, Longcope and Tait, 1968; Osborn and Yannonne, 1971; Resko, 1971). Androgens are also produced by the ovaries (Osborn and Yannonne, 1971), but in lesser amounts, and cyclically. The adrenal androgens could therefore underlie the baseline levels of sexual receptivity seen in intact females throughout the cycle.

The activity of the adrenal cortex was therefore suppressed by dexamethasone sodium phosphate, administered to oestradiol-treated ovariectomised female monkeys (Everitt and Herbert, 1969 b). This synthetic steroid acts on the adrenal by inhibiting ACTH secretion from the pituitary. The result of this treatment was a marked decrease in female sexual receptivity. However, this was reversed by very small amounts of testosterone propionate (100ug, 200ug/day) or androstenedione (100, 200, 400ug/day), but not by two other adrenal cortex secretions, cortisol and progesterone.

The role of the adrenal androgens in the maintenance of sexual receptivity was demonstrated finally by adrenalectomy of ovariectomised female monkeys (Everitt, Herbert and Hamer, 1972). The animals were maintained on cortisol and given oestradiol as usual. They were found to be unreceptive to males. They refused more often, presented less and initiated and accepted a lower proportion of sexual behaviour with males, who nonetheless found them attractive, as evidenced by their high

male acceptance ratios (see also Everitt and Herbert, 1972, p 250).

Small amounts (200, 400ug/day) of androstenedione restored sexual receptivity to levels not significantly different from the pre-adrenalectomy condition. Androstenedione is a major adrenal androgen (Resko, 1971), and is probably converted by peripheral tissue to the more "potent" androgenic steroid testosterone (Baird et al, 1968). Relatively small amounts of testosterone, as such, are secreted by the adrenals, but the use of a "precursor" hormone which can subsequently be converted by target tissue to another more active hormone is not unusual in the endocrine system, and perhaps avoids the necessity for high levels of "potent"androgen in the systemic plasma (Baird, 1972). The major adrenal androgen in terms of quantity is dehydroepiandrosterone (DHA) but this was not effective in restoring sexual behaviour to adrenalectomised females (Everitt et al., 1972). DHA is usually viewed as a "weak" androgen (Dorfman and Shiply, 1956, cited in Everitt and Herbert, 1972) and so this finding is consistent with its known androgenic properties.

In these experiments, no peripheral changes could be detected after either adrenalectomy or androgen replacement. The females were already receiving oestradiol, so that the sex skins were red and the vaginas fully cornified, and no additional effects of androgens on the female genitalia were detected. In particular, neither the length nor the width of either the glans or the shaft of the clitoris was changed significantly (Everitt et al, 1972). This is no doubt due to the small amounts of androgens used, since large amounts of testosterone propionate could cause clitoral hypertrophy and increased sensitivity (Trimble and Herbert, 1968).

1.4.7. Summary of Sexual Attractiveness and Receptivity

A summary of the effects of gonadal and adrenal hormones on sexual behaviour in rhesus monkeys in the context of the menstrual cycle has

been given by Everitt and Herbert (1972), on the basis of these findings. The discussion referred to a diagram (p 251), which is reprinted below:-

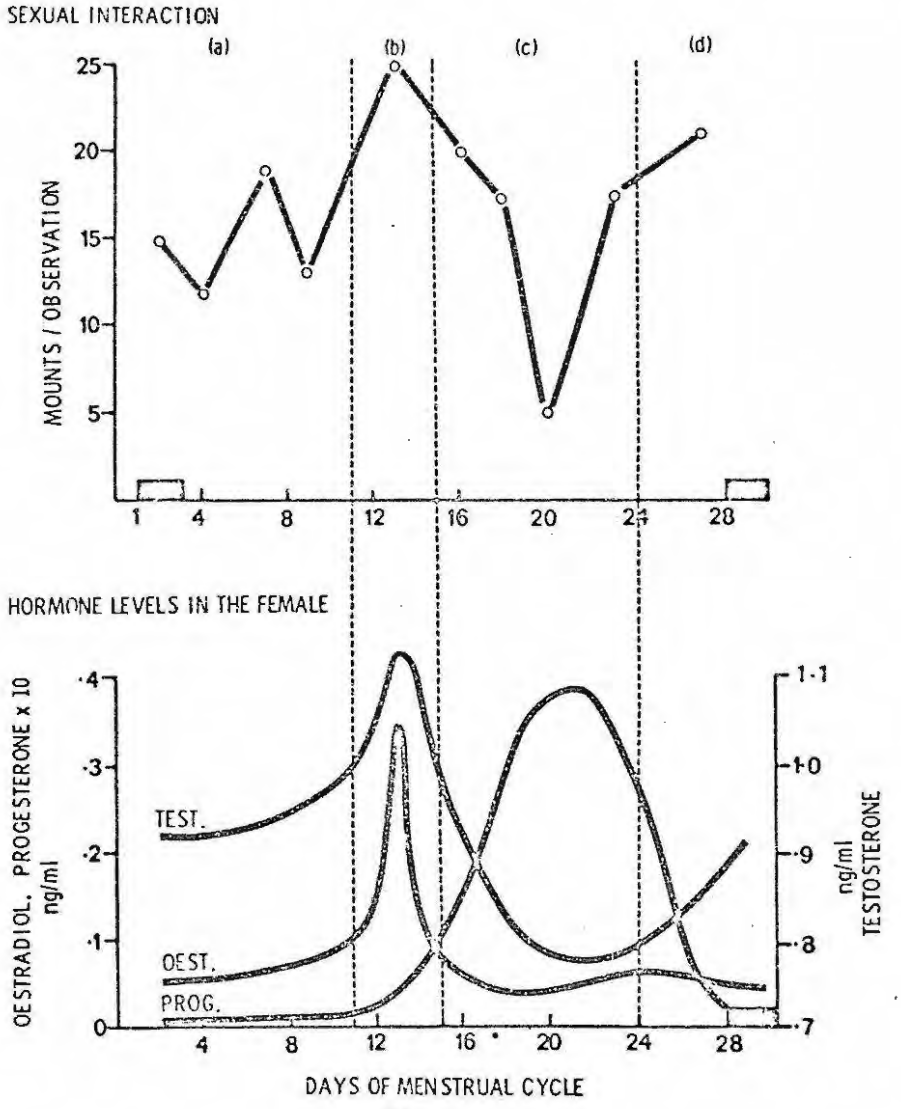


Fig. 3.

The relationship between sexual interaction and hormone levels in the female rhesus monkey.

Above: Sexual interaction measured as mounts/observation period based on data from Herbert 1967.

Below: An outline of hormonal levels in the female rhesus monkey based on data of Knobil et al. 1967; 1971 and Hess & Resko 1972.

(a) — Increasing attractiveness.
(b) — Maximum attractiveness, maximum receptivity.
(c) — Drecreasing attractiveness, ? decreasing receptivity.
(d) — Increasing attractiveness, ? increasing receptivity.
▨ — Menstrual bleeding.

During the first 10 days of the cycle (the early follicular phase), the systemic plasma of the female contains increasing levels of oestrogen (Hotchkiss, Atkinson and Knobil, 1971). This is associated with increasing levels of female attractiveness, and hence sexual stimulation of the male. At the same time, increasing levels of androgens from the ovaries are super-imposed on the higher levels of androgens from the adrenals. Androgen levels therefore increase (Hess and Resko, 1973), and so female sexual receptivity increases. At mid-cycle, both oestrogen and androgen levels are at a maximum, and hence sexual activity in the pair will reach a peak.

In the luteal phase of the cycle, progesterone antagonises the effects of oestrogen on the vaginal tract of the female. In addition, it is possible that progesterone could lower the levels of androgen in the blood (Hess and Resko, 1973). For either reason, or for both, the level of sexual activity in the pair will decrease markedly. Further, it has been suggested that progesterone could compete with androgens for receptor sites in the brain (in the chicken - Meyer, 1972; in the mouse - Erpino and Chappelle, 1971; Erpino, 1973) or antagonise the effects of androgens on other behaviours such as marking (in the gerbil - Griffo and Lee, 1973). In this way, progesterone could also lower sexual receptivity in female monkeys. This was, of course, suggested by earlier workers, and is quite consistent with their findings (Michael, Herbert and Saayman, 1966; Michael, Saayman and Zumpe 1967 a, b, 1968).

The final point to be noted about this explanatory diagram is that androgen levels never decline to zero, because the adrenals continue to secrete some androgens throughout the cycle. This may provide the basis for the chronic sexual receptivity of female higher primates including women. The"emancipation" of the primate brain from hormone

control, suggested forty years ago, would then be seen as the result of an evolutionary development whereby one part of sexual behaviour, namely, female receptivity, is controlled primarily by the adrenals rather than by the ovaries.

1.5 Androgens

1.5.1. Androgens in Sub-Primate Mammals

Androgens have been administered to female sub-primates in two contexts, usually closely related. First, a considerable body of data is now available on androgenisation of bodily tissue plus the brain and genital tract during foetal development (reviews by Short, 1972; Reinisch, 1974). Secondly, androgens administered in adulthood to androgenised animals activate male patterns of sex behaviour. These two actions of androgens are usually referred to as the "organising" and the "activating" actions respectively.

If androgen is present during "critical stages" of foetal development, permanent masculinisation takes place in both the reproductive anatomy and the brains of the foetal animals. This period can be as short as twelve hours or less in the developing rat brain (Arai and Gorski, 1968 a, b, c), and is usually controlled by relatively small amounts of testicular androgens. The critical period for such androgenisation varies from species to species. In the hamster (Swanson and Crossley, 1971), the mouse (Edwards, 1969) and the rat (Grady, Phoenix and Young, 1965), it is in about the first postnatal week. In the guinea pig (Phoenix, Goy, Gerall and Young, 1959), the rhesus monkey (Young, Goy and Phoenix, 1964), and women (Ehrhardt and Money, 1967) it is during gestation.

If androgen is administered experimentally during the critical period, the probability that the individual will subsequently show "masculine type" sexual and related behaviours under activating androgens in adult life is increased markedly, irrespective of whether the animal was a genetic male or female. Such early androgenisation at the

same time reduces the probability that the animal will subsequently show "female type" behaviours under exogenous oestrogen stimulation as an adult.

No corresponding "organising" role for oestrogens in female-type neural structures seems to exist. On the contrary, absence of androgens in the critical periods allows the development of neural structures appropriate to female sexual behaviours and female hypothalamic cyclicity, at least in rodents (for example, Harris, 1964; and evidence reviewed by Short, 1972).

Androgens have been implanted directly into the brains of sub-primates (see for example, Davidson, 1972). Palka and Sawyer (1966) placed testosterone implants in the posterior hypothalamus (ventromedial/pre-mamillary area) of female rabbits and elicited oestrous behaviour. This non-specificity of hormone effects on oestrous behaviour in sub-primates is consistent with the early but remarkably insightful suggestion of Eayrs (1952, cited in Everitt, 1970), that sex hormones lower the threshold for evoking behavioural responses more by virtue of the structure of the nervous system on which they act than because of any other properties they have.

It is now well established that systemically administered testosterone activates sexual receptivity in female sub-primates as well. This is true for the rat (Beach, 1942; Beyer and Komisaruk, 1971), the rabbit (Beyer, Mc Donald and Vidal, 1970) and the cat (Green, Clemente and de Groot, 1957). Furthermore, it is now clear that testosterone stimulates not only the eliciting of lordosis, but also the female's willingness to seek sexual contact with a virile male. This is true on measures such as moving to the male in the open field, crossing an electric grid to reach the male and in running and choosing a male rather than a female

in a run (Meyerson, Lindström, Nordström and Agmo, 1973).

1.5.2. Androgenisation in Primates

Androgenisation of the brain during the critical periods of development does more than alter the responsiveness of neural tissue to testosterone in adult life. Permanent functional changes probably take place in the brain as well. Evidence here is based mainly on the behaviour as juveniles and young adults of pseudohermaphrodite rhesus monkeys (Goy, 1968), since it is clear that androgenisation of the brain and reproductive tract takes place in higher primates as well. This is not easy to achieve experimentally, because injecting testosterone into pregnant rhesus monkey mothers can cause abortion of the foetus. But Goy, Phoenix and co-workers at Oregon have achieved"pseudohermaphrodite" monkeys by exposing genetic females to testosterone (5mg to 25 mg/day) in utero for the last third of normal pregnancy. At birth these animals had ovaries but no visible vagina, a well-developed penis and a scrotum which was well-developed but empty (Young, Goy and Phoenix, 1964).

The Oregon pseudohermaphrodites showed patterns of infant and juvenile behaviour intermediate between male and female infants (Goy, 1968). Marked sexual dimorphism of play, play-initiation and threatening behaviour is typical of infant and juvenile rhesus monkeys. A related finding is that pigtailed macaque mothers treat their male and female infants differently, being increasingly punitive of males so that greater "maternal independence" is seen in male infants than in females (Jensen, Bobbitt and Gordon, 1967, 1968).

Researchers have waited with eagerness for the full reports of the behaviour of these pseudohermaphrodites as adults. These are now available (Eaton, Goy and Phoenix, 1973). Seven of them were bilaterally ovariectomised and tested with oestrogen-treated stimulus females, and

then given long periods (30 weeks) of exogenous testosterone. The conclusion was that even before "activating" testosterone, the pseudohermaphrodites showed levels of aggressiveness significantly higher than control females. Together with their masculinised juvenile behaviour (Phoenix, Goy and Resko, 1968), this was taken by Eaton et al (1973) as evidence that "the adult rhesus brain was functionally modified by prenatal androgen" (p 120). After testosterone treatment pseudohermaphrodites showed "male type" sexual behaviour and mounted more than did control females, but these results were not significant because of extreme individual variability. However, patterns of intromission and even ejaculation were recorded for a pseudohermaphrodite, something which is impossible for a non-androgenised female.

In humans, androgenisation of the female foetus is "strikingly similar" to what happens in animal studies (Reinisch, 1974), particularly the Oregon studies on monkeys. Androgenisation of a genetic female can occur in the adrenogenital syndrome (Ehrhardt, Epstein and Money, 1968; Ehrhardt, Evers and Money, 1968), by means of a genetic defect in the enzymes controlling the biosynthesis of cortisol, which results in increased ACTH secretion by the pituitary due to a failure of negative feedback and therefore to the secretion of relatively large amounts of androgen from the adrenal. In progesterone-induced hermaphroditism (Ehrhardt and Money, 1967) synthetic progestins administered in the control of abortion led to virilisation of the female foetus in a few cases. Ehrhardt and her fellow-workers have found that tomboyish behaviour, male-type interests and career choices and, surprisingly, high I.Q. scores as a group characterise such androgenised women.

1.5.3. The Site of Action of Adrenal Androgens in the Brains of Female Monkeys

In normal (non-androgenised) female monkeys, it is seen from the above that adrenal androgens could exercise control over sexual receptivity, while gonadal oestrogens (and progesterone) could control her attractiveness. No effects of adrenal androgens on peripheral tissue could be detected (Everitt, Herbert and Hamer, 1972) and therefore the site of action of these steroids must be presumed to be central. Everitt and Herbert (1974, in preparation) have addressed themselves to the question "Where in the central nervous system of the female monkey do these androgens act, to influence sexual receptivity?"

Very small quantities (105 - 280 ug) of testosterone propionate were fused to thin stainless steel tubes and implanted down stereotaxically-placed cannulae screwed into the skulls of adrenaloctomised, ovariectomised female monkeys (Everitt and Herbert, 1974, in preparation). The females remained attractive to males throughout the experiments (oestradiol at 25ug/day in oil), but were unreceptive prior to testosterone implantation. An X-ray picture in fig. 4 illustrates the intracranial implant in situ.

Implants into the anterior hypothalamus/preoptic area, from the optic chiasm to the cranial border of the ventromedial nucleus, reversed the effects of adrenalectomy by restoring female sexual receptivity. When implants of cholesterol were made into the same loci, the females were unreceptive once more. Similar implants of testosterone into the pulvinar nucleus of the thalamus, or the cerebral cortex, were without effect. And finally, implants of the same size were made into the posterior hypothalamus (i.e. the premamillary and mamillary areas or pretectal areas), but in spite of their very close proximity to the anter-

Figure 4 X-ray plate showing intracranial implant of testosterone in situ. Note tip of implant projecting from end of cannula. Note site of implant in hypothalamus.

ior hypothalamus/preoptic area, they were without effect on sexual receptivity. No peripheral effects of these very small amounts of testosterone implanted into the brain were detected.

This work has therefore established fairly precisely at least one of the important sites of action of the adrenal androgens in the brains of female monkeys, namely, the anterior hypothalamus/preoptic area. This area accords well with the site of action of oestrogen in rodents, since implants of oestrogen in this area evoke oestrous behaviour in ovariectomised rats (Lisk, 1966).

The work would also suggest that androgens do not act on the nervous system of monkeys by being converted first into oestrogens, as has been suggested by McDonald and co-workers for the rat (McDonald and Meyerson, 1973) and the rabbit (McDonald, Beyer and Vidal, 1970). This follows from the fact that adrenalectomised females were quite unreceptive, even though they were given oestradiol systemically every day as usual. It was only when testosterone was implanted into the anterior hypothalamic area in these relatively minute quantities that sexual receptivity was restored.

This evidence would not, of course, exclude the possibility that androgens are converted to oestrogenic compounds once they enter the cell, via, for example, a cell membrane which allows incorporation of androgens but not oestrogens. It has recently been shown that oestrone and even oestradiol can in fact be formed from androstenedione in vivo, in isolated rhesus monkey brain preparations perfused via the carotid arteries (Flores, Naftolin, Ryan and White, 1973).

1.6. Brain Monoamines

What these studies have not shown is how the adrenal androgens act on the anterior hypothalamus (and possibly other brain areas as well) to control female sexual receptivity. As mentioned above, attention has recently been focussed on the biogenic amines as possible mediators of the action of steroids on neural tissue. The evidence that suggests that monoamines and particularly 5-hydroxytryptamine could be involved in this action will therefore be presented.

Biogenic amines are derivatives of amino acids. The two major divisions of monoamines active in the brain are the indoleamines and the catecholamines. It is clear that in the hypothalamic area 5-hydroxytryptamine (5HT) is the major biologically active indoleamine, but the possible roles of 5HT precursors and metabolites in the brain have not yet been elucidated. The major catecholamines active in hypothalamic tissue are dopamine and noradrenalin, but again it is likely that precursors and related substances such as adrenalin play a role in the functioning of the brain.

1.6.1. Catecholamines.

The catecholamines are derived from the amino-acid L - tyrosine. The rate - limiting step in the synthesis is the hydroxylation of L-tyrosine by tyrosine hydroxylase to L-dopa. L-dopa is converted by aromatic L-amino acid decarboxylase to dopamine (DA), which acts as an inter-neuron transmitter in the brain in its own right. Dopamine is converted by dopamine B-oxidase to noradrenalin (NA).

In general, more is known of the pathways and actions of DA and NA than of adrenalin. Further enzymatic action by phenylethanolamine N-methyl transferase (PNMT) leads to the formation of adrenalin. Recent

immuno-histochemical techniques have demonstrated well-defined adrenalin neural pathways in the rat brain (Hökfelt, Fuxe, Goldstein and Johansson, 1973). Clearly adrenalin is an important neurotransmitter in the pons, mid-brain and diencephalon as well, as it is in other areas such as the spinal cord (Hökfelt, Fuxe, Goldstein and Johansson, 1974).

Catecholamines are degraded by monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT). For example, degradation of dopamine by both MAO and COMT gives the CSF metabolite homovanillic acid (HVA).

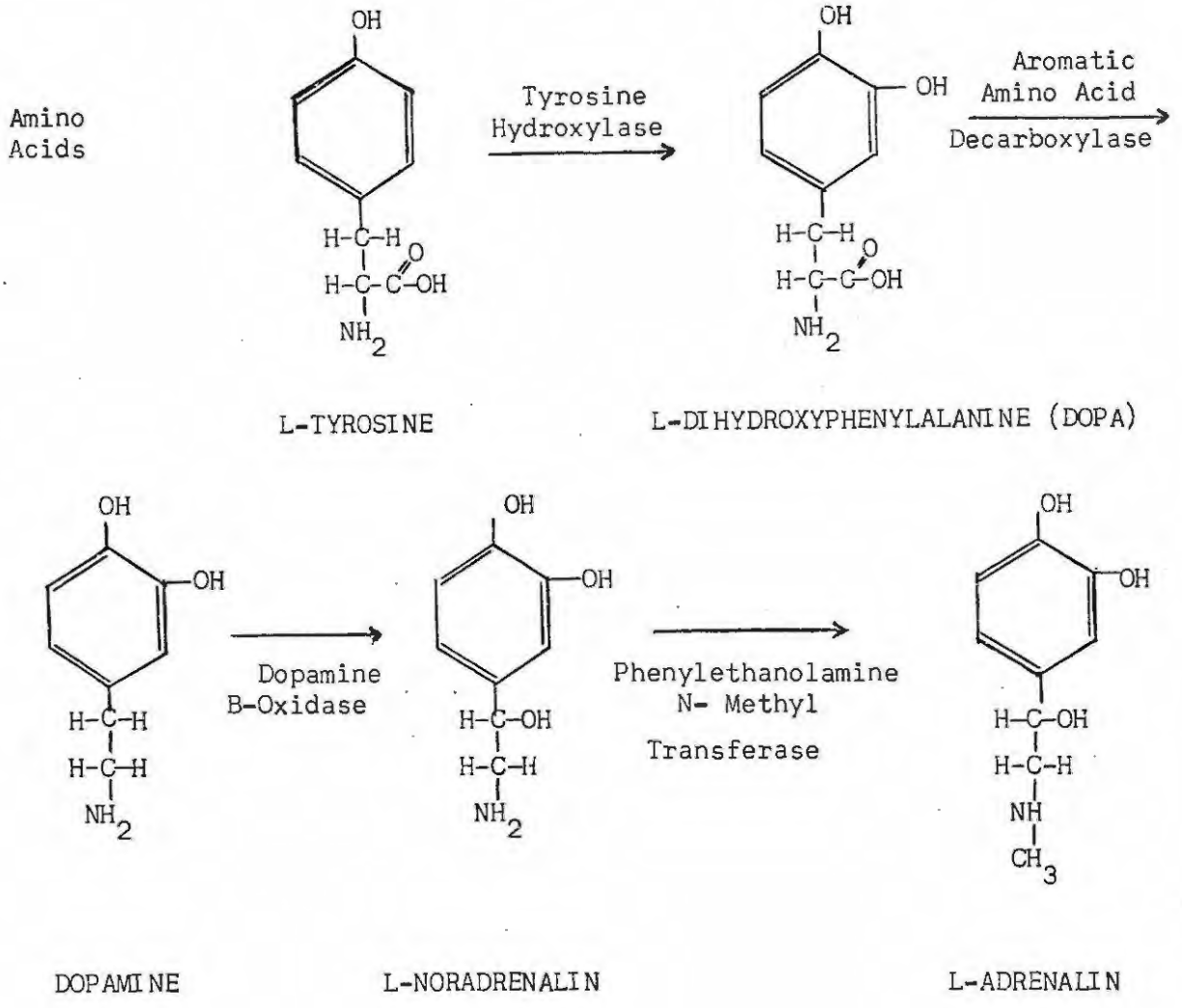


Figure 5. Biosynthesis of Catecholamines

The catecholamines adrenalin and noradrenalin act in two quite different ways in the body. First, they are both secreted from the adrenal medulla into the blood stream, and act as circulating hormones. Adrenalin has a marked effect on the body in adapting to sudden emergencies. It constricts blood vessels to the intestines and kidneys and to the skin, but dilates blood vessels supplying muscles and heart. It increases heart rate and accelerates respiration, as well as increasing glycogen break-down to glucose. Noradrenalin has effects rather similar to those of adrenalin, but does not affect glycogen metabolism and it also exerts considerable control over blood pressure by constricting blood vessels to skeletal muscle. Secondly, in the brain and neural tissue, NA and adrenalin act as inter-neuron transmitter substances. In this role, the action of synaptic noradrenalin on post-synaptic membranes is clearly separated from the action of NA from the systemic plasma, presumably by the blood-brain barrier (Barchas et al, 1972).

1.6.2. Indoleamines

5HT is derived from the amino acid tryptophan in the plasma. Tryptophan is crucially important in the synthesis of protein, since the body has stores of tryptophan smaller than those of any other amino acid in the general tissue. In this way the plasma levels of tryptophan limit the rate of protein synthesis. To get into the body, tryptophan enters the liver, and the rate of this entry is limited by the enzyme tryptophan pyrrolase.

The rate limiting step in the biosynthesis of 5HT out of tryptophan is the 5-hydroxylation of tryptophan by the enzyme tryptophan hydroxylase to form 5-hydroxytryptophan (5HTP). This immediate precursor of 5HT is almost immediately decarboxylated by aromatic L-amino acid decarboxylase to 5HT. The 5HT synthesis inhibitor (PCPA) acts by inhibiting the hydroxylation of tryptophan to 5HTP.

5HT is catabolised by MAO to form an intermediate oxidative deaminated aldehyde product. This is then oxidised to 5-hydroxyindole-3-acetic acid (5HIAA), the main metabolite of 5HT in the CSF, or else it could be reduced by alcohol dehydrogenase to 5-hydroxytryptophol.

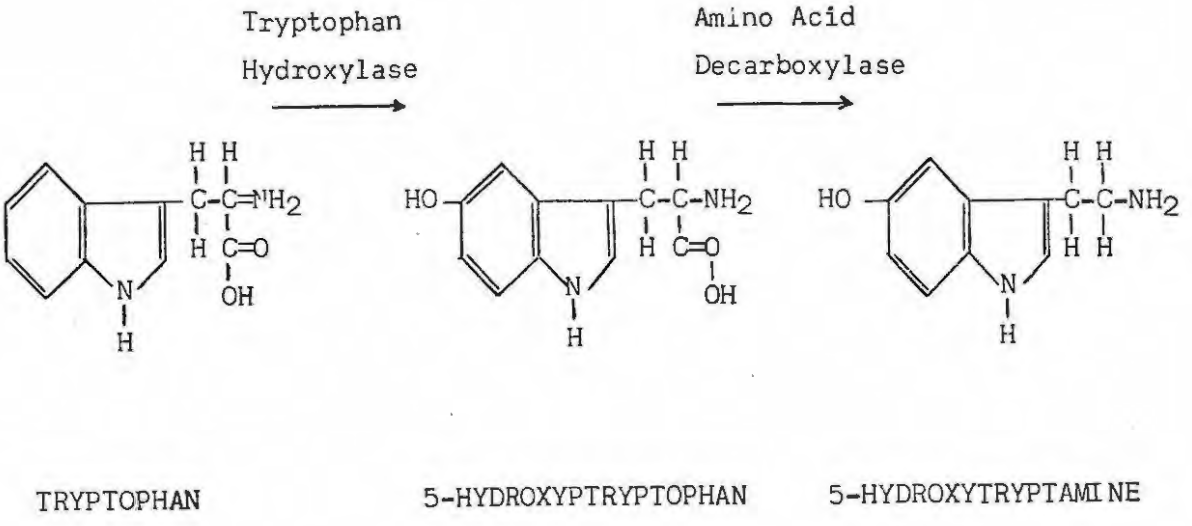


Figure 6. Biosynthesis of 5-HT

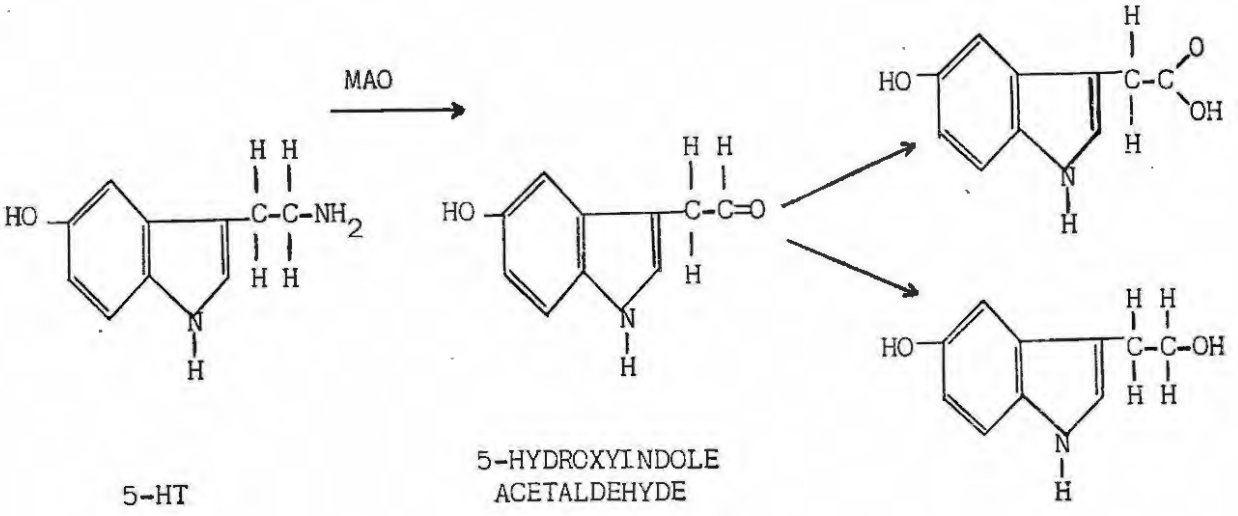


Figure 7. Metabolism of 5-HT

An exception to this general pattern of synthesis and metabolism of 5HT is provided by the pineal gland of most mammals. The pineal is rich in 5HT (see Herbert, 1971), but it can also transform 5HT to methoxyindole compounds such as melatonin. It is also possible that these substances are secreted into the CSF and could act on 5HT-containing neurons in, for example, the optic and infundibular recesses of the third ventricle (Symington, Marks and Ryan, 1972; Knowles, 1972).

1.6.3. Anatomical Distribution of Monoamines

In the brain, the biogenic amines are found in the neuron, as distinct from the glia or vascular tissue. They are highly concentrated in the synapses, and it is probable that the amines (or the enzymes of their synthesis) are transported down the axon and stored in the terminal vesicles. (Dahlström and Fuxe, 1964). From there, they are released into the synaptic cleft to act on the post-synaptic membrane. Most of the transmitter is inactivated again by reuptake into the presynaptic endings, at least in the case of the catecholamines, but some transmitter is metabolised by MAO and COMT and is lost.

The biogenic amines are found in specific nerve fibre pathways in the brain (Andén, Dahlström, Fuxe and Larsson, 1965). The distribution of these pathways is shown in fig. 8.

1.6.3.1. Dopamine

The dopamine-containing neurons are found in high densities in only a few areas of the brain. The nigroneostriatal fibres run from the substantia nigra to the caudate nucleus and putamen, and, less densely, to the globus pallidus. These fibre tracts have received considerable attention because functional deficits in this dopaminergic system are strongly suspected in many (but not all) cases of Parkinson's disease (see, for

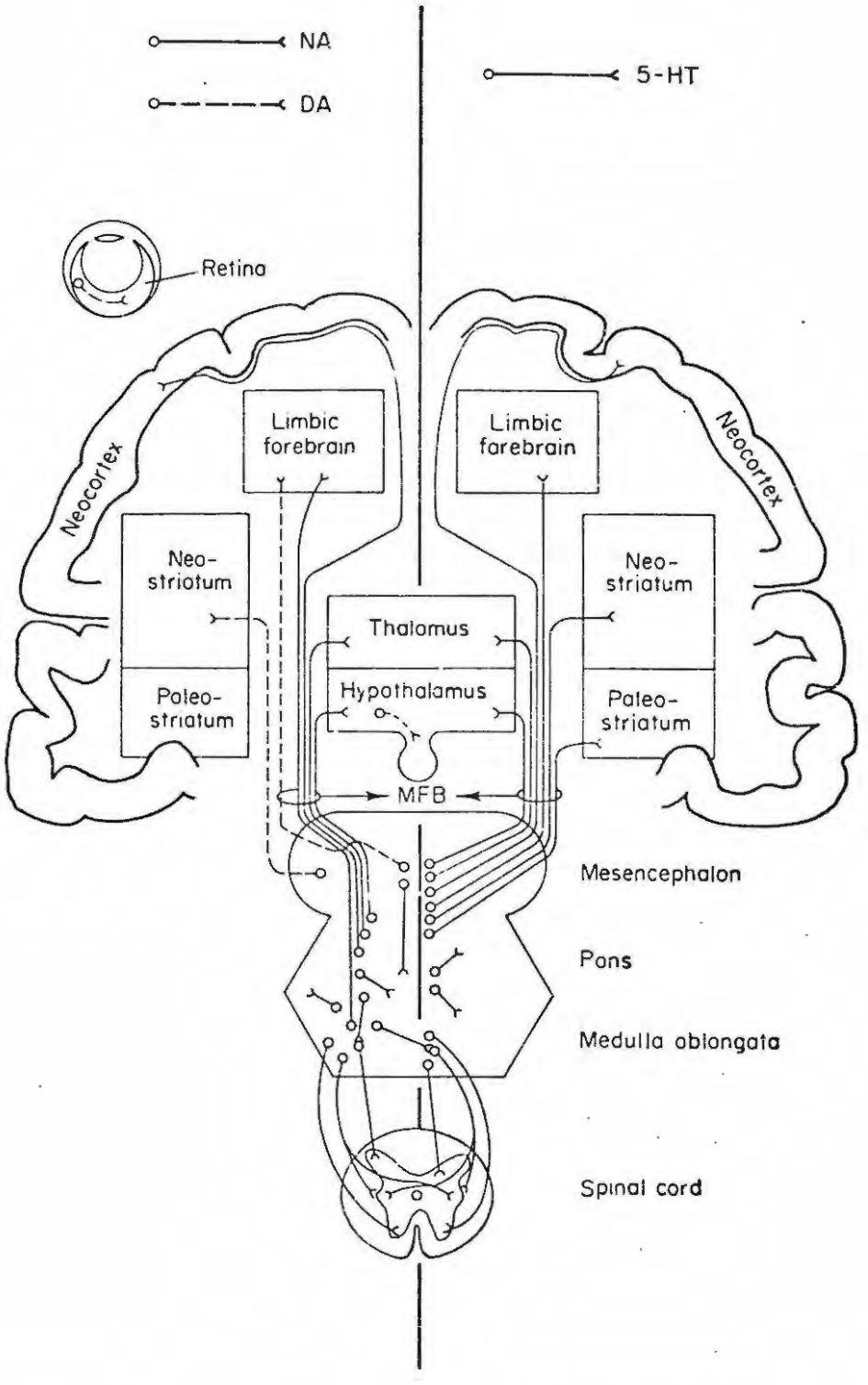


Fig. 8. Schematic representation of the distribution of norepinephrine (NE), dopamine (DA), and serotonin containing (5-HT) fiber tracts in the brain. MFB refers to the median forebrain bundle of the hypothalamus. This figure is taken from Andén *et al.* (1966).

example, Goodwin, 1971). The second important dopaminergic system is in the tuberoinfundibular tract, running from cell bodies in the arcuate nucleus of the medial hypothalamus down to terminals in the hypothalamo-hypophyseal portal vessel system of the median eminence. There is also a dopamine-containing system running to the limbic forebrain, probably from the interpeduncular nucleus in the midbrain (Andén, Dahlström, Fuxe, Larsson, Olson and Ungerstedt, 1966).

1.6.3.2. Noradrenalin

NA systems are more widespread. The cell bodies of the NA-containing neurons are located in the pons and medulla oblongata (Fuxe and Hökfelt, 1969). From the venterolateral tegmental area, the main outflow of axons is the medial forebrain bundle, but there are NA pathways in the cerebellum, amygdala and neocortex as well. From the pons and medulla, two main NA pathways ascend, a dorsal one from the locus coeruleus and a ventral one from other cell bodies in the reticular formation (Dahlström and Fuxe, 1964). The cells of the locus coeruleus are probably not direct controllers of neuroendocrine processes (Fuxe, Hökfelt and Jonsson, 1970), but are almost certainly involved in sleep (Jouvet, 1969). The ventral pathway, on the other hand, innervates the hypothalamus, the preoptic area and the limbic cortex (see fig. 8), and is therefore likely to be directly involved in the control of reproductive functioning. There is a high density of NA-containing nerve endings in these areas (Fuxe and Hökfelt, 1969, 1970), particularly in the hypothalamus and preoptic area. The fact that NA is found extensively in the so-called "old" brain suggests a role for this neural transmitter in emotional and drive behaviours (Barchas et al, 1972, p 238).

Attention has been drawn to the fact that each NA-containing neuron gives off a huge number of collaterals (Fuxe and Hökfelt, 1970). A

neuron in the locus coeruleus could give rise to a number of ascending fibres but could also innervate a dense network of fibres in the pons and medulla. This extensive anatomical branching means that activating a NA-containing neuron could set off a large number of neuroendocrine processes, such as occur at periods of oestrus. The ascending fibres from the neurons in the locus coeruleus run in the dorsal mesencephalic pathways and finally ramify in the thalamus and cortex (see fig. 8). But these cells also ramify in the reticular formation, so that by means of this branching network, arousal and cortical "awareness" could be influenced.

1.6.3.3. Adrenalin

As noted above, Swedish workers have recently traced adrenalin (A) pathways in the rat brain as well, using specific immunofluorescence against PNMT, the enzyme converting NA to A (Hökfelt et al, 1973, 1974), The cell bodies of the A neurons are found in the ventrolateral reticular formation of the rostral medulla, very close to some of the NA cell bodies. The nerve endings of these cells are often in the periventricular areas of the brain stem, but it is clear that some reach the hypothalamus as well. However, the actual amounts of adrenalin in the rat brain are small, being estimated at only 5 per cent. of NA and A (Hökfelt et al, 1973, 1974).

1.6.3.4. 5-Hydroxytryptamine

The cell bodies of the 5HT-containing neurons lie mostly in the midline of brain stem and the caudal midbrain, the nuclei of the raphe (Fuxe, Hökfelt and Ungerstedt, 1968). Like the NA-containing neurons, they also give rise to extensive networks of collaterals, and therefore activity in one neuron can influence widely-separated areas of the brain (Fuxe and Hökfelt, 1970). This extensive anatomical branching is con-

sistent with the role proposed for 5HT neurons in sleep and arousal (Jouvet, 1969, 1973).

The fibres ascend medially in the midbrain, but swing more laterally to enter the medial forebrain bundle (see fig. 8). The nerve endings of these fibres have not been completely established (Fuxe and Hökfelt, 1970), but clearly they sweep up into the limbic forebrain and corpus striatum as well. The highest density of 5HT-containing synapses is in the hypothalamus. The primary area is the suprachiasmatic nucleus, suggesting a role for 5HT in LH releasing factor release. But 5HT nerve endings are also in the retrochiasmatic area and in the median eminence.

1.7. Monoamines and Hormonal Effects on the Nervous System

The reasons for suspecting that monoamines might mediate the effects of ovarian and adrenal steroids on neural tissue are mostly indirect ones. But some data derived from direct measurement are available.

1.7.1. Gonadotrophin Releasing Factors

First, monoaminergic neurons in the hypothalamus are involved in the regulation of gonadotrophin release from the anterior pituitary (Kordon and Glowinski, 1972). Radioactive oestradiol has been shown to concentrate in the preoptic area and ventromedial hypothalamus of female rats (Pfaff, 1973). The areas of the hypothalamus which concentrate oestradiol are also the ones in which small implants of oestradiol affect gonadotrophin release from the pituitary (McGuire and Lisk, 1969). These areas are also known to be areas of great monoamine concentration (Fuxe and Fökfelt, 1967; see also above).

Further evidence comes from the fact that noradrenalin levels in the hypothalamus of the female rat are lowest at oestrus. Administering oestrogen decreases these levels, and ovariectomy increases them (Stefano and Donoso, 1967). More recently, Hale and Symington (1972) have shown that dopamine affects gonadotrophin releasing factors in vitro in the bovine hypothalamus, and in vivo in oestrogen/progesterone-treated ovariectomised rats. On the basis of their experiments at the University of Rhodesia, Hale and Symington propose that dopamine may stimulate the cell bodies of the gonadotrophin releasing factor neurons of the arcuate nucleus in the hypothalamus, but may inhibit their terminals at the hypothalamo-hypophyseal portal vessel system of the stalk median eminence. These findings all combine to suggest that monoamine neurons are central

to the control of gonadotrophin releasing factors from the hypothalamus, under feedback from ovarian steroids in the systemic plasma. The suggestion has been made specifically for the medio-basal hypothalamus by Kordon, Gogan, Henry and Rotsztejn (1973) and Labhsetwar (1971).

1.7.2. Synaptosome Studies

Secondly, Janowsky and Davis (1970) have shown that oestradiol and progesterone block the uptake of labelled noradrenalin into synaptosomes. In vitro preparations of synaptosomes are made from homogenised rat brains by ultracentrifugation and sucrose gradient separation (see also Janowsky et al, 1972). The fact that ovarian steroids can affect the physiological activity of these axon terminal preparations is suggestive in the present context. However, it should be noted that no evidence exists to show that ovarian steroids can be selectively bound by synaptosomes and therefore the mechanism of this action is unclear at the present time.

1.7.3. The Amine Hypothesis of Affective Illness and the Premenstrual Syndrome

Thirdly, there is now considerable indirect evidence implicating brain monoamines in various psychiatric disorders, notably depression (Coppen, 1967) and perhaps even the schizophrenias. The monoamine hypothesis of affective illness (Ashcroft et al, 1965) has required considerable modification in the light of more recent evidence (Ashcroft et al, 1972) since it was first proposed. But it remains influential in providing the theoretical framework for the treatment of both unipolar and bipolar depressive patients.

Consistent with the amine hypothesis are those cases where monoamine oxidase inhibitors and tricyclic antidepressants, which raise amine levels in the brain, have relieved depression. The monoamine

precursors tryptophan and L-dopa have also been used to relieve some cases of depression, but L-dopa has also been known to bring on depression and even suicide in other patients (Cherington, 1970; evidence reviewed by Goodwin, 1971). A deficiency of 5HT in the brain causes clinical depression (see Carpenter, 1970). But Carpenter also notes that much of the evidence which shows that depletion of 5HT causes depression is equally convincing for the thesis that catecholamine depletion causes depression. What does seem established is that some cases of depression have been successfully treated by either indoleamine or catecholamine precursor administration. Against the amine hypothesis is a recent finding by Coppen et al (1972) that low levels of 5HT metabolite measured in the CSF of depressed and manic patients did not change on recovery of the patient.

The monoamine hypothesis of affective disorders is not itself sufficient to implicate these neural transmitters in the effects of hormones on behaviour. But taken together with the undoubted effects of the menstrual cycle on women's moods, emotions and psychiatric states, the argument is more compelling. Increases in anxiety, depression and irritability are commonly reported in women during premenstrual periods and during menstruation (Janowsky and Davis, 1970). Women's crimes of violence, actual and threatened suicides, depressions requiring hospitalisation and even onsets and relapses of schizophrenia and manic depressive psychoses are all more frequent during the premenstrual/menstrual period than at other times of the cycle (Janowsky, Gorney, Castelnuovo-Tedesco and Stone, 1969). These periods of psychological and psychiatric lability are associated with periods of rapidly changing oestrogen and progesterone titres in the blood. In contrast, the follicular phase of the menstrual cycle is characterised by steady (or steadily increasing) levels of ovarian steroids, and (significantly) this is also a period of relative

emotional and psychiatric stability in women.

Supportive evidence comes from similar findings in late pregnancy and in the post-partum period. This period is characterised by great changes in progesterone levels in the blood and is also a period of marked emotional lability. Furthermore, oral contraceptives can cause depression (Grant and Mears, 1967), particularly those utilising high proportions of progestins relative to oestrogen (Grant and Pryse Davies, 1968). Both the ingesting and withdrawal of oral contraceptives would cause changes in hormone levels ir the body.

However, the amine hypothesis of affective illness is by no means unequivocal. It should also be noted that there is no direct physiological evidence to show that the fluctuating levels of ovarian hormones which seem to be linked with emotional instability and depression in the premenstrual period do in fact act via monoaminergic neurons in the brains of women.

1.7.4. Sex Differentiation

Fourthly, evidence exists from the work of Ladosky and co-workers in Brazil that 5HT levels in the brains of neonatal rats show sex differences during critical periods of development. Ladosky and Gaziri (1970) measured 5HT in the midbrains and forebrains of rat puppies. Males and females had exactly the same 5HT concentrations when measured on day 1, day 4 and day 8 after birth. But on day 12 after birth, the concentrations of 5HT in the female brains were significantly higher than those found in male litter mates. Testosterone propionate prevented this rise of 5HT on day 12 in female rats injected with the hormone on the day of birth. Males castrated at birth resembled their female litter mates rather than their male siblings in the amounts of 5HT found in brain slices during day 12.

This work has been both confirmed and extended by Giulian, Pohorecky and McEwen (1973). They found that, "levels of 5HT in the female brain were significantly higher than those in the male on postnatal days 10, 12 and 14, but not on days 2, 4, 8, or on days 16 and 25" (p 1329). Further, Ladosky's findings on the effects of early androgenisation of female pups and castration of male pups, were confirmed. Giulian et al also found that oestrogens given on the day of birth led to a precocious 5HT rise in female rats (by day 8), and also led to an atypical 5HT rise in the brains of male pups. Neither oestrogen nor androgens given on day 20 had any effect on 5HT levels measured on day 25.

Since it is well established that "critical periods" exist during sex differentiation of the brain, and since gonadal steroids are responsible for such sex differentiation (1.5.1. and 1.5.2. above), Ladosky suggests that 5HT levels are affected by the same processes whereby gonadal steroids androgenise the brain. Ladosky finds it significant that "testosterone liberated by the neonatal gonad may modify the metabolism of brain serotonin only on day 12, which period corresponds to the time of sexual differentiation of the structures that control gonadotrophin secretion" (p 168). This view is supported by Giulian et al, who state that "these effects of gonadal hormones on brain 5HT levels suggest an involvement of the gonads in maturation of the serotonin system of the brain" (p 1329). The same considerations may also apply to the catecholaminergic systems of the brain (Ladosky, pers. comm.).

1.7.5. Substitution of Monoamine Depletors for Progesterone

Finally, there is early evidence from Meyerson (1964 a, b, c; 1966) to suggest that in oestrogen-primed ovariectomised rats it is possible to substitute monoamine-depleting substances such as reserpine

and tetrabenazine for progesterone, in activating female sexual behaviour. Pharmacological agents which increase monoamine levels in the brain, such as the MAO inhibitors and the tricyclic antidepressant drugs, actually decrease sexual behaviour in oestrogen/progesterone-treated ovariectomised rats. Taken together, these results led Meyerson to suspect that monoamines (particularly 5HT) were implicated in the neural mechanisms whereby progesterone facilitates lordosis and other sexual behaviours in female rats. Specifically, he suggested that 5HT-containing neurons in the diencephalon served an inhibitory function, and that progesterone acted to lower 5HT activity in these neural pathways. This hypothesis of Meyerson has given rise to an extensive literature, and forms the starting point for the present investigation into the possible role of 5HT in mediating the effects of adrenal androgens on sexual receptivity in female monkeys.

1.8. 5HT and Sexual Behaviour

Reserpine facilitates copulatory behaviour in male rats (Dewsbury and Davis, 1970). But a major problem about accepting evidence from reserpine, tetrabenazine and MAO inhibitor studies is the lack of specificity of action. Reserpine causes a long-lasting depletion of monoamines from nerve terminals, and it blocks the uptake of NA into neurons (Barchas et al, 1972). But it affects all monoamines. MAO inhibitors such as iproniazid, nialamide and pargyline all "retard" sex behaviour in male rats (Dewsbury, Davis and Jansen, 1972). But again MAO inhibitors lead to increases of all the brain monoamines. Even administration of specific precursors like tryptophan or 5HTP is not conclusive, since 5HT may increase to the extent that catecholamines are displaced by amine displacement (Weissman and Harbert, 1972), and it would then not be clear which action had produced the possible behavioural changes observed.

For this reason, a drug which inhibits the synthesis of a particular monoamine in a fairly specific way is a valuable tool of research. Parachlorophenylalanine (PCPA) is a fairly selective depletor of 5HT in the body and brains of animals, since it inhibits the action of tryptophan hydroxylase (Koe and Weissman, 1966), and it has now been used extensively in rats, rabbits, cats, monkeys and humans to study the effects of selective inhibition of 5HT on behaviour. However, PCPA does inhibit the activity of catecholamines as well (Koe and Weissman, 1966), and this point is taken up in detail below (4.4.)

1.8.1. Males

There is now little doubt that PCPA causes dramatic increases in sexual activity in male rats, cats and rabbits. Shillito (1969, 1970)

gave PCPA to juvenile and adult male rats and found increased mounting behaviour among the adults in the cage. In the juveniles, PCPA increased chasing, rolling and grooming of other animals, to the extent of causing hair loss in the groomees. She concluded that these juvenile behaviours were the precursors of adult male sexual behaviours, and that 5HT inhibited male sexual behaviour in rats since the increased mounting behaviours disappeared once she administered 5HTP.

These findings on male rats have subsequently received considerable support (Benkert and Eversmann, 1972; Bertolini, 1971; Gessa, 1970; Gessa, Tagliamonte, Tagliamonte and Brodie, 1970; Gawienowski, Merker and Danon, 1973; Salis and Dewsbury, 1971; Sheard, 1969; Tagliamonte, 1972; Tagliamonte, Tagliamonte, Gessa and Brodie, 1969). Treating a cage-full of male rats with PCPA causes "compulsive" sexual behaviour (Gessa, 1970) and multiple mountings are seen. For example, Gessa (1970) produced a photograph of six male rats all mounting in chain formation.

Gessa and Tagliamonte's group at the National Institute of Health in Bethesda have indicated that PCPA is most effective in producing this compulsive homosexual mounting pattern in male rats in the presence of testosterone (Gessa, Tagliamonte, Tagliamonte and Brodie, 1970). Malmnäs and Meyerson (1971) have shown the essential role of testosterone for full copulatory behaviour in male rats paired with receptive females and given PCPA. But Shillito and co-workers have shown that a single dose of PCPA is sufficient to induce mounting in castrated male rats, and even in adrenalectomised castrated male rats, so clearly PCPA can have this effect even in the absence of all known sources of androgen (Bond, Shillito and Vogt, 1972).

When PCPA is given with an MAO inhibitor (pargyline), which will presumably increase catecholamines in the presence of inhibited 5HT path-

ways, then the behaviours are most marked (Tagliamonte, Tagliamonte and Gessa, 1971). This led Gessa's group to propose an inhibitory role for 5HT and a stimulatory role for CA neurons in male sex behaviour. Tagliamonte et al, (1969) have also ruled out the possibility that PCPA causes compulsive mounting in male rats by inhibiting pineal indole hormones by noting "sexual excitement" in pinealectomised rats after PCPA "at about the same frequency as intact animals" (Gessa, 1970, p 623).

Compulsive homosexual mounting after PCPA has also been seen in male rabbits (Perez - Cruet, Tagliamonte, Tagliamonte and Gessa, 1971), and Gessa (1970) produced a photograph of chain mountings in a group of male rabbits. Perez - Cruet et al found that their rabbits even mounted a cat after PCPA. These findings have been confirmed by workers outside the NIH group as well (Sjoerdsma, 1970).

PCPA has the same effects in male cats (Hoyland, Shillito and Vogt, 1970). At Cambridge the cats were housed in groups and very seldom showed any mounting behaviour except when one of the females was at oestrus. But after PCPA, male cats mounted other cats and even two male kittens showed mounting behaviour. However, a puzzling result has come from the laboratories of Dement and co-workers in California. In April, 1970, "hypersexuality" and increased aggressiveness is reported in 26 male cats given PCPA (Ferguson, Hendrickson, Cohen, Mitchell, Barchas and Dement, 1970). This result is in accord with other work on cats, rats and rabbits. Yet in November, 1970, in the same journal, a study on 12 male cats treated with PCPA and tested in a standardised test situation, in which "sexual performance was either unchanged or diminished" (p 868) is reported (Zitrin, Beach, Barchas and Dement, 1970). It is not clear why two such conflicting results should have been obtained by closely associated (and even the same) workers, although Zitrin et al (1970) recognise a possible methodological weakness.

Whalen and Luttge (1970) have noted that PCPA can cause compulsive homosexual mountings in male rodents and rabbits, but have gone on to argue that this finding is uninteresting, since they found that PCPA does not affect "normal" heterosexual activity in rats. This suggestion caused some disquiet, and subsequent workers, such as the NIH group, have been at pains to deal with the objection. It now seems clear that Whalen and Luttge were wrong. Bertolini (1971) has shown that PCPA enhances male-male ("abnormal") mountings very markedly, but that it also enhances male-female ("normal") mountings. Salis and Dewsbury (1971) showed that PCPA can definitely facilitate normal heterosexual patterns of male sexual behaviour in rats. Malmnäs and Meyerson (1971) report that both the number of males mounting and the number of mounts per minute increased when PCPA was given to testosterone-treated castrated male rats. Further confirmation comes from Mitler, Morden, Levine and Dement (1971), who showed that PCPA on its own increased heterosexual mounting and copulation in intact male rats paired with sexually receptive females, while Ahlenius et al (1971) found increased intromissions and shortened ejaculation latencies in intact male rats treated with PCPA and with PCPA + pargyline and paired with females.

Gessa et al (1971, and elsewhere) have pointed out that Whalen and Luttge's mistake came in the criterion used for "sexual enhancement", when they used rats which were "vigorous copulators" and measured the number of ejaculations which occurred before "sexual satiation". Without PCPA these rats averaged 7 ± 0.9 ejaculations per test, and Whalen and Luttge base their statements on the fact that this number of ejaculations per test was not increased by PCPA. But it is not clear what the upper limit for the number of ejaculations per test would be, and whether any advance on this performance could be expected. Gessa et al (1971) wryly remark that to look for any increase under these conditions might be like

"screening anti-depressant drugs on normally happy subjects".

1.8.2. Females

Meyerson's original work on central monamine depletion by tetrabenazine and reserpine led to the suggestion that 5HT was inhibitory for sexual behaviour patterns in female rats (Meyerson, 1964 a, b, c, 1966, 1968). An MAO inhibitor given with 5HTP, the immediate precursor of 5HT, inhibits the lordosis reflex in female rats (Meyerson, 1964 b). Supportive evidence came from the fact that PCPA facilitated sexual receptivity in oestrogen-treated ovariectomised female rats (Meyerson and Lewander, 1970). Recently, this finding has been confirmed (Ahlenius, Engel, Eriksson, Modigh and Södersten, 1972; Zemlan, Ward, Crowley and Margules, 1973). Zemlan et al also elicited lordosis by placing two 5HT-receptor blockers (methysergide and cinanserin hydrochloride) directly into the medial preoptic area and into the anterior and posterior hypothalamus of female rats.

Meyerson has extended these findings to the female hamster. MAO inhibitors decreased lordotic responding in oestrogen/progesterone-treated ovariectomised hamsters, and 5HTP-loading with a sub-effective dose of pargyline inhibited lordosis as well. In contrast to the female rat, however, reserpine could not be substituted for progesterone in activating oestrous behaviour in the oestrogen-treated ovariectomised female hamster (Meyerson, 1970).

In the female cat, PCPA elicits typical "pro-oestrous" behaviour patterns of treading and rubbing, and if the cat was stroked, then it arched its back and reflected the tail to one side (Hoyland, Shillito and Vogt, 1970). These effects were reversed by 5HTP. However, these results should be interpreted with caution, since the authors mention that

many of the cats in the Cambridge colony normally responded to humans by treading and rubbing, in contrast to others which avoided contact with humans.

Södersten and co-workers in Sweden have also shown increased lordosis in oestrogen-treated ovariectomised female rats after tetrabenazine and after CA-synthesis inhibition by alpha-methyl-p-tyrosine (AMPT) (Ahlenius, Engel, Eriksson and Södersten, 1972). They have also shown increased lordosis in castrated male rats (treated with oestrogen) after tetrabenazine (Larsson and Södersten, 1971), after AMPT (Södersten and Ahlenius, 1972) and after PCPA (Eriksson and Södersten, 1973). While such "heterolous" sex behaviour patterns in male rats should not be accorded too much weight in a consideration of "normal" female sexual behaviour, these results do serve to show the extent to which 5HT and monoamine depletion affects sexual behaviour in appropriate animal models. Finally, levels of 5HT and its precursor (tryptophan) in homogenised mouse forebrains and midbrains have been found to fluctuate with the oestrous cycle (Greengrass and Tonge, 1971). In these brain slices, the "midbrain" includes the thalamus, the hypothalamus and striatum, and 5HT was at a maximum in the forebrain and midbrain at dioestrus. During proestrus, tryptophan levels fell and at oestrus 5HT was at a minimum in both forebrain and midbrain homogenates. Since forebrain levels fluctuated as well, Greengrass and Tonge interpreted this as implicating 5HT in the control of oestrous behaviours as well as in the neuroendocrine events of ovulation. Their findings are, of course, consistent with the view that 5HT is inhibitory for female sexual behaviour. Supportive evidence comes from the finding that oestrogen and progesterone affect 5HT levels in the midbrains of ovariectomised female rats (Tonge and Greengrass, 1971). Although the effects were more marked on catecholamines, oestrogen and progesterone did affect 5HT in midbrain and hindbrain homogenates

as well. However, a factor casting considerable doubt on the value of these results is that oestradiol and progesterone were both found to increase the levels of 5HT in the midbrain and hindbrain sections, whereas the earlier report by Greengrass and Tonge (1971) showed 5HT to be minimal at oestrus, when endogenous oestrogen and progesterone titres in the blood are likely to be high. The work clearly shows that ovarian hormones have effects on midbrain 5HT levels, but must be regarded as being tentative at this stage.

More recently, Everitt, Fuxe and Hökfelt (1974, in preparation and personal communication) have shown that inhibition of DA and 5HT, by either receptor blockade of DA or synthesis inhibition of DA and 5HT does stimulate oestrous behaviour in oestrogen/progesterone treated ovariectomised rats. These workers have also been able to show that oestrogen and progesterone affect 5HT levels in the brains of ovariectomised rats, with oestrogen increasing and progesterone decreasing 5HT turn-over rates (Everitt, personal communication).

1.9. The Present Experiments

From the above, it seemed that enough evidence on sub-primate mammals existed to suggest that adrenal and ovarian hormones could also affect the sexual receptivity of female monkeys via 5HT-containing neural systems. It seemed likely that the anterior hypothalamus and preoptic areas, in particular, would be involved, but the monoamine-containing pathways of the midbrain and brain-stem (such as those in the nucleus of the raphe) could be generally implicated. The present experiments were therefore designed to examine this possibility. At the same time, the effects of manipulating 5HT levels on social behaviours such as grooming and proximity could be studied.

In addition, the role of 5HT in some aspects of the male monkey's sexual behaviour could be examined, using the techniques developed to study female monkeys. In particular, the possibility that testicular androgens acted on the male brain via 5HT-containing neural structures was investigated. Finally, the suggestion that 5HT played an inhibitory role in some aspects of sexual behaviour (reviewed above) made it worth examining whether 5HT was involved in the characteristic "refractory period" which follows ejaculation in male monkeys such as the rhesus and the baboon (Saayman, 1970).

CHAPTER 2

MATERIALS AND METHODS

2.1. Animals

Nineteen adult male and 24 adult female rhesus monkeys Macaca mulatta were used. The males weighed between 9.7 kg and 16.4 kg, with a mean weight of 12.4 kg. All were obtained from dealers and had been caught in the wild, except for five which were colony bred. The colony bred males were used only for biochemical studies and were not used in the behavioural experiments (see Harlow, 1962). Fourteen males were intact, and five were bilaterally castrated.

The females weighed between 4.6 kg and 10.9 kg, with a mean weight of 7.1 kg. All were obtained from dealers and had been caught in the wild. All females were bilaterally ovariectomised at least several months before the experiments started, and ten of them were also bilaterally adrenalectomised.

2.2. Operative Techniques

Three major surgical operations were used, namely, ovariectomy and adrenalectomy of females and castration of males.

2.2.1. Anaesthesia

Animals were first tranquillised by intramuscular injections of either phencyclidine hydrochloride (Sernylan, Parke-Davis and Company, 10mg/kg) or ketamine hydrochloride (Ketalar, Parke-Davis and Company, 7 - 8mg/kg). The latter tranquilliser proved to be most satisfactory in this work.

Full surgical anaesthesia was achieved with either pentobarbitone sodium (Nembutal, Abbot Laboratories Limited), administered by slow intravenous injection until anaesthesia was deep, or latterly with halothane gas.

Throughout all surgical procedures, full aseptic routine was followed and no post-operative sepsis resulted. Prophylactic treatment with a long-acting penicillin was given routinely after each operation (Triplopen, Glaxo Limited, 1 mega unit) by deep intramuscular injection.

2.2.2. Bilateral Ovariectomy

All females used were bilaterally ovariectomised. The abdomen was opened through a mid-line incision from above the pubis to the umbilicus. Care was taken not to extend the incision too far towards the pubis. The intestines were retracted and the ovaries exposed. Two thread ligatures were passed, one round the ovarian pedicle and one round the mesovarium (Everitt, 1970). The ovary was removed by excising between the two ligatures, care being taken to remove the

whole ovary. The abdomen was then closed in successive layers. Interrupted chromic gut sutures were used to close first the peritoneum, then the muscle and then the subcutaneous tissue (usually only a few sutures). The skin was closed with interrupted thread sutures which were removed eight days later. The sutured wound was protected with a spray coating of "plastic skin" (Nobecutane, Evans Medical Limited) and routine prophylactic penicillin given. Animals were allowed at least two months to recover before testing was started.

2.2.3. Bilateral Adrenalectomy

Ten females were also bilaterally adrenalectomised. This was done in one operation by a surgeon and three assistants. A midline incision was made from the xiphisternum to a point below the umbilicus towards the pubis. The left adrenal gland is more accessible than the right and this was usually removed first. It was exposed by retraction of the large and small intestine to the opposite side by one of the assistants. The surgeon then teased out the gland and surrounding fat and part of the perirenal fascia with the diathermy apparatus. Usually some large veins entering the left renal vein and the inferior vena cava were either ligated with thread or cauterised.

The right adrenal gland was approached by reflecting the liver upwards until the upper part of the right kidney could be reached. The gland lies immediately next to the angle between the right renal vein and the inferior vena cava. The danger of traumatising either vein during removal of the gland is considerable. A single large adrenal vein usually enters the inferior vena cava, and this was ligated with thread. The gland was then separated very carefully from the inferior vena cava using the diathermy apparatus.

A visual inspection was made to confirm that both glands had been

completely removed. The abdominal wound was sutured together in layers as in the case of ovariectomy, and penicillin given.

2.2.4. Post Operative Care

50 mg of cortisol (as hydrocortisone sodium succinate, Boots) was given by intramuscular injection immediately before the operation. The animals were watched carefully during the immediate post-operative period, and kept warm with an infra-red lamp. They were given a further injection of 20 mg of cortisol later in the day.

The next day they were given 40 mg of cortisol in morning and afternoon doses of 20 mg each, and this was gradually reduced over a period of seven days to the maintenance dose of 20 mg per day given every afternoon into alternate legs.

Although the animals were very weak in the immediate post-operative period, all ten made speedy and complete recoveries. They were allowed free access to food, water and saline during recovery, and their feeding and defaecation returned to normal within a week to ten days. No fatalities or adrenal insufficiencies resulted. Indeed, the speed with which animals returned to "normal" after major abdominal surgery was remarkable.

2.2.5. Bilateral Castration of Males

Five males were bilaterally castrated. The scrotum was prepared and incised fairly extensively to give access to the testes, the fascial coverings and the spermatic cords. Each testis was partially freed from the fascial layers and the spermatic cord exposed. The cord was then twisted and thread ligatures were passed round it to occlude the pampiniform plexus and the testicular artery. Incision was made between the ligatures and the testes were removed. What remained of the fascial layers was trimmed and chromic gut sutures were used to close subcutaneous

tissue where necessary. Chromic gut was used to close the scrotum, and Nobecutane and penicillin administered as usual. No sepsis resulted and all animals made good recoveries.

2.3. Behavioural Observations

Fourteen females and 6 males were used in the behavioural experiments. Females were housed individually in cages (33"x30"x40" high) in a communal monkey house. The males were housed in "double cages" (see fig. 9), which could be divided by a movable mesh (or solid) partition secured by a stout catch. The male was confined to half his cage and a female was then placed in the other side (see fig. 10).

Each female was paired with a male (always the same one) for 30 minute observation periods. Observations were carried out daily for 10 days, and the order in which animals were tested was always the same. No pair was ever tested more than once a day. Females were transferred to the male's cage by means of a small (18"x16"x18" high) carrier box (see fig. 11), with which they were fully familiar since it was used for their daily injections (see fig. 12). The observer then took up his position behind a one-way-vision mirror and curtaining system (see figs. 13 and 14) to observe the behaviour of the pair.

Each female was allowed to adapt to the experimental situation before observations began. This process was done in two stages. Leaving the divider in place, the observer watched for signs of aggression between the animals. Once he had satisfied himself that it was safe to proceed, the divider was removed and five introductory tests of 30 minutes each were given on the next five days. Such tests were given only to pairs not familiar with each other or with the test situation (five pairs in these experiments).

During the introductory tests, the observer learnt as much as he could about the two animals. In particular, he noted whether the two animals were compatible enough for quantifiable behavioural interaction

Figure 9 Male rhesus monkey in double (observation) cage. Note partition to be removed once female is introduced to empty half of cage.

Figure 10 Introduction of female to male's double cage at start of behavioural observations.

Figure 11 Female rhesus monkey in carrier box.

Figure 12 Injection of female rhesus monkey in carrier box.
Note sliding door, to allow immobilisation of monkey.

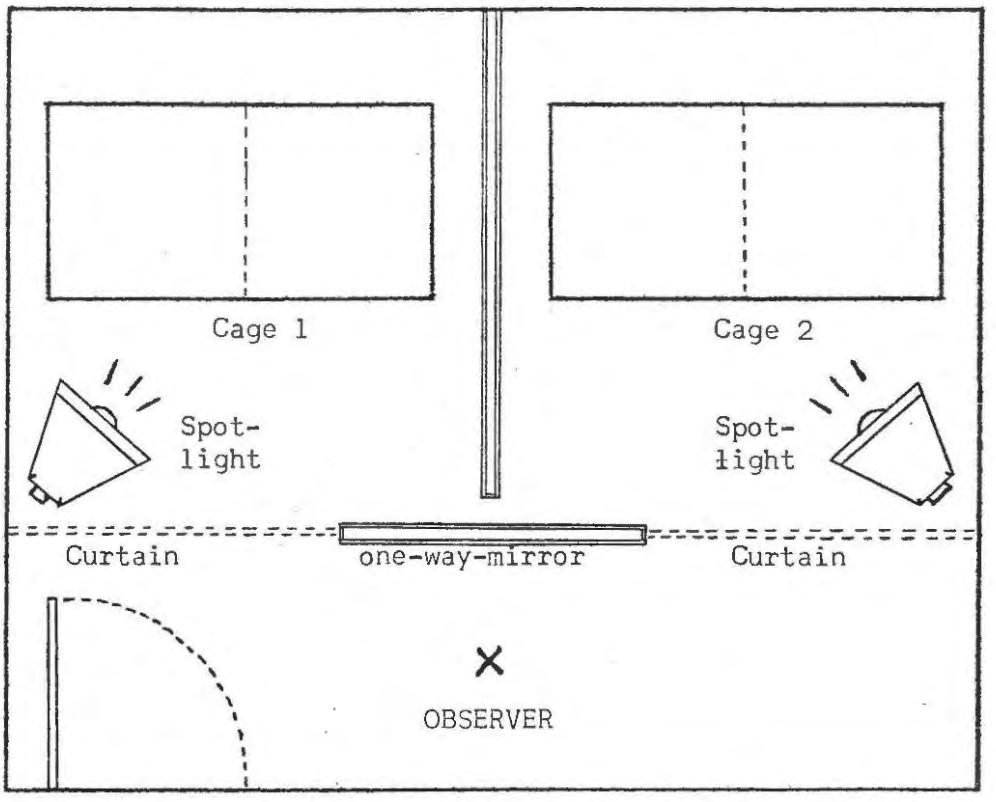

Cage 1
Cage 2
Spot-
light
Spot-
light
Curtain
one-way-mirror
Curtain
OBSERVER

Figure 13 Schematic diagram of observation room used for behavioural observations.

Figure 14 Behavioural observations recorded from behind one-way-vision mirror.

to occur. Sometimes this condition was not met, in which case the female was paired with a second male. This happened with three females in these experiments, but as they were also unsatisfactory with a second and even with a third male, they were discarded and did not feature in the results.

2.3.1. Measurements of Behaviour

The rhesus male is a multiple mounter. During most mounts, the penis is intromitted into the vagina, and a number of pelvic thrusts are delivered (see fig. 15). Between each mount, the animals may sit together or groom each other. The series of mounts continues until ejaculation, but after ejaculation no further mounting activity is shown by the male for a time, usually for at least 15 minutes (the "refractory period"). Ejaculation is typified by a prolonged intromission by the male during which thrusting stops and the back is arched. Spasmodic muscle contraction is seen and semen is discharged. During the male's ejaculation, the female usually shows an "ejaculatory response" (see fig. 16, and 2.3.2.1. below). Individual males vary considerably, both in their mounting rates and in the length of the refractory period following ejaculation (see also Michael and Saayman, 1967). It is not unusual for a male to mount up to twice a minute, with each intromission lasting about two seconds, during which time five to ten thrusts are delivered. Some males average twelve or more thrusts per mount, and could ejaculate twice or even three times in one thirty minute observation period.

This relatively stereotyped pattern of behaviour in the rhesus monkey lends itself to quantitative study. A standardised scoring system was used to measure the various components of the behaviour of the pair. This system has been developed over the last ten years at Birmingham University and elsewhere (see Herbert, 1970; Michael and Zumpe, 1968),

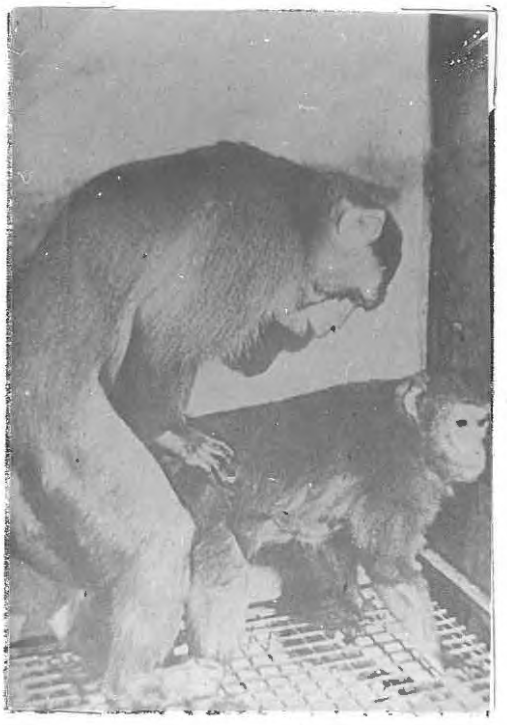


Figure 15 Male rhesus mounting receptive female. Note position of male feet on female ankles.

Figure 16 Female ejaculatory response. Note spasm of male musculature during ejaculation and the three female ejaculatory responses of head turn (Δ) lipsmack (Θ) and hand clasp (C).

and has been used to quantify individual components of the sexual, social and aggressive behaviours of the pair, as well as the time and order in which they occur.

The female is placed in the observation cage, the divider removed and the observer retires behind the one-way mirror. He then starts the stop-clock, and remains as silent and motionless as possible while completing the score sheet (see fig. 14). The observation period is divided arbitrarily into thirty second intervals, running from a quarter past the minute to a quarter to the minute. All the intervals thus refer to the thirty-seconds around either the minute or the half minute, except for the first and last divisions, which are only fifteen seconds long.

2.3.2. Components of Behaviour Studied

Sexual, social and aggressive behaviours were recorded.

2.3.2.1. Sexual Behaviour

Initiations

Sexual behaviour may be initiated either by the male or the female. The male-initiated mounts are usually preceded by the male approaching the female from the rear and clasping her hips with his hands to position himself behind her. If such a clasp was not followed by a mount then a "clasp" was scored. During the mount, the male grips the back of the female's legs with his feet, and thus the entire weight of the male body is supported by the female (see fig. 15). An incomplete form of mount, for example, if the male slips off the female, is scored as a "half mount". Neither clasps nor half mounts figure in the results.

The female-initiated mounts are usually preceded by a sexual

invitation from the female. Three forms of invitation were scored. The "classic" female presentation consists of a posture in which the female stands on all fours and presents her hindquarters to the male, often reflecting the tail to one side and adopting a very slight crouch (see fig. 17). In this position, male mounting and intromission is facilitated. A second possibility is for the female to stand or sit and "head-duck". During this gesture, the head is bobbed up and down in a more or less vertical plane (see fig. 18). Sometimes the gesture is accompanied by a hand slap of the cage floor. Thirdly, the female could give a "hand-reach", whereby she reaches back and touches the male with her hand (see fig. 19). Very occasionally, the female may mount the male, apparently to induce him to mount (Everitt, 1970). No attempt was made to distinguish between various intensities of sexual invitation. They were scored as single presentations irrespective of the form in which they occurred. Presentations are either accepted or refused by the male. A male could refuse a female sexual presentation or invitation by ignoring the gesture, or, more commonly, by lipsmacking and either grooming the female, touching the female or presenting himself for grooming.

Refusals

A female could refuse a male initiation by either jumping away or threatening him (see fig. 20) or, more commonly, by sitting down when clasped or refusing to get up from a prone position. This usually follows a period of being groomed by the male. If the male made a definite clasp or approach, and the female actively refused the mount, a female refusal was scored.

Thrusts

The number of pelvic thrusts made during each mount was recorded, providing intromission had occurred. It is not uncommon for a male to make a number of small pelvic movements while trying to insert the penis

Figure 17 Classic female presentation. Note slight crouch, reflection of tail and turning of genitalia towards the male (foreground).

Figure 18 "Head duck" form of female invitation to mount. The head is bobbed rapidly in a vertical plane.

Figure 19 "Hand reach" form of female invitation to mount. The female reaches back to touch the male, or slap the floor of the cage.

Figure 20 Female refusal. Note grimace on face of male, a gesture of "appeasement" or "conciliation", often accompanied by lipsmacking.

into the vagina, but these are to be distinguished from intromitted pelvic thrusts which are much deeper. Likewise during ejaculation, a number of supernumerary thrusts are made during the ejaculatory spasm, but these were not recorded.

Ejaculatory Response

During the male's ejaculation, the female often makes an ejaculatory response. She could turn her head to face the male, lipsmack and reach with her hand to clasp the male's arm, flank, leg or even face and genitalia (see fig. 16). An arbitrary one point was scored for each of the three possible gestures of head-turn (Δ), lipsmack (θ) or hand reach (C). The maximum ejaculatory response score is thus three points.

2.3.2.2. Social Behaviour

Grooming and grooming invitations as well as proximity behaviour were recorded to yield indices of social behaviour. The time spent by the male grooming the female or the female grooming the male was measured to the nearest half minute. Grooming could either be started by an animal moving into proximity with the other member of the pair and grooming (see fig. 21), or else in response to a definite grooming invitation. Grooming invitations were scored as either accepted or refused depending on whether or not the invitee started grooming within half a minute of the invitation being made. Grooming invitations were distinguished from sexual invitations in that usually an animal lies down in front of the invitee and offers parts of the body for attention (see fig. 22). Lipsmacking often accompanies such a gesture.

Proximity is measured as the time the pair spent within arm's length of one another, to the nearest half minute.

Figure 21 Female/male grooming behaviour. Male is relaxed and faces away from female (no eye-contact).

Figure 22 Grooming of throat region. The male has risen from a prone position and presented the throat for grooming. Again, note absence of direct staring (threat).

2.3.2.3. Aggressive behaviour

Aggressive behaviour usually takes the form of threatening, and actual physical contact between the two members of the pair is hardly ever made (see fig. 20). Aggressive episodes were quantified simply in terms of the number of half minute intervals in which such an act occurred. The direction of the aggression was scored, but no attempt was made to grade it according to intensity. Aggression is very seldom recorded in the male/female pair situation.

When a particular behaviour takes place, it is entered in appropriately marked columns against the half-minute interval in which it occurred. A typical sequence of behavioural events is shown in the example of a score sheet given in fig. 23.

2.3.3. Interpretation of the Scoring System

In this case, the male mounted the female immediately the divider was removed and delivered five thrusts. This is entered as a male-initiated mount, with a five, in the first division (i.e. within the first 15 seconds). No behaviour took place in the next half minute but in the half minute round minute 1, the male moved to the female and sat next to her. This is recorded in column 8 as "Proximity". In the next half minute, the female presents to the male, who accepts the presentation by mounting and delivering five thrusts (column 1). After that the female presents again, but this time the male refuses the presentation (column 3) and instead starts to groom the female (column 6). Grooming continues for another minute.

During the half minute around minute $3\frac{1}{2}$, the female again presents. But the male refuses, and instead presents himself for grooming, which the female accepts (column 4). After half a minute, the male sits up and

Figure 23. Example of score sheet used for behavioural observations.

♀ 1886 with ♂ 1935
Test No. 6 (O.B.) 27/6/1973

Column 1 Mounts and thrusts	2 3 Initiation ♂ ♀	4 5 Invitation ♂ ♀	6 7 Grooming ♂ ♀	8 Prox.		9 10 Aggression ♂ ♀
0 5♂					♂ to ♀	
1				✓	♂ to ♀	
5♀				✓	(∴) classic	
2	R		✓	✓		
			✓	✓		
3			✓	✓		
	R	A	✓	✓		
4			✓	✓		
6♂				✓	♂ thr. ♀	✓
5						
	R					
6						
10 ♂E					3 ♀ (Δ, Θ, C)	
etc.						
27			✓	✓	♀ to ♂	
			✓	✓		
28			✓	✓		
			✓	✓		
29			✓	✓		
			✓	✓		
30						

clasps the female, who allows mounting and six thrusts (column 1). The female now attempts to groom the male, but instead he threatens her (column 9) and moves away (column 8). Around minute $5\frac{1}{2}$, the male moves to the female, and clasps, but she refuses by sitting down (column 2). But a minute later, the male tries again, the female accepts and the male mounts to deliver 10 thrusts and ejaculates (column 1). At ejaculation, the female shows a strong ejaculatory response by turning her head, lipsmacking and clutching the male's shoulder with her hand. Her ejaculatory response is thus scored as 3 (Δ , Θ , C).

After that no more interaction takes place, since the female gets up on the perch and the male sits and cleans his genitalia. Eventually, the female gets down and invites grooming. The male accepts and grooms until the end of the test.

From this score sheet, the various measures of sexual, social and aggressive behaviour are computed and derived. For example, the animals spent a total of 7 minutes in proximity to one another. The male groomed the female for $4\frac{1}{2}$ of these minutes. The female presented 3 times and the male accepted only one third of these presentations.

2.3.3.1. Computed indices

The score sheet yields the following computed indices:-

For the female:- (1) total number of female presentations, both accepted and refused by the male (2) number of presentations to the first ejaculation, both accepted and refused by the male (3) total number of female refusals (4) number of female refusals to the first ejaculation (5) female's ejaculatory response.

For the male:- (6) total number of mounts during the test, both male and female initiated (7) number of mounts to the first ejaculation, both

male and female initiated (8) time to the initiation of sex behaviour, i.e. to the first mount (9) ejaculation time, the time in minutes from the first mount until the occurrence of the first ejaculation (10) total number of thrusts.

Social behaviours:- (11) total proximity (12) total grooming by both male and female (13) total grooming invitations, accepted and refused, by both male and female (14) total aggressive episodes, both male and female.

These indices are then expressed as means per test for the series of 10 observation periods, except for the female refusals and the aggressive episodes which are given as totals per 10 tests.

2.3.3.2. Derived indices

From the computed indices, the following indices are derived for the whole series of 10 tests:- (1) Percentage of the number of mounts to the first ejaculation which were initiated by the female. Together with the mean number of presentations per test, and the inverse of the total number of refusals, this measure yields a good index of the sexual receptivity of the female. (2) Female acceptance ratio to the first ejaculation. This is given by the expression:- total number of successful male initiations / the total number of male initiations made, expressed as a percentage, and provides a further index of female sexual receptivity. (3) Mean thrusts per intromitted mount. This is given by the total number of thrusts / the total number of intromitted mounts for each test, expressed as a mean for 10 tests. (4) Mean mounting rate. This is given by the total number of mounts less the ejaculatory mount / the time between the first mount and the ejaculatory mount (excluding the half minute in which ejaculation occurred for each test) expressed as a mean for 10 tests. (5) Sexual Performance Index (SPI). This is given

by the mean thrusts per mount multiplied by the mean mounting rate. This yields a measure of male sexual activity (Michael and Saayman, 1967), BUT THE INDEX IS NOT USED IN THE ANALYSIS OF DATA GIVEN IN CHAPTER 3 BELOW. (6) Male acceptance ratio. This is given by the total number of female presentations accepted by the male over the total number of female presentations made, expressed as a percentage, and yields a measure of the attractiveness of the female to the male. IN ADDITION, THE NUMBER OF EJACULATIONS MADE BY THE MALE IN TEN TESTS WAS SCORED.

If ejaculation did not occur in all ten tests, indices dealing with ejaculation were calculated on the basis of data obtained only from the tests in which ejaculation did occur.

2.3.4. Treatments for Behavioural Observations

2.3.4.1. Maintenance treatments.

The ten adrenalectomised females were maintained on cortisol, given as hydrocortisone sodium succinate (Boots) at the rate of about 3.0mg/kg body weight per day. Animals received 20 mg per day by intramuscular injection into alternate legs. In addition, they received deoxycorticosterone trimethylacetate (Percorten, Ciba), at the rate of 25 mg once a month by deep intramuscular injection. The animals remained in excellent health throughout the experiments and no adrenal insufficiency resulted.

2.3.4.2. Hormone treatments

Hormone treatment was started from five to seven days before the start of behavioural observations, and continued every day for the duration of the test series. Oestradiol was administered as Oestradiol benzoate (Dimenformon, Organon), dissolved in arachis oil and 10 per cent. benzyl alcohol, either 15 ug or 25 ug per day. This was given to females whenever they took part in behavioural observations, to make them attractive to males (Herbert, 1966), and was given at the end of each afternoon's testing by intramuscular injection into alternate legs.

2.3.4.3. Pharmacological treatments

Two pharmacological agents were given to manipulate the levels of 5HT in the brains of the females:-

First, para-chlorophenylalanine methylester hydrochloride (PCPA, Hassle, Goteborg) was dissolved in Sorenson's phosphate buffer (pH 7.8). Females received either 75 mg/kg or 100 mg/kg either subcutaneously or by intramuscular injection. Treatments started four days before tests were due to begin and thence every fourth day until the end of the test series. Females therefore received four PCPA injections in all. PCPA is a selective inhibitor of 5HT synthesis (Koe and Weissman, 1966).

Secondly, L-5-hydroxytryptophan methylester hydrochloride (5HTP, Calbiochem, San Diego) was dissolved in Sorenson's phosphate buffer (pH 7.8). Females received 20 mg/kg subcutaneously or by intramuscular injection. Treatments started two days before tests were due to begin and continued every second day until the end of the test series. Females therefore received six 5HTP injections in all. 5HTP is the substance whose synthesis is inhibited by PCPA (1.6.2. above).

2.3.5. Animals used for Behavioural Observations

Eight ovariectomised females were tested with four males while receiving either 15 ug or 25 ug oestradiol per day. They were then bilaterally adrenalectomised and retested with the same males while receiving the same amounts of oestradiol as before. Two other adrenalectomised females (1265, 1992), which had been used in a previous experiment (Everitt and Herbert, 1974) were then added to give a total of 10 adrenalectomised animals. These two animals had brain implant cannulae in position, but were in excellent health and were quite unreceptive since they had not had any hormonal treatment, other than cortisol, for several months.

All ten of these females then received oestradiol as before but

with PCPA, and were retested with the same males. The females used for the adrenalectomy/PCPA experiments were 1886, 2259, 1992, 1265, 2263, 2219, 2261, 2260, 1887, 2264. The males used were 1935, 2092, 2272, 2273. The pairings were as follows:-

Female	1886	paired	with	male	1935
"	2259	"	"	"	2272
"	1992	"	"	"	1935
"	1265	"	"	"	2092
"	2263	"	"	"	1935
"	2219	"	"	"	2092
"	2261	"	"	"	2092
"	2260	"	"	"	1935
"	1887	"	"	"	2273
"	2264	"	"	"	2273

Five of these 10 females were then given oestradiol and PCPA as before but with 5HTP as well, and retested. The females used for the PCPA+ 5HTP experiment were 1265, 1886, 2259, 2260, 2261.

Four (different) ovariectomised females were tested with three males while receiving 15 ug oestradiol per day. They were then given oestradiol as before but with 5HTP, and retested. The females were 315, 316, 317, 321. The males used were 1935, 1263 and 2088. The pairings used were as follows:-

Female	315	with	male	1935
"	316	"	"	1264
"	317	"	"	2088
"	321	"	"	1264

2.4. Biochemical Experiments

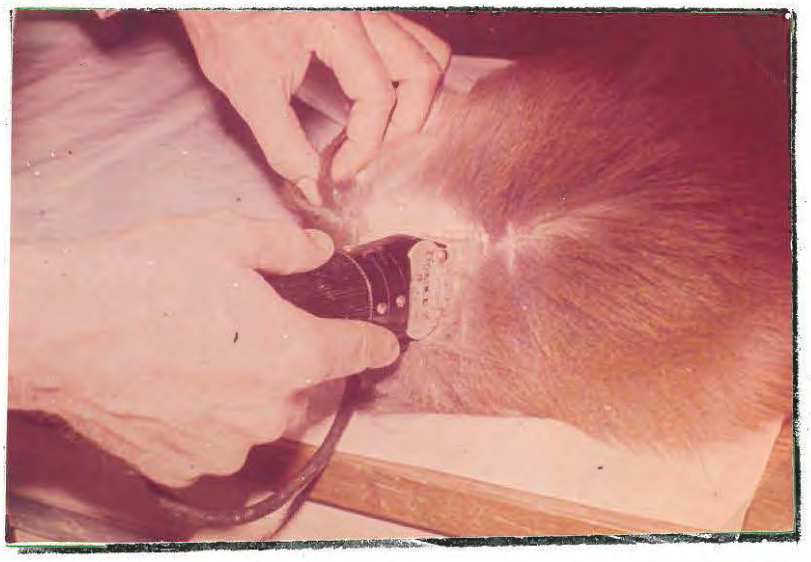
Twenty one females and fifteen males were used in the biochemical experiments. All the females were ovariectomised, and seven were also adrenalectomised. These seven had previously taken part in the behavioural observations described above. Ten males were intact, but five had been castrated.

2.4.1. Withdrawing Cerebrospinal Fluid

Cerebrospinal fluid (CSF) was withdrawn from the cisterna magna of monkeys under ketamine hydrochloride anaesthesia (Ketalar, 7-10mg/kg). In order to minimise the effects of circadian rhythms in CSF metabolites, tapping was done at the same time of day every time CSF was taken. For females, tapping was done between 0900 and 1100 hours BST on the day of assay, while males were operated on between 1400 and 1600 hours BST on the day of assay.

After the animal was anaesthetised, the hair on the back of the skull and neck was shaved clean (see fig. 24). The animal was brought to the operating table and an assistant laid it flat on its side. He then grasped the head with the right hand and the lumbar region of the back with his left, and bent the neck and spine into a curved position. This facilitated the insertion into the cisterna magna of a Howard Jones "21" children's spinal needle (Thackeray), of the sort used in pediatric lumbar puncture (see fig. 25). Great care was taken to keep on the midline, and not to insert the needle too far. The central trochar was then removed, and clear CSF dripped from the needle (see fig. 26). The CSF was then very slowly drawn off, using a glass syringe which had been sterilised after washing in 10 per cent. HCL (see fig. 27).

Potentially this is an hazardous operation, and monkeys could



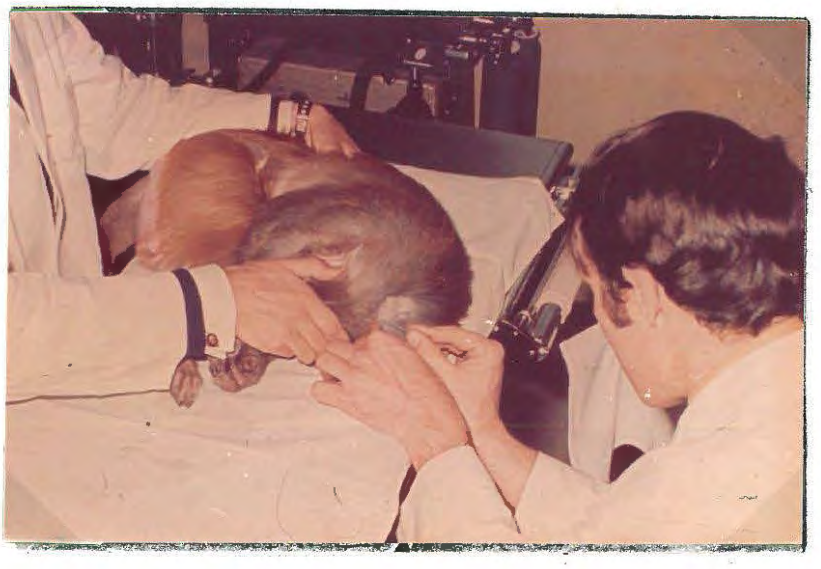
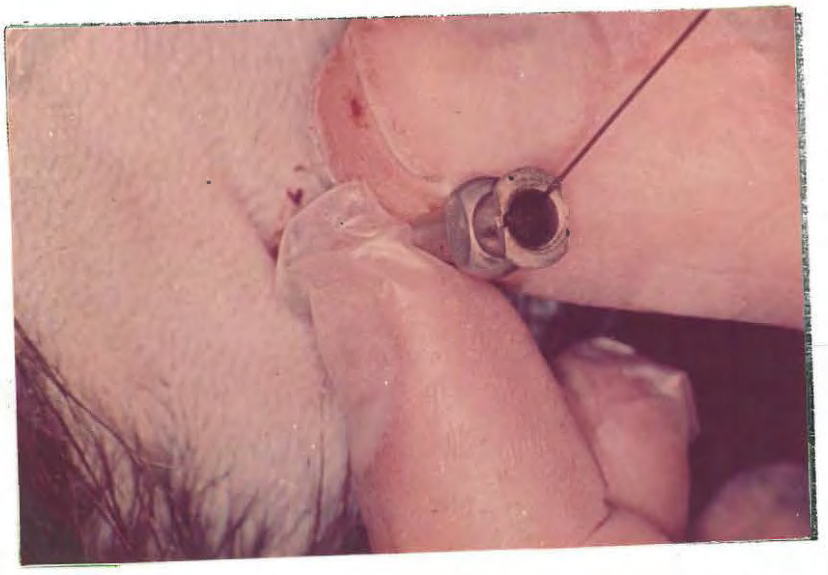


Figure 24 Extraction of cerebrospinal fluid from the cisterna magna :

1. Shaving the neck.

Figure 25 Extraction of cerebrospinal fluid from the cisterna magna :

2. Insertion of needle. Note full surgical anaesthesia of monkey and full aseptic routine.



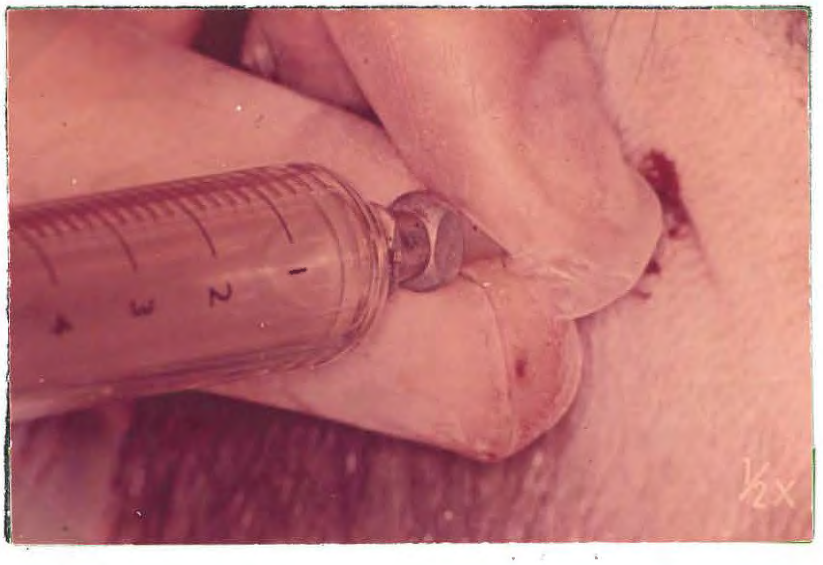

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Figure 26 Extraction of cerebrospinal fluid from the cisterna magna :

3. Removal of trochar.

Figure 27 Extraction of cerebrospinal fluid from the cisterna magna :

4. Drawing off of CSF into a sterilised glass syringe.

suffer serious neural lesions of the lower brain stem/upper spinal cord. The X-ray picture in fig. 28 shows the successful insertion of a tapping needle, and the gross anatomy of the region tapped. These lesions could result in paralysis of the lower extremities, or adversely affect respiratory and other vital functions, and could even be fatal. In practice, however, the operation was repeated many times without injury to the animals (483 cisternal punctures were made during the course of these experiments).

Occasionally CSF could not be withdrawn and in these cases the operator would take out the needle and start again. If CSF could still not be obtained, the operation was discontinued, since repeated probings of this area would put the animal at risk. On four occasions, temporary damage to the monkeys must have been done, since in the post-operative period the animals (two females and two males) had difficulty in moving about the cages and in using their hindquarters. This was most noticable two days after the operation, when they were not able to get up on the perch. But all four animals then showed gradual improvement over the next ten days and subsequently made complete recoveries. It was thought likely that these motor deficits were caused by internal haemorrhage in the region of the cisterna magna. Spontaneous recovery in a relatively short space of time after the operation would, perhaps, support this diagnosis rather than one of direct injury to the cord itself.

The CSF was immediately pipetted out into polypropylene centrifuge tubes, which had been carefully washed in 10 per cent. HCL, rinsed in double distilled water and dried in an oven. Usually about 4 or 5 mls. of CSF were collected from each animal. This was immediately pipetted into 1 ml aliquots in each tube, and ascorbic acid added (see below). The tubes were then stood in ice until assay. Usually four or five monkeys were tapped in one day.

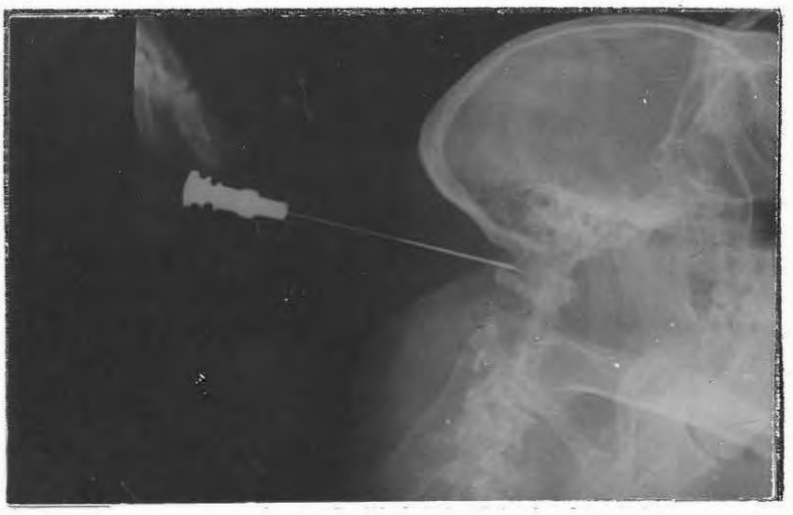

Figure 28 X-ray plate showing spinal needle inserted into the cisterna magna, just before drawing of CSF begins.

2.4.2. The Assay

The CSF was assayed for 5 hydroxyindole-3-acetic acid (5HIAA), the primary metabolite of 5HT in the CSF (Bowers, 1970) immediately after the last animal had been tapped. The method used was the spectrophotofluorimetric method of Ashcroft and Sharman (1962), modified slightly to speed up certain routine steps in the assay.

2.4.2.1. Equipment

An Aminco-Bowman Spectrophotofluorimeter (SPF, Cat No. 4-8100, American Instrument Company, Silver Spring) was used. The instrument is in three functional parts, an optical unit, a Xenon lamp ballast and Xenon lamp control, and a photomultiplier/microphotometer.

The optical unit consists essentially of a Xenon lamp (with housing and a blower) to deliver a beam for activation of the compound to be measured. This is delivered through a monochromator (grating type)onto the sample, contained in a quartz curvette, by lifting a cell shutter. The resulting fluorescence is directed via a second monochromator on to the photomultiplier by lifting a photomultiplier shutter.

The light is then transformed by the photomultiplier to a weak electrical signal, which is amplified by the photometer and displayed on a self-contained meter on the face of the photometer. The meter bears an arbitrary scale of 1 to 100 in convenient divisions.

A schematic drawing to show the arrangement of these components in the Aminco-Bowman SPF is given in fig. 29. A photograph of the machine is given in fig. 30.

Centrifuging was done in a Servall centrifuge. Temperature, time and rate of spinning could all be preset.

Figure 29 Schematic diagram of the Aminco-Bowman Spectrophotofluorimeter.

** activating light
* fluorescent light

Figure 30 Aminco-Bowman Spectrophotofluorimeter, Cat. No. 4-8100, American Instrument Company, Silver Spring.

All glassware was carefully washed in 10 per cent. HCL and rinsed twice in double distilled water before being oven dried.

Pipetting was done with four adjustable Finnpipettes (Jencons (Scientific) Limited, Hemel Hempstead), preset to deliver exact quantities of reagent. Finnpipettes were fitted with disposable polypropylene jets. Disposable pasteur glass pipettes were used in the final stage of the assay.

2.4.2.2. Reagents

A solution of 2 per cent. ascorbic acid in double distilled water was further diluted to make a 0.2 per cent. solution. Ascorbic acid was always made fresh on the day of assay.

Solutions of 10 per cent. Zn SO4 and 10 per cent. NaOH were mixed and kept in the laboratory at shelf temperature.

Standard solutions of 5HIAA were mixed fresh on the day of assay. First, 0.01 gms of 5HIAA (5-Hydroxyindole-3-acetic acid, Sigma) were added to 5 ml of 2 per cent. ascorbic acid, and a 50 ml flask filled with double distilled water. This gave a 100 ug per ml standard solution. A 1 ug per ml standard solution was then prepared from this by pipetting 1 ml of the 100 ug/ml solution into 10 ml of 2 per cent. ascorbic acid and filling a 100 ml flask with distilled water. Solutions could be kept at 4^{o}c in a fridge, but were usually used immediately.

The final reagent used was a mixture of hydrochloric acid + ascorbic acid, mixed as near to the time of use as possible. This was prepared by carefully adding 12.5 ml of 0.2 per cent. ascorbic acid to 37.5 ml of concentrated HCL.

2.4.2.3. Method

Ascorbic acid was added to CSF immediately after CSF had been with-

drawn from the animal and pipetted out into centrifuge tubes at the rate of 2 mg per ml of CSF. Separate weighing of dry powder into each polypropylene centrifuge tube was so time-consuming that a solution of ascorbic acid was prepared and the appropriate quantities were then pipetted into the tubes. The total volume per tube was brought to 1.2 ml of CSF + ascorbic acid solution.

Standards

For each assay, a set of standard values was also obtained, since the meter on the photometer gave readings on an arbitrary scale. This meant that each day at the same time as the CSF was assayed for 5HIAA, the following tubes were also prepared:-

Blanks: four blanks were made up of 1.2 ml of 0.2 per cent. ascorbic acid on its own.

Internal Standards were made up of 1.0 ml of CSF + 2 mg ascorbic acid + a measured amount of 1 ug/ml standard 5HIAA solution to give a total volume of 1.2 ml and the required ascorbic acid concentration.

External Standards were usually four tubes of each of three external standards made up, and labelled 200, 100 and 50. These referred to ng/ml of 5HIAA, being respectively 200 ng/ml 100 ng/ml and 50 ng/ml of 5HIAA pipetted into appropriate volumes of 0.2 per cent. ascorbic acid.

Precipitation of proteins. To each tube was added 250 ul of 10 per cent. $ZnSO_4$, and 50 ul of 10 per cent. NaOH, and the reagents thoroughly mixed on a bench mixer. Sixteen tubes were prepared at a time. The tubes were transferred to the Serval centrifuge and spun at 20°C for 20 minutes at 10,000 rpm.

1 ml of supernatant was then pipetted into clean glass test tubes.

Fluorimetry. A stop watch was started and 500 ul of hydrochloric/

ascorbic acid mixture were added to the first two test tubes, and mixed.

Between 10 and 15 minutes after the addition of the acid mixture (time is crucial at this stage), the contents of the tubes were transferred by pasteur pipette to the quartz curvette. Reading was done at 298 activation and 530 fluorescence on slit 5, with the Aminco-Bowman SPF at maximum sensitivity.

The process was repeated for all remaining tubes.

Calculation of the standard curve. The object of this step was to produce a reading on the arbitrary scale of the meter which corresponded to 200 ng/ml, 100 ng/ml and 50 ng/ml of 5HIAA. Readings for each tube of CSF could then be referred to the standard curve and calculated in ng/ml. With experience and the use of Finnpipettes, it was possible to obtain a standard curve which was seldom more than a few ng/ml out (2x "50"="100"=$\frac{1}{2}$x"200"). In cases of slight discrepancy, the line of best fit on graph paper was drawn, and the scale reading corresponding to 100 ng/ml was read.

The mean of four readings for the "Blanks" was calculated, and used to correct all standard and CSF readings.

Calculation of 5HIAA in the CSF. Since CSF readings were usually repeated three times for each animal, the mean of the readings was taken and referred to the standard curve. The internal standard reading (usually one ml of CSF) provided a check on both the CSF reading and the standard curves, since 100 ng of 5HIAA were added to one ml of CSF to make up the internal standard.

2.4.3. Treatments for Biochemical Experiments

For the females, sampling the CSF would have interfered with the behavioural observations being made, and therefore no CSF was taken from

animals during a behavioural series. CSF was taken at the end of a series or else treatments given were repeated where necessary. This did not arise for males in that different males were used for behavioural and biochemical experiments.

Females

2.4.3.1. The effects of PCPA and PCPA + 5HTP on 5HIAA levels

In order to monitor the effects of PCPA and 5HTP treatments on 5HT in the brain, the following measures were made (1) with no hormones, at least two months after ovariectomy and/or adrenalectomy (2) after oestradiol treatment of 15 ug/day for 10 days (3) after 4, 8 and 12 days on PCPA, i.e. after one, two and three injections of PCPA (4) after one and two weeks following withdrawal of PCPA + oestradiol treatment (5) after 2 days and 12 days of PCPA/5HTP with oestradiol treatment (6) one week after withdrawal of PCPA/5HTP + oestradiol treatment.

2.4.3.2. The effects of 5HTP on 5HIAA levels.

In order to monitor the effects of 5HTP alone on 5HT in the brains of oestradiol-treated ovariectomised females, measures were made (1) with no hormones, at least two months after ovariectomy (2) after oestradiol treatment, of 15 ug/day for 10 days (3) after 2 days and 12 days of oestradiol + 5HTP treatment (4) one week after the withdrawal of oestradiol + 5HTP treatment.

2.4.3.3. The effects of gonadal steroids on 5HT in the brain.

In order to monitor the effects of steroid hormones on 5HT in the brain, as measured by 5HIAA in the CSF, the following measures were made (1) with no hormones, at least two months after ovariectomy (2) with 15 ug/day oestradiol for 10 days (3) with 15 ug oestradiol per day as

before but with 15 mg progesterone in oil (Progesterone, Evans Medical Limited) for 10 days (4) with 250 ug and 400 ug per day testosterone propionate (Testosterone Propionate, Evans Medical Limited) for 10 days.

2.4.3.4. Turn-over rates of 5HT in the brain

In order to monitor the turn-over rates of 5HT in the brain as measured by the rate of accumulation of 5HIAA in the CSF over two hours, the probenecid test was used (Korf and Van Praag, 1970; 1971; Bowers, 1970). CSF was withdrawn as usual and then probenecid sodium (Merck, Sharpe and Dolme, Hoddesdon, 20 mg/kg.) in phosphates (pH 7.7 to 7.9) was given by slow intravenous injection. Exactly two hours later CSF was again withdrawn.

After assay, 5HT turn-over was expressed as the percentage increase of 5HIAA over the two hour period, using the first reading as base.

The same steroid hormone treatments were repeated in the same females to give turn-over rates of 5HT as well as levels of 5HIAA after each treatment.

Males

2.4.3.5. The effects of androgens on 5HT in the brain

In order to monitor the effects of androgens on 5HT levels and turn-over rates as measured by 5HIAA levels in the CSF, bilaterally castrated males were left for six months after the operation in individual cages in the communal monkey house. CSF was then withdrawn, a probenecid test administered and the two tappings of CSF assayed for 5HIAA. Animals were then given 10 mg/day testosterone propionate for 10 days and the probenecid test repeated.

2.4.3.6. The effects of ejaculation on 5HT in the brain

In order to monitor the effects of recent ejaculations on 5HT levels and turn-over rates in the brain, males were housed in individual cages in the communal monkey house for at least six months. CSF was then withdrawn, a probenecid test administered and both tappings of CSF assayed for 5HIAA. After a week to ten days, animals were taken to the behaviour observation laboratory and placed in the large observation cages. A fully receptive and attractive (oestradiol-treated) female was then placed with the male as for a normal behavioural observation period. The observer retired behind the one-way mirror. As soon as the male ejaculated, the observer separated the two animals and immediately anaesthetised the male. CSF was then withdrawn, a probenecid test administered and both tappings of CSF assayed for 5HIAA. The first tapping of CSF was always done within at most five minutes of the animal ejaculating, and the second tapping two hours after that.

Control:

To test for possible order effects in this experiment, five of the animals were tested in reverse order, namely, ejaculation first and "baseline" ten days later.

2.4.5. Animals Used for Biochemical Experiments

Females

2.4.5.1. Effects of PCPA on 5HIAA levels.

Nine ovariectomised females were used to study the effects of PCPA on 5HIAA levels in the CSF. Seven of these were also adrenalectomised, i.e. seven of the ten animals that had taken part in the behavioural observations. Two other animals who were ovariectomised but not adrenalectomised, were also given the PCPA treatments (1864 and 2267), to see whether there were any marked differences in the effect of PCPA on non-

adrenalectomised animals. The animals used were 1886, 1887, 2259, 2260, 2261, 2263, 2264 and 1864, 2267.

2.4.5.2. Effects of 5HTP on PCPA treated animals

Five of the above animals then received the normal PCPA treatment but with 5HTP as well. The animals used were 1886, 2259, 2260, 2261, 2263.

2.4.5.3. Effects of 5HTP alone on 5HIAA

The same four ovariectomised females that had taken part in the behavioural experiments to measure the effects of 5HTP alone on sexual receptivity, were also used to measure the effects of 5HTP on 5HIAA levels in the CSF. The animals used were 315, 316, 317, 321.

2.4.5.4. Effects of gonadal steroids on 5HT levels and turn-over rates in the brain

Ten ovariectomised females were used. Eight of these were not used at all for any experiments other than these hormonal ones. But two animals, (1864, 2267) were subsequently used as controls in the PCPA biochemical experiments, since they were not adrenalectomised. However, the hormonal experiments had been completed, and these two animals had not received any hormones for at least two months prior to being used in the PCPA experiments. The animals used were 1091, 1080, 1353, 1520, 1754, 1811, 2266, 2268, and 1864, 2267.

Males

2.4.5.5. Effects of androgens on 5HT levels and turn-over rates in the brain.

Five males were used, all of whom were bilateral castrates. These males were colony bred (Messrs. Pfizers Limited), and their numbers were

2286, 2287, 2288, 2289, 2290.

2.4.5.6. Effects of ejaculation on 5HT in the brain

Ten males were used, all of whom were intact and had been caught in the wild. Their numbers were 1263, 1264, 1654, 1994, 2088, 2089, 2272, 318, 325, 326.

2.5 Experimental Design and Statistical Treatment

The designs and statistical treatments used for the behavioural and biochemical experiments were as follows:-

2.5.1. Behavioural Experiments

The male/female pair must form the unit of analysis. This is because an individual male might well change his behaviour depending on the female with which he is paired (see Herbert, 1968; Everitt and Herbert 1969 a, b; Michael and Saayman, 1967). If the same pairing is kept throughout all treatment conditions, such sources of variability are reduced to a minimum.

Ten tests were given under each treatment condition. Comparisons are therefore between matched pairs of tests on each treatment. All the data for a pair were first subjected to a two-way analysis of variance. An assumption of normality in the distribution of scores for the ten tests was not justified in these circumstances, and therefore ordinal analysis was carried out using Friedman's two-way analysis of variance (Siegel, 1956).

In those cases where the χ_r^2 statistic was associated with a probability of $p < 0,05$, differences between individual treatments were tested by the Wilcoxon matched-pairs signed-rank test (see also Siegel, 1956).

The following comparisons were thus made using the Wilcoxon test:-

(1) Oestradiol alone versus adrenalectomy + oestradiol, to determine the effects of adrenalectomy on sexual receptivity.

(2) Adrenalectomy + oestradiol versus adrenalectomy + oestradiol + PCPA, to test the effects of PCPA.

(3) Adrenalectomy + oestradiol + PCPA versus adrenalectomy + oestradiol + PCPA + 5HTP, to test the effects of 5HTP.

(4) Adrenalectomy versus PCPA + 5HTP, to test whether 5HTP returned behaviour to levels not significantly different from these obtained before PCPA was given.

In addition, in four pairs, oestradiol versus oestradiol + 5HTP to test the effects of 5HTP alone.

These comparisons were made for each of the measures of sexual behaviour, grooming and social behaviour used. In all, there were seventeen measures used, with fourteen pairs of animals and (usually) three or four treatment conditions, altogether totalling 720 hours of observation.

Fig. 31 below illustrates just one example of such a comparison. The behavioural measure "Female presentations" is taken in the case of female 1886 (always paired with male 1935). The two treatments to be compared are "oestradiol" versus "adrenalectomy + oestradiol".

Under oestradiol, in test one, the female made ten presentations. In test two, she made five presentations, etc. After adrenalectomy, however, in test one she made only one presentation. In test two she made two, etc. The mean number of presentations she made under oestradiol was 75/10 = 7.5, and after adrenalectomy it was only 20/10 = 2.0.

After Friedman's two-way analysis of variance (assuming there were other treatment conditions as well), the oestradiol scores were compared with the adrenalectomy scores using the Wilcoxon test.

In addition to this analysis for each individual pair of animals, using the above tests, further analysis was carried out on the "grouped data". This was to test for the overall effects of each treatment on all the animals. For example, for animal 1886 above, the mean scores of 7.5 (oestradiol) and 2.0 (adrenalectomy) would be entered in the

the results table. In the "Results" section below, behavioural data are all presented in this form:-

	Oestradiol	Adrenalect.		Difference	Rank of Difference
1	10	1		+9	$9\frac{1}{2}$
2	5	2		+3	$3\frac{1}{2}$
3	10	2		+8	$7\frac{1}{2}$
4	5	2		+3	$3\frac{1}{2}$
5	10	3		+7	6
6	5	2		+3	$3\frac{1}{2}$
7	10	2		+8	$7\frac{1}{2}$
8	5	3		+2	1
9	10	1		+9	$9\frac{1}{2}$
10	5	2		+3	$3\frac{1}{2}$
Totals	75	20			= 0
Means	7.5	2.0			

n = 10. t = 0 p < 0.01 (Significant)
indicator : * *

Figure 31. Example of comparison using Wilcoxon matched-pairs signed-rank test.

Note that means (oestradiol and adrenalectomy) are entered in the first two columns of fig. 32, with indicator * * (i.e. p < 0.01).

PRESENTATIONS PER TEST

GROUPED DATA

(illustrative data only)

Treatment Animal No.	Oestradiol	Adrenalectomy	PCPA	5HTP
1886	7.5	2.0 **	5.2 **	
2259	3.0	3.0	3.0	
1992	7.2	2.1 *	3.4	
1265	6.0	4.3	6.5 *	
etc.	.	.	.	
	.	.	.	
	.	.	.	
Totals	40	25	35	
Means	4.0	2.5	3.5	
t df Significance				

Figure 32. Example of typical summary table, giving means / treatment for all ten animals.

Grouped data then subjected to analyses of variance and paired t-tests.

Thus for each behavioural measure (such as female presentations), a table was drawn up giving scores which were means for ten tests under each treatment condition. Next to each score (mean) is an indicator, indicating whether the differences were significant at the appropriate confidence limit on the Friedman/Wilcoxon analysis carried out as above (no indicator = not significant, * = $p < 0.05$, ** = $p < 0.01$).

Then, in addition, the scores (means) for all the animals under one treatment condition (say, "oestradiol") were totalled and compared with the corresponding scores for the next treatment (say adrenalectomy). Because the data compared refer to the means of ten tests in each case, the data were much more normally distributed (a graph plot and visual inspection). To make each comparison, paired "t" tests were therefore used. Since previous work (Everitt, Herbert and Hamer, 1972) had already shown that sexual receptivity in female rhesus monkeys is reduced by adrenalectomy, and since there was evidence to suggest that PCPA facilitates sexual behaviour in rodents and other non-primate mammals, one-tailed tests were applied.

The assumption of homogeniety of variance appears justified in this case. The data refer to the same pairings of animals throughout the experiments. Following Hays (1963, p 322 ff), no prior, separate tests for homogeneity of variance were therefore carried out, other than visual inspection (see also Everitt, 1970; Dixon, 1973).

In Chapter 4 (below), it is stressed that such overall comparisons of the "grouped data", while illuminating, are to be interpreted with great care. For example, it could happen that after adrenalectomy, seven out of the ten females present significantly less than they did before the operation (Wilcoxon, $p < 0.01$ for each, say). It could also be that a paired "t" test, on the "grouped" data including the other three animals,

shows a difference between the two treatments which does not reach the required (one-tailed) significance level ($p < 0.05$). However, this need not be interpreted as indicating that the treatment produced no effect on sexual receptivity in these females. It is quite possible that the three females which did not respond to the operation by presenting less, did start refusing the male more. In which case, the sexual receptivity of all ten animals was certainly reduced by the operation. But this reduction in 'receptivity' was not shown in the same way in all ten cases.

2.5.2. Biochemical Experiments

In the biochemical experiments, the experimental design amounted to a simple "before and after treatment" comparison, with each animal acting as its own control.

Data for all 5HIAA levels were in ng/ml of CSF, and so scores could be compared directly. However, turn-over rates of 5HT in the brain were expressed as percentages, and so these had first to be transformed (arc-sine) to convert the percentages to angles, before comparisons were made (see also Everitt, 1970).

Females

2.5.2.1. The effects of PCPA and 5HTP on 5HIAA levels

Since PCPA is a known depletor of brain 5HT (Koe and Weissman, 1966) and since 5HTP is the immediate precursor of 5HT and the substance whose synthesis is inhibited by PCPA, the predicted direction of change of these treatments was clear. A one-way analysis of variance was carried out on the means of each treatment condition, namely, oestradiol, PCPA (4 days), PCPA + 5HTP (2 days) and oestradiol alone (after 7 days

off 5HTP). Where the F statistic (n = 9, df Treat. = 3, df Error = 32) was associated with a probability of 0.05 or less, paired "t" tests were used for the individual relevant comparisons.

To test the effects of PCPA and 5HTP on 5HIAA levels in the CSF, the following comparisons were made:-

(1) Oestradiol alone versus oestradiol + PCPA (after 4 days).

(2) Oestradiol + PCPA versus oestradiol + PCPA + 5HTP (2 days).

(3) Oestradiol + PCPA + 5HTP versus oestradiol alone (7 days off 5HTP).

Comparisons were not made with PCPA after 8 days and 12 days, since these gave levels of 5HIAA even lower than at 4 days. Similarly, 5HTP levels after 12 days were obviously higher than after 2 days.

2.5.2.2. Effects of 5HTP alone on 5HIAA levels

To test the effects of 5HTP alone on 5HIAA levels in the CSF of the four animals given 5HTP, the same analysis was carried out. The following comparisons were made:-

(1) Oestradiol versus oestradiol + 5HTP (2 days).

(2) Oestradiol versus oestradiol + 5HTP (12 days).

(3) Oestradiol versus oestradiol (after 7 days off 5HTP).

Because the sample size is so small, the assumptions governing the analysis are not justified in this case. But the effects of 5HTP were marked, and so it is not clear that a larger "n" would achieve very much more. The results of these experiments are therefore included for completeness.

2.5.2.3. Effects of steroids on 5HT levels and turn-over rates in the brain

Both the levels of 5HIAA in the CSF and the turn-over rates of 5HT

in the brain, as measured by the rate of accumulation of 5HIAA in the CSF, were compared under the various hormonal treatment conditions.

Since there was no clear prediction about what effects such gonadal steroid treatments would have on 5HIAA levels, the analysis was by two-way analysis of variance. In these cases where the F statistic was associated with a probability of 0.05 or less, the relevant differences between means of treatment conditions were tested by paired "t" test, as follows:-

(1) No hormones versus oestradiol.

(2) No hormones versus testosterone.

(3) Oestradiol versus oestradiol + progesterone.

(4) Oestradiol versus return to no hormones.

(5) Testosterone versus return to no hormones.

Males

2.5.2.4. Effects of androgens on 5HT levels and turn-over rates in the brain

The following comparison was made for both (1) the levels of 5HIAA in the CSF and (2) the turn-over rates of 5HT in the brain as measured by the rates of accumulation of 5HIAA in the CSF:-

No hormones (at least 6 months after castration) versus testosterone propionate replacement.

2.5.2.5. Effects of ejaculation on 5HT in the brain

The following comparison was made for both levels and turn-over rates of 5HT in the brain:-

Base line (after housing in individual cages) versus immediately after ejaculation.

In all four cases, paired "t" tests were used since there was only one comparison to make.

2.6. Calculator

Analysis was done by means of a Hewlett-Packard Model 10 series 9810A programmable calculator.

CHAPTER 3

RESULTS

Results are presented in four parts.

In part 3.1, the results of all the behavioural observations on male/female pairs of monkeys are given, both before and after pharmacological treatments.

In part 3.2, the effects of these pharmacological treatments on 5HIAA levels in the CSF are given.

In part 3.3, the effects of physiological doses of gonadal steroids on 5HIAA levels and 5HT turn over rates are presented.

In part 3.4, the results of the experiments conducted on the males are detailed.

PART 3.1.

3.1. Behavioural Observations on Male/Female Pairs

Once the divider was removed and the female allowed to interact with the male, the usual behaviour observed was that of copulation and grooming. Aggressive behaviour was only observed once in all the tests which were conducted (720 hours of observation).

Individual males vary considerably in their patterns of copulatory and grooming behaviour. Some males hardly ever groom a female, and will not sit next to her during the mounting sequence. Others will either groom or allow grooming for the full 30 minutes of the test period, interrupting this grooming only to mount the female and thrust. Mounting rates and thrusting rates are usually typical of a particular male irrespective of his female partner (see also Michael and Saayman, 1967), providing the female is reasonably receptive.

Most males will invite grooming from a female at some stage of the test period. Perhaps the most frequent stage will be after the ejaculatory mount. It is quite common for a male to dismount, clean his penis and then be groomed by the female. If the female is not sexually receptive, a male might modify both his sexual and his grooming behaviour, and examples of this will be given below.

3.1.1. The Effects of Bilateral Adrenalectomy, PCPA and PCPA + 5HTP on Sexual Behaviour

Results in this section are given under two headings, namely Female Sexual Behaviour and Male Sexual Behaviour.

Female Sexual Behaviour

3.1.1.1. Presentations

Since this is one of the most important measures of female sexual receptivity, it will be dealt with in some detail.

Oestradiol versus adrenalectomy

In all eight females for which the first comparison is possible, a statistically significant decrease in the mean number of presentations per test was recorded (female 2261, $p < 0.05$, all others $p < 0.01$), following adrenalectomy (Table 1). In the case of females 1992 and 1265, no preadrenalectomy data were available (see 2.3.5. above). Bilateral adrenalectomy likewise reduced the mean number of presentations per test before the male's first ejaculation (Table 2). Thus the decrease in presentations was not due to any effect of the male's ejaculation.

Taking the "grouped data" for all females, Table 1 shows that after adrenalectomy the mean number of presentations per test decreased significantly (mean of 6.525 decreased to 1.03, df = 16, "t" 2.7279, $p < 0.01$, one-tailed test). Table 2 shows that the same applied to the number of presentations to the male's first ejaculation ($df=16$, $t=2.2175$, $p < 0.05$).

It is clear therefore that adrenalectomy reduced the mean number of presentations made by the females, both per test and to the male's first ejaculation. Not only was this decrease significant in every individual case, but the overall effect on all animals was significant as well.

Adrenalectomy versus PCPA

Table 1 shows that PCPA caused a significant increase in the mean

Table 1 - Mean number of presentations per test

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	20.6	1.1 **	16.1 **	8.3 **	**
2259	9.5	1.3 **	3.7 **	1.0 *	n/s
1992	n/d	0.9	5.8 **		
1265	n/d	4.1	10.9 **	4.6 **	n/s
2263	2.2	0.0 **	0.4		
2219	5.3	1.0 **	2.5 *		
2261	3.3	0.5 *	1.8 *	0.2 **	n/s
2260	1.3	0.0 **	0.0	0.0	n/s
1887	6.7	1.3 **	1.9		
2264	3.3	0.1 **	1.2 *		
Means	6.525	1.03	4.30	2.82	
t		2.7297	2.013	0.6169	
df		16	18	13	
Significance		$p < 0.01$	$p < 0.05$	n/s	

* $p < 0.05$

** $p < 0.01$

Wilcoxon matched-pairs signed-ranks test

Table 2 - Mean number of presentations per test to the male's first ejaculation

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	19.5	0.7 **	16.1 **	8.3 **	**
2259	5.3	1.0 **	3.4 **	1.0 *	n/s
1992	n/d	0.9	5.6 **		
1265	n/d	4.0	10.5 **	4.6 **	n/s
2263	1.9	0.0 **	0.4		
2219	2.5	0.6 **	2.4 *		
2261	1.9	0.5 *	1.5	0.2 **	n/s
2260	1.2	0.0 **	0.0	0.0	n/s
1887	6.0	1.3 **	1.8		
2264	3.3	0.1 **	1.2 *		
Means	5.2	0.91	4.29	2.82	
t		2.2175	2.0139	0.5658	
df		16	18	13	
Significance		$p < 0.05$	$p < 0.05$	n/s	

* $p < 0.05$

** $p < 0.01$

Wilcoxon matched-pairs signed-ranks test

number of presentations per test in seven of the ten females. In the case of three of these, the increase was significant at the 0.05 level, and in the other four $p < 0.01$. This increase in presentations per test is also reflected in the number of presentations to the first ejaculation by the male, except that here the presentations made by female 2261, while they increased, did not reach the required significance level (Table 2).

The overall effect of PCPA on all ten animals was to increase the number of presentations per test significantly (mean of 1.03 per test was increased to 4.43, $df = 18$, $t = 2.013$, $p < 0.05$). The same applied to the number of presentations to the first ejaculation (0.91 increased to 4.29, $df = 18$, $t = 2.0139$, $p < 0.05$).

This is one of the important findings in these experiments, and will be discussed in detail below. Furthermore, the three females (2263, 2260, 1887) which did not show significant increases in the number of presentations they made deserve comment. In each case, although they did not present more, they did refuse less (see Table 4 Total number of refusals, below). These three animals were thus "non-presenters".

PCPA versus 5HTP

In five animals PCPA + 5HTP was then administered, and Tables 1 and 2 show that in four of the five females, 5HTP significantly reduced the number of presentations per test and to the first ejaculation ($p < 0.01$ in each case). The fifth female (2260) was one of the "non-presenters", and it should be noted that her refusals increased markedly with 5HTP treatment (Table 4, below).

Overall, the effects of 5HTP were not significant both for the number of presentations per test and to the first ejaculation.

Adrenalectomy versus 5HTP

In four of the five animals given 5HTP, there were no significant differences between the PCPA + 5HTP-treated condition and adrenalectomy alone, before PCPA, either in the mean number of presentations or in the mean number of presentations to the first ejaculation. This tends to confirm what the PCPA versus 5HTP comparison shows. In the fifth animal (1886), the presentation rate was not reversed completely by 5HTP given with PCPA, and was significantly higher after 5HTP than it was after adrenalectomy alone.

3.1.1.2. Percentage of female initiated mounts to the male's first ejaculation

Table 3 shows that after adrenalectomy there was a significant decrease in the percentages of mounts to the male's first ejaculation which were initiated by the female, in seven of the eight females for which data are available. In the eighth female (2261) the percentage was decreased but not significantly (25.76 per cent. down to 16.25 per cent).

This effect was reversed by PCPA. In six of the ten females, the percentage was significantly increased. In three of the remaining four, the percentages went up but not significantly, and in female 2260 there was no change.

5HTP in turn reversed the effects of PCPA in three of the five animals (1886, 2259, 1265, $p < 0.01$). In the other two animals, one was not presenting at all at this stage (2260), and the other initiated a lower proportion of mounts than under PCPA alone.

When PCPA and 5HTP scores were compared with adrenalectomy alone, it was clear that there were no significant differences in the scores of three of the five animals. In the other two (1886 and 1265), the differences were significant, showing that 5HTP in these two animals had not

Table 3 - Percentage of female initiated mounts to the male's first ejaculation

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	72.58	5.56 **	68.20 **	35.20 **	**
2259	59.65	14.75 **	42.31 **	21.13 *	n/s
1992	n/d	13.23	35.00 **		
1265	n/d	41.67	93.33 **	65.90 *	*
2263	16.98	0.00 **	2.25		
2219	30.00	14.93 **	12.50 *		
2261	25.76	16.25	21.54	6.45	n/s
2260	16.36	0.00 **	0.00	0.00	n/s
1887	60.53	20.34 **	33.33		
2264	41.40	1.75 **	18.00 *		
Means	40.4075	12.848	32.646	25.736	
t		3.993	1.959	0.4431	
df		16	18	13	
significance		$p < 0.01$	$p < 0.05$	n/s	

* $p < 0.05$

** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test.

Measure is given by :- number of mounts initiated by female / total number of mounts (to the first ejaculation) as a percentage.

completely reversed the effects of PCPA, by decreasing the scores to the adrenalectomy - alone condition.

As might be expected, this derived score (percentage of female initiated mounts to the first ejaculation) therefore reflects the fact that presentations made by the females decreased after adrenalectomy, were partly restored by PCPA and were in turn decreased by PCPA + 5HTP.

The overall effect of adrenalectomy on all ten animals was to decrease significantly the percentage of female initiated mounts (from 40.4075 to 12.8480, df = 16, t = 3.993, $p < 0.01$). PCPA reversed this effect significantly (12.848 up to 32.646, df = 18, t = 1.9590, $p < 0.05$). But 5HTP had no significant overall effect.

3.1.1.3. Total female refusals during ten tests

Table 4 shows that the three females (2263, 2260, 1887) which did not present significantly less after adrenalectomy did refuse the male significantly more often. In all three cases, this was reversed by PCPA. This was significant in two cases (2263, $p < 0.05$, 2260, $p < 0.01$) and was very marked in the case of 2260.

In four of the five animals treated with 5HTP, refusals went up. This was significant in the cases of 2259 ($p < 0.05$) and 2260 ($p < 0.01$) and was very marked in the case of 2260.

Overall, adrenalectomy increased the number of refusals per animal from 0.111 to 11.9. PCPA reduced this again from 11.9 to 1.80, while 5HTP increased this markedly from 1.80 to 26.0. However, the effects of adrenalectomy and PCPA were not significant (df=16, t=1.6014 and df 18, t=1.436 respectively). The effect of 5HTP was significant (df 13, t=2.1027, $p < 0.05$).

Comparing PCPA + 5HTP scores with adrenalectomy alone shows that overall the five animals refused more after 5HTP than after adrenalectomy alone (total 130 refusals as against 82). But the difference was not significant overall, and individually, was only significant in the case

Table 4 - Total female refusals during ten tests

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	0	3	0	6	n/s
2259	0	1	1	19 *	**
1992	n/d	0	0		
1265	n/d	4	0	0	n/s
2263	0	7 *	0 *		
2219	0	2	2		
2261	1	4	1	13	n/s
2260	0	70 **	10 **	92 **	n/s
1887	0	28 **	4		
2264	0	0	0		
Means	0.111	11.9	1.80	26.0	
t		1.6014	1.436	2.1027	
df		16	18	13	
Significance		n/s	n/s	$p < 0.05$	

* $p < 0.05$

** $p < 0.01$

Wilcoxon matched-pairs signed-ranks test

of female 2259.

Since it is rare for a female to refuse a male when intact (Table 4, oestradiol condition, one refusal in 80 tests), these results are important.

3.1.1.4. Female acceptance ratio, to the male's first ejaculation

Female acceptance ratios to the male's first ejaculation depend on the number of mounting attempts made by the male and the number of female refusals. Table 5 shows that the female acceptance ratio for female 2260 decreased significantly after adrenalectomy ($p < 0.01$), increased significantly after PCPA ($p < 0.01$) and decreased significantly after 5HTP ($p < 0.01$).

The changes in the number of refusals made by female 2260 (Table 4 above) after each treatment are thus reflected in this derived score. However, it is relatively unusual for a female to refuse a male (see 1.4.5. above) and this is the most marked case of the female acceptance ratio changing with treatments.

Although the overall differences are not significant it should be noted that this derived score does reflect the changes which occurred in female sexual behaviour after adrenalectomy and PCPA. The female acceptance ratio fell from an average of 99.75 (practically 100 per cent.) down to 84.846. This was increased by PCPA to 95.074, close to preoperative levels. The decrease again after 5HTP, on the other hand, was significant ($df=13$, $t = 2.3243$, $p < 0.05$). In no case was there a significant difference between 5HTP and adrenalectomy alone. Overall, the female acceptance ratio was slightly lower, but this difference was not significant.

Table 5 - Female acceptance ratios to the male's first ejaculation

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	100	96.8	100	94.6	n/s
2259	100	98.6	95	70.3	n/s
1992	n/d	100	100		
1265	n/d	91.3	100	100	n/s
2263	100	90.67	100		
2219	100	97.06	95		
2261	98	95	98	69	n/s
2260	100	36.36 **	76.74 **	13.21 **	n/s
1887	100	62.67	86		
2264	100	100	100		
Means	99.75	86.846	95.074	69.422	
t		1.7368	1.1675	2.3243	
df		16	18	13	
Significance		n/s	n/s	$p < 0.05$	

Measure is given by: number of male mounting attempts accepted by female/total number of male mounting attempts, as a percentage.

* = $p < 0.05$
** = $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

3.1.1.5. Female ejaculatory response

During the male's ejaculation, the female makes an ejaculatory response (see 2.3.2.1. above).

Table 6 shows that after adrenalectomy the ejaculatory response of the female was decreased in six of the seven females for which data were available. In the case of three, these decreases were significant (2260, $p < 0.05$; 2219, 2261 $p < 0.01$). Taking the overall data for all animals, the decrease was significant (df15, $t = 2.0715$, $p < 0.05$).

After PCPA, in seven of the nine females for which data were available, the ejaculatory response increased again, and in the case of three animals these increases were significant. Overall, the increase was significant (df16, $t = 2.0785$, $p < 0.05$).

Finally, 5HTP decreased the response significantly, when compared with PCPA alone, in four of the five females treated with PCPA + 5HTP. In addition, the overall decrease was significant at the 0.01 confidence level. Only in the case of 2259 was the PCPA and 5HTP treated condition significantly different from adrenalectomy alone.

Table 6 - Female ejaculatory response

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	2.3	2.0	2.0	2.0	n/s
2259	2.0	2.0	2.0	1.0 **	**
1992	n/d	1.9	2.1		
1265	n/d	2.6	3.0	2.2 **	n/s
2263	2.0	0.9	1.4		
2219	3.0	2.1 **	3.0 **		
2261	3.0	1.9 **	3.0 *	1.1 **	n/s
2260	1.9	0.5 *	2.2 **	0.2 **	n/s
1887	2.0	1.4	2.0		
2264	2.0	n/d	n/d		
Means	2.275	1.7	2.3	1.3	
t		2.0715	2.0785	2.7131	
df		15	16	12	
Significance		$p < 0.05$	$p < 0.05$	$p < 0.01$	

* $p < 0.05$

** $p < 0.01$

Wilcoxon matched-pairs signed-ranks test

3.1.1.6. Summary of the effects of adrenalectomy, PCPA and PCPA + 5HTP on female sexual behaviour

After bilateral adrenalectomy the females presented significantly less often to the males. This was true when each female was analysed individually and also when all females were analysed as a group ($p < 0.01$). The two closely related indices of behaviour, namely, mean number of presentations to the first ejaculation ($p < 0.05$) and the percentage of female initiated mounts to the first ejaculation ($p < 0.01$), reflected this decrease.

In addition, three of the females refused the male significantly more often, which meant that the female acceptance ratio declined. The female's ejaculatory response also tended to go down after adrenalectomy, and this was significant in three cases.

PCPA reversed these effects, and this is the important finding of these experiments. In seven cases, the females presented significantly more often to the males, and the remaining three females refused him significantly less often than before PCPA. PCPA tended to restore the female's ejaculatory response and in three cases this change was significant. The derived scores confirmed this finding.

Finally, in the five females treated with PCPA + 5HTP, 5HTP in turn reversed these effects of PCPA. Four presented significantly less often to the male, and the remaining one refused the male significantly more often. 5HTP reduced the female ejaculatory response significantly in four of the five animals.

When comparing 5HTP with adrenalectomy alone, it was clear that there were no significant differences on most of the measures used. The exception was female 1886, who presented more under 5HTP than she had

after adrenalectomy and before PCPA ($p < 0.01$). Female 2259 refused more after 5HTP than after adrenalectomy and her ejaculatory response was lower ($p < 0.01$).

The exact relationship of these measures to female sexual "receptivity" will be discussed in detail below.

Male's Sexual Behaviour

Although treatments were given only to females in these experiments, the sexual receptivity (or otherwise) of female rhesus monkeys can have marked effects on the behaviour of the male. It is therefore important to analyse the sexual and other behaviour of the male in at least as much detail, in order to tease out any possible "secondary" effects of the surgical and pharmacological treatments used.

3.1.1.7. Mean number of mounts per test

Table 7 shows that after adrenalectomy the number of mounts made per test declined in all eight cases for which data are available. In four of these the decline was significant ($p < 0.01$ in each case) and the overall decline was significant (11.4 down to 6.72, $p < 0.01$). However, PCPA administered to the females did not restore the number of mounts made per test in any consistent way. In the case of 1886 and 1992, PCPA did significantly increase the number of mounts ($p < 0.01$). But in other cases the number of mounts declined. Overall, PCPA increased the number of mounts slightly, but this was not significant.

The addition of 5HTP to PCPA did reduce the mean number of mounts per test, in all five cases. But this change was significant in only two cases (1886, $p < 0.05$, 2260 $p < 0.01$), and was not significant overall.

In all, this index of sexual behaviour of male/female pairs of rhesus monkeys appears to have given rather variable results, and is not as sensitive as others used. Possible reasons for this are discussed below.

Table 7 - Mean number of mounts per test

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	18.6	9.0 **	15.4 **	12.5 *	*
2259	10.4	8.3	6.1	4.2	**
1992	n/d	7.1	12.4 **		
1265	n/d	7.2	6.6	5.4	*
2263	10.6	6.8	8.9		
2219	15.9	7.0 **	8.0		
2261	13.9	6.2 **	8.0	3.1	*
2260	7.2	4.0 **	3.8	1.4 **	**
1887	7.6	5.9	3.6		
2264	7.0	5.7	5.0		
Means	11.4	6.72	7.78	5.32	
t		3.2336	0.8398	1.1476	
df		16	18	13	
Significance		$p < 0.01$	n/s	n/s	

* $p < 0.05$

** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

3.1.1.8. Mean ejaculation time

Table 8 shows that the mean time taken from the first intromitted mount to the male's first ejaculation also yielded rather variable results. Much depended on the particular pair of animals, and on the behaviour adopted by the male once the female became either unreceptive or receptive again.

In six of the eight cases for which data are available, adrenalectomy increased the mean ejaculation times. In four of these cases (2259, 2219, 2261, 2260) this change was significant. However, in two cases (1886, 1887), the mean ejaculation time actually decreased. In the case of 1886 this decrease was significant.

Likewise PCPA did not lead to consistent changes in this index. In four cases (2259, 1265, 2261, 2260), the mean ejaculation time went down (2260, $p < 0.05$). In the other six cases, the index went up and in three of these the change was significant (1886, 1992, 2219, $p < 0.01$).

In the five cases where PCPA + 5HTP was given, only one change reached significance, female 1886, where the index went down ($p < 0.05$). In the cases of 2259 and 2261, the index went up, which was in the same direction as after adrenalectomy for these two animals. But in the other two cases, results were variable; again possible reasons for this will be discussed below (4.2.5.) No overall difference was significant.

3.1.1.9. Mean time to initiation of the first mount

Table 9 shows that no clear pattern emerged in the time taken to initiate the first mount of a mounting sequence, under the various treatment conditions. It might perhaps be expected that the "latency to mount" after the divider is removed would yield a measure of the female's receptivity or otherwise (Everitt, personal communication). But except for female

Table 8 - Mean ejaculation time

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	12.70	8.25 **	14.65 **	11.55 *	*
2259	3.72	6.30 **	5.65	16.33	n/s
1992	n/d	6.80	10.80 **		
1265	n/d	8.45	8.20	5.40	**
2263	8.25	13.10	13.50		
2219	3.35	4.30 *	6.95 **		
2261	2.85	7.43 **	6.75	8.00	n/s
2260	4.05	6.70 *	3.30 *	3.30	n/s
1887	7.50	6.69	10.00		
2264	8.20	12.25	19.50		
Means	6.3275	8.027	9.93	8.916	
t		1.1772	1.0833	0.3742	
df		16	18	13	
Significance		n/s	n/s	n/s	

Measure is given by the number of minutes (to the nearest half minute) from the first mount of a mounting sequence to the male's ejaculatory mount.

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

Table 9 - Mean time to initiation of the first mount

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	2.15	1.55	4.95 **	6.30	**
2259	0.05	0.05	0.15	11.75	n/s
1992	n/d	1.95	4.30 **		
1265	n/d	8.95	5.45	4.05	**
2263	1.55	9.95 **	13.06 *		
2219	1.00	4.95 **	7.40		
2261	2.25	5.90	5.35	12.20	n/s
2260	3.05	9.80 *	7.95	20.25 **	*
1887	1.30	1.50	n/d		
2264	2.20	3.40	6.95		
Means	1.6938	4.8	6.1733	10.91	
t		2.2974	0.8323	1.8491	
df		16	17	12	
Significance		$p < 0.05$	n/s	$p < 0.05$	

Mean number of minutes (to the nearest completed half minute) from the start of the test to the first mount.

* $p < 0.05$

** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

2260, no such pattern could be discerned. Female 2260 will be discussed in detail below, since this pairing yielded the clearest example seen in these experiments of the male adapting his behaviour to accommodate changes in the female's sexual receptivity. For the time being, it should be noted that initiation times lengthened after adrenalectomy ($p < 0.05$) in the case of female 2260. They then shortened again after PCPA (although not significantly) and then lengthened considerably after 5HTP ($p < 0.01$). In the cases of both 2260 and 2259, initiation times after 5HTP were much longer than they had been after adrenalectomy alone.

3.1.1.10. Mean number of thrusts per intromitted mount

Table 10 shows that no clear pattern emerged after the various treatment conditions in the mean number of thrusts per intromitted mount. Sometimes adrenalectomy raised the thrusting rate, but it seemed to be as likely to lower it. The same applied to PCPA and PCPA + 5HTP. Certainly no convincing changes in this index of sexual behaviour were found under these conditions. Indeed, the index was remarkable for the fact that it remained relatively constant across all four treatment conditions. This would be expected if thrusting rates were to depend on the individual characteristics of the male rather than on the sexual receptivity of the female in any one pair.

3.1.1.11. Mean male mounting rate

Table 11 shows that in all cases for which data are available, adrenalectomy lowered the rate at which males mounted females. In six of these females the change was significant, and overall the change was significant ($p < 0.01$). However, PCPA did not always increase the mounting rate when it was given to adrenalectomised animals. Indeed, in only two females was the mounting rate increased significantly after PCPA, and even in these two cases the rate did not reach that of the preadrenalectomy

Table 10 - Mean number of thrusts per intromitted mount

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	6.02	5.71	4.81 **	5.13	n/s
2259	6.56	6.43	6.16	5.81	n/s
1992	n/d	4.53	4.24		
1265	n/d	4.34	6.56 **	5.57	*
2263	5.27	4.71 **	5.13 *		
2219	8.39	5.31 **	6.07 **		
2261	7.87	8.94 *	6.81 **	7.23	n/s
2260	7.47	7.28	8.56	10.10	n/s
1887	6.48	7.05	6.75		
2264	5.72	5.86	4.85		
Means	6.7225	6.016	5.994	6.768	
t		1.1449	0.0361	0.9142	
df		16	18	13	
Significance		n/s	n/s	n/s	

The measure is given by total number of thrusts /total intromitted mounts

* $p < 0.05$

** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

Table 11 - Mean Male Mounting Rate

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	1.39	0.98 **	1.00	1.05	n/s
2259	1.34	0.85 *	0.80	0.42	n/s
1992	n/d	0.91	1.07		
1265	n/d	0.77	0.72	0.82	n/s
2263	1.17	0.69 **	0.84		
2219	1.57	1.37 *	1.08		
2261	2.05	0.41 **	0.99 **	0.65	n/s
2260	1.24	0.46 **	0.81 **	0.51	n/s
1887	0.97	0.93	0.83		
2264	0.74	0.72	0.57		
Means	1.3088	0.8090	0.8710	0.6900	
t		3.1743	0.6152	1.6982	
df		16	18	13	
Significance		$p < 0.01$	n/s	n/s	

Measure given by the total number of mounts less the ejaculatory mount / the ejaculation time.

* $p < 0.05$ Wilcoxon matched-pairs signed-ranks test.

** $p < 0.01$

"receptive" state. In the same way, 5HTP did not reverse the effects of PCPA in any convincing way. There were no significant differences between adrenalectomy and 5HTP conditions.

3.1.1.12. Mean male acceptance ratio

Table 12 shows that with only one exception there were no significant changes in the male acceptance ratios throughout all treatment conditions. As this ratio is one of the primary measures of the "attractiveness" of a female, measuring the "stimulus value" of the female's presentations to the male (Everitt, 1970), it is important to note that this did not vary significantly between treatment conditions. Indeed, the one female where this ratio did change (1265) is not an exception either, in the sense that the change was in the "right direction", as will be discussed under 4.2.5. below. The same applies to the overall scores.

3.1.1.13. (Appendix). Total number of ejaculations made by the male in ten tests

The total number of ejaculations made by the male in ten tests under each of the treatments administered to the female is shown in Appendix 1, facing page 138 below. The number of ejaculations declined in some females when the females became unreceptive after adrenalectomy (females =2259, 2263, 2261 and 2264). However, only in the case of female 2259 were the changes significant (Friedman's two-way analysis of variance). Further, only in the case of females 2259 and 2263 did the number of ejaculations increase again after administration of PCPA. In neither case was the change significant.

In contrast to some males in the Birmingham colony (Herbert, pers comm), the males used in these particular experiments seldom ejaculated more than once per 30 minute observation period. The exceptions were male 2272 (with female 2259) and male 2092 (with female 2261, base line condition).

Appendix 1 : Total Number of Ejaculations made by the Male of a Male/Female Pair of Rhesus Monkeys in Ten Observational periods (30 minutes each) under each of the Treatments administered to the Female.

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP
1886	10	10	10	10
2259	16	8**	12	3 **
1992	n/d	10	10	
1265	n/d	10	10	10
2263	10	5	7	
2219	10	10	10	
2261	15	10	10	7
2260	10	10	10	8
1887	10	8	4	
2264	10	5	3	
Means	11.375	8.6	8.6	7.6

Animal	Oestradiol	5HTP
315	10	10
316	10	10
317	10	10
321	10	3 **
Means	10	8.25

* p 0.05 Wilcoxon matched-
** p 0.01 pairs signed-ranks test

Table 12 - Mean male acceptance ratio

Animal	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	69.23	57.14	65.20	61.44	n/s
2259	66.23	80.00	67.65	100.00	n/s
1992	n/d	100.00	78.60		
1265	n/d	75.00	52.00 **	76.00 *	n/s
2263	94.74	n/d	90.00		
2219	76.00	n/d	91.67		
2261	94.74	100.00	93.33	100.00	n/s
2260	75.00	n/d	n/d	n/d	n/s
1887	83.33	92.31	66.67		
2264	87.90	100.00	75.00		
Means	80.8963	86.35	75.5689	84.36	
t		0.7652	1.4119	0.9369	
df		13	14	11	
Significance		n/s	n/s	n/s	

Measure given by the number of presentations accepted by the male / the total number of presentations made by the female, expressed as a percentage.

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

3.1.1.13. Summary of the effects of adrenalectomy, PCPA and PCPA + 5HTP on male sexual behaviour

On the whole, no overall picture emerged from these indices of male sexual behaviour under the various treatments given to females. The number of mounts per test declined after adrenalectomy, but did not change again with either PCPA or 5HTP. Individual differences among males were most marked, and it will be valuable to discuss these in detail below.

What is important to notice in this section is that none of the treatment conditions appeared to make any significant changes in the male acceptance ratios. This would inidcate that the "stimulus value" of the female's presentations to the male remained at high and constant levels throughout all the experiments.

3.1.2. The Effects of Adrenalectomy, PCPA and PCPA + 5HTP on Male and Female Grooming and Social Behaviour

Measures of grooming, social and agonistic behaviour were made, but as only one isolated incident of threatening behaviour occurred in all the tests scored (when female 1886 threatened male 1935 after 5HTP treatment), no further analysis of agonistic behaviour was possible. Results in this section will therefore deal only with grooming, invitations to grooming and proximity.

Grooming and social behaviour is dictated to a large extent (although not exclusively) by the particular behavioural characteristics of the male. It is therefore important to note the identity of the male in the case of each pairing. For the sake of emphasis, the number of each male is therefore included in the tables which follow (see also 2.3.5 above).

3.1.2.1. Male Grooming

Table 13 shows that none of the treatments given to females had any regular effects on male grooming, either individually or overall. Since males 2092, 2272 and 2273 were all virtually non-groomers, the only pairs of interest involve the four females paired with male 1935. In the case of females 2263 and 2260, male grooming went up significantly after adrenalectomy. In these two cases, it could perhaps be argued that grooming was acting as a "substitute" activity for copulatory behaviour once the female had become less sexually receptive and was refusing the male (see 3.1.1.3 Female refusals, above). Certainly these were the two females that refused males, and their grooming invitations did go up at the same time (3.2.4. below). But in the case of female 1886, male grooming went down, and in any case these changes did not reverse systematically after PCPA. So there is not sufficient evidence to substantiate or refute such a "substitution" hypothesis.

Table 13 - Male grooming

Female	Male	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	1935	2.3	0.6	2.4	1.15	n/s
2259	2272	0.0	0.0	0.5	0.0	n/s
1992	1935	n/d	0.0	4.05 **		
1265	2092	n/d	0.0	0.0	0.0	n/s
2263	1935	7.50	15.20 **	15.80		
2219	2092	0.0	0.0	0.0		
2261	2092	0.0	0.0	0.0	0.0	n/s
2260	1935	10.9	24.1 **	24.1	27.25	n/s
1887	2273	0.0	0.0	0.0		
2264	2273	0.0	0.0	0.2		
Means		2.5875	3.99	4.7	5.68	
t			0.423	0.196	0.184	
df			16	18	13	
Significance			n/s	n/s	n/s	

Mean number of minutes (to the nearest completed half minute) spent grooming by the male, per observation period.

* $p < 0.05$

** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test.

3.1.2.2. Female grooming

In contrast to male grooming, female grooming did tend to vary in a systematic way with successive treatments. Table 14 shows that when the females were "sexually unreceptive" (adrenalectomy and 5HTP treatment), they tended to groom the male less than when they were "receptive" (oestradiol and PCPA). This happened in all cases where the male was one which allowed grooming (1935 and 2092), but was most marked in the case of females 2260 and 2219. Female 2260 groomed an average of 11.5 minutes per test under oestradiol. After adrenalectomy this figure dropped to 3.95 ($p < 0.01$). PCPA reversed this significantly, her grooming increasing to 5.9 minutes per test ($p < 0.01$). 5HTP in turn reversed this to levels very similar to those of adrenalectomy before PCPA (2.6 minutes per test, $p < 0.01$). Female 2219 dropped from a mean of 21.6 minutes per test to 13.55 minutes per test after adrenalectomy ($p < 0.01$). PCPA restored this to 20.05 minutes per test ($p < 0.01$). Similar changes, but not as marked, tended to occur in the other five females. However, it should be noted that although the overall means showed the same trend, in no case was the change significant. In the three females paired with "non-groomers" (males 2272 and 2273), no such changes were possible.

3.1.2.3. Male invitations to groom

The usual grooming invitation consists of either lying or posturing in front of the potential groomer. Sometimes the head was thrown right back and the jaw and throat offered for grooming. Grooming invitations were often accompanied by lip-smacking, and were easy to recognise and distinguish from sexual advances (male) and sexual presentations (female).

Table 15 shows that no pattern was apparent in male grooming invitations across the four treatments given to females.

Table 14 - Female grooming

Female	Male	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	1935	11.70	4.85 **	6.40	9.50	**
2259	2272	0.0	0.0	2.75	0.0	n/s
1992	1935	n/d	9.0	9.65		
1265	2092	n/d	7.5	10.35	9.90	n/s
2263	1935	13.15	11.5	12.45		
2219	2092	21.60	13.55 **	20.05 **		
2261	2092	15.35	5.95 **	3.40	1.45	**
2260	1935	11.50	3.95 **	5.90 *	2.60 **	n/s
1887	2273	0.0	0.0	0.0		
2264	2273	0.0	0.0	0.0		
Means		9.1625	5.63	7.095	4.69	
t			1.1396	0.5876	0.7583	
df			16	18	13	
Significance			n/s	n/s	n/s	

Mean number of minutes (to the nearest completed half minute) spent grooming by the female, per observation period.

* $p < 0.05$

* * $p < 0.01$ Wilcoxon matched-pairs signed-ranks test.

Table 15 - Male grooming invitations

Female	Male	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	1935	1.8	0.5	0.0	0.3	n/s
2259	2272	0.1	0.0	0.4	0.1	n/s
1992	1935	n/d	0.6	0.9		
1265	2092	n/d	2.7	1.5	2.7	n/s
2263	1935	1.5	6.0 **	0.1 **		
2219	2092	3.6	1.1 **	1.4		
2261	2092	2.0	1.7	0.8	0.8	n/s
2260	1935	0.8	1.4	0.0 *	0.0	*
1887	2273	0.0	0.0	0.0		
2264	2273	0.0	0.0	0.0		
Means		1.225	1.4	0.51	0.78	
t			0.2292	1.4568	0.62	
df			16	18	13	
Significance			n/s	n/s	n/s	

* $p < 0.05$

** $p < 0.01$

Wilcoxon matched-pairs signed-ranks test.

3.1.2.4. Female grooming invitations

Like female grooming, and in contrast to the males, female grooming invitations did tend to vary in a systematic way with treatments. Table 16 shows that when some females were "sexually unreceptive", they invited grooming more often. For example, females 2259 and 2260 showed this very clearly. They invited significantly more often after adrenalectomy, but less often after PCPA. Then after 5HTP they invited significantly more often again. Female 2263 showed the same pattern (both differences, $p < 0.01$). In the other females, either the same pattern emerged, but the differences were not significant, or else no change resulted. It could be that in some females at least, rather than present or accept a sexual advance from the male, the female will invite grooming. It is interesting to note that the actual amount of grooming done by the female goes down (Table 14) when she is unreceptive, but her grooming invitations tend to go up. Again, however, there were no overall significant differences.

3.1.2.5. Proximity

Table 17 shows that overall, adrenalectomy led to a decrease in the amount of time the female spent in close proximity to the male. This reversed to a small extent with PCPA, and reversed in turn with 5HTP. However, none of these overall changes was significant.

Females 1887, 2264 and 2259 were paired with non-grooming males, who often actively avoided proximity with their females after ejaculation.

Of the other seven females, only 2219 showed a pattern of proximity behaviour which seemed to follow her sexual "receptivity" in any systematic way. Her proximity declined significantly after adrenalectomy ($p < 0.01$) and increased significantly again after PCPA ($p < 0.05$).

Table 16 - Female grooming invitations

Female	Male	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Adrenalectomy v 5HTP
1886	1935	1.3	0.0	0.0	0.1	n/s
2259	2272	0.0	2.3 **	0.0 **	0.8 *	*
1992	1935	n/d	0.1	0.1		
1265	2092	n/d	0.7	0.0	0.5	n/s
2263	1935	1.5	6.5 **	3.4 **		
2219	2092	0.0	0.2	0.0		
2261	2092	0.0	0.1	0.0	0.1	n/s
2260	1935	0.3	8.2 **	2.7 **	7.1 **	n/s
1887	2273	0.0	0.0	0.2		
2264	2273	0.0	0.0	0.0		
Means		0.3875	1.81	0.64	1.72	
t			1.2981	1.1248	0.9923	
df			16	18	13	
Significance			n/s	n/s	n/s	

* $p < 0.05$

** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

Female 2263 showed similar changes, but not significantly. But on the whole this measure of social behaviour gave variable results across treatments.

Table 17 - Mean number of minutes (to the nearest half minute) spent by a male/female pair of monkeys in close proximity (i.e. within arm's length of one another).

Female	Male	Oestradiol	Adrenalectomy	PCPA	PCPA + 5HTP	Oestradiol v 5HTP
1886	1935	28.85	14.05 **	25.35 **	27.35	**
2259	2272	14.50	10.80 *	9.15	1.05 **	**
1992	1935	n/d	20.15	24.65 **		
1265	2092	n/d	7.85	10.60	10.95	n/s
2263	1935	28.50	27.35	29.25 *		
2219	2092	28.15	20.30 **	22.75 *		
2261	2092	23.65	6.20 **	4.40	2.70	n/s
2260	1935	26.65	28.45	30.00 *	30.00	*
1887	2273	9.10	6.10	2.85 *		
2264	2273	7.30	3.90 **	1.90		
Means		20.8375	14.515	16.09	14.41	
t			1.4750	0.3431	0.2537	
df			16	18	13	
Significance			n/s	n/s	n/s	

* $p < 0.05$

** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test.

3.1.2.6. Summary of the effects of adrenalectomy, PCPA and PCPA + 5HTP on male and female grooming and social behaviour

Of the four males used in these experiments, only one was a systematic groomer. Male 1935 often interacted with his female for most of the 30-minute observation period, either as groomer or groomee. He was paired with four females, 1886, 1992, 2263 and 2260, and it is clear from the results that all the male grooming and much of the other grooming and social interaction was accounted for by these pairs.

Male 2092 was content to act as groomee, but would never do any grooming of a female himself. He was paired with 1265, 2219 and 2261. Male 2273 was a complete non-groomer. He neither groomed nor allowed grooming, and would actively avoid proximity or grooming interaction with the female immediately after ejaculation. He was paired with females 1887 and 2264. Male 2272 was very similar to 2273 except that he did allow grooming on two occasions. He was paired with female 2259.

Probably because of the individual characteristics of the males, no overall differences were found in any of the five measures of grooming and social behaviour used. Certainly no real patterns emerged for male grooming and male grooming invitations. There was a hint that females showed less proximity behaviour with males when they were sexually "unreceptive", but this pattern was seen clearly in only two animals, and the overall means, although reflecting this trend, were not significantly different across treatments.

There was also quite a strong hint that females invited grooming more frequently when they were not sexually "receptive" (i.e. after adrenalectomy and 5HTP treatment). This suggested that some females will invite grooming rather than present or accept a sexual advance from a male. This pattern was seen clearly in three females, and the trend,

although not significant, was apparent overall.

This increase in female grooming invitations during unreceptivity appeared to be linked to a decline of female grooming during these periods, but it was emphasised that this trend was not clear cut, and no reliance should be placed on the latter finding.

3.1.3. The Effects of 5HTP alone on Sexual Behaviour

Results in this section are given under two headings, namely, Female sexual behaviour and Male sexual behaviour. Only four females were used. The females were ovariectomised, but otherwise intact (2.3.5. above).

Female Sexual Behaviour

3.1.3.1. Presentations

Table 18 shows that in all four females, 5HTP caused a decrease in the number of presentations made per test. This change was significant for all females except female 316, and was significant overall. Table 19 shows that the same applied to the mean number of presentations to the male's first ejaculation ($t = 2.2811$, $p < 0.05$).

3.1.3.2. Percentage of female initiated mounts to the male's first ejaculation

Table 20 shows that the decline in presentations made by females 315, 317 and 321 after 5HTP was reflected in the derived index of the percentage of mounts to the male's first ejaculation which were initiated by the female. This change was significant for all three females, but not overall.

3.1.3.3. Total female refusals and female acceptance ratios to the male's first ejaculation

Tables 21 and 22 show that none of these females refused the male, and only one refusal was recorded in 40 hours of observations. Therefore the female acceptance ratio to the male's first ejaculation was always 100 per cent. (the one refusal was after ejaculation).

3.1.3.4. Female ejaculatory response

Table 23 shows that the female ejaculatory response did go down after 5HTP in three animals, but only in the case of 317 was this change significant.

TABLE 18

Mean number of presentations per test (5HTP alone)

Animal	Oestradiol	5HTP
315	2.3	0.7 **
316	1.8	1.2
317	3.4	1.0 *
321	6.2	0.9**
Means	3.425	0.95
t		2.5025
df		6
Significance		$p < 0.05$

TABLE 19

Mean number of presentations to the male's first ejaculation (5HTP alone)

Animal	Oestradiol	5HTP
315	2.3	0.7**
316	1.2	1.0
317	3.4	1.0 *
321	5.9	0.9**
Means	3.2	0.9
t		2.2811
df		6
Significance		$p < 0.05$

* $p < 0.05$
** $p < 0.01$

Wilcoxon matched-pairs signed-ranks test

Table 20 - Percentage of female initiated mounts to the male's first ejaculation (5HTP alone)

Animal	Oestradiol	5HTP
315	15.44	6.36 *
316	18.97	15.00
317	22.41	10.00 *
321	62.79	25.00 **
Means	29.9025	14.09
t		1.3433
df		6
Significance		n/s

* $p < 0.05$
** $p < 0.01$

Wilcoxon matched-pairs signed-ranks test.

TABLE 21 - Total female refusals (5HTP alone)

Animal	Oestradiol	5HTP
315	0	0
316	0	0
317	0	0
321	0	1
		n/s

TABLE 22 - Female acceptance ratios to the male's first ejaculation (5HTP alone)

Animal	Oestradiol	5HTP
315	100	100
316	100	100
317	100	100
321	100	100
		n/s

Table 23 - Female ejaculatory response (5HTP alone)

Animal	Oestradiol	5HTP
315	1.0	0.6
316	3.0	2.1
317	2.0	0.9 **
321	1.8	2.0
Means	1.95	1.40
t		0.5417
df		6
Significance		n/s

Means per test

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

3.1.3.5. Summary of the effects of 5HTP alone on female sexual behaviour

5HTP administered to oestradiol-treated females caused a decrease in the number of presentations made in all four females, and these changes were significant in the case of three of them and were significant overall. The same applied to the mean presentation rate to the male's first ejaculation. The derived index of percentage of female initiated mounts to the male's first ejaculation reflected this change. 5HTP tended to lower female ejaculatory responses but this trend was not clear cut or convincing. None of the females was a "refuser", so no change was seen in refusal rates or female acceptance ratios.

3.1.3.6. The effects of 5HTP alone on male sexual behaviour

Since 5HTP had no clear effect on any of the six measures of male sexual behaviour analysed, results are presented together and not discussed individually. It can be seen from Table 24 that the mean number of mounts per test decreased a little after 5HTP, which is in the same direction as after adrenalectomy and when 5HTP was added to PCPA. But this change was significant only in the case of female 321 and actually increased in the case of 316. Mean ejaculation time (Table 25) and mean time to initiation of the first mount (Table 26) both went up after 5HTP, but not significantly. In fact, only 321 (time to initiation) was a significant change. Thrusts per mount (Table 27) and mounting rate (Table 28) stayed virtually unchanged, perhaps confirming that these two measures are dependent more on the individual characteristics of the male than on the sexual "receptivity" of the female. Male acceptance ratios did not change (Table 29).

TABLE 24 - Mean number of mounts per test (5HTP alone)

Animal	Oestradiol	5HTP
315	12.3	11.0
316	5.8	7.1
317	6.1	5.5
321	10.0	2.4**
Means	8.575	6.5
t		0.8688
df		6
Significance		n/s

TABLE 25 - Mean ejaculation time (5HTP alone)

Animal	Oestradiol	5HTP
315	11.05	10.95
316	3.00	3.30
317	6.20	3.90
321	5.35	11.20
Means	6.4	7.3375
t		0.3415
df		6
Significance		n/s

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

TABLE 26 - Mean time to initiation of the first mount (5HTP alone)

Animal	Oestradiol	5HTP
315	4.05	5.80
316	0.00	0.00
317	8.05	7.25
321	0.25	19.65**
Means	3.0875	8.1750
t		1.1188
df		6
Significance		n/s

TABLE 27 - Mean number of thrusts per intromitted mount (5HTP alone)

Animal	Oestradiol	5HTP
315	4.88	4.92
316	6.34	6.16
317	4.41	5.43*
321	4.39	5.60
Means	5.005	5.5275
t		0.9943
df		6
Significance		n/s

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

Table 28 - Mean male mounting rate (5HTP alone)		
Animal	Oestradiol	5HTP
315	1.02	0.95
316	1.64	1.58
317	0.81	0.79
321	1.53	0.78
Means	1.25	1.025
t		0.8189
df		6
Significance		n/s

Table 29 - Mean male acceptance ratio (5HTP alone)		
Animal	Oestradiol	5HTP
315	82.61	100.00
316	91.67	90.00
317	50.00	40.00
321	93.22	66.67
Means	79.3750	74.1675
t		0.3113
df		6
Significance		n/s

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

Table 30 - Male grooming (5HTP alone)		
Animal	Oestradiol	5HTP
315	4.45	3.05
316	6.50	14.60 **
317	12.35	7.65
321	2.65	14.25 **
Means	6.4875	9.8875
t		0.9739
df		6
Significance		n/s

Table 31 - Female grooming (5HTP alone		
Animal	Oestradiol	5HTP
315	10.95	15.70
316	15.25	7.25 *
317	3.15	2.05
321	3.00	6.55 *
Means	8.0875	7.8875
t		0.0481
df		6
Significance		n/s

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

3.1.3.7. Summary of the effects of 5HTP alone on male sexual behaviour

Apart from a slight trend towards a lower mean number of mounts per test, 5HTP did not have any marked effect on the components of male sexual behaviour measured. As in the case of adrenalectomy, PCPA and PCPA + 5HTP treatments, therefore, the important point to note in these results is that the male acceptance ratios were unchanged after 5HTP, showing that the "stimulus value" of the female's presentations did not change with treatment and so changes in males' activity could not be responsible for any "secondary" change in the female's sexual behaviour.

3.1.4The Effects of 5HTP alone on Male and Female Grooming and Social Behaviour

Although there were some individual changes in grooming, grooming invitations and proximity behaviour after 5HTP, which were significant, there were no overall significant changes. Indeed, no pattern could be detected in any of the measures except in female grooming invitations (Table 33). When the female became sexually unreceptive, she invited grooming more frequently. This happened in each case, but the change was significant only for female 316 ($p < 0.05$) and 321 ($p < 0.01$). This trend therefore confirms that found after adrenalectomy and PCPA + 5HTP treatment (3.1.2.6. above).

Table 32 - Male grooming invitations (5HTP alone)			Table 33 - Female grooming invitations (5HTP alone)		
Animal	Oestradiol	5HTP	Animal	Oestradiol	5HTP
315	1.0	2.2	315	0.1	0.2
316	1.2	0.1 *	316	0.4	1.7 *
317	2.2	1.4	317	1.0	2.2
321	0.7	1.3	321	1.0	4.2**
Means	1.275	1.25	Means	0.625	2.075
t		0.0462	t		1.6937
df		6	df		6
Significance		n/s	Significance		n/s

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

Table 34 - Proximity (5HTP alone)		
Animal	Oestradiol	5HTP
315	23.95	22.35
316	25.20	27.95
317	28.30	20.35 **
321	16.80	23.45 *
Means	23.5625	23.525
t		0.0129
df		6
Significance		n/s

* $p < 0.05$
** $p < 0.01$ Wilcoxon matched-pairs signed-ranks test

3.1.4.1. Summary of the effects of 5HTP alone on male and female grooming and social behaviour

Female grooming invitations tended to increase after 5HTP. This finding is consistent with earlier findings that female grooming invitations increased when the female became sexually unreceptive after adrenalectomy and PCPA + 5HTP treatments. No other consistent changes were observed.

PART 3.2.

Whenever females were treated with PCPA or 5HTP, they were also given oestradiol, to ensure that biochemical measures of 5HIAA in the cisternal CSF were made under conditions identical to those used for the behavioural experiments. All comparisons to measure the effects of pharmacological treatments are thus referred to the oestradiol-treated condition as base.

3.2. The Effects of PCPA, 5HTP and Recovery Periods on the Levels of 5HIAA in the CSF of Female Monkeys

No marked differences were seen (by visual inspection) in the cases of the two non-adrenalectomised females (1864 and 2267), and therefore all nine results are included together.

3.2.1. PCPA Treatment

Table 35 shows that PCPA in the doses used was effective in lowering the levels of 5HIAA in the cisternal CSF of female monkeys. Individual animals varied a little as to the actual percentage decreases they showed, but one injection of PCPA usually reduced the levels of 5HIAA to half the oestradiol (base line) level. Overall, the mean level of 5HIAA (ng/ml of CSF) was reduced from 68.216 ng/ml to 31.936 ng/ml, a drop of 53.18 per cent. This change was significant at the $p < 0.002$ level. After 8 days (or two injections) of PCPA, the mean level had dropped further to 28.906 ng/ml, which was only 42.37 per cent. of the oestradiol-treated condition. After three injections (12 days) the levels of 5HIAA averaged 27.238 ng/ml or 39.93 per cent. of base line. Naturally, these levels were also significantly different from base line.

Table 35 - Effects of PCPA, 5HTP and recovery periods on the levels of 5HIAA in the CSF of female monkeys

Animals	No Hormones	Oestradiol	PCPA			off PCPA		PCPA + 5HTP		off PCPA + 5HTP
		15 ug/day for 10 days	4 days	8 days	12 days	7 days	14 days	2 days	12 days	7 days
1886	77.662	68.961	40.000	23.148	22.917	50.980	61.749	68.889	90.794	68.868
1864	83.818	76.475	44.444	32.749	26.389	32.353	54.663	74.211		30.682
2267	68.512	58.333	27.778	28.431	30.833	30.000	45.552	84.211		27.272
2261	64.583	62.245	26.667	34.259	25.067	41.176	44.816	63.207	114.151	30.189
2263	63.380	56.082	30.000	18.519	22.062	36.275	38.095	65.409	110.377	34.906
2260	80.394	78.571	32.500	42.596	36.208	47.059	58.712	73.043	129.524	33.019
2264	66.161	64.912	23.768	28.571	28.571	22.327	34.285	54.717		29.921
2259	66.111	63.265	31.132	26.415	24.528	36.792	37.107	54.043	93.684	32.353
1887	90.041	85.100	31.132	25.472	28.571	38.947	40.632	94.000		30.250
Means	73.407	68.216	31.936	28.906	27.238	37.323	46.179	70.192	107.706 / 65.825	35.273
Percentage of oestradiol level		100.00%	46.82%	42.37%	39.93%	54.71%	67.70%	102.90%	163.62%	51.71%

Scores in ng 5HIAA/ml of CSF.
Comparisons : Oestradiol v PCPA (4 days) $t = 9.2166$, $df = 16$, $p < 0.002$.
PCPA v PCPA + 5HTP (2 days) $t = 0.3626$, $df = 16$, not significant.

3.2.2. Recovery from PCPA

Recovery from PCPA was slow. Seven days after the last PCPA injection, 5HIAA levels in the CSF were still markedly depressed. They averaged 37.323 ng/ml, which was only 54.71 per cent. of the oestradiol-treated condition.

Even after 14 days, the levels averaged only 46.179 ng/ml, which is 67.70 per cent. of the oestrogen-treated level.

3.2.3. PCPA + 5HTP Treatment

A number of pilot experiments were conducted to find the minimum dose of 5HTP which should be added to the PCPA dose used in order to restore 5HIAA levels to those of the oestradiol-treated condition. The actual dose used (20 mg/kg every second day) was established in this way. Table 35 shows that it was effective. After the second injection (2 days), levels were 70.192 ng/ml, which is 102.9 per cent. of the oestradiol level and not significantly different from it (df16, $t = 0.626$).

There was an increase of 5HIAA in the CSF during the 10 day period of behavioural observations. This is shown by the increase in levels after 12 days of 5HTP + PCPA. For these five animals, the average oestradiol level was 65.825 ng/ml. An average of 107.706 ng/ml therefore represents an increase to 163.62 per cent. of the oestrogen levels. This will be discussed in detail under 4.3.2.2. below.

3.2.4. Recovery from PCPA + 5HTP.

Seven days after the last PCPA + 5HTP injection, the effects of 5HTP had completely disappeared. The average 5HIAA level was 35.273 ng/ml, which is only 51.71 per cent. of the oestrogen-treated condition

and almost identical to the level found seven days after PCPA alone had been stopped (54.71 per cent. of base). It therefore seems clear that 5HTP cleared very rapidly from the system, leaving presumably, only the effects of PCPA on 5HIAA levels in the CSF.

3.2.5. Summary of the Effects of PCPA, 5HTP and Recovery Periods on the Levels of 5HIAA in the CSF

PCPA reduced the levels of 5HIAA in the cisternal CSF of female monkeys by 53.18 per cent. on average, after only one injection. Each successive injection lowered the levels still further. Recovery from PCPA was slow and two weeks later the levels of 5HIAA were still depressed (67.7 per cent. of base). Doses of 5HTP (20 mg/kg every second day) to animals given the usual PCPA treatment restored 5HIAA in the CSF to levels not significantly different from the average oestradiol-treated condition. 5HTP cleared very rapidly from the system however, since one week later only the inhibitory effects of PCPA were apparent.

Seven of the nine animals used in these experiments had also taken part in the behavioural experiments reported in part one of the results above. It is therefore important to note that the effects of PCPA on 5HIAA levels in the CSF are known for the majority of the animals which showed increased sexual receptivity after PCPA in the behavioural experiments.

3.2.6. 5HTP alone

Table 36 shows that 5HTP markedly increased the levels of 5HIAA in the CSF of the four females treated with 5HTP alone. After two days of 5HTP (two injections), the mean levels of 5HIAA had increased by 72.8 per cent. from 51.757 ng/ml to 89.445 ng/ml. This change was significant ($p < 0.01$). After the ten behavioural observations, these levels had increased to a mean of 157.579, an increase of 204.46 per cent.

Again, 5HTP cleared very rapidly from the system, and after 7 days the levels were not significantly different from the oestradiol-treated condition.

Table 36 - Effects of 5HTP alone on the levels of 5HIAA in the CSF of female monkeys

Animals	No Hormones	Oestradiol	5HTP 2 days	5HTP 12 days	Off 5HTP 7 days
315	47.059	46.316	83.750	168.868	63.636
316	52.942	49.474	81.132	162.500	54.832
317	75.000	60.620	109.270	128.421	62.500
321	51.250	50.620	83.629	170.526	54.545
Mean	56.563	51.757	89.445	157.579	58.878
Percentage increase over oestradiol level			72.82%	204.46%	13.76%

Scores in ng 5HIAA/ml of CSF. Females ovariectomised and given oestradiol (15 ug/day for 10 days), 5HTP (20 mg/kg every second day).

Comparisons : Oestradiol v 5HTP $t = 5.1483$, $df = 6$, $p < 0.01$: Oestradiol v off 5HTP, $t = 1.8108$, $df = 6$, not significant.

3.2.7. Summary of the Effects of 5HTP alone on 5HIAA levels in the CSF

5HTP as given to four females in the behavioural experiments markedly increased the levels of 5HIAA in the CSF. After two days the increase was 72.82 per cent. ($p < 0.01$) and after twelve days it was 204.46 per cent. ($p < 0.002$). However, 5HTP cleared rapidly from the system and after a week levels were not significantly different from base line oestradiol levels.

Again, it should be noted that these results refer to the four females in which this same regimen of 5HTP had induced sexual unreceptivity in the behavioural experiments.

PART 3.3

3.3 The Effects of Gonadal Steroids on 5HIAA Levels and 5HT Turn-over Rates

3.3.1. The Effects of Oestradiol, Oestradiol + Progesterone and Testosterone Propionate on Levels of 5HIAA

Table 37 shows that none of the gonadal steroids used produced any significant effects on 5HIAA levels in the CSF. Analysis of variance showed no significant differences between means. However, visual inspection of the raw data suggested that further experiments should be conducted. The overall means after oestradiol treatment went down from 75.9105 ng/ml to 69.2413 ng/ml. This effect was antagonised by the addition of progesterone to oestradiol, and the average level of 5HIAA in the CSF of the ten animals after progesterone rose again to 79.5554 ng/ml. Testosterone propionate reduced the levels of 5HIAA to a mean of 66.122 ng/ml, as against the no hormone level of 75.9105.

3.3.2. Summary of Effects of Gonadal Steroids on 5HIAA Levels

Although none of the gonadal steroids used produced any significant effects, there was a suggestion that oestradiol and testosterone lowered 5HIAA levels in the CSF and that progesterone antagonised the effects of oestradiol in this respect. Because of this hint, it was decided to conduct further experiments on the turn-over rates of 5HT in the brain, as measured by the probenecid test.

3.3.3. The Effects of Oestradiol, Oestradiol + Progesterone and Testosterone Propionate on 5HT Turn-over Rates

In contrast to the effects on 5HIAA levels given above (3.3.1.), turn-over rates of 5HT after probenecid were markedly affected by gonadal

Table 37 - Effects of oestradiol, oestradiol + progesterone and testosterone propionate on the levels of 5HIAA in the CSF of female monkeys

Animal	No Hormones	Oestradiol Benzoate	Oestradiol + Progesterone	Testosterone Propionate	Return to No Hormones
1754	72.160 (3)	65.137 (4)	67.345 (2)	57.338 (2)	69.405 (2)
1811	67.687 (4)	55.916 (4)	67.157 (2)	54.825 (1)	56.863 (1)
2266	73.854 (2)	74.854 (4)	73.913 (1)	66.667 (1)	61.765 (1)
1353	69.696 (4)	61.430 (3)	84.375 (1)	67.170 (2)	71.369 (2)
1080	82.663 (4)	76.427 (3)	96.548 (2)	68.091 (2)	81.405 (2)
1520	109.476 (4)	94.833 (3)	118.403 (1)	94.515 (2)	92.877 (2)
1864	87.175 (4)	80.286 (4)	90.971 (1)	75.063 (2)	76.552 (1)
2268	63.498 (3)	57.084 (3)	63.147 (2)	54.453 (2)	62.745 (1)
2267	68.578 (3)	58.333 (1)	64.130 (1)	61.571 (2)	62.500 (1)
1091	64.318 (1)	68.113 (1)	69.565 (1)	61.527 (1)	62.745 (1)
Mean	75.9105	69.2413	79.5554	66.122	69.850

Scores in ng 5HIAA/ml of CSF. Figures in parenthesis give number of assays making up score.

Analyses of variance : DF treat. = 4, DF error = 45, F = 1.5913, not significant.

steroid treatment. Table 38 shows that oestradiol lowered the turn-over rates of 5HT on this measure from a mean of 207.1 per cent. to a mean of 149.75 per cent. ($p < 0.002$). In every case, the turn-over rate was lowered. Progesterone given with oestradiol antagonised this effect, raising the turn-over rate to 177.375 per cent. on average ($p < 0.05$). It should be noted that progesterone did not restore the turn-over rate completely to the no hormone condition.

Testosterone propionate produced a very marked effect on turn-over rates, reducing the measure from a mean of 207.1 per cent. to 130.58 per cent ($p < 0.002$). Furthermore, when the turn-over rates after testosterone propionate were compared with those on return to no hormones, the effect was quite clear (mean 206.1588 per cent., $p < 0.002$). Similarly, turn-over rates after oestradiol were significantly different from those on return to no hormones ($p < 0.002$).

The mean for no hormones before gonadal steroid treatment was 207.1 per cent. On return to no hormones after gonadal steroids, the mean was 206.16 per cent. So clearly the assay system and techniques used were reliable. Approximately eight months separated these two sets of measures.

Table 38 - Effects of oestradiol, oestradiol + progesterone and testosterone propionate on 5HT turnover rates in female monkeys

Animal	No Hormones		%	Oestrogen		%	Progesterone		%	Testosterone		%	No Hormones (return to base)		%
1754	64.706	200.000	209.09	65.789	165.789	152.02	68.826	220.652	220.59	52.083	136.805	162.67	72.727	238.636	228.13
1811	57.163	182.558	219.36	57.282	130.097	127.12	61.956	144.927	133.92	54.825	126.316	130.40	56.863	152.941	168.96
2266	75.000	205.814	174.42	70.550	166.990	136.70	73.913	182.913	147.06	66.667	151.754	127.63	61.765	208.824	238.09
1353	78.261	247.826	216.66	77.174	179.348	132.39	84.375	201.042	138.27	78.125	210.417	169.33	72.727	209.091	187.50
1080	93.478	302.174	223.25	80.072	214.130	167.42	91.667	243.750	165.91	84.028	179.167	113.22	81.818	214.773	162.50
1520	113.725	314.240	176.32	94.560	234.783	148.29	118.403	325.000	174.48	97.917	197.917	102.13	71.591	232.197	224.34
1864	83.907	252.326	200.72	83.495	225.243	169.77	90.971	258.696	184.38	65.625	133.333	103.17	76.552	238.306	211.30
2268	63.953	215.116	236.37	66.304	152.174	129.51	60.417	164.583	172.41	54.167	123.967	128.86	62.745	209.804	234.38
2267	68.056	204.167	200.00	58.333	166.667	185.37	64.130	198.913	210.17	59.167	140.833	138.03	62.500	193.437	209.50
1091	62.745	197.549	214.81	68.113	169.565	148.95	69.565	227.174	226.56	61.527	141.758	130.40	62.745	186.275	196.88

Scores are ng 5HIAA/ml of CSF before, and 2 hours after, intravenous injections of probenecid (20 mg/kg in phosphate buffer). Third column gives percentage increase of 5HIAA in CSF over 2 hours.

Statistical comparisons

Analysis of variance : df Treat. = 4, df Error = 45, F = 18.7453, $p > 0.01$.

Comparisons :
- No Hormones v Oestradiol, $t = 6.5430$, df = 18, $p > 0.002$.
- No Hormones v Testosterone, $t = 8.1209$, df = 18, $p > 0.002$.
- Oestradiol v OB + Progesterone, $t = 2.2725$, df = 18, $p > 0.05$.
- Oestradiol v Return to NH, $t = 5.4124$, df = 18, $p > 0.002$.
- Testosterone v Return to NH, $t = 6.8825$, df = 18, $p > 0.002$.

Means (of % turnovers)

No Horm.	207.10
O.B.	149.75
Prog.	177.37
T.P.	130.58
No Horm. (return)	206.16

3.3.4. Summary of the Effects of Gonadal Steroids on 5HT Turn-over Rates

Both oestradiol and testosterone propionate lowered the turn-over rates of 5HT in the brains of female monkeys, as measured by the two-hour probenecid test. These effects were both significant ($p<0.002$ in each case).

Progesterone antagonised the effects of oestradiol in this respect, and the effect was significant ($p < 0.05$). Comparisons with a return to no hormones condition confirmed the effects of oestradiol and testosterone.

No differences were found between the measures of turn-over rates under no hormones before gonadal steroid treatments and after them, indicating that the assay system was reliable and that the treatments had no permanent effects on the 5HT neurons of the brain.

PART 3.4

3.4. The Males

Results in this section are presented in two parts :- (3.4.1.) the effects of castration and subsequent testosterone propionate replacement on 5HIAA levels and 5HT turn-over rates in the brain and (3.4.2.) the effects of recent ejaculation on 5HIAA levels and 5HT turn-over rates in the brain.

3.4.1. The Effects of Testosterone Propionate Replacement on 5HIAA Levels and 5HT Turn-over Rates in Castrates

Table 39 shows that replacement with 10 mg/day testosterone propionate for 10 days had no significant effect on the levels of 5HIAA in the CSF of castrated male monkeys. There seemed to be no clear pattern emerging, although overall the mean level increased slightly. Table 40 shows that no significant differences were found in the rate of accumulation of 5HIAA in the CSF, with the two hour probenecid test. Again, the overall mean increased, but the results were variable and not significant.

Table 39 - Effects of testosterone propionate replacement on 5HIAA levels in the CSF of castrated male monkeys

Animal	Castrated	Testosterone propionate replacement
2286	56.667	59.583
2287	66.333	86.250
2288	64.000	101.562
2289	73.000	72.500
2290	54.000	54.687
Mean	62.800	74.916

Scores in ng 5HIAA/ml of CSF. Testosterone propionate 10 mg/day for 10 days.

Comparison : $t = 1.3059$, $df = 18$, not significant.

Table 40 - Effects of testosterone propionate replacement on 5HT turn-over rates in the brains of castrated males

Animal	Castrated			Testosterone replacement		
	Before	After Probenecid	% Increase	Before	After Probenecid	% Increase
2286	56.667	144.005	154.12	59.583	145.418	144.06
2287	66.333	167.663	152.76	86.250	262.925	204.84
2288	64.000	171.994	168.74	101.562	280.128	175.82
2289	73.000	231.001	216.44	72.500	215.006	196.56
2290	54.000	139.558	158.44	54.687	179.866	228.90
Mean	62.800	170.844	170.100	74.916	216.669	190.036

Scores are ng 5HIAA/ml of CSF before, and 2 hours after, intravenous injections of probenecid (20 mg/kg in phosphate buffer). Third column gives percentage increase over 2 hours.

Comparison : $t = 1.0710$, $df = 8$, not significant.

3.4.2. The Effects of Recent Ejaculation on 5HIAA Levels and 5HT Turn-over Rates

Table 41 shows that recent ejaculation had no significant effect on the levels of 5HIAA in the CSF of male monkeys, when compared with the base-line levels after separation from the females. The five males who received the two treatments in reverse order (i.e. ejaculation first) are marked "E" on the table. However, no differences were observed and therefore all results are included together.

Table 42 shows that recent ejaculation had no significant effects on the rate of accumulation of 5HIAA in the CSF with the probenecid test.

Table 41 - Effects of recent ejaculation on 5HIAA levels in the CSF of male monkeys

Animal	No Treatment	Recent Ejaculation	
1263	49.767	50.000	E
1264	69.207	74.777	E
1654	47.434	47.500	E
1994	53.750	51.639	
2088	69.945	69.761	E
2089	80.000	73.770	
2272	50.000	52.500	E
318	42.411	40.176	
325	43.501	43.716	
326	41.379	59.016	
Mean	54.7394	56.2855	

Scores in ng 5HIAA/ml of CSF. E indicates animal was tested first after ejaculation and then under no treatment.

Comparison : $t = 0.2659$, $df = 18$, not significant.

Table 42 - Effects of recent ejaculation on 5HT turn-over rates in the brains of male monkeys

Animal	No Treatment			Recent Ejaculation		
	Before	after Probenecid	% Increase	Before	after Probenecid	% Increase
1263	49.767	167.963	237.50	50.000	158.333	216.67
1264	69.207	247.904	258.34	74.777	235.491	214.92
1654	47.434	169.518	257.38	47.500	150.000	215.79
1994	53.750	177.500	230.23	51.639	158.197	206.35
2088	69.945	212.022	204.56	69.761	175.066	150.75
2089	80.000	215.000	168.75	73.770	223.497	202.96
2272	50.000	148.361	196.72	52.500	152.500	190.48
318	42.411	125.000	194.73	40.176	136.081	238.71
325	43.501	116.711	168.29	43.716	138.798	217.50
326	41.379	124.138	200.00	59.016	172.678	192.60
Mean	54.739	170.421	211.65	56.286	170.064	204.69

Scores are ng 5HIAA/ml of CSF before, and 2 hours after, intravenous injections of probenecid (20 mg/kg in phosphate buffer). Third column gives percentage increase over 2 hours. Comparison : $t = 0.5467$, $df = 18$, not significant.

3.4.3. Summary of the Effects of Androgen Replacement and Recent Ejaculation on 5HIAA Levels in the CSF and 5HT turn-over Rates

Testosterone propionate had no significant effects on the levels of 5HIAA in the CSF or on the turn-over rates of 5HT in the brains of castrated male monkeys. Nor did recent ejaculation have any significant effects on either 5HIAA levels or on 5HT turn-over rates.

CHAPTER 4

DISCUSSION OF RESULTS

It is not known what part biogenic amines play in the sexual receptivity of female primates. The results reported above contribute to an understanding of the role of 5HT in the sexual receptivity of female rhesus monkeys. However, caution should be exercised in the interpretation of any results involving primate behaviour. Humans and non-human primates are typically adaptable, and great individual differences between animals can always be expected when experiments are conducted on these species.

In interpreting the results of the experiments conducted here, two additional issues are of central importance. The first concerns the male/female pair used in the behavioural observations, and the second concerns the notion of female sexual receptivity. Both have been given comprehensive coverage by Everitt (1970), among others.

OUTLINE OF DISCUSSION OF RESULTS

The results of these experiments are discussed in six main parts.

(1) The two issues mentioned above are considered. Section 4.1. deals with the male/female pair used, while section 4.2. deals with female receptivity.

(2) The salient points of the results given in chapter 3 are selected and emphasised (section 4.3.).

(3) Problems associated with the use of PCPA to deplete 5HT in the brain are considered in detail, namely, the possibility that PCPA acts by depleting catecholamines rather than 5HT (section 4.4), and that it acts via a non-specific sensitising mechanism (section 4.5). Section 4.6. reviews other experiments in which PCPA has been given to monkeys.

(4) The effects of gonadal steroids on 5HT turn-over rates are indicated. The significance of these effects is that androgens could act on 5HT-containing neural systems in the brain to control female sexual receptivity in primates (section 4.7).

(5) The essentially negative results of the investigations into the role of 5HT in female grooming and social behaviours (section 4.8) and male reproductive processes (section 4.9) are discussed in detail.

(6) The results of this study are correlated, and the conclusions stated.

4.1. Field Studies, Captive Groups and Male/Female Paired Observations

Detailed accounts of both the advantages and disadvantages of studying male/female pairs of rhesus monkeys placed together to interact for half-hour test periods in an empty cage, have been given by Everitt (1970), by Herbert (1970) and by Dixon (1973).

4.1.1. Field Studies

Field studies allow the animals to be studied in their natural environment. In this respect the field study is ideal. The stimuli affecting the behaviour of the animal come from the environment to which the animal is adapted by evolution. In particular, the social stimuli impinging on the animal from a feral group are the crucial ones which typically shape and maintain its behaviour (cf Skinner, 1969). Certainly many primates live in large groups, and the social and other stimuli impinging on an animal are extremely complex.

However, this very complexity of social organisation militates against accurate measurement of any particular behaviour. So many social interactions are possible in a big primate troop that a large number of variables could be acting on any one member of the group. Furthermore, it is not always possible to keep the animals under continuous observation in the field. In arboreal species such as the New World monkeys this is especially true. In species inhabiting nearly impenetrable forests such as the Budongo forest (Reynolds and Reynolds, 1965; Marler, 1972), it may be possible to observe sexual behaviour only at rare intervals. In those species lacking a definite sexual swelling, it is not always possible to determine the various stages of the female's menstrual cycle.

4.1.2. Small Captive Groups

Monkeys could be studied in small groups which will obviate some of the difficulties of field studies. Providing adult feral animals are used, the chances of observing full reproductive behaviour are good. Small captive groups have been used by, for example, Scruton and Herbert (1972) and Dixon (1973) in Miopithicus talapoin, Gartlan and Brain (1968) in Cercopithecus aethiops, Rowell (1967) in Papio anubis, Goldfoot (1971) in Macaca nemistrina and Hinde and Rowell (1962), Herbert (1968) and Everitt and Herbert (1969 a, b) in Macaca mulatta.

In small captive groups, the interactions between social factors and endocrine events in shaping an animal's behaviour can be examined. In this way, the effects of castration and hormone replacement are studied in a social setting. Thus Dixon (1973) was able to examine (in commendable detail) the interaction between dominance, the amounts of aggression and sexual behaviour and the testosterone levels in the blood of males in small groups of talapoin monkeys. An important finding was that:- "Sexual behaviour in subordinate males, either intact or testosterone-treated castrates, could be activated if the top-ranking male were removed. This procedure resulted in the second-ranking male ... showing significantly increased levels of sexual activity; that of the third male, who was least dominant, remained in abeyance. However, when the second male was removed, the third began to show mounting activity, though in this case stimulation was less marked" (Dixon et al, 1973, p 57).

Clearly then, social factors such as dominance play a crucial role in the actions of hormones on behaviour. In this case, sexual behaviour can be completely inhibited by the presence of a more dominant male, irrespective of the hormonal condition of the animal. This example is

taken to illustrate the point, but further consideration of caged group work will be given below.

However, many disadvantages still remain, even with small caged groups. Such groups are usually formed of adult animals purchased as they come from dealers, and they need not be members of the same wild troop. The compositions of the groups, as well as the laboratory environments, are therefore often abnormal (see also Dixon, 1973). This abnormality of composition could interact with hormonal treatments in a misleading way. In other words, complex interactions from the members of the group could obscure the effects of hormone or other experimental manipulations on the sexual behaviour of the animal.

4.1.3. Paired Observations

Jolly (1972) has argued forcibly that far more sexual behaviour is obtained out of caged primates than is usually seen in the wild, and that this reflects the impoverished social environment of the laboratory situation. While this is no doubt true, the main justification for paired observations in the laboratory lies in the necessity for adequate control over all those extraneous variables which would otherwise cut across and confound the study of hormonal influences on sexual behaviour. Furthermore, the aim of paired observations is not to reproduce the full complexity of the feral environment, but to "dissect out" (Herbert, 1970) those parts of the animals' sexual behaviour which are affected by hormones and which are under study. The clear peaks of sexual interaction seen at mid-cycle in pairs of rhesus monkeys in the laboratory are important, even if the overall level of sexual activity for all stages of the cycle is higher than it would have been in the wild. This point is referred to again, but clearly field and laboratory studies are complementary in the study of primate sexual behaviour (see also Dixon et al, 1973).

For these reasons, paired observations were used in these experiments. The rigorous behavioural measurements made under such standardised, constant and controlled experimental conditions made it possible to detect the often subtle effects of monoamine manipulation. Indeed, much of the available evidence on female sexual attractiveness (Michael and Keverne, 1968) and female sexual receptivity (Everitt, Herbert and Hamer, 1972) in rhesus monkeys was obtained by means of these testing procedures. While extrapolations from such artificial laboratory conditions to the wild should always be made with due caution, in practice it is surprising how often the findings of the laboratory have been applicable in the wild (Saayman, 1970). For example, patterns of mounting observed in the paired-observation cage are effectively the same as those observed in wild rhesus troops, as described by, for instance, Lindburg (1971). Housing the animals separately also eliminates the problem of behaviours occurring prior to the behavioural tests.

4.2. What is Receptivity?

Considerable attention has been given to the question of what constitutes sexual receptivity in female primates (Herbert, 1970; Everitt and Herbert, 1972; Dixon et al, 1973), and the difficulties involved should not be underestimated. The primary measures used are the number of presentations made by each female per test, and the number of times she refuses male mounting attempts. But these measures by themselves are not adequate measures of female receptivity. Only changes that occur when parallel changes in male sexual behaviour can be accounted for, yield valid conclusions about female receptivity. In particular, the sexual attractiveness of the female to the male should not vary, so that a crucial requirement of these experiments was that the females should be on the same oestradiol regimen whenever they were tested with males. But a number of imponderables remain, such as whether a more receptive female is thereby more "attractive" to a male in some secondary way?

4.2.1. Presentations

A female will present to a male in response to male aggression (Kempf, 1917; Zuckerman, 1932, cited in Herbert, 1970; Marais, 1969). However, juvenile male baboons will also present to dominant males (see also Dixon, 1973) which means that such presentations are primarily responses to aggression, or are social gestures of submission, and are to be distinguished from sexual presentations. It is therefore essential to record all incidents of aggression in the behavioural observations. No cases of male aggression towards females were seen in over 720 hours of observation, so female presentations in response to direct male aggression can be specifically excluded in these experiments.

This does not mean that more subtle cues are not operating in

complex male/female sexual interactions. On the contrary, it is very likely that the male does influence the rate of female sexual presentations in a number of ways. For example, the male often "threatens away" just prior to mounting a female (Zumpe and Michael, 1970). In this behaviour, the male will stare and head-duck in a threatening way at a point outside the cage, as if at an imaginary opponent. The behaviour can be directed anywhere outside the cage, and is not specifically aimed at the observation mirror. It has been interpreted by Michael, Zumpe and co-workers as a "displacement activity", due in part to tension generated by the presence of another animal in the male's "home" cage. No anthropomorphic interpretation will be attempted here, but it remains the case that the female will often join in such "threatening away", and may present sexually shortly after the male displays this behaviour.

In a similar way, a male may give less direct cues to the female which might lead to her presenting. For example, Everitt (1970) has pointed out that "subtle cues" such as the male glancing at the female, posturing or lip-smacking may all ".....function to assure her that her presentations or movements towards the male would not be met with aggression" (p 149). As Everitt rightly points out, the extent to which these factors influence the female's presentation rate is largely "imponderable", and is certainly very difficult to measure. At the same time of course, a very unreceptive female is unlikely to be prompted to present by such small and subtle male cues.

It is usual in this work to assume (Herbert, 1970; Everitt, 1970) that no one form of presentation is any more effective than any other in inducing a male to mount. In other words, a classic presentation is not a more intense or "better" type of presentation than a head-duck

or a hand-reach (2.3.2.1. above). The assumption is made to obviate the need to "grade" presentations in any order of effectiveness, and is justified by the tendency for a particular female to have a preferred way of presenting (Everitt, personal communication; personal observation). Should her sexual receptivity increase, she will then increase the frequency of her particular form of presentation, i.e. she will not change to another form. For example, female 1886 used a hand action ("thump") on the floor of the cage, with head-ducks, to invite the male to mount, while female 1265 seldom showed anything but classic presentations.

A presentation was scored as such providing some time had elapsed between one head-duck or presentation and the next. A classic presentation held for a long time was thus scored as only one presentation, as was a series of hand-reaches or head-ducks without an interval between each movement.

Withdrawing oestrogen or giving progesterone to a female makes her unattractive (see 1.4.4. above). Attention has already been drawn to the fact that this could result in the female presenting more frequently than she did before. However, this could hardly be interpreted to mean that oestradiol inhibits sexual receptivity in female monkeys and that withdrawing it increases presentation rates. Clearly the effects of oestrogen withdrawal or progesterone administration are to reduce male initiations, which sometimes results in the female presenting more to counteract the effect.

4.2.2. Sex Preference

The effects of the male on female presentation rates are even more clearly seen in the sex preference experiments of Everitt and Herbert (Herbert 1968; Everitt and Herbert, 1969 a, b; Herbert, 1970). In these, two ovariectomised females are placed together in a cage with a

male. Even if both receive the same amount of oestrogen, the male invariably shows a distinct preference for one. He then spends his time copulating, grooming and sitting with this female, excluding the non-favourite, who is often threatened by the favourite and "spends much of her time sitting by herself in a corner of the cage" (Herbert, 1970, p 132).

If the preferred female is then taken off oestrogen (or given progesterone), the male shifts his attention to the second one. The remarkable point to be noted here is that the second female then responds to this increased attention by presenting more to the male, and perhaps even threatening the former favourite. As pointed out by Herbert (1970), this change in her presentation rate and other behaviours cannot be the result of any hormonal treatment, which has remained constant throughout the experiment, but is due to the male changing his behaviour towards the former non-favourite.

If both females are untreated, the male still interacts (but at a lower level of sexual activity) with the favourite and ignores the non-favourite. The males determine which female shall be the favourite; once he has made his choice, the favourite shows increased sex behaviour and may threaten the other female. However, his choice could be influenced by the behaviour of the female, since in one case a clear favourite proved to be sexually unreceptive"... and was eventually rejected in favour of one of the non-favourites" (Herbert, 1970, p 133).

These elegant and important experiments have been supported by the recent findings of Phoenix (1973) on rhesus, and by the findings of Goldfoot (1971) on groups of three Macaca nemistrina females placed with a male, described in detail above (1.4.3.1). Essentially, Goldfoot found that the highest scores on all measures of sexual behaviour (in-

cluding ejaculations) were recorded when the alpha female was at mid-cycle. When the gamma female was at mid-cycle, the males copulated (but did not ejaculate) with the alpha female. However, if both alpha and beta females were removed, the male copulated and ejaculated with gamma, who also showed increased sex behaviour. Phoenix showed that ejaculation of male rhesus is very much a function of the particular female with which he is paired.

Clearly then, males have sexual preferences which do not depend only on the hormonal states of the female. Furthermore, some males do not initiate much sexual behaviour, or are relatively slow to do so (for example, male 2273 in the experiments reported here). A female paired with such a male could well present more than she might do were she paired with a more vigorous initiator. Certainly a female will sometimes refuse one male but copulate readily with another (personal observation, female 2259). The rationale for treating the male/female pair as the unit of analysis lies precisely in these (and other) individual differences and sexual preferences (see also Michael and Saayman, 1967). The strength of the experimental design used here lies in the fact that these differences and preferences are specifically controlled for by retaining the same pairing throughout all treatment conditions.

4.2.3. Refusals

The number of times a female refuses a male depends on her sexual receptivity. But it also depends on the frequency of male mounting attempts, since "... a male making many mounting attempts is perhaps more likely to be refused than one making a few, both in terms of giving the female more cause and more opportunity to do so" (Everitt, 1970, p 150). Refusals may also depend on preferences, as mentioned above, and on the vigour with which a male attempts to mount, a quality which is very difficult to measure. The derived index, the female acceptance

ratio, is an important one in assessing female receptivity, since it expresses the proportion of male initiations accepted or refused by the female and thereby takes account of some of these factors.

4.2.4. Ejaculatory Response

Controversy has surrounded the exact status of the ejaculatory response. On one extreme, Zumpe and Michael (1968) have argued that the clutching reaction of the female monkey at the time of male ejaculation is the homology of orgasm in women. Zumpe noted that the clutching reaction is hormone dependent, since it decreased after ovariectomy, was restored by oestrogen and was again decreased to very low levels by progesterone. [~~But her findings are hardly surprising, since ovariectomy and progesterone treatment both have marked effects on the male's sexual activity via a considerable reduction in the female's sexual attractiveness (1.4.5. above), and therefore both treatments are bound to affect the female's ejaculatory response, if only because the male no longer mounts, thrusts and ejaculates with her at anything like the level he did when she was intact at mid-cycle, or oestrogen-treated.~~]

Zumpe's reasons for drawing the homology with human orgasm are that some females showed "withdrawal reactions" from the male after ejaculation which were so vigorous that the female collided with the side of the cage. In 3 out of 13 females, apparently involuntary pelvic movements continued for a few seconds after this, with the female sitting down, before she started grooming the male. But her main evidence comes from a motion picture record of the clutching reaction which she claims occurs before the ejaculatory spasm of the male. Furthermore, she claims that in females which have not been paired with males for some weeks, and which have (presumably) a high level of sex "drive", the female orgasm can occur long before male ejaculation, so much so that the male is not able to continue

thrusting.

Support for the view that the rhesus clutching reaction is consummatory sexual behaviour or orgasm has been given by Saayman (1970), following his detailed study of a large troop of wild baboons. The withdrawal reaction and copulation call of the female baboon follows intromitted mounts with pelvic thrusts from the male, and typically the female will leap away from the male and run a few paces, while giving the distinctive vocalisation referred to as the copulation call (Bolwig, 1959; Hall, 1962). The behaviour is seemingly an involuntary reaction, and it is note-worthy that it occurred more frequently when females were mounted by mature males and less frequently when they were mounted by sub adult and juvenile males. This lead Saayman to conclude that "... adequate physical stimulation from a fully developed mature male was necessary for the full expression of the consummatory response" (p 107). Certainly, the behaviour occurred less frequently in flat females, as against inflating and swollen females, as would be expected from Saayman's finding that the number of male thrusts per mount was lowest with flat females.

Against this view, Herbert, Everitt and Scruton (personal communication) all report that extensive observations on rhesus monkeys' sexual behaviour have not supported Zumoe's findings. In particular, they have not observed a female kept away from males for a number of weeks undergoing "orgasm" to the extent that further male thrusting is impossible. Zumpe reports that a male may even show aggression towards a female should this happen repeatedly. Certainly, in all the cases where the ejaculatory response was recorded in these experiments, it occurred exclusively on the male's ejaculatory mount. Ejaculation was easily recognised by the deeper thrusts of the ejaculatory mount, the characteristic spasm of the

musculature, a straightened back, the emission of semen and the short supernumerary thrusts.

Since there is at least some doubt as to whether the clutching reaction of the female is the homology of human orgasm, the term ejaculatory response is preferred. On its own, it is not a definitive measure of sexual receptivity. However, in these experiments it was found to covary with the presentation rates and inversely with refusal rates, confirming earlier work by, for example, Everitt (1970).

Taken together, therefore, presentation rates, refusals, the ejaculatory response and the derived indices of female acceptance ratio and percentage of mounts initiated by the female, give a compound measure of female sexual receptivity. But as emphasised above, these measures are only valid when possible parallel changes in male behaviour are both accurately recorded and accounted for. This was done in these experiments by recording six components of male sexual behaviour and three components of grooming and social behaviour.

4.2.5. Male Sexual Behaviours

In the male behaviours, the most important finding was that the male acceptance ratio did not decline, showing that the females remained attractive to the males (3.1.1.12). The number of mounts a male makes per test could give a measure of his sexual activity. But Herbert (1965, 1970, 1973) has shown that it is not a reliable one. For example, a male paired with an unattractive female may make many mounts, but not ejaculate. Or else, a male in which the dorsal (sensory) nerves of the penis have been transected may also make a large number of mounts. This is not to be interpreted simply as an increase in his sexual activity, since such males show "grossly abnormal, shallow, ataxic thrusts" (Herbert

1973, p 295), and their ejaculation times are prolonged. Such a male eventually stops mounting the female, and even when she is reintroduced to him 18 months later, sexual activity, including mounting, is at very low levels. Interestingly enough, introducing a different female to the male restores his mounting behaviour for a while, but it soon declines once more.

Herbert has interpreted these findings as indicating "that the stimuli transmitting a female's attractiveness to a male eventually become inoperative if sensory input from his penis is interrupted" (Dixon et al, 1973, p 45). Furthermore, in the case of unattractive females (for example, without oestrogen), it is thought likely that "the female's stimulus (value) was sufficient to cause the male to mount, but not to raise the mounting rate or the number of thrusts per mount to a critical level necessary for ejaculation".(Everitt, 1970, p 151). This would explain a large number of mounts observed with unattractive females, and is supported by similar comments made by Saayman (1970) on ejaculation and grooming in baboons. It is also possible that oestrogen-dependent changes in the quality of the female's vagina, which are known to affect the male via olfactory mechanisms (1.4.5. above), could also act via the sensory afferent input from the glans of the male penis during copulation leading to ejaculation.

But by far the most important changes noted in male sexual behaviour in these experiments were those which showed the great adaptability of certain males to the sexual receptivity of the female. Once the receptivity of the female had been altered by the experimental manipulations, the male appeared to adapt his behaviour to the situation. The clearest example was the case of male 1935 when paired with female 2260. After adrenalectomy, female 2260 stopped presenting to the male and started to

refuse him. Whenever he tried to mount, she would roll over and present for grooming. Eventually the male would clasp her hips and attempt to lift her onto all fours. If he succeeded in mounting and intromitting, he would then sometimes thrust 13 or 14 times in rapid succession and ejaculate in one mount. This was in such marked contrast to the behaviour recorded when the female was not refusing him that there was no doubt about the fact that the male's behaviour had changed specifically to accomodate the altered situation. During the oestradiol series, he averaged 7.2 mounts per test and 7.47 thrusts per intromitted mount. During the 5HTP series, he averaged only 1.4 mounts per test, but 10.1 thrusts per intromitted mount.

When 1935 was paired with 1886, a similar pattern of male adaptation was noted. In the oestradiol series, he averaged 18.6 mounts per test. This declined significantly to 9.0 per test after adrenalectomy, increased significantly to 15.4 per test when she became receptive again on PCPA and then declined significantly to 12.5 per test after 5HTP. In this case, the explanation for the changes may perhaps be different. This female, when receptive, presented at an exceptionally high rate (averaging 20 presentations per test), and this rate may have exceeded the mounting rate optimal for the male. In this case, the decline in presentation rates (Table 1) which accompanied her relative unreceptivity after adrenalectomy and 5HTP could have caused the decline in the number of mounts the male made per test (Table 6), since this male tended to accept female presentations by mounting even if he had not yet achieved penile erection following the previous mount.

A different sort of adaptation was seen in the case of male 2273. Once female 1887 stopped presenting to him and refused him, he simply withdrew from further contact with her, made no further initiation attempts

and sat up on the perch ignoring the female. Such a marked inhibition of the male's sexual activity by the female's changed receptivity was unusual, and frustrating in that it made further analysis of this pair's behaviour impossible.

Male 2092 was adaptable as well. He was the only male who would copulate with a female up on the perch. If a female was unreceptive, she would often sit on the perch staring outside the cage and ignoring the male completely. The other males would usually display a number of soliciting behaviours directed at the female in this situation. They would lipsmack at her, threaten away or attempt to hoist her down from the perch by clutching her hips. But male 2092 would climb up on the perch and sit with the female, and then mount, intromit and thrust if and when the opportunity arose.

These changes in male behaviour, particularly the adaptations by the male to the female's changed receptivity, are open to a certain amount of subjective and even anthropomorphic interpretation. However, the quantitative measurements used in this study make it possible to state in objective terms which changes took place (for example, 1935 with 2260), and the above serves to draw attention to the changes noted in Chapter 3.

Overall, male sexual behaviour did not alter reliably, because of these adaptations of behaviour (see 3.1.1.13 above). Mounts per test declined after adrenalectomy, but did not recover again after PCPA or 5HTP, pointing perhaps to a further male adaptation of behaviour. Perhaps the best conclusion to draw is that male behaviour in these experiments was very much a function of the individual male, his particular behavioural characteristics and his individual adaptation to the loss or gain of sexual receptivity by his female partner.

4.3. Treatments

4.3.1. Behavioural

4.3.1.1. Bilateral adrenalectomy led to a definite loss of sexual receptivity in all eight of the females tested, thus supporting earlier findings from this same laboratory (Everitt, 1970; Everitt, Herbert and Hamer, 1972). Females presented significantly less often to males, both individually and as a group (3.1.1.1.). This was reflected in the derived index of the percentage of female initiated mounts (3.1.1.2). In addition, three females refused the male significantly more often, while the female's ejaculatory response also tended to go down. (3.1.1.3; 3.1.1.5).

4.3.1.2. PCPA completely or partially restored sexual receptivity in all ten adrenalectomised females treated with it. As noted in Chapter 3, this is the important finding of these experiments. In seven of the ten females, presentation rates were increased significantly by PCPA over the adrenalectomy condition, and the remaining three females were the ones who refused the male significantly less after PCPA. This means that all ten females were made more receptive on either one or other of the two primary measures of sexual receptivity. The derived scores both reflected these increases of receptivity, and PCPA tended to restore the ejaculatory response in females as well. The latter change was significant in three cases.

4.3.1.3. 5HTP was administered to two groups of females. In the five adrenalectomised PCPA-treated females, 5HTP led to a definite loss of receptivity in all five animals. Four of them presented significantly less than when on PCPA alone, and the fifth one, female 2260 who was not a presenter in any case, refused the male significantly less. The

ejaculatory response went down significantly in four of the five animals.

Further evidence to show that 5HTP reversed the effects of PCPA on sexual receptivity comes from the comparison (3.1.1.6. above) between 5HTP and adrenalectomy alone. There were no significant differences between the two treatment conditions on any of the measures used, with two exceptions. The first was female 1886, who presented significantly more after 5HTP than she did after adrenalectomy, and the second was female 2259, who refused significantly more after 5HTP, and had a lower ejaculatory response than she had after adrenalectomy. However, it is noted that these differences are inconsistent in that they are in opposite directions, and therefore they are probably not crucial differences.

In the four ovariectomised oestrogen-treated females, 5HTP caused a decrease in sexual receptivity. All four females presented less after 5HTP, and the change was significant in three of these, and was significant overall. These females did not refuse males, and although 5HTP tended to reduce the ejaculatory response, the trend was not significant or convincing.

4.3.2. Biochemical

4.3.2.1. PCPA on 5HIAA levels in the CSF

A strong point of the experimental design in these experiments is that parallel biochemical measurements could be made of the actual effects on 5HT of the doses of PCPA used in the behavioural experiments. The PCPA dose regimen used was certainly effective in lowering 5HT activity, as measured by 5HIAA levels in the CSF. Interestingly enough, PCPA seemed tc lower the levels of 5HIAA as much in the two non-adrenalectomised control animals as it did in the seven adrenalectomised ones which

had taken part in the behavioural experiments, and for this reason the results were included together (Table 35).

One injection reduced 5HIAA levels in the CSF by 53.18 per cent. on average, to 46.82 per cent.of the oestradiol-treated condition. Two injections reduced it by 57.63 per cent. to 42.37 per cent. of base-line, and after 3 injections or 12 days of PCPA the levels were down to 39.93 per cent. of base-line. As noted (3.2.2. above) recovery from PCPA was slow.

4.3.2.2. 5HTP + PCPA on 5HIAA levels.

The dose of 5HTP was titrated with the usual PCPA dose to give 5HIAA levels not significantly different from the pre-PCPA condition. The dose of 20 mg/kg every second day achieved this after the second day. However, it should be noted that there was a slow build-up of 5HIAA in the CSF over the 10 days of the behavioural observations, until after 12 days or 6 injections of 5HTP, the levels stood at 163.62 per cent. of base-line. Purely from the point of view of experimental design, it would obviously have been preferable had this level not crept up during the ten days of behavioural testing. However, since the object was to show that restoring 5HIAA levels to at least their pre-PCPA levels reversed the effects of PCPA on sexual receptivity, this is not a major criticism. There was no clear evidence that overall sexual receptivity was greater at the beginning of 5HTP treatment than later. It seemed better to get a restoration of 5HIAA as soon as possible and to use a standard regimen of 5HTP + PCPA than to attempt adjustments of dosage during an experimental series of ten tests. Clearly no cisternal tapping could be done on animals during behavioural tests.

5HTP given on its own to the four non-adrenalectomised animals

raised the 5HIAA levels in the CSF to very high levels. After 2 days the increase was 72.82 per cent. going up to an increase of 204.46 per cent. after 12 days.

4.4. PCPA and Catecholamines

The main conclusion of these experiments, that PCPA is able to restore sexual receptivity in unreceptive female monkeys, is therefore consistent with the known effects of PCPA on sexual behaviour in sub-primate mammals (1. 8. 2. above). PCPA is a selective inhibitor of 5HT synthesis and it lowered 5HIAA levels in the CSF of these monkeys. The tentative conclusion is, therefore, that PCPA facilitated sexual receptivity by depleting brain 5HT (see Meyerson, 1964 a, b, c).

It should be noted that an alternative interpretation has been forcibly argued by Södersten, Ahlenius and co-workers in Sweden (Ahlenius, Engel, Eriksson and Södersten, 1972; Ahlenius, Engel, Eriksson, Modigh and Södersten, 1972). PCPA affects catecholamines as well as 5HT in the brain (Koe and Weissman, 1966). In fact, PCPA decreases catecholamines in the rat brain almost immediately, but the levels recover again within 26 hours, while the effects of PCPA on 5HT are slower and much longer lasting. After PCPA, 5HT levels in the rat brain are still markedly depressed after 26 hours (Ahlenius et al, 1972). Södersten and his co-workers suggest that it is only in its anti-catecholaminergic effects that PCPA is active in inducing sexual behaviour in male and female rats, because the primary facilitatory effect of PCPA on sexual behaviour is during the time of catecholamine depression.

But two factors in these findings would suggest an anti-serotoninergic effect of PCPA on sexual behaviour rather than a catecholamine effect. First, 5HTP (the immediate precursor of 5HT) reversed the sexual receptivity induced by PCPA at the same time that it restored 5HIAA in the CSF to pre-PCPA levels. 5HTP would not be expected to restore catecholamines; indeed, 5HTP would be more likely to further deplete catecholamine central stores by amine displacement (Weissman and Harbert, 1972). Therefore the

effect would not be in the right direction. If anything, 5HTP should have enhanced the facilitatory effects of PCPA on sexual receptivity.

Secondly, if the time course of action of PCPA is similar in monkeys and rats, the dose regimen used in these experiments would further argue against any possible CA effect on sexual behaviour. The effects of PCPA on 5HIAA levels were marked a full 4 days after the first injection, and persisted for at least 7 days into the recovery period. This finding is quite consistent with the findings of Maas, Redmond and Gauen (1972) on urine levels of 5HIAA after PCPA in stump-tailed macaques Macaca speciosa.

For these two reasons, it is likely that PCPA was active in inducing sexual receptivity in female monkeys by depleting 5HT rather than CA. This basic disagreement between Södersten's work and the present findings could reflect simple species differences, since oestrous behaviours such as lordosis are controlled in rats by oestrogen and progesterone, and there is no reason to suppose that this marked difference in hormonal control of sexual behaviour is not carried over into monoamine mechanisms of control as well.

However, very recently, Everitt, Fuxe and Hökfelt (1974, in press) have carried out controlled studies of the effects of amine depletion on sexual behaviour in oestrogen-treated ovariectomised (and adrenalectomised) rats "using treatments which are rather more precise in their action on DA, NA and 5HT transmission in the CNS" (p 1). Everitt has found that "PCPA continued to effect sexual receptivity to the same extent 26-28 and 48-50 hours after injection, when 5HT levels are much depressed, but CA levels have returned to normal". (p 3).

This work by Everitt and co-workers is consistent with earlier

work by Meyerson and Lewander (1970) and Zemlan et al (1973), and is of course, contrary to the work of Ahlenius and Södersten. It therefore seems unlikely that the primary action of PCPA on sexual behaviour is catecholaminergic, particularly in the monkey.

Recently, Eriksson and Södersten (1973) have suggested that monoaminesynthesis inhibitors such as PCPA or AMPT could induce lordosis in oestrogen-treated ovariectomised rats not by a direct action on neural tissue as suggested by Meyerson (1964 a, b, c, and elsewhere), but by an action on the pituitary/adrenal system via ACTH, as suggested earlier by, for example, Feder and Ruf (1969). ACTH secretion in the rat may be inhibited by a CA brain circuit (Ganong, 1972), and therefore the suggestion would be that central CA depletion by AMPT or PCPA might free enough ACTH from the pituitary to stimulate significant amounts of adrenal progesterone. This progesterone would then facilitate lordosis and related sexual behaviours in oestrogen-primed rats.

Eriksson and Södersten found that PCPA or AMPT facilitated lordosis in ovariectomised oestrogen-treated rats but did not do so when rats were adrenalectomised as well. However, this explanation is clearly not applicable in the case of the monkey. First, progesterone inhibits sex behaviour in pairs of monkeys (1.4.7.). Secondly, the monkeys used were adrenalectomised. Furthermore, Everitt et al (1974) have recently published results which contradict those of Eriksson and co-workers on this point, so clearly further definitive study to settle the issue is required.

4.5. The Problem of Specificity of 5HT Depletion

PCPA increased sexual receptivity in these female monkeys by reducing the activity of 5HT-containing neurons, rather than by acting on CA-containing neurons. But any conclusion that such PCPA-reduced 5HT activity disinhibited specifically the sexual behaviour of the female, rather than any other behaviour, would be premature. It remains possible that lowering 5HT in the CNS causes some more general sensitising effect on the animal (Weissman and Harbert, 1972), so that the thresholds of response to all incoming stimuli are lowered. The animal would then be more responsive to any particular set of environmental stimuli impinging on it, and in the particular paired situation used here, the incoming stimuli would be primarily sexual. In this way, the suggested general sensitising effect would manifest itself in terms of greater sexual activity because of the particular environment to which the animal is restricted.

4.5.1. 5HT and Sleep and Arousal

The argument is a strong one, since there is no doubt that 5HT is involved in sleep and arousal. Lesions in the nuclei of the raphe, in the caudal midbrain and rostral pontine regions, cause marked insomnia in cats (Jouvet, 1969). Such lesions of the dorsal and medial raphe nuclei of the brainstem destroy the perikarya of most of the 5HT-containing neurons projecting to the forebrain of the rat (Cohen and Bowers, 1972), and result in great reductions of 5HT in the forebrain and basal diencephalon. In cats, it takes 10 to 13 days for the 5HT nerve endings to become depleted of transmitter, following raphe system lesions (Jouvet, 1969), but they do eventually lose all their 5HT.

Levels of 5HT in the rat brain are always highest during periods

of light, and lowest during the periods of darkness (Hery, Rouer and Glowinski, 1972). Since the periods of darkness are activity periods for rats, this daily variation in brain 5HT metabolism is closely related to normal circadian rhythms of sleep. 5HT itself does not cross the blood-brain barrier, but both tryptophan and 5HTP have been reported to lead to sedation and sleep (Delorme, Froment and Jouvet, 1966).

PCPA has been shown to decrease sleep in a number of animals. In rats, there is a decrease of both slow-wave sleep and paradoxical sleep after PCPA (Mouret, Bobillier and Jouvet, 1968). Jouvet's suggestion has been that 5HT-containing neurons, particularly in the nuclei of the raphe, are responsible for the onset of slow-wave sleep. Destruction of the raphe nuclei, or reduction of 5HT activity in the brain by PCPA, would thus free the reticular activating system (RAS) from inhibitory control, and would serve to keep the cortex in an active, aroused state of insomnia for many hours. In this way, the lower frequency of paradoxical sleep observed (Jouvet, 1969) would follow from the reduction in slow-wave sleep (Mouret et al, 1968), since paradoxical sleep usually follows slow-wave sleep.

PCPA also blocks slow-wave sleep in cats, so that the cats stay awake for periods of over 60 hours (Delorme et al, 1966; Jouvet, 1969). These findings have received extensive support (for example, Koella, Feldstein and Czicman, 1968; Dement, 1969). Furthermore, the effects of PCPA in this respect are completely reversed by 5HTP, and indeed normal sleep patterns follow if 5HTP is given in balanced quantities with PCPA (Dement, 1969).

PCPA has been given to rhesus monkeys, where it was found that any dose above 600 mg/kg caused great changes in sleep patterns (Weitzman, Rapport, McGregor and Jacoby, 1968). The amount of non-REM sleep (slow

wave sleep) decreased considerably, but the amount of REM (or paradoxical) sleep stayed the same as before PCPA. Weitzman et al confirmed biochemically that PCPA did reduce 5HT in seven areas of the brain, particularly in the rostral brain-stem (reduction of 40 per cent.), as required by Jouvet's hypothesis. No total insomnia was seen in Weitzman's monkeys, and in this respect the monkey was different from the cat.

Unfortunately, little evidence is available on the effects of PCPA on human sleep. Some evidence is available on the effects of PCPA on the sleep patterns of the chronically ill (Wyatt, 1970), but interpretation of clinical material of this sort is always difficult. PCPA was given to 11 patients, of whom 7 were carcinoid tumour patients and 2 had Huntington's chorea, in a double-blind comparison with placebo controls. The finding was that PCPA reduced REM sleep by 20 per cent. to 70 per cent., but did nothing to slow-wave sleep. One patient given 2000mg/day for $2\frac{1}{2}$ years took only 33-1/3rd per cent. of his normal amounts of REM sleep. 5HTP given to 4 of the patients tended to reverse these effects, and given to normal controls it increased REM sleep.

Wyatt's results are, of course, contrary to the findings on animals, where PCPA acts primarily on non-REM sleep. However, Wyatt did find that there was no rebound effect following REM sleep deprivation by PCPA in humans, and this is consistent with findings on animals by other workers. Increased REM sleep during the recovery period is a feature of conventional REM sleep deprivation techniques (Dement, Henry, Cohen and Ferguson, 1967; Jouvet, 1969), but is not seen after PCPA.

It seems clear, therefore, that 5HT plays a central role in the control of sleep and arousal, since depleting 5HT produces changes in the patterns of REM and non-REM sleep and may even produce total insomnia. Recently it has been suggested that 5HT inhibits catecholamine-

induced arousal in rats (Mabry and Campbell, 1973), since PCPA increased locomotor activity in rats given reserpine (Mabry and Campbell, 1973) or L-dopa (Chruscial and Herman, 1969).

4.5.2. 5HT and Pain

Tenen (1967) found that PCPA facilitated the acquisition of an avoidance conditioning response involving foot shock in rats, and argued that it did this by increasing the rat's sensitivity to pain. Subsequently Brody (1970) confirmed and extended Tenen's work, and showed that PCPA facilitates both active and passive avoidance learning. In further experiments, Tenen (1968) has shown that PCPA does increase a rat's sensitivity to pain as measured by a modified Evans "flinch-jump" test of pain sensitivity. Furthermore, PCPA antagonised the analgesic effects of morphine in rats and did this not by neutralising morphine directly but by reducing 5HT in the brain. This was clear because PCPA concentrations in the body are maximum 3 hours after an intraperitoneal injection of PCPA, at which stage 5HT levels have not yet been decreased. 5HT is at a minimum 2 days after the injection, but by then PCPA levels in the body are reduced (Koe and Weissman, 1966). Tenen found no antagonistic effect of PCPA on morphine after 3 hours but a large effect after 2 days. Similar results were found with other analgesics such as methadone and meperidine.

4.5.3. 5HT and Sensory Thresholds

Brody (1970) has extended these findings to other sensory modalities. He found that PCPA also increases locomotor activity in rats in response to flashing lights and loud noises, and that PCPA prolonged the periods of thirst in rats whose drinking had been inhibited by quinine. In all, he argued that 5HT-depleted rats were more reactive to external stimuli than were controls. The effects of PCPA are therefore rather

subtle, since spontaneous locomotor activity is not markedly elevated in rats given PCPA (Koe and Weissman, 1966; Tenen, 1967; Brody 1970). Rather it seems as if PCPA lowers the thresholds of reaction, at least to the particular auditory, visual, taste and pain stimuli used by Brody and other workers.

Further support for Brody's suggestion comes from the fact that PCPA seems to increase motor activity in an activity chamber if the animals are left in it for a long time (Fibiger and Campbell, 1971). Taken together with the unremarkable effects of PCPA on spontaneous motor activity, this has been interpreted (Weissman and Harbert, 1972), to mean that "the novelty of the activity box is sustained (by PCPA)". PCPA-treated rats seem to eat normally, but Brody's finding of increased sensitivity to quinine and dextrose, and Sheard's (1969) finding that PCPA affected a rat's consumption of alcohol solutions, could mean that taste or smell thresholds are decreased by 5HT depletion.

4.5.4. 5HT and electroconvulsive shock and self-stimulation of the brain

In addition, PCPA is reported to lower the threshold for convulsions following electroconvulsive shock in rats (Koe and Weissman, 1968), and to facilitate electrical self-stimulation of the brain (Gibson, McGeer and McGeer, 1970). It does this by lowering the threshold of electrical current required to reinforce self-stimulation (Gibson et al, 1970) or by pushing up the rates of self-stimulation after PCPA (Poschel and Ninteman, 1971).

4.5.5. 5HT and Aggression

In Sheard's (1969) work with PCPA, it was reported that mouse-killing in rats was stimulated by PCPA and that this was reversed by 5HTP. The work is supported by Di Chiara, Lamb and Spano (1971), who

found that olfactory bulb lesions and sectioning of the tracts led to mouse-killing behaviour and that PCPA increased this behaviour in the brain-lesioned animals. However, serious doubts are raised about the findings of Di Chiara et al by the fact that tests in which the mouse was killed by the rat were an hour long, whereas those in which no muricide took place were only 15 minutes long.

It is also likely that muricide is not a good measure of aggression, since it is a rather specialised and unusual behaviour for rats. Clearly predation cannot be equated with aggression, nor is it clear that rats with extensive brain lesions are the best models on which to test the effects of 5HT-depletion on aggression. The same argument applies to the suggestion by Di Chiara et al that PCPA enhances septal-lesion-induced aggressiveness in rats.

4.5.6. Hyperresponsiveness after 5HT Depletion

In the light of these findings, Brody (1970), Weissman and Harbert (1972) and others have argued forcibly that depleting 5HT in the brains of some animals (mainly the rat) make the animal hyperresponsive to incoming stimuli from the environment. The findings on pain and sensory thresholds reviewed briefly above are consistent with this hypothesis, and perhaps the taking of less sleep after PCPA is consistent with it as well.

However, it should be noted that such a generalised effect on responsiveness to environmental stimuli does not exclude the possibility that depleting 5HT could have some more specific effects on certain behaviours at the same time. The wide distribution of 5HT neurons and terminals in the brain (1.6.3.4. above) suggest that wide-ranging changes in behaviour would be seen after 5HT-depletion by PCPA. But equally,

5HT terminals are more densely packed in certain areas of the brain such as the hypothalamus than in others, so there is no a priori reason why specific effects on certain behaviours, such as sexual behaviour, should not be noted as well.

An example could be the 5HT-containing neurons of the anterior hypothalamus, known to be implicated in the control of body temperature. In a series of elegant experiments since 1968, Myers and co-workers have been able to perfuse the rostral hypothalamus of monkeys via implanted cannulae, and measure the activity of monoaminergic neurons in response to temperature changes (Myers and Sharpe, 1968; Myers and Yasksh, 1969; Myers, 1970; Myers and Beleslin, 1971). Cooling the air to -5°C round the body of an unanaesthetised rhesus monkey in a restraining chair leads to the release of 5HT from the anterior hypothalamus/preoptic area. Myers has interpreted this finding as indicating that 5HT could be the transmitter involved in the anterior hypothalamic neurons which subserve heat production when the body is subjected to extreme cold (Myers and Beleslin, 1971). The point at issue is that a general depletion of 5HT in the brain may or may not act on such a specific controlling system, in addition to (or as a part of) any general effects it may have on behaviour.

4.5.7. More Specific Effects of 5HT Depletion

Against the suggestion that depleting 5HT in the brain merely sensitises the animal in some general way, is the extensive literature on rats, rabbits, cats and hamsters, reviewed above (1.8.1. and 1.8.2.), which shows that PCPA causes multiple mountings in male rats and rabbits caged together (see Gessa, 1970). An explanation of why PCPA-induced depletion of 5HT causes male rats and rabbits to mount each other, rather than to fight each other more or run about more, would have to be given

before a pure sensitisation hypothesis could be sustained. Indeed, even in the case where male/female sexual behaviour is increased in rats paired together after PCPA, the argument loses some of its force when it is pointed out that ovarian hormones themselves do no more than "lower the threshold for sexual behaviour" in the female (or the male), and that under certain circumstances PCPA is able to mimic the effects of ovarian steroids in this respect.

The point to be made here is that some general sensitising effect of lowered 5HT in the brain, and a more specific effect on sexual behaviour, need not be mutually exclusive. For example, oestrogen increases behaviours such as locomotion and aggression in female rats, and alters a number of other parameters such as olfactory, tactile and auditory thresholds (see also Gradwell, Everitt and Herbert, 1974, in press). Yet the specific role of oestrogen in the control of sexual behaviour in rodents can hardly be doubted.

In fact, the effects of oestrogen on some behaviours such as general activity can be so marked under certain circumstances that its general activating effects are noted before any specific effects on sexual behaviour are seen. In a recent experiment, oestradiol benzoate (10 ug/day in oil) was shown to increase significantly the activity levels of a female black and red tamarin Saguinus nigricollis paired with a male, as measured by the number of gross body movements she made in a 30-minute observation period (Gradwell and Stones, 1974, in preparation). This was found even though no significant effects on specific behaviours such as copulation, grooming and invitations to groom could be detected. So that clearly it is possible for a steroid such as oestradiol to stimulate activity levels and to have a specific effect on sexual behaviour, and for the two to be divorced on occasions. It is therefore likely that the same thing could apply in the case of 5HT-depletion in the brain.

4.5.8. The Present Experiments

The results of the present experiments would support such an argument. There were no increases in aggression in females treated with PCPA (3.1.2. above). Only one case of aggression was recorded in all observations, and that was under 5HTP. However, it should be pointed out that female aggression in paired-observations of rhesus monkeys is rare in any case, and this probably reflects the fact that males are clearly dominant over females in rhesus troops.

Again, there were no consistent increases in grooming behaviour or proximity behaviours (3.1.2.6. above). No subjective impression of hyperactivity in these monkeys was gained, but no quantitative measures are available to support this impression. Generally, the fact that no great hyperactivity in the cage was observed in monkeys after PCPA accords well with the data on spontaneous locomation recorded by other observers (Tenen, 1967; Brody, 1970) for the rat.

4.6. Other PCPA Experiments on Monkeys

The work of Weitzman et al (1968) on PCPA and sleep in the rhesus has already been discussed. PCPA has also been given to monkeys by Redmond, Maas and co-workers at the University of Illinois, and by Boelkins at the University of California. It should be noted that the findings of these workers would appear to be in conflict with the effects of PCPA on sexual receptivity in female monkeys observed in these experiments. However, the three studies differ on so many points of experimental design that they are not strictly comparable, and this no doubt accounts for the inconsistent results obtained.

First, Maas, Redmond and Gauen (1973) studied small groups of Macaca speciosa for an hour a day, but the monkeys were housed together all the time. The chief measure used was that of "mean initiated social interactions per hour", which included aggressive episodes, attacks, grooming and auto-grooming, social huddling, social-sexual presentations and copulations. Four female monkeys were studied,but two of these became so ill that treatment had tc be discontinued. PCPA made no significant difference to the mean initiated social interactions per hour in the other two females.

A fuller description of the effects of PCPA on these monkeys is given by Redmond, Maas, Kling, Graham and Dekirmenjian (1971). The two that became ill were adipsic, aphagic and ataxic. They showed weight loss and hair loss and became so debilitated that they are described as being close to death, and had to be force-fed with fluids through a naso-gastric tube. A "gaunt, masklike face" is described in one animal.

As mentioned, the present experiments and those of Redmond et al are therefore not comparable. In particular, Redmond et al gave doses

of PCPA which are huge in relation to the 75 mg/kg every fourth day used in these experiments. They gave 150 mg/kg every day for two weeks and for five weeks, so that in 12 days a 10 kilogram monkey would have received 18 grams of PCPA as against the 2 grams used here. However, Redmond et al did give the PCPA by nasogastric intubation, so that the effective dose difference may not have been quite as great as 18 to 2.

Boelkins (1973) gave PCPA to three immature female crab-eating macaques Macaca fascicularis, and concluded that, if anything, the drug reduced "socially-orientated" behaviours. In particular, "the results suggest that PCPA treatment is not necessarily correlated with increased levels of agonistic or sexual behaviours" (p 361). However, the use of immature females in tests of sexual and other behaviours calls for some explanation, since at least one possible yardstick of maturity is in terms of sexual maturity. Furthermore, the three females are described as having had "extensive laboratory social experience", perhaps indicating that they had grown up together in that cage, although this point is not made clear in the report.

Clearly Boelkins used doses of PCPA which are on a different scale from those used here. He injected 500 mg/kg every day for 10 days, after 7 days of oral PCPA. A 10 kilogram monkey would thus have received 50 grams of injected PCPA plus large oral doses of PCPA as against the 2 grams used in these experiments.

The very high doses alone might be enough to account for the different results obtained, particularly if the effects of PCPA are different when it is given in large doses. Certainly the fact that Redmond's monkeys became so ill is suggestive in this respect, since no such symptoms were seen following the relatively small amounts given to animals in these experiments. Boelkins and Redmond et al studied small groups housed to-

gether continuously, rather than pairs put together for half hour interaction periods. They also used different species of macaque, while Boelkins' monkeys were immature. Neither Boelkins nor Redmond et al designed their scoring measures specifically to measure sexual behaviour, and so the emphasis of the three studies is different. Taken together, these factors probably preclude direct comparisons of the effects of PCPA on female sexual behaviour in the three studies.

A final point to be made is that all these workers gave PCPA to normal animals. In these experiments, by contrast, PCPA was given to monkeys made unreceptive by withdrawing hormones (androgens). It could be that "PCPA may be unable to stimulate behaviour to supra-normal levels in intact animals but be capable of restoring normal levels in hormone-deprived ones. A parallel finding has been reported to follow the administration of increasing doses of testosterone to castrated male guinea pigs (Young, 1961), in which this hormone stimulated mounting behaviour to a maximum determined by the animals' genetic constitution". (Gradwell et al, 1974).

4.7. The Effects of Gonadal Steroids on 5HT Turnover Rates

Oestradiol and testosterone propionate both lowered the levels of 5HIAA in the CSF of ovariectomised monkeys and progesterone tended to antagonise the effects of oestradiol in this respect, but none of these changes was significant (3.3.2. above). However, oestradiol and testosterone propionate both caused significant decreases in the turnover rates of 5HT in the brain, as measured by the two-hour probenecid test, while progesterone increased these turnover rates significantly when it was added to oestradiol (3.3.4. above). This means that the reduced turnover of 5HT under oestradiol and testosterone only showed up when the transport of 5HIAA out of the CSF was blocked by probenecid.

A possible explanation for these results could be that 5HIAA is cleared from the CSF through the arachnoid villi and out into the blood stream at more or less the same rate as it enters the CSF. If this is the case, then a reduced flow of 5HIAA through the CSF under oestradiol and testosterone would not be reflected in the levels of 5HIAA present in the pool of CSF at any one tapping. However, if the active transport of it out of the CSF is blocked (by probenecid), then the different rates of accumulation of 5HIAA under hormones and no hormones would be clearly seen.

CSF was taken from the cisterna magna of monkeys rather than by lumbar puncture. The latter technique exposes the animal to far less danger of permanent injury or death than does a cisternal puncture (2.4.1. above), but there is some doubt about whether lumbar CSF gives a reliable measure of brain 5HT activity. The spinal cord is itself rich in 5HT-containing neurons and much of the 5HIAA measured in lumbar CSF could therefore be of spinal origin (Bulat and Zivkovic, 1971). Bulat and Zivkovic have pointed out that brain 5HT is only a small proportion of total body 5HT, since the gut and spinal cord, inter alia, are rich in

this neural transmitter. They injected 5HIAA into the cisterna magna of cats and found that it did not appear in the lumbar CSF, but was (presumably) taken out of the arachnoid villi without flowing downwards. In contrast, 5HIAA of spinal cord origin was reflected in spinal CSF. Bulat and Zivkovic therefore query whether lumbar CSF from mental patients gives an accurate measure of 5HT activity in the brain.

On the other hand, Van Praag, Korf and fellow workers in Holland have always proceeded as if lumbar CSF does measure brain monoamine activity, and Ashcroft (personal communication), on the basis of his research, has concluded that it is safe to use lumbar punctures to monitor brain monoamines in humans. Certainly there is an ethical problem in tapping from the cisterna magna of human patients, thereby exposing the patient to great (and perhaps unjustified) risks (2.4.1. above). But experimentors working with animals have tended to use cisternal puncture to obtain CSF to monitor brain function because of the work of Bulat and Zivkovic and others, and this was done in these experiments.

4.7.1. The Probenecid Test

Probenecid blocks the efflux of both 5HIAA and HVA from the CNS into the blood (Guldberg, Ashcroft and Crawford, 1966). The resulting accumulation of these acids in the brain has been shown to give a measure of the turnover rates in the CNS of the respective monoamines 5HT and DA (Neff, Tozer and Brodie, 1967; Bowers, 1970). It has been used extensively in human mental patients, particularly sufferers of endogenous depression (Roos and Sjöström, 1969; Van Praag, Korf and Puite, 1970; Van Praag and Korf, 1971; Korf and Van Praag, 1971; Bowers, 1972; Van Praag, Flentge, Korf, Dols and Schut, 1973), and in Parkinson's patients (Olsson and Roos, 1968; Lakke, Korf, Van Praag and Schut, 1972; Lakke, Korf, Hoorntjie, Van Praag and Schut, 1973).

Some depressed patients show a subnormal rate of accumulation of 5HIAA in the CSF on the probenecid test (Van Praag and Korf, 1971). This has enabled Van Praag and co-workers to screen out patients likely to benefit from 5HTP treatment, on the assumption that the low 5HIAA accumulation is the result of deficient 5HT activity (Van Praag, Korf, Dols and Schut, 1972; Van Praag, Korf and Schut, 1973). Clearly the rationale for such treatment lies in the amine (and particularly 5HT) hypothesis of the causes of affective illness (1.7 above).

Lakke et al (1972, 1973) have stated that probenecid enables them to predice the chances of alleviating Parkinson's disease with L-dopa therapy in a particular patient. If the HVA accumulation in the CSF after probenecid is low, it would indicate that dopamine is not being cleared from the CNS at a pathologically high rate and the chances of recovery after L-dopa treatment are therefore good. A high HVA accumulation would be contra-indicative of L-dopa alleviation of symptoms. The work of Godwin-Austin, Kantamaneni and Curzon (1971) is an essential agreement with this point.

4.7.2. Problems of the Specificity of Action of Probenecid

However, it must be acknowledged that at this stage insufficient research has been directed at the possibility that probenecid influences central monoamine metabolism in other ways as well. Clearly it does block the transport of HVA and 5HIAA out of the brain and CSF, but only recently has attention been given to the suggestion that it also affects monoamine precursors and synthesis pathways (Korf, Van Praag and Sebens, 1972; Lewander and Sjödström, 1973).

Korf et al (1972) showed that in rats probenecid lowered the levels of tryptophan in the blood, but raised them in the brain. They suggested that probenecid might interfere with the binding of tryptophan to serum

albumins, and also showed that probenecid lowered blood tryptophan levels in man. More recently Lewander and Sjödström (1973) have found that probenecid raises the levels of free (non-protein bound) tryptophan in the blood of human senile psychosis patients. These findings, if substantiated, are important, since Fernstrom and Wurtman (1971) have shown that increased tryptophan levels in the blood and brain lead to increased 5HT concentrations in the brain of rats within about 60 minutes. Furthermore, Tagliamonte, Tagliamonte, Perez-Cruet, Stern and Gessa (1971) have suggested a close covariance between brain tryptophan levels and brain 5HT turnover rates. So that if it is confirmed that probenecid does increase brain tryptophan levels, as suggested by Korf et al (1972), then the accumulation of 5HIAA in the CSF after probenecid would be due in part to a faster turnover rate of 5HT in the brain.

However, these findings do not yet present a self-consistent and complete picture of the effects of probenecid on 5HT or DA metabolism. While Korf et al (1972) suggested that probenecid increases brain tryptophan concentrations in rats, Van Praag et al (1973) found that it did not raise the levels of tryptophan in the CSF of depressed human patients, although normal loading with oral tryptophan does. It is possible that tryptophan metabolism was not normal in these depressed patients. Furthermore, tryptophan loading increased HVA in the CSF of these patients, suggesting some amine displacement of dopamine from DA-containing neurons. In all, the suggestions of Van Praag and co-workers which they invoke to explain their findings on probenecid must remain speculative, until further work is done to clarify the exact effects of probenecid on monoamine metabolic pathways. In particular, their suggestion that exogenously administered tryptophan accumulating in the brain is metabolised differently from the way in which endogenous pools of brain tryptophan are synthesised, and that dopaminergic neurons would have to synthesise 5HT out

of trytophan, would seem to require further amplification.

The problems of the specificity of action of probenecid, while important, are peripheral to the present experiments. Clearly, the important finding here was that after oestradiol and testosterone, 5HT turnover rates on the probenecid test were lower than on the same test under no hormones or after progesterone had been added to oestradiol. It might be suggested that oestradiol and testosterone reacted with probenecid in ways different from the way in which progesterone reacted with probenecid, and in this way decreased 5HT activity in the brain. But such an explanation is very unlikely. It is known that steroid hormones on their own affect 5HT (for example, Greengrass and Tonge, 1971; see 1.7. and 1.8.2. above). This would indicate that probenecid is not necessary for these effects of steroid hormones on 5HT.

Very recently, Everitt et al (1974, in press) and Everitt (personal communication) have used the H22/54 depletion model to measure NA and 5HT turnover rates under gonadal hormones in rats. Their preliminary results suggest that oestrogen increases 5HT turnover rates and progesterone decreases them. Their final conclusions are awaited with interest, since they could be related to the present discussion on the problems of specificity in the probenecid test. On the other hand, it should be noted that oestrogen and (particularly) progesterone could possibly have very different effects in rodents from what they have in monkeys. For example, oestrogen stimulates oestrous behaviour in the female rat, whereas the main action of oestrogen in female monkeys seems to be on the male. Furthermore, progesterone facilitates oestrous behaviour in rats, but, if anything, it inhibits sexual receptivity in female monkeys and women (1.4.7. above). It is therefore quite possible that these findings reflect species differences, since there is no good reason to suppose that this marked difference in

hormonal control of sexual behaviour is not carried over into monoamine mechanisms of control as well" (4.4, page 205, above).

4.7.3. Discussion Summary of the Effects of Gonadal Steroids on 5HT Activity in the Brains of Female Monkeys

Testosterone propionate lowered the turnover rates of 5HT in the periventricular areas of the brain, as measured at the cisterna magna. These results are consistent with the suggestion that adrenal androgens act on the anterior hypothalamus/preoptic area of the brain, and possibly on other neural sites, to control the female monkey's sexual receptivity. Certainly the results of these experiments do not exclude this possibility, suggested by the work reviewed above (1.5.3.). The area in question is rich in 5HT (1.6.3.4. above). The suggestion is therefore that adrenal androgens are taken up by cells in this area to disinhibit sexual receptivity.

Oestradiol lowers the turnover rates of brain 5HT as well. This result is consistent with the role of oestrogens in a feed-back circuit on the hypothalamus to control the release of LHRF and FSHRF into the hypothalamo-hypophyseal portal vessel system. Gonadotrophin releasing factors are released from neurons the perikarya of which are in the basal hypothalamus, and this area is well innervated by 5HT-containing axon terminals (1.6.3.4. above).

The finding that progesterone increases 5HT turnover rates in oestradiol-treated monkeys is consistent with the SUGGESTION that progesterone can cause female monkeys to become unreceptive (1.4.5. and 1.4.7. above). Progesterone could increase the turnover rate of 5HT either by antagonising the effects of oestrogen or by some direct effect of its own on 5HT-containing neurons. It has already been suggested that luteal progesterone may antagonise the effects of adrenal androgens on sexual receptivity

in monkeys (1.4.7. above). If it is the case that adrenal androgens increase female receptivity by disinhibiting 5HT neural systems in the brain, then the increase of 5HT turnover rates by progesterone would clearly work in the opposite direction, and serve to inhibit sexual receptivity. This suggestion is consistent with the finding that it is those compounds containing high proportions of progesterone, rather than those containing high proportions of oestrogens, which lead to the highest incidence of loss of libido complaints in women taking oral contraceptive pills (Grant and Mears, 1967; Grant and Pryse-Davies, 1968).

Progesterone has not been studied in nearly the same detail as have oestrogens and androgens in female monkeys, and more work on the effects of progesterone on both sexual attractiveness and (particularly) sexual receptivity is required.

The exact way in which gonadal hormones are taken up by monoamine-containing neurons to influence their firing rates is perhaps still a moot point. In particular, it should be noted that the suggested sites of action of hormones are primarily those brain areas rich in axon terminals (see also Pfaff, 1973). Although Janowsky and Davis (1970) have shown effects of gonadal steroids on synaptosomes in vitro, the mechanism of this action is by no means clear (see 1.7.2. above). Davidson (1972) has suggested that gonadal steroids could act on a neuron in at least three ways:- (1) by modifying the membrane potential by their presence (2) by activating or inhibiting the firing of neurons in some direct way, perhaps by modifying synaptic transmission, and (3) by changing the firing thresholds of the cell in response to incoming stimuli. The determination of which of these possibilities is the most likely is beyond the scope of the present discussion, other than to point out that the result of such an hormonally-induced modification of the neuron is seen in the increased or decreased turnover rates of the neural transmitter used by the cell to communicate with other cells.

4.8. Grooming and Social Behaviour

Heterosexual grooming takes up a large proportion of the time available for social interactions in adult members of wild rhesus monkey and baboon troops. Thus Kummer (1968 a) noted that in the hamadrayas, "of all social activity, mutual grooming takes up the largest amount of an adult baboon's time". (p 44). There is a diurnal rhythm of grooming in the baboon, with peaks in the early morning and late afternoon when the animals are not feeding (Hall, 1962; Saayman, 1971).

Mutual grooming is very definitely part of the sexual behaviour of rhesus monkeys and baboons. But it also "serves to define an individual's position within the highly organised primate society. Thus grooming behaviour occupies a position intermediate between a specifically sexual and a more generally social type of activity" (Michael and Herbert, 1963, p 500). This point has been emphasised by Sade (1965), who argued that the behavioural events occurring on either side of a grooming episode must be carefully analysed in order to detect the exact significance of any grooming episode. The grooming could, for example, be part of a sexual behaviour sequence, or to placate aggression, or it might occur in the context of general social interaction. Only a very careful analysis of the events leading up to the grooming sequence, as well as those following it, will serve to determine its exact function.

Heterosexual grooming is prominent during consort pair associations between adult male and female baboons (Hall and De Vore, 1965; Kummer, 1968 a; Saayman, 1971) and rhesus monkeys (Carpenter, 1942; Southwick et al, 1965; Lindburg, 1971). The males of both these species are multiple mounters, and courtship and copulation is spread over a period of time. One of the primary purposes of grooming behaviour in the consort pair association could be to ensure "close physical contact between the partners

during the intervals between mounts, thus influencing the successful outcome of mounting invitations and consequently facilitating the attainment of the ejaculatory threshold" (Saayman, 1971, p 162).

It is certainly the case that one partner may groom the other in the intervals between mounts (Michael and Herbert, 1963). Furthermore, both mounting rates and thrusting rates in a copulatory series must exceed a certain critical rate for ejaculation to take place (Michael and Saayman, 1967 a). Sometimes a male will not ejaculate with an unattractive female if the mounting rate is too low. However, mutual grooming is almost always seen during the sexual refractory period following ejaculation as well, so that clearly grooming does more than ensure physical proximity for the attainment of the ejaculatory threshold.

4.8.1. Grooming Behaviour and the Menstrual Cycle

Mutual grooming has been shown to vary with the menstrual cycle in rhesus monkeys. Michael and Herbert (1963) reported that males groomed females progressively more during the follicular phase of the menstrual cycle, reaching a peak at mid-cycle. Male grooming then declined during the luteal phase and was minimal at the time of menstruation. Female grooming on the other hand was minimal at mid-cycle and reached a peak during the late luteal and early follicular phases.

These reports were confirmed in a later paper by Michael, Herbert and Welegalla (1966), who also reported that bilateral ovariectomy abolished the rhythms of grooming activity observed in the intact animal. Ovariectomy reduced the grooming of the males to the low levels typical of the luteal phase of the cycle, while oestradiol restored it to levels typical of mid-cycle. Progesterone antagonised the effects of oestradiol in this respect.

However, Rowell (1968) has pointed out that the results of Michael

et al are misleading as far as female patterns of heterosexual grooming are concerned, since they would indicate that the hormonal conditions in the female's body at mid-cycle are antagonistic to female grooming, and that she actually grooms more during the luteal phases of her cycle. This is probably not so. Michael's results are based on observations of pairs of monkeys placed together for one hour a day in a bare cage. Rowell correctly argues that since rhesus monkeys do not groom each other at the same time, the increased male grooming observed at mid-cycle would prohibit the female from grooming the male at that time. The more of the limited hour of social interaction taken up by male grooming, the less time is available for female grooming. The restricted social environment of paired tests, together with male/female dominance patterns in rhesus society, are therefore sufficient to explain the reciprocal male/female grooming interactions reported by Michael and co-workers.

Rowell's own findings on groups of baboons housed together continuously in a fairly large enclosure in Uganda show that while male grooming did increase in the late follicular phases of the female cycle, and peak at mid-cycle, so did the female's grooming of the male. Her findings have been convincingly verified by Saayman (1971), who showed that grooming interactions which involved cycling female chacma baboons in the wild were significantly influenced by the menstrual cycle. Inflating females groomed subadult males proportionately more than did other females, and fully swollen females groomed adult males more than did other females. Furthermore, cycling females groomed adult males more when they themselves were fully swollen than at any other stage of the cycle. This usually took place in the consort pairs they formed with adult males at this time.

The most active groomers of all in the baboon troops observed by Saayman were the flat females in the late luteal phase of the cycle. But most of their grooming involved either other females or juveniles. This

pattern of female grooming therefore appeared to vary with the cycle as follows:- as the female entered the follicular phase and her sex skin started to turgesce, she would groom, and be groomed by, the juvenile and subadult males. At mid-cycle she would groom adult males, usually while part of a consort pair. After the break-up of the consort pair, she would return to her usual female grooming partners.

These findings are consistent with other reports on grooming in wild troops of baboons (Hall and De Vore, 1965) and rhesus monkeys (Carpenter, 1942; Lindburg, 1971). Clearly then, under more natural conditions both male and female grooming reach a peak at mid-cycle. In addition, male/female grooming in this context is closely related to sexual behaviour and the cyclical variations in grooming behaviour parallel the variations in sexual behaviour. It was therefore of interest in these experiments to discover whether grooming is a product of the female's increased sexual attractiveness at mid-cycle or of her increased sexual receptivity at that time. The experiments were designed so that female attractiveness was held constant throughout all the treatments given to alter her receptivity, and therefore it is possible to make a statement on this point.

4.8.2. Grooming and Attractiveness or Receptivity

However, it is clear from the results of these experiments (3.2.1.6. above) that there were no marked parallel changes in female grooming when she became sexually unreceptive. Male grooming did not vary with the female's sexual receptivity in any consistent way (3.1.2.1. above), nor did male grooming invitations (3.1.2.3. above). There was a hint that some females invited grooming more (3.1.2.4. above), and groomed less (3.1.2.2. above), when they were unreceptive (i.e. after adrenalectomy or 5HTP), and were less inclined to sit with the male (3.1.3.5. above). But none of these trends was significant, and therefore these experiments have not been able to demonstrate a clear link between grooming behaviour

and female sexual receptivity.

The most likely explanation for the cycles of grooming observed in rhesus and baboon troops in the wild therefore remains one in terms of female sexual attractiveness. At mid-cycle the female will be maximally attractive in terms of pheromone production from a fully cornified vagina (1.4.5. above). This is probably one of the factors influencing the adult male to form a consort pair at this stage. It is, of course, likely that the female will also be fully receptive at this point (1.4.6. above). The male will spend more time in close proximity to the attractive female, at least in relation to other non-cycling females in the troop. This will provide the opportunity for increased female grooming of adult males, which would not otherwise be available, because male/female partners usually alternate in the role of groomer and groomee during a protracted bout of grooming.

It is possible that grooming could facilitate the attainment of the ejaculatory threshold in this way; at least it will do nothing to inhibit ejaculation. Under certain conditions, grooming is not only related to sexual behaviour, but may even be sexually arousing. In the hamadryas baboon, grooming is often focussed on the perineal area (Kummer, 1968 a) and it has been suggested that the "picking reaction", whereby a finger is used to probe an area of skin, has sexual significance (see also Sparks, 1967; Anthoney, 1968). Certainly male rhesus monkeys show penile erections while grooming, and will then mount shortly after the erection. But the same thing happens in the absence of inter-mount grooming, and so clearly grooming is not necessary for sexual arousal.

Any explanation of grooming behaviour in terms of sexual behaviour says nothing about other commonly observed forms of grooming, such as female/female grooming. It is noticeable that highly aggressive mon-

keys in which there is a clear linear dominance hierarchy, such as the rhesus and baboon, engage in a great deal of grooming. Monkeys in which a more relaxed atmosphere prevails, and which have no clear dominance hierarchy, such as the howler, are noted for an absence of grooming. (Carpenter, 1934; 1965). Indeed, Altmann (1959) reports extensive bot infestations on the skins of howlers on Barro Colorado, indicating that these monkeys do not groom even in the interests of skin hygiene.

However, there are so many exceptions to any generalisation in terms of grooming and aggression or dominance, that any such statement is of limited value. The langur is not an aggressive animal and has no clearly defined dominance hierarchy, yet they allogroom for up to 4 or 5 hours a day (Jay, 1965). The bonnet macaque grooms a great deal, but such grooming does not appear to be related to dominance status at all (Simonds, 1965). The gorilla is neither aggressive nor a great groomer, and in this respect resembles the howler monkey (Schaller, 1965).

Analyses of the significance of various grooming behaviours in terms of energy models reminiscent of Lorenz's (1935) "hydraulic model" or Tinbergen's (1951) "hierarchical model", and the early formulations of the European Ethologists, are probably not explanatory at all. For example, Terry (1970) has suggested that grooming serves to reduce "tension" (sic) in groups of primates. But as Page (personal communication) has pointed out, the introduction of such an hypothetical energy construct does no more than state what has already been observed, namely, that some monkeys are groomed more when they are injured, after giving birth, during a copulatory sequence or when threatened by a more dominant animal. The hypothetical construct would appear to add nothing to the explanation.

In a similar way, these experiments do not allow any statement to

be made on the suggestions of Anthoney (1968) on the ontogeny of various baboon grooming and greeting behaviours. Rhesus monkeys do pick through the fur of the groomee and appear to carry small particles or objects to their lips and mouth with the hands. They do sometimes lower the mouth briefly to touch the fur of the groomee on the site being groomed, and they do lipsmack while grooming their partners. But nothing in these observations would indicate that such behaviour develops from nipple sucking, or is in any way related to lipsmacking at the erect penis (p 361). Neither these experiments nor those of Anthoney carried out deprivation of nipple sucking in infant monkeys, which would seem to be necessary in order to test whether lipsmacking and grooming behaviour are in fact adult forms of nipple sucking.

4.9. The Experiments on the Males

The results of the experiments on the females are consistent with the view that adrenal androgens act via 5HT-containing neural systems in the brain to control female sexual receptivity in monkeys. In the male, androgens are secreted in large quantities from the Leydig cells of the testes, and are active in both an organising action (1.5. above) and an activating action in male sexual behaviour. It is therefore possible that androgens act on the male brain by reducing the activity of 5HT-containing neurons as well.

4.9.1. The Effects of Testosterone Replacement in Castrate Males

However, there were no significant effects on 5HIAA levels or 5HT turnover rates when 10 mg of testosterone propionate were given each day for ten days to male rhesus monkeys bilaterally castrated at least six months previously (3.4.1. above). These experiments have therefore been unable to demonstrate any role for 5HT-containing neural systems in the androgenic control of sexual behaviour in male monkeys, at least in the doses used here.

It remains possible that under larger dose regimens and other experimental conditions such effects might be apparent. However, it should be noted that "in all mammalian species studied, some components of male sexual behaviour persist after castration. Since these behaviours are displayed after all residual androgens of testicular origin have been metabolised, they cannot be attributed to the concurrent action of the gonadal hormones" (Phoenix, Slob and Goy, 1973, p 472). Castration in adult men has variable effects on sexual capacity and motivation, ranging from no loss of sexual capacity to an almost total loss (Money, 1961).

Only very recently has a systematic study of the effects of castra-

tion and replacement therapy on the sexual behaviour of adult male subhuman primates been carried out (Phoenix, Slob and Goy, 1973; Resko and Phoenix, 1972). Phoenix and co-workers have shown that sexual behaviour does continue for some time after postpubertal castration in male rhesus monkeys. "Fifty percent of the males achieved intromission 1 year after castration" (Phoenix et al, 1973, p 472). However, certain aspects of sexual behaviour such as the motivation for sexual contact, did appear to be less than in the precastration condition, since testosterone replacement was able to restore "whatever aspects of behaviour were affected".

Resko and Phoenix (1972) suggest that the continuing sexual behaviour of male rhesus monkeys might be related to the relatively small amounts of androgens (probably adrenal) which they were able to detect in the systemic plasma of the Oregon monkeys as late as 55 weeks after castration. However, there was no correlation between the amounts of circulating testosterone, from whatever source, and the frequency of sexual behaviours, although Resko and Phoenix point out correctly that this does not exclude the possibility that the adrenal androgens are important in maintaining male sex behaviours. Clearly different animals could have differential responses to the same low level of circulating steroid, and many other factors (particularly dominance) are almost certainly related to whether or not sexual behaviour continues after castration. For example, Wilson and Vessey (1968) found that castration reduced sexual behaviour in free ranging male rhesus monkeys, but that one male copulated (with ejaculation) 7 years later. However, such free-ranging studies are clearly not able to control for dominance factors, and a male might not even attempt copulation if he is very low on the dominance hierarchy. Dominance and aggression are at least partially related to testosterone levels in the blood, at least in rhesus monkeys (Rose, Holaday and Bernstein, 1971; Rose, Gordon and Bernstein, 1972).

The point to be made here is that the exact role of testicular testosterone in the control of sexual behaviour in male rhesus monkeys and men is not established. Apart from considerations of dominance already mentioned it is clear that male rhesus monkeys have sexual preferences (Everitt and Herbert, 1969), and their ejaculation frequency is very much a function of their particular female partners (Phoenix, 1973). No clear role for 5HT in the effects of testicular androgens on the brains of rhesus monkeys could be shown within the limited scope of this experiment. But the extent to which testicular androgens themselves control the adult male primate brain requires further research.

4.9.2. The Effects of Ejaculation

The ejaculatory mount of a series of mounts by a male rhesus monkey is followed by a period of sexual quiescence called the refractory period (see also Carpenter, 1942; Altmann, 1962). The pattern of a series of mounts culminating in an ejaculatory mount and a refractory period is also seen in the chacma baboon in South Africa, although it is possible that not all baboons show this pattern (Hall and De Vore, 1965).

The length of the refractory period has been timed by Saayman (1970) in free-ranging chacma baboons in the Northern Transvaal. The period between the ejaculatory mount of one mounting series and the first mount of the next series was timed at a mean of 56.0 minutes, with a standard deviation of 27.9 minutes. Often the refractory period is spent in reciprocal male/female grooming.

The experiments above, together with other work, have shown that 5HT-containing neurons could be involved in the inhibitory control of sexual behaviour patterns and sexual motivation in female mammals (see 1.8 above). It is therefore possible that 5HT-containing neural systems

are similarly involved in the inhibitory control of male sexual behaviour. In particular, the hypothesis would be that 5HT-containing neural systems are highly active during the refractory period, or shortly after ejaculation, causing the remarkable inhibition of sexual behaviour seen at that time.

Yet it is clear that no consistent effects of very recent ejaculation on either 5HIAA levels in the CSF or on 5HT turnover rates in the periventricular areas of the brain could be detected under these experimental conditions (3.4.2. above). CSF was withdrawn within five or ten minutes of the male ejaculating with the teaser female. But neither the levels of 5HIAA in the CSF nor the increases of 5HIAA over 2 hours were significantly different from the base line conditions.

It is possible that CSF was withdrawn from the cisterna magna too soon after ejaculation for any possible effects on 5HIAA to be detected. However, a special effort was made to withdraw the CSF as soon after ejaculation as possible, since in the paired situation a rhesus male will sometimes start a second mounting sequence within about ten minutes of the previous ejaculation. Indeed, some males in the Birmingham colony, such as 2092, regularly ejaculate twice in half an hour with a receptive and attractive female, and one male has been known to ejaculate three times (and once he ejaculated four times) in half an hour (Herbert, personal communication). Clearly the amount of metabolite present in the CSF does not give an exact temporal monitor of 5HT-neuron activity, but an effect on either the immediate levels of 5HIAA or on the rate of accumulation of 5HIAA over 2 hours might perhaps have been expected.

The conclusion must therefore be that these experiments could not demonstrate a role for 5HT in the refractory period following ejaculation in male rhesus monkeys.

4.10. Correlation

PCPA reverses or tends to reverse the negative effects of adrenalectomy on sexual receptivity in female monkeys. Although no specific monitoring of CA-containing neurons was carried out, it is probable that PCPA did this by lowering the "serotoninergic tone" (Meyerson, 1972) of the brain, rather than by acting on any CA-containing neurons. Certainly PCPA lowered 5HT in the brain as measured by the levels of 5HIAA in the CSF. 5HTP was able to reverse both these effects of PCPA, in that after PCPA + 5HTP the sexual receptivity of the females was not significantly different from the adrenalectomised (pre-PCPA) condition, and 5HIAA levels in the CSF were restored to normal and above-normal levels.

5HTP on its own increased the levels of 5HIAA in the CSF and inhibited sexual receptivity, supporting the idea of an inhibitory role for 5HT in the sexual receptivity of female monkeys.

Adrenal androgens are THOUGHT to control sexual receptivity in female monkeys. Small doses of a potent androgen, testosterone propionate, did lower the turnover rates of 5HT in the CNS, as measured by the 2-hour probenecid test. However, the same was true of oestradiol, so that these experiments were not able to distinguish between oestrogen-controlled neural mechanisms of sexual receptivity (Michael and Saayman, 1968; Michael, 1968, and elsewhere) and androgen-controlled mechanisms (Everitt and Herbert, 1969 a, b; 1972).

The findings on oestrogen are, however, consistent with its role in other diencephalic mechanisms, such as in the control of ovulation and in the neurons of the hypothalamo-hypophyseal portal vessel system. The rich 5HT-innervation of the hypothalamus and the dense packing of 5HT-terminals in the stalk/median eminence region are suggestive in this respect.

It remains possible that in lowering the activity of 5HT-containing neurons in the brain, PCPA simply increased the general sensitivity of the animal to all incoming stimuli. Certainly 5HT is involved in sleep and arousal (Jouvet, 1969) and it is possible, indeed even likely, that depleting 5HT from the brain lowers the thresholds of responsiveness to incoming sensory stimuli such as sounds, tastes, smells and pains. So that the effects of PCPA on sexual receptivity could be due to such lowered thresholds of response to environmental cues (which in these experiments were predominantly sexual).

However, such general sensitising effects do not exclude the possibility that lowered 5HT disinhibits sexual receptivity as well as other behaviours. A clear parallel for such a dual action exists in the case of the ovarian steroid oestradiol. The specific role of oestradiol in the sexual behaviour of the female rat is not in doubt, but this hormone also affects such parameters as aggressive behaviour, running activity and a number of sensory thresholds.

To take the argument a step further, it could hardly be claimed that adrenal androgens are not important in controlling sexual receptivity in female monkeys because they have other effects as well. Yet these experiments have shown that testosterone propionate lowers 5HT turn-over rates in the brains of female monkeys. The same argument could therefore be used against androgens having any specific effect on sexual receptivity, since by lowering 5HT they would (on the argument) be simply making the animal more responsive to environmental cues, especially sexual ones.

Clearly nothing in these results excludes the possibility that adrenal androgens control sexual receptivity in female monkeys by lowering the activity of 5HT-containing neurons in the anterior hypothalamus/preoptic area, and possibly in other brain areas as well.

The finding that progesterone increases brain 5HT turn-over rates lowered by oestradiol suggests either that progesterone acts against some of the effects of oestradiol in the control of ovulation or that it is involved in the control of female sexual receptivity. The finding is consistent with either role. It is, of course, likely that progesterone is involved in at least these two control systems in the female higher primate body.

The experimental design used here made it possible to test whether male and female grooming and social behaviours vary in parallel with female sexual receptivity, since the hormonal treatments subserving female sexual attractiveness were held constant throughout all the treatments given specifically to alter the female's sexual receptivity. However, it was clear that grooming and social behaviours did not vary with female receptivity in any marked or consistent way, and it therefore remains likely that the prime reason for the mid-cycle peaks of female/male grooming observed in the wild is to be found in the mechanisms of female attractiveness.

No clear role for 5HT-containing neural systems in the action of testosterone on the brains of male rhesus monkeys could be shown. This is perhaps not surprising in view of the rather variable effects of castration and testosterone replacement on primate males. Clearly circulating testicular androgens are not the sole determinants of sexual behaviour in male rhesus monkeys and men. Perhaps more surprising is the failure to demonstrate any role for 5HT-containing neurons in the post-ejaculatory refractory period of male rhesus monkeys. Other work, including these experiments, suggests that 5HT is involved in inhibitory systems in the control of sexual behaviour in female mammals, and it might therefore have been expected that a post-ejaculatory period refractory for sexual behaviour in the male would be under serotoninergic control.

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