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Influence of age and of testosterone on the response of male rats to parachlorophenylalanine

VALERIE J. BOND, ELIZABETH E. SHILLITO AND MARTHE VOGT

A.R.C. Institute of Animal Physiology, Babraham, Cambridge

Summary

1. Castrated male rats and male rats that had been castrated as well as adrenalectomized, showed hypersexual behaviour 24 h after treatment with parachlorophenylalanine (PCPA), as did intact rats.

2. A dose of PCPA 100 mg/kg was sufficient to induce mounting behaviour; this dose lowered the cerebral 5-hydroxytryptamine (5-HT) to about 50% in 24 h and further to 40% in 72 hours.

3. Groups of juvenile male rats treated chronically with PCPA 100 mg/kg or 50 mg/kg, or with testosterone propionate 1·25 mg, showed hair loss after three weeks of treatment (6 injections), because of increased social interaction.

4. Groups of intact male rats 9–11 weeks old given testosterone propionate 1·25 mg subcutaneously, showed mounting behaviour 3–5 h after the injection which was indistinguishable from the behaviour seen 24 h after treatment with PCPA 100 mg/kg. The 5-HT content of the brain was not altered by testosterone.

5. The number of rats which showed mounting after PCPA treatment did not change with age, but the younger rats made more mounts in the observation time than rats more than three months old.

6. The age of castration (3 weeks or 4 months) did not influence the results.

Introduction

An increase in sexual behaviour has been observed in male rats treated with parachlorophenylalanine (PCPA) (Sheard, 1969; Shillito, 1969, 1970; Tagliamonte, Tagliamonte, Gessa & Brodie, 1969) and in both male and female cats (Ferguson, Henriksen, Cohen, Mitchell, Barchas & Dement, 1970; Hoyland, Shillito & Vogt, 1970). This increase is thought to be related to the depletion of cerebral 5-hydroxytryptamine (5-HT) produced by treatment with PCPA (Koe & Weissman, 1966), particularly as treatment with 5-hydroxytryptophan (5-HTP; a precursor of 5-hydroxytryptamine) stops the increased sexual behaviour as long as the amine concentrations are raised.

As PCPA produces changes in behaviour in young rats as well as in older, mature animals, it was decided to investigate the effect of this compound on castrated rats to determine if testosterone was involved in the increased sexual activity. While these experiments were continuing, Gessa, Tagliamonte, Tagliamonte & Brodie (1970) reported that in castrated male rats weighing 300–320 g, no sexual activity occurred after treatment with PCPA. They also found that the combination of testosterone with PCPA produced an even greater increase in sexual

behaviour in both castrated and intact male rats than PCPA given to intact rats. Gessa *et al.* (1970) always used older animals and observed a much lower incidence of sexual activity than reported by Shillito (1969). It seemed possible that age might be a factor in this behavioural change and some results with different age groups are presented. As some testosterone is produced from the adrenals, observations have also been made on rats which were adrenalectomized and castrated before treatment with PCPA. Malmnäs & Meyerson (1971) have also reported some work with PCPA in adrenalectomized and castrated rats.

Methods

1. Castrated and castrated-adrenalectomized rats

The experiments were carried out on male albino Wistar rats supplied by A. Tuck & Son (Laboratory Animal Breeding Station, Rayleigh, Essex) as three week old weanlings. The animals were kept in groups of eight or ten rats to a cage under reversed daylight conditions. In this lighting schedule, a red light was on from 10.00–22.00 h and a white light was on from 22.00–10.00 h. The rats had food and water *ad libitum*. Three groups of rats were castrated at three weeks of age (groups 1, 3 and 4) and three groups were adrenalectomized and castrated at three weeks of age (groups 5, 6 and 7). One group was castrated at 5 weeks (group 2) and one group at 4 months (group 8). With the exception of group 8 they were all left for two weeks to recover from the operation and for any circulating testosterone to be eliminated. Observations were always made on groups of eight animals except for group 8 in which there were seven animals. The adrenalectomized rats were given drinking water containing betamethasone 0·3 mg/l. (groups 5 and 6) or 0·15 mg/l. (group 7). Some unoperated rats were kept as controls to the groups which had been castrated, or castrated and adrenalectomized.

During the experiments, each group of rats was put into an observation cage which measured 91·5 cm × 122 cm, under the conditions described by Shillito (1970). The rats were left for three days and then observed on two successive days without treatment. Observations were made for one hour following the change from white to red light at 10·00 h, when the rats were usually very active. After observation on the second day, all the animals were injected intraperitoneally with

TABLE 1. *Treatment, age and number of rats in each group which showed mounting behaviour after treatment with parachlorophenlyalanine*

Treatment group	Group No.	Age at surgery (weeks)	Age at which observed (weeks)	Dose of PCPA (mg/kg)	No. of rats mounting in the group
Castrated only	1	3	5, 6, 10, 11, 14, 15	316	5/8
	2	5	8, 9	316	7/8
	3	3	5, 6, 14, 15	316	6/8
	4	3	12, 13, 27	316	7/8
	8	16	17, 18	100	5/7
Castrated and adrenalectomized	5	3	5, 6, 10, 11	100	5/8
	6	3	5, 6, 14, 15	100	5/8
	7	3	12, 13, 15	100	6/8
Intact	9	No surgery	6, 7	100	6/8
	10		7, 8	100	5/8
	11		16, 17	100	6/7

N.B. The number and identity of rats mounting in the groups did not change with repeated observation.

PCPA (Pfizer Ltd.) at a dose of either 316 mg/kg or 100 mg/kg. The drug was given as a suspension in 1% Tween 80. If the rats weighed under 150 g the concentration was 31·6 mg/ml or 10 mg/ml. When the rats weighed more than 150 g the concentration was 63·2 mg/ml or 20 mg/ml. On some days 5-HTP (Roche Products Ltd.) was injected intraperitoneally 60 min before observation started. 5-HTP was given as a solution in saline at a concentration of 0·5 mg/ml. Observations were made at 10·00 h on the following three days. Two forms of social interaction were recorded; the incidence of mounting and the number of times one rat lay on top of another. The rats were marked individually and the identities of the two animals involved in any interaction were recorded where possible.

The details of the ages at which the rats were observed and the number of experiments are shown in Table 1.

2. *Concentrations of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

The effect of PCPA 100 mg/kg on the concentrations of 5-HT and 5-hydroxyindol-3-ylacetic acid (5-HIAA) in the brain of the rats was determined fluorimetrically (Ahtee, Sharman & Vogt, 1970) at various times after drug treatment. The forebrain of the rats was used after removal of the pineal, and the cerebellum was used for recoveries and for the blank.

In some experiments, the rats treated with PCPA were given injections of 5-HTP 5 mg/kg intraperitoneally and their behaviour was observed 60 min later. On other occasions rats were treated with PCPA: those showing mounting behaviour were then given 5-HTP 5 mg/kg which stopped the mounting. When the abnormal sexual behaviour recommenced, the rats were killed and the 5-HT in the brain was assayed and compared with that of rats treated only with the PCPA. Estimates of brain 5-HT were also made 2 h after treatment with 5-HTP.

3. *Hair loss in rats*

An experiment to investigate whether low doses of PCPA given chronically would produce hair loss as a result of increased social interaction in grouped juvenile rats as reported by Shillito (1970) for higher doses was carried out with three groups of rats. At three weeks of age, the rats were divided into three groups of ten rats to a cage and kept under reversed daylight conditions. The rats from one cage were injected intraperitoneally with PCPA 100 mg/kg, and a second group was given a dose of 50 mg/kg. The third group received subcutaneous injections of testosterone propionate 1·25 mg in arachis oil at a concentration of 1·25 mg/ml. All the rats were treated twice a week for three weeks, so that they received six injections. They were killed four days after the last injection, and in two rats from each group the brain was removed for estimation of 5-HT and 5-HIAA concentrations in the forebrain. The testes were removed from all the animals and weighed in pairs. The bodies of the animals were examined for hair loss.

4. *Comparison of PCPA treatment with testosterone treatment*

Two groups of intact male rats aged 9–11 weeks were kept under the same conditions as the castrated rats and were given subcutaneous injections of testosterone

propionate 1·25 mg in arachis oil at a concentration of 1·25 mg/ml. Observations were made for 3–5 h after injection and again 24 h later, and the number of social interactions was recorded. Three weeks later the same rats were treated with PCPA 316 mg/kg and were observed for the next three days to compare the degree of hypersexuality induced by this treatment with that following testosterone administration. Two other groups were treated first with PCPA 316 mg/kg and three weeks later with testosterone 1·25 mg.

Results

1. Castrated and castrated-adrenalectomized rats

The castrated rats behaved differently from the intact rats in that they were less active and the start of their activity did not always coincide with the light change.

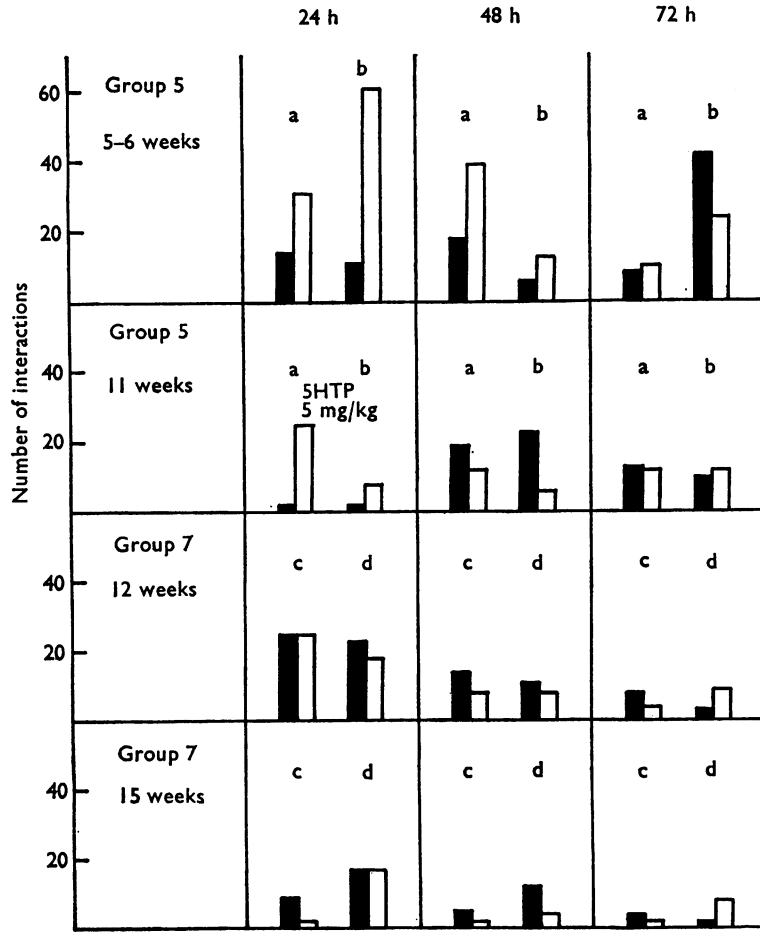

FIG. 1. Number of interactions during 1 h, in which rats mounted (shaded columns) or lay on top of one another (open columns). Experiments with groups of eight rats treated with parachlorophenylalanine 100 mg/kg i.p. and observed for a one hour period at 24 h, 48 h and 72 h later. Those animals treated with 5-hydroxytryptophan (5-HTP) were treated i.p. 60 min before observations started. The letters above the columns refer to the same group of rats thus (a) group 5 adrenalectomized and castrated at 3 weeks of age; (b) intact controls of the same age as group 5; (c) group 7 adrenalectomized and castrated at 3 weeks of age; (d) intact controls of the same age as group 7. The ages of the rats at the time of drug treatment are shown in column 1 of the figure.

When the rats were active they showed no mounting and very little other social interaction. Twenty-four hours after treatment with PCPA both at 316 mg/kg and at 100 mg/kg, the castrated rats became active as soon as the lights changed and they showed increased interaction, chasing and lying on top of one another and also mounting each other. In some young rats, the mounting pattern was incomplete during the first days but it seemed to mature with time. Some rats seemed to resist being mounted and rolled over onto their backs so that, when such rats were present, the incidence of lying over each other was increased. The rats which had been adrenalectomized and castrated behaved in a similar way to those that had been castrated only, but occasionally they appeared to be more active.

The increase in mounting and lying-over behaviour seen in castrated rats treated with PCPA was higher in the young rats than in rats over 3 months old; in rats 5 and 6 weeks old mounting stopped on the third day after the injection, with the exception of rats that had been both adrenalectomized and castrated in which it persisted. In most cases the interactions were more frequent in the observation period 24 h after drug treatment than in the subsequent observation periods; in these they decreased over the next two days.

When old, large rats were treated with PCPA 316 mg/kg, they appeared to be ill and became less active than usual. It was possible that the large volume of

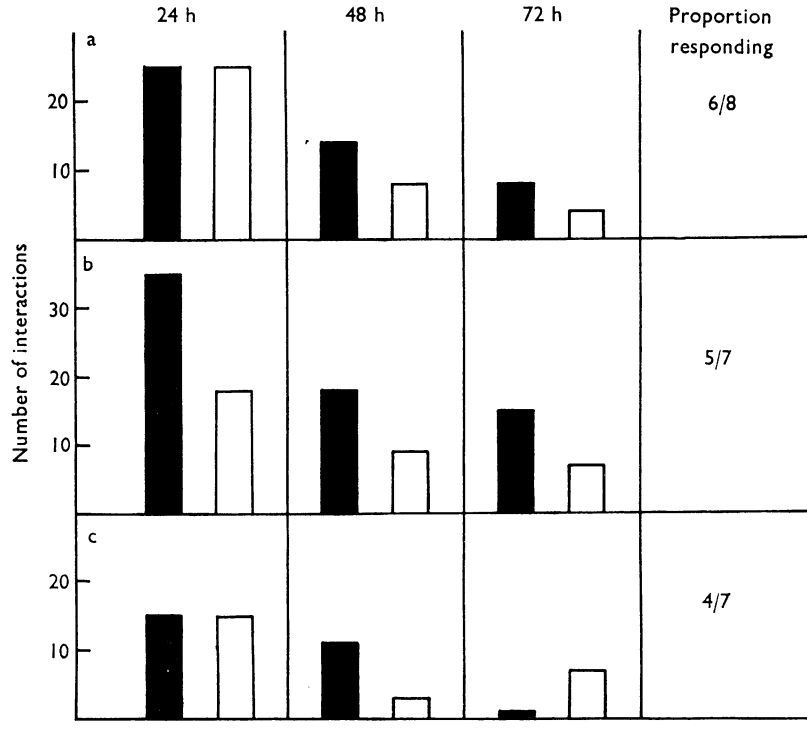

FIG. 2. Number of interactions during 1 h, in which rats of 3 months or over mounted (shaded columns) or lay on top of one another (open columns) after treatment with para-chlorophenylalanine 100 mg/kg i.p.; the rats were observed for one hour at 24 h, 48 h and 72 h later. (a) Castrated and adrenalectomized at 3 weeks and not observed until 3 months of age; (b) castrated at 4 months and observed 5 days later; (c) intact rats 4 months old. The ratio in the last column gives the proportion of animals in the group showing hypersexual behaviour.

drug was the cause of the illness and so three daily injections of PCPA 100 mg/kg were given instead. Observations were still made every day and it was found that a single injection of 100 mg/kg was able to induce an increase in sexual behaviour in the castrated rats. This was also found to be true of intact rats, and this single dose was used for all further work with the castrated and adrenalectomized rats.

Examples of the number of interactions after PCPA administration are shown in Figure 1. The number of mounts observed during the first hour after light change was very variable in different groups regardless of whether the rats were intact or castrated. Some groups were so hypersexual that the rats would mount each other continually and in lines so that one rat mounted a rat which was mounting yet another animal. In other groups the incidence of mounting would be low, although the number of animals showing this behaviour was not necessarily low. The older the animals the sooner the sexual activity declined during the observation time. Younger animals would continue to mount throughout the hour whereas some of the older groups were only active in the first half hour. The age at which the rats were castrated did not influence the number of rats in the group which showed sexual behaviour after treatment with PCPA. Table 1 summarizes these results, giving the number of animals in each group which showed mounting at the first experiment. In those groups which were watched more than once, the identity of animals mounting after PCPA treatment did not change. There were always some rats which never mounted during the periods of observation. The length of time between the day of surgery and the day of observation did not appear to have any effect on the number of rats showing sexual behaviour (Fig. 2). The hypersexual behaviour could be prevented by injecting the rats with 5-HTP. At 10 mg/kg the rats were sedated and inactive; after 5 mg/kg 5-HTP they were normally active but did not mount (Fig. 1).

2. *Concentrations of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid*

The concentrations of 5-HT and 5-HIAA in the brains of rats that had been adrenalectomized and castrated, were not different from those of intact rats. PCPA 100 mg/kg reduced the concentration of 5-HT to about 50% of control values within 24 h (Table 2) and this was reduced further to about 40% 72 h after injection of the drug. With the higher dose of 316 mg/kg the concentration of 5-HT has been shown to fall to 32% of the control value 24 h after injection (Shillito, 1970) and the greatest reduction (to 11%) occurs three days after this dose (Koe & Weissman, 1966), so the smaller dose did not produce such a great depletion of 5-HT and yet hypersexual behaviour was observed.

TABLE 2. *Concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-ylacetic acid (5-HIAA) in the forebrain of rats treated once with parachlorophenylalanine (PCPA) 100 mg/kg i.p.*

Treatment group	Time after PCPA (h)	5-HT (ng/g)	5-HIAA (ng/g)
Intact controls	—	436±25	189±14 (18)*
Castrated and adrenalectomized controls	—	418±42	162±19 (4)
Intact; PCPA 100 mg/kg	24	224±19	64± 5 (11)
Intact; PCPA 100 mg/kg	48	215±25	96±39 (7)
Intact; PCPA 100 mg/kg	72	177± 9	59± 2 (5)

* Mean±S.E. (number of estimates). The estimates have been corrected for losses in the analytical procedure.

In the experiments in which 5-HTP was injected and 5-HT concentrations were estimated at intervals until sexual activity recommenced (see section 2 of **Methods**), sexual activity started again about 3 h after injection. Two hours after the PCPA-treated animals were injected with 5-HTP 5 mg/kg, the brain 5-HT concentration had risen from 50% of that of control animals to about 75%. But by 3 h after the 5-HTP administration the 5-HT concentration had returned to about 50% of the control and mounting behaviour was again apparent. At 3 h the 5-HT concentrations were lowered to 47% of the control value and this was sufficient to induce mounting behaviour again. The 5-HT concentrations in these animals were very similar to those of rats given PCPA only but the 5-HIAA concentrations were higher because of the recent availability of newly formed 5-HT. Thus one rat treated with PCPA only, had a 5-HT value of 129 ng/g and a 5-HIAA value of 49 ng/g as compared with a rat treated 3 h previously with 5-HTP 5 mg/kg which had a 5-HT value of 135 ng/g and a 5-HIAA value of 95 ng/g.

3. Hair loss in rats

Intact rats treated chronically with PCPA 100 mg/kg or 50 mg/kg were very active and showed much social interaction in the form of chasing and lying over one another and mounting. After three injections of PCPA spaced over 8 days, the group of rats receiving 100 mg/kg showed hair loss in patches and this increased during the following days so that the rats were naked down their sides. After four injections both the other groups of rats which were receiving either PCPA 50 mg/kg or testosterone, looked dishevelled. Although there were no bare patches they looked as if some hair had been removed. After the sixth injection, when all the rats treated with PCPA 100 mg/kg had lost hair and showed bald patches of skin, 8/10 rats given PCPA 50 mg/kg had bald patches and 6/10 of the animals given testosterone were similarly affected. The latter group sometimes showed bare patches of skin at the sites of the subcutaneous injections, but these were not taken into consideration when the hair loss was estimated.

The weights of the rats, averaged for each group at the time the animals were killed, showed the testosterone group to be the heaviest at a weight of 79·6 g. The rats given PCPA 100 mg/kg weighed 72·7 g and the rats given PCPA 50 mg/kg were the lightest at 67·1 g. The mean weights of the testes in the two groups given PCPA were very similar being 1·05 g $\pm$ 0·076 (PCPA 100 mg/kg) and 1·03 g $\pm$ 0·011 (PCPA 50 mg/kg) whereas the testosterone treated group had smaller testes, the average weight being 0·78 g $\pm$ 0·058 ($\pm$S.E.).

The concentrations of cerebral 5-HT and 5-HIAA in animals from each group were estimated. The results (Table 3) showed that the chronic testosterone treat-

TABLE 3. *Concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindol-3-ylacetic acid (5-HIAA) in the forebrain of rats treated chronically with parachlorophenylalanine (PCPA) or testosterone*

Treatment group	5-HT (ng/g)	5-HIAA (ng/g)
Untreated control	416	237
Testosterone propionate, 6 × 1·25 mg s.c.	409	270
	426	255
PCPA 6 × 100 mg/kg i.p.	136	129
	146	141
PCPA 6 × 50 mg/kg i.p.	182	137

The estimates have been corrected for losses in the analytical procedure. (Six injections of either drug were spaced over three weeks).

ment produced no change in 5-HT or 5-HIAA, while chronic PCPA treatment reduced the 5-HT concentrations to about 35% of the control, and the 5-HIAA concentration to about 55% of the control.

4. *Comparisons of PCPA treatment with testosterone treatment*

When a group of 9–11 week old intact male rats was treated with testosterone and observed 24 h later, the animals appeared to be more active and behaved like very young rats chasing and making contact with each other and grooming frequently; the incidence of mounting was very low. However, when observations were made three hours after the subcutaneous injection of testosterone, mounting was observed and this behaviour was very similar to that of the same animals seen after treatment with PCPA 3 weeks previously (Fig. 3). The highest incidence of mounting was observed 4–5 h after the injection of testosterone and in this group 6/8 of the rats were affected. Reversing the order of treatments did not influence the results.

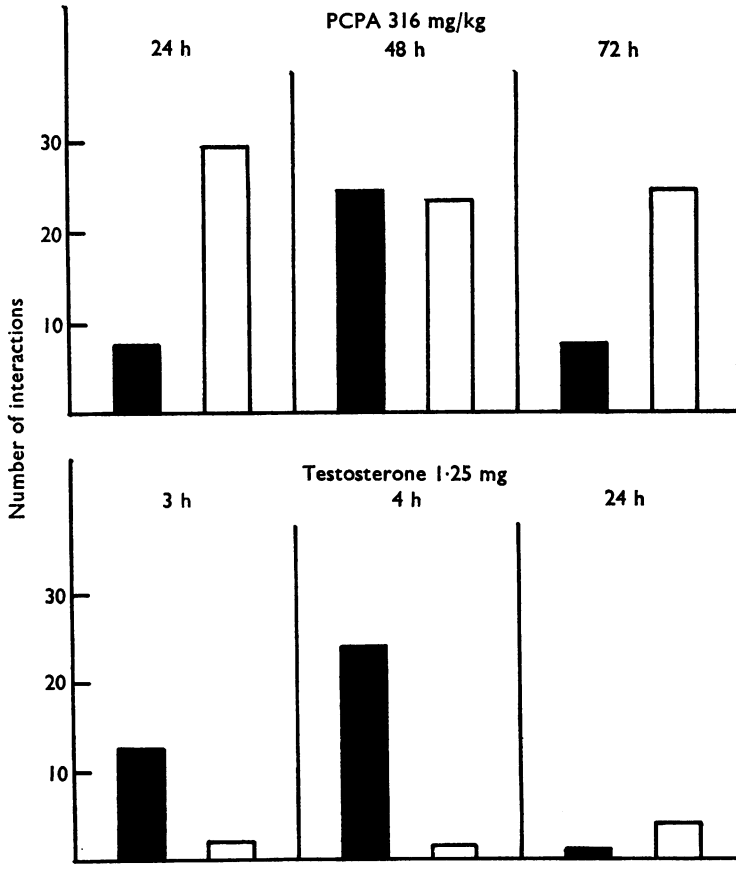

FIG. 3. Number of interactions during 1 h, in which rats mounted (shaded columns) or lay on top of one another (open columns). Experiments with one group of 8 rats treated with PCPA 316 mg/kg i.p. and observed for a one hour period at 24 h, 48 h and 72 h later. The same rats 3 weeks later were treated s.c. with testosterone propionate 1·25 mg in 1 ml arachis oil and observed for one hour periods at 3 h, 4 h and 24 h later.

Discussion

The observations demonstrating that sexual behaviour in male rats could be stimulated by changes in brain 5-HT, raised several queries relating to the role of testosterone in relation to the brain amine. Beach (1944) suggested that the motor acts involved in courtship and mating were governed by a central excitatory mechanism whose responsiveness was greatly increased by androgens. When the testes were removed from male rats before puberty, the animals showed a low level of sexual responsiveness and infrequent and incomplete mating activity. Injections of testosterone restored normal behaviour. Castration after puberty induced a waning of vigour and frequency of sexual behaviour, and again treatment with testosterone restored sexual activity to normal.

In a group of normal male rats, hypersexual behaviour in the form of mounting each other was produced by treatment with testosterone. Experiments reported in this paper show that this hypersexual behaviour was very similar to that seen after treatment with PCPA. In fact, 3–5 h after a subcutaneous injection of 1·25 mg testosterone, the rats showed an increase in mounting and lying on top of one another which was indistinguishable from the change in behaviour seen 24 h after PCPA 100 mg/kg. It is also possible to produce hypersexual behaviour in a group of castrated males by treatment with larger doses of testosterone than those given to intact males (Beach, 1944). Yet the degree of hypersexual behaviour produced by PCPA in castrated rats is similar to that produced in intact males by the same dose of PCPA. It is interesting that young rats treated with testosterone show an increase in chasing and lying over one another, as do rats that have been injected with PCPA. In fact, in juvenile rats undergoing chronic treatment with testosterone, this increase in social interactions can cause hair loss in the same way that chronic treatment with PCPA results in hair loss. However, the rats treated with testosterone showed no change in the concentrations of 5-HT and 5-HIAA in the brain whereas the rats given PCPA 100 mg/kg showed a fall in cerebral 5-HT to 33% of the control level.

The observations described in this paper show that the depletion of 5-HT in the brain by PCPA need not be greater than 50% to be accompanied by hypersexual behaviour in grouped male rats. Furthermore, the incidence of sexual behaviour was always observed to be greatest 24 h after treatment with PCPA when the depletion of 5-HT is not yet at its maximum. It is possible that fatigue may be an additional factor in these results, because rats which become very active sexually on the first day show a reduced activity on the following days. However, it seems to be the change in the concentration of brain amine which triggers the increase in sexual behaviour rather than the persistently low concentrations.

There is a clear influence of age on the behavioural change produced by PCPA. Young rats of 5–10 weeks showed a greater increase in sexual activity than rats of more than 12 weeks. This might be related to overall activity, because the young animals were more active than those that were three months old. In the older groups of rats the results were more variable because some groups were more active than others; however, the age at which castration had been carried out seemed to have no influence on the action of the PCPA (Fig. 2). Gessa *et al.* (1970) reported no increase in sexual behaviour in castrated rats treated with PCPA. They used old rats weighing 300–320 g, which is equivalent to the twelve week old animals used in these laboratories, so it is possible that only a low

response occurred. The rats were not observed in reversed daylight conditions and were not living in groups all the time, so these conditions may also be responsible for the different results. Gessa *et al.* (1970) and also Malmnäs & Meyerson (1971) used several repeated doses of PCPA before observing the rats. The latter workers measured the activity of castrated rats treated with PCPA and reported that although they showed increased mounting behaviour they were sluggish in an activity cage. It is possible that this low activity was the result of the administration of PCPA chronically instead of as a single dose. These same workers used rats castrated as adults and found that PCPA supplemented the hormone treatment which restored to normal the copulatory responses to female rats. They concluded that PCPA in the male rat increases the hormone-activated response, and yet the experimental results reported in the present paper seem to demonstrate that the PCPA is acting on a central mechanism which will function in the absence of any testosterone.

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REFERENCES

AHTEE, L., SHARMAN, D. F. & VOGT, M. (1970). Acid metabolites of monoamines in avian brain; effects of probenecid and reserpine. *Br. J. Pharmac.*, **38**, 72–85.

BEACH, F. A. (1944). Experimental studies of sexual behaviour in male mammals. *J. clin. Endocr.*, **4**, 126–133.

FERGUSON, J., HENRIKSEN, S., COHEN, H., MITCHELL, G., BARCHAS, J. & DEMENT, W. (1970). Hypersexuality and behavioural changes in cats caused by administration of *p*-chlorophenylalanine. *Science*, **168**, 499–501.

GESSA, G., TAGLIAMONTE, A., TAGLIAMONTE, P. & BRODIE, B. (1970). Essential role of testosterone in the sexual stimulation induced by p-chlorophenylalanine in male animals. *Nature, Lond.*, **227**, 616–617.

HOYLAND, V. J., SHILLITO, E. E. & VOGT, M. (1970). The effect of parachlorophenylalanine on the behaviour of cats. *Br. J. Pharmac.*, **40**, 659–667.

KOE, B. K. & WEISSMAN, A. (1966). *p*-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.*, **154**, 499–516.

MALMNÄS, C. O. & MEYERSON, B. J. (1971). *p*-Chlorophenylalanine and copulatory behaviour in the male rat. *Nature, Lond.*, **232**, 398–400.

SHEARD, M. H. (1969). The effect of *p*-chlorophenylalanine on behaviour in rats: relation to brain serotonin and 5-hydroxyindoleacetic acid. *Brain Research*, **15**, 524–528.

SHILLITO, E. E. (1969). The effect of *p*-chlorophenylalanine on social interactions of male rats. *Br. J. Pharmac.*, **36**, 193–194*P*.

SHILLITO, E. E. (1970). The effect of parachlorophenylalanine on social interactions of male rats. *Br. J. Pharmac.*, **38**, 305–315.

TAGLIAMONTE, A., TAGLIAMONTE, P., GESSA, G. & BRODIE, B. (1969). Compulsive sexual activity induced by *p*-chlorophenylalanine in normal and pinealectomized male rats. *Science, N.Y.*, **166**, 1433–1435.

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