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# The effect of *para*chlorophenylalanine on social interaction of male rats

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## Summary

1. Juvenile male rats treated with *para*chlorophenylalanine showed hair loss round the head and neck extending down the chest and abdomen.

2. Treated isolated rats did not have this loss of hair, while untreated animals living in the same cage as treated rats lost their hair. The loss therefore seems to be caused by increased social behaviour. This consists of a greater frequency of chasing each other, rolling over and social grooming.

3. Adult male rats show an increase in mounting after treatment with *para*-chlorophenylalanine, and this change in behaviour was counteracted by treatment with 5-hydroxytryptophan.

4. It is concluded that 5-hydroxytryptamine inhibits sexual behaviour in male rats. The increase in social interaction seen in juvenile rats may be the behavioural precursor of adult sexual behaviour.

5. Atropine 2·5 mg/kg blocked all forms of social interaction in adult male rats, although other activity was not altered.

## Introduction

Since Koe & Weissman (1966) reported that *para*chlorophenylalanine lowered 5-hydroxytryptamine in the brain of rodents and did not lower noradrenaline or dopamine concentrations, several workers have used this compound to determine a possible role for 5-hydroxytryptamine in the brain. Koe & Weissman reported no gross changes in behaviour in rats or mice injected with the compound. Tenen (1967) found that rats treated with *para*chlorophenylalanine showed a faster acquisition of a conditioned avoidance response and this was correlated with increased sensitivity to pain. In a further study Tenen (1968) found that *para*chlorophenylalanine antagonized the analgesic effects of morphine. Woolley & van der Hoeven (1963) found an improvement in the learning ability of mice in a T-maze, when depleted of 5-hydroxytryptamine by other methods. This was in contrast to Tenen (1967), who found no effect on rat learning in a T-maze. An alteration in patterns of sleep produced by changes in concentrations of 5-hydroxytryptamine in the brain has been reported by Matsumoto & Jouvet (1964) in cats and rats; Torda (1967) and Mouret, Bobillier & Jouvet (1968) have found a loss of sleep in rats given *para*chlorophenylalanine. These workers found that paradoxical sleep disappears totally when slow wave sleep and brain 5-hydroxytryptamine are at the minimum level. Paradoxical sleep then reappears after 52 hr and is longer than normal, whereas slow wave sleep is diminished for much longer, and recovers with

no rebound, in direct correlation with recovery of normal brain 5-hydroxytryptamine. They reported that circadian variations were absent when 5-hydroxytryptamine was 90% below control levels. Weitzman, Rapport, McGregor & Jacoby (1968) have also found abnormal sleep patterns in monkeys given *para*chlorophenylalanine.

When 3 week old male rats were treated with *para*chlorophenylalanine for periods of 2 weeks, it was noticed that they showed a loss of hair round the chin and shoulders which sometimes extended down the ventral side. In addition their vibrissae were absent or shorter than usual, and looked as if they had been chewed off. The rats were kept in groups of ten animals to a cage and they were often seen to groom each other and pull each other about, particularly after being handled. Although control groups of rats also groomed each other they did not show any marked loss of hair, and so the question arose whether the hair loss was the result of increased social behaviour or whether it was caused by a dietary or other deficiency related to the drug treatment. The animals used moulted from the juvenile pelage to adult pelage during the experimental treatment and this might also have contributed to the excessive loss of hair. It was decided to investigate whether this phenomenon could be explained by changes in social behaviour.

## Methods

The experiments were carried out on male albino rats supplied by Tucks, usually as weanlings 3 weeks of age or 7–14 weeks of age when older rats were used. The *para*chlorophenylalanine was given by intraperitoneal injection as a suspension in 1% Tween 80, a behaviourally inactive detergent. Two concentrations were made. They were: 31·6 mg/ml and 63·2 mg/ml. The solution containing 31·6 mg/ml was given to rats weighing less than 150 g and that containing 63·2 mg/ml was used in rats over this weight. The total dose depended on the duration of the experiment. The first dose of 316 mg/kg was given on a Friday, and the next two, of 100 mg/kg, the following Monday and Wednesday. When observations were made for longer periods, the same sequence of three injections a week was repeated two or three times. Koe & Weissman (1966) showed that with this dosage the cerebral 5-hydroxytryptamine reached a low concentration on the third day of treatment, and that further doses of 100 mg/kg kept it low. This was confirmed in this laboratory by R. O'Keeffe, who found that the brain concentration of 5-hydroxytryptamine in treated rats was reduced to 13·6% of normal after one week of treatment. Control groups of rats were given the vehicle alone.

### *Tests of social behaviour*

Male weanlings were divided at random into seven groups. The rats in each group were kept together in one cage with the exception of the rats in groups 6 and 7. They were treated as follows for 2 or 3 weeks.

(1) Ten rats injected with 1% Tween 80 (controls) (repeated 4 times).

(2) Ten rats injected with *para*chlorophenylalanine (repeated 4 times).

(3) Ten rats, five of which were treated with *para*chlorophenylalanine and five with 1% Tween 80 (repeated twice).

(4) Five rats treated with *para*chlorophenylalanine.

(5) Eight rats, five of which were treated with *para*chlorophenylalanine and three with 1% Tween 80.

(6) Four rats treated with *para*chlorophenylalanine but kept in individual cages.

(7) Four rats injected with 1% Tween 80 and kept in individual cages.

Injections were also given to four groups of adult males 10–12 weeks old, kept in groups of eight rats to a cage, similar to groups 1 and 2 above.

In the second and third weeks of treatment, encounter experiments were carried out to observe in more detail the social behaviour of the animals. The rats were adapted to conditions of reversed daylight before observation by first leaving them in darkness for 2 days, then lighting the cages at night and covering them during the day. Observations were made in red light during the rats' most active phase, which was now in the daytime. Later, all rats were kept in reversed daylight with white light from 22.00 hr to 10.00 hr and red light from 10.00 hr to 22.00 hr for a week before observations started.

The encounter experiments used four animals living in a cage made from four rectangular plastic bowls, two joined together and all connected to a central circular bowl by plastic drain pipes (Fig. 2). Four days before observations were made, the rats were marked for identification and put into the cage, one animal to each rectangular bowl measuring 27 cm × 33 cm × 13 cm deep, the exit of which had been blocked by a rubber bung. At the beginning of the experiment all the rubber bungs were removed so that the rats could move through the whole cage. Observations were made for the next hour and the number of interactions in which one animal lay on top of another was recorded for each minute.

After adult rats had been used in the basin cage for encounter experiments, it was decided to observe a larger group of animals living together all the time in a large cage. Groups of eight adult males, 7–11 weeks old, were watched in a cage which measured 91·5 cm × 122 cm, and was covered with a Perspex lid. The cage contained sawdust and hay and the area was divided up by plastic partitions and an inverted basin provided a nest box. Water and food was freely available and the animals were kept in reversed daylight as described above, and had been in the room for a week before observations started. In contrast to the previous groups, after they had been in the observation cage for 3 days these rats were watched on 2 successive days without any treatment and were then given 316 mg/kg *para*chlorophenylalanine by intraperitoneal injection. In some groups four out of eight rats were treated, in others all eight rats were given the drug. They were then watched for the following 3 days. Koe & Weissman (1966) showed that the brain 5-hydroxytryptamine reached its lowest level (11% of normal) 3 days after treatment with *para*chlorophenylalanine 316 mg/kg; estimations of brain 5-hydroxytryptamine by Mr. P. Tegerdine in this laboratory showed that as early as 24 hr after an injection of this dose the levels were reduced to 32% of the control values. Some groups of rats were given various doses of 5-hydroxytryptophan by intraperitoneal injection, one hour before being observed. This usually restores the 5-hydroxytryptamine levels in treated rats (Jéquier, Lovenberg & Sjoerdsma, 1967). 5-Hydroxytryptophan was also given to some rats 24 hr after treatment with *para*chlorophenylalanine and to some rats 48 hr after treatment. On some days atropine was given, 30 min before observation, to rats which had already had *para*chlorophenylalanine.

The rats kept in the conditions of reversed daylight with a 12 hr cycle showed a burst of activity just as the lights changed from white to red ; therefore these groups of animals were watched for one hour following the light change, that is from 10.00–11.00 hr. Various forms of social interactions were recorded at the time they occurred ; most of these were lying on top of one another or mounting. Some groups of eight 4 week old rats were also observed in this way.

## Results

### *Loss of hair related to grouping of animals*

Loss of hair occurred in most of the young male rats, kept in groups of ten, when some or all had been given *para*chlorophenylalanine (groups 2, 3, 4 and 5 of **Methods**) (Fig. 1 and Table 1). The extent of hair loss was less in the groups containing five treated animals only and it did not occur in any animal kept in isolation. In the mixed groups many of the treated and the control rats showed

TABLE 1. *Number of rats showing loss of hair and chewed vibrissae; effect of grouping and treatment with* para*chlorophenylalanine*

| Group | No. of rats | Treatment | No. of rats affected |
|---|---|---|---|
| 1 | 10 | 1% Tween 80 (control) | 0/10 in 4 groups |
| 2 | 10 | *Para*chlorophenylalanine | 8/10 |
| | 10 | *Para*chlorophenylalanine | 9/10 |
| | 10 | *Para*chlorophenylalanine | 7/10 |
| | 10 | *Para*chlorophenylalanine | 8/10 |
| 3 | 10 | 5 1% Tween 80 | 2/5 |
| | | 5 *Para*chlorophenylalanine | 3/5 |
| | 10 | 5 1% Tween 80 | 4/5 |
| | | 5 *Para*chlorophenylalanine | 5/5 |
| 4 | 5 | *Para*chlorophenylalanine | 3/5 |
| 5 | 8 | 5 *Para*chlorophenylalanine | 2/5 |
| | | 3 1% Tween 80 | 1/3 |
| 6 | 4 in individual cages | 1% Tween 80 | 0/4 |
| 7 | 4 in individual cages | *Para*chlorophenylalanine | 0/4 |

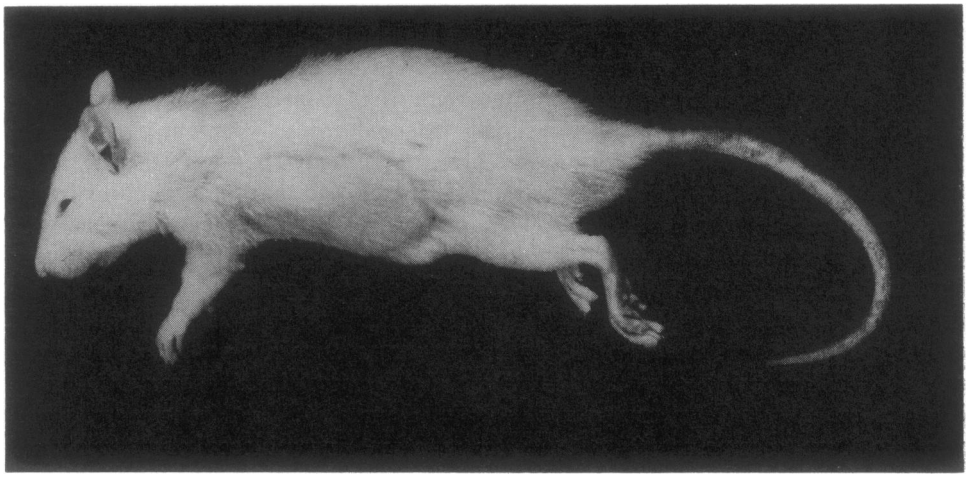

FIG. 1. Example of hair loss in a young juvenile rat treated with *para*chlorophenylalanine.

bald patches and loss of vibrissae (Table 1). Old rats that were treated showed no loss of hair, and on those animals which were kept in groups for some time with continued drug treatment, the hair grew again, usually after 3 weeks.

These results indicated that the loss of hair associated with drug treatment was brought about by some social behaviour which was related to the number and the age of the animals in the group. It could not have been a direct effect of the drug on the hair because, among mixed groups, untreated rats also lost their hair.

Encounter experiments were then carried out to determine whether this change in social behaviour was related to social grooming, to general activity or to aggression, and what influence age had on the behaviour of the rats.

Preliminary observations on the behaviour of treated and untreated rats showed that a period of isolation before the experiment was necessary if frequent social interactions were to be observed in a limited time. Rats spend quite some time investigating any new cage, so they were placed 4 days before the experiment in the cages (Fig. 2) so that they had some time in which to become familiar with parts of the apparatus. On removal of the stoppers, the rats could then mingle and interact with each other without the disturbance of handling and the effect of a completely new environment.

The first observations on young rats showed that most often social grooming occurred while one rat lay on its back with another rat lying on it, sometimes at right angles to each other. The rats took up this posture after an initial period of chasing and rolling over each other ; they showed no signs of aggression and they did not appear to be apprehensive. This contrasts with the description by Grant & Mackintosh (1963) of these postures as being aggressive in adult rats. The rat underneath lay very still and relaxed while the rat on top nibbled the fur under the chin and round the shoulders and head ; on some occasions the rat underneath also

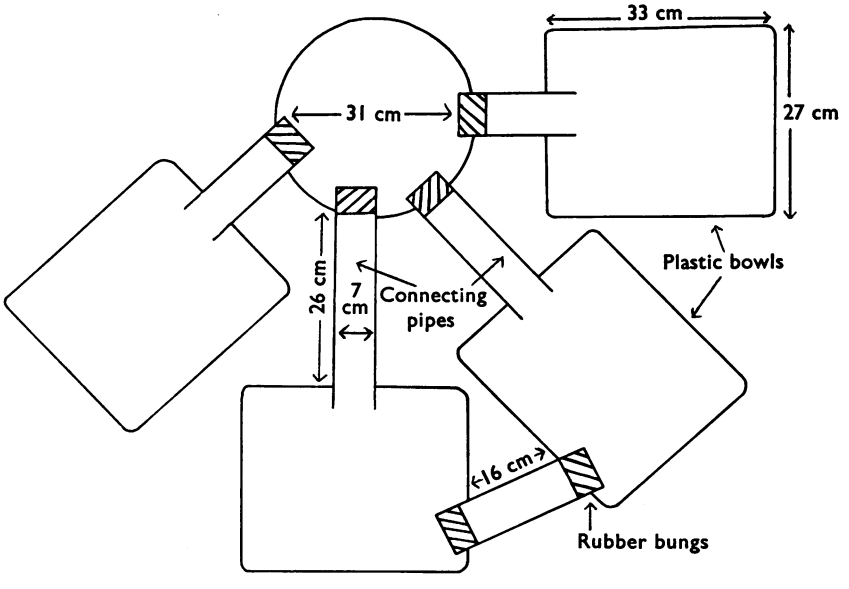


FIG. 2. Plan of the cage used in the encounter experiments with four rats. The rubber bungs in the pipes (shaded) were removed to allow the rats to mix after having lived in isolation.

nibbled the rat on top. Although this behaviour was seen in both treated and control rats, animals from groups which showed hair loss lay on top of one another more frequently than rats which did not show loss of hair. It seemed possible that this social grooming could be the main behaviour pattern responsible for hair loss, which is why the number of interactions in which one animal lay on top of another was recorded over a period of an hour.

### *Encounter experiments with four rats*

When all the plugs were removed and the animals were allowed to move through the observation cage (Fig. 2), the first few minutes were spent in exploratory behaviour and there were no social activities. After this the behaviour of the rats varied with age.

#### *Juvenile rats*

All juvenile males, less than 6 weeks old, very quickly started to chase and nose each other, to tumble over each other and eventually to lie on top of one another, sometimes grooming. There seemed to be no tension between the rats which were very active and energetic. Periods of social interacting alternated with periods of feeding, self-grooming or nosing around. The rats did not defend their own cage against the other rats, but often one or two animals initiated the chasing and rolling over. The number of times the control rats lay on top of one another totalled about sixty for the group of four in one hour ; the rate was typically once or twice a minute. The treated juveniles interacted much more frequently, sometimes more than 100 times in the hour ; the number of interactions was usually two or three in a minute and sometimes reached six. Social grooming also occurred more often in the treated than in the controls. As a result of this excessive grooming the hair was pulled out and the bare patches were frequently licked by other rats, and this possibly hindered re-growth. It was noticed that rats with less hair loss and intact vibrissae took less part in the social behaviour of the group than the very bald animals and those without vibrissae. The encounter frequency is shown in Fig. 3 and Table 2.

#### *Adult rats*

Adult males more than 6 weeks old showed more aggressive components in their behaviour towards one another. The rats tried to avoid lying down, and side kicks with the hind feet and sparring between two rats occurred before one rat managed to get on top of the other. The total amount of activity was much less than with juvenile rats, and the treated animals were less, not more, active than the controls. The number of social interactions was sometimes very low except for mounting, which occurred and could become very frequent, particularly in the older treated males of 14 weeks. Attempts to mount also led to one rat lying on top of the other, but the interaction was clearly different from that seen in young rats in previous experiments. Because of this, Table 2 only shows interactions not resulting from sexual behaviour, and summarizes the number of such encounters.

*Encounter experiments with eight rats*

*Adult rats*

The adult rats, aged 10–12 weeks, living in the large cage were very active when the lights changed. They moved freely round the cage, climbing on the partitions, eating and drinking and also showing social reactions in the form of chasing and lying on top of each other and grooming. These interactions again occurred without tension and did not appear to be aggressive ; they generally took place within the

TABLE 2. *Number of interactions in* 1 *hr in the basin cage* (*Fig.* 2)*; treated rats had been injected with* para*chlorophenylalanine*

| Age | Controls | Treated |
|---|---|---|
| 5 weeks | 60 | 135 |
| | 69 | 104 |
| | 59 | 94 |
| | 62 | 108 |
| 14 weeks | 35 | 8 |
| | 34 | 18 |

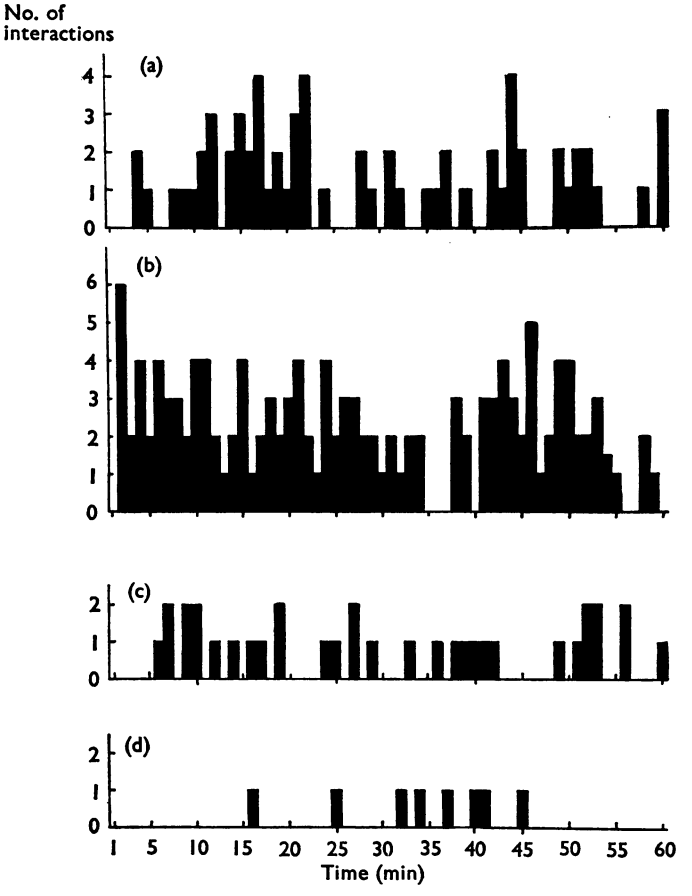


FIG. 3. Incidence of social interactions when one rat lay on top of another, during the encounter experiments with four rats. (a) Juvenile males (controls) ; (b) juvenile males treated with *para*chlorophenylalanine ; (c) adult males (controls) ; (d) adult males treated with *para*chlorophenylalanine.

first 30 min of observation and on average occurred about 14 times in one hour among the untreated group. It was unusual to observe any mounting behaviour in the untreated groups, but 24 hr after treatment with *para*chlorophenylalanine 316 mg/kg, this behaviour pattern occurred frequently, and it continued throughout the hour of observation. When only four rats in the group were treated, only these animals behaved in this way. When eight animals were treated they were all affected and although they began mounting at different times, the records show that over 3 days each rat had mounted another at some time. The results are shown in Fig. 4. This increase in mounting was associated with a reduction in the number of times one rat lay on top of another rat, and it was apparent that a change had occurred in both the mounting and the mounted rat. In untreated rats it was observed that the approaching animal put its front feet on the back of the second rat, which then turned over and lay on its back. In contrast, among treated rats, the second rat would stand still and allow the first rat to mount. When 5-hydroxytryptophan was given to the treated rats, mounting occurred either not at all or much less frequently than with *para*chlorophenylalanine alone (Fig. 4). Tenen (1967) used a 75 mg/kg dose of 5-hydroxytryptophan, but this dose and also 50 mg/kg sedated the animals, so that activity of all kinds was reduced. When the dose was reduced to 25 mg/kg, 20 mg/kg and 10 mg/kg, the activity of the rats was

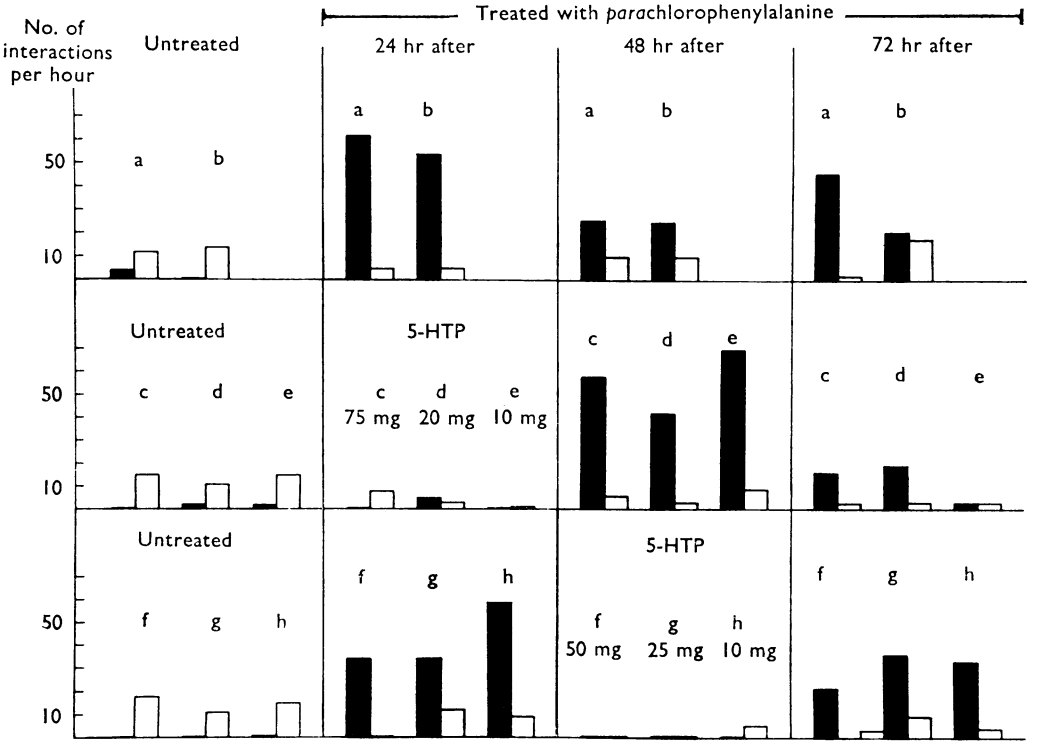


FIG. 4. Number of interactions in 1 hr in which adult male rats 10–12 weeks old lay on top of one another or mounted. Experiments with groups of eight rats untreated and then given *para*chlorophenylalanine sometimes followed by 5-hydroxytryptophan (5-HTP) 10 mg, 20 mg, 25 mg, 50 mg and 75 mg/kg and watched for 3 days. The letters above the columns refer to the same group of rats. Shaded columns, Mounting behaviour ; open columns, lying on top of another rat.

normal but mounting did not occur and "lying on top behaviour" was reduced. On the day after treatment with 5-hydroxytryptophan, the treated rats resumed mounting behaviour.

When atropine 2·5 mg/kg was given to *para*chlorophenylalanine-treated adult rats 30 min before observations started, the change in behaviour was very remarkable. The rats moved around the cage apparently quite normally, eating and drinking, but there was no social interaction of any kind. They behaved as if no other rat was present.

*4 week old rats*

When groups of eight, 4 week old rats were watched in the large cage, the same type of interactions seen in the smaller groups—chasing, rolling over and lying on top of one another—were observed. This activity was sometimes very intense and two observers were necessary for the first 20 min of the observation time in order to record the encounters accurately. The young rats were not so active in the later

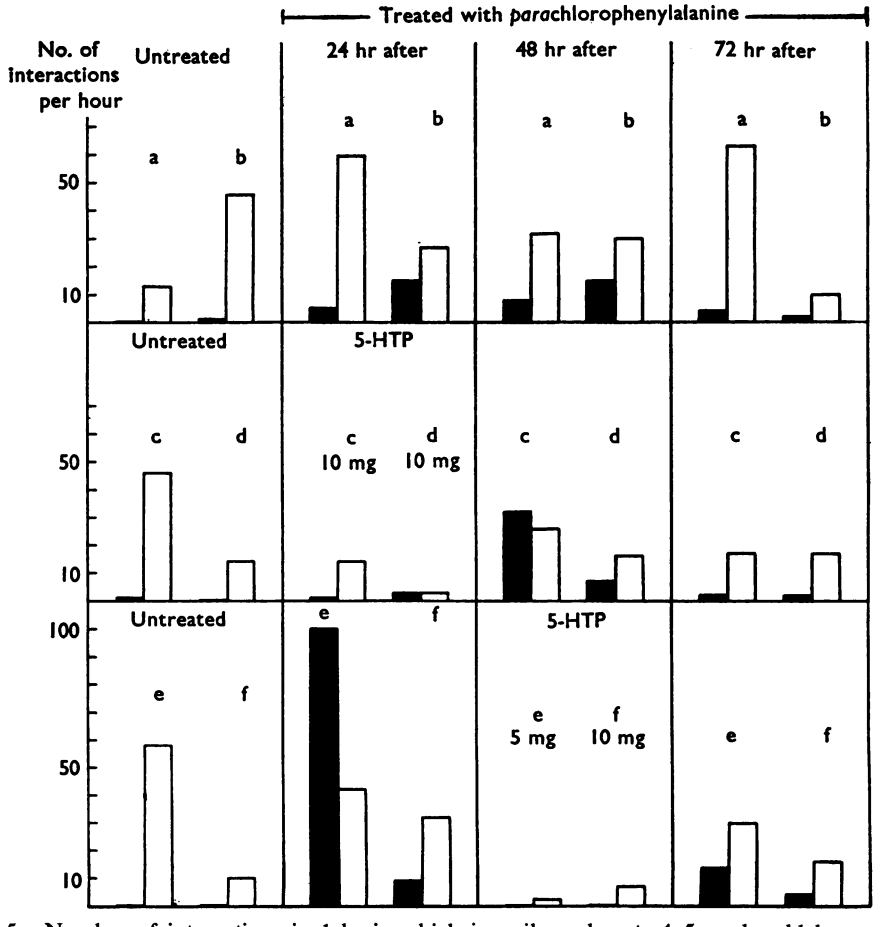


FIG. 5. Number of interactions in 1 hr in which juvenile male rats 4–5 weeks old lay on top of one another or mounted. Experiments with groups of eight rats untreated and then given *para*chlorophenylalanine, sometimes followed by 5-hydroxytryptophan (5-HTP) 10 mg and 5 mg/kg and watched for 3 days. The letters above the columns refer to the same group of rats. Shaded columns, Mounting behaviour; open columns, lying on top of another rat.

part of the hour as were the adult animals. After treatment with *para*chlorophenylalanine two effects were noticed: some rats showed an increased frequency of chasing and lying on top of one another, while others started mounting behaviour. The frequency of mounting increased as the rats got older, so that at 5 weeks of age, mounting behaviour was quite prominent after treatment with the drug (Fig. 5). Treatment with 5-hydroxytryptophan prevented this increase in both lying on top of another rat and in mounting.

When rats which had been treated with *para*chlorophenylalanine earlier were given atropine 2·5 mg/kg 30 min before observations were started, they stopped all interactions for about half an hour and were very sedated. After that period the rats became normally active, interacting as before. When atropine 1 mg/kg was given to the young treated rats, however, there was no obvious sedation, activity appeared normal, but social interaction was completely absent.

## Discussion

By giving *para*chlorophenylalanine to mammals, further evidence has been adduced that 5-hydroxytryptamine is probably involved in several cerebral mechanisms (Mouret *et al.*, 1968 ; Tenen, 1967). The observations reported in this paper suggest that another effect of 5-hydroxytryptamine is to inhibit sexual behaviour in adult male rats. Even when the 5-hydroxytryptamine was reduced to only 20% that of normal, the animals showed a marked increase in mounting behaviour. Replacement of the 5-hydroxytryptamine by administering 5-hydroxytryptophan restored behaviour to normal.

It seems that the chasing, rolling over and social grooming of the young male rats is the behavioural precursor of the adult male behaviour. This activity was increased considerably by administration of *para*chlorophenylalanine, so much so that the young groups of rats lost hair and vibrissae. This was an indication of increased social interaction and not an effect of the drug on metabolism, as was shown by the fact that treated juvenile rats kept in isolation did not lose hair at all. When young rats were watched over a period from 4 to 6 weeks of age, it was possible to see the development of the behaviour changing from the initial stages, where the rat underneath lies on its back with another rat on top with social grooming, to genuine mounting, when the mounted rat stands still and its partner is clasping and thrusting.

Singer (1968) found that atropine blocked or produced a severe decrement of sexual behaviour in rats, and it was interesting to know whether atropine would block the sexual behaviour that had been induced by *para*chlorophenylalanine. The results of giving atropine in as small a dose as 2·5 mg/kg were very striking. The adult male rats showed no reactions to each other although their activity and behaviour patterns of investigating, feeding and drinking appeared normal. It was thus all social interactions, not only sexual behaviour, that was blocked. The juvenile rats seemed to be more sensitive to atropine, because 2·5 mg/kg sedated them but 1 mg/kg did not interfere with their activity apart from blocking all social interaction.

Several observations have been made on female rats, both adult and juvenile, but no consistent changes in behaviour have been obtained. Treatment with *para*chlorophenylalanine does not seem to interfere with the oestrous cycle and it may

be the fact that female rats only show sexual behaviour when in oestrous, that makes it difficult to observe any increase in these behaviour patterns.

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