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Behavioural Effects in Adulthood of Neonatal Manipulation of Brain Serotonin Levels in Normal and Androgenized Females

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WILSON, C. A., I. GONZALEZ AND F. FARABOLLINI. *Behavioural effects in adulthood of neonatal manipulation of brain serotonin levels in normal and androgenized females.* PHARMACOL BIOCHEM BEHAV **41**(1) 91–98, 1992.—5HT concentrations in the hypothalamus are higher in females than males over the second week of life and this differentiation is testosterone-dependent. We have investigated the possible influence of 5HT over this period on the development of systems that control adult behaviour, in particular those influenced by neonatal testosterone. Neonatal androgenization (250 μg/pup testosterone propionate; TP; on day 1 postpartum) induced a masculine pattern of behaviour in females ovariectomised in adulthood and bearing a TP implant. The neonatal treatment reduced exploration, motor activity and female sexual behaviour and increased anxiety, orientation toward the incentive female and male sexual behaviour. Depletion of 5HT by pCPA (100 mg/kg days 8–16 postpartum) enhanced the TP-induced increment in locomotion and female sexual behaviour and increased sexual orientation toward the incentive female, while 5HTP (20 mg/kg days 8–16 postpartum) antagonised the reduction in exploration by TP. Thus 5HT may normally exert an inhibitory control on the action of neonatal testosterone on exploration, motor activity and sexual behaviour. Neonatal PCPA treatment also had a marked anxiolytic effect which was independent of the presence of T as it was noted in normal and androgenized females and previously has been observed in intact males. This might indicate a primary control by a serotonergic system on the development of the systems controlling anxiety.

Sexual differentiation 5-Hydroxytryptamine Locomotion Exploratory behaviour
Sexual behaviour and orientation Elevated plus-maze test for anxiety

IT is well established in rodents that over the last few days in utero and first days after birth, androgens induce anatomical differentiation in specific brain areas, which in turn are the cause of functional (including behavioural) sexual dimorphism in adulthood (3, 26, 36). The link between the hormone action and anatomo-functional sexual differentiation is most likely to be a variety of neurotransmitters, amongst which 5-hydroxytryptamine (5HT; serotonin) is an important candidate. Sex differences in brain levels of 5HT have been reported, particularly in the second week of life and this is under the control of the previous androgen environment. Thus levels of 5HT and 5-hydroxyindole acetic acid (5HIAA) are higher in the whole hypothalamus and preoptic area of the female compared to the male during the first 12 to 14 days after birth and perinatal androgenization (in the first week postpartum) of the female, results in masculinization of the 5HT system, with a reduction in indolamine concentrations (13, 14, 21, 31, 38). It has been suggested that the reduced 5HT concentration in the male has a functional significance in that it removes an inhibitory influence of the serotonergic system on testosterone in the neonatal period (20,38).

5HT has an organizational influence on neuronal circuits (22) affecting subsequent behaviour in adulthood (24,25). In a previous report, we have shown that depletion of 5HT by para-chlorophenylalanine (pCPA) in male rats in the second week of life (but not the first) increased reactivity to environmental and social cues and decreased anxiety (11). Male sexual behaviour only showed a tendency to increase although we and others have previously found an enhancement with the same treatment (17,38). In adulthood, there appears to be a reciprocal relationship between 5HT and testosterone in the control of male sexual behaviour [see (37)] and in addition, 5HT seems to have a general effect in increasing responsiveness to the environment (7,19).

In females, the effect of manipulation of 5HT in the neonatal period is less well-known. Female sexual behaviour, i.e., lordotic activity, is not affected by neonatal manipulation of 5HT either in female or male rats (14,38). However in androgenized females the reduced lordotic activity normally observed, was potentiated by depletion of 5HT and antagonised by raising the 5HT levels (with 5-hydroxytryptophan; 5HTP). Thus 5HT seems to affect lordosis only in the presence of concomitant neonatal testosterone (38).

A variety of behavioural activities in rodents are sexually

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differentiated beside sexual behaviour. In particular, it is well established that females show greater locomotor and exploratory activity and exhibit less emotionality and show a greater preference for novelty (2, 5, 15, 30). Although there is clear evidence for the organizational role of androgens in determining some of these sex differences, the role of serotonin and its interaction with hormones is still to be clarified.

This investigation is concerned with the organisational effects of 5HT on subsequent behavioural activity, in the presence and absence of neonatal testosterone. Brain 5HT activity has been manipulated over the second week of life (days 8–16) in females with and without prior androgenization. Comparisons have been made on various aspects of behaviour including exploratory and motor activities, anxiety, sexual motivation and sexual performance. In this way a broad behavioural profile has been obtained allowing a parallel analysis of the various behaviours and their interrelationships.

METHOD

Twelve litters born on the same day to Wistar rats (bred at St. George's Hospital Medical School) were randomized and culled such that each dam had a litter of 6 males and 6 females. The animals were kept throughout life in a reversed lighting regime 12 hours on:12 hours off (lights off 11.00 hours) and observed under red illumination. Temperature was maintained at 22°C and the rats were fed on Breeding Diet No. RM3 (Special Diet Services, Lillyco; Surrey).

On the day of birth, each pup of 6 of the litters received 250 μg/pup testosterone propionate (TP; Sigma Chemical Co., Dorset) subcutaneously (SC) in 0.1 ml corn oil. The pups of the remaining 6 litters received 0.1 ml/pup corn oil. On days 8 to 16 daily, each of 2 litters from the TP- and oil-treated groups were treated with either 20 mg/kg 5HTP (Sigma) or 100 mg/kg pCPA (given as 120 mg of the methyl ester; Sigma). Both drugs were injected SC in 1 ml/kg saline and the remaining 4 litters received 1 ml/kg saline SC.

The litters were weaned on day 21 and the females were housed in groups of 5 or 6. The males were not used in these experiments. On day 60 all the females were ovariectomized under halothane and nitrous oxide (May & Baker Ltd.; Dagenham) and then housed individually each with a female of similar age, ovariectomized 3 weeks before who served as a "neutral" companion. On day 66, a silastic implant containing TP (10 mm in length; internal diameter 1.5 mm; Dow Corning tubing 602-285; Midland, MI) was placed under the skin of each of the test rats. The experimental design with the days on which animals were treated and tested is shown in Table 1.

TABLE 1

TREATMENTS AND TESTING SCHEDULE

Pretreatment (day 1 after birth)	Treatment (over days 8–16)	Day 60	Day 66	Days 90–110
(10) Oil	Saline	OVX	TP implant	
(5) Oil	5HTP	OVX	TP implant	
(12) Oil	pCPA	OVX	TP implant	
				Behavioral testing
(12) TP	Saline	OVX	TP implant	
(13) TP	5HTP	OVX	TP implant	
(11) TP	pCPA	OVX	TP implant	

No. of rats in each group in parentheses. OVX: ovariectomised; TP: testosterone propionate.

Behavioural Testing

Behavioural testing started at day 90 after birth and was completed within 3 weeks. The order of testing was as follows: The combination of the holeboard and plus maze tests were applied first. One week later the animals were subjected to the sexual orientation test followed immediately by the test for female sexual behaviour which took place in a different room. A few days after each test for female behaviour the rats were tested for masculine sexual behaviour. Two tests for male behaviour were carried out with a fully receptive female "teaser" and two tests were observed in the home cage with the neutral companion. The sexual orientation and sexual behaviour tests were carried out under red lighting and the holeboard and elevated plus-maze test in a dim white light (360 radiometric lux).

The Holeboard and Plus-Maze Tests

Each of these tests were carried out for 5 minutes and the holeboard test was always first:

The holeboard was a black Perspex box (60 × 60 × 35 cm) with four holes (3.8 cm diameter) equally spaced on the floor. The latter was divided into 36 squares. Frequency and duration of head-dipping and frequency of rearing were recorded with a microcomputer (Epson, PX-4), whereas locomotor activity was recorded in parallel as number of line-crossings. Number of boluses were counted at the end of the test.

The elevated plus-maze consisted of 2 open arms (50 × 10 cm) and two enclosed arms of the same size with 40 cm high walls arranged so that the arms of the same type were opposite each other. The apparatus was wooden and elevated to a height of 62 cm. The measures recorded were frequency and duration of arm visits, separately, for open and closed arms. Both apparatus were thoroughly cleaned at the end of every test.

Sexual orientiation test. The test for sexual orientation was carried out in a circular arena of 90 cm diameter, surrounded by a 30 cm high wall. Two small wire-mesh cages (15 cm^2) were fixed into the wall such that the front of each cage was "flush" with the wall and the 2 cages were opposite each other. They contained two stimulus animals, an intact sexually experienced male and a receptive female [ovariectomized bearing a subcutaneous 7 mm Silastic implant containing oestradiol benzoate (OB; Sigma)]. Animals had been adapted to the apparatus (in the absence of stimulus animals) for 10 minutes on 2 consecutive days prior testing. During the 10-minute test the following measures were taken: frequency and duration of visits to an area (30 × 15 cm) in front of each stimulus animal, and time spent investigating it.

Female sexual activity. In this test the rats were placed with a series of vigorous males each in an observation arena and subjected to 20 mounts. The number of lordotic responses were then assessed and expressed as a lordosis quotient (No. of lordoses/No. of mounts × 100%).

Male sexual activity. This behavioural test was carried out in two ways:

1) In the same room and conditions in which the female sexual activity was observed. The test animals were placed in observation arenas and a receptive "teaser" female (see Sexual Orientation test for details) was introduced 3 minutes later. The latency to first mount and number of mounts (with or without intromissions) performed by the test female were recorded over the following 10 minutes. This test was repeated 2 days later and the results of the second test are presented.

2) The test animals were observed for 10 minutes in their own cages, which they shared with a neutral companion. The

TABLE 2

EFFECT OF NEONATAL SEROTONIN MANIPULATION IN FEMALE RATS WITH AND WITHOUT PRETREATMENT ON DAY 1 [ACTIVITY IN THE 5-MINUTE HOLEBOARD TEST (MEANS ± SE)]

	Females			Androgenized Females		
	Saline (10)	5HTP (5)	pCPA (12)	Saline (10)	5HTP (12)	pCPA (9)
Head-Dipping						
Frequency	6.6 ± 0.9	7.8 ± 1.4	5.5 ± 1.0	3.2 ± 0.4†	8.4 ± 1.6*	4.5 ± 1.0‡
Duration (min)	10.1 ± 1.7	12.4 ± 2.1	10.7 ± 2.5	6.2 ± 0.9	15.3 ± 4.2*	10.1 ± 3.2
Rearing						
Frequency	25.9 ± 2.5	30.0 ± 4.5	25.6 ± 2.5	21.4 ± 2.6	24.3 ± 2.9†	17.2 ± 3.4†‡
Locomotion						
No. crosses	231 ± 22	199 ± 18	207 ± 13	195 ± 21	188 ± 18	137 ± 20*†‡
Boluses						
(No.)	1.6 ± 0.6	0.6 ± 0.6	1.6 ± 0.7	3.6 ± 0.6†	4.9 ± 0.6†	3.8 ± 0.9†

$p<0.05$ or 0.01 *compared to saline group of the same pretreatment; †compared to corresponding group of the other pretreatment; ‡compared to other treatment groups of same pretreatment. Number of rats in each group in parentheses.

neutral companion was removed for the 24 hours prior to the test and then replaced at the beginning of the test. The mount latency and number of mounts were recorded as well as aggressive episodes, scoring the number of fights shown by the experimental subject. The test was repeated 2 days later and a mean of the results of the two tests are presented.

Statistical Analysis

The data obtained from the holeboard and plus-maze tests, and from the tests for feminine and masculine sexual behaviour were processed by multi-trial analysis of variance (ANOVA) for two factors: Sex (normal, androgenized) and Treatment (saline, 5HT, pCPA).

Data obtained from the sexual orientation test were subjected to three-way ANOVA, involving the factors, Sex and Treatment and the factor Incentive (male, female) as a repeated measure.

Separate one-way ANOVA for the factor Treatment was performed on normal and androgenized females for measures of masculine and feminine sexual behaviour. Ninety-five percent confidence for mean test was used for post hoc comparisons between groups.

Fisher's Exact tables were used to assess the significance of differences in proportion of rats showing particular activities.

RESULTS

Nonsocial Behaviour

The results of the holeboard and the plus-maze are shown respectively in Tables 2 and 3.

Holeboard test. In the two-way ANOVA for the holeboard parameters (Table 4, left panel), the main factor "Sex" was significant for Rearing, Locomotion and Boluses and marginally

TABLE 3

EFFECT OF NEONATAL SEROTONIN MANIPULATIONS IN FEMALE RATS WITH AND WITHOUT TESTOSTERONE TREATMENT ON DAY 1 [ACTIVITY IN THE 5-MINUTE ELEVATED PLUS-MAZE TEST (MEAN ± SE)]

	Females			Androgenized Females		
	Saline (10)	5HTP (5)	pCPA (12)	Saline (10)	5HTP (12)	pCPA (11)
Closed Arms						
% Time	51.0 ± 5.7	59.2 ± 4.7	43.7 ± 5.3‡	68.6 ± 5.8†	56.0 ± 4.7*	55.4 ± 5.1*†
No. Entries	9.3 ± 0.9	9.4 ± 0.9	8.3 ± 0.7	5.5 ± 1.3†	8.1 ± 0.9	6.8 ± 0.8
Open Arms						
% Time	6.7 ± 2.3	5.2 ± 1.7	13.5 ± 2.4*‡	2.3 ± 1.3†	5.5 ± 1.2	7.3 ± 1.6*†
No. Entries	2.9 ± 0.9	2.6 ± 1.0	4.5 ± 0.8*‡	1.3 ± 0.6†	2.5 ± 0.5	2.3 ± 0.6†
Total Entries						
No.	12.2 ± 1.1	12.0 ± 1.0	12.8 ± 1.3	6.8 ± 1.8†	10.6 ± 1.2	9.1 ± 1.1†

$p<0.05$ *compared to saline group of the same pretreatment; †compared to corresponding group of the other pretreatment; ‡compared to other treatment group of same pretreatment. Number of rats in each group in parentheses.

TABLE 4

TWO-WAY ANOVA (F AND p VALUES) FOR MEASURES OBTAINED IN THE HOLEBOARD AND PLUS-MAZE TESTS

	Holeboard					Plus-Maze				
	HD					OP		CL		
	FR	D	RE	LO	BO	No. En.	%Time	No. En.	%Time	TE
Main Factors										
"Sex" (df=1,52)	2.1	0.1	6.2	6.9	20	5.9	6.3	7.9	4.9	10.1
	<0.1	n.s.	<0.01	<0.01	<0.0001	<0.02	<0.01	<0.007	<0.03	<0.002
Treatment	5.3	2.3	1.7	2.3	0.2	1.7	5.6	1.3	1.9	1.3
(df=2,52)	<0.008	<0.1	<0.1	<0.1	n.s.	<0.1	<0.006	n.s.	<0.1	n.s.
Interaction										
"Sex" × Treatment	1.3	0.6	0.2	1.1	1.3	0.9	1.3	0.9	1.5	0.8
(df=2,52)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

HD: head dip; RE: rears; LO: locomotion; BO: boluses; OP: open arm; CL: closed arm; TE: total entries; FR: frequency; D: duration; En: entries.

significant for frequency of Head-dipping: the main factor "Treatment" was highly significant for frequency of head-dipping and marginally significant for rearing, locomotion and duration of head-dipping. The significance of the factor Sex was due to overall lower rates of rearing, locomotion and head-dipping and higher number of boluses in androgenized females (results of post hoc single comparisons shown in Table 2). As for the treatments, manipulation of serotonin had no effect on any of the parameters in normal females; in the androgenized females, treatment with 5HTP restored levels of head-dipping to those of normal females. Treatment with pCPA decreased locomotion with respect to androgenized, saline- and 5HTP-treated females; rearing also showed a tendency to decrease.

Plus-maze test. Two-way ANOVA applied to the plus-maze data (Table 4, right panel), showed significances for both factors Sex and Treatment for the entries and time in the open arms. The factor Sex was also significant for entries and time on the closed arms and for total entries. Androgenized females, on the whole, entered less and spent less time on the open arms than normal females; conversely, they entered more and spent more time in the closed arms and had lower total entries (results of single comparisons in Table 3). As for treatments, pCPA had the same effect in both androgenized and normal females, consisting in increased time on the open arms, accompanied by decreased time into closed arms. 5HTP decreased the time into closed arms, only in androgenized females.

Sexual Activity

Sexual orientation test. Three-way ANOVA was applied to the measures obtained in the sexual orientation test, with Sex, Treatment and Incentive as main factors and Incentive as a repeated measure. Both factors Sex and Treatment were significant for time in front of the incentives (Table 5). The factor Incentive was significant for the time investigating and marginally significant for the time in front; the interaction Sex × Incentive was also significant and the interaction Sex × Treatment × Incentive marginally significant for both measures. This indicates that the preference for the incentive animals depended on the "Sex" of the experimental animal; similarly the effect of the treatments depended on "Sex" of the experimental animal and on the sex of the incentive animal.

Results of single comparisons in Table 6 show that androgenized, saline-treated females did not differ from normal females for the time spent in front of the incentives which was equally distributed between male and female. However, time spent by the androgenized females in investigating the incentive male was significantly lower compared to the normal females. As indicated by the significance of the factor Sex and the interaction Sex × Incentive, overall the androgenized females spent more time investigating the incentive females and less time with the incentive males, compared to the normal females. This was mainly due to the effect of the treatments, which differed in the normal and androgenized groups as shown by the interaction Sex × Treatment × Incentive. 5HTP and pCPA both resulted in increased time investigating the female and pCPA also increased the time spent in the female area by androgenized animals. In normal females, 5HTP decreased the time spent in front of the incentive female.

Feminine sexual behaviour. The results of tests on female sexual behaviour expressed as lordosis quotient are shown in Table 7. The number of animals showing lordosis is also reported. In the two-way ANOVA for factors Sex and Treatment applied to Lordosis Quotient, only Sex was significant, $F(1,5) = 8.6$, $p<0.005$. This was due to overall lower lordosis quotients in androgenized females, accompanied by reduced proportion of animals showing behaviour (control 24/27; androgenized 22/34;

TABLE 5

THREE-WAY ANOVA FOR REPEATED MEASURES (F AND p VALUES) APPLIED TO SEXUAL ORIENTATION TEST

	Time Investigating		Time in Front	
	F	p	F	p
Main Factors				
1) Sex (df=1,55)	0.5	n.s.	4.8	<0.03
2) Treatment (df=2,55)	0.8	n.s.	5.1	<0.009
3) Incentive (df=1,55)	6.3	<0.01	2.2	<0.1
Interactions				
1×2 (df=2,55)	2.1	<0.1	1.2	n.s.
1×3 (df=1,55)	23.4	<0.0001	6.3	<0.01
2×3 (df=2,55)	2.3	<0.1	0.3	n.s.
1×2×3 (df=2,55)	2.6	<0.08	2.5	<0.09

TABLE 6

EFFECT OF NEONATAL SEROTONIN MANIPULATION IN FEMALE RATS WITH AND WITHOUT TESTOSTERONE PRETREATMENT ON DAY 1 [IN THE SEXUAL ORIENTATION TEST (MEAN ± SE)]

	Females				Androgenized Females			
	Total Time in Front (s)		Time Investigating (s)		Total Time in Front (s)		Time Investigating (s)	
	Female	Male	Female	Male	Female	Male	Female	Male
Saline	198 ± 8.7	182 ± 16	82 ± 7	98 ± 15	192 ± 21	190 ± 11	78 ± 10	56 ± 6†
5HTP	142 ± 11*	177 ± 27	59 ± 7	101 ± 23	217 ± 13†	150 ± 12	119 ± 10*†	52 ± 8†
pCPA	178 ± 16	197 ± 20	93 ± 12	80 ± 8	243 ± 16*†	165 ± 17	117 ± 12*†	61 ± 10

$p<0.05$ *compared to saline group of the same pretreatment; †compared to corresponding group of the other pretreatment. All times recorded in seconds. Number of rats in each group as shown in Tables 2 and 3.

$p<0.03$). This effect of neonatal testosterone was further potentiated by pCPA the latter group exhibiting a significantly lower lordosis quotient compared to the corresponding group of the oil pretreatment ($p<0.05$). In addition, the proportion of androgenized animals showing lordosis was lower in the pCPA-treated group compared to the 5HTP-treated group (pCPA group 4/11; 5HTP group 11/12; $p<0.01$).

Masculine sexual behaviour. Two-way ANOVA for factors Sex and Treatment, applied to the measures of masculine sexual behaviour obtained in the two types of tests, with the female teaser (A) and with the companion (B), only gave rise to significances for the number of mounts confined to the factor Sex [Test A: $F(1,55)=6.7$, $p<0.01$; Test B: $F(1,55)=7.3$, $p<0.009$]. This was due to overall higher levels of mounting in both tests in the androgenized with respect to normal females, as shown by data reported in Table 7.

The latency to mounting was slightly different for the factor Sex ($F=2.1$, $p<0.1$) in the test with the companion, due to overall shorter latencies in the androgenized females. In addition, the proportion of animals showing mounting behaviour toward the companion was slightly higher in the androgenized groups (androgenized females 25/34; normal females 15/27; $p=0.14$) as was the proportion showing episodes of aggression (androgenized females 14/34; normal females 5/27; $p=0.06$).

Single comparisons of mounting frequency toward the teaser (Test A) between the treatment and saline groups within each pretreatment set indicated that both 5HTP and pCPA reduced mounting in normal females and this was also shown by the significant difference between the corresponding treatment groups in the normal and androgenized females. In tests carried out with the companion, 5HT manipulation did not exert any obvious effect in either pretreatment group.

DISCUSSION

5-Hydroxytryptamine concentrations in the hypothalamus are higher in females than in males in the second week of life. This sexual differentiation is testosterone-dependent and since 5HT is known to influence growth and differentiation of neuronal systems, it is possible that 5HT acts to influence some of the an-

TABLE 7

EFFECT OF NEONATAL SEROTONIN MANIPULATION IN FEMALE RATS WITH AND WITHOUT TESTOSTERONE PRETREATMENT ON DAY 1 [MASCULINE AND FEMININE SEXUAL BEHAVIOUR (MEANS ± SE)]

	Females			Androgenized Females		
	Saline (10)	5HTP (5)	pCPA (12)	Saline (11)	5HTP (12)	pCPA (11)
Masculine Sexual Behaviour						
A) With Teaser (2nd Test)						
No. animals showing the behaviour	9/10 (90%)	5/5 (100%)	10/12 (83%)	9/11 (82%)	12/12 (100%)	11/11 (100%)
No. mounts	18.0 ± 3.7	6.8 ± 2.1*	9.8 ± 2.5*	19.1 ± 3.8	20.3 ± 2.8†	18.1 ± 2.9†
Mount Latency (s)	109 ± 58	109 ± 34	177 ± 62	146 ± 68	54 ± 13	69 ± 17
B) With Companion (Mean of 2 tests)						
No. animals showing the behaviour	4/10 (40%)	3/5 (60%)	8/12 (67%)	8/11 (73%)	9/12 (75%)	8/11 (73%)
No. mounts	1.2 ± 0.6	1.9 ± 1.1	3.9 ± 1.1	5.3 ± 1.7*	6.8 ± 1.8*	6.1 ± 1.8
Mount Latency (s)	365 ± 55	408 ± 84	268 ± 61	278 ± 67	244 ± 57*	256 ± 54
No. animals showing aggressive episodes	1/10 (10%)	1/5 (20%)	3/12 (25%)	4/11 (36%)	3/12 (25%)	7/11 (64%)
Feminine Sexual Behaviour						
No. animals showing lordosis	9/10 (90%)	4/5 (80%)	11/12 (92%)	7/11 (64%)	11/12 (92%)	4/11 (36%)‡
Lordosis Quotient	75.5 ± 9.1	77.0 ± 11.7	74.2 ± 8.1	58.6 ± 8.9	61.2 ± 7.0	41.8 ± 11.2†

$p<0.05$ *compared to saline group of the same pretreatment; †compared to corresponding group of the other pretreatment; ‡compared to other treatment group of same pretreatment. Number of rats in each group in parentheses.

drogenizing effects of neonatal T (see introduction for references). If this hypothesis is correct, manipulation of 5HT in the neonatal period might affect androgen-dependent physiological functions in adulthood and this possibility has been investigated. In a previous report (11) we have shown that neonatal depletion of 5HT, by pCPA, in males altered certain aspects of behaviour (reducing anxiety and increasing offensive aggressive behaviour and slightly enhancing male sexual behaviour). These findings in males, however, did not distinguish whether these effects were due to gender and/or the interaction of 5HT with neonatal T. The present studies were designed to see whether similar manipulation of neonatal 5HT would be effective in normal females or females after neonatal T treatment. The results obtained showed that 5HT manipulation over the second week postpartum in females, could indeed interact with neonatal testosterone and alter aspects of behaviour subsequently in adulthood. In addition, some forms of behaviour were altered by 5HT manipulation that were independent of testosterone.

In the first place, our data show a general masculization of the behavioral profile in androgenized females, both for social and nonsocial aspects of behaviour, indicating that under the influence of neonatal T, masculine differentiation takes place in parallel for a number of behaviours. Circulating hormones in adulthood are not involved as in these experiments both the normal and androgenized females received the same treatment (i.e., a TP implant).

Comparing androgenized females with normal females on nonsocial behaviour, the former displayed reduced exploratory and motor activity, a reduction in time spent in the open arms of the plus maze and increased boluses, the latter two parameters indicating increased anxiety. Such behavioral patterns are similar to that described by others, in males (2,15) emphasising the dependence of behavioral sex differences on neonatal T (5, 6, 27, 33). Manipulation of 5HT in the neonatal period had different effects on the various aspects of nonsocial behaviour, and on normal and androgenized females. Depletion of 5HT, with pCPA, decreased levels of anxiety in both normal and androgenized females as noted by the increase in time spent in the open arms. This was not due any increase in general activity since locomotion did not alter in the normal rats and was actually reduced in the androgenized group. A similar finding of reduced anxiety was noted previously in males treated with pCPA in the second week postpartum (11). These findings taken together indicate that the reduction in anxiety induced by 5HT depletion is independent of gender and the presence of testosterone neonatally. It seems, therefore, that 5HT has a direct effect over the neonatal period on systems controlling anxiety. In adulthood 5HT is known to modulate levels of anxiety too (19).

Other aspects of nonsocial behaviour such as locomotion and exploration were also modulated by neonatal 5HT manipulation, but only in the presence of testosterone. pCPA increased the decrement in locomotion and rearing and 5HTP antagonised the reduction in exploration, only in androgenized females. This indicates that over the neonatal period T has a primary organising effect on locomotion and exploration and that a serotonergic system can act to inhibit this effect. This interrelationship has been suggested before (20,38).

Previous reports have shown that neonatal 5HT has different effects on the ontogeny of reactivity to novel stimuli (25), analgesia (32) and level of locomotion (24). The changes in locomotion were observed in the open-field test in which the final result is a net change in exploration and emotional drive, which tend to act in opposition (23,24). In our experiments these components have been dissociated, firstly by using the holeboard test in which exploration and locomotion can be measured separately (12) and then by measuring the emotional component, i.e., anxiety, by the elevated plus maze test (23). The differential effects of 5HT on aspects of nonsocial behaviour, indicates there are independent mechanisms controlling these various aspects. Similarly, 5HT manipulation had different effects on various parameters of sexual behaviour which also changed in the presence and absence of T.

In these experiments sexual activity was assessed by noting sexual motivation, and the performance of both masculine and feminine behaviour. On the whole, neonatal androgenization induced masculinization of sexual behaviour with reduced female sexual behaviour, increased male sexual behaviour and increased orientation toward an incentive female.

Sexual motivation in rats, as measured by the sexual orientation test, depends on sexual experience, hormonal treatment in adulthood and the neonatal hormonal environment. Normal, saline-treated females did not show any preference between the stimulus male and female, probably because they were sexually naive (8). In androgenized saline-treated females, the preference in terms of time in front of the stimulus animal was unchanged, but time spent investigating the stimulus male was significantly reduced, so that preference was proportionately shifted in favour of the stimulus female, which is in agreement with a previous report (9). The preference for the female stimulus was further potentiated in the androgenized animals by 5HT manipulation in either direction; increased by 5HTP or decreased by pCPA. It is possible that the 5HT depletion acted in synergy with the neonatal T, in line with the suggested reciprocal inhibitory effect of the two systems (20, 38, 45). In the case of 5HTP, the increased preference for the stimulus female could be nonspecific and depend on a general increased motivation to explore, as observed in the holeboard test, once again only in androgenized animals. It seems that masculine-like sexual motivation is primarily determined by neonatal T and that 5HT can only act to modify its effect. As an additional, but not exclusive possibility, 5HT manipulation may have different effects in different hormonal environments; it is interesting, for example, to note that 5HTP has opposite effects in the two pretreatment groups, increasing the preference for the female in the androgenized rats and proportionately decreasing the same in normal females. The direction of the effect may, therefore, be determined by the hormones present at birth.

Neonatal T induced a reduction in female sexual behaviour and this was potentiated by depletion of 5HT, while 5HTP tended to antagonize it. This indicates, once again, the inhibitory effect of a serotonergic system on the action of testosterone 5HT manipulation had no effect in normal females confirming our previous findings (38) and indicates that 5HT has no effect on the development of feminine sexual behaviour.

Androgenized females showed overall higher levels of mounting both directed toward the teaser (unfamiliar receptive female) and to the familiar cage companion (an ovariectomized female). This is in agreement with the described pattern of behaviour in androgenized females (34) and may be at least in part, due to the sensitizing effect of neonatal T treatment toward the TP implants given in adulthood (4). Manipulation of 5HT in these animals did not affect mounting behaviour, as also previously observed (38). The lack of effect occurred in spite of the fact that the animals showed more interest in the stimulus female in the sexual orientation test (see Table 6). This seems to indicate that the performance of masculine sexual behaviour and the sexual motivation, although both controlled by neonatal androgens are independent systems which can be differentially affected during neonatal development.

In contrast to the androgenized females, in normal females, neonatal 5HT had an effect on development of mounting behaviour, since manipulation of 5HT (in either direction) reduced the

number of mounts directed toward a teaser. The fact that neonatal increase and reduction of 5HT have similar effects has been noted before (18,20). In this particular case, the effect of 5HTP could be due to raised 5HT activity in the direction typical of a female, which could reduce the response of T given in adulthood. As for pCPA, when given neonatally, it enhances male sexual behaviour in males (17), reduces male sexual behaviour in females [see Table 7 and (38)], while it has no effect on male sexual behaviour in androgenized females (see Table 7). This suggests that the response of the androgenized female is halfway between the two extremes of the opposite responses shown by normal males and females and emphasizes the fact that neonatal T does not induce complete masculinization of the females, and that the effect of pCPA on mounting behaviour is different according to gender and the presence or absence of neonatal T.

Interestingly, 5HT manipulation did not significantly affect mounting activity in normal females tested with a familiar companion, and if anything pCPA tended to increase mounting. The difference between the results obtained with the teaser and the companion might be explained not only by the difference in the partners, but also by the different environments. The test with the companion took place in the familiar home cage, where the companion was removed and reintroduced after an absence of 24 hours. This gave rise to a complex pattern of social behaviour including mounting often accompanied by episodes of aggression. Earlier reports show that aggression in females depends on the environment and experience, besides the hormonal milieu (1) and that aggression and mounting are enhanced in parallel by chronic testosterone treatment (similar to that used in our experiments) (35).

Certain caution must be exercised in the interpretation of these results. It is assumed throughout that pCPA is acting centrally to reduce the synthesis of 5HT by inhibition of tryptophan hydroxylase. However, the pCPA was administered systemically and so may have affected peripheral as well as central systems. It is known, for instance, that it modifies a number of serotonergic pathways involved in the transmission of sensory inputs (10). In addition, pCPA has a number of other effects besides inhibition of tryptophan hydroxylase although they are transient, i.e., enhanced release of 5HT, inhibition of catecholamine synthesis (37).

In conclusion, taking the above provisos into account, we have shown that in the female rat, in the second week of life, 5HT has organizing effects which appear to be different on various aspects of behaviour. Whereas in the case of anxiety, 5HT seems to be a primary controlling factor independent of the presence of T, in other forms of behaviour an interaction between a 5HT system and neonatal T has been shown, suggesting that serotonergic and T-dependent neuronal mechanisms are inter-related over the neonatal period. In particular, the results indicate that 5HT has an inhibitory effect on the action of neonatal T since 5HT depletion by pCPA enhances the decrement in locomotion, the increment in sexual orientation toward the incentive female and the reduction in lordotic responses induced by androgenization. Conversely, 5HTP antagonizes the reduction in exploration induced by T. This does not rule out the possibility of a direct organising role of 5HT even in females although in a different perinatal period. For example, administration of pCPA to pregnant rats results in an increase in the volume of the sexually dimorphic nucleus (SDN) of the POA in female offspring similar to that normally seen in males (16), indicating that 5HT is involved in the development of sexual differentiation in the prenatal phase.

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