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The Effect of Neonatal Manipulation of Hypothalamic Serotonin Levels on Sexual Activity in the Adult Rat

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WILSON, C. A., J. R. PEARSON, A. J. HUNTER, P. A. TUOHY AND A. P. PAYNE. *The effect of neonatal manipulation of hypothalamic serotonin levels on sexual activity in the adult rat.* PHARMACOL BIOCHEM BEHAV **24**(5) 1175–1183, 1986.—5HT and 5HIAA levels were altered in neonatal males and females by treating them with 100 mg/kg p-chlorophenylalanine (PCPA) or 20 mg/kg 5-hydroxytryptophan (5HTP) over either days 1 to 7 or 11 to 16 of life. Androgenized females (testosterone propionate given on day 1) were also treated over days 1 to 7. In adulthood the effects of these treatments on sexual behaviour were observed. None of the treatments affected feminine sexual behaviour in either sex. Reduction of 5HT activity by PCPA enhanced masculine sexual behaviour in males and potentiated the defeminizing effect of exogenous testosterone in females. Increasing 5HT by 5HTP antagonised the defeminizing effect of exogenous testosterone. These findings indicate that the lower levels of hypothalamic 5HT and 5HIAA seen in the neonatal males may have some physiological importance, since 5HT appears to antagonise the androgenizing and defeminizing effects of testosterone.

Sexual behaviour Sexual differentiation Serotonin 5HTP PCPA

THE influence of gonadal steroids during the neonatal period on sexual differentiation of the hypothalamus and subsequent adult reproductive function and behaviour is well established [2, 3, 19, 28]. The biochemical mechanisms by which steroids exert their effect on the CNS are still obscure. It is thought likely that the steroids influence neuronal organization since there are steroid-dependent sexually dimorphic neuronal structures in specific hypothalamic and amygdaloid areas [1, 15, 29, 32]. It follows from this that neurotransmitter activity might also be different in the sexes and may well be involved in sexual differentiation. This hypothesis is supported by the fact that administration of pharmacological agents known to alter neurotransmitter activity, can affect sexually differentiated behaviour in adulthood [8, 21, 33].

Ladosky and Gaziri [26] were the first to show that levels of 5-hydroxytryptamine (5HT; serotonin) in the whole brain were higher in female than male rats in the second postnatal week (probably due to differences in monoamine oxidase activity [11]) which in turn may depend on the neonatal steroid environment [12,26], later steroidal manipulation having no effect [12]. More recent reports show that the sex difference in 5HT concentration on day 12 is significant in the hypothalamus [13] and, in particular, in the medial preoptic area [36]. These findings have not always been confirmed and no sex differences in 5HT were noted in the hypothalamus by Wilson and Agrawal [39] or in the preoptic area and midbrain raphe by Watts and Stanley [38]. This may be due to a shift in the time in which those differences are seen in different strains. There may even be two periods of sex difference in 5HT concentration since brain levels of 5HT and decarboxylase activity are reported to be higher in female brains on days 1 and 2 of life as well as day 12 [17, 18, 31], although again this has not been confirmed in all reports [12, 26, 39]. Since 5HT systems have an influence on proliferative differentiation of neuronal and glial precursor cells in the developing nervous system [27] and assuming there is a sexually differentiated 5HT activity induced by the neonatal steroids, perhaps 5HT may be involved in the sexual differentiation of neuronal structures.

We have attempted to manipulate brain 5HT levels over days 1 to 7 or 11 to 16 of postnatal life and note the effect of this on sexual behaviour in adulthood in both sexes and androgenized females. Days 1–7 incorporate part of the period over which the steroids exert their masculinizing effect on the hypothalamus and days 11–16 is the period when 5HT activity shows sex differences. Since 5HT activity is greater in the female one might predict that raising 5HT levels would have a feminizing effect while a reduction

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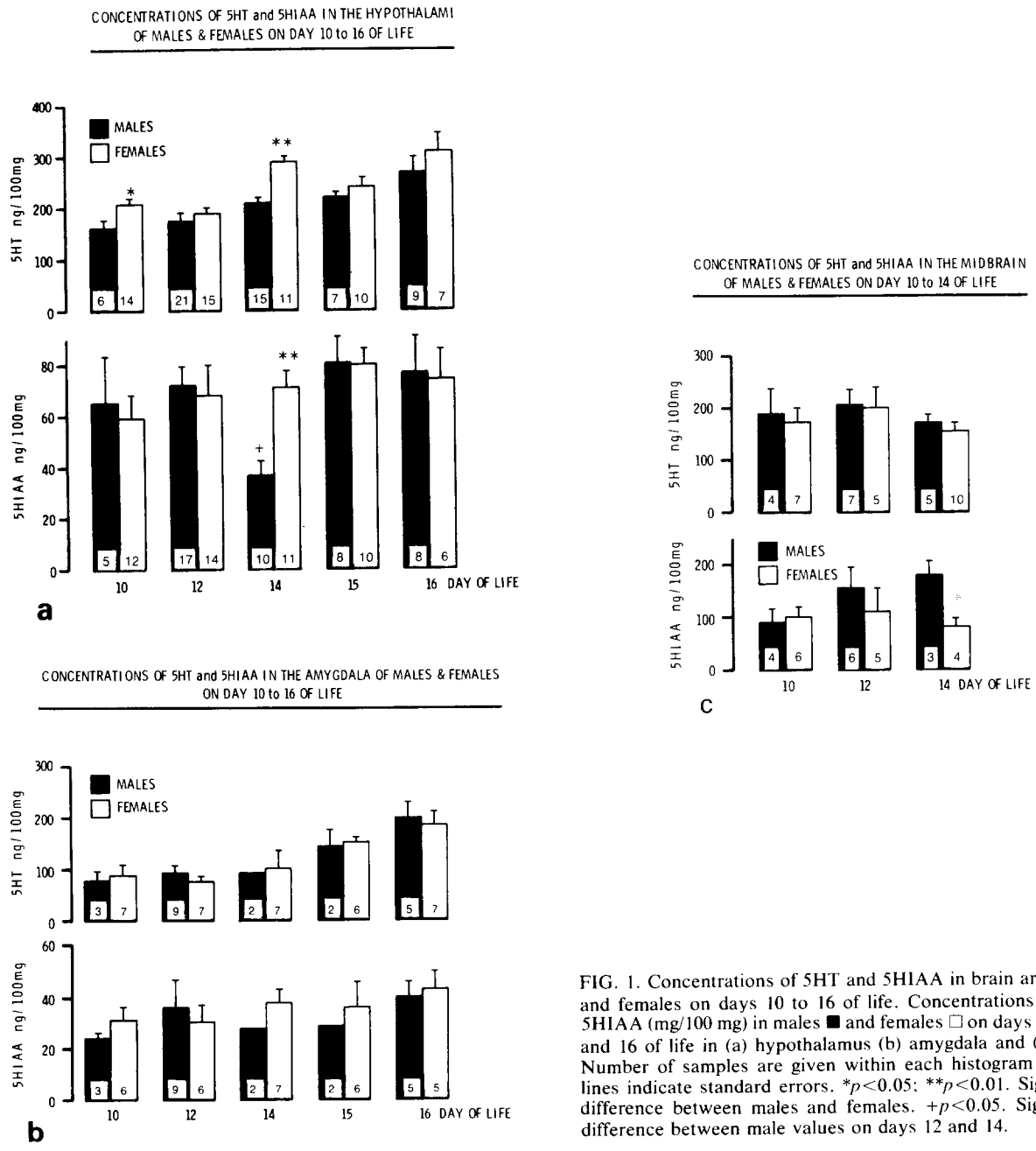

FIG. 1. Concentrations of 5HT and 5HIAA in brain areas of males and females on days 10 to 16 of life. Concentrations of 5HT and 5HIAA (mg/100 mg) in males ■ and females □ on days 10, 12, 14, 15 and 16 of life in (a) hypothalamus (b) amygdala and (c) midbrain. Number of samples are given within each histogram and vertical lines indicate standard errors. *$p<0.05$; **$p<0.01$. Significance of difference between males and females. +$p<0.05$. Significance of difference between male values on days 12 and 14.

would enhance masculine activity. Furthermore these manipulations may modify the masculinizing action of exogenous testosterone given to neonatal females.

METHOD

Experiment 1

The litters, born on the same day of 19 mothers (Wistar strain bred in the Animal House of St. George's Hospital Medical School) were randomised and culled within 24 hours of birth, such that each litter consisted of 4 or 5 males and 4 or 5 females. The pups of at least 3 litters were then autopsied on days 10, 12, 14, 15 and 16 after birth (day of birth= day 0) and their hypothalami, amygdala and midbrains dissected [14] and stored at −80°C until assayed for indole compounds.

Experiment 2

The litters of 14 mothers were randomised and culled as above. The pups were treated either over days 1 to 7 after birth or 11 to 16 after birth with pharmacological agents known to augment or deplete 5HT (details below). Two litters from each treatment group were autopsied at the end of the treatment period, i.e., either on day 8 or 17 and their hypothalami removed and stored at −80°C until assayed for indole compounds.

TABLE 1

EFFECT OF 5HTP AND PCPA ADMINISTERED NEONATALLY ON HYPOTHALAMIC CONCENTRATIONS OF 5HT AND 5HIAA

	Concentration of hypothalamic indoles (ng/100 mg ± SEM)					
	Males			Females		
Treatment (days)		5HT	5HIAA		5HT	5HIAA
			Day 8			
1 to 7						
0.05 ml saline	(8)	145 ± 13.4	45 ± 5.1	(10)	134.5 ± 7.3	40 ± 4.3
20 mg/kg 5HTP	(9)	287 ± 19.8‡	82 ± 9.9‡	(10)	302 ± 13.1‡	88 ± 10.5‡
100 mg/kg PCPA	(6)	76 ± 14.3†	34 ± 2.0*	(7)	64 ± 13.8†	40 ± 6.0
150 mg/kg PCPA	(6)	40 ± 4.9‡	29 ± 11.0*	(6)	52 ± 3.8†	37 ± 15.7
			Day 17			
11 to 16						
0.05 ml saline	(6)	192.5 ± 10.9	65 ± 8.3	(10)	181 ± 14.1	64 ± 5.3
20 mg/kg 5HTP	(8)	257 ± 10.0‡	78 ± 8.7	(8)	259.5 ± 25.3†	69 ± 11.2
100 mg/kg PCPA	(7)	64 ± 4.1‡	12 ± 8.0‡	(8)	74 ± 11.0‡	15 ± 6.7‡

Significance of difference between treated groups and saline controls: *$p<0.05$; †$p<0.01$; ‡$p<0.001$.

TABLE 2

EFFECT OF NEONATALLY ADMINISTERED 5HTP AND PCPA ON GROWTH AND DEVELOPMENT

	Treatment over days 1 to 7			Treatment over days 11 to 16		
	Saline	5HTP	PCPA	Saline	5HTP	PCPA
Body weight on week 19 of life (g ± SEM)						
Females:	276 ± 1.9 (17)	276 ± 2.0 (14)	275 ± 2.5 (12)	276 ± 2.3 (17)	279 ± 3.3 (21)	279 ± 2.0 (22)
Males:	403 ± 6.8 (18)	410 ± 8.9 (16)	420 ± 7.2 (12)	418 ± 6.0 (20)	411 ± 6.1 (21)	421 ± 5.2 (23)
Day of vaginal opening (days ± SEM)	38.8 ± 0.88 (17)	37.6 ± 1.1 (14)	37.7 ± 0.45 (12)	36.5 ± 0.8 (17)	35.8 ± 0.59 (21)	35.7 ± 0.43 (22)
% showing oestrous cycles	82%	86%	64%	93%	82%	95%
Hypothalamic indole concentration on weeks 28–30 of life (ng/100 mg ± SEM)						
Females:						
5HT	353 ± 46.6 (6)	not done	331 ± 40.6 (6)	320 ± 29 (4)	303 ± 19.4 (5)	328 ± 12.3 (5)
5HIAA	110 ± 6.5 (5)	not done	94 ± 13.1 (6)	76 ± 16.5 (4)	67.8 ± 3.9 (5)	71.6 ± 2.9 (7)
Males:						
5HT	300 ± 20.1 (6)	not done	314 ± 39 (5)	367 ± 31.9 (5)	290 ± 18 (6)	325 ± 34.3 (6)
5HIAA	121 ± 7.1 (6)	not done	117 ± 8.3 (5)	73.6 ± 34 (6)	85.7 ± 6.2 (6)	67.8 ± 6.0 (6)

Neonatal treatments consisted of 0.05 ml/saline/rat, 20 mg/kg 5HTP and 100 mg/kg PCPA.

TABLE 3

THE EFFECT OF NEONATAL ADMINISTRATION OF 5HTP AND PCPA ON SEXUAL BEHAVIOUR IN ADULT FEMALE RATS

	Treatment over days 1 to 7			Treatment over days 11 to 16		
	Saline	5HTP	PCPA	Saline	5HTP	PCPA
(a) Feminine Behaviour (Mean of 3 tests)						
No. of rats showing Lordosis	21/30 (70%)	12/17 (70.5%)	8/10 (80%)	15/18 (85%)	19/21 (89%)	17/22 (77%)
Lordosis Quotient median (range)	62 (0–100)	68 (0–100)	51 (0–100)	61 (0–199)	76 (0–100)	63 (0–100)
No. showing Ear-wiggling (EW)	28/30 (93%)	17/17 (100%)	10/10 (100%)	15/18 (83%)	19/21 (90%)	17/22 (77%)
Average EW ± SEM	6.8 ± 0.56	8.3 ± 0.50	2.3 ± 0.30‡	2.5 ± 0.19	2.8 ± 0.16	2.4 ± 0.22
(b) Masculine Behaviour (1st test)						
No. rats showing male behaviour	15/30 (50%)	9/17 (53%)	7/10 (70%)	12/18 (67%)	19/21 (90%)	8/22 (36%)†
Mount Latency mean sec ± SEC	361 ± 47	199 ± 8*	221 ± 88	93 ± 52	73 ± 17	245 ± 4.2‡
No. of Mounts	27.6 ± 6.3	24.6 ± 5.0	28.9 ± 45	34.6 ± 5.0	39.2 ± 0.89	19.8 ± 3.2†
No. of Intromission-like movements	0.81 ± 0.48	1.3 ± 0.64	0.9 ± 0.41	4.3 ± 0.93	3.0 ± 0.89	2.1 ± 0.40

Neonatal treatments consisted of 0.05 ml/rat saline, 20 mg/kg 5HTP an 100 mg/kg PCPA.
Significance of difference between saline and treated groups days 1 to 7: *$p<0.05$; ‡$p<0.001$ (t-test).
Significance of difference between saline and treated groups days 11–16:
No. of rats showing male behaviour, ‡$p<0.001$ (Chi^2 test).
Mount latency, ‡$p<0.001$ ($F(5,58)=6.5$) (Scheffe's test after one way analysis of variance).
Number of mounts, †$p<0.01$ ($F(2,58)=6.64$) (Scheffe's test after one way ahalysis of variance).

Experiment 3

The litters of 33 mothers were randomised and culled as above and treated either over days 1 to 7 or 11 to 16 after birth with pharmacological agents known to augment or deplete 5HT (details below). Pups were weaned on day 21 and the sexes separated and housed in cages of 6 to 8 in a reversed lighting environment (lights off 11.00–23.00 hr). All the rats were weighed weekly and the time of vaginal opening noted in the females. Vaginal smears were taken daily from the day of vaginal opening for the following 6 weeks. A test for feminine sexual behaviour was then carried out on the late afternoon of proestrus and the following day the rats were ovariectomized under Halothane anaesthesia (May & Baker & Co. Ltd., Kent); this occurred between days 80 and 93 of life. Three further tests for feminine sexual behaviour were then carried out after suitable hormone treatment (see the Behavioural tests section, below), at weekly intervals starting 3 weeks after ovariectomy. At the end of all their tests for feminine activity, silastic implants containing testosterone (T) were placed subcutaneously into each animal (10 mm long; 1.5 mm internal diameter; Dow Corning tubing 602-285, Midland, MI) and they were tested twice for masculine behaviour with a one week interval between tests.

When all the tests of the females were completed, testing of the males started; at this time the animals were 20 weeks old. The males were subjected to two tests for masculine sexual behaviour with a one week interval, and then they were castrated under Halothane anaesthesia and a testosterone silastic implant was placed under the skin (parameters as described above). Three weeks later testing re-commenced and the animals received 3 tests with a one week interval between each test. The testosterone implant was then removed and the castrated males were tested for feminine behaviour after suitable hormone treatment (see the Behavioural tests section, below); they received 3 tests with one week intervals between each test.

Experiment 4

The litters of 24 mothers were randomised and culled as described above and the pups of 6 litters for each treatment were injected subcutaneously on day 1 of life with either 0.05 ml corn oil, 125,250 or 500 μg of testosterone propionate (TP; Sigma Chemical Co. Ltd., Dorset, UK) dissolved in 0.05 ml corn oil. Two litters at each hormone level were also given the neonatal treatments described below over days 1–7 after birth. Weaning, housing and vaginal smears were carried out as described for Experiment 3. At 12 weeks all the females were ovariectomized and tested for feminine and then masculine behaviour as described for Experiment 3. The males were not used in this experiment.

Neonatal Treatments

The litters were injected daily subcutaneously either between days 1–7 or 11–16 after birth (day of birth=day 0). They received either 0.05 ml saline (0.9% w/v NaCl) or 20 mg/kg 5-hydroxytryptophan (5HTP; Sigma Chemical Co. Ltd., Dorset, UK) or 100 mg/kg or 150 mg/kg p-chlorophenylalanine methyl ester (PCPA; Sigma Chem. Co. Ltd., Dorset, UK).

In Experiment 2 each treatment was administered to 2 litters. In Experiment 3 saline was given to 8 litters days 1–7

TABLE 4

THE EFFECT OF NEONATAL ADMINISTRATION OF 5HTP AND PCPA ON SEXUAL BEHAVIOUR IN ADULT MALE RATS

	Treatment over days 1–7			Treatment over days 11–16		
	Saline	5HTP	PCPA	Saline	5HTP	PCPA
(a) Masculine Behaviour (2nd test)						
No. of rats showing male behaviour	18/25 (72%)	11/14 (78%)	9/11 (81%)	17/20 (86%)	18/20 (90%)	13/22 (59%)
Mount Latency sec ± SEM	168.5 ± 45	94 ± 17	65 ± 20†	107 ± 25	216 ± 42	157 ± 67
No. of Mounts mean ± SEM	7.6 ± 2.5	3.9 ± 1.0	9.9 ± 2.0	21 ± 3.0	25 ± 5.6	14 ± 9.0*
No. of Intromissions mean ± SEM	9.1 ± 1.6	13.3 ± 2.6	8.6 ± 0.9	7 ± 0.9	8 ± 1.0	6 ± 1.0
Ejaculatory Latency sec ± SEM	535 ± 95	519 ± 96	489 ± 37	556 ± 80	547 ± 75	388 ± 62
Refractory Period sec ± SEM	338 ± 18	355 ± 10	333 ± 20	304 ± 11	315 ± 12	314 ± 14
(b) Feminine Behaviour (Mean of 3 tests)						
No. showing Lordosis behaviour	14/25 (56%)	9/14 (60%)	6/11 (54.5%)	13/20 (65%)	16/21 (76%)	6/23* (26%)
Lordosis Quotient	18.5 (0–75)	21 (0–100)	17 (0–73)	23 (0–100)	23 (0–100)	5† (0–85)
No. showing Ear-wiggling	8/25 (32%)	5/14 (36%)	3/10 (27%)	6/20 (30%)	6/21 (28%)	1/23 (4%)
av. EW ± SEM	1.6 ± 0.44	2.6 ± 0.68	1.0 ± 0.70	1.4 ± 0.5	1.6 ± 0.8	1.0

Neonatal treatments consisted of 0.05 ml/rat saline, 20 mg/kg 5HTP an 100 mg/kg PCPA.
Significance of difference between saline and treated groups days 1 to 7: †$p<0.01$ (t-test).
Significance of difference between saline and treated groups days 11 to 16:
Number of mounts. *$p<0.05$ ($F(2,61)=4.1$) (Scheffe's test after one way analysis of variance).
No. showing female behaviour, *$p<0.05$ (Chi^2 test).
Lordosis Quotient, ‡$p<0.001$ ($F(2,61)=6.6$) (Levy's Multiple Comparison test after Kruskall Wallis one way analysis of variance).

and to 4 litters days 11–16; 5HTP was given to 6 litters days 1–7 and to 4 litters days 11–16 and 100 mg/kg PCPA was given to 3 litters days 1–7 and to 6 litters days 11–16. In Experiment 4 each treatment was given to 2 litters at each concentration of the neonatal testosterone treatment.

Behavioural Tests

Feminine behaviour. Feminine sexual activity was tested 4 hours into the dark period under red illumination by placing animals with sexually vigorous intact males and noting their lordotic responses to 20 mounts. The results were expressed as a lordosis quotient (LQ) = [(No. of lordoses)/(No. of mounts)] × 100. Soliciting behaviour was noted by observing the occurrence of ear-wiggling, hopping and darting. Intact females were tested on the late afternoon of proestrus. Ovariectomised females were primed with 2 μg oestradiol benzoate (OB) plus 0.5 mg progesterone (P) given 48 and 4 hours before testing respectively, in 0.1 ml corn oil subcutaneously. Castrated males were primed with 20 μg OB plus 0.5 mg P given 48 and 4 hours before testing respectively.

Masculine behaviour. Male sexual behaviour was observed by placing each animal in an arena (approximately 2 feet in diameter) and 5 minutes later introducing an ovariectomized female made sexually receptive by a subcutaneous silastic implant of OB (7 mm long, 1.5 mm internal diameter, Dow Corning tubing 602-285 Midland, MI). The latency to mount and to ejaculate and the length of the post-ejaculatory refractory period was noted, as well as the number of mounts and intromissions preceding ejaculation. Tests were terminated at 15 minutes if no activity was exhibited, and at 30 minutes if ejaculation had not occurred.

Indole Compound Assays

The hypothalami of pups killed between days 8 and 17 of life and of adults autopsied at the end of the experiment were assayed for 5HT and 5HIAA by the fluorescent assay of Curzon and Green [6]. The recovery for 5HT and 5HIAA was 105% and 76% respectively and the intra- and interassay coefficients of variance was 6.0% and 10.2% for 5HT and 4.3% and 17.7% for 5HIAA.

Statistics

The number of animals showing particular activities in each group was compared by the Chi^2 test.

Parametric results were tested for significant differences by Scheffe's test after one-way analysis of variance, and nonparametric results by Levy's Multiple Comparison test after Kruskal Wallis one-way analysis of variance. In Experiment 4 co-analysis of variance for quantal bioassays was used to assess the slopes of the dose response curves and the significance of their displacement [10].

TABLE 5

EFFECT OF 5HTP AND PCPA INJECTED OVER DAYS 1 TO 7 ON SEXUAL BEHAVIOUR IN ADULT ANDROGENIZED FEMALES

Neonatal TP treatment	Saline days 1–7	5HTP days 1–7	PCPA days 1–7
(a) Masculine Behaviour	No. of animals showing mounting behaviour		
—	0/9 (0%)	3/11 (27%)	2/14 (14%)
125 μg	5/9 (55%)	2/9 (22%)	5/15 (33%)
250 μg	7/12 (58%)	3/10 (30%)	4/13 (13%)
500 μg	12/18 (67%)	7/22 (32%)	6/15 (40%)
(b) Feminine Behaviour	No. of animals showing lordotic behaviour		
—	9/9 (100%)	11/11 (100%)	15/15 (100%)
125 μg	4/11 (37%)	6/11 (54.5%)	3/15 (20%)
250 μg	2/10 (20%)	4/12 (33%)	1/14 (7%)
500 μg	1/18 (5%)	2/22 (9%)	0/15 (0%)

Neonatal treatments consisted of 0.05 ml/rat saline, 20 mg/kg 5HTP, 100 mg/kg PCPA.

RESULTS

Experiment 1: Changes in Levels of Indole Compounds in Brain Areas Over Days 10–16 (Fig. 1)

Figure 1a, b, c show changes in 5HT and 5HIAA levels in the hypothalami, amygdala and midbrain areas in males and females over days 10 to 16. In the hypothalamus, 5HT levels were significantly higher in the female than in the male on days 10 and 14 and female 5HIAA levels were also higher on day 14. Although very few samples were assayed (samples were lost in the dissection), 5HIAA levels were significantly lower in the midbrain area of the females compared to males on day 14. There were no significant sex differences in indole levels in the amygdala during this period.

Experiment 2: The Effect of Neonatally Administered 5HTP and PCPA on Hypothalamic Indole Activity (Table 1)

Table 1 shows that daily injections of 5HTP significantly raised hypothalamic 5HT concentration in both sexes the day after the end of treatment, over either days 1 to 7 or 11 to 16. Over the former period 5HIAA levels were also significantly increased. Daily injections of PCPA over the two periods of treatment reduced hypothalamic 5HT in both sexes. PCPA (100 mg/kg) reduced 5HIAA concentration in males treated over days 1–7 and in both sexes treated over days 11–16, but had no effect on 5HIAA levels in females treated on days 1–7. The higher dose of 150 mg/kg PCPA was also ineffective in reducing 5HIAA levels.

Experiment 3: The Effect of Neonatally Administered 5HTP and PCPA on:

(a) Growth and Development (Table 2). PCPA (150 mg/kg) given over days 1–7 had a deleterious effect on the pups, which were underweight and sickly; no further work was carried out at this dose. 5HTP (20 mg/kg) and 100 mg/kg PCPA administered over days 1–7 and 11–16 had no significant effect on growth rates. Table 2 shows that at 19 weeks of age, the body weight of the rats in the treated groups were not significantly different from the relevant saline-treated controls. The treatments had no significant effect on the day of vaginal opening or the percentage of female rats showing normal oestrous cycles. Attempts were made to smear the

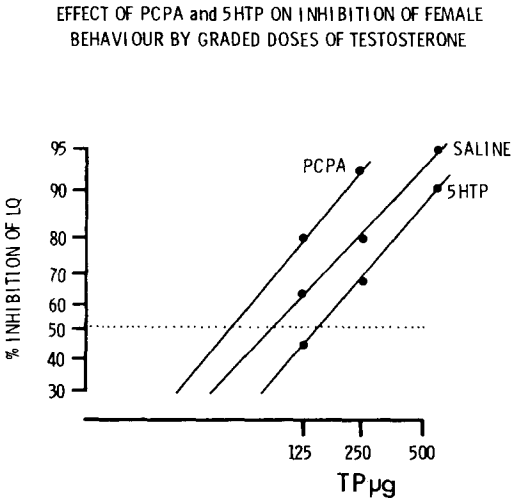

FIG. 2. Females were treated with testosterone proprionate on day 1 of life. The dotted line indicates the dose inhibiting Lordosis Quotients (LQ) by 50% (ID_{50}).

neonatally androgenized females; but of the 128 females treated with TP on day 1, the vagina opened in only 4 animals before the 9th week of life (i.e., 3%). Vaginal opening in these 4 rats was delayed and occurred between the 7th and 8th week of life; and after that they were in constant oestrus. (Three of the 4 rats had in addition been treated with PCPA.) The neonatal treatment with TP (at all doses) produced visible masculinization of the female phallus in approximately 50% of the animals.

The neonatal manipulation of brain 5HT had no effect on hypothalamic indole concentrations in adulthood.

(b) Sexual behaviour in females (Table 3). When the intact females were tested for receptivity on the day of proestrus, they all showed the maximum lordosis quotient (LQ) of 100%. After ovariectomy and steroid priming, the LQ was similar in all the groups (Table 3a shows the mean of 3 tests). Even though all the females exhibited ear-wiggling, PCPA treatment over days 1 to 7 significantly reduced its frequency, indicating a reduction in soliciting behaviour.

The results of the first test for masculine behaviour in the females are shown in Table 3b, as too few animals exhibited mounting behaviour in the second test. Neonatal treatment over days 1 to 7 of life had no effect on the number of females showing masculine behaviour, nor on the number of mounts and number of times the females exhibited intromission-like patterns of movement. However 5HTP over this period significantly reduced the latency to first mount. 5HTP treatment over days 11 to 16 had no effect on any of the parameters, but PCPA over this period significantly reduced the number of females showing masculine behaviour and reduced the level of activity in these animals, as they had a significantly longer mount latency and a reduced number of mounts.

(c) Sexual behaviour in males (Table 4). The neonatal treatments had no effect on masculine sexual behaviour in intact males and so the results are not shown. Table 4a shows masculine activity in the rats after castration and testosterone replacement. Only the results of the second tests are shown, as in the first test only 30% of the anixals exhibited any behaviour, and the results of the third test were similar to those shown. The neonatal treatment with 5HTP over days 1 to 7 or 11 to 16 had no effect on masculine behaviour in adulthood, while PCPA treatment had a small stimulatory effect; in the group treated over days 1 to 7 mount latency was significantly reduced and in the rats treated over days 11 to 16 there were significantly fewer mounts preceding ejaculation.

5HTP treatment had no effect on feminine sexual activity in the males, nor did PCPA given on days 1 to 7. However, when PCPA was given over days 11 to 16, there was a significantly reduced number of males exhibiting lordotic responses.

Experiment 4: The Effect of Neonatally Administered 5HTP and PCPA on Behaviour in Androgenized Females (Table 5 and Fig. 2)

Treatment with 125, 250 and 500 μg TP induced just over half the rats to exhibit masculine sexual behaviour and treatment with either PCPA of 5 HTP had no significant effect on this, although there was a tendency for both treatments to reduce the percentage of rats showing masculine activity. In the females treated neonatally with 125 μg TP, PCPA significantly reduced the number of mounts with and without intromission patterns in those females showing masculine activity; (saline M + I (5) 57.4±12.9; 5HTP M + I (2) 99.5 (76–123); PCPA M + I (5) 28.4±10.2; (number) mean ±SEM). The PCPA treated group was significantly different from the other groups, $p<0.05$, $F(2,9)=5.2$.

Neonatal TP reduced the percentage of female rats showing lordotic activity in a dose-dependent manner. Figure 2 shows the dose-response curve was displaced to the left and right by PCPA and 5HTP treatments respectively, which meant that PCPA enhanced and 5HTP antagonised the defeminizing effect of TP. The PCPA and 5HTP dose response curves were parallel with each other and so the displacement between them could be estimated and was found to be significant ($p<0.05$). The neonatal dose of TP inhibiting female behaviour in 50% of the animals (ID_{50}) was 135±37 μg/rat in the 5HTP-treated group and 36.5±15.4 μg/rat in the PCPA-treated group; these were significantly different ($p<0.05$). The slopes of the dose-response curves of both the 5HTP and PCPA treated groups were significantly different from that of the saline-treated group ($p<0.05$) indicating that both treatments had a significant effect on the action of neonatal testosterone.

DISCUSSION

Reports in the literature have shown that there is a significant sexual difference in 5HT brain concentration over days 10 to 14 of life, particularly in the hypothalamus and preoptic area [12, 13, 26, 36]. We have also shown a sex difference in hypothalamic 5HT concentration with significantly higher levels in the female on days 10 and 14; the reason for the lack of a significant difference on day 12 is not clear. 5HIAA did not change dramatically in the female over days 10 to 16, but on day 14 there was a significant fall in the males, indicating that while 5HT synthesis rose around day 14 in females, the release of 5HT was inhibited in males. Midbrain 5HIAA concentration was lower in females than in males on day 14. This may be due to an autoregulatory effect of increased hypothalamic activity, since the midbrain and hypothalamus contain the cell bodies and nerve terminals respectively, of the same serotonergic tract (see [36]). Wilson and Agrawal [39] found lower levels of 5HT in the neonatal female midbrain. There was no sexual difference in the amygdala area confirming previous findings [13].

In order to investigate the physiological significance of the sexual difference in indoleamine activity in the neonatal brain, 5HT concentrations were manipulated over the first week of life when testosterone induces sexual differentiation of the hypothalamus and then over days 11 to 16 which is the period covering the sexual dimorphism in hypothalamic 5HT levels. The treatment consisting either of 5HTP (the precursor of 5HT) or PCPA (an inhibitor of 5HT synthesis; [25]) induced significant changes in 5HT levels in the expected way in all the groups. 5HIAA was also altered as expected, except there was no significant change after 100 mg/kg PCPA on days 1–7 or after 20 mg/kg 5HTP on days 11–17. It was not possible to use a higher dose of PCPA since 150 mg/kg was toxic, but it is possible that the dose of 5HTP was too low since others have used 100 mg/kg 5HTP without ill effect [13]. The treatments did not affect growth in either sex, although there was a tendency for the PCPA treated males to be heavier than the controls. Hole [20] has shown that PCPA treatment over the first 7 weeks of life induces a significant increase in body weight after week 9. In the females vaginal opening and oestrous cycles were not affected by the treatments; others have reported that PCPA delays vaginal opening, but has no effect on subsequent cyclicity [9,23]. The treatments had no effect on the androgenizing action of exogenous neonatal testosterone in delaying vaginal opening or inducing masculinization of the phallus.

Manipulation of hypothalamic 5HT activity over days 1 to 7 or 11 to 16 had no effect on feminine sexual behaviour in females. The only significant change noted was a reduction in soliciting behaviour (average frequency of ear wiggling) in females treated with PCPA; and this confirms the work of Hyyppa *et al.* [23]. The lack of effect of 5HTP confirms the results of Gladue *et al.* [13] who administered a higher dose of 5HTP (100 mg/kg) over days 9 to 11 of life. 5HTP had no effect on masculine behaviour in males, but PCPA had a slight stimulatory effect in that it reduced ML (when administered on days 1 to 7) and decreased number of mounts to ejaculation (when administered on days 11 to 16). This increase in activity was not as marked as that noted by Hyyppa *et al.* [23] when PCPA was given over days 2 to 6.

Observations on the heterotypical sexual behaviour in

males and females showed that PCPA had an inhibitory effect in both sexes, while 5HTP appeared to stimulate masculine behaviour in females (since the ML was reduced) and had no effect on feminine behaviour in males. Hole [20] has shown that PCPA treatment over the first 7 weeks of life reduces arousal in a variety of behavioural tests. The reduction in heterotypical sexual activity in the PCPA treated groups may, therefore, be due to a reduced state of arousal, while 5HTP may have caused some increase in arousal. This change in arousal state by the neonatal treatments is presumably not sufficient to influence homotypical sexual activity.

In homotypical sexual activity, neonatal 5HT does not appear to have any influence on the development of feminine behaviour and seems to be inhibitory for masculine behaviour. Possibly the fall in 5HT activity on day 14 in males specifically removes an antagonistic influence on the androgenizing and defeminizing activity of neonatal testosterone, while the rise in 5HT activity in the females may have a protective effect against any circulating testosterone. In order to see if 5HT and testosterone are mutually antagonistic, the effect of neonatal indolamine manipulation on the androgenizing effects of exogenous neonatal testosterone in females was studied. The treatments had the expected effect on the defeminizing activity of testosterone activity respectively. However, both treatments antagonized the masculinizing effects of testosterone. This finding is similar to that of Jarzab and Dohler [24] using tryptophan (a precursor of 5HT) and PCPA. Others have also shown that both 5HTP and PCPA can antagonise the action of neonatal testosterone in inducing constant oestrous [35,37]. Perhaps the unexpected effect of PCPA is specific to this drug and masks any action due to reduction in 5HT levels; for instance it may mimic an effect of stress [7] or affect another neuronal system [5]. 5HTP, on the other hand, exerted the expected effect and antagonised the action of testosterone. This was presumably due to raised levels of 5HT, although an interesting alternative suggestion may be an effect via the β-adrenergic system. Raum and Swerdloff [34] have shown that increased β-adrenergic activity antagonises the masculinizing effects of testosterone. It is also known that serotonergic and β-adrenergic systems have a mutually positive modulatory effect [4,30]. Hypothalamic noradrenaline concentrations (the endogenous β-agonist) are also sexually differentiated in the neonatal period [22] with higher levels in the female on days 5 [31] and 12 [39] of life.

In conclusion, we have confirmed the neonatal sex differences in hypothalamic 5HT and have also shown that 5HIAA levels are lower in the male hypothalamus, indicating that the release of 5HT is reduced in the neonatal male. The hypothesis that raised 5HT activity will induce feminization and reduced 5HT will have a masculinizing effect is not supported by our results, since feminine sexual behaviour was not affected by manipulation of neonatal brain 5HT. Instead, it appears that neonatal 5HT activity may specifically antagonise the effects of neonatal testosterone, since reducing 5HT activity with PCPA over either the first or second week of life enhances the masculinizing effect of endogenous testosterone in males and the defeminizing effect of exogenous testosterone in females; raising 5HT, on the other hand, antagonises the masculinizing and defeminizing effects of exogenous testosterone. As testosterone is not involved in the development of female behaviour, manipulation of neonatal brain 5HT did not affect feminine activity in females that did not receive exogenous testosterone.

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REFERENCES

1. Arai, Y. Synaptic correlates of sexual differentiation. *Trends Neurosci* **4:** 291–293, 1981.
2. Beach, F. A. Hormonal modification of sexually dimorphic behaviour. *Psychoneuroendocrinology* **1:** 3–23, 1975.
3. Booth, J. E. Sexual differentiation of the brain. In: *Oxford Reviews of Reproductive Biology*, vol 1, edited by C. A. Finn. Oxford: Oxford University Press, 1979, pp. 58–158.
4. Brunello, N., M. L. Barbaccia, D.-M. Chuang and E. Costa. Down-regulation of β-adrenergic receptors following repeated injections of desmethylimipramine: permissive role of serotonergic axons. *Neuropharmacology* **21:** 1145–1149, 1982.
5. Coen, C. W., M. C. Coombs, P. M. J. Wilson, E. M. Clement and P. C. B. MacKinnon. Possible resolution of a paradox concerning the use of p-chlorophenyl alanine and 5-hydroxytryptophan: Evidence for a mode of action involving adrenaline in manipulating the surge of luteinizing hormone in rats. *Neuroscience* **8:** 583–591, 1983.
6. Curzon, G. and A. R. Green. A rapid method for the determination of 5-hydroxyindole acetic acid in small regions of the rat brain. *Br J Pharmacol* **39:** 653–655, 1970.
7. Dahlof, G.-G., E. Hard and K. Larsson. Influence of maternal stress on the development of the fetal genital system. *Physiol Behav* **20:** 193–195, 1978.
8. Dorner, G., G. Hinz, F. Docke and R. Tonjes. Effects of psychotrophic drugs on brain differentiation in female rats. *Endokrinologie* **70:** 113–123, 1977.
9. Fajer, A. B., D. Hoffman and E. Shillito. Inhibitory effect of p-chlorophenylalanine on the sexual maturation of female rats. *J Reprod Fertil* **22:** 379–380, 1970.
10. Finney, D. J. *Statistical Methods in Biological Assay*, 3rd edition. High Wycombe: Charles Griffin & Co. Ltd., 1978, pp. 349–368.
11. Gaziri, L. C. J. and W. Ladosky. Monoamine oxidase variation during sexual differentiation. *Neuroendocrinology* **12:** 249–256, 1973.
12. Giulian, D., L. A. Pohorecky and B. S. McEwen. Effects of gonadal steroids upon brain 5-hydroxytryptamine levels in the neonatal rat. *Endocrinology* **93:** 1329–1335, 1973.
13. Gladue, B. A., R. R. Humphrys, J. F. DeBold and L. G. Clemens. Ontogeny of biogenic amine systems and modification of indole levels upon adult sexual behaviour in the rat. *Pharmacol Biochem Behav* **7:** 253–258, 1977.
14. Glowinski, J. and L. L. Iversen. Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. *J Neurochem* **13:** 655–699, 1966.
15. Gorski, R. A., R. E. Harlan, C. D. Jacobson, J. E. Shryne and A. M. Southam. Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. *J Comp Neurol* **193:** 529–539, 1980.
16. Gotz, F. and G. Dorner. Homosexual behaviour in prenatally stressed male rats after castration and oestrogen treatment in adulthood. *Endokrinologie* **76:** 115–117, 1980.
17. Hardin, C. M. Sex differences in serotonin synthesis from 5-hydroxytryptophan in neonatal rat brain. *Brain Res* **59:** 437–439, 1973.
18. Hardin, C. M. Sex differences and the effects of testosterone injections on biogenic amine levels of neonatal rat brain. *Brain Res* **62:** 286–290, 1973.

19. Harlan, R. E., J. H. Gordon and R. A. Gorski. *Sexual Differentiation of the Brain: Implications for Neuroscience*, vol 4, edited by D. M. Schneider. New York: Raven Press, 1979, pp. 31–71.
20. Hole, K. Behaviour and brain growth in rats treated with p-chlorophenylalanine in the first weeks of life. *Dev Psychobiol* **5:** 157–173, 1972.
21. Hull, E. M., J. K. Nishita, D. Bitran and S. Dalterio. Perinatal dopamine-related drugs demasculinize rats. *Science* **224:** 1011–1013, 1984.
22. Hyyppa, M. and U. K. Rinne. Hypothalamic monoamines after the neonatal androgenization, castration or reserpine treatment of the rat. *Acta Endocrinol* **66:** 317–324, 1971.
23. Hyyppa, M., P. Lampiren and P. Lehtinen. Alterations in the sexual behaviour of male and female rats after neonatal administration of p-chlorophenylalanine. *Psychopharmacologia* **25:** 152–161, 1972.
24. Jarzab, B. and K. D. Dohler. Serotonergic influences on sexual differentiation of the rat brain. *Prog Brain Res* **61:** 119–126, 1984.
25. Koe, B. K. and A. Weissman. p-Chlorophenylalanine: a specific depletor of brain serotonin. *J Pharmacol Exp Ther* **154:** 499–516, 1966.
26. Ladosky, W. and L. C. J. Gaziri. Brain serotonin and sexual differentiation of the nervous system. *Neuroendocrinology* **6:** 168–174, 1970.
27. Lauder, J. M., J. A. Wallace, H. Krebs, P. Petrusz and K. McCarthy. In vivo and in vitro development of serotonergic neurons. *Brain Res Bull* **9:** 605–625, 1982.
28. MacLusky, N. G. and F. Naftolin. Sexual differentiation of the central nervous system. *Science* **211:** 1294–1303, 1981.
29. Matsumoto, A. and Y. Arai. Sexual dimorphism in 'wiring pattern' in the hypothalamic arcuate nucleus and its modification by neonatal hormonal environment. *Brain Res* **190:** 238–242, 1980.
30. Nimgaonkar, V. L., A. R. Green, P. J. Cowen, D. J. Heal, D. G. Grahame-Smith and J. F. W. Deakin. Studies on the mechanisms by which clenbuterol, a β-adrenoceptor agonist, enhances 5HT-mediated behaviour and increases metabolism of 5HT in the brain of the rat. *Neuropharmacology* **22:** 739–749, 1983.
31. Ortega-Corona, B. G., N. Esparza-Avalos and A. Sosa. Sex differences in hypothalamic monoamine levels of the neonatal rat. In: *Catecholamines: Basic & Clinical Frontiers*, vol 1, edited by E. Usdin, I. J. Kopin and J. Barchas. New York: Pergamon Press, 1979, pp. 812–814.
32. Raisman, G. and P. M. Field. Sexual dimorphism in the hemophil of the preoptic area of the rat and its dependence on neonatal androgen. *Brain Res* **53:** 1–29, 1973.
33. Ramirez, D. A., H. F. Carrer and A. G. Nasello. Prenatal amphetamine exposure: Ovulation, sexual behaviour and hypothalamic monoamine content in rats. *Pharmacol Biochem Behav* **11:** 605–609, 1979.
34. Raum, W. J. and R. S. Swerdloff. The role of hypothalamic adrenergic receptors in preventing testosterone-induced androgenization in the female rat brain. *Endocrinology* **109:** 273–278, 1981.
35. Reznikov, A. G., N. D. Nosenko and L. P. Demkiv. New evidences for participation of biogenic monoamines in androgen-dependent sexual differentiation of hypothalamic control of gonadotrophin secretion in rats. *Endokrinologie* **73:** 11–19, 1979.
36. Simerly, R. B., L. W. Swanson and R. A. Gorski. Demonstration of a sexual dimorphism in the distribution of serotonin-immunoreactive fibers in the medial preoptic nucleus of the rat. *J Comp Neurol* **225:** 151–166, 1984.
37. Vaughan, M. K., G. H. Vaughan and W. K. O'Steen. Oestrous cycle changes after neonatal injections of 5-hydroxytryptamine in normal and androgen-sterilized female rats. *J Endocrinol* **45:** 141–142, 1969.
38. Watts, A. G. and H. F. Stanley. Indoleamines in the hypothalamus and area of the midbrain in raphe nuclei of male and female rats throughout post-natal development. *Neuroendocrinology* **38:** 461–466, 1984.
39. Wilson, W. E. and A. K. Agrawal. Brain regional levels of neurotransmitter amines as neurochemical correlates of sex-specific ontogenesis in the rat. *Dev Neurosci* **2:** 195–200, 1979.