Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 3, pp. 591–601, 1994

0091-3057/94 $6.00 + .00

Interactions Between 5-Hydroxytryptamine (5-HT) and Testosterone in the Control of Sexual and Nonsexual Behaviour in Male and Female Rats

M. I. GONZALEZ,* F. FARABOLLINI,† E. ALBONETTI† AND C. A. WILSON‡[1]

*Department of Physiology, University of Madrid, Spain
†Institute of Physiology, University of Siena, Siena, Italy
‡Department of Obstetrics & Gynaecology, St. George's Hospital Medical School, London

Received 18 February 1993

GONZALEZ, M. I., F. FARABOLLINI, E. ALBONETTI AND C. A. WILSON. *Interactions between 5-hydroxytryptamine (5-HT) and testosterone in the control of sexual and nonsexual behaviour in male and female rats.* PHARMACOL BIOCHEM BEHAV 47(3) 591–601, 1994.—Two 5-hydroxytryptamine$_2$ (5-HT$_2$) agents, ritanserin and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) (both at 0.25 mg/kg IP), were administered to castrated males bearing graded testosterone implants (empty, 2.5-, 5-, and 10-mm length) and to normal and neonatally androgenized ovariectomized females bearing 10-mm testosterone implants. The results indicate that testosterone stimulates male sexual behaviour and appears to have a dose-related anxiolytic effect, but no effect on other nonsexual activities. 5-HT and testosterone had opposite effects on male sexual behavior, with ritanserin (5-HT antagonist) enhancing activity in both sexes and DOI (5-HT agonist) inhibiting behaviour in males, the latter being testosterone-dependent. Independent of testosterone, ritanserin reduced locomotion and exploration and increased anxiety in males, while DOI increased locomotion and exploration in both sexes. Ritanserin had a gender-specific effect on anxiety which was independent of testosterone, since in castrated males it was anxiogenic whether they bore testosterone implants or not, while in females it was anxiolytic whether the females were neonatally androgenized (250 μg/pup testosterone proprionate [TP] on day 1) or not. These results show that 5-HT and testosterone have opposite effects on male sexual behaviour and these may be interrelated. In adulthood, their effects on nonsexual activities are not inversely related and are independent of each other in contrast to the relationship seen in the neonatal period.

5-Hydroxytryptamine Testosterone Locomotion Exploratory behaviour
Elevated plus-maze test for anxiety Male sexual behaviour

IN previous reports we have shown in the rat an interaction at the neonatal level between testosterone and 5-hydroxytryptamine (5-HT) on the development of systems that control adult behaviour. In particular, manipulation of brain 5-HT activity in the second week postpartum in both males and androgenized females indicated that 5-HT exerts an antagonist effect on the masculinizing action of neonatal testosterone on exploration, locomotion, and sexual behaviour (9,36,38). Thus the occurrence of a transient reduction in hypothalamic 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the second week of life in normal males (23,38) may have a physiological significance in removing an inhibitory influence, so that the organizational actions of testosterone in the brain can occur unimpeded (21,36,38).

In adulthood, male sexual behaviour is dependent on the presence of testosterone (32), and 5-HT (acting on 5-HT$_2$ receptors) reduces male sexual activity (12,17) and so may be acting to inhibit the action of testosterone in adulthood as well as neonatally. We have therefore investigated the possibility that 5-HT may antagonize the activational as well as the

[1] Requests for reprints should be addressed to Professor C. A. Wilson, Robert Barnes Laboratory, Department of Obstetrics & Gynaecology, St. George's Hospital Medical School, London SW17 ORE, UK.

organizational effects of testosterone on sexual behaviour, locomotion, exploration, and anxiety. Testosterone affects several nonsexual behavioral activities including aggression and scent-marking (10). Although it has no effect on open-field activity, direct measurements on the effect of testosterone in adulthood on exploration and anxiety have not been reported as far as we know. In these experiments, the interaction of pharmacological manipulation of 5-HT with testosterone given in adulthood was studied in males, females, and androgenized females.

There are many conflicting reports on the behavioral effects of 5-HT, mainly due to the variety of receptor subtypes that mediate its various effects. In these experiments, we have used a selective 5-HT_2 agonist and antagonist in an attempt to simplify the interpretation of the results.

MATERIAL AND METHODS

Experiment 1 (Males)

Forty Wistar male rats eight weeks old (bred at St. George's Hospital Medical School) were orchidectomized under halothane and nitrous oxide (May & Baker Ltd., Dagenham, UK) and at the same time a silastic implant was placed under the skin of each rat, containing testosterone proprionate (TP; Sigma Chemical Co., Dorset, UK). The TP implants (i.d. 1.5 mm; Dow Corning tubing 602-285, Midland, MI) were either 10.0, 5.0, or 2.5 mm in length filled with TP or empty 10-mm implants. This gave rise to four groups of males with approximately normal (17 pmol/l; 5 ng/ml), half normal, quarter normal, and negligible circulating levels (0.05 pmol/l; 0.18 ng/ml) of T (37).

Experiment 2 (Females)

Eight litters born on the same day to Wistar rats (bred at St. George's Hospital Medical School) were randomized and culled to five males and five females each. On the day of birth, half of the pups received 250 μg/pup TP subcutaneously in 0.1 ml corn oil. The other half received 0.1 ml corn oil. The litters were weaned on day 21. The males were not used in this experiment. On day 60, all the females were ovariectomized under halothane and nitrous oxide. At the same time, a 10-mm silastic implants (i.d. 1.5 mm; Dow Corning tubing 602-285) was placed under the skin of each rat; half of the implants contained TP, the other half were empty.

In both experiments, the animals were housed five of the same group and sex to a cage in a reversed lighting regime (12 : 12 h, light off at 0800) with the temperature maintained at 22°C. The experimental design is shown in Table 1.

Pharmacological Treatment

On days 81–87 after birth (see Table 1), 1 h before behavioural testing half of the animals of each group were injected IP with 0.25 mg/kg ritanserin, a 5-HT_2 antagonist; the other half of the animals were subjected to IP administration of 1 ml/kg saline. Two days later, animals previously treated with ritanserin were injected with saline, while animals previously treated with saline were injected with ritanserin.

On days 88–94 the same treatment schedule was followed but 0.25 mg/kg 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI) was administered instead of ritanserin.

Drug or saline administration occurred about 3 h into the dark cycle.

Behavioural Testing

From days 65 to 80 after birth, animals were familiarized with the experimental setting and the testing apparatus by having each animal placed in the hole-board box and on the elevated plus maze for 5 min on two separate occasions at approximately one-week intervals.

On the day of testing each animal was subjected to hole board, elevated plus maze, and male sexual activity tests in sequence. All the tests were observed 4 h into the dark cycle under red illumination.

Hole board. The animals were placed for 5 min in a black perspex box (60 × 60 × 35 cm) with four holes (3.8 cm diameter) equally spaced on the floor, which was divided into 36 squares. Frequency and duration of head-dipping, locomotion (number of line-crossings), and frequency of rearing were recorded.

Elevated plus maze. The maze consisted of two 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 for 5 min were frequency and duration of open or closed arms visits.

Male sexual activity. The animals were placed in an observation arena (50–60 cm diameter), and 3–4 min later a receptive female (ovariectomized, bearing a 7-mm silastic implant containing oestradiol benzoate, Sigma) was introduced to the arena and the following parameters noted:

1. Time taken between introduction of female and first mount (mount latency, ML).
2. Time taken between introduction of female and first intromission (intromission latency, IL).
3. Number of mounts to ejaculation (M).
4. Number of intromissions (I).
5. Time between first mount and ejaculation (ejaculation latency, EL).
6. Time between ejaculation and the first mount of the next cycle of sexual activity (refractory period, RP).

The test was terminated after the refractory period, after 30 min if there was no ejaculation, or after 15 minutes if there were no mounts.

In experiment 2 with the female rats, only number of mounts was noted, and the test was terminated at 10 min if there were no mounts.

Statistical Analysis

For each behavioural parameter, data were analyzed with multivariate analysis of variance (MANOVA). For males the main factors were 1) testosterone level (TP level) with four levels (10-, 5-, 2.5-mm, and empty implants), and 2) drug treatment with two levels (saline and drug). For females the main factors were 1) neonatal treatment (two levels: TP or oil), 2) pretreatment (two levels: TP or empty implants), and 3) drug treatment (two levels: saline or drug).

For each parameter two distinct analyses were carried out, one with data obtained after treatment with ritanserin and the other with data recorded after treatment with DOI.

When appropriate, post hoc comparisons were carried out with t tests. Paired t tests or t tests for independent samples were used, depending on the groups to be compared ($p < 0.05$).

TABLE 1

TREATMENTS AND TESTING SCHEDULE

Experiment I (Males)			
Day 60 After Birth	Days 65–80	Days 81–87	Days 88–94
(9) ORCH + T10 (9) ORCH + T5 (10) ORCH + T2.5 (10) ORCH + Empty	Familiarization with behavioral test apparatus	Ritanserin + Behavioral testing	DOI + Behavioral testing

Experiment II (Females)				
Day 1	Day 60 After Birth	Days 65–80	Days 81–87	Days 88–94
TP	(10) OVX + T10 (10) OVX + Empty	Familiarization with behavioral test apparatus	Ritanserin + Behavioral testing	DOI + Behavioral testing
Oil	(10) OVX + T10 (10) OVX + Empty			

Number of rats in each group in parentheses. ORCH = orchidectomised; T10, T5, T2.5 = testosterone proprionate implants of 10-, 5-, and 2.5-mm length; Empty = 10-mm empty implant, OVX = ovariectomised.

RESULTS

Experiment 1 (Males)

Hole board test. The main factor TP level was not significant for any of the hole board parameters, nor was there any interaction between testosterone and drug treatments (Table 2). On the contrary, the main factor drug treatment showed some significances with both drugs. Ritanserin reduced locomotion, head-dipping frequency, and head-dipping duration with no effect on rearing (Tables 2 and 3). Although the interaction between drug treatment and TP level was not significant, it is of interest that these inhibitory effects were clearly not present in the T10 group.

DOI increased Head-Dipping frequency and Head-Dipping duration and reduced Rearing, but had no effect on Locomotion (Tables 2, 3).

TABLE 2

THREE-WAY ANOVA (F and p Values) APPLIED TO MALES (Ritanserin and DOI): HOLE BOARD AND PLUS-MAZE TEST

	Hole Board				Plus Maze		
	Locomotion	H-D Frequency	H-D Duration	Rearing	% Time in Open Arms	No. Open Entries	Total Entries
Ritanserin							
Main factors							
1) T Level	0.94	0.15	0.84	0.21	1.21	2.04	0.54
(df = 3, 34)	NS	NS	NS	NS	NS	NS	NS
2) Drug treatment	11.6	9.6	4.7	0.00	1.24	0.03	3.23
(df = 1, 34)	$p < 0.001$	$p < 0.004$	$p < 0.03$	NS	NS	NS	NS
Interaction							
1 × 2	2.03	0.75	0.95	0.49	1.5	2.91	1.23
(df = 3, 34)	NS	NS	NS	NS	NS	$p < 0.05$	NS
DOI							
Main factors							
1) T Level	1.96	0.15	0.7	1.39	2.23	2.46	1.39
(df = 3, 34)	NS	NS	NS	NS	NS	NS	NS
2) Drug treatment	0.35	6.87	14.1	8.07	0.19	0.72	0.07
(df = 1, 34)	NS	$p < 0.01$	$p < 0.006$	$p < 0.008$	NS	NS	NS
Interaction							
1 × 2	1.65	0.01	0.13	0.23	0.29	0.54	1.11
(df = 3, 34)	NS	NS	NS	NS	NS	NS	NS

H-D = head-dipping, T Level = testosterone level (i.e., size of testosterone implant), Drug Treatment = saline, DOI, or ritanserin.

TABLE 3

ACTIVITY OF MALE RATS IN THE 5-MIN HOLE BOARD TEST

TP Level	Treatment	Locomotion (no. of squares crossed)	Head-Dipping		Rearing Frequency
			Frequency	Duration (seconds)	
Empty	Sal	142.5 ± 13.7	8.6 ± 1.2	15.4 ± 2.5	12.4 ± 1.9
(10)	Rit	106.1 ± 21.8	5.2 ± 1.2*	9.7 ± 2.5*	8.1 ± 2.0
T2.5	Sal	180.5 ± 26.1	8.3 ± 1.7	13.1 ± 3.1	15.9 ± 2.7
(10)	Rit	130.9 ± 19.0	5.3 ± 0.9	9.1 ± 1.8	9.7 ± 1.6
T5	Sal	182.1 ± 12.6	8.4 ± 1.3	16.3 ± 3.8	17.3 ± 2.8
(9)	Rit	133.7 ± 13.6	6.8 ± 0.9	15.9 ± 3.6	11.8 ± 2.2
T10	Sal	147.4 ± 22.8	7.3 ± 1.4	13.8 ± 2.9	17 ± 3.5
(9)	Rit	155.9 ± 23.3	6.7 ± 0.9	13.8 ± 2.5	14.8 ± 2.8
Empty	Sal	132.0 ± 17.8	8.5 ± 1.5	17.1 ± 4.0	11.9 ± 18
(10)	DOI	153.5 ± 12.3	10.4 ± 1.2	25.3 ± 2.6	13.3 ± 1.8
T2.5	Sal	188.7 ± 20.7	9.1 ± 1.3	17.6 ± 3.8	23.5 ± 2.0
(10)	DOI	182.9 ± 22.7	11.1 ± 1.8	24.7 ± 4.8	16.2 ± 2.3
T5	Sal	181.7 ± 15.7	8.1 ± 1.0	13.5 ± 2.4	17.5 ± 2.8
(9)	DOI	154.5 ± 17.6	8.9 ± 1.8	25.6 ± 5.7	11.5 ± 2.5
T10	Sal	174.3 ± 19.1	8.4 ± 1.4	12.7 ± 2.8	19.3 ± 2.9
(9)	DOI	194.3 ± 26.8	10.5 ± 1.5	14.9 ± 2.6	18.4 ± 2.3

All results expressed as mean ± SE. Number of rats in each group in parentheses. T10, T5, T2.5 = testosterone proprionate implants of 10-, 5-, or 2.5-mm length; Sal = saline 1 ml/kg IP; Rit = ritanserin 0.25 mg/kg IP; DOI 0.25 mg/kg IP. *$p < 0.05$ (paired t test).

TABLE 4

ACTIVITY OF MALE RATS IN THE 5-MIN ELEVATED PLUS-MAZE TEST

T Level	Treatment	Open Arms		No. Total Entries
		% Time	No. Entries	
Empty	Sal	6.1 ± 2.7	2.0 ± 0.8	9.3 ± 1.5
(10)	Rit	2.2 ± 1.3	0.9 ± 0.5	7.6 ± 2.2
T2.5	Sal	13.9 ± 5.8	3.2 ± 0.9	10.5 ± 1.6
(10)	Rit	6.6 ± 2.5	2.4 ± 0.8	7.8 ± 1.6
T5	Sal	14.4 ± 4.2	3.3 ± 0.9	11.3 ± 1.5
(9)	Rit	6.2 ± 2.7	2.0 ± 0.6	10.4 ± 0.5
T10	Sal	14.1 ± 5.9	3.3 ± 1.2	9.5 ± 1.9
(9)	Rit	14.0 ± 3.8	3.7 ± 0.9	10.3 ± 1.5
Empty	Sal	14.1 ± 3.7	3.7 ± 0.7	9.7 ± 1.0
(10)	DOI	17.8 ± 5.0	4.2 ± 0.9	10.8 ± 1.1
T2.5	Sal	25.5 ± 3.6	6.1 ± 0.9	13.4 ± 1.7
(10)	DOI	26.2 ± 5.8	6.5 ± 1.3	13.6 ± 1.5
T5	Sal	19.4 ± 6.5	4.3 ± 1.2	12.2 ± 1.1
(9)	DOI	17.2 ± 6.7	3.2 ± 1.1	9.8 ± 1.4
T10	Sal	28.8 ± 7.1	6.1 ± 1.5	13.2 ± 2.0
(9)	DOI	36.5 ± 7.8	6.5 ± 1.5	14.3 ± 2.5

All results expressed as mean ± SE. Number of rats in each group in parentheses. T10, T5, T2.5 = testosterone proprionate implants of 10-, 5-, or 2.5-mm length; Sal = saline 1 ml/kg IP; Rit = ritanserin 0.25 mg/kg IP; DOI 0.25 mg/kg IP.

Elevated plus-maze test. MANOVA did not show any significance for the two main factors TP level and drug treatment on the parameters measured in the plus maze. In the case of ritanserin, however, there was a significant interaction between the two factors for the number of entries into open arms, an indication that the treatment had different effects according to TP level (Table 2). Ritanserin decreased number of entries in all groups except T10 (Table 4).

Masculine sexual behaviour. While the presence of testosterone proved to be essential for the occurrence of male sexual activity, as shown by its absence in the empty implant group (Table 5), MANOVA indicated that increasing levels of T did not influence the various parameters of sexual behaviour in the ritanserin experiment. This is shown by the lack of significance of the factor TP level (Table 6). The main factor drug treatment was significant for the number of mounts (Table 6) due to an overall decrease in the number of mounts independent of the level of testosterone (Table 5). Ritanserin also reduced the latencies to intromission and ejaculation in the T2.5 group as indicated by comparison of the saline and ritanserin treatments by paired *t* tests (Table 5).

As indicated by the significance of the main factor drug treatment, DOI had an overall effect in increasing intromission latency independent of TP level (Tables 5 and 6). In the case of ejaculatory latency and refractory period, a significant interaction between drug treatment and TP level was found (Table 6), suggesting a differential effect of the drug according to the TP level. Paired *t* tests applied to saline and DOI for each TP level showed that both parameters were increased by DOI in the group bearing the 5-mm TP implant, but not in the other groups (Table 5).

Experiment 2 (Females)

Hole board test. As indicated by the significance of the main factor neonatal for both the ritanserin- (Table 7) and DOI-treatment experiments (Table 8), neonatal testosterone increased (see Table 9) head-dipping frequency and rearing independently of the pretreatment and treatments.

In both sets of experiments, testosterone implants in adulthood (pretreatment) significantly increased rearing independently of the neonatal androgenization (Table 9), as indicated by the significance of the main factor pretreatment and the lack of interaction with the factor neonatal (Tables 7 and 8). The pretreatment with testosterone also increased locomotion: This result was, however, confined to subjects treated with DOI (Tables 8 and 9) and was mainly due to the effect of the drug.

As for drug treatments, ritanserin had no effect on any of the parameters (Tables 7 and 9). DOI increased locomotion and head-dipping frequency and duration independently of neonatal androgenization and subsequent pretreatment, as indicated by the significance of the main factor drug treatment in absence of any interaction (Tables 8 and 9).

Elevated plus-maze test. MANOVA did not show any significance for the main factors neonatal and pretreatment in the experiment with ritanserin (Table 7): This indicates that neither neonatal treatment with testosterone nor its implant in adulthood has an effect on the parameters measured in this test.

In the experiment with DOI (Table 8) the main factor neonatal was found to be significant in the absence of any interaction: Therefore neonatal testosterone increased the number of total entries independently of subsequent pretreatment and treatment.

In the case of ritanserin (Table 7), MANOVA also showed a significance for the factor drug treatment for percentage time and entries into the open arms due to overall increased levels of the two parameters independently of previous manipulations (neonatal and pretreatment) (Table 10). DOI had no effect on any of the parameters (Tables 8 and 10).

TABLE 5

MALE SEXUAL BEHAVIOUR IN MALE RATS

TP Level	Treatment	Mounts		Intromissions		Ejaculation Latency (s)	Refractory Period (s)
		Latency (s)	Number	Latency (s)	Number		
Empty	Sal	900 ± 0	0 ± 0	900 ± 0	0 ± 0	–	–
(10)	Rit	900 ± 0	0 ± 0	900 ± 0	0 ± 0	–	–
T2.5	Sal	31.3 ± 10.6	13.9 ± 4.1	48.7 ± 14.4	12.4 ± 2.5	313.9 ± 83.4	273.3 ± 18.0
(10)	Rit	9.0 ± 2.2	7.2 ± 1.8	12.5 ± 2.2*	7.6 ± 0.8	105.8 ± 16.7*	251.5 ± 26.8
T5	Sal	5.9 ± 1.7	11.5 ± 2.7	14.1 ± 5.5	13.8 ± 2.4	274.4 ± 62.8	271.9 ± 23.1
(9)	Rit	7.7 ± 4.1	6.8 ± 1.2	11.8 ± 5.1	13.1 ± 1.7	245.2 ± 57.0	327.9 ± 21.6
T10	Sal	3.3 ± 0.5	7.1 ± 2.3	13.7 ± 5.8	11.5 ± 2.4	195.9 ± 58.4	313.0 ± 18.3
(9)	Rit	4.1 ± 1.7	6.5 ± 1.6	6.2 ± 0.8	8.7 ± 1.7	116.8 ± 29.0	323.0 ± 9.7
Empty	Sal	900 ± 0	0 ± 0	900 ± 0	0 ± 0	–	–
(10)	DOI	900 ± 0	0 ± 0	900 ± 0	0 ± 0	–	–
T2.5	Sal	22.3 ± 5.9	12.1 ± 3.5	51.2 ± 17.3	10.5 ± 1.8	273.9 ± 55.6	285.7 ± 23.7
(10)	DOI	55.2 ± 15.4	10.1 ± 4.2	85.7 ± 40.9	6.2 ± 1.6	866.2 ± 258	486.8 ± 97.0
T5	Sal	7.0 ± 1.8	10.6 ± 4.1	15.5 ± 4.9	12.7 ± 1.3	282.4 ± 49.0	305.8 ± 21.4
(9)	DOI	31.7 ± 11.8	25.0 ± 8.9	42.0 ± 13.8	10.5 ± 2.5	1178.1 ± 268.2*	661.7 ± 101.0*
T10	Sal	4.2 ± 0.7	5.0 ± 1.1	8.8 ± 2.1	8.1 ± 1.6	132.0 ± 29.9	298.5 ± 17.3
(9)	DOI	4.7 ± 0.5	5.0 ± 1.7	9.2 ± 1.1	9.1 ± 1.5	128.7 ± 29.0	291.5 ± 13.7

All results expressed as mean ± SE. Number of rats in each group in parentheses. T10, T5, T2.5 = testosterone proprionate implants of 10-, 5-, or 2.5-mm length; Sal = saline 1 ml/kg IP; Rit = ritanserin 0.25 mg/kg IP; DOI 0.25 mg/kg IP. *$p < 0.05$ (paired *t* test).

TABLE 6

THREE-WAY ANOVA (F and p Values) APPLIED TO MALES (Ritanserin and DOI): SEXUAL BEHAVIOUR TEST

	Mounts		Intromissions		Ejaculation Latency	Refractory Period
	Latency	Number	Latency	Number		
RITANSERIN						
Main factors						
1) T Level	1.33	0.77	1.51	1.03	1.55	0.6
(df = 2, 24)	NS	NS	NS	NS	NS	NS
2) Drug treatment	1.04	6.85	0.73	0.01	0.24	3.47
(df = 1, 24)	NS	$p < 0.01$	NS	NS	NS	NS
Interaction						
1 × 2	0.57	1.27	0.42	2.87	0.48	1.72
(df = 2, 24)	NS	NS	NS	NS	NS	NS
DOI						
Main factors						
1) T Level	1.77	2.94	0.32	2.5	6.33	5.18
(df = 2, 24)	NS	NS	NS	NS	$p < 0.003$	$p < 0.02$
2) Drug treatment	3.99	1.59	6.94	1.83	18.02	14.5
(df = 1, 24)	NS	NS	$p < 0.01$	NS	$p < 0.0003$	$p < 0.0008$
Interaction						
1 × 2	0.95	1.45	1.92	1.06	4.08	4.13
(df = 2, 24)	NS	NS	NS	NS	$p < 0.03$	$p < 0.02$

T Level = testosterone level (i.e., size of testosterone implant), Drug Treatment = saline, DOI, or ritanserin.

Masculine sexual behaviour. Neonatal testosterone and pretreatment with a testosterone implant increased masculine sexual activity (number of mounts) in females as indicated by the significance of the main factors neonatal and pretreatment in the MANOVA in both sets of experiments (Tables 7, 8, and 11). In the experiment with DOI there was also an interaction between the two factors (Table 8): The increase in number of mounts in the TP implant with respect to empty implant

TABLE 7

THREE-WAY ANOVA (F and p Values) APPLIED TO FEMALES IN THE FIRST WEEK OF TESTING (Ritanserin)

	Hole Board				Plus Maze			Sex
	Locomotion	H-D Frequency	H-D Duration	Rearing	% Time in Open Arms	No. Open Entries	Total Entries	No. Mounts
Main factors								
1) Neonatal	0.207	4.455	2.192	6.022	0.634	2.380	1.123	10.046
(df = 1, 36)	NS	$p < 0.05$	NS	$p < 0.05$	NS	NS	NS	$p < 0.01$
2) Pretreat	2.823	0.012	0.50	4.122	4.036	0.51	0.055	71.834
(df = 1, 36)	NS	NS	NS	$p < 0.05$	NS	NS	NS	$p < 0.001$
3) Treat	1.39	1.32	0.31	0.76	4.29	4.33	3.60	7.85
(df = 1, 37)	NS	NS	NS	NS	$p < 0.05$	$p < 0.05$	NS	$p < 0.01$
Interactions								
1 × 2	1.79	0.99	0.09	1.24	2.94	3.13	0.16	0.279
(df = 1, 36)	NS	NS	NS	NS	NS	NS	NS	NS
1 × 3	0.23	0.31	0.02	1.17	0.32	0.17	0.06	1.74
(df = 1, 37)	NS	NS	NS	NS	NS	NS	NS	NS
2 × 3	0.01	0.02	0.96	0.00	0.18	0.34	0.46	0.04
(df = 1, 37)	NS	NS	NS	NS	NS	NS	NS	NS
1 × 2 × 3	0.01	0.02	1.84	0.09	2.33	0.33	0.09	1.36
(df = 1, 37)	NS	NS	NS	NS	NS	NS	NS	NS

H-D = head-dipping, Neonatal = neonatal androgenizing treatment, Pretreat = testosterone implant, Treat = treatment with ritanserin.

TABLE 8

THREE-WAY ANOVA (F and p Values) APPLIED TO FEMALES IN THE SECOND WEEK OF TESTING (DOI)

	Hole Board				Plus Maze			Sex
	Locomotion	H-D Frequency	H-D Duration	Rearing	% Time in Open Arms	No. Open Entries	Total Entries	No. Mounts
Main factors								
1) Neonatal	1.92	15.189	3.299	4.976	1.155	2.479	6.735	5.228
(df = 1, 36)	NS	$p < 0.001$	NS	$p < 0.05$	NS	NS	$p < 0.05$	$p < 0.05$
2) Pretreat	4.84	0.98	0.091	5.047	2.554	2.64	2.42	63.555
(df = 1, 36)	$p < 0.05$	NS	NS	$p < 0.05$	NS	NS	NS	$p < 0.001$
3) Treat	4.34	8.30	5.23	1.02	0.04	0.13	0.97	2.57
(df = 1, 37)	$p < 0.05$	$p < 0.01$	$p < 0.05$	NS	NS	NS	NS	NS
Interactions								
1 × 2	2.74	1.74	0.065	0.107	1.095	0.46	0.055	6.11
(df = 1, 36)	NS	NS	NS	NS	NS	NS	NS	$p < 0.05$
1 × 3	0.03	0.06	1.41	1.50	0.00	0.13	0.03	0.00
(df = 1, 37)	NS	NS	NS	NS	NS	NS	NS	NS
2 × 3	0.02	1.67	2.72	0.53	0.14	0.01	0.15	2.66
(df = 1, 37)	NS	NS	NS	NS	NS	NS	NS	NS
1 × 2 × 3	0.03	0.03	0.40	0.54	0.76	0.01	0.29	0.00
(df = 1, 37)	NS	NS	NS	NS	NS	NS	NS	NS

H-D = head-dipping, Neonatal = neonatal androgenizing treatment, Pretreat = testosterone implant, Treat = treatment with DOI.

was in fact greater in androgenized than in normal animals (Table 11).

Concerning drug treatments, ritanserin tended to increase the number of mounts independently of previous testosterone manipulation (Tables 7 and 11). DOI had no effect on mounting behavior (Tables 8 and 11).

DISCUSSION

5-HT has a number of different effects on various aspects of behaviour due to the multiplicity of its receptor's subtypes; we have here restricted our observations to the effects exerted at 5-HT_2 receptors. DOI and ritanserin are a relatively selective 5-HT_2 agonist (16) and antagonist (24), respectively [al-

TABLE 9

ACTIVITY OF FEMALE RATS IN THE 5-MIN HOLE BOARD TEST

Neonatal Treatment	Pretreatment	Treatment	Locomotion (no. of squares crossed)	Head-Dipping		Rearing Frequency
				Frequency	Duration (s)	
TP	Empty	Sal	176.2 ± 16.2	7.7 ± 1.7	10.6 ± 2.6	27.1 ± 3.9
	(10)	Rit	166.4 ± 20.7	6.0 ± 1.0	7.6 ± 1.6	23.5 ± 4.7
	TP implant	Sal	181.6 ± 18.2	6.7 ± 1.0	6.1 ± 1.0	29.4 ± 6.0
	(10)	Rit	171.7 ± 19.5	5.5 ± 0.9	8.6 ± 1.7	24.4 ± 3.6
Oil	Empty	Sal	170.5 ± 37.3	5.0 ± 1.3	10.8 ± 3.2	14.7 ± 3.1
	(10)	Rit	144.5 ± 19.0	4.5 ± 1.0	10.7 ± 3.2	14.6 ± 2.8
	TP implant	Sal	215.0 ± 18.5	5.6 ± 0.7	10.1 ± 2.1	22.8 ± 3.4
	(10)	Rit	194.4 ± 17.9	5.1 ± 0.6	8.9 ± 1.5	23.7 ± 3.5
TP	Empty	Sal	157.7 ± 20.7	6.1 ± 0.7	4.8 ± 0.8	19.2 ± 3.5
	(10)	DOI	185.2 ± 15.7	10.1 ± 1.6	9.6 ± 1.7	18.7 ± 2.8
	TP implant	Sal	167.2 ± 20.5	7.1 ± 1.0	7.0 ± 1.3	29.4 ± 5.3
	(10)	DOI	194.0 ± 25.7	8.5 ± 1.6	7.2 ± 1.3	20.5 ± 4.3
Oil	Empty	Sal	110.7 ± 25.1	2.5 ± 0.7	5.1 ± 2.0	11.3 ± 4.5
	(10)	DOI	129.7 ± 24.9	5.7 ± 1.3	18.2 ± 8.1	10.6 ± 2.6
	TP implant	Sal	171.6 ± 18.6	5.2 ± 0.7	9.1 ± 1.7	18.2 ± 3.1
	(10)	DOI	198.7 ± 16.9	6.5 ± 0.9	11.8 ± 2.5	19.8 ± 3.3

All results expressed as mean ± SE. Number of rats in each group in parentheses. TP = testosterone proprionate 250 μg/rat SC on day 1 postpartum, Oil = corn oil 0.1 ml/rat SC on day 1 postpartum, TP Implant = testosterone proprionate implant 10-mm length, Empty = empty implant 10-mm length, Rit = ritanserin 0.25 mg/kg IP, DOI 0.25 mg/kg IP, Sal = 1 ml/kg IP.

TABLE 10
ACTIVITY OF FEMALE RATS IN THE 5-MIN ELEVATED PLUS-MAZE TEST

Neonatal Treatment	Pretreatment	Treatment	Open Arms		No. Total Entries
			% Time	No. Entries	
TP	Empty	Sal	15.4 ± 4.1	3.8 ± 1.1	11.9 ± 2.3
	(10)	Rit	17.2 ± 3.8	4.3 ± 0.9	14.1 ± 1.7
	TP implant	Sal	11.3 ± 2.5	3.1 ± 0.5	10.4 ± 1.5
	(10)	Rit	22.4 ± 5.1	3.6 ± 0.4	11.0 ± 0.9
Oil	Empty	Sal	4.6 ± 2.2	1.3 ± 0.5	8.0 ± 1.8
	(10)	Rit	9.6 ± 2.8	2.4 ± 0.6	10.1 ± 1.3
	TP implant	Sal	16.3 ± 2.9	3.3 ± 0.7	11.2 ± 1.0
	(10)	Rit	18.1 ± 3.1	3.7 ± 0.6	12.7 ± 1.2
TP	Empty	Sal	10.1 ± 3.0	3.7 ± 0.9	13.2 ± 1.9
	(10)	DOI	11.1 ± 4.5	3.8 ± 1.4	13.1 ± 2.1
	TP implant	Sal	12.9 ± 4.2	4.7 ± 0.9	13.6 ± 1.6
	(10)	DOI	13.5 ± 4.2	4.6 ± 1.3	15.2 ± 1.8
Oil	Empty	Sal	7.7 ± 3.7	1.4 ± 0.7	7.4 ± 1.7
	(10)	DOI	4.9 ± 3.9	1.8 ± 1.2	8.6 ± 2.0
	TP implant	Sal	10.5 ± 3.0	3.6 ± 1.0	11.1 ± 1.3
	(10)	DOI	12.0 ± 3.8	4.0 ± 1.1	12.0 ± 1.7

Results expressed as mean ± SE. Number of rats in each group in parentheses. TP = testosterone proprionate 250 μg/rat SC on day 1 postpartum, Oil = corn oil 0.1 ml/rat SC on day 1 postpartum, TP Implant = testosterone proprionate implant 10-mm length, Empty = empty implant 10-mm length, Rit = ritanserin 0.25 mg/kg IP, DOI 0.25 mg/kg IP, Sal = saline 1 ml/kg IP.

though both act on the 1C receptor as well (20)], and these agents have been used to alter 5-HT activity in these experiments.

A variety of behavioral activities in rodents are sexually differentiated besides sexual behaviour, T in the perinatal period being the main determinant of the masculine behavioral profile (18,35). Males show lower locomotory activity than females, and this is also true of females which have been androgenized over the neonatal period (3–5,33). In adulthood, however, T does not exert a significant effect on locomotion, since in our experiments there was no significant difference in untreated castrated males and those bearing T implants, nor in androgenized and normal females with and without T implants. Others have shown that certain nonsexual behaviours including locomotion and social interaction are unaffected by the absence of T in adulthood (10,30).

Manipulation of 5-HT activity showed that a reduction in 5-HT activity by ritanserin reduced locomotory behaviour in males (see Table 2), but this effect was not present in the group with T10 implants. Ritanserin affected neither the androgenized nor the normal females. DOI had no effect on locomotion in any of the male groups, but it reduced rearing. In females, DOI induced an overall increased locomotion irrespective of neonatal T or the presence of T in adulthood.

These findings are different from those reported in the literature where DOI and another 5-HT agonist, quipazine, reduced locomotory activity in male rats (26,39). Ritanserin, which reversed the effect of DOI, had no effect by itself in intact males (29). It is of interest, therefore, that in the present experiments the effect of ritanserin seemed to be masked in the castrated males bearing T10 implants, which produce near physiological circulating levels of T as seen in intact rats (37).

Reports in the literature indicate that the effect of 5-HT on spontaneous locomotion is a complex one and chemical

TABLE 11
MASCULINE SEXUAL BEHAVIOUR IN FEMALES

Neonatal Treatment	Pretreatment	Treatment	No. Mounts
TP	Empty	Sal	2.2 ± 0.9
	(10)	Rit	10.0 ± 3.8*
	TP implant	Sal	23.0 ± 2.9
	(10)	Rit	27.9 ± 3.6
Oil	Empty	Sal	0
	(10)	Rit	0.2 ± 0.1
	TP implant	Sal	26.7 ± 1.7
	(10)	Rit	26.0 ± 5.0
TP	Empty	Sal	0.7 ± 0.1
	(10)	DOI	0.6 ± 0.1
	TP implant	Sal	28.5 ± 6.0
	(10)	DOI	22.7 ± 4.1
Oil	Empty	Sal	0.6 ± 0.4
	(10)	DOI	0.8 ± 0.5
	TP implant	Sal	22.2 ± 4.0
	(10)	DOI	15.2 ± 3.5

Results expressed as mean ± SE. Number of rats in each group in parentheses. TP = testosterone proprionate 250 μg/rat SC on day 1 postpartum, Oil = corn oil 0.1 ml/rat SC on day 1 postpartum, TP Implant = testosterone implant 10-mm length, Empty = empty implant 10-mm length, Rit = ritanserin 0.25 mg/kg IP, DOI 0.25 mg/kg IP, Sal = saline 1 ml/kg IP.

lesions of brain 5-HT systems can affect locomotion in either direction, possibly according to the pathways involved (11,34). Changes in locomotion have been measured concomitantly with changes in social and sexual activity, as change in locomotion might be an important component of the latter two behaviours. In fact, it changes independently from sexual behaviour (19), and both increases and decreases in locomotion have been associated with impaired social interaction (11). In the test mostly used in these reported experiments (i.e., the open field), locomotion is the net result of explorative and emotional drives, acting in opposition. The open field fails to distinguish these components, while in the hole board test, used in this experiment, locomotion is solely associated with exploration.

Exploratory behaviour is sexually differentiated, with a greater activity in females than in males and androgenized females (3,4). The effect of neonatal androgens in the females was partially prevented by neonatal administration of the 5-HT precursor 5-HTP, indicating a possible antagonism between T and 5-HT (36). In adulthood, the presence or absence of T implants in males or females did not modify exploratory activity, as shown by the frequency and duration of head-dipping in the hole board test (present results). Turning to the drug treatments in males, we have found that the 5-HT_2 antagonist ritanserin decreased duration and frequency of head-dipping, while DOI, the agonist, had the opposite effect. Once again the effect of ritanserin tended to be masked when T levels were high, as in the males bearing the T10 implant. In females, exploration was on the whole increased by DOI, both in terms of frequency and duration of head-dipping. This effect was independent of previous androgenization and of the pretreatment with T.

Other authors have indicated that 5-HT inhibits exploratory activity in adulthood (14,39). This discrepancy with our results may be due to the lack of novelty in our tests (15,25). Wing et al. (39) only obtained their results when the rats were unfamiliar with the test; in our experimental design, the animals had been familiarized with the testing apparatus prior to the drug treatments, and so any effect of the drug was not related to novelty.

The levels of emotionality might be the basis of the 5-HT-induced behavioral modifications, particularly as 5-HT modulates anxiety-related behaviours in adulthood (7). In the present experiments, animals were tested in the plus-maze test, selectively measuring levels of anxiety. In this test, blockade of 5-HT activity is anxiolytic, as seen after administration of parachlorophenylalanine, 5-HT_1 and 5-HT_2 antagonists, and 5-7 dihydroxytryptamine lesions, while 5-HT_2 agonists are anxiogenic (6,22). There are conflicting reports on the effect of ritanserin on anxiety; Critchley and Handley (8) showed that it increased entries into the open arms, while Pellow et al. (28) found the opposite. Gardner (13) has reviewed several other reports on the effects of ritanserin on anxiety which are also conflicting.

In our experiments, ritanserin differentially affected activity in the plus maze in males and in females. In males, the drug interacted with T levels, since the number of entries into the open arms was decreased in all groups except in that with the T10 implant (see Tables 2 and 4). In females, ritanserin increased both entries and percent of time on the open arms irrespectively of androgenization and treatment with T at adulthood. Thus the genetic sex of the animal but not the neonatal androgenization played a role in the response to 5-HT depletion. A proviso must be added here, that unfortunately the neonatal treatment had a very modest (albeit significant) effect on sexual behaviour and so it is possible that the dose (i.e., 250 μg/pup TP) on day 1 postpartum was insufficient to exert a significant effect on other aspects of behaviour or response. DOI did not affect activity in the plus maze in either males or females.

The low level of significant effects in the plus-maze test may be due to a variety of factors such as repetition of the testing, which was carried out twice before drug treatments started and was further repeated during the two weeks of the drug treatments. Validation of the plus-maze test by Pellow et al. (27) indicated that repetition does not affect response. However, in our experiments an overall increase in time spent in the open arms was observed after the second treatment (DOI/saline), one week after the first treatment (Rit/saline) (one-way ANOVA for the two saline groups, $F = 2.78$, $p < 0.01$), possibly due to the familiarity with the test itself.

When regression analysis was applied to the two saline groups we obtained a significant correlation between levels of testosterone and percentage time on open arms ($R = 0.34$, $p = 0.04$) for the saline/DOI group, suggesting that testosterone in adulthood has an anxiolytic effect in males.

It is well established that neonatal androgenization induces masculinization and defeminization of sexual behaviour in adulthood in both sexes (18). The presence of T in adulthood is also essential for male sexual activity in both males and females. Thus, in males, castration will eventually abolish all aspects of sexual behaviour (32), and these can be reinstated, in full, by replacement of T at concentrations well below those seen in the normal intact rat (1). Circulating T also has an important influence on other forms of behaviour including aggression and dominance (2).

These findings on the effect of T on male sexual behaviour were replicated in our experiments, since we showed that in castrated males sexual behaviour was completely abolished and returned in full in the presence of the T2.5 implant, which only reinstated circulating plasma levels to a quarter (2 pmol/l; 0.6 ng/ml) of the physiologically normal concentration seen in intact rats (8 pmol/l; 2.4 ng/ml) (37). Thus the groups bearing T2.5, T5, and T10 implants all showed similar sexual activity, although latencies to mount, intromission, and ejaculation tended to be longer in the T2.5 group. MANOVA showed no interaction on behaviour between T levels and ritanserin, but there was an interaction between T and DOI.

Reports in the literature are almost unanimous in suggesting that 5-HT_2 activity exerts an inhibitory effect on male sexual behaviour (12,17), and a variety of selective 5-HT_2 agonists and antagonists have been shown to inhibit and stimulate male sexual behaviour, respectively. In our experiments on males, ritanserin and DOI had no effect on sexual behaviour in castrated rats, and this confirms many previous reports that drugs rarely affect sexual behaviour in the absence of T (31). In the groups bearing the range of T implants, behaviour was so vigorous that further enhancement could not be observed easily, although in the T2.5 group ritanserin reduced latencies to intromission and ejaculation and at all levels of T it reduced the number of mounts required to reach ejaculation. The expected inhibitory effect of DOI was observed in that latency to intromission was increased in all T-implant groups, and latency to ejaculation and length of the refractory period were also increased, although only in the T5 implant group.

In females, enhancement of male behaviour is shown by an increase in the number of mounts exhibited when placed with another normal receptive female. In spite of the low levels of mounting, MANOVA showed that frequency of mounts was increased by neonatal androgenization, and that

the presence of T in adulthood greatly enhanced mounting behaviour in both the androgenized and normal females.

Ritanserin increased mount frequency in the female groups, MANOVA indicating that its effect was independent of both neonatal and adult T treatment. DOI, on the other hand, had no effect on male behaviour in females. Perhaps the predicted inhibitory effect of DOI could not be manifested on the relatively low levels of masculine activity in these females.

In summary, therefore, when sexual activity was at such a level that changes induced by 5-HT manipulation could be seen, there was an inverse relationship between 5-HT activity and male sexual behaviour in both males and females, and this was in some cases related to the level of T in the animal.

It is interesting to note that the effect of 5-HT manipulation on masculine sexual activity appeared independent of the effects on nonsocial behaviours. Thus, the potentiating effect of the 5-HT_2 antagonist ritanserin on male sexual activity observed in both sexes was coupled with reduced locomotion and exploration and increased anxiety in males, but in females with decreased anxiety with unmodified locomotion and exploration.

In this investigation we set out to see if the presence of T in adulthood influenced various aspects of behaviour and whether or not 5-HT and T interacted in their control of these behaviours. We found that T in adulthood is essential for the occurrence of male sexual behaviour but had no effect on locomotion and exploratory behaviour in either sex. In males, testosterone had an anxiolytic effect, but only when the animals had become familiar with the test apparatus. It is of interest that the effects of the 5-HT_2 antagonist ritanserin on reducing locomotion and exploration and increasing anxiety, while not requiring the presence of T, seemed to be masked by the highest dose of T (i.e., the T10 implant). Apart from this, there was no interaction between T and ritanserin in reducing locomotion and exploration in males (it had no effect in females) and DOI in stimulating locomotion and exploration in both sexes. DOI had no effect on anxiety, but ritanserin had a gender-specific effect, being anxiogenic in males and anxiolytic in females—the latter whether treated with 250 μg/pup TP on day 1 postpartum or not.

In conclusion, manipulation of 5-HT in adulthood had differential effects on various aspects of behaviour, dependent on the sex of the animal but not on neonatal androgenization (induced by 250 μg/pup TP). There may be an inverse relationship and interaction between 5-HT and T on male sexual behaviour, but in contrast to the findings noted in the neonatal period (9,36), there was no inverse relationship or testosterone dependence seen on nonsexual aspects of behaviour in adulthood.

ACKNOWLEDGEMENTS

We are grateful for support by the NATO International Scientific Exchange Programme.

REFERENCES

1. Albert, D. J.; Jonik, R. H.; Watson, N. V.; Gorzalka, B. B.; Walsh, M. L. Hormone-dependent aggression in male rats is proportional to serum testosterone concentration but sexual behaviour is not. Physiol. Behav. 48:409–416; 1990.
2. Albert, D. J.; Walsh, M. L.; Gorzalka, B. R.; Siemens, Y.; Louie, H. Testosterone removal in rats results in a decrease in social aggression and a loss of social dominance. Physiol. Behav. 36:401–407; 1986.
3. Archer, J. Rodent sex differences in emotional and related behaviour. Behav. Biol. 14:451–479; 1975.
4. Beatty, W. W. Gonadal hormones and sex differences in nonreproductive behaviours in rodents: Organizational and activational influences. Horm. Behav. 12:112–163; 1979.
5. Blizard, D. A.; Lippman, H. R.; Chen, J. J. Sex differences in open-field behaviour in the rat: The inductive and activational role of gonadal hormones. Physiol. Behav. 14:601–608; 1975.
6. Briley, M.; Chopin, P.; Moret, C. Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat. Psychopharmacology 101:187–189; 1990.
7. Brockkamp, C. L. E.; Berendsen, H. H. G.; Jenck, F.; Van Delft, A. M. L. Animal models for anxiety and response to serotonergic drugs. Psychopathology 22(Suppl. 1):2–12; 1989.
8. Critchley, M. A. E.; Handley, S. C. Effects in the X-maze anxiety model of agents acting at 5-HT_1 and 5-HT_2 receptors. Psychopharmacology 93:502–506; 1987.
9. Farabollini, F.; Hole, D. R.; Wilson, C. A. Behavioral effects in adulthood of serotonin depletion by p-chlorophenylalanine given neonatally to male rats. Int. J. Neurosci. 41:187–199; 1988.
10. Fielder, T. J.; Peacock, N. R.; McGivern, R. F.; Swerldhoff, R. S.; Bhasin, S. Testosterone dose-dependency of sexual and nonsexual behaviors in the gonadotrophin-releasing hormone antagonist-treated male rat. J. Androl. 10:167–173; 1989.
11. File, S. E.; Deakin, J. F. W. Chemical lesions of both dorsal and median raphe nuclei and changes in social and aggressive behavior in rats. Pharmacol. Biochem. Behav. 12:855–859; 1980.
12. Foreman, M. M.; Hall, J. L.; Love, R. L. The role of the 5-HT_2 receptor in the regulation of sexual performance of male rats. Life Sci. 45:1263–1270; 1989.
13. Gardner, C. R. Potential use of drugs modulating 5-HT activity in the treatment of anxiety. Gen. Pharmacol. 19:347–356; 1988.
14. Geyer, M. A.; Peterson, L. R.; Rose, G. J. Effects of serotonergic lesions on investigating responding by rats in a holeboard. Behav. Neural Biol. 30:160–177; 1980.
15. Geyer, M. A.; Russo, P. V.; Masten, V. L. Multivariate assessment of locomotor behavior; pharmacological and behavioral analyses. Pharmacol. Biochem. Behav. 25:277–288; 1986.
16. Glennon, R. A.; McKenny, J. D.; Lyon, R. A.; Titeler, M. 5-HT_1 and 5-HT_2 binding characteristics of 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopiopane analogues. J. Med. Chem. 29:194–199; 1986.
17. Gorzalka, B. B.; Mendelson, S. D.; Watson, N. V. Serotonin receptor subtypes and sexual behavior. Ann. N. Y. Acad. Sci. 600:435–444; 1990.
18. Goy, R. W.; McEwen, B. S. Sexual differentiation of the brain. Cambridge, MA: MIT Press; 1980.
19. Hillegaart, V.; Ahlenius, S.; Larsson, K. Effects of local application of 5-HT into the median and dorsal raphe nuclei on male rat sexual and motor behavior. Behav. Brain. Res. 33:279–286; 1989.
20. Hoyer, D. Molecular pharmacology and biology of 5-HT_{1C} receptors. Trends Pharmacol. Sci. 9:89–94; 1988.
21. Johnson, H. M.; Payne, A. P.; Gilmore, D. P.; Wilson, C. A. Neonatal serotonin reduction alters the adult feminine sexual behavior of golden hamsters. Pharmacol. Biochem. Behav. 35:571–575; 1990.
22. Kshama, D.; Horishikeshaven, H. J.; Shanbhogue, R.; Munonyedi, U. S. Modulation of base-line behavior in rats by putative serotonergic agents in three ethoexperimental paradigms. Behav. Neural. Biol. 54:234–253; 1990.
23. Ladosky, W.; Gaziri, L. C. J. Brain serotonin and sexual differentiation of the nervous system. Neuroendocrinology 6:168–174; 1970.
24. Leysen, J. E.; Gommeren, W.; van Gompel, P.; Wynants, J.; Janssen, P. F. M.; Laduron, P. M. Receptor-binding properties in vitro and in vivo of ritanserin. A very potent and long acting serotonin-5_2 antagonist. Mol. Pharmacol. 27:600–611; 1985.
25. Mittman, S. M.; Geyer, M. A. Effects of 5-HT_{1A} agonists on

locomotor and investigatory behaviors in rats differ from those of hallucinogens. Psychopharmacology 98:321–329; 1989.
26. Paznik, A.; Stefanski, R.; Paejkow; Kostowski, W. Serotonergic mechanisms in the nucleus accumbens affected by chronic desipramine treatment. Pharmacol. Biochem. Behav. 39:43–48; 1991.
27. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
28. Pellow, S.; Johnston, A. L.; File, S. E. Selective agonists and antagonists for 5-hydroxytryptamine receptor subtypes, and interactions with yohimbine and FG 7142 using the elevated plus-maze test in the rat. J. Pharm. Pharmacol. 39:915–928; 1987.
29. Pranzatelli, M. R.; Jappay, E.; Snodgrass, S. R. Effects of 5-HT receptor subtype-selective drugs on locomotor activity and motor habituation in the DHT adult rat model. Pharmacol. Biochem. Behav. 27:497–504; 1987.
30. Primus, R. J.; Kellogg, C. K. Gonadal hormones during puberty organise environment-related social interaction in the male rat. Horm. Behav. 24:311–323; 1990.
31. Sachs, B. D.; Meisel, R. L. The physiology of male sexual behaviour. In: Knobil, E.; Neill, J. D., eds. in chief. The physiology of reproduction. New York: Raven Press; 1988:1393–1485.
32. Sodersten, P.; Hansen, S.; Enoreth, P.; Wilson, C. A.; Gustaffson, J. A. Testosterone in the control of rat sexual behaviour. J. Steroid Biochem. 12:337–346; 1950.
33. Stevens, R.; Goldstein, R. Effects of neonatal testosterone and estrogen on open-field behaviour in rats. Physiol. Behav. 26:551–553; 1981.
34. Vanderwolf, C. H. A general role for serotonin in the control of behaviour: Studies with intracerebral 5,7-dihydroxytryptamine. Brain Res. 504:192–198; 1989.
35. Ward, I. L.; Ward, O. B. Sexual behaviour differentiation: Effects of prenatal manipulations in rats. In: Adler, N.; Pfaff, D.; Goy, R. W., eds. Handbook of behavioral neurobiology. New York: Plenum Press; 1985:77–98.
36. Wilson, C. A.; Gonzalez, I.; Farabollini, F. Behavioral effects in adulthood of neonatal manipulation of brain serotonin levels in normal and androgenized females. Pharmacol. Biochem. Behav. 41:91–98; 1991.
37. Wilson, C. A.; Herdon, H. J.; Barclay, L. C.; Clayton, R. W. Effect of testosterone on gonadotrophin-releasing hormone receptors in the castrated rat: Preliminary evidence for a stimulatory effect of testosterone on gonadotrophin function in the male rat. J. Endocrinol. 108:441–449; 1986.
38. Wilson, C. A.; Pearson, J. R.; Hunter, A. J.; Tuohy, P. A.; Payne, A. P. The effect of neonatal manipulation of hypothalamic serotonin levels on sexual activity in the adult rat. Pharmacol. Biochem. Behav. 24:1175–1183; 1986.
39. Wing, L. L.; Tapson, G. S.; Geyer, M. A. 5-HT_2 mediation of acute behavioral effects of hallucinogens in rats. Psychopharmacology 100:417–425; 1990.