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Neonatal Organizational Effects of the 5-HT_2 and 5-HT_{1A} Subsystems on Adult Behavior in the Rat

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GONZÁLEZ, M. I., E. ALBONETTI, A. SIDDIQUI, F. FARABOLLINI AND C. A. WILSON. *Neonatal organizational effects of the 5-HT_2 and 5-HT_{1A} subsystems on adult behavior in the rat.* PHAMACOL BIOCHEM BEHAV **54**(1) 195–203, 1996.—Males, females, neonatally androgenized females, and neonatally castrated males were treated over the second week of life with 0.25 mg/kg of either the 5-HT_2 agonist 1-(2,5-dimethoxy-3-iodophenyl)-2-aminopropane HCl (DOI), the 5-HT_2 antagonist ritanserin (Rit), the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), or the 5-HT_{1A} antagonist WAY100135 (WAY). Exploration, anxiety, sociosexual preferences, and sexual behavior were measured in adulthood. Agents acting on 5-HT_{1A} receptors do not appear to affect organization of any of the behavioral systems studied. DOI increased exploratory activity but in females only, which suggests that testosterone antagonizes the stimulatory effect of 5-HT_2 activity on exploration. Neonatal ritanserin selectively reduced anxiety in females, and DOI had a similar effect in androgenized females. This indicates that neonatal 5-HT_2 activity is anxiogenic in normal females, anxiolytic in androgenized females, and has no effect on anxiety in males. Males and androgenized females both showed a preference for the female teaser that was abolished by the 5-HT_2 agonist, DOI. These results point out that 5-HT_2 activity selectively suppresses heterosexual preference induced in the presence of neonatal testosterone. DOI also reduced both male sexual behavior in males and female sexual behavior in androgenized females. Thus, the 5-HT_2 system antagonizes the action of testosterone in stimulating heterosexual orientation and sexual activity, and this is independent of genetic sex.

5-Hydroxytryptamine (5-HT) 5-HT receptors (5-HT_2, 5-HT_{1A}) Testosterone Exploratory behavior
Elevated plus-maze for anxiety Sexual preference Sexual behavior

IT IS WELL known that in adult rodents 5-hydroxytryptamine (5-HT; serotonin) influences a number of different behaviors, ranging from agonism (5,11,38) and sexual activity (20) to anxiety-related behaviors (7,22). In addition, several reports indicate that 5-HT activity in the neonatal period, and especially during the second week of life, is involved in the organization of adult behavior (10,18,23,25,39,41), especially playing a role in the sexual differentiation of both social and nonsocial behaviors (39,41). In particular, there appears to be an interaction between 5-HT and androgens, which may be important in inducing brain sexual differentiation (21,25, 39,41).

Neonatal 5-HT depletion by *p*-chlorophenylalanine (pCPA) has been reported to decrease anxiety, enhance heterosexual preference, and raise aggression in male rats (10,23,41). In females, reports are confined to sexual behavior and are more controversial. pCPA reduced receptivity in golden hamsters (25), while it had no effect in rats (41). The interplay between neonatal testosterone (T) and 5-HT was selectively studied in neonatally androgenized females, that is, rats belonging to the genetic female sex, with experimentally induced male-like circulating levels of T on day 1 after birth. 5-HT depletion by pCPA in these animals enhanced the defeminizing effects of exogenous T, conversely raising 5-HT levels with 5-HTP, reduced the T effect. This suggests that during the neonatal period 5-HT may antagonize the androgenizing effects of endogenous T (41).

5-HT acts on a number of receptor subtypes, and there is increasing evidence that in the adult 5-HT_2 activity is anxiogenic (8,34) and involved in the inhibitory control of male sexual activity (15,19,20) and aggression (5,38).

5-HT_{1A} receptors also appear to be involved in many kinds

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of behavior. 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), and other partial agonists have inhibitory effects on total locomotor activity (1). They have sexually dependent effects on sexual behavior, being inhibitory on lordosis in females and stimulatory on ejaculatory behavior in males (1,28). They are also anxiogenic in various experimental animal models (9), although they can be anxiolytic or ineffective in others (29,32)

In the present investigation the organizational effects of the 5-HT_2 and 5-HT_{1A} subsystems and their possible interaction with neonatal T on the adult behavior of male and female rats were studied in three separate experiments. For this purpose, animals were administered during the second week of life with either the 5-HT_2 agonist (DOI), the 5-HT_2 antagonist ritanserin (Rit), the 5-HT_{1A} agonist 8-OH-DPAT, or the 5-HT_{1A} antagonist WAY100135. Sociosexual preferences, sexual behavior, exploration, and anxiety were measured in adulthood. We were particularly interested in studying sexual behavior both in its motivational (sexual orientation) and consummatory aspects. The recording of exploration helped to verify whether sexual orientation merely reflected a general motivation to investigate novel stimuli or was underlied by a specific sexual motivation. Anxiety was also measured, as it is known to affect the reactivity to social and environmental stimuli (24,26).

METHOD

Procedure

In all experiments litters born to Wistar rats bred at St. George's Hospital Medical School were randomized and culled so that each dam had six males and six females.

Experiment 1

On the day of birth litters were randomly assigned to one of three different groups (males, normal females, androgenized females). The androgenized females (n = 23) were injected subcutaneously (SC) with 250 μg/pup testosterone propionate (TP; Sigma Chemical Co., Dorset) in 0.05 ml corn oil on the day of birth. Normal females (n = 25) and males (n = 26) received corn oil only.

On days 8–16 after birth, a third of each group was treated daily with 0.25 mg/kg of the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-HCL (DOI; Res. Biochem. Inc., Natick, MA); another third received 0.25 mg/kg of the 5-HT_2 antagonist ritanserin (Rit; Res. Biochem. Inc.). The doses used were those reported to produce behavioral effects when administered in adults (3,29) or in preweanling rat pups (16). Both drugs were injected SC in 1 ml/kg saline. The remaining third of each group received saline only.

Experiment 2

On the day of birth, the females were randomly assigned to normal or androgenized females groups. The androgenized females (n = 34) were injected subcutaneously (SC) with 250 μg/pup testosterone propionate (TP; Sigma Chemical Co.) in 0.05 ml corn oil on the day of birth. The normal females (n = 33) received only vehicle. The males of these litters were not used in this experiment.

On days 8–16 after birth, a third of each group, normal females and androgenized females was treated daily with 0.25 mg/kg of the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) (Res. Biochem. Inc.); another third received 0.25 mg/kg of the 5-HT_{1A} antagonist WAY100135 (WAY, *N-tert*-butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropionamide dihydrochloride) (Wyeth Research Centre, Taplow, Bucks, UK). The doses used were those reported to produce behavioral effects when administered in adults (1,8,29) or in preweanling rat pups (16). Both drugs were injected SC in 1 ml/kg saline. The remaining third of each group received saline.

Experiment 3

On the day of birth, the males of all the litters were randomly assigned to one of three different groups: intact males (n = 6), sham-castrated males (n = 22), and castrated males (n = 19). Before surgery on the day of birth the males of the last two groups were anesthesized by placing them in a freezer at −20°C for 10 min. Surgery consisted of a small nick midscrotum and externalizing the testes in both castrated and sham groups, removal of the testes in the former, and replacement under the skin in the latter, then the cut was stitched together. Afterwards, the animals were returned to the mother so lactation followed its normal course. The females of these litters were not used in this experiment.

On days 8–16 after birth a third of the sham-castrated and castrated males was treated daily with 0.25 mg/kg of the 5-HT_{1A} agonist 8-OH-DPAT; another third received 0.25 mg/kg of the 5-HT_{1A} antagonist WAY100135. Both drugs were injected SC in 1 ml/kg saline. The remaining third of each group received saline. (The intact males (n = 6) received saline only.) The experimental design in the schedule of treatments is represented in Table 1.

In the three experiments animals were weaned on day 21 and housed as same-sex groups of five to six. They were kept throughout life in a reversed light : dark regimen (light off at 0800 h). Temperature was maintained at 22°C; food and water were provided ad lib.

In adulthood normal females were checked daily for the phase of their estrous cycle by taking vaginal smears. In androgenized females, the vagina remained closed.

Behavioral Testing

In the three experiments, behavioral testing started when the experimental animals were about 90 days old. On the first testing day, animals underwent the holeboard test and in sequence the elevated plus-maze test for the assessment of locomotion and exploration or anxiety, respectively (13,22). One week later, that is, the eighth testing day, the Sexual Orientation test was carried out, followed immediately by the Sexual Behavior test (39). Female sexual behavior was measured in females and androgenized females; male sexual behavior was measured in males and castrated males. All the tests were carried out between 1100 and 1700 h (i.e., 3 h into the dark period) under dim red light.

Holeboard Test

The apparatus consisted of a black Perspex box (60 × 60 × 35 h cm) with four holes, 3.8 cm in diameter, equally spaced on the floor. Test duration was 5 min. Behaviors recorded were head-dipping duration (HD DUR; total duration of all the head-dipping acts recorded; s) and locomotion (LOC; number of squares crossed).

Elevated Plus-Maze Test

The plus-maze consisted of four wooden arms 50 cm long and 10 cm wide, 62 cm high from the floor. Two opposite

arms were provided with 40 cm high walls (closed arms); the remaining two had no walls (open arms). Test duration was 5 min. Items recorded were total time spent in either open or closed arms. Time spent in the open arms as a percentage of total time spent into both open and closed arms (%OP) was then computed.

Sexual Orientation Test

This test was carried out in a circular arena (90 cm diameter) with a 30 cm high Perspex 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 two cages were opposite to each other. Each box housed a stimulus animal: a sexually experienced male and a receptive female. The test animals were familiarized with the apparatus by placing them in the arena without the stimuli animals for a 10-min period on each of the 2 days before the actual test. Test duration was 5 min. Frequency of investigations and time spent investigating either stimulus animal were recorded. The percentages of time investigating each stimulus with respect to the total time spent investigating either stimuli were computed. To reduce the investigation of both stimuli to a single figure, a ratio was calculated as % duration investigating the female stimulus : % duration investigating the male stimulus.

Sexual Behavior Test

Each subject was placed into an observation arena (50 cm diameter); 3 to 4 min later the teaser was introduced and recording was started.

Females. Each subject was confronted with a vigorous male until 20 mounts had been achieved. Lordosis frequency was recorded and expressed as the lordotic quotient (number of lordosis/number of mounts × 100). The females were tested on the day of proestrus.

Males. Each subject was confronted with a receptive female. Latency to first mount (MT LAT), number of mounts to ejaculation (No. MT), latency to first intromission (INT LAT), number of Intromission to ejaculation (No. INT), time between first mount to ejaculation (ejaculatory latency, EJ LAT), and time between ejaculation and the first mount of the second cycle of sexual activity (Refractary period, RP) were recorded. The test was terminated after the refractary period, or after 30 min if there was no ejaculation, or after 15 min if there were no mounts.

Statistical Analysis

Data from Experiment 1, Experiment 2, and Experiment 3 were analyzed separately.

Data recorded in the holeboard and plus-maze were analyzed by two-way ANOVA, factor 1 being sex (three levels: males, females, androgenized females) in Experiment 1 and androgenization (two levels: normal females, androgenized females) in Experiment 2. In Experiment 3, where a two-way ANOVA with one isolated control group of intact animals was carried out (42), factor 1 was castration (two levels: sham-castrated males and castrated males). Factor 2 was treatment (three levels: saline, DOI, Rit in Experiment 1; three levels: saline, WAY100135, 8-OH-DPAT in Experiments 2 and 3).

TABLE 1

EXPERIMENTAL SCHEDULES FOR EXPERIMENTS 1, 2, AND 3

Experiment	Animal Group	Pretreatment on Day 1 (s/c)	Treatment on Days 8-16 (s/c)
1	Males (n = 26)	Oil	Saline (n = 10)
			DOI (n = 9)
			Ritanserin (n = 7)
	Females (n = 25)	Oil	Saline (n = 6)
			DOI (n = 10)
			Ritanserin (n = 9)
	Androgenized Females (n = 23)	TP	Saline (n = 7)
			DOI (n = 9)
			Ritanserin (n = 7)
2	Females (n = 33)	Oil	Saline (n = 11)
			8-OH-DPAT (n = 9)
			WAY100135 (n = 11)
	Androgenized Females (n = 34)	TP	Saline (n = 10)
			8-OH-DPAT (n = 12)
			WAY100135 (n = 12)
3	Males (n = 6)	–	Saline (n = 6)
	Sham-castrated males (n = 22)	Sham castration	Saline (n = 10)
			8-OH-DPAT (n = 6)
			WAY100135 (n = 6)
	Castrated males (n = 19)	Castration	Saline (n = 5)
			8-OH-DPAT (n = 6)
			WAY100135 (n = 8)

The experimental schedules for experiments 1, 2, and 3. On day 1, oil was given at 0.05 ml/pup s/c, and testosterone propionate (TP) at 250 μg/pup in 0.05 ml oil s/c.

On days 8-16 postpartum, rats received daily either 1 ml/kg saline s/c or s/c injections of DOI, ritanserin, 8-OH-DPAT, or WAY10035 all at 0.25 mg/kg. These doses were chosen as active concentrations from previous literature reports (1,3,8,29).

For the sexual orientation test, a two-way ANOVA was carried out (factors 1 and 2 being as above) for the ratio between the percentage of time spent investigating female stimulus : male stimulus.

Female sexual behavior. Since this test was carried out only in females and androgenized females, it was analyzed with a two-way ANOVA, factor 1 being sex, with two levels (females and androgenized females), factor 2 being treatment, as specified above, for Experiments 1 and 2.

Male sexual behavior. As this test was only carried out in males and castrated males, one-way ANOVA was used, with three levels (saline, DOI, Rit) in Experiment 1, and two-way ANOVA, factor 1 being castration with two levels (sham-castrated, castrated), factor 2 being treatment as described above, in Experiment 3.

Planned comparisons were carried out whenever appropriate (42). Nonnormal data were subjected to logarithmic transformation. The angular transformation was carried out on percentages whose ranges varied between near zero and 30% (31).

RESULTS

The animal groups and treatments in Experiments 1, 2, and 3 are summarized in Table 1.

Holeboard Test

Means, standard errors (SE), and results of planned comparisons for locomotor activity and duration of head dipping are shown in Table 2 for Experiment 1, Table 3 for Experiment 2, and Table 4 for Experiment 3.

Experiment 1 (Table 2)

Head dipping (HD) (duration). ANOVA resulted in a significant effect for both main factors, sex, $F(2, 71) = 6.18$, $p = 0.003$, and treatment, $F(2, 71) = 6.09$, $p = 0.003$, and a significant sex × treatment interaction, $F(4, 71) = 2.82$, $p = 0.03$. Planned comparisons showed that in females HD durations were higher after neonatal administration of DOI than after saline or ritanserin, with no difference between different treatments in males or androgenized females.

Locomotion (LOC). No significant differences were obtained for any of the groups and treatments.

Experiment 2 (Table 3)

HD, duration. No differences were found for any of the groups and treatments.

Locomotion (LOC). ANOVA resulted in a significant effect for the main factor androgenization, $F(1, 61) = 9.58$, $p = 0.003$, and a significant (androgenization × treatment) interaction, $F(2, 61) = 4.40$, $p = 0.01$. Planned comparisons proved that in normal females both WAY100135- and 8-OH-DPAT-treated subjects ambulated significantly more than the saline-treated ($p = 0.006$ and $p = 0.01$, respectively); in addition, both WAY100135- and 8-OH-DPAT-treated normal females ambulated significantly more than the correspondent groups of androgenized females ($p = 0.001$ and $p = 0.007$, respectively).

Experiment 3 (Table 4)

No significant differences were found for the two parameters measured.

TABLE 2

ACTIVITY IN THE 5 MIN HOLEBOARD AND PLUS-MAZE TESTS (MEANS ± SEM) (EXPERIMENT 1)

		Holeboard		Plus-Maze
	Treatment	Total LOC	HD DUR	% OP DUR
Males	Saline ($n = 10$)	233.6 ± 19.3	26.5 ± 2.9	3.5 ± 2.1
	DOI ($n = 9$)	238.6 ± 8.7	31.9 ± 4.5	0.7 ± 0.7
	RIT ($n = 7$)	262.6 ± 17.0	29.2 ± 2.7	3.1 ± 1.2
Females	Saline ($n = 6$)	240.6 ± 37.2	25.8 ± 6.4	6.0 ± 2.5
	DOI ($n = 10$)	267.7 ± 13.7	60.5 ± 5.5*†	1.6 ± 0.8†
	RIT ($n = 9$)	277.8 ± 14.2	41.2 ± 4.5	13.4 ± 6.1‡
Androgenized Females	Saline ($n = 7$)	254.6 ± 26.7	36.2 ± 6.5	4.5 ± 1.6
	DOI ($n = 9$)	238.9 ± 12.5	42.4 ± 6.0	12.0 ± 5.3‡
	RIT ($n = 7$)	244.8 ± 15.3	49.0 ± 7.4	3.9 ± 1.8

TOTAL LOC = number of ambulations; HD DUR = head dipping duration (in seconds); %OP DUR = time spent into the open arms as a percentage of total time spent into open and closed arms.

*$p < 0.05$ vs. same-sex, saline.

†$p < 0.05$ vs. same-sex, ritanserin.

‡$p < 0.05$ vs. corresponding groups, same treatment.

TABLE 3

ACTIVITY IN THE 5 MIN HOLEBOARD AND PLUS-MAZE TESTS (MEANS ± SEM) (EXPERIMENT 2)

		Holeboard		Plus-Maze
	Treatment	Total LOC	HD DUR	%OP DUR
Females	Saline (n = 11)	216.0 ± 12.2	45.0 ± 5.9	6.3 ± 2.5
	WAY (n = 11)	266.7 ± 14.1*†	37.7 ± 3.1	8.6 ± 1.5
	8-OH-DPAT (n = 9)	266.9 ± 4.4*†	37.2 ± 7.3	8.4 ± 3.2
Androgenized Females	Saline (n = 10)	228.0 ± 12.7	34.4 ± 7.7	4.0 ± 1.4
	WAY (n = 12)	207.9 ± 15.0	38.5 ± 5.4	6.0 ± 2.7
	8-OH-DPAT (n = 12)	212.2 ± 13.7	40.5 ± 4.7	7.1 ± 1.8

TOTAL LOC = number of ambulations; HD DUR = head dipping duration (in seconds); %OP DUR = time spent into the open arms as a percentage of total time spent into open and closed arms.

*$p < 0.05$ vs. same-sex, saline.

†$p < 0.05$ vs. same-treatment, androgenized females.

Elevated plus-maze test. Means, SE, and results of planned comparisons after a two-way ANOVA for percentage time spent into the open arms with respect to total time spent in arms (%OP dur) are shown in Table 2 for Experiment 1, Table 3 for Experiment 2, and Table 4 for Experiment 3.

Experiment 1 (Table 2)

ANOVA, carried out on data subjected to the angular transformation, resulted in a significant interaction between the two main factors, sex and treatment, $F(4, 69) = 2.75$, $p = 0.03$. Whereas in males no effect of the drugs was noted, in normal females, scores were lower in the DOI than Rit group ($p < 0.01$). Moreover, the scores of the Rit group were higher than the corresponding groups of males and androgenized females. Turning to the agonist, in androgenized females the DOI group scores were higher than for corresponding groups of normal males and females.

Experiments 2 and 3 (Tables 3 and 4)

No differences were found for any of the groups or treatments with the 5-HT$_{1A}$ drugs. Although it appears that the males (castrated or intact) in Experiment 3, had lower scores

TABLE 4

ACTIVITY IN THE 5 MIN HOLEBOARD AND PLUS-MAZE TESTS (MEANS ± SEM) (EXPERIMENT 3)

		Holeboard		Plus-Maze
	Treatment	Total LOC	HD DUR	%OP DUR
Males Intact	Saline (n = 6)	213.0 ± 12.3	22.0 ± 4.2	0.9 ± 0.4
Males Sham	Saline (n = 10)	218.8 ± 13.1	19.1 ± 3.0	4.7 ± 1.3
	WAY (n = 6)	198.6 ± 17.4	23.6 ± 5.6	2.5 ± 0.8
	8-OH-DPAT (n = 6)	239.5 ± 12.7	26.6 ± 5.5	1.0 ± 1.0
Males Castrated	Saline (n = 5)	222.0 ± 28.9	13.2 ± 2.4	2.2 ± 1.3
	WAY (n = 8)	259.2 ± 19.2	26.3 ± 3.0	2.3 ± 1.0
	8-OH-DPAT (n = 6)	218.0 ± 24.0	19.5 ± 3.3	3.5 ± 1.6

TOTAL LOC = number of ambulations; HD DUR = head dipping duration (in seconds); %OP DUR = time spent into the open arms as a percentage of total time spent into open and closed arms.

than the females (normal or androgenized) in Experiment 2, statistical comparisons could not be made as data were obtained in different experiments.

Sexual Orientation Test

Experiment 1 (fig. 1). The results (means, SE, and results of planned comparisons) obtained after a two-way ANOVA applied to the ratio of percentages of time investigating the male or the female stimuli rats with respect to the total time spent investigating both stimuli are reported in Fig. 1.

Females in all instances investigated the female stimulus less than the male stimulus; males and androgenized females on the whole investigated the female stimulus more than the male. Planned comparisons proved that in males and androgenized females the preference towards the female stimulus was antagonized by the treatment with DOI, when the investigation became equally distributed between the two stimuli, as shown by the ratio being closer to the value of one.

Experiment 2. Significances were found for the main factors androgenization, $F(1, 59) = 4.36$, $p = 0.03$, and treatment, $F(2, 59) = 3.63$, $p = 0.03$. Planned comparisons showed that, irrespective of treatment, the female stimulus was investigated for longer by the androgenized females than the normal female ($p = 0.02$), while the opposite was true for the male stimulus ($p = 0.04$). Irrespective of androgenization, the investigation of the female stimulus was lower in the 8-OH-DPAT-treated groups [ratios of female : male (F : M) investigation for normal females = 1.58 ± 0.37; androgenized females = 1.53 ± 0.14] compared to the saline-treated females (ratio of F : M investigation for normal females = 1.87 ± 0.6; androgenized females = 3.08 ± 0.67, $p = 0.03$) and to the WAY- (ratio of F : M investigation for normal females = 1.7 ± 0.28; androgenized females = 2.16 ± 0.26, $p = 0.05$).

Experiment 3. No effect of androgenization or the treatments were found.

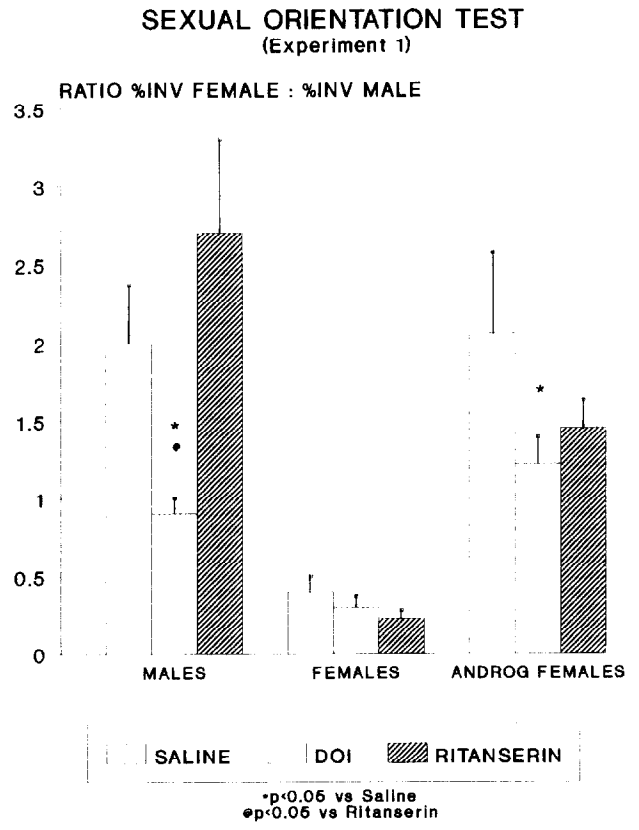

FIG. 1. Experiment 1. Effect of the 5-HT_2 agonist (DOI) and antagonist (ritanserin) on the ratio of the % time investigating female stimulus : % time investigating male stimulus (sexual orientation test). *$p < 0.05$ vs. saline; @$p < 0.05$ vs. ritanserin.

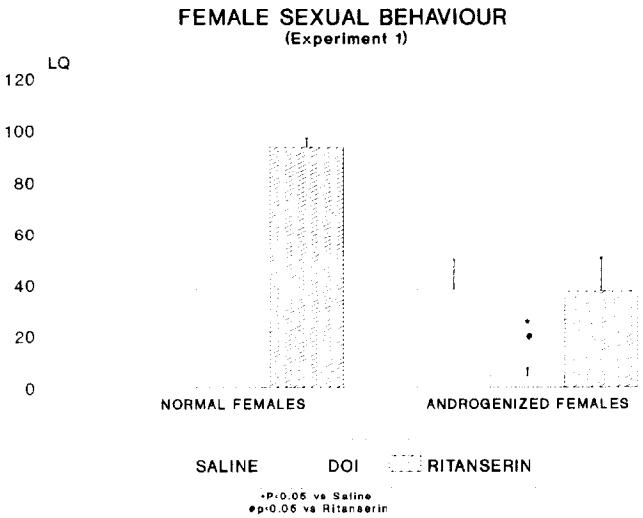

FIG. 2. Experiment 1. Effect of the 5-HT_2 agonist (DOI) and antagonist (ritanserin) on lordosis quotient (LQ) (female sexual behavior test). *$p < 0.05$ vs. saline; @$p < 0.05$ vs. ritanserin.

Female Sexual Behavior Test

Experiment 1 (fig. 2). Means, SE, and results of planned comparisons are reported in Fig. 2.

Two-way ANOVA carried out on lordosis quotients (LQ) of females and androgenized females resulted in a significant effect for both main factors sex, $F(1, 37) = 130.01$, $p = 0.0001$, and treatment, $F(2, 37) = 5.44$, $p = 0.008$. Irrespective of treatment, LQ were higher in normal females than androgenized females; irrespective of sex, they were lower in animals treated with DOI than either saline or ritanserin. However, this is only due to a lower level of LQ in the androgenized females, i.e., DOI only lowered LQ in the presence of neonatal testosterone.

Experiment 2. No significant effects were seen after the 5-HT_{1A} drugs.

Male Sexual Behavior Test

Means, SE, and results of planned comparisons for the most relevant measures of male sexual behavior are shown in Fig. 3 for Experiment 1.

Experiment 1 (fig. 3). Due to their nonnormal distributions, data were transformed logarithmically. In this experiment (5-HT_2 drugs), one-way ANOVA gave a significant result for mount latency (MT Lat), $F(2, 25) = 10.81$, $p < 0.001$. Post hoc comparisons demonstrated that latencies were longer for the DOI-treated group than for the other groups.

Significant results were also obtained for Intromission la-

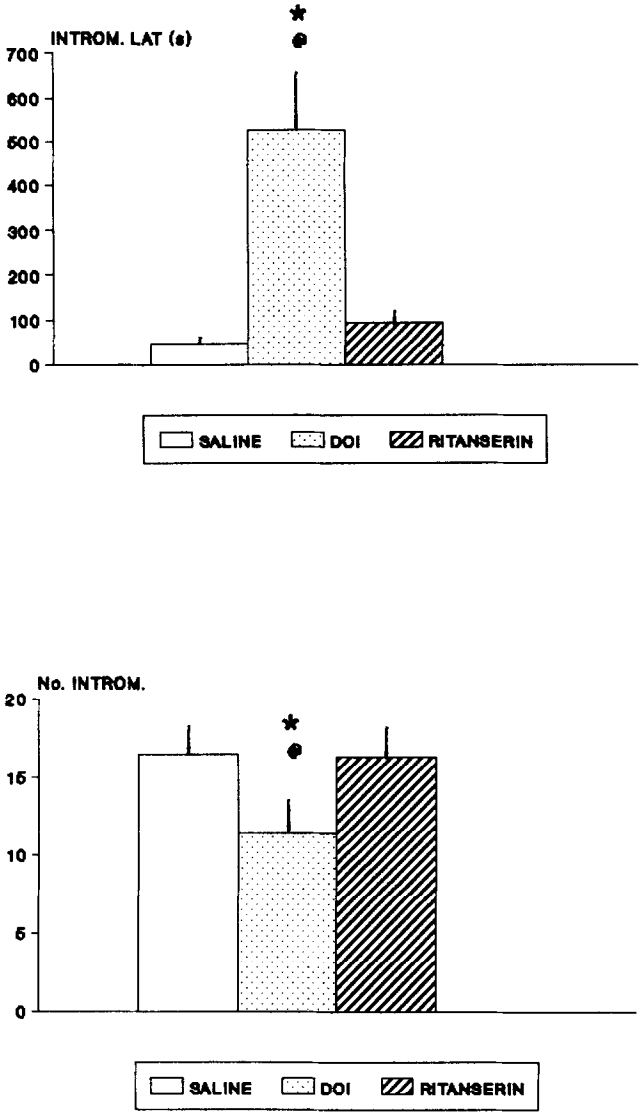

FIG. 3. Experiment 1. Effect of the 5-HT$_2$ agonist (DOI) and antagonist (ritanserin) on Intromission Latency (s) and number of intromissions to reach ejaculation (male sexual behavior test). *$p < 0.05$ vs. saline; @$p < 0.05$ vs. ritanserin.

tency (INT LAT), $F(2, 52) = 10.15, p < 0.001$, and for number of Int, $F(2, 52) = 11.05, p < 0.001$. Means, standard errors, and results of planned comparisons for these parameters are shown in Fig. 3. Intromission latencies were longer and number of intromissions lower in the DOI-treated group than in the other groups.

As for ejaculation latency, no significant difference was found.

Experiment 3. The castrated animals did not show any sexual activity. The sham-castrated males showed low levels of activity, with no differences between treatments (5-HT$_{1A}$ drugs).

DISCUSSION

In previous studies we have manipulated endogenous levels of 5-HT over the second week of life and shown it has organizational effects on adult behavior, depending on the behavioral parameter, genetic sex, and presence of circulating testosterone in the neonatal period (10,39,41). In this study we have started to investigate the receptor subtype that might mediate these actions of 5-HT and have administered either more selective 5-HT$_2$ or 5-HT$_{1A}$ agonists or antagonists.

The following serotonergic drugs were employed; DOI, an agonist known to act on 5-HT$_2$ receptors, ritanserin, a 5-HT$_2$ antagonist (36), 8-OH-DPAT, a 5-HT$_{1A}$ agonist (43), and WAY100135, a 5-HT$_{1A}$ antagonist (14).

Turning first to observations on sexual orientation, which is indicative of sexual motivation, we have shown that saline-treated males and androgenized females both showed a preference for the female stimulus. This was not altered by the two antagonists, ritanserin and WAY, but was abolished by the 5-HT$_2$ agonist DOI both in males and androgenized females and was decreased by the 5-HT$_{1A}$ agonist, 8-OH-DPAT, but only in the androgenized females. This indicates that 5-HT activity in the neonatal period selectively suppresses heterosexual preference induced in the presence of neonatal testosterone, and it seems to be antagonizing the testosterone effect independent of genetic sex. Note, we have considered the androgenized female to be behaviorally masculinized and, therefore, her preference for a female is heterosexual. Previously, we have shown in male rats that reduction in endogenous 5-HT over the neonatal period nonselectively induced by pCPA increased heterosexual preference, supporting the concept of antagonism between 5-HT and testosterone (10). In a previous study, androgenized females showed an equal interest in male and female stimuli, but in that experiment they were ovariectomized as adults and also received testosterone in adulthood (39). In that same study there was also no difference in baseline preference in the normal females; in the present study the normal females of the first two experiments showed a preference for the male teaser or no preference at all, respectively, proving that different batches of animals can differ in this basic behavior. None of the serotonergic agents, whether acting on 5-HT$_{1A}$ or 5-HT$_2$ receptors, affected sexual orientation in the normal females.

Not only heterosexual preference, but also male sexual activity was depressed in males by the 5-HT$_2$ agonist. This is consistent with previous results where neonatal 5-HT depletion by pCPA facilitated male sexual behavior (41). Neonatal administration of 5-HT$_{1A}$ agents did not affect male sexual behavior in any of the groups, so these results suggest a selective inhibitory role of the 5-HT$_2$ system on the neonatal organization of male sexual behavior. We show here that this is coupled with a disappearance of sexual preference. The inhibitory effect of 5-HT$_2$ activity on male sexual behavior in adulthood is well established [see (20)], and in particular, systemic administration of DOI reduces male behavior in adults (37) while we have shown that ritanserin can enhance adult male sexual activity (19).

Female sexual receptivity was markedly reduced by administration of neonatal androgen, confirming many other reports [see (6)]. The 5-HT$_{1A}$ and 5-HT$_2$ agents had no effect in the normal female; but DOI reduced receptivity further in the androgenized female. Thus, DOI reduced both male behavior

in males and female behavior in androgenized females, showing that it has an inhibitory effect on sexual activity, seemingly only in the presence of neonatal testosterone. The inhibitory effect of DOI on female behavior conflicts with an earlier finding, when we showed that neonatal 5-HTP attenuated the testosterone-induced inhibition of lordosis (41). However, 5-HTP increases 5-HT activity at all receptor types, and so its effect on 5-HT_2 receptors may be masked by some other receptor activation. In adulthood, both 5-HT_2 and 5-HT_{1A} receptors are involved in the control of sexual behavior, and their effects are sex dependent. Thus, 5-HT_2 inhibits male and stimulates female sexual activity (20,27,40), while 5-HT_{1A} has the opposite effects being inhibitory in females and stimulatory in males (2,28,35).

Turning to exploration of the environment, as assessed in adulthood by head-dipping duration in the holeboard test (13), DOI increased exploratory activity in females, but had no effect in males or androgenized females. Ritanserin and the 5-HT_{1A} drugs had no effect on exploration in any of the groups. Thus, although in normal females DOI increased the motivation to explore the environment, it did not change the sexual motivation and their lordotic activity. Conversely, DOI decreased preference for heterosexual conspecifics and sexual activity in both males and androgenized females but did not affect their exploration of the environment. This shows that neonatal 5-HT_2 activity differentially affects sociosexual investigation and environmental exploration according to the sex of the animal and the neonatal presence of testosterone. In particular, DOI only showed its inhibitory effect on heterosexual orientation and sexual activity in the presence of neonatal testosterone, independent of the genetic sex (i.e., both in males and androgenized females). On the other hand, it only showed its stimulatory effect on exploration in the absence of neonatal testosterone, i.e., in normal females. A possible explanation is that over neonatal period, 5-HT_2 activity antagonizes the stimulatory effect of testosterone on sexual activity and testosterone antagonises the stimulatory effect of 5-HT on exploration.

One can speculate on the mechanisms of this mutual antagonism. Steroids are known to alter 5-HT receptor synthesis, density, and affinity (4,5,33), so the presence of testosterone may well affect the action of a 5-HT agent. The converse situation, where 5-HT alters the actions of testosterone, is most likely an intracellular interaction, where the stimulation of the membrane-bound 5-HT receptor initiates intracellular changes that affect the genomic response to testosterone.

Increased exploration by DOI in normal females was not paralleled by reduced emotionality. Anxiety was assessed in the elevated plus-maze system, in which the percentage time spent in the open arms is an inverse measure of anxiety (22). While the 5-HT_{1A} agents had no effect in any of the sexes, neonatal ritanserin selectively reduced anxiety in females and DOI had a similar effect, but only in androgenized females. This shows that neonatal 5-HT_2 activity can have differential effects on anxiety in normal and androgenized females, with no effect in males. In previous studies (10,39) we have shown that a reduction in endogenous 5-HT through a nonselective compound (pCPA) is anxiolytic in males, females, and androgenized females; in this study, this effect was only reproduced by ritanserin in females, but not in the other sexes. It is possible that while the activation of a 5-HT_2 system in the neonatal period is anxiogenic in normal females, the organizational effect of 5-HT on anxiety in males or in the presence of neonatal testosterone, may be mediated by other receptor subtypes.

Anxiety cannot be regarded as a unitary concept, and it is important to refer to the test employed (12), because what is measured, for instance, in the elevated plus-maze test is not the only form of anxiety in animals. Indeed, using this test, conflicting results have been reported on the influence of 5-HT on anxiety in adulthood. Although there are indications that a reduction in 5-HT activity can reduce anxiety (5, 26), studies with more specific ligands are not consistent with this view. In particular, while the 5-HT_2 antagonist ritanserin was anxiogenic, the 5-HT_{1A} agonist 8-OH-DPAT was anxiogenic in some cases (8) and anxiolytic or ineffective in others (29,32).

Summarizing our previously reported studies, it appears that 5-HT inhibits the organizational effects of testosterone on locomotion and exploration (which testosterone normally reduces) and male sexual activity and heterosexual orientation (which it normally enhances). Neonatal 5-HT also appears to have a genetic and testosterone-independent anxiogenic effect. In this study we hoped to elucidate, at least in part, the receptor subtypes mediating the serotonergic effects.

Agents acting on 5-HT_{1A} receptors do not appear to substantially affect the organization of any of the behavioral systems studied. On the other hand, some of the previous effects noted after manipulation of endogenous 5-HT may be mediated by 5-HT_2 receptors. The 5-HT_2 system antagonises the action of testosterone in stimulating heterosexual orientation and sexual activity and this is independent of genetic sex. Similarly, testosterone appears to antagonise the stimulatory effect of 5-HT_2 activity on exploration. Unfortunately, the effect of DOI on exploration in neonatally castrated males was not noted and so its dependence on genetic sex is not known. In agreement with our previous findings, the effects of 5-HT manipulation on sexual activity proved to be independent of the effects on exploration and also locomotion and anxiety. Previous findings indicate that reduction of 5-HT, by pCPA, was anxiolytic in both sexes and independent of testosterone. This indicates an anxiogenic effect of 5-HT, which as shown in these experiments, may be a 5-HT_2 effect in females, whereas other receptors may mediate the anxiogenic effect in the presence of testosterone.

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