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Citalopram combined with WAY 100635 inhibits ejaculation and ejaculation-related Fos immunoreactivity

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Abstract

The role of 5-HT (5-hydroxytryptamine, 5-HT)$_{1A}$ receptor activation in the sexual side-effects, in particular delayed ejaculation, of selective serotonin reuptake inhibitors (SSRIs) was studied. Male Wistar rats were treated for 15 days with vehicle, the SSRI citalopram (10 mg/kg/day p.o.), the 5-HT$_{1A}$ receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide 3HCL (WAY 100635, 0.1 mg/kg/ day s.c.), or both drugs combined. Sexual behavior was assessed weekly. One h after the last sexual behavior test, rat brains were processed for Fos-immunohistochemistry. Acute and chronic citalopram mildly inhibited ejaculation, which was strongly augmented by co-administration of WAY 100635. WAY 100635 alone did not alter sexual behavior. Brain sites associated with ejaculation showed reduced Fos-immunoreactivity in rats treated with both citalopram and WAY 100635. Citalopram reduced Fos-immunoreactivity in the arcuate hypothalamic nucleus, an area that might link serotonergic neurotransmission to ejaculation.

Keywords: Selective serotonin reuptake inhibitor; Ejaculation; Antidepressant; c-fos; 5-HT$_{1A}$

1. Introduction

The Selective Serotonin Reuptake Inhibitors (SSRIs) citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline are useful therapeutic agents for the treatment of depression, but are also associated with a high incidence of sexual dysfunction including delayed ejaculation (Rosen et al., 1999).

The antidepressant effect of all SSRIs occurs through blockade of the serotonin (5-hydroxytryptamine, 5-HT) transporter, which results in elevated extracellular serotonin levels (Hiemke and Hartter, 2000). Since serotonin inhibits sexual behavior (Ahlenius and Larsson, 1998; Lorrain et al., 1997; Marson and McKenna, 1992), elevated serotonin levels are thought to cause the SSRI-induced delayed ejaculation.

However, although all SSRIs inhibit serotonin reuptake and increase serotonin levels in a similar manner (Nutt et al., 1999), they vary in their degree of delaying ejaculation: chronic treatment with paroxetine or fluoxetine strongly delays ejaculation in humans (Waldinger et al., 1998; Waldinger and Olivier, 1998) and rats (Cantor et al., 1999; Frank et al., 2000; Vega et al., 1998; Waldinger et al., 2002), whereas citalopram and fluvoxamine appear to affect sexual function to a lesser extent in humans (Waldinger and Olivier, 1998; Waldinger et al., 2001) and rats (Ahlenius and Larsson, 1999). These findings led to the theory that

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fluoxetine and paroxetine affect the neurobiological substrate involved in ejaculation somehow different from citalopram and fluvoxamine.

The 5-HT_{1A} receptor might be involved in this difference, since 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and flesinoxan strongly facilitate ejaculation in rats (Ahlenius et al., 1981; Coolen et al., 1997; Haensel and Slob, 1997). Possibly, the degree of 5-HT_{1A} receptor activation during chronic SSRI-treatment determines the severity of potential sexual side effects.

To test the hypothesis that activation of the 5-HT_{1A} receptor prevents inhibition of ejaculation during acute and chronic treatment with citalopram, we co-administered citalopram with the silent and selective 5-HT_{1A} receptor antagonist WAY 100635 (Fletcher et al., 1996) and studied the effects on sexual behavior.

The brain areas where serotonin and 5-HT_{1A} receptors influence ejaculation are not yet known. The staining of Fos, the protein product of the immediate-early gene *c-fos*, has been used to investigate neural activation following copulation and ejaculation (Coolen et al., 1996; Greco et al., 1996, 1998; Pfaus and Heeb, 1997; Veening and Coolen, 1998) and acute and chronic administration of SSRIs (Jongsma et al., 2002; Lino-de-Oliveira et al., 2001; Veening et al., 1998). To investigate how the drug-treatments altered the activation pattern in the CNS following sexual behavior, the pattern and number of Fos immunoreactive cell nuclei throughout the brain and spinal cord were studied.

2. Materials and methods

2.1. Animals

Adult male (n=60, 250–300 g and 3 months of age at the start of the experiment) and female (n=100) Wistar rats (Harlan, Zeist, the Netherlands) were used. The animals arrived at the laboratory at least 14 days prior to the start of the experiments, in order to adapt to the laboratory environmental condition and a reversed light/dark cycle (12:12 h, lights off at 6.30 am). Food and tap water were available ad libitum. Males were housed individually and the females two per cage. Females were sterilized by ligation of the oviducts and served as stimulus animals. Sexual receptivity was reliably induced by subcutaneous administration of 50 μg estradiol benzoate dissolved in 0.1 ml arachidis oil 36 h prior to testing. Following the Dutch law on the Protection of Animals, the Animal Ethical Committee of the University of Nijmegen approved of the studies.

2.2. Drugs

N-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide 3HCL (WAY 100635, Wyeth-Ayerst, Princeton, NJ) was dissolved in 0.9% NaCl and injected subcutaneously in a dose of 0.1 mg/kg and a volume of 1 ml/kg.

Citalopram hydrobromide (kindly provided by Lundbeck, Copenhagen, Denmark) was dissolved in 1% methylcellulose and administered orally in a dose of 10 mg/kg and a volume of 5 ml/kg.

2.3. Behavioral observations

All sessions were performed between 10:00 h and 15:30 h, in a red-lighted room. The same procedure was used for all sessions: male rats were placed in a rectangular mating arena (40×50×65 cm) with wood shavings on the floor and a Perspex front. After 10 min of habituation a receptive female entered the arena and free contact was allowed for thirty min. The first two weekly sessions were used as training and no observer was present. In the next two weekly sessions the number of ejaculations was counted. From the animals that had reached 2 or 3 ejaculations in 30 min, 32 rats were selected for the experiment and randomly assigned to the four experimental groups (n=8 per group). Every day between 16:00 h and 18:00 h, each rat received two injections within one min, in a combination depending on the experimental group: saline (s.c.)+methylcellulose (p.o.), WAY 100635 (s.c.)+methylcellulose (p.o.), saline (s.c.)+citalopram (p.o.) or WAY 100635 (s.c.)+citalopram (p.o.). On testing days, both injections were given 55–60 min prior to the behavioral testing of the rat. One rat was discarded from the co-administration group because of a failed injection.

In total three behavioral tests of 30 min were run 1 h after the 1st, the 8th and the 15th injection (days 1, 8 and 15). The total number of ejaculations, mounts and intromissions were counted using event recording software of The Observer (Noldus, the Netherlands). The ejaculation latency (time from first mount or intromission to ejaculation), post-ejaculatory interval (time from ejaculation to next mount or intromission), mount frequency (number of mounts prior to ejaculation), intromission frequency (number of intromissions prior to ejaculation), mount latency (time from the start of the test to the first mount) and the intromission latency (time from the start of the test to the first intromission) were calculated. All parameters were analyzed for the first and second ejaculatory cycle.

2.4. Immunohistochemistry

One hour after the end of the behavioral test on day 15, males were anesthetized using an overdose of sodium pentobarbital (60 mg/ml, 0.2 ml/kg, i.p.) and perfused transcardially with 0.1 M phosphate buffered saline (PBS, pH 7.3) followed by fixative (4% paraformaldehyde in PBS, pH 7.2). Brains were removed and postfixed for 24 h at 4 °C before the paraformaldehyde was replaced by 30% sucrose in phosphate buffer.

Coronal sections (40 μm) were cut using a freezing microtome and collected in PBS-containing tubes. All steps of the immunohistochemistry described below were performed at room temperature.

First, sections were rinsed in PBS, soaked in 30% H_2O_2 for 30 min and rinsed 3×20 min in PBS. After 30 min of preincubation with PBS containing 0.1% bovine serum albumin and 0.5% Triton-X-100, sections were incubated overnight in the same medium with an *c-fos* antiserum raised in rabbit (Santa Cruz, USA, dilution 1:20,000). The next day, the sections were rinsed 3×20 min in PBS and incubated for ninety min in donkey anti-rabbit antibody (Biotin SP conjugated, Jackson Immuno Research, USA, diluted 1:400). Sections were rinsed 3×20 min and incubated for ninety min in ABC-elite (Vector elite 1:800 in PBS, prepared 60 min in advance, Brunschwig Chemie, the Netherlands). Again, the sections were rinsed 3×20 min in PBS. Then, sections were stained using a chromogen solution consisting of 0.02% 3,3′ -diaminobenzidine tetrahydrochloride (DAB) and 0.03% Nickel-Ammonium in 0.05M Tris-buffer (pH 7.6): exactly 10 min of incubation without, and 10 min with 30% H_2O_2. This resulted in a blue-black staining. All sections were rinsed 3×20 min in PBS and mounted on gelatin chrome alum-coated glass slides, dried overnight, cleared in xylene, embedded with Entellan (Merck, Germany) and coverslipped.

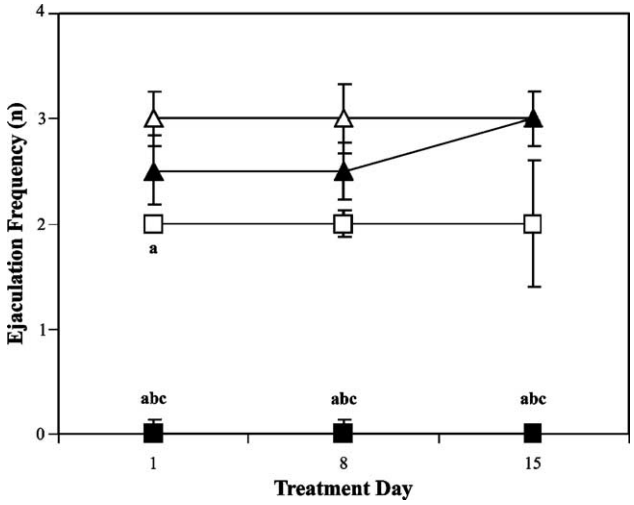

Fig. 1. The effects of vehicle (open triangles), WAY 100635 (0.1 mg/kg/day s.c., black triangles), citalopram (10/mg/kg/day p.o., open squares) or co-administration of WAY 100635 and citalopram (black squares) after acute, 8-day and 15-day treatment on the ejaculation frequency of rats in a 30-min test with a receptive female. Data are medians±standard error of the median; a=different from the vehicle-treated group, b=different from the WAY-treated group, c=different from the citalopram-treated group; $P<0.05$.

Immunoreactive cell nuclei were quantified using the software program Neurolucida (Brightfield, USA). Numbers of Fos-immunoreactive nuclei were counted in homologous square fields (using a grid size of 100×100 μm) displaying a

Table 1
The effects of vehicle, WAY 100635 (0.1 mg/kg/day s.c.), citalopram (10/mg/kg/day p.o.) or co-administration of WAY 100635 and citalopram after 1-day, 8-day and 15-day treatment on the ejaculation frequency (EF) ejaculation latency (EL), post-ejaculatory interval (PEI), mount frequency (MF), intromission frequency (IF) and intromission latency (IL) of sexually experienced male rats in a 30-min test with a receptive female

Day	Parameter	Treatment			
		Vehicle (n=8)	WAY 100635 (n=8)	Citalopram (n=8)	WAY+citalopram (n=7)
1	EF (n)	3.00±0.26	2.50±0.33	2.00±0.00[a]	0.00±0.14[a,b,c]
	EL (s)	340.16±69.06	444.00±101.91	454.90±54.40	1800.00±38.78[a,b,c]
	PEI (s)	282.45±10.48	320.71±20.20	364.98±8.73[a]	–
	MF (n)	13.00±2.11	12.00±4.23	12.00±0.85	–
	IF (n)	11.50±1.85	10.00±0.71	7.00±0.99	–
	ML (s)	26.80±12.85	8.02±0.33[a]	9.53±2.92	17.63±25.52[b]
	IL (s)	7.50±3.67	11.90±1.33	18.13±12.69	231.78±283.18[a,b,c]
8	EF (n)	3.00±0.33	2.50±0.26	2.00±0.13	0.00±0.14[a,b,c]
	EL (s)	288.69±61.54	319.06±57.73	486.45±48.27	1800.00±14.75[a,b,c]
	PEI (s)	269.85±24.20	280.95±30.16	355.56±25.02	–
	MF (n)	9.50±3.23	8.50±1.39	19.50±3.96	–
	IF (n)	7.00±1.06	8.50±0.59	6.50±1.39	–
	ML (s)	6.37±5.39	5.80±1.47	10.36±4.45	28.67±7.96
	IL (s)	8.24±1.16	9.92±2.52	6.73±4.82	37.68±52.88[a,b,c]
15	EF (n)	3.00±0.26	3.00±0.26	2.00±0.59	0.00±0.00[a,b,c]
	EL (s)	371.41±47.22	382.53±49.00	624.20±374.16	1800.00±0.00[a,b,c]
	PEI (s)	276.99±10.31	331.70±19.28	322.03±16.00	–
	MF (n)	12.50±2.64	16.00±2.37	21.00±6.01	–
	IF (n)	11.00±12.25	8.00±0.86	8.00±0.67	–
	ML (s)	5.11±0.88	5.91±1.29	10.36±2.89[a]	12.63±32.20[a,b]
	IL (s)	8.38±1.03	29.20±11.68	10.60±2.33	42.18±292.82[a,c]

$P<0.05$.

Data are medians±standard error of the median.

[a] Different from the vehicle-treated group.

[b] Different from the WAY-treated group.

[c] Different from the citalopram-treated group.

Table 2
The effects of vehicle, WAY 100635 (0.1 mg/kg/day s.c.) or citalopram (10/mg/kg/day p.o.) after 1-day, 8-day and 15-day treatment on the second ejaculation latency (EL), post-ejaculatory interval (PEI), mount frequency (MF) and intromission frequency (IF) of sexually experienced male rats in a 30-min test with a receptive female

Day	Parameter	Treatment		
		Vehicle (n=8)	WAY 100635(n=8)	Citalopram (n=8)
1	EL (s)	190.65±25.92	193.67±32.29	378.87±63.27
	PEI (s)	377.40±4.37	429.82±13.46	429.94±25.87
	MF (n)	7.50±2.31	4.50±2.74	11.00±1.97
	IF (n)	4.50±0.59	3.00±0.00	4.00±0.56
8	EL (s)	150.60±30.55	183.65±16.97	297.45±50.18
	PEI (s)	339.49±25.07	430.32±15.77	389.67±6.13
	MF (n)	6.00±1.27	7.00±1.45	11.50±1.22
	IF (n)	4.00±0.71	3.00±0.13	4.00±0.23
15	EL (s)	201.91±37.52	213.94±10.56	546.95±130.74
	PEI (s)	394.42±20.43	437.81±12.60	–
	MF (n)	10.50±4.09	9.00±2.12	29.00±7.34
	IF (n)	4.50±0.59	4.00±0.56	4.00±0.33

Data are medians±standard error of the median.

representative density of stained cells. Some series of brain slices did not yield representative staining and were removed from quantification.

Fos expression was quantified in the following brain areas known to be involved in sexual behavior: the medial preoptic nucleus, the rostral and caudal medial division of the posterior bed nucleus of the stria terminalis, the dorsal parvicellular part of the paraventricular hypothalamic nucleus, the posterodorsal part of the medial amygdaloid nucleus, the parvicellular part of the medial subparafascicular thalamic nucleus and the sacral parasympathetic nucleus at the L6-S1 level of the spinal cord. Since these areas are activated in relation with ejaculation, the citalopram-treated animals that did not ejaculate in the last sexual behavior test were excluded from the comparison.

Fos expression was further quantified in all areas showing substantial fos-immunoreactivity in most of the rats in at least one experimental group: the prelimbic cortex, the ventral part of the lateral septal nucleus, the lateral division of the dorsal bed nucleus of the stria terminalis, the medial parvicellular part of the paraventricular hypothala-

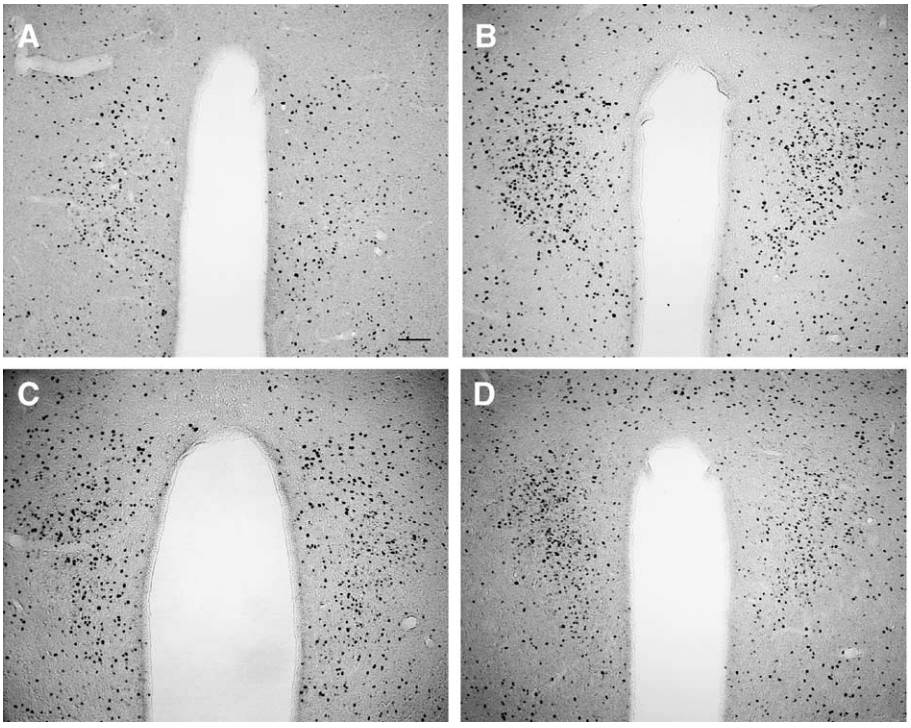

Fig. 2. Fos-positive nuclei in medial parvicellular as well as the dorsal parvicellular part of the paraventricular hypothalamic nucleus (Bregma −1.80 mm) of rats that were treated for 15 days with vehicle (A), WAY 100635 (B), citalopram (C) or co-administration of WAY 100635 and citalopram (D) and perfused 1h after a sexual behavior test. Scale bar=100 μm.

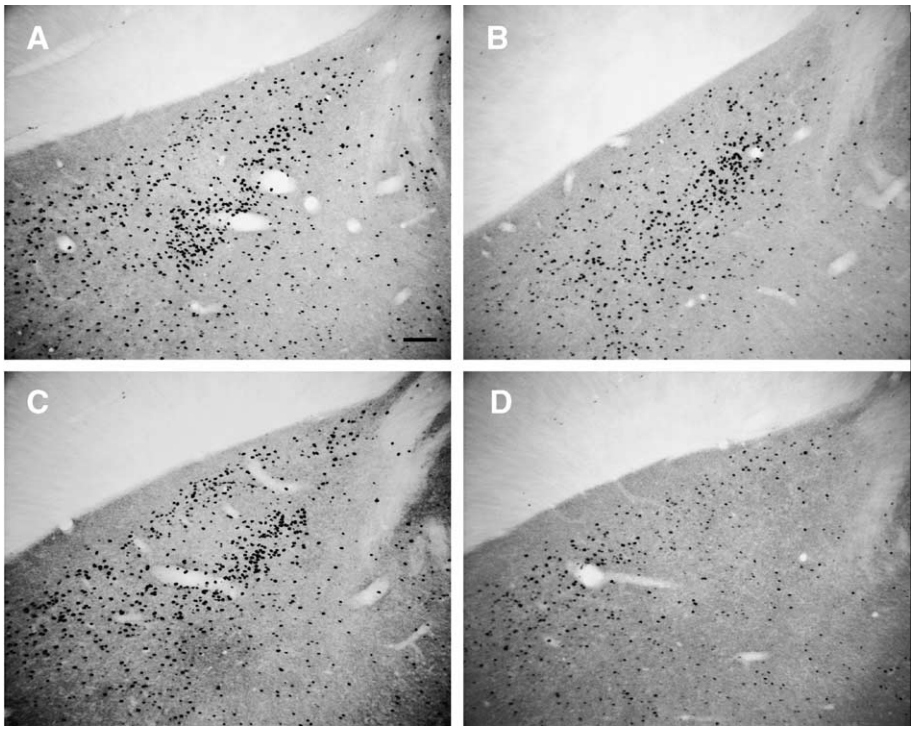

Fig. 3. Fos-positive nuclei in the posterodorsal division of the medial amygdaloid nucleus (Bregma −3.14 mm) of rats that were treated for 15 days with vehicle (A), WAY 100635 (B), citalopram (C) or co-administration of WAY 100635 and citalopram (D) and perfused 1 h after a sexual behavior test. Scale bar=100 μm.

mic nucleus, the arcuate hypothalamic nucleus, the lateral part of the central amygdaloid nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, the ventral part of the premammillary nucleus, the apical subnucleus of the interpeduncular nucleus, the ventrolateral periaqueductal gray, the compact part of the nucleus incertus, the locus

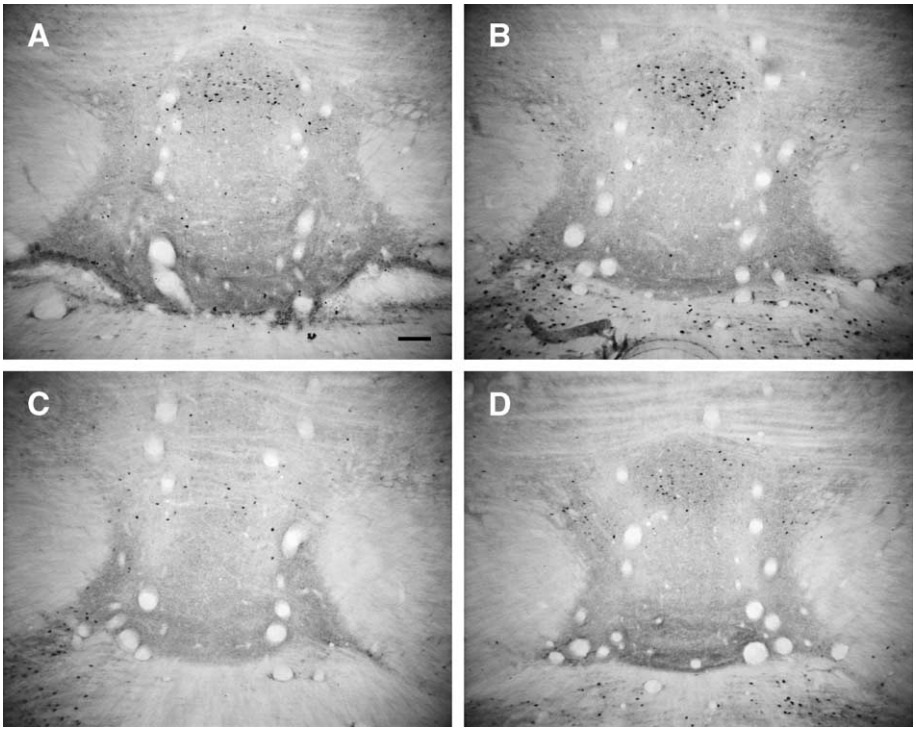

Fig. 4. Fos-positive nuclei in the apical division of the interpeduncular nucleus (Bregma −6.72 mm) of rats that were treated for 15 days with vehicle (A), WAY 100635 (B), citalopram (C) or co-administration of WAY 100635 and citalopram (D) and perfused 1 h after a sexual behavior test. Scale bar=100 μm.

coeruleus and the medial part of the nucleus of the solitary tract.

2.5. Statistical analysis

All behavioral data were analyzed using the Kruskall Wallis test and, in case of overall significant differences, with the Mann–Whitney test.

The immunocytochemical data were analyzed using Univariate Analysis of Variance (ANOVA), further post-hoc comparisons were made using the Student–Neuman–Keuls test.

In order to find common changes in activated areas of individual animals, a correlation analysis was performed on the numbers of Fos-immunoreactive cell nuclei using Pearson's correlation coefficient. The level of significance in all tests was $P<0.05$.

3. Results

3.1. Sexual behavior

An overview of the sexual behavior of the four treatment groups is shown in Table 1.

Analysis with the Kruskall–Wallis test showed that there were group differences on the first treatment day in ejaculation frequency ($\chi^2=15.857$; $P=0.001$), ejaculation latency ($\chi^2=13.225$; $P=0.004$), post-ejaculatory interval ($\chi^2=9.534$; $P=0.023$), mount latency ($\chi^2=9.188$; $P=0.027$) and intromission latency ($\chi^2=15.366$l; $P=0.002$). Further analysis with the Mann–Whitney test revealed that the group treated with both WAY 100635 and citalopram had a reduced ejaculation frequency ($P<0.01$, Fig. 1) and an increased ejaculation latency and intromission latency ($P<0.01$) than all the other groups, and a higher mount latency than the group treated with WAY 100635 only ($P=0.015$). The WAY 100635-treated group had a lower mount latency compared to the vehicle-treated group ($P=0.021$). Citalopram alone reduced EF ($P=0.036$) and increased postejaculatory interval compared to vehicle on day 1 ($P=0.004$).

After one week of drug-treatment, the Kruskall–Wallis test showed group differences in ejaculation frequency ($\chi^2=18.521$; $P=0.000$), EL ($\chi^2=16.873$; $P=0.001$) and intromission latency ($\chi^2=8.621$; $P=0.035$). Further analysis with the Mann–Whitney test showed that the co-administration group had a reduced ejaculation frequency ($P<0.005$) and an increased ejaculation latency ($P<0.005$)

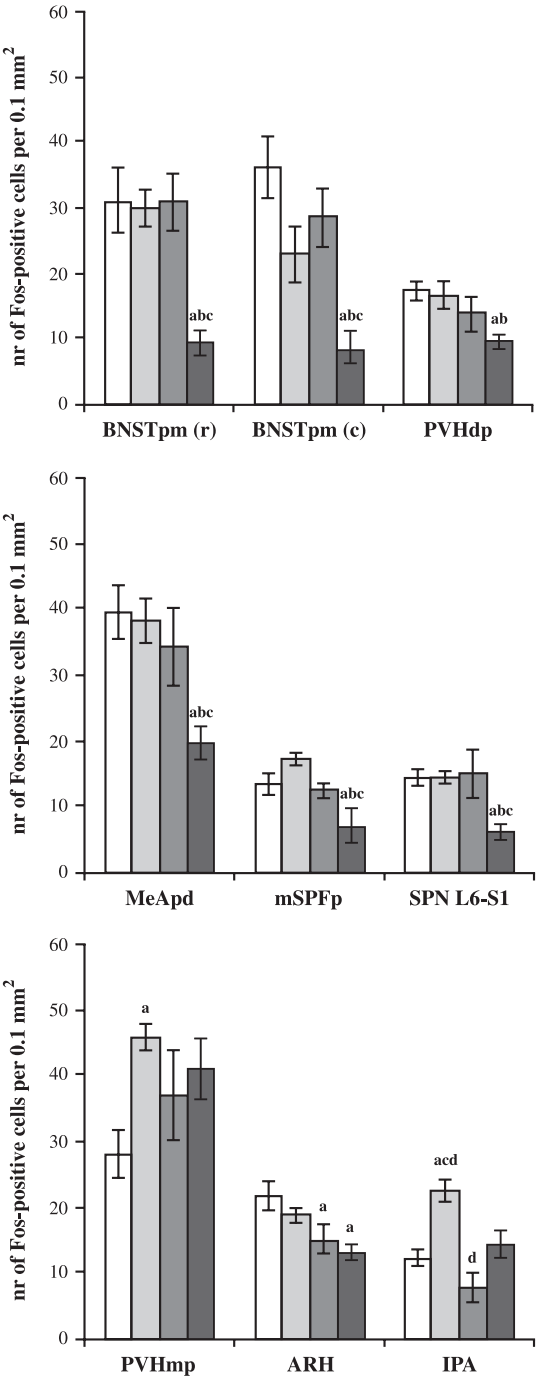

Fig. 5. The number of fos-immunoreactive nuclei in the rostral and caudal bed nucleus of the stria terminalis, medial division, posterior part (BNSTpm r/c); paraventricular hypothalamic nucleus, dorsal parvicellular part (PVHdp); medial amygdaloid nucleus, posterodorsal part (MEApd), the medial subparafascicular thalamic nucleus, parvicellular part (mSPFp), the sacral parasympathetic nucleus at the L6-S1 level of the spinal cord (SPN), the paraventricular hypothalamic nucleus (PVHmp), the arcuate hypothalamic nucleus (ARH) and the apical subnucleus of the interpeduncular nucleus (IPA) of rats treated for 15 days with vehicle (open bars), WAY 100635 (0.1 mg/kg/day s.c., light grey-filled bars), citalopram (10/mg/kg/day p.o., grey-filled bars) or co-administration of WAY 100635 and citalopram (dark grey-filled bars) and perfused 1 h after a sexual behavior test. Data are means±standard error of the mean: a=different from the vehicle-treated group, b=different from the WAY-treated group, c=different from the citalopram-treated group, d=different from the co-administration group; $P<0.05$.

and intromission latency ($P<0.05$) compared to all other groups. There were no differences between the other groups.

Two weeks of treatment resulted in differences in ejaculation frequency ($\chi^2=17.318$; $P=0.001$), ejaculation latency ($\chi^2=15.102$; $P=0.002$), mount latency ($\chi^2=10.245$; $P=0.017$) and intromission latency ($\chi^2=9.337$; 0.025). According to the Mann–Whitney test, the co-administration group had a reduced ejaculation frequency ($P<0.05$) and an increased ejaculation latency ($P<0.05$) compared to all other groups. The co-administration group differed in mount latency from the vehicle- and WAY 100635-treated groups ($P<0.05$) and in intromission latency from the vehicle- and citalopram-treated groups ($P<0.05$). Citalopram increased mount latency compared to vehicle-treatment ($P=0.036$).

No significant differences on any day existed between the experimental groups in the second ejaculatory cycle (Table 2).

3.2. Immunohistochemistry

The combination of drug administration and the concomitant performance of sexual behavior preceding perfusion led to substantial *c-fos* expression. Figs. 2, 3 and 4, showing respectively the paraventricular hypothalamic nucleus, the posterodorsal division of the medial amygdaloid nucleus and the apical division of the interpeduncular nucleus, are illustrations of this expression.

The Univariate ANOVA revealed significant differences between the experimental groups in the number of Fos-positive in several areas (Fig. 5): the rostral ($F=8.052$, $P=0.001$) and caudal ($F=9.462$, $P=0.000$) medial division of the posterior bed nucleus of the stria terminalis, the dorsal parvicellular part of the hypothalamic paraventricular nucleus ($F=5.519$, $P=0.005$), the posterodorsal division of the medial amygdaloid nucleus ($F=6.263$, $P=0.003$), the parvicellular part of the medial subparafascicular thalamic nucleus ($F=6.663$, $P=0.003$), the sacral parasympathetic nucleus at the L6/S1 level ($F=9.784$, $P=0.000$), the medial parvicellular part of the paraventricular hypothalamic nucleus ($F=3.637$, $P=0.034$), the arcuate hypothalamic nucleus ($F=4.965$, $P=0.011$) and the apical subnucleus of the interpeduncular nucleus ($F=13{,}226$, $P=0.000$).

Further analysis with the post-hoc Student–Neuman–Keuls test showed that the number of Fos-immunoreactive nuclei was strongly reduced in the co-administration group compared to all other experimental groups in the rostral and caudal medial division of the posterior bed nucleus of the stria terminalis, the posterodorsal division of the medial amygdaloid nucleus, the parvicellular part of the medial subparafascicular thalamic nucleus and the sacral parasympathetic nucleus at the L6/S1 level ($P<0.05$). Furthermore, the co-administration group showed less *c-fos* expression in the dorsal parvicellular part of the hypothalamic paraventricular nucleus compared to animals treated with either vehicle or WAY 100635 ($P<0.05$), but not to animals treated with citalopram.

Injections with citalopram alone or in combination with WAY 100635 reduced Fos-immunoreactivity in the arcuate hypothalamic nucleus compared to vehicle-treatment ($P<0.05$). Animals that had received injections with WAY 100635 alone showed more Fos-immunoreactivity than vehicle-treated rats in the medial parvicellular part of the paraventricular hypothalamic nucleus ($P<0.05$).

WAY 100635 increased the number of Fos-positive neurons in the apical subdivision of the interpeduncular nucleus compared to all other groups, whereas citalopram decreased Fos-immunoreactivity. In all other areas quantified, no significant differences existed between the experimental groups (Table 3).

A correlation analysis (Table 4) applied on the number of Fos-immunoreactivity neurons in the quantified brain areas of individual animals, in order to find co-varying changes,

Table 3
The number of Fos-immunoreactive nuclei in the prelimbic area (PrL), the ventral part of the lateral septal nucleus (LSV), the lateral division of the dorsal bed nucleus of the stria terminalis (BNSTld), medial preoptic nucleus (MPN), the lateral part of the central amygdaloid nucleus (CEAl), the dorsomedial part of the ventromedial hypothalamic nucleus (VMHdm), the ventral premammillary nucleus (PMv), the ventrolateral periaqueductal gray (PAGvl), the compact part of the nucleus incertus (NIc), the locus coeruleus (LC) and the medial part of the nucleus of the solitary tract (NTSm) of sexually experienced rats treated for 15 days with vehicle, WAY 100635 (0.1 mg/kg/day s.c.), citalopram (10/mg/kg/day p.o.) or co-administration of WAY 100635 and citalopram, 1 h after a 30-min sexual behavior test with a receptive female

Brain Area	Treatment			
	Vehicle ($n=5$)	WAY 100635 ($n=6$)	Citalopram ($n=5$)	WAY+Citalopram ($n=6$)
PrL	18.40±2.14	21.17±1.88	22.40±2.39	22.50±1.41
LSV	13.60±2.40	21.20±1.37	16.60±2.33	18.67±2.26
BNSTld	19.20±2.18	26.33±4.20	23.80±2.51	20.00±1.91
MPN	39.25±4.39	37.25±3.82	37.20±8.01	23.50±2.63
CEAl	24.00±1.76	26.33±2.25	22.00±2.94	22.20±1.76
VMHdm	30.00±4.00	33.17±2.13	29.00±2.78	31.00±2.91
PMv	28.20±2.17	20.80±2.12	26.50±4.84	28.40±1.44
PAGvl	23.00±1.00	23.67±2.86	25.60±3.67	20.33±2.74
NIc	17.00±2.50	22.50±3.96	23.00±2.75	15.80±0.76
LC	20.40±2.82	19.00±2.93	19.60±1.75	16.40±1.50
NTSm	14.80±3.44	17.17±1.04	17.60±2.14	19.00±1.89

Data are means±SEM.

Table 4
In brain areas activated by sexual behavior and ejaculation (the medial preoptic area (MPN); rostral and caudal bed nucleus of the stria terminalis, medial division, posterior part (BNSTpm r/c); paraventricular hypothalamic nucleus, dorsal parvicellular part (PVHdp); medial amygdaloid nucleus, posterodorsal part (MEApd), the medial subparafascicular thalamic nucleus, parvicellular part (mSPFp) and the sacral parasympathetic nucleus at the L6-S1 level of the spinal cord (SPN), the numbers of Fos-positive cells are correlated

Correlation	MPN	BNSTpm (r)	BNSTpm (c)	PVHdp	MEApd	mSPFp
BNSTpm (r)	0.829[a]					
BNSTpm (c)	0.591[a]	0.615[a]				
PVHdp	0.796[a]	0.604[a]	0.659[a]			
MEApd	0.715[a]	0.647[a]	0.710[a]	0.852[a]		
mSPFp	0.418[b]	0.525[b]	0.534[b]	0.543[a]	0.565[a]	
SPN (L6-S1)	0.446[b]	0.401[b]	0.371	0.549[a]	0.413[b]	0.466[b]

[a] $P<0.01$.
[b] $P<0.05$.

resulted in strong and significant correlations between the medial preoptic area, the rostral and caudal medial division of the posterior bed nucleus of the stria terminalis, the dorsal parvicellular part of the paraventricular hypothalamic nucleus, the posterodorsal part of the medial amygdaloid nucleus and the parvicellular part of the medial subparafascicular thalamic nucleus ($0.418<R^2<0.852$, $P<0.05$). Fos-immunoreactivity in the sacral parasympathetic nucleus at the L6/S1 level was strongly correlated with the dorsal parvicellular part of the paraventricular hypothalamic nucleus ($R^2=0.549$, $P<0.01$), less strongly with the other ejaculation-related areas ($0.401<R^2<0.466$, $P<0.05$) and not significantly with the caudal medial division of the posterior bed nucleus of the stria terminalis ($R^2=0.371$, $P=0.81$).

4. Discussion

4.1. Sexual behavior

Acute and chronic administration of the 5-HT_{1A} receptor antagonist WAY 100635 alone had no relevant effect on sexual behavior, which is consistent with previous findings using WAY-100635 in doses up to 0.6 mg/kg s.c. (Ahlenius and Larsson, 1999) These results show that under basal conditions 5-HT_{1A} receptors do not play a crucial role in sexual behavior, either because ejaculation can be achieved via other pathways, or because pre- and post-synaptic 5-HT_{1A} receptors are not significantly activated during copulation.

Acute injection with the SSRI citalopram (10 mg/kg p.o.) reduced the ejaculation frequency and increased the post-ejaculatory interval in sexually experienced male Wistar rats, but this effect disappeared after 8 and 15 days of treatment. On day 15, citalopram slightly increased the mount latency. These mild sexual side effects might have been stronger if a higher dose of citalopram was used. However, (Cremers et al., 2000a; Hjorth et al., 1997) found that equivalent doses of citalopram at least doubled extracellular 5-HT levels in the ventral hippocampus, indicating a relevant effect of the SSRI on serotonergic neurotransmission. Moreover, Ahlenius and Larsson (1999) showed that acute injection with citalopram in doses up to 40 mg/kg s.c. did not change sexual behavior.

When citalopram and the 5-HT_{1A} receptor antagonist WAY 100635 (0.1 mg/kg s.c.) were co-administered, 75% of the animals failed to ejaculate within 30 min on days 1 and 8, and 100% failed to ejaculate on day 15. In addition, the intromission latency was strongly increased on all test days and the mount latency was increased on day 15. These results are consistent, although more pronounced, with the findings of Ahlenius and Larsson (1999), who demonstrated an inhibition of sexual behavior in male Wistar rats after acute co-administration of WAY 100635 (0.04 and 0.08 mg/kg, s.c.) and citalopram (10 mg/kg, s.c.). The present study shows that the effect of treatment with WAY 100635 and/or citalopram on sexual behavior does not change after chronic treatment, indicating that these drugs fail to induce long-term alterations of the neurobiological substrate underlying sexual behavior.

Several explanations are possible for the synergistic effects of WAY 100635 and citalopram on sexual behavior. In vivo microdialysis studies have shown that WAY 100635 significantly facilitates the elevation of serotonin levels by acute citalopram treatment through blockade of the negative feedback signal of the somatodendritic 5-HT_{1A} autoreceptor (Cremers et al., 2000a; Hjorth et al., 1997). It is possible that in order to inhibit ejaculation, serotonin levels need to exceed a certain threshold that is not reached by either citalopram or WAY 100635 alone. However, 5-HT_{1B} receptor antagonists augment the citalopram-induced elevation of serotonin levels even more (Cremers et al., 2000a), while decreasing the inhibition of ejaculation induced by co-administration of WAY 100635 with citalopram (Ahlenius and Larsson, 1999). This suggests that elevation of serotonin levels by blockade of presynaptic receptors is not sufficient to affect ejaculation, and that postsynaptic receptors at least play a role.

By elevating serotonin levels throughout the central nervous system, SSRIs increase the activation of different subtypes of postsynaptic 5-HT receptors. This results in conflicting effects on sexual behavior, since ejaculation is facilitated by postsynaptic 5-HT_{1A} receptors (Ahlenius et al., 1981; Coolen et al., 1997; Fernandez-Guasti and

Escalante, 1991; Haensel and Slob, 1997) and inhibited by postsynaptic 5-HT_{1B} and 5-HT_{2C} receptors (Ahlenius and Larsson, 1998; Fernandez-Guasti et al., 1992; Fernandez-Guasti and Rodriguez-Manzo, 1992; Foreman et al., 1989; Hillegaart and Ahlenius, 1998; Waldinger et al., 1998). When the facilitating 5-HT_{1A} receptors are blocked, for example by WAY-100635, the netto effect of SSRI-induced elevation of 5-HT levels might be inhibition of ejaculation via 5-$HT_{1B/2C}$ receptors. This could also explain the differences in sexual side effects between paroxetine and fluoxetine on one hand and citalopram on the other hand: chronic administration of paroxetine and fluoxetine cause desensitization of postsynaptic 5-HT_{1A} receptors (D'Souza et al., 2002; Hensler, 2003; Kantor et al., 2001; Li et al., 1997), whereas chronic citalopram treatment has less pronounced effects on 5-HT_{1A} receptors (Arborelius et al., 1996; Auerbach and Hjorth, 1995; Chaput et al., 1986; Cremers et al., 2000b; Gundlah et al., 1997; Hjorth and Auerbach, 1999; Invernizzi et al., 1995; Moret and Briley, 1996). Although it is unknown whether SSRI-induced desensitization also affects 5-HT_{1A} receptors involved in ejaculation, it provides an interesting approach for future research.

4.2. Immunohistochemistry

Animals treated with both WAY 100635 and citalopram showed significantly less Fos-immunoreactivity compared to the other experimental groups in the rostral and caudal medial division of the posterior bed nucleus of the stria terminalis, the posterodorsal part of the medial amygdaloid nucleus, the parvicellular part of the medial subparafascicular thalamic nucleus and the sacral parasympathetic nucleus at the L6-S1 level of the spinal cord. These areas are known to express c-fos when an ejaculation has occurred (Coolen et al., 1996, 1997, 2003; Greco et al., 1996, 1998), which is consistent with the behavioral results. The dorsal parvicellular part of the paraventricular hypothalamic nucleus showed a similar activation pattern. Moreover, the number of Fos-immunoreactive cell nuclei in the dorsal parvicellular part of the paraventricular hypothalamic nucleus was strongly correlated with all known ejaculation-related areas suggesting that this area participates in the neural circuitry that is activated by ejaculation (Coolen et al., 2003).

There is evidence to assume that the ejaculation-related areas are activated by the genitosensory signal that an ejaculation has occurred, rather than to cause ejaculation (Coolen et al., 1996, 1997, 2003; Parfitt and Newman, 1998). Therefore, it was not surprising that the amount of Fos-immunoreactivity appeared to be related to the ejaculation frequency. Therefore, this group of brain areas provided no further clues about the location where drugs interact with the neural network to inhibit ejaculation.

Chronic citalopram treatment as well as co-administration reduced Fos-immunoreactivity in the arcuate hypothalamic nucleus, which coincided with increased mount latencies in these experimental groups. The arcuate nucleus is innervated by serotonergic fibers (Paxinos, 1995) and expresses postsynaptic 5-HT_{1A} (Aznar et al., 2003; Collin et al., 2002), 5-HT_{1B} (Makarenko et al., 2002) and 5-HT_{2C} (Clemett et al., 2000) receptors. It is connected with the medial preoptic area, the medial amygdala, the bed nucleus of the stria terminalis and the paraventricular nucleus, and is thought to integrate information about metabolism with reproductive activity, using galanin-like peptide, neuropeptide Y and gonadotropin-releasing hormone as messengers (Gottsch et al., 2004; Magoul et al., 1994). Taken together, the arcuate hypothalamic nucleus is in an excellent location to connect the serotonergic system with the reproductive system.

The apical interpeduncular nucleus, a brain area that sends projections to the hippocampus, septum and raphe nuclei (Montone et al., 1988) and is dense in 5-HT_{1A} receptor labeling (Kia et al., 1996), was strongly activated by chronic treatment with WAY 100635. Co-administration of citalopram reversed this effect (Fig. 4, Table 3). The medial parvicellular part of the paraventricular hypothalamic area showed a similar activation following chronic WAY 100635 treatment, but (co-) administration of citalopram did not alter this activity. Apparently, blocking the 5-HT_{1A} receptor changes activity in these areas, but since WAY 100635 alone did not affect any parameter of sexual behavior it is unlikely that these areas are involved in male copulation.

Substantial Fos-immunoreactivity was visible in the prelimbic area, the ventral part of the lateral septal nucleus, the lateral division of the dorsal bed nucleus of the stria terminalis, the lateral part of the central amygdaloid nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, the ventral premammillary nucleus, the ventrolateral periaqueductal gray, the compact part of the nucleus incertus, the locus coeruleus and the medial part of the nucleus of the solitary tract. However, the different drug treatments had no effect on the number of immunoreactive nuclei in these brain areas, indicating that they are activated as a result of a common experience such as general activity, sexual arousal or receiving injections.

The distribution of Fos-immunoreactivity and treatment-induced differences in Fos-immunoreactivity do not correspond with previous findings (Jongsma et al., 2002). In that study the effects of acute administration of WAY 100635 and/or citalopram on c-fos expression were analyzed. Drug-dependent differences were found in the prefrontal cortex, the central amygdala, the ventromedial hypothalamic nucleus, the dorsal raphe nucleus and other areas. A synergistic effect of WAY 100635 and citalopram was found in the paraventricular hypothalamic nucleus. The differences with the present study can be attributed to the addition of sexual behavior. Furthermore, (sub)chronic treatment with serotonergic agents has repeatedly been found to attenuate or change Fos-immunoreactivity com-

pared to acute treatment (Li and Rowland, 1996; Lino-de-Oliveira et al., 2001; Veening et al., 1998), probably because the brain habituates to the treatment, and/or the Fos-inducing mechanisms become desensitized.

In summary, our results show that the weak inhibition of ejaculation by citalopram is strongly augmented by the silent and selective 5-HT_{1A} receptor antagonist WAY 100635. 5-HT_{1A} receptors seem to play a minor role in sexual behavior when serotonin turnover is normal, but prove to be crucial for ejaculation when serotonin levels are elevated. A combination of elevated serotonin levels and reduced 5-HT_{1A} receptor activation, for example through desensitization of this receptor, could therefore be responsible for the sexual side effects reported by men treated with SSRIs.

In general, Fos-immunoreactivity reflected the occurrence of an ejaculation rather than revealing putative locations where serotonergic neurotransmission and ejaculatory behavior interact with each other. However, the arcuate hypothalamic nucleus showed altered Fos-immunoreactivity after chronic citalopram treatment with or without WAY-100635, and needs to be investigated as a possible location for the interaction of serotonergic drugs with male copulatory behavior.

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References

Ahlenius, S., Larsson, K., 1998. Evidence for an involvement of 5-HT_{1B} receptors in the inhibition of male rat ejaculatory behavior produced by 5-HTP. Psychopharmacology (Berl.) 137, 374–382.

Ahlenius, S., Larsson, K., 1999. Synergistic actions of the 5-HT_{1A} receptor antagonist WAY-100635 and citalopram on male rat ejaculatory behavior. Eur. J. Pharmacol. 379, 1–6.

Ahlenius, S., Larsson, K., Svensson, L., Hjorth, S., Carlsson, A., Lindberg, P., Wikstrom, H., Sanchez, D., Arvidsson, L.E., Hacksell, U., Nilsson, J.L., 1981. Effects of a new type of 5-HT receptor agonist on male rat sexual behavior. Pharmacol. Biochem. Behav. 15, 785–792.

Arborelius, L., Nomikos, G.G., Hertel, P., Salmi, P., Grillner, P., Hook, B.B., Hacksell, U., Svensson, T.H., 1996. The 5-HT_{1A} receptor antagonist (S)-UH-301 augments the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic treatment with citalopram. Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 630–640.

Auerbach, S.B., Hjorth, S., 1995. Effect of chronic administration of the selective serotonin (5-HT) uptake inhibitor citalopram on extracellular 5-HT and apparent autoreceptor sensitivity in rat forebrain in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 597–606.

Aznar, S., Qian, Z., Shah, R., Rahbek, B., Knudsen, G.M., 2003. The 5-HT_{1A} serotonin receptor is located on calbindin- and parvalbumin-containing neurons in the rat brain. Brain Res. 959, 58–67.

Cantor, J.M., Binik, Y.M., Pfaus, J.G., 1999. Chronic fluoxetine inhibits sexual behavior in the male rat: reversal with oxytocin. Psychopharmacology (Berl.) 144, 355–362.

Chaput, Y., de Montigny, C., Blier, P., 1986. Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 333, 342–348.

Clemett, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P., Fone, K.C., 2000. Immunohistochemical localisation of the 5-HT_{2C} receptor protein in the rat CNS. Neuropharmacology 39, 123–132.

Collin, M., Backberg, M., Onnestam, K., Meister, B., 2002. 5-HT_{1A} receptor immunoreactivity in hypothalamic neurons involved in body weight control. NeuroReport 13, 945–951.

Coolen, L.M., Peters, H.J., Veening, J.G., 1996. Fos immunoreactivity in the rat brain following consummatory elements of sexual behavior: a sex comparison. Brain Res. 738, 67–82.

Coolen, L.M., Peters, H.J., Veening, J.G., 1997. Distribution of Fos immunoreactivity following mating versus anogenital investigation in the male rat brain. Neuroscience 77, 1151–1161.

Coolen, L.M., Veening, J.G., Wells, A.B., Shipley, M.T., 2003. Afferent connections of the parvocellular subparafascicular thalamic nucleus in the rat: evidence for functional subdivisions. J. Comp. Neurol. 463, 132–156.

Cremers, T.I., de Boer, P., Liao, Y., Bosker, F.J., den Boer, J.A., Westerink, B.H., Wikstrom, H.V., 2000a. Augmentation with a 5-HT(1A), but not a 5-HT(1B) receptor antagonist critically depends on the dose of citalopram. Eur. J. Pharmacol. 397, 63–74.

Cremers, T.I., Spoelstra, E.N., de Boer, P., Bosker, F.J., Mork, A., den Boer, J.A., Westerink, B.H., Wikstrom, H.V., 2000b. Desensitisation of 5-HT autoreceptors upon pharmacokinetically monitored chronic treatment with citalopram. Eur. J. Pharmacol. 397, 351–357.

D'Souza, D.N., Zhang, Y., Garcia, F., Battaglia, G., Van De Kar, L.D., 2002. Destruction of serotonergic nerve terminals prevents fluoxetine-induced desensitization of hypothalamic 5-HT(1A) receptors. Psychopharmacology (Berl.) 164, 392–400.

Fernandez-Guasti, A., Escalante, A., 1991. Role of presynaptic serotonergic receptors on the mechanism of action of 5-HT_{1A} and 5-HT_{1B} agonists on masculine sexual behaviour: physiological and pharmacological implications. J. Neural Transm.: Gen. Sect. 85, 95–107.

Fernandez-Guasti, A., Rodriguez-Manzo, G., 1992. Further evidence showing that the inhibitory action of serotonin on rat masculine sexual behavior is mediated after the stimulation of 5-HT_{1B} receptors. Pharmacol. Biochem. Behav. 42, 529–533.

Fernandez-Guasti, A., Escalante, A.L., Ahlenius, S., Hillegaart, V., Larsson, K., 1992. Stimulation of 5-HT_{1A} and 5-HT_{1B} receptors in brain regions and its effects on male rat sexual behaviour. Eur. J. Pharmacol. 210, 121–129.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanhope, K.J., Critchley, D.J., Childs, K.J., Middlefell, V.C., Lanfumey, L., Corradetti, R., Laporte, A.M., Gozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent 5-HT_{1A} receptor antagonist. Behav. Brain Res. 73, 337–353.

Foreman, M.M., Hall, J.L., Love, R.L., 1989. The role of the 5-HT_2 receptor in the regulation of sexual performance of male rats. Life Sci. 45, 1263–1270.

Frank, J.L., Hendricks, S.E., Olson, C.H., 2000. Multiple ejaculations and chronic fluoxetine: effects on male rat copulatory behavior. Pharmacol. Biochem. Behav. 66, 337–342.

Gottsch, M.L., Clifton, D.K., Steiner, R.A., 2004. Galanin-like peptide as a link in the integration of metabolism and reproduction. Trends Endocrinol. Metab. 15, 215–221.

Greco, B., Edwards, D.A., Michael, R.P., Clancy, A.N., 1996. Androgen receptor immunoreactivity and mating-induced Fos expression in forebrain and midbrain structures in the male rat. Neuroscience 75, 161–171.

Greco, B., Edwards, D.A., Zumpe, D., Michael, R.P., Clancy, A.N., 1998. Fos induced by mating or noncontact sociosexual interaction is colocalized with androgen receptors in neurons within the forebrain,

midbrain, and lumbosacral spinal cord of male rats. Horm. Behav. 33, 125–138.
Gundlah, C., Hjorth, S., Auerbach, S.B., 1997. Autoreceptor antagonists enhance the effect of the reuptake inhibitor citalopram on extracellular 5-HT: this effect persists after repeated citalopram treatment. Neuropharmacology 36, 475–482.
Haensel, S.M., Slob, A.K., 1997. Flesinoxan: a prosexual drug for male rats. Eur. J. Pharmacol. 330, 1–9.
Hensler, J.G., 2003. Regulation of 5-HT_{1A} receptor function in brain following agonist or antidepressant administration. Life Sci. 72, 1665–1682.
Hiemke, C., Hartter, S., 2000. Pharmacokinetics of selective serotonin reuptake inhibitors. Pharmacol. Ther. 85, 11–28.
Hillegaart, V., Ahlenius, S., 1998. Facilitation and inhibition of male rat ejaculatory behaviour by the respective 5-HT_{1A} and 5-HT_{1B} receptor agonists 8-OH-DPAT and anpirtoline, as evidenced by use of the corresponding new and selective receptor antagonists NAD-299 and NAS-181. Br. J. Pharmacol. 125, 1733–1743.
Hjorth, S., Auerbach, S.B., 1999. Autoreceptors remain functional after prolonged treatment with a serotonin reuptake inhibitor. Brain Res. 835, 224–228.
Hjorth, S., Westlin, D., Bengtsson, H.J., 1997. WAY100635-induced augmentation of the 5-HT-elevating action of citalopram: relative importance of the dose of the 5-HT1A (auto)receptor blocker versus that of the 5-HT reuptake inhibitor. Neuropharmacology 36, 461–465.
Invernizzi, R., Bramante, M., Samanin, R., 1995. Extracellular concentrations of serotonin in the dorsal hippocampus after acute and chronic treatment with citalopram. Brain Res. 696, 62–66.
Jongsma, M.E., Sebens, J.B., Bosker, F.J., Korf, J., 2002. Effect of 5-HT(1A) receptor-mediated serotonin augmentation on Fos immunoreactivity in rat brain. Eur. J. Pharmacol. 455, 109–115.
Kantor, S., Graf, M., Anheuer, Z.E., Bagdy, G., 2001. Rapid desensitization of 5-HT(1A) receptors in Fawn-Hooded rats after chronic fluoxetine treatment. Eur. Neuropsychopharmacol. 11, 15–24.
Kia, H.K., Miquel, M.C., Brisorgueil, M.J., Daval, G., Riad, M., El Mestikawy, S., Hamon, M., Verge, D., 1996. Immunocytochemical localization of serotonin$_{1A}$ receptors in the rat central nervous system. J. Comp. Neurol. 365, 289–305.
Li, B.H., Rowland, N.E., 1996. Effect of chronic dexfenfluramine on Fos in rat brain. Brain Res. 728, 188–192.
Li, Q., Muma, N.A., Battaglia, G., Van de Kar, L.D., 1997. A desensitization of hypothalamic 5-HT_{1A} receptors by repeated injections of paroxetine: reduction in the levels of G(i) and G(o) proteins and neuroendocrine responses, but not in the density of 5-HT_{1A} receptors. J. Pharmacol. Exp. Ther. 282, 1581–1590.
Lino-de-Oliveira, C., Sales, A.J., Del Bel, E.A., Silveira, M.C., Guimaraes, F.S., 2001. Effects of acute and chronic fluoxetine treatments on restraint stress-induced Fos expression. Brain Res. Bull. 55, 747–754.
Lorrain, D.S., Matuszewich, L., Friedman, R.D., Hull, E.M., 1997. Extracellular serotonin in the lateral hypothalamic area is increased during the postejaculatory interval and impairs copulation in male rats. J. Neurosci. 17, 9361–9366.
Magoul, R., Ciofi, P., Tramu, G., 1994. Visualization of an efferent projection route of the hypothalamic rat arcuate nucleus through the stria terminalis after labeling with carbocyanine dye (DiI) or proopiomelanocortin-immunohistochemistry. Neurosci. Lett. 172, 134–138.
Makarenko, I.G., Meguid, M.M., Ugrumov, M.V., 2002. Distribution of serotonin 5-hydroxytriptamine 1B (5-HT(1B)) receptors in the normal rat hypothalamus. Neurosci. Lett. 328, 155–159.
Marson, L., McKenna, K.E., 1992. A role for 5-hydroxytryptamine in descending inhibition of spinal sexual reflexes. Exp. Brain Res. 88, 313–320.
Montone, K.T., Fass, B., Hamill, G.S., 1988. Serotonergic and non-serotonergic projections from the rat interpeduncular nucleus to the septum, hippocampal formation and raphe: a combined immunocytochemical and fluorescent retrograde labelling study of neurons in the apical subnucleus. Brain Res. Bull. 20, 233–240.
Moret, C., Briley, M., 1996. Effects of acute and repeated administration of citalopram on extracellular levels of serotonin in rat brain. Eur. J. Pharmacol. 295, 189–197.
Nutt, D.J., Forshall, S., Bell, C., Rich, A., Sandford, J., Nash, J., Argyropoulos, S., 1999. Mechanisms of action of selective serotonin reuptake inhibitors in the treatment of psychiatric disorders. Eur. Neuropsychopharmacol. 9 (Suppl. 3), S81–S86.
Parfitt, D.B., Newman, S.W., 1998. Fos-immunoreactivity within the extended amygdala is correlated with the onset of sexual satiety. Horm. Behav. 34, 17–29.
Paxinos, G., 1995. The rat nervous system, second edition. Academic Press, Sydney, Australia.
Pfaus, J.G., Heeb, M.M., 1997. Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. Brain Res. Bull. 44, 397–407.
Rosen, R.C., Lane, R.M., Menza, M., 1999. Effects of SSRIs on sexual function: a critical review. J. Clin. Psychopharmacol. 19, 67–85.
Veening, J.G., Coolen, L.M., 1998. Neural activation following sexual behavior in the male and female rat brain. Behav. Brain Res. 92, 181–193.
Veening, J.G., Coolen, L.M., Spooren, W.J., Joosten, H., van Oorschot, R., Mos, J., Ronken, E., Olivier, B., 1998. Patterns of c-fos expression induced by fluvoxamine are different after acute vs. chronic oral administration. Eur. Neuropsychopharmacol. 8, 213–226.
Vega, M.J., Larsson, K., Eriksson, E., 1998. The selective serotonin reuptake inhibitor fluoxetine reduces sexual motivation in male rats. Pharmacol. Biochem. Behav. 60, 527–532.
Waldinger, M.D., Olivier, B., 1998. Selective serotonin reuptake inhibitor-induced sexual dysfunction: clinical and research considerations. Int. Clin. Psychopharmacol. 13 (Suppl. 6), S27–S33.
Waldinger, M.D., Berendsen, H.H., Blok, B.F., Olivier, B., Holstege, G., 1998. Premature ejaculation and serotonergic antidepressants-induced delayed ejaculation: the involvement of the serotonergic system. Behav. Brain Res. 92, 111–118.
Waldinger, M.D., Zwinderman, A.H., Olivier, B., 2001. SSRIs and ejaculation: a double-blind, randomized, fixed-dose study with paroxetine and citalopram. J. Clin. Psychopharmacol. 21, 556–560.
Waldinger, M.D., van de Plas, A., Pattij, T., van Oorschot, R., Coolen, L.M., Veening, J.G., Olivier, B., 2002. The selective serotonin re-uptake inhibitors fluvoxamine and paroxetine differ in sexual inhibitory effects after chronic treatment. Psychopharmacology (Berl.) 160, 283–289.