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Effects of chronic treatment with fluvoxamine and paroxetine during adolescence on serotonin-related behavior in adult male rats

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Abstract Selective Serotonin Reuptake Inhibitors (SSRIs) are designed to treat adults, but are increasingly prescribed for adolescents. SSRIs might cause permanent changes in serotonin-related behavior in adolescents, since their serotonergic system is still developing.

Male Wistar rats were treated with paroxetine (15 mg/kg p.o.) or fluvoxamine (30 mg/kg p.o.) throughout adolescence. After a washout period their behavior in the elevated plus-maze, prepulse inhibition test, Forced swimming test and elevated T-maze were studied. In addition, the effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on sexual behavior and lower lip retraction were measured. Paroxetine mildly inhibited weight gain during treatment. Both SSRIs caused a reduction in ejaculation frequency and in time spent on the open arm of the elevated plus-maze in adult rats. Fluvoxamine slightly increased

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avoidance latency in the elevated T-maze compared to paroxetine. No differences between the groups were found in the other tests. Apparently, chronic treatment with SSRIs during adolescence may cause mild changes in adult behavior.

1. Introduction

Selective Serotonin Reuptake Inhibitors (SSRIs) are a family of antidepressants that is increasingly used for its efficacy and safety in adults suffering from depression, anxiety or obsessive—compulsive disorder. However, not only adults suffer from depression: estimations of the prevalence of depression amongst adolescents range between 1% and 25% (Kessler et al., 2001; van Dulmen et al., 2002). Consequently, SSRIs are often prescribed to children and adolescents, despite a lack of demonstrated efficacy and safety data in this subgroup (Courtney, 2004; Lynch et al., 2001; Murray et al., 2004; Rushton et al., 2000). Besides the risk of side effects caused by SSRIs during treatment in adolescents (Wilnes et al., 2003), SSRIs might also disturb the development of the central nervous system that is still proceeding during adolescence (Spear, 2000). This could cause long-term effects that are revealed in adulthood.

Adolescence is the ontogenic transition from the dependence of youth to the (relative) independence of adulthood that many species undergo and includes puberty, which is the onset of sexual maturation. In male rats, adolescence roughly lasts from postnatal day 28 to postnatal day 60 (Spear, 2000) with puberty occurring around postnatal day 45 (Engelbregt et al., 2000).

During this time period the serotonergic system undergoes several changes: the density of serotonergic transporters increases in the frontal cortex, the nucleus accumbens and the caudate putamen, and decreases in the brain stem throughout adolescence (Moll et al., 2000; Tarazi et al., 1998); the release of serotonin from the raphe nuclei is increased in adolescent rats compared to adult rats (Knoll et al., 2000); serotonin levels in the hippocampus, striatum, brain stem and cortex are increased compared to juvenile and adult rats (Chen et al., 1997); the percentage of serotonergic varicosities forming synapses in the basal forebrain increases throughout adolescence (Dinopoulos et al., 1997) and 5-HT_{1A} receptor binding is increased in the hippocampus, cerebral cortex, midbrain and brainstem in adolescent rats compared to adult rats (Xu et al., 2002). In addition, the dopaminergic and noradrenergic systems, which are closely linked to the serotonergic system, are also undergoing some changes during adolescence (Benes et al., 2000; Choi et al., 1997). One possible function of this temporary upregulation of the serotonergic system might be pruning, which means that synapses and perhaps receptors are 'selected' from a surplus to retain in adulthood, depending on the experiences and environment of the adolescent rats (Andersen and Navalta, 2004). An additional function might be the serotonin-mediated onset of puberty in male (Shishkina and Dygalo, 2000) and female (Monroy et al., 2003) rats.

Exposure to environmental variables that affect the serotonergic system during its development, for example SSRI-treatment which causes elevated serotonin levels (Bymaster et al., 2002), might cause 'miswiring' in the central nervous system that leads to disturbances later in life. In particular behavior in which serotonergic neurotransmission plays a crucial role, including sexual behavior (Ahlenius and Larsson, 1997; Hull et al., 2004; Marson and McKenna, 1992), anxiety (Bagdy, 1998; Graeff, 2002), depression (Berendsen, 1995; Nutt et al., 1999) and schizophrenia (Kusljic et al., 2003), can be expected to be vulnerable to SSRI-treatment during adolescence.

To investigate this, adolescent male Wistar rats were treated chronically (30 days) with vehicle (methylcellulose, 5 ml/kg/day p.o.), paroxetine (15 mg/kg/day p.o.) or fluvoxamine (30 mg/kg/day p.o.). The doses of SSRIs were selected based on the reported significant effects of similar or equivalent doses of these SSRIs on serotonin levels in the brain (Bosker et al., 1995; Bymaster et al., 2002). Following a washout period of 20 days, sexual behavior, anxiety-like behavior in the elevated plus-maze and the elevated T-maze, depressive-like behavior in the Forced swimming test and schizophrenic symptoms in the Prepulse Inhibition Test were tested in the now adult rats. An additional experiment was performed to investigate 5-HT_{1A} receptor functioning in these rats by testing the effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT (0.0; 0.1; 0.2 and 0.4 mg/kg s.c.) on sexual behavior and lower lip retraction. The effects of the SSRIs on bodyweight were measured during the treatment and during behavioral testing.

2. Experimental procedures

2.1. Animals

Male Wistar rats ($n=48$, 55—67 g and 21 ± 2 days of age at the start of the experiment) from Harlan CPB (Zeist, the Netherlands) arrived at the laboratory 2 weeks before the start of the experiment in order to adapt to a reversed light/dark cycle (12 : 12 h, lights off at 6:30 am). They received food and tap water ad libitum. Prior to and during drug treatment the rats were group housed ($n=8$) and weighed daily. After drug treatment, throughout behavioral testing, the rats were individually housed and weighed weekly.

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

Paroxetine (tablets containing 20 mg, Genthon BV, Nijmegen, the Netherlands) and Fluvoxamine (tablets containing 50 mg, Cetrafarm, Etten-Leur, the Netherlands) were obtained from the pharmacy of the Radboud University

Nijmegen Medical Centre. The required amount of tablets were crushed every day and suspended in 1% methylcellulose. All rats received a daily oral injection with methylcellulose in a volume of 5 ml/kg, containing paroxetine (15 mg/kg, $n=16$) or fluvoxamine (30 mg/kg, $n=16$) or methylcellulose (vehicle, $n=16$), from postnatal day 33 to postnatal day 62.

For some behavioral tests the rats were injected subcutaneously with the selective 5-HT_{1A} receptor agonist (±)-8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT, Sigma-Aldrich Chemie, Germany), dissolved in saline in a dose of 0.0 ("saline"), 0.1, 0.2 or 0.4 mg/kg, injected in a volume of 1 ml/kg.

2.3. Behavioral observations

Starting 20 days after the last drug injection, a cascade of behavioral tests was performed with the adult rats. All tests took place in the dark phase between 10.00 and 16.00 h in red-lighted or dimly lit rooms. Before every test the animals were allowed to habituate for at least 10 min in the test room.

2.3.1. Sexual behavior

The sexual performance of the rats was studied in three consecutive tests, the first 20 or 21 days, the second 27 or 28 days and the third 33 or 34 days after the last drug injection.

Each rat was placed in a rectangular mating arena ($40\times50\times65$ cm) made from wood with wood shavings on the floor and a Perspex front. The rat was allowed to habituate for 15 min, and then a female stimulus rat (sterilized by ligation of the oviducts and made sexually receptive by subcutaneous administration of 50 μg estradiol benzoate dissolved in 0.1 ml arachidis oil 36 h prior to testing) was introduced in the arena. Free contact was allowed for 30 min. In this period an observer scored all mounts, intromissions and ejaculations using event-recording software "The Observer" (Noldus, Wageningen, the Netherlands). Afterwards the total number of ejaculations ('ejaculation frequency') was determined, and the time from first mount or intromission to ejaculation ('ejaculation latency'), time from ejaculation to next mount or intromission ('post-ejaculatory interval'), number of mounts prior to ejaculation ('mount frequency') and number of intromissions prior to ejaculation ('intromission frequency') were calculated for each ejaculation.

2.3.2. Elevated plus-maze

Open, elevated alleys evoke greater avoidance responses than elevated closed alleys. Voluntary passage onto the open arms of an elevated, plus-shaped maze is associated with neurobiological changes indicative of decreased anxiety (Hogg, 1996).

The behavior of the rats in the elevated plus-maze was investigated 23 or 24 days after the last drug injection. The black plastic maze consisted of two open arms (10×50 cm) and two closed arms ($10\times50\times50$ cm) radiating from a center platform (10×10 cm) and was elevated 50 cm above floor level. Each rat was placed on the center platform facing an open arm.

The behavior of the rats during the 5 min test was videotaped and quantified afterwards by measuring the time spent in the open arms, the closed arms and the center platform, calculating the percentage of time spent on the open arms versus the closed arms, counting the number of entries in each compartment and calculating the percentage of entries in the closed arms relative to the open arms. After each 5 min test the maze was cleaned with 2% stafilex and 70% alcohol.

2.3.3. Prepulse inhibition of the startle response

In the prepulse inhibition test, a weak sensory event (prepulse) inhibits the startle response to a startling stimulus, a process that is called "sensorimotor gating". Sensorimotor gating is disturbed in patients suffering from schizophrenia (Braff et al., 2001).

The behavior of the rats in the prepulse inhibition test was measured 31 days after the last SSRI injection. The prepulse inhibition experiments were performed in four acoustic startle chambers of San Diego Instruments. Every cage contains a plexiglas tube (8.2 cm in diameter, 25 cm in length) resting on a plastic frame. A piezoelectric accelerometer mounted under the tube detected and transduced the motion of the tube. Stimulus delivery was done using SR-LAB software, via a speaker mounted 10 cm above the cylinder. The computer software also digitized, rectified, and recorded the response of the accelerometer; with 100 ms readings collected beginning at stimulus onset. Startle amplitude was defined as the average of 100 readings. The whole system was mounted within a sound-attenuating chamber. Throughout the startle session, a background noise of 70 dB was maintained. The experiment started with a 5-min habituation session with background noise in the startle system. After this habituation period, ten blocks of five trials were delivered to measure prepulse inhibition. Each of these blocks consisted of one startle trial (120 dB, 20 ms broad band burst), one no-stimulus condition, and three different prepulse/startle pairs administered pseudo randomly. In these pairings, the prepulse was 3, 5, or 10 dB above background. These prepulses were always 20 ms broadband burst and always followed by the 120 dB startle pulse 100 ms later. The interval between two trials was between 10 and 20 s. The startle amplitude was calculated as the mean of 10 delivered startle trials. The degree of prepulse inhibition (in percentage) was calculated as $(1-\text{average startle amplitude on prepulse trial}/\text{average startle amplitude on startle trial})\times100$.

2.3.4. Forced swimming test

When rodents are forced to swim in an inescapable situation, they typically display an immobile posture, which is said to reflect a state of "behavioral despair". Antidepressant treatments have been shown to reduce immobility time in the forced swim test (Connor et al., 2000).

The behavior of the rats in the Forced swimming test was investigated 35 and 36 days after the last drug injection. The test consisted of a training session of 15 min and a test session of 5 min, 24 h later. For both the training and the test session, each rat was placed in a

Perspex cylinder (25 cm high and 20 cm diameter) filled with water at 25 °C up to 22 cm, so that the rat could not reach the floor without diving. The behavior of the rat was videotaped and quantified afterwards by measuring the time spent trying to climb out of the cylinder ('climbing'), swimming around ('swimming') and staying immobile ('floating'). After every session the rats were dried with a towel and the cylinder was emptied, cleaned and refilled with fresh water.

2.3.5. Elevated T-maze

In the elevated T-maze test, rats learn in a series of trials to stay in the closed arm of a maze in order to avoid the fearful open arms, which is thought to be related to generalized anxiety disorder. In the last trial the rats escape from the open arm to the closed arm, which is thought to be associated with panic disorder (Graeff et al., 1998).

The behavior of the rats in the elevated T-maze was investigated 56 days after the last drug injection. The elevated plus-maze described above was used, but one closed arm was made inaccessible. Each rat was placed at the end of the accessible closed arm and the time until the rat entered the open arm with all four paws was measured, with a maximum of 10 min. As soon as the rat entered the open arm, it was picked up and placed again at the end of the closed arm. This was repeated 4 times (trial 1—5). In the sixth trial, the rat was placed at the end of the open arm and the time until the rat entered the closed arm with all four paws was measured. After 6 trials the maze was thoroughly cleaned with 2% stafilex and 70% alcohol.

2.3.6. 8-OH-DPAT

Injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT induces retraction of the lower lip (Berendsen et al., 1989) and, despite the occurrence of flat body posture, strongly accelerates ejaculation (Ahlenius et al., 1981) in rats. These parameters can be used to measure the sensitivity of 5-HT_{1A} receptors to 8-OH-DPAT.

24 out of the 48 rats ($n=8$ per group) from the study were tested in an additional 4-week experiment, starting 14 weeks after the last SSRI injection. All rats received one injection with 8-OH-DPAT or saline per week, so that all rats received all doses (saline or 0.1, 0.2 or 0.4 mg/kg 8-OH-DPAT s.c.) once in a semi-random order. 20 min after every injection, the rats were tested in a sexual behavior test as described above. 5 and 50 min after the injection (before and after the sexual behavior test), the rats were placed in a clear perspex cylinder as used in the Forced swimming test, which was in turn placed on top of a slanted mirror so that occurrence of lower lip retraction (LLR) could be videotaped. Afterwards, the LLR was scored as absent (0), half present (0.5) or fully present (1).

2.4. Statistics

Behavioral data were analyzed with a Univariate Analysis of Variance (ANOVA) or, when data were not normally distributed, with the non-parametric Kruskall—Wallis test and the Mann Whitney test. All statistical analyses were performed using the Statistical Package for the Social Sciences version 12.0 (SPSS Inc., Chicago IL, USA). The level of significance in all tests was set at $P<0.05$.

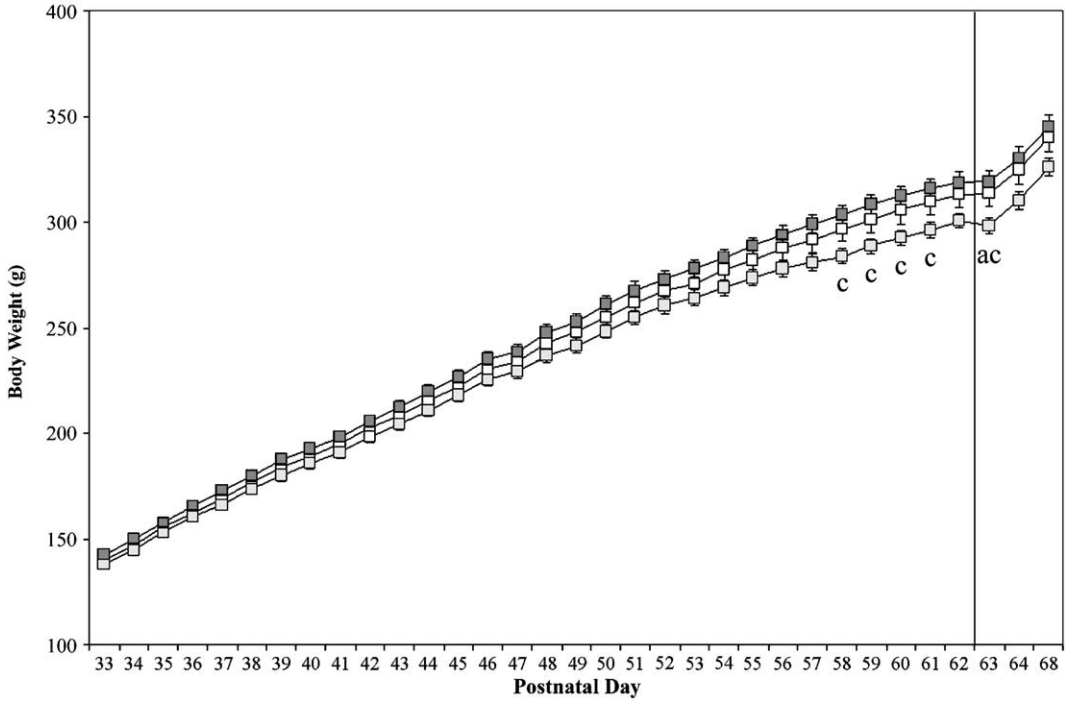

Figure 1 The effects of treatment with vehicle (white squares), paroxetine (15 mg/kg/day p.o., light-grey squares) or fluvoxamine (30 mg/kg/day p.o., dark-grey squares) from postnatal day 33 to postnatal day 62 (adolescence) on the body weight of male Wistar rats. Data are means ± standard error of the mean; a = different from vehicle, c = different from fluvoxamine; $P<0.05$.

3. Results

Body weight of the rats was measured daily during SSRI-treatment (Fig. 1). Overall significant differences in body weight were found on postnatal day (PND) 57 ($F(2,42)=3.431$; $P=0.042$), PND 58 ($F(2,42)=3.936$; $P=0.027$), PND 59 ($F(2,42)=3.965$; $P=0.027$), PND 60 ($F(2,42)=3.601$; $P=0.036$), PND 61 ($F(2,42)=3.664$; $P=0.034$) and PND 63 ($F(2,42)=4.082$; $P=0.024$). No differences were present on PND 62 or any of the other treatment days. Further post-hoc analysis demonstrated that paroxetine-treated animals had a significantly lower body weight in the last phase of SSRI-treatment compared to fluvoxamine-treated animals ($P<0.05$). On the first day after SSRI-treatment (PND 63), paroxetine-treated animals had a significantly reduced body weight compared to both fluvoxamine- and vehicle-treated animals ($P<0.05$). No significant differences between the experimental groups in body weight were found during the behavioral testing.

Sexual behavior was monitored in three successive weekly tests (Fig. 2). Analyses with the Kruskall–Wallis test revealed that the experimental groups differed significantly in the number of ejaculations reached in the test in week 3 ($\chi^2=7.785$; $P=0.020$), but not in the other two tests ($P>0.558$) or in ejaculation latency ($P>0.387$), mount frequency ($P>0.056$) or intromission frequency ($P>0.234$) in any of the three tests. Further post-hoc analysis showed that pretreatment with paroxetine or fluvoxamine significantly reduced the ejaculation frequency to two ejaculations compared to three ejaculations following pretreatment with vehicle ($P<0.05$).

In the elevated plus-maze (Fig. 3), a significant difference in the time spent in the open arms ($\chi^2=6.240$; $P=0.044$) and the percentage of time spent in the open arms versus the closed arms ($\chi^2=7.300$; $P=0.026$) was found, but not in the absolute ($P=0.131$) and relative ($P=0.052$) number of entries in the open arms. Further post-hoc analysis revealed that adult rats pretreated with paroxetine or fluvoxamine reduced the time spent in the open arms compared to vehicle treatment in adult rats.

In the elevated T-maze (Fig. 4) an overall significant difference was found in the second trial ($\chi^2=6.285$; $P=0.043$), but not in any other trial including the escape trial ($P>0.210$). Further post-hoc testing revealed that adult rats pretreated with fluvoxamine needed significantly more time to enter the open arm in the second trial compared to adult rats pretreated with paroxetine.

The experimental groups did not differ in any parameter measured in the prepulse inhibition test (Table 1),

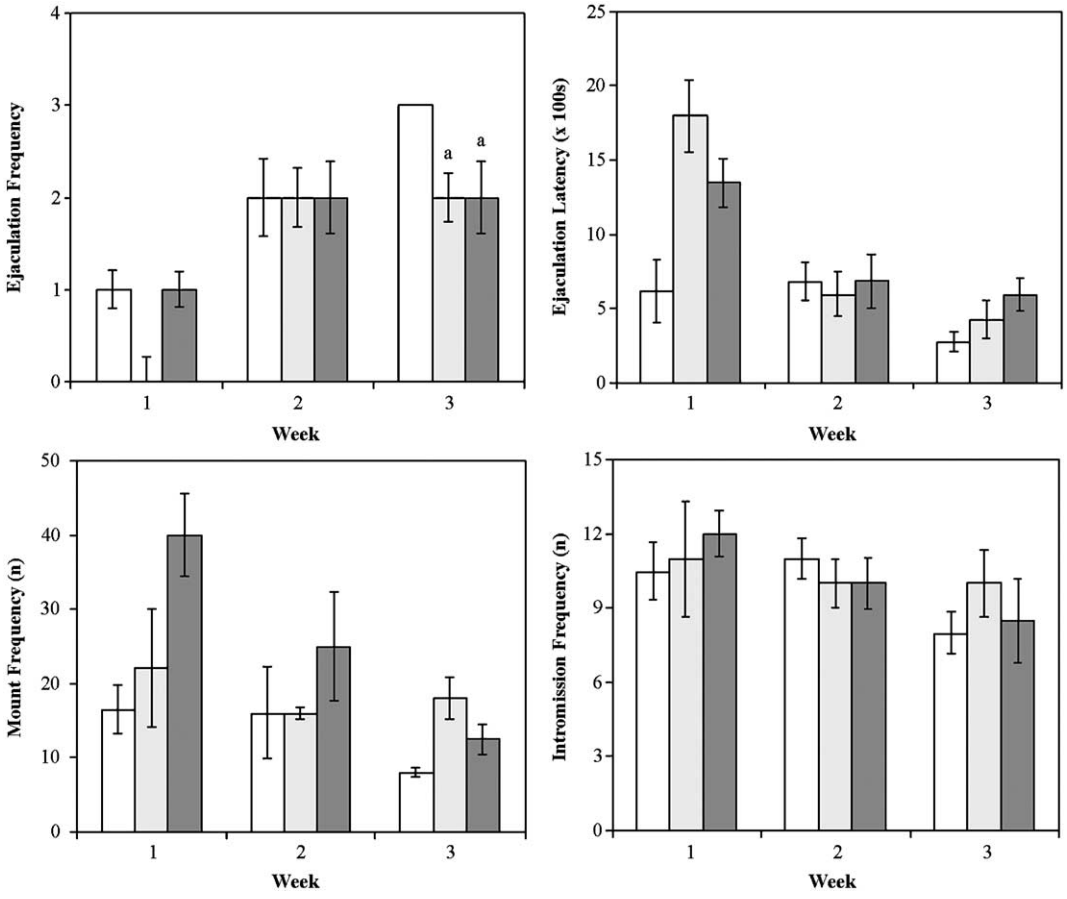

Figure 2 The effects of chronic treatment with vehicle (white bars), paroxetine (15 mg/kg/day p.o., light-grey bars) or fluvoxamine (30 mg/kg/day p.o., dark-grey bars) during adolescence on the ejaculation frequency, ejaculation latency, mount frequency and intromission frequency of adult male Wistar rats in three weekly 30 min tests with a receptive female. Data are medians ± standard error of the median; a = different from vehicle; $P<0.05$.

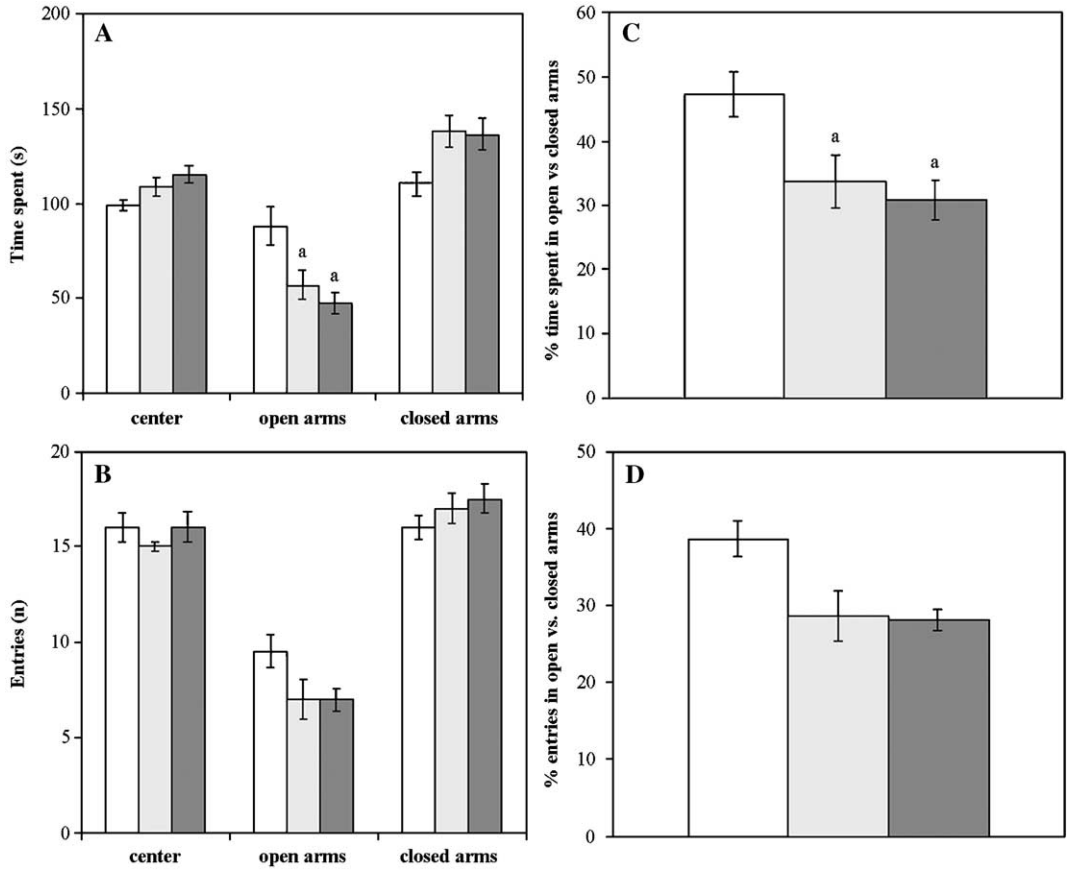

Figure 3 The effects of chronic treatment with vehicle (white bars), paroxetine (15 mg/kg/day p.o., light-grey bars) or fluvoxamine (30 mg/kg/day p.o., dark-grey bars) during adolescence on the time spent (A) and number of entries (B) in the center and open or closed arms, and the percentage of time spent (C) and number of entries (D) in the open vs. the closed arms of the elevated plus-maze, of adult male Wistar rats. Data are medians ± standard error of the median; a = different from vehicle; $P<0.05$.

and the forced swim test (Table 2). 8-OH-DPAT facilitated sexual behavior (Table 3) and strongly induced lower lip retraction (Table 4), but there were no significant differences between the experimental groups.

4. Discussion

In general, the effects of chronic treatment with SSRIs during adolescence on body weight and serotonin-related

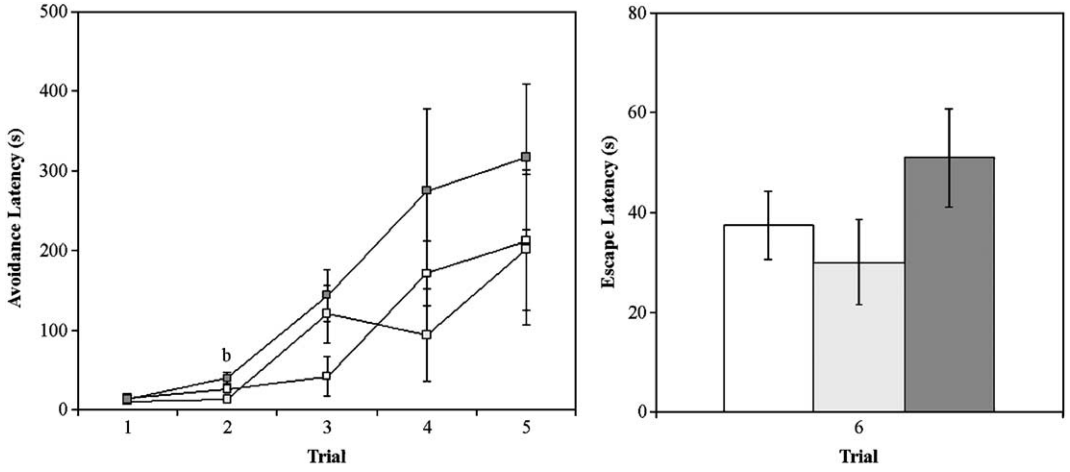

Figure 4 The effects of chronic treatment with vehicle (white squares and bars), paroxetine (15 mg/kg/day p.o., light-grey squares and bars) or fluvoxamine (30 mg/kg/day p.o., dark-grey squares and bars) during adolescence on the avoidance latency (trial 1—5) and escape latency (trial 6) of adult male Wistar rats in the elevated T-maze. Data are medians ± standard error of the median; b = different from paroxetine; $P<0.05$.

Table 1 The absolute and relative inhibition of the startle response to a sound stimulus of 120 dB by a prepulse of 0, 3, 5 or 10 dB of adult male Wistar rats that were treated chronically with vehicle, paroxetine (15 mg/kg p.o.) or fluvoxamine (30 mg/kg p.o.) during adolescence

Parameter	Treatment: prepulse (dB[A])	Vehicle	Paroxetine (15 mg/kg/day p.o.)	Fluvoxamine (30 mg/kg/day p.o.)	*P*-value
Startle	0	206.70 ± 26.23	156.10 ± 39.12	194.45 ± 23.26	.792
	3	98.20 ± 7.59	90.15 ± 33.74	89.85 ± 12.48	.881
	5	87.80 ± 6.92	85.55 ± 21.94	62.25 ± 12.97	.472
	10	54.45 ± 3.68	54.60 ± 8.55	40.80 ± 10.54	.883
Prepulse inhibition (%)	0	0	0	0	–
	3	51.47 ± 5.26	44.64 ± 6.18	42.53 ± 4.63	.811
	5	62.58 ± 3.77	69.12 ± 4.68	68.86 ± 4.16	.472
	10	80.03 ± 3.12	77.53 ± 3.01	77.93 ± 3.11	.883

Data are medians ± standard error of the median.

behavior of male Wistar rats were very mild. Similar or equivalent doses of paroxetine and fluvoxamine have been reported to increase serotonin levels in the brain significantly (Bymaster et al., 2002). Serotonin levels are already increased in male adolescent rats compared to juvenile and adult rats (Chen et al., 1997; Knoll and Miklya, 1995) and this may have prevented more vigorous consequences of SSRI-treatment in this period. However, some effects were seen on body weight, sexual behavior and anxiety-like behavior.

4.1. Body weight

Chronic treatment with paroxetine slightly but significantly reduced weight gain. Similar results in rats were obtained in a previous study (Konkle and Bielajew, 1999) and might be explained by an increased activation of 5-$HT_{1B/2C}$ receptors that reduce food intake (Simansky, 1996). Interestingly, fluvoxamine did not cause a reduction in body weight gain, indicating that there are differences between the two SSRIs in their effect on serotonin receptors involved in feeding behavior.

4.2. Sexual behavior

Chronic treatment with paroxetine and fluvoxamine during adolescence led to a reduced number of ejaculations in the third of three weekly 30 min sexual behavior tests. The rats were naıve at the start of the sexual behavior tests and displayed a learning curve throughout the three sessions (Pfaus et al., 2001). No differences between the experimental groups existed in the first two tests, although a strong trend in increased ejaculation latency in the SSRI-treated rats can be observed in the first test. In the third test vehicle-treated rats were still improving their ejaculation frequency, whereas paroxetine- and fluvoxamine-treated rats remained on the level of the second test, resulting in a significant difference. In general, serotonin inhibits sexual behavior and ejaculation (Hull et al., 2004; Marson and McKenna, 1992), and the reduced ejaculation frequency and trend to increased ejaculation latency could reflect increased serotonergic neurotransmission. Another possibility is that the SSRI-treated rats experience increased anxiety to approach the female.

4.3. Anxiety-like behavior

Chronic treatment with paroxetine or fluvoxamine during adolescence led to increased anxiety-like behavior in the elevated plus-maze in adult rats. In addition, fluvoxamine-treatment during adolescence led to a longer avoidance latency time in the second trial of the elevated T-maze compared to paroxetine-treatment. Numerous reports show that behavior of the rat on the elevated plus-maze and elevated T-maze is modulated by the serotonergic system. Activation of 5-HT_{1B}, 5-HT_{2C} and 5-HT_4 receptors by systemic injection of receptor agonists reduces the

Table 2 The percentage of time spent swimming, climbing or immobile in the Forced swimming test during a 15 min training session and a 5 min test session 24 h later of adult male Wistar rats that were treated chronically with vehicle, paroxetine (15 mg/kg p.o.) or fluvoxamine (30 mg/kg p.o.) during adolescence

Session	Treatment: % time spent	Vehicle	Paroxetine (15 mg/kg/day p.o.)	Fluvoxamine (30 mg/kg/day p.o.)	*P*-value
Training	Swimming	15.64 ± 1.87	13.24 ± 1.59	16.93 ± 3.40	.589
	Climbing	4.97 ± 0.46	4.83 ± 0.60	4.97 ± 0.53	.977
	Floating	78.23 ± 2.14	80.75 ± 2.04	76.76 ± 3.65	.610
Test	Swimming	15.95 ± 2.24	15.99 ± 2.35	15.94 ± 3.40	1.000
	Climbing	11.70 ± 2.06	8.94 ± 1.75	15.38 ± 3.10	.200
	Floating	71.99 ± 3.35	74.72 ± 4.04	68.39 ± 3.47	.489

Data are means ± standard error of the mean.

Table 3 The effects of 8-OH-DPAT (0.0; 0.1; 0.2 and 0.4 mg/kg s.c.) on the ejaculation frequency (EF), ejaculation latency (EL), mount frequency (MF) and intromission frequency (IF) in a 30 min sexual behavior test of adult male Wistar rats treated chronically with vehicle, paroxetine or fluvoxamine during adolescence

Parameter	Treatment: 8-OH-DPAT	Vehicle	Paroxetine (15 mg/kg/day p.o.)	Fluvoxamine (30 mg/kg/day p.o.)	*P*-value
EF	Saline	3.50 ± 0.33	3.50 ± 0.30	3.50 ± 0.33	.973
	0.1 mg/kg s.c.	4.00 ± 0.53	3.00 ± 0.14	4.00 ± 0.26	.517
	0.2 mg/kg s.c.	4.00 ± 0.14	4.00 ± 0.28	4.50 ± 0.33	.108
	0.4 mg/kg s.c.	4.50 ± 0.26	4.00 ± 0.23	4.00 ± 0.33	.657
	P-value:	0.164	0.232	0.230	
EL	Saline	246.95 ± 90.44	220.61 ± 46.68	163.44 ± 45.44	.801
	0.1 mg/kg s.c.	122.82 ± 41.38	216.90 ± 30.21	228.60 ± 58.79	.697
	0.2 mg/kg s.c.	83.44 ± 41.71	119.85 ± 65.80	125.67 ± 83.15	.356
	0.4 mg/kg s.c.	98.07 ± 50.37	55.50 ± 14.54	0.00 ± 55.52	.702
	P-value	0.099	0.057	0.164	
MF	Saline	8.00 ± 5.01	5.50 ± 1.60	4.50 ± 2.84	.343
	0.1 mg/kg s.c.	2.50 ± 1.58	2.00 ± 1.27	2.00 ± 0.66	.615
	0.2 mg/kg s.c.	0.00 ± 0.56	2.00 ± 1.13	2.00 ± 0.73	.198
	0.4 mg/kg s.c.	1.50 ± 0.59	0.00 ± 0.46*,&,#	0.00 ± 1.25	.110
	P-value:	0.077	0.004	0.220	
IF	Saline	7.00 ± 0.46	5.00 ± 0.69	6.50 ± 0.59	.141
	0.1 mg/kg s.c.	4.00 ± 0.33	3.00 ± 1.13	6.00 ± 1.45	.641
	0.2 mg/kg s.c.	2.00 ± 1.13*	3.00 ± 0.42	2.50 ± 0.73*&	.743
	0.4 mg/kg s.c.	1.50 ± 0.92*	2.00 ± 0.46	0.00 ± 0.33*&	.193
	P-value:	0.007	0.179	0.000	

*Different from saline; &different from 0.1 mg/kg 8-OH-DPAT; #different from 0.2 mg/kg 8-OH-DPAT ($P<0.05$).
Data are medians ± standard error of the median.

time spent on the open arm of the elevated plus-maze (Lin and Parsons, 2002; Setem et al., 1999; Smriga and Torii, 2003). 5-$HT_{2B/2C}$ receptor agonists increase, and 5-$HT_{2B/2C}$ receptor antagonists decrease the avoidance latency in the elevated T-maze (Graeff et al., 1998; Zangrossi et al., 2001). 5-HT_{1A} receptor agonists increase the time spent on the open arm of the elevated plus-maze (Collinson and Dawson, 1997) and reduce the avoidance latency in the elevated T-maze (Zangrossi et al., 2001).

Therefore, the results suggest that chronic SSRI-treatment during adolescence in rats leads to sustained changes in 5-HT neurotransmission or functioning of 5-HT receptors involved in anxiety-like behavior. Consistently, the density of serotonin transporters in the frontal cortex was persistently increased in 90-day old Wistar rats that had been treated with fluoxetine (5 mg/kg/day via drinking water) for two weeks starting at postnatal day 25, while no effect was found when treatment started at postnatal day 50 (Wegerer et al., 1999). Since serotonergic neurotransmission in the frontal cortex is involved in anxiety (Graeff et al., 1996), increased 5-HT transporter density might underlie the increased anxiety in the rats treated with SSRIs in the present study. Interestingly, treatment with MDMA ("ecstacy") during adolescence caused a twofold increase in the time that rats spent on the open arms of the elevated plus-maze, one week

Table 4 The effects of 8-OH-DPAT (0.0; 0.1; 0.2 and 0.4 mg/kg s.c.) on the relative lower lip retraction, 5 or 50 min after injection, of adult male Wistar rats treated chronically with vehicle, paroxetine or fluvoxamine during adolescence

After	Treatment: 8-OH-DPAT	Vehicle	Paroxetine (15 mg/kg/day p.o.)	Fluvoxamine (30 mg/kg/day p.o.)	*P*-value
5 min	Saline	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.000
	0.1 mg/kg s.c.	0.75 ± 0.13*	0.50 ± 0.14*	0.50 ± 0.13*	0.589
	0.2 mg/kg s.c.	0.50 ± 0.13*	1.00 ± 0.14*	0.75 ± 0.13	0.168
	0.4 mg/kg s.c.	0.75 ± 0.13*	1.00 ± 0.07*	1.00 ± 0.03*	0.544
	P-value:	<0.001	<0.001	<0.001	
50 min	Saline	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.000
	0.1 mg/kg s.c.	0.50 ± 0.13*	0.50 ± 0.14*	0.50 ± 0.13*	0.993
	0.2 mg/kg s.c.	0.50 ± 0.13*	1.00 ± 0.14*	0.50 ± 0.13*	0.172
	0.4 mg/kg s.c.	0.75 ± 0.13*	1.00 ± 0.07*,&	1.00 ± 0.13*,&	0.704
	P-value:	<0.001	<0.001	<0.001	

*Different from saline; &different from 0.1 mg/kg 8-OH-DPAT; ($P<0.05$).
Data are medians ± standard error of the median.

after discontinuation of the drug treatment (Piper and Meyer, 2004). Chronic MDMA treatment leads, amongst other things, to reductions in serotonin and serotonin-immunoreactive fibers in the neocortex, striatum and hippocampus (Lyles and Cadet, 2003). Apparently, a drug-induced increase in serotonergic neurotransmission during adolescence leads to opposite effects on anxiety-like behavior compared to a decrease, after discontinuation of the drug treatments.

4.4. 5-HT_{1A} receptor

Since 5-HT_{1A} receptor agonists facilitate ejaculation in the sexual behavior test (Ahlenius et al., 1981; Haensel and Slob, 1997) and reduce anxiety-like behavior in the elevated plus-maze (Collinson and Dawson, 1997), alterations in functioning of this receptor could underlie the differences between the experimental groups. However, no differences between the experimental groups were found in the lower lip retraction and acceleration of ejaculation caused by injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT. This indicates that SSRI-treatment during adolescence had no long-term effect on 5-HT_{1A} receptor functioning. Since no differences in ejaculation frequency following saline-injection in the additional test were found between the experimental groups, in contrast to the previous sexual behavior test ten to fourteen weeks earlier, it cannot be excluded that impaired 5-HT_{1A} receptor functioning caused the behavioral effects in the first tests.

4.5. Discontinuation syndrome

It is possible that the increased anxiety-like behavior and decreased ejaculation frequency are not caused by a developmental 'miswiring' of the brain, but are a temporary effect of the discontinuation of the drug treatment. Results from human studies have shown that 5 to 8 days after sudden discontinuation of chronic paroxetine-treatment, patients score higher in a self-rating anxiety test than during the treatment (Rosenbaum et al., 1998). Another study reported that 4% of paroxetine-treated patients suffers from anxiety as a withdrawal symptom (Coupland et al., 1996). Although in the present study the rats underwent a three-week washout period before the behavioral tests, discontinuation-effects cannot be excluded completely.

4.6. Conclusion

In conclusion, treatment with SSRIs during adolescence leads to some mild effects on sexual behavior and anxiety-like behavior in adult rats, indicative of altered serotonergic neurotransmission. It remains to be investigated whether the effects of chronic SSRI-treatment on ejaculation and anxiety-like behavior are permanent or temporary, whether they are specific for treatment during adolescence, and whether 5-HT_{1A} receptors play a role.

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