Supplemental Information

Methods

Sucrose Preference

The sucrose preference test consisted of a two-bottle choice paradigm (1). This paradigm has been used extensively to assess the effects of stress-induced anhedonia (2). Rats were habituated to drink water from two bottles for 5 days. At the start of the experiment, rats were exposed to ascending concentrations of sucrose (0, 0.125, 0.25, 0.5, and 1% wt/vol) for 2 days per sucrose concentration. Water and sucrose consumption were measured at 8:00 and 17:00 hours each testing day at which time the position of the sucrose bottle (left or right) was counterbalanced between the Fluoxetine- (FLX) and vehicle- (VEH) treated groups, across cages and days (see Figure S4, below). The preference for sucrose over water was used as a measure for rats' sensitivity to reward.

Locomotor Activity

Spontaneous locomotor activity was assessed in an open-field (OF) apparatus that consisted of a square box (63 x 63 x 26 cm) that rats can explore freely. This apparatus is fully automated (Florida State University Psychology Department engineering group), and records the rats' locomotor activity as 'distance traveled' in cm.

Elevated Plus Maze

FLX- and VEH- treated rats were tested for 5 min on the elevated plus maze (EPM), a behavioral model of anxiety-like behavior. The maze was made of gray plastic and consisted of two perpendicular, intersecting runways (12 cm wide X 100 cm long). One runway had tall walls (40 cm high) or "closed arms," and the other one had no walls or "open arms." The arms were connected together by a central area, and the maze was elevated 1 m from the floor. Testing was conducted between 9 AM and 1 PM under controlled light conditions (~90 lux). At the beginning of the 5-min observation, animals were placed in the central area, facing one of the open arms, and the cumulative time spent and number of entries into the open arms was recorded (3).

Forced Swim Test

The forced swim test (FST) is a 2-day procedure in which rats are forced to swim under conditions in which they cannot escape. On the first day, rats are forced to swim. Initially, they engage in escape-like behaviors but eventually adopt a posture of immobility in which they make only the movements necessary to maintain their head above water. When retested 24 h later, rats become immobile very quickly; however, antidepressant treatment between the forced swim exposures can significantly increase their escape-like behaviors, an effect that has been correlated with antidepressant activity in humans (4). At the start of the experiment, rats were placed in plastic cylinders (75 x 30 cm) filled to 54 cm depth with 25˚C water and forced to swim for 15-min. At the end of this period, rats were removed from the water, dried with towels, and placed in a warmed enclosure for 30-min. All cylinders were emptied and cleaned between rats. Twenty-four h after the forced swim, rats were retested for 5-min under identical conditions, and sessions were videotaped. In this study, the latency (sec) to become immobile, total immobility (sec), and behavioral counts (floating, swimming, and climbing) were the dependent variables [see (5,6)]. Behavioral counts were rated at 5-sec intervals during the 5-min retest. Latency to immobility was defined as the time at which the rat first initiated a stationary posture that did not reflect attempts to escape from the water (7). To qualify as immobility, this posture had to be clearly visible and maintained for ≥2.0 sec.

Sexual Behavior

The sexual behavior experiments were carried out as previously reported (8-10). Rats were housed in a separate room maintained on a 12-h light/dark cycle (lights on between 24:00 and 12:00 h). Sexual behavior was assessed under red light conditions between 13:00-18:00 h in a circular arena (60 cm) containing wood chips on the floor. Each male was given a 5 min acclimation period to the testing arena. Testing started at the end of the acclimation period by the introduction of a receptive female to the arena. Testing sessions (at PD80 and PD90, respectively) lasted 90-min (see Figure S6 below). Behaviors recorded were mount latency (ML), elapsed time between introduction of the female and the first display of mounting, ejaculation latency (EL), time between first mount and first ejaculation, ejaculation frequency (EF), and total number of ejaculations. For rats that either did not display mounting behavior or failed to reach an ejaculation during the test session, the ML and EL was given as 90 min [similarly to (9)]. Sprague-Dawley ovariectomized female rats (Charles River, Raleigh, NC) were used in these experiments. Receptivity of the females was induced by injection of estradiol benzoate [50

mg, subcutaneously (sc)] and progesterone (500 mg, sc) 48 and 4-6 h before testing, respectively. One week prior to the experiment, the females were tested for one intercourse session with an experienced male. Prior to testing, female receptivity was verified by the exhibition of lordosis, in the presence of the experienced male, and accepted intromission. Each female was used to test only one experimental male.

Results

Effects of FLX on Basal Locomotor Activity

Chronic VEH- or FLX (10 mg/kg, b.i.d.) exposure during adolescence did not influence distance traveled (cm) in the open field 24 h after treatment (Short-term; Figure S3-A; *n*=10/group), or in adulthood (Long-term; Figure S3-B; *n*=14-15/group).

Basal Locomotor Activity 24 h After Day 1 of FST

Because changes in FST performance can be influenced by differences in motor activity, separate groups of VEH- and FLX-treated rats were tested in the OF 24 h after day 1 of FST (*n*=6/group). No changes in locomotor activity were evident in rats tested either short- or long-term (Figure S3 C-D) after treatment.

Table S1. Experimental/Treatment Design for Adolescent rats treated with Fluoxetine. Adolescent rats received fluoxetine (FLX; 10 mg/kg) or vehicle (VEH) intraperitoneal injections [twice daily (b.i.d.)] from PD35-49. Rats were tested in no more than 2 behavioral paradigms in the order of testing and time interval between tests as depicted in the Table. Rats were tested either 24 h (Short-term; PD50) or 20+ days after the last injection (Long-term; PD70+). Rats assigned to sex behavior were tested at PD80 and PD90, respectively. Rats in groups 8 and 9 were re-exposed to FLX as adults (>PD60): those in the acute condition (group 8) received a single injection of FLX or VEH at PD69, whereas those in the chronic condition (group 9) received a single injection of FLX or VEH for 5 consecutive days (PD65-69; see also Figure S5). Latency to start feeding behavior testing started at PD70.

Group	Day 35-49	Interval	TEST 1	Interval	TEST 2
1	VEH or FLX, b.i.d.	24 hours	FST	X	X
2	VEH or FLX, b.i.d.	24 hours	Sucrose Preference	20 days	FST
3	VEH or FLX, b.i.d.	24 hours	EPM	20 days	Sucrose Preference
4	VEH or FLX, b.i.d.	24 hours	Latency to Start Feeding	20 days	EPM
5	VEH or FLX, b.i.d.	24 hours	Novel Object Approach	20 days	Latency to Start Feeding
6	VEH or FLX, b.i.d.	20 days	Locomotor Activity	24 hours	Novel Object Approach
7	VEH or FLX, b.i.d.	24 hours	Locomotor Activity	30 and 40 days	Sex Behavior

Group	Day 35-49	Interval	Fluoxetine Re-exposure	Interval	TEST PD70
8	VEH or FLX, b.i.d.	20 days	Acute: PD69	24 hrs	Latency to Start Feeding
9	VEH or FLX, b.i.d.	16 days	Chronic: PD65-69	24 hrs	Latency to Start Feeding

FST, forced swim test; EPM, elevated plus maze

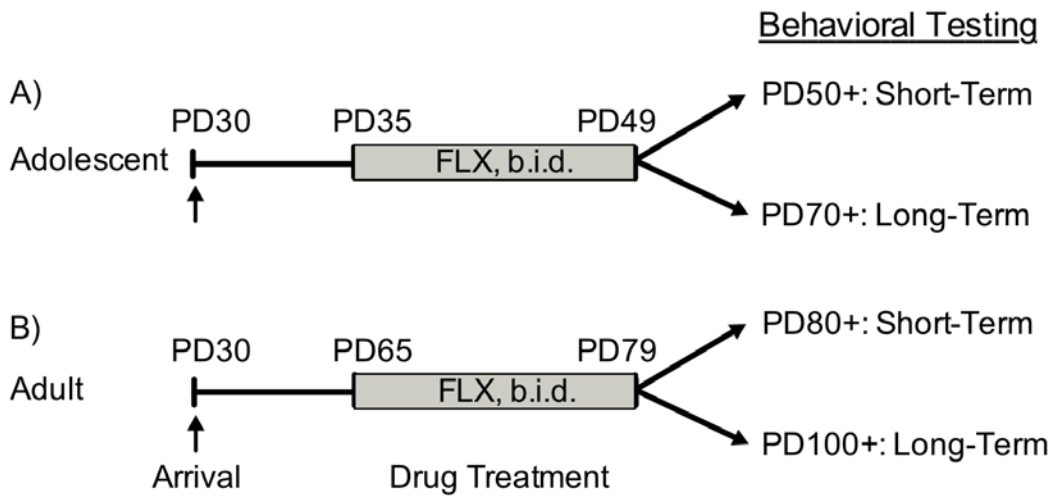

Figure S1. Timeline of developmental experimental procedures. All rats arrived in the laboratory on postnatal day (PD) 30. Rats were randomly assigned to receive fluoxetine (FLX) from (***A***) PD35-49 (adolescence) or from (***B***) PD65-79 (adulthood). All rats received intraperitoneal injections of FLX (10 mg/kg) or VEH twice daily (b.i.d.) 4 hrs apart (900 and 1300 hrs, respectively). Behavioral testing of rats treated during adolescence (***A***) was conducted either 24 hr after the last injection (PD50; Short-term) or when they reached adulthood (PD70; Long-term). Rats receiving FLX in adulthood (***B***) were tested either 24 hr (PD80; Short-term) or 21 days (PD100; Long-term) after the last injection.

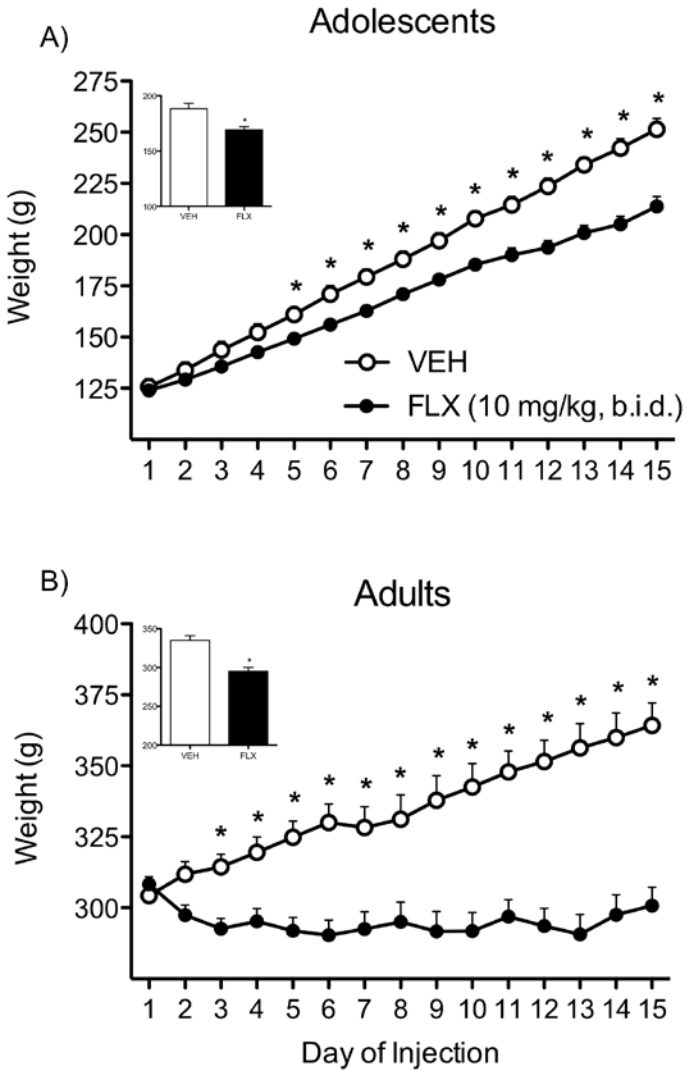

Figure S2. Effects of repeated (15 days) exposure to fluoxetine (FLX; 10 mg/kg, b.i.d.) on weight gain. Data were analyzed by mixed-design (within: day of injection, between: FLX) repeated measures ANOVA followed by post hoc test. (***A***) Adolescents (n=18/group): body weight increased across days regardless of condition, and FLX treatment resulted in significantly lower weight gain, starting on day 5 of drug exposure, when compared to control rats. (***B***) Adults (n=7-8/group): similar pattern of results was obtained from the adult rats treated with FLX, resulting in lower weight gain starting on day 3 of drug exposure as compared to controls. *Significantly different when compared to VEH-treated controls (p<0.05). Data are presented as average weight gain across days and drug treatment (mean ± SEM, in grams).

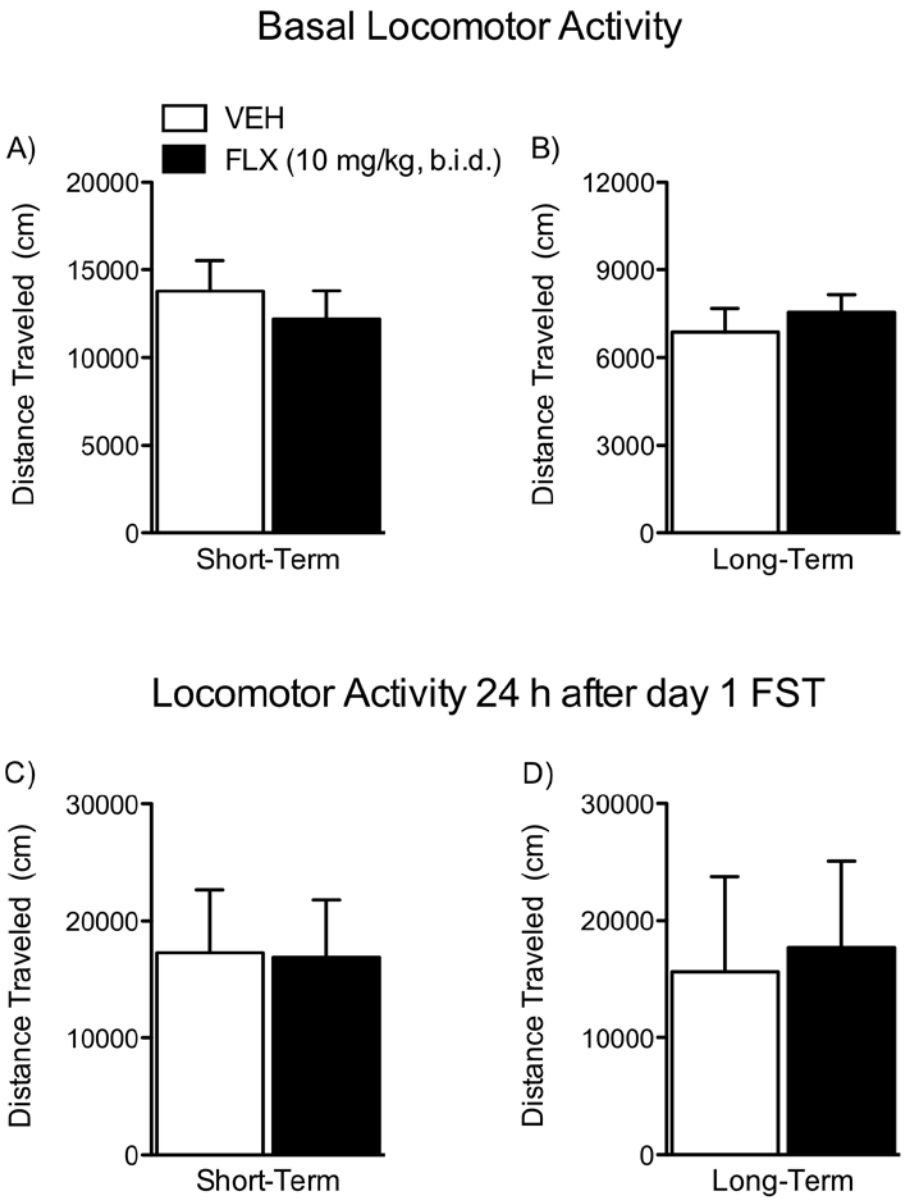

Figure S3. Exposure to FLX [10 mg/kg (twice daily; b.i.d.)] during adolescence did not affect total basal locomotor activity in the open field in rats tested either 24 hr (**A:** Short-term; n=10/group) after the last injection, or when tested in adulthood (**B:** Long-term; n=14-15/group). Similarly, FLX treatment did not affect locomotor activity 24 h after day 1 (***C*** and ***D***) of forced swimming (FST; n=6/group).

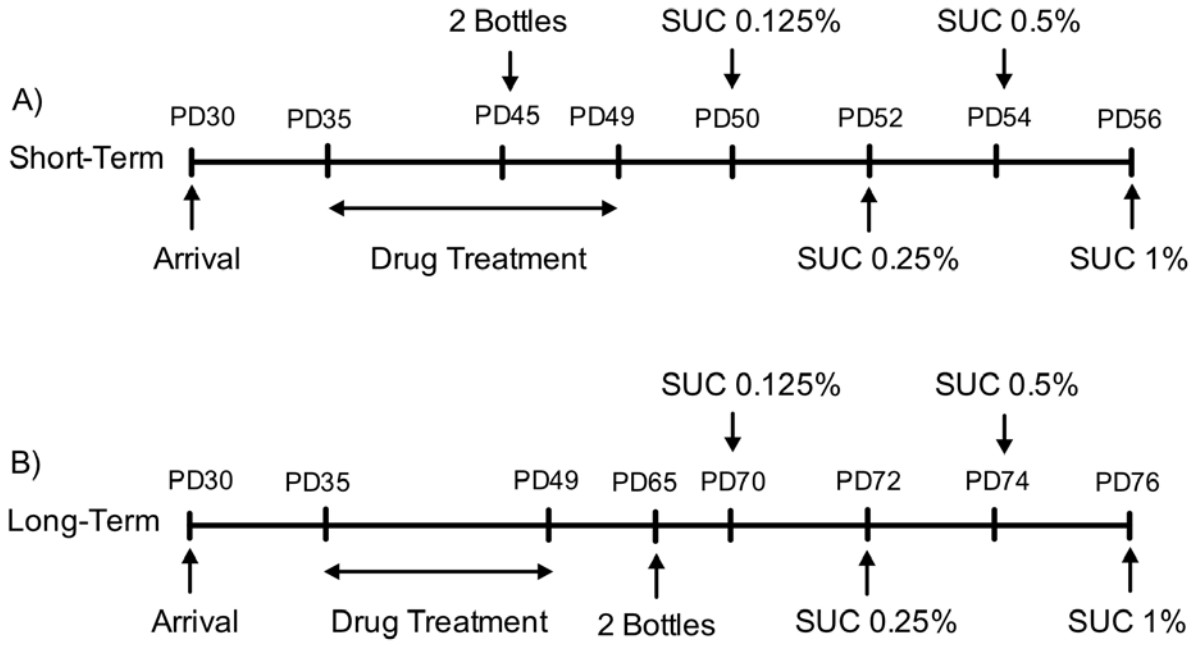

Figure S4. Timeline: Sucrose Preference testing. All rats arrived in the laboratory on postnatal day (PD) 30. Rats were randomly assigned to receive fluoxetine (10 mg/kg twice daily) or vehicle from PD35-49. Rats assigned to the Short-term testing condition (***A***) were habituated to drink water from two water bottles starting on PD45 for five consecutive days. Twenty-four hrs after the last injection (PD50), rats were introduced to ascending concentrations of sucrose (SUC; 0.125, 0.25, 0.5 and 1%; two days per concentration). Rats assigned to the Long-term condition (***B***) were acclimated to drink water from two different bottles starting at PD65. At PD70, these rats were introduced to the same ascending concentrations of sucrose.

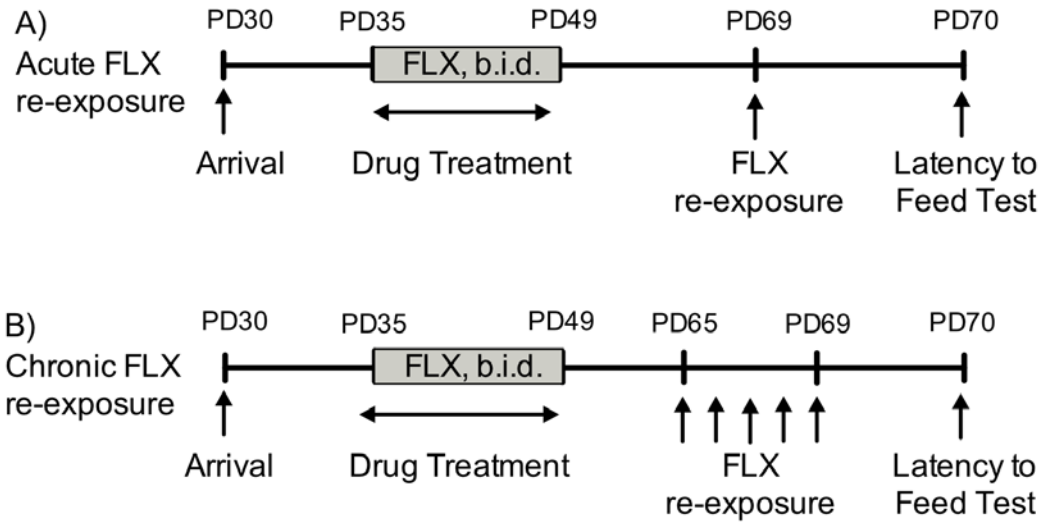

Figure S5. Fluoxetine Re-exposure Timeline: Latency to Feed in a Novel Environment test. Rats arrived in the laboratory on postnatal day (PD) 30. Rats were randomly assigned to receive either fluoxetine [FLX; 10 mg/kg twice daily (b.i.d.) ; n=12] or vehicle (n=12) from PD35-49. After treatment, rats were randomly assigned to either an acute (***A***) or chronic (***B***) FLX re-exposure treatment as adults. Rats in the Acute FLX re-exposure group received a single FLX (10 mg/kg) or vehicle injection on PD69, whereas the rats in the chronic FLX re-exposure group received once daily injections of FLX (10 mg/kg) or vehicle for five consecutive days (PD65-69). All rats were then tested on the latency to start feeding in a novel environment test on PD70.

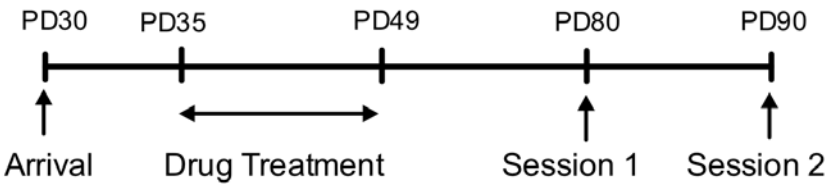

Figure S6. Timeline: Sexual Behavior testing. All rats arrived in the laboratory on postnatal day (PD) 30. Rats were randomly assigned to receive either fluoxetine (10 mg/kg twice daily; n=10) or vehicle (n=10) from PD35-49. All rats were tested on sexual copulatory behaviors at PD80 (Session 1) and again at PD90 (Session 2).

1. Bolaños CA, Willey MD, Maffeo ML, Powers KD, Kinka DW, Grausam KB, *et al.* (2008): Antidepressant treatment can normalize adult behavioral deficits induced by early-life exposure to methylphenidate. *Biol Psychiatry* 63: 309-16.
2. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987): Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* 93: 358-64.
3. Bolaños CA, Perrotti LI, Edwards S, Eisch AJ, Barrot M, Olson VG, *et al.* (2003): Phospholipase Cgamma in distinct regions of the ventral tegmental area differentially modulates mood-related behaviors. *J Neurosci* 23: 7569-76.
4. Cryan JF, Markou A, Lucki I (2002): Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* 23: 238-45.
5. Carlezon WA Jr, Mague SD, Andersen SL (2003): Enduring behavioral effects of early exposure to methylphenidate in rats. *Biol Psychiatry* 54: 1330-7.
6. Detke MJ, Rickels M, Lucki I (1995): Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology (Berl)* 121: 66-72.
7. Lucki I (1997): The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol* 8: 523-32.
8. Bolaños CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ (2003): Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biol Psychiatry* 54: 1317-29.
9. Grønli J, Murison R, Fiske E, Bjorvatn B, Sørensen E, Portas CM, *et al.* (2005): Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiol Behav* 84: 571-7.
10. Eaton RC, Markowski VP, Lumley LA, Thompson JT, Moses J, Hull EM (1991): D2 receptors in the paraventricular nucleus regulate genital responses and copulation in male rats. *Pharmacol Biochem Behav* 39: 177-81.