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The effects of dopaminergic/serotonergic reuptake inhibition on maternal behavior, maternal aggression, and oxytocin in the rat☆

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Abstract

Studies using dopaminergic and serotonergic agonists or antagonists implicate involvement of these systems in various aspects of early maternal behavior and postpartum aggression towards an intruder in rats, both of which are associated with the presence of oxytocin in specific brain regions. It is unclear however, if or how long-term uptake inhibition of either neurotransmitter system alone or in combination, affects oxytocin system dynamics or maternal behavior/aggression. Pregnant women frequently take drugs (antidepressants, cocaine) that induce long-term reuptake inhibition of dopamine and/or serotonin, thus it is important to understand these effects on behavior and biochemistry. Rat dams were treated throughout gestation with amfonelic acid, fluoxetine, or a combination of both, to investigate effects of reuptake inhibition of dopamine and serotonin systems respectively, on maternal behavior, aggression and oxytocin. The more appetitive aspects of maternal behavior (nesting, licking, touching) and activity were increased by the low dose of amfonelic acid, high dose of fluoxetine, or the high dose combination more than other treatments. Aggression was decreased by amfonelic acid and somewhat increased by fluoxetine. Dopamine uptake inhibition appears to have a strong effect on hippocampal oxytocin levels, while receptor dynamics may be more strongly affected by serotonin uptake inhibition.

Keywords: Amfonelic acid; Fluoxetine; Dopamine; Serotonin; Reuptake inhibition; Oxytocin; Maternal behavior; Maternal aggression

1. Introduction

Though there has been considerable interest in the biological basis of maternal behavior for some time, there are few systematic studies that have examined reuptake inhibition of specific neurotransmitter systems considering how many women use drugs during pregnancy, both legal (antidepressants, anti-anxiety medications, or antipsychotics) and illegal (cocaine), that act as reuptake inhibitors of norepinephrine, dopamine, and/or serotonin (Cooper et al., 1996; Kessler et al., 1994; Ritz et al., 1990; Thomas and Palmiter, 1997). This is particularly relevant clinically in light of new research on the effects of prenatal exposure to SSRIs (Moses-Kolko et al., 2005), and the fact that currently the use of broad spectrum antidepressants which inhibit reuptake of all three systems simultaneously, are being proposed for treatment of depression (Skolnick et al., 2003; Beer et al., 2004). Furthermore, the treatment of clinically depressed schizophrenic and Parkinson's patients who are pregnant can also result in reuptake inhibition of multiple neurotransmitter systems (Lemke et al., 2004; Sawabini and Watts, 2004; Quintin and Thomas, 2004). Given these facts, the study of the effects of simultaneous reuptake inhibition of various neurotransmitter systems on maternal behavior requires exploration.

Animal models may prove more useful for these studies given the difficulties of multiple variable controls in human studies. Reports using rodent models have found that

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serotonin and dopamine can both impact various aspects of the oxytocin system, and given the peptide's likely role in maternal behavior onset and aggression in rodents, gestational uptake inhibition of one or a combination of these systems could subsequently alter these behaviors (Bagdy, 1996; Cunningham et al., 1992; Giordano et al., 1990; Hansen, 1994; Honda et al., 1985; Kendrick et al., 1992; Lubin et al., 2003a,b; Sarnyai and Kovacs, 1994; Van de Kar et al., 1995). Oxytocin has been shown to be extremely important to the onset of maternal behavior, though perhaps not as vital to the maintenance of maternal behavior and evidence suggests it plays a role in maternal aggression as well (Fahrbach et al., 1985; Lubin et al., 2003b; Pedersen et al., 1985, 1994; Van Leengoed et al., 1987). Pedersen et al. (1982) demonstrated that oxytocin administration to ovariectomized rats can induce and perpetuate maternal behavior, while Lubin et al. (2003b) demonstrated that oxytocin antagonist administration into the central amygdala increases maternal aggression. Johns et al. (2004) found that gestational treatment with fluoxetine (a serotonin reuptake inhibitor) resulted in a reduction in oxytocin receptor affinity and an upregulation of oxytocin receptor number in the amygdala. Several brain regions are suggested as being relevant to maternal behavior and/or aggression in the rat, including the medial preoptic area (MPOA), ventral tegmental area (VTA), hippocampus, and amygdala, and reductions in oxytocin in these areas correlate with deficits in maternal behavior and/or aggression (Ferris et al., 1992; Gaffori and Le Moal, 1979; Johns et al., 1995, 1997; Kimble et al., 1967; Lubin et al., 2003b; Numan and Smith, 1984; Numan, 1994a,b).

The direct effects of dopamine (DA) and serotonin (5-HT) reuptake inhibition, alone or in combination, occurring during pregnancy on subsequent maternal behavior are poorly understood. The serotonin system has been loosely linked to maternal behavior, with research suggesting that serotonin agonists alter peripheral oxytocin release, which is important for lactation (Bagdy et al., 1992; Bagdy and Kalogeras, 1993; Saydoff et al., 1991; Uvnas-Moberg et al., 1996), and that reduced serotonin levels result in increased aggression (Coccaro, 1989, 1992; Olivier and Mos, 1992; Olivier et al., 1995). Furthermore, De Almeida and Lucion (1994) found that when injected acutely (ICV), serotonin receptor agonists reduce maternal aggression, yet do not impair normal maternal behavior.

Manipulations of the dopamine system have been more strongly associated with alterations in various aspects of maternal behavior. Dopamine system stimulation through the use of agonists, particularly D2 receptor agonists, has been shown to promote the release of peripheral oxytocin (Amico et al., 1992, 1993; Crowley et al., 1992; Parker and Crowley, 1992), while administration of dopamine antagonists results in a significant disruption in pup retrieval, nest building, and motor activity in general (Byrnes et al., 2002; Giordano et al., 1990; Keer and Stern, 1999; Silva et al., 2001, 2003; Stern and Keer, 1999). As Stern and Keer (1999) suggest, this could potentially be due to the dopamine system involvement in motivation and reward. A 1.5 mg/kg dose of the dopaminergic reuptake inhibitor, amfonelic acid, enhanced maternal behaviors on postpartum day (PPD) 1 and decreased maternal aggression on PPD 6 in gestationally treated rat dams and oxytocin levels were significantly increased in the amygdala of the less aggressive AFA treated dams on PPD 8 (Johns et al., 1995, 1996).

We are unaware of any other studies examining the effect of both separate and simultaneous reuptake inhibition of dopamine and serotonin on maternal behavior, aggression, and oxytocin levels in rodents. Our novel approach seems particularly timely given recent findings on prenatal exposure to SSRIs and proposed treatments for depression (Moses-Kolko et al., 2005; Beer et al., 2004). This study reports the effects of gestational treatment with several doses of relatively selective reuptake inhibitors for dopamine and serotonin neurotransmitter systems, on maternal behavior/aggression and oxytocin using a previously established rodent model of behavioral testing.

2. Methods

2.1. Subjects

Virgin female Sprague–Dawley Rats (200–230 g) were group housed in a temperature and humidity controlled room for a 7-day habituation period prior to breeding. Females were then individually housed with a sexually active male until conception was noted by the presence of a sperm plug. On the day a sperm plug was discovered, designated as gestation day 0, the female was removed from the breeding cage, randomly assigned to treatment or control groups, individually housed, and provided food (Purina Rat Chow) and water ad libitum (with the exception of the yoke-fed controls, see Treatment). Singly housed pregnant females were maintained on a reversed 12 : 12 h light cycle (lights out at 0900 hours) for 8 days, then transferred to a room with a regular 12 : 12 h light cycle (lights on at 0700 hours) for the remainder of the experiment, a procedure that generally results in the majority of dams delivering their litters during daylight hours (Mayer and Rosenblatt, 1998).

2.2. Treatment

Upon determination of pregnancy, females were randomly assigned to one of eleven treatment groups, or as a surrogate. Surrogate dams received no treatment but were weighed every 5 days. Throughout gestation (GD 1–20), all treatment groups received daily subcutaneous (sc) injections on alternating flanks of the following drugs: 0.9% normal saline (SAL) in a 1 ml/kg volume at 9:00 A.M. and 4:00 P.M.; 0.625, 1.25, or 2.5 mg/kg amfonelic acid (AFA, Research Biomedicals Inc., Natick, MA) dissolved in a pH 10 solution (0.1 ml 1 N NaOH and 0.6 ml of 0.1 N HCL in

distilled water) once daily at 9:00 A.M. and a normal saline injection at 4:00 P.M.; 2 mg/kg, 4 mg/kg or 8 mg/kg fluoxetine hydrochloride (FLU, Sigma, St. Louis MO, USA) dissolved in distilled water at 9:00 A.M. and a normal saline injection at 4:00 P.M.; or a combined treatment of an AFA injection on one flank and a FLU injection on the opposite flank at 9:00 A.M. (i.e. low dose AFA/FLU=AFA 0.625/FLU 2 mg/kg; medium AFA/FLU=AFA 1.25/FLU 4 mg/kg; high AFA/FLU=AFA 2.5/FLU 8 mg/kg), followed by a normal saline injection at 4:00 P.M. Single daily doses of treatment drugs or the combination of drugs were given because of their long half-life. Afternoon injections of saline were given to mimic the stress of twice daily injections used in our current behavioral testing paradigm, and injections were given on alternate flanks to minimize skin damage. Dams were weighed daily. Food consumption was measured for FLU and yoke-fed saline groups.

Two separate saline injected control groups were used, a yoke-fed control (SALY) group to control for the anorexic effects of FLU administration, while another saline control group (SAL) served as a control for the AFA treatment dams and had free access to food. The SALY group was given only as much food as the average FLU and AFA/FLU exposed dams ate on the same particular gestational day. AFA does not generally produce anorexia, but when paired with FLU may produce this effect.

AFA was chosen as the dopaminergic reuptake inhibitor because of its non-amphetamine-like properties, and because unlike other more common DA reuptake inhibitors, it has few effects on the noradrenergic system. AFA has been described as very selective in vivo for protecting dopamine storage pool content (Fuller and Perry, 1981). It has also been suggested that AFA has abuse potential because of its reinforcing effects, which may be even greater than some other stimulants and opioids (Izenwasser and Kornetsky, 1989; Porrino et al., 1988). Amfonelic acid has few effects on the adrenergic systems, and at the doses in the present study, there are no alpha-adrenergic effects and no effect on serotonin turnover (personal communication, Brian McMillen, Ph.D.). The 2.5 mg/kg dose of AFA produces significant DA uptake blockade for about 12 h after injection (half life 8–12 h) and produces no toxic effects on rat dams (Johns et al., 1995).

FLU was chosen as the serotonergic reuptake inhibitor, because while it is selective to the serotonergic system, it is not selective to one specific receptor subtype (Cooper et al., 1996). FLU could potentially bind to DA and NE reuptake sites at 10 mg/kg, so the highest dose in this study (8 mg/kg) was chosen to avoid these effects. FLU has a half-life (including its metabolites) of approximately 8–15 h in rats (Raap and Van de Kar, 1999), has been successfully administered to pregnant rats (Montero et al., 1990), and has been used by women throughout gestation (Goldberg and Nissim, 1994).

All procedures were conducted under federal and University Institutional Animal Care and Use Committee (IACUC) guidelines for humane treatment of laboratory subjects.

2.3. Maternal behavior testing

Upon delivery of their last pup, designated as PPD 1, the dams were brought in their home cage to a 10×12 ft observation room. The home cage was placed into a $24 \times 16 \times 20$ in dimly lit testing cubicle, designed to reduce environmental distractions during testing, and the subject's pups were removed. Gestational length, weight gain, litter size and weight, and gender of pups were recorded. Eight male pups born within 12 h to a surrogate dam were placed in a warm cage above the test cubicles while the dam to be tested habituated to the room and test chamber for 30 min. After the habituation period, 10 pieces of nesting material (paper towel strips) were placed at the rear of the cage and the 8 male surrogate pups were placed in the front of the cage. Untreated male surrogate pups were used to eliminate the possible effects of differential pup treatment due to prenatal drug exposure or gender preference. Videotaping with a VHS recorder with low light sensitivity began as soon as the pups were placed into the cage and continued for 30 min. Any apparent physical danger to pups would have resulted in removal of the dam and exclusion of the data from analysis, but this did not occur during behavioral testing. The 11 behaviors of interest, which have been previously described (Hofler et al., 2003), focus primarily on maternal pup-directed behavior displayed by the dam, as well as general activity. Behaviors included: nest-build (dam manipulates paper strips with her mouth or paws); touch/sniff pups (dam touches pups with her front paws or nose); retrieve pups (dam retrieves 2, 6 or 8 pups from the front to the back of the cage); self-groom (dam grooms herself with her tongue or paws); rest off/lie on (dam rests away from the pups or lies flat on top of pups); crouch (dam stands over the pups with her back arched in the nursing position with stiff straight legs and head lowered); lick pups (dam licks the pups); rear/sniff (dam rears on hind legs and sniffs the cage or air); and other (any behavior other than those designated above). Following maternal behavior testing, dams and their surrogate litters were returned to the colony until PPD 6. Dams and litters were monitored daily to assure pups were being fed.

2.4. Maternal aggression testing

On PPD 6, dams and their litters were brought in their home cage to the behavioral observation room where pups and dams were weighed. Dams and litters were then returned to their home cages, and the cage placed in the testing cubicle (as used for maternal behavior testing) and a 5-min chamber habituation period ensued. Following the habituation period, a smaller male intruder (175–190 g) was placed in the cage on the end opposite the dam and her litter, and the session was videotaped for a 10-min

period. No session had to be discontinued because of excessive aggression from either the intruder male or dam. Following testing, the male was removed from the cage, and the dam and pups were returned to the colony room. A new male was used for each test so that previous experience of the intruder would not affect their behavior. The 11 aggressive behaviors of interest, which have been previously described (Lomas et al., 2002; Lubin et al., 2003b) included: push/box/kick (dam pushes or kicks the intruder); maternal behavior (dam licks pups, retrieves, or crouches over pups); rough groom (dam grooms intruder male roughly, usually around head, neck, or back); self-groom (dam grooms herself); lateral/front threat (dam threatens male while approaching from the side, or threatens face to face); fight/attack (a quick lunge by the female usually followed by rolling, biting and fur-pulling directed towards the neck and back regions of the intruder); rear/sniff (dam rears on hind legs and sniffs the top or sides of cage); nip/bite (dam nips or bites male, but not as part of a fight attack); chase male (female chases intruder); aggressive posture (dam stands over a submissive intruder with extended front paws pinning him down); and other (any behavior other than those included in the categories above). Following testing, dams and litters were returned to the colony.

2.5. Brain dissection

On PPD 7, at approximately 9:00 A.M., one day following aggression testing, dams were killed by decapitation. The entire brain was removed and the whole MPOA, hippocampus, amygdala, and VTA were dissected on ice, weighed, and rapidly frozen and stored at −80 °C for later oxytocin radioimmunoassay (RIA) as previously described (Johns et al., 1997). Briefly, brains were coronally sectioned from the ventral side rostral to the optic chiasm (approximately A7100 according to Konig and Klippel, 1963) and just caudal to the optic chiasm (approximately A5800) to define the preoptic–anterior hypothalamic area. The MPOA was dissected by making a horizontal cut ventral to the anterior commissure and vertical cuts inferior to the lines of lateral ventricles. The brains were sectioned once again just caudal to the tuber cinereum (approximately A3800) to define the medial basal hypothalamus. The amygdala was removed from these two sections. The VTA was dissected from the caudal section by making dorso-ventral cuts medial to the optic tracts with a dorsal cut at the ventral extent of the central gray and the whole hippocampus was then removed from the caudal remainder of the brain.

2.6. Oxytocin radioimmunoassay

Brain region tissues were homogenized in cold buffer (19 mM monobasic sodium phosphate, 81 mM dibasic sodium phosphate, 0.05 M NaCl, 0.1% BSA, 0.1% Triton 100, 0.1% sodium azide, pH 7.4) and centrifuged at 3000 $\times g$ for 30 min. Oxytocin immunoreactive content was assayed in the supernatant according to a protocol from Peninsula Labs (Belmont, CA). Samples and standards (0.5–500 pg) were incubated in duplicate for 16–24 h at 4 °C with rabbit anti-oxytocin serum. They were then incubated for 16–24 h at 4 °C with [125I]-Oxytocin after which time normal rabbit serum and goat anti-rabbit IgG serum were added and incubated 30 min at room temperature. The [125I]-Oxytocin bound to the antibody complex was separated from free by a 30-min centrifugation at 4 °C. The radioactivity in the pellet was measured using a LKB CliniGamma counter, which calculates the picogram content of OT in each sample from the standard curve. The intra-assay coefficient of variance was 4.05% and inter-assay coefficient of variance was 8.95%. The standard curve range was 1–128 pg/tube, and the sensitivity of the assay was approximately, 0.5 pg/tube. Picograms of oxytocin per milligram of tissue were analyzed and compared for differences between groups.

2.7. Data analyses

Taped sessions were scored by two independent observers blind to treatment condition with inter- and intra-reliability set at 90% or better concurrence for frequency and latency, and 80% or better for duration of behaviors displayed by the dam. An "in house" computer program was used to score and determine the frequency, duration, latency, and sequence of all relevant behaviors displayed by the rat dams as they occurred on tape. If a particular behavior of interest was not exhibited by a dam, she was assigned a frequency and duration of 0, and the highest possible latency for the behavior (1800 s for maternal behavior, 600 s for maternal aggression). One-way between-groups Analyses of Variance (ANOVA) were employed to analyze differences in gestational measures, maternal behavior/aggression and oxytocin levels. Tukey (HSD) tests were employed for all post hoc analyses. Behaviors and oxytocin levels of AFA treated dams were statistically compared to the SAL control group in one set of ANOVAs, while behaviors and oxytocin levels of FLU and AFA/FLU treated dams were compared to their yoke-fed saline controls (SALY) in a second set of ANOVAs. A third between-groups ANOVA, excluding control groups, followed by specific post hoc analyses were done for separate comparisons of the effects of AFA vs. AFA/FLU only and FLU vs. AFA/FLU on maternal behavior and aggression measures. Statistical significance was set at less than or equal to the 0.05 level ($p \leq 0.05$). Data for significant effects is presented in tables as least squares means ± standard error. Statistically significant group means are designated in bold print with appropriate letters (small case subscripts for 0.05, large for 0.01), and differences are described in the text as being different at the $p \leq 0.05$ (if they were between 0.02 and 0.05) and $p \leq 0.01$ level.

Group data is presented in text first for separate treatments and their respective control group, followed by group comparisons of AFA/FLU to AFA and then to FLU exclusive of control groups. Behaviors assessed during maternal behavior testing are grouped as pup-directed, non-pup directed, and activity and those for maternal aggression testing are designated as aggressive, defensive, and activity related behaviors. Group differences in oxytocin levels (picograms/milligram tissue) in each of the brain regions of interest are presented last.

3. Results

3.1. Gestational variables

As illustrated in Table 1, there were no differences between AFA, FLU, AFA/FLU treated dams and their respective controls, SAL or SALY, on gestation length, litter size and weight, male and female pup ratio, and culled litter weight gain. Dams exposed to high doses of AFA/FLU gained less weight during pregnancy than dams exposed to low doses of AFA/FLU, FLU, or yoke-fed saline dams [$F(3,32)=6.30$, $p\leq 0.02$]. Dams treated with medium doses of AFA gained significantly less weight during pregnancy than dams exposed to low doses of AFA [$F(3,34)=3.039$, $p\leq 0.04$], but this effect may be partially due to the fact that there were fewer pups born to this group.

3.2. Maternal behavior: AFA vs. SAL

3.2.1. Pup directed maternal behaviors

As indicated in Table 2, there was a significant overall effect of AFA treatment on the duration of crouching [$F(3,32)=6.11$, $p\leq 0.01$], and latency to lick pups [$F(3,32)=6.586$, $p\leq 0.01$]. Post hoc analyses determined that low dose AFA treated dams crouched for a shorter duration than medium dose AFA ($p\leq 0.02$) and saline treated ($p\leq 0.01$), dams and that they began licking their pups sooner than all other groups ($p\leq 0.01$).

3.2.2. Non-pup directed and activity behaviors

There was a significant treatment effect on the frequency [$F(3,32)=3.67$, $p\leq 0.02$], duration [$F(3,32)=3.47$, $p\leq 0.03$], and latency [$F(3,32)=4.487$, $p\leq 0.01$] of self-grooming and on the frequency [$F(3,32)=2.987$, $p\leq 0.05$] and duration [$F(3,32)=4.17$, $p\leq 0.01$] of "other" behaviors. Low dose AFA treated dams self-groomed for a longer duration than medium dose ($p\leq 0.05$) and saline treated dams ($p\leq 0.05$) and did so more frequently ($p\leq 0.05$, both groups). Medium dose AFA treated dams also began to groom themselves later in the session than high dose AFA treated dams ($p\leq 0.01$). Low dose AFA treated dams performed "other" behaviors more frequently ($p\leq 0.04$) and for a longer duration ($p\leq 0.01$) than saline treated dams.

3.3. Maternal behavior: FLU vs. SALY

3.3.1. Pup directed maternal behaviors

As shown in Table 3, all FLU treatment dams non-significantly tended to crouch (with the exception of crouch duration of FLU low) and nest-build less, while they would touch/sniff and lick pups more often than SALY treated dams. There was a significant effect of treatment on the frequency of touch/sniff pups [$F(6,64)=6.56$, $p\leq 0.01$]. FLU high dose treatment dams had a higher frequency of touch/sniff pups than SALY ($p\leq 0.01$), FLU low ($p\leq 0.01$), and FLU med groups ($p\leq 0.05$).

3.3.2. Non-pup directed and activity behaviors

There was a significant treatment effect on the latency [$F(6,63)=8.47$, $p\leq 0.01$] to self-groom and duration [$F(6,63)=11.63$, $p\leq 0.01$] of "other" behaviors. Low dose FLU treated dams began self-grooming later than FLU med, FLU high, and SALY ($p\leq 0.01$) treated dams. FLU high dose dams performed "other" behaviors for a longer

Table 1
Gestational measures

		Numbers of dams	Gestation length	Dam wt gain (g)	Litter size	Number of males	Number of females	Litter wt PPD 1 (g)	Litter wt gain (g)
Amfonelic acid	0.625 mg/kg	10	21.4±0.3	156.9±9.7	14.4±0.9	6.9±0.6	7.8±0.7	93.9±5.6	59.1±4.4
	1.25 mg/kg	10	21.6±0.3	118.4±9.7*	12.8±0.9	7.3±0.6	5.5±0.7	82.6±5.6	60.3±4.4
	2.5 mg/kg	10	21.1±0.3	130.6±10.3	13.6±0.9	6.4±0.6	7.2±0.7	86.8±5.6	58.1±4.4
	Saline	9	21.0±0.3	147.1±10.3	14.1±1.0	8.9±0.7	5.2±0.7	89.7±5.9	60.8±4.6
Fluoxetine	2.0 mg/kg	10	21.2±0.1	150.6±6.4	15.0±0.8	7.2±0.7	7.8±0.6	91.5±4.7	59.3±3.6
	4.0 mg/kg	13	21.0±0.1	135.9±5.3	14.0±0.7	7.7±0.6	6.3±0.5	83.6±4.1	56.3±3.3
	8.0 mg/kg	11	21.5±0.1	133.2±5.8	13.6±0.7	7.1±0.6	6.5±0.6	82.5±4.5	61.2±3.6
Amfonelic acid/ fluoxetine	0.625/2.0 mg/kg	10	21.2±0.1	158.1±6.1	13.8±0.8	7.6±0.7	6.3±0.6	90.1±4.7	68.9±3.6
	1.25/4.0 mg/kg	9	21.1±0.1	136.9±6.4	13.1±0.8	6.7±0.7	6.4±0.6	81.2±4.9	65.8±4.1
	2.5/8.0 mg/kg	8	21.0±0.2	119.9±6.8*	13.7±0.8	8.1±0.7	5.6±0.6	79.4±4.9	61.8±3.8
	Yoke-fed saline	11	21.2±0.2	151.2±5.8	15.1±0.7	7.9±0.6	7.0±0.6	96.5±4.5	64.3±3.5

Mean±SEM of all gestational measures. Group means designated with asterisks were statistically significant from groups designated in the Results section at the $p\leq 0.05$ level.

Table 2
AFA vs. SAL: maternal behavior

Postpartum day 1 maternal behavior			Amfonelic acid			Saline
			0.625 mg/kg	1.25 mg/kg	2.5 mg/kg	
Pup-directed maternal behaviors	Crouch	Frequency	7.2±1.6	4.1±1.9	6.0±1.6	7.6±1.7
		Duration (s)	$824.8\pm131.4_{m,S}$	1477.9±157.1	1294.3±131.4	1581.8±138.5
		Latency (s)	257.4±67.8	234.8±81.0	172.4±67.8	122.5±71.4
	Nest-build	Frequency	11.6±3.0	3.1±3.5	0.3±3.0	2.4±3.1
		Duration (s)	92.1±24.1	13.6±28.8	1.4±24.1	12.0±25.4
		Latency (s)	1032.0±249.1	685.8±297.8	1479.8±249.1	1219.3±262.6
	Touch/sniff	Frequency	18.4±1.7	15.0±2.0	14.3±1.7	13.1±1.8
		Duration (s)	85.8±8.3	78.3±10.0	71.6±8.3	73.5±8.8
		Latency (s)	17.0±5.1	19.0±6.1	4.8±5.1	15.5±5.4
	Lick pups	Frequency	3.4±0.9	0.0±1.0	0.9±0.9	0.2±0.9
		Duration (s)	27.3±8.2	0.0±9.8	9.5±8.2	0.7±8.6
		Latency (s)	$619.9\pm189.4_{M,H,S}$	1800.0±226.4	1541.3±189.4	1421.4±199.7
Non-pup directed	Self-groom	Frequency	$6.4\pm1.2_{m,s}$	1.3±1.4	3.7±1.1	1.6±1.2
		Duration (s)	$87.8\pm18.0_{m,s}$	8.5±21.6	42.8±18.0	18.4±19.0
		Latency (s)	414.0±178.0	$1129.9\pm212.7_{H}$	218.5±178.0	835.2±187.6
Activity	Other	Frequency	$46.6\pm6.3_{s}$	27.9±7.5	27.4±6.3	21.5±6.3
		Duration (s)	$580.1\pm103.2_{S}$	168.8±123.4	345.9±103.2	97.8±103.2
		Latency (s)	4.0±3.1	7.6±3.7	1.2±3.1	11.7±3.1

Mean±SEM of selected maternal behaviors on postpartum day 1. Means with capitalized subscripts differ at $p \leq 0.01$ while lower case subscripts differ at $p \leq 0.05$. Different letters indicate differences as follows: L (l) indicates groups significantly different from low dose of that drug treatment, M (m) indicates groups significantly different from medium dose of that respective drug treatment, H (h) indicates groups significantly different from high dose of that respective drug treatment, and S (s) indicates groups significantly different from saline control.

duration than FLU low ($p \leq 0.01$), FLU med ($p \leq 0.01$), and SALY ($p \leq 0.01$) treated dams.

3.4. Maternal behavior: AFA/FLU vs. SALY

3.4.1. Pup directed maternal behaviors

The high dose AFA/FLU treated dams generally performed several pup-directed behaviors (lick pups, touch/sniff) at higher rates or for a longer duration compared to other dams (see Table 3). There was a significant effect of treatment on the duration of crouching [$F(6,63)=2.66$, $p \leq 0.03$], frequency [$F(6,63)=3.79$, $p \leq 0.01$] and latency [$F(3,35)=3.85$, $p \leq 0.02$] of lick pups, and the frequency [$F(3,35)=6.26$, $p \leq 0.01$] and duration [$F(3,35)=4.83$, $p \leq 0.01$] of touch/sniff pups. AFA/FLU high dose treated dams crouched for a shorter duration than AFA/FLU medium dose treatment dams ($p \leq 0.05$) and began to lick pups sooner than saline treated dams ($p \leq 0.05$), and more frequently than low ($p \leq 0.05$) and medium ($p \leq 0.01$) dose AFA/FLU, as well as SALY ($p \leq 0.01$) treated dams. AFA/FLU high dose dams also touched pups more frequently than low dose AFA/FLU ($p \leq 0.05$) and SALY ($p \leq 0.01$) dams, and for a longer duration than SALY dams ($p \leq 0.05$).

3.4.2. Non-pup directed and activity behaviors

There was a significant treatment effect on the duration of "other" behaviors [$F(6,63)=11.63$, $p \leq 0.01$]. The high dose AFA/FLU treated dams performed "other" behaviors longer than AFA/FLU low dose ($p \leq 0.05$), AFA/FLU medium dose ($p \leq 0.01$), and SALY treated dams ($p \leq 0.01$).

3.5. Maternal behavior: AFA/FLU vs. AFA and FLU

3.5.1. Pup directed maternal behaviors

Table 4 represents differences between AFA and AFA/FLU, as well as differences between FLU and AFA/FLU, exclusive of saline controls. There were significant between groups treatment effects on the frequency [$F(8,78)=4.15$, $p \leq 0.01$] and duration [$F(8,78)=2.81$, $p \leq 0.01$] of touch–sniff pups, frequency of lick pups [$F(8,77)=2.82$, $p \leq 0.01$], and the frequency [$F(8,77)=2.17$, $p \leq 0.04$] and duration [$F(8,77)=3.30$, $p \leq 0.01$] of crouching.

3.5.1.1. AFA/FLU vs. AFA. AFA/FLU high dose treated animals touched their pups more frequently and for a longer duration than AFA high dams ($p \leq 0.05$), and licked their pups more frequently than AFA low dose treated dams ($p \leq 0.05$). Differences were also evident in AFA/FLU low dose treated dams, which crouched more frequently than AFA medium treated dams ($p \leq 0.05$).

3.5.1.2. AFA/FLU vs. FLU. AFA/FLU high dose treated animals touched their pups more frequently than FLU low dose dams ($p \leq 0.05$), while AFA/FLU low dose treated dams touched their pups less than FLU high dose treated dams ($p \leq 0.05$). Furthermore, AFA/FLU high dose treated dams touched their pups for a longer duration than FLU low ($p \leq 0.01$) and FLU medium ($p \leq 0.05$) dose treated dams, and crouched for a shorter duration than FLU low treated dams ($p \leq 0.05$). AFA/FLU high dose treated dams also licked their pups more frequently than FLU low, and FLU medium ($p \leq 0.05$) dose treatment dams.

Table 3
FLU and AFA/FLU vs. SALY maternal behavior

Postpartum day 1 maternal behaviors			Fluoxetine			Yoke-fed saline	Amfonelic acid/fluoxetine		
			2.0 mg/kg	4.0 mg/kg	8.0 mg/kg		0.625/2.0 mg/kg	1.25/4.0 mg/kg	2.5/8.0 mg/kg
Pup-directed maternal behaviors	Crouch	Frequency	6.3±1.6	5.0±1.5	9.4±1.5	10.4±1.5	11.3±1.5	7.7±1.5	8.1±1.7
		Duration (s)	1587.1±151.5	1290.6±137.0	1206.2±137.0	1354.8±137.0	1382.3±143.7	1444.1±143.7	782.0±160.6$_{m}$
		Latency (s)	125.0±136.2	338.0±123.2	225.4±123.2	303.2±123.2	296.0±129.2	259.9±129.2	163.0±144.4
	Nest-build	Frequency	2.8±1.9	1.4±1.7	2.9±1.7	5.0±1.7	3.2±1.8	3.9±1.8	7.9±2.0
		Duration (s)	12.4±18.2	2.0±16.5	18.8±16.5	24.3±16.5	5.9±17.3	15.6±17.3	74.6±19.3
		Latency (s)	1107.4±268.1	1131.6±242.5	1157.3±242.5	658.7±242.5	998.0±254.3	681.3±254.3	758.2±284.3
	Touch/sniff	Frequency	13.9±1.8	15.9±1.6	23.5±1.6$_{l,M,y}$	12.7±1.6	15.2±1.7	17.1±1.7	23.6±1.9$_{l,Y}$
		Duration (s)	52.2±12.7	73.9±11.0	99.8±11.5	70.7±11.5	76.4±12.0	79.5±12.0	127.9±13.5$_{y}$
		Latency (s)	29.2±9.8	22.6±8.5	9.7±8.9	18.1±8.9	21.7±9.3	16.2±9.3	14.1±10.4
	Lick pups	Frequency	0.3±0.9	0.2±0.8	1.8±0.8	0.0±0.8	0.9±0.8	0.5±0.8	4.9±0.9$_{l,M,Y}$
		Duration (s)	0.5±4.8	0.8±4.3	11.2±4.3	0.0±4.3	0.9±4.6	0.7±4.6	13.0±5.1
		Latency (s)	1231.0±255.7	1330.7±231.3	873.6±231.3	1800±231.3	1281.6±242.6	985.3±242.6	693.7±271.2$_{y}$
Non-pup directed	Self-groom	Frequency	0.7±1.9	3.6±1.7	4.2±1.7	4.6±1.7	5.1±1.8	5.9±1.8	3.8±2.0
		Duration (s)	5.3±29.0	64.8±26.2	30.7±26.2	53.8±26.2	58.3±27.5	63.0±27.5	24.0±30.7
		Latency (s)	1486.6±149.0$_{M,H,Y}$	441.0±134.7	252.8±134.7	302.8±134.7	495.0±141.3	538.2±141.3	306.7±158.0
Activity	Other	Frequency	22.1±5.1	25.8±4.6	37.2±4.6	27.7±4.6	28.9±4.8	28.0±4.8	40.9±5.4
		Duration (s)	110.8±55.4	135.2±50.1	386.4±50.1$_{L,M,Y}$	152.1±50.1	147.2±52.6	121.2±52.6	614.0±58.8$_{l,M,Y}$
		Latency (s)	15.7±72.1	165.8±65.2	4.0±65.2	4.0±65.2	0.6±68.4	8.9±68.4	0.4±76.5

Mean±SEM of selected maternal behaviors on postpartum day 1. Means with capitalized subscripts differ at $p \leq 0.01$ while lower case subscripts differ at $p \leq 0.05$. Different letters indicate differences as follows: L (l) indicates groups significantly different from low dose of that drug treatment, M (m) indicates groups significantly different from medium dose of that respective drug treatment, H (h) indicates groups significantly different from high dose of that respective drug treatment and Y (y) indicates groups significantly different from saline control.

Table 4
AFA and FLU vs. AFA/FLU maternal behavior

Postpartum day 1 maternal behaviors			Amfonelic acid			Amfonelic acid/fluoxetine			Fluoxetine		
			0.625 mg/kg	1.25 mg/kg	2.5 mg/kg	0.625/2.0 mg/kg	1.25/4.0 mg/kg	2.5/8.0 mg/kg	2.0 mg/kg	4.0 mg/kg	8.0 mg/kg
Pup-directed maternal behaviors	Crouch	Frequency	7.2±1.5	4.1±1.7$_{l}$	6.0±1.5	11.3±1.5	7.7±1.5	8.1±1.6	6.3±1.5	5.0±1.4	9.4±1.4
		Duration (s)	824.8±145.1	1477.9±173.5	1294.3±145.1	1382.3±145.1	1444.1±145.1	782.0±162.3	1587.1±153.0$_{h}$	1290.6±138.4	1206.2±138.4
		Latency (s)	257.4±110.7	234.8±132.3	172.5±110.7	296.0±110.7	259.9±110.7	163.0±123.8	125.0±116.7	338.0±105.5	225.4±105.5
	Nest-build	Frequency	11.6±2.4	3.1±32.8	0.3±2.4	3.2±2.4	3.9±2.4	7.9±2.7	2.8±2.5	1.4±2.3	2.9±2.3
		Duration (s)	92.1±21.4	13.6±25.6	1.4±21.4	5.9±21.4	15.6±21.4	74.6±23.9	12.4±22.5	2.0±20.4	18.8±20.4
		Latency (s)	1032.0±252.1	685.8±301.3	1479.8±252.1	998.0±252.1	681.3±252.1	758.2±281.8	1107.4±265.7	1131.6±240.4	1157.3±240.4
	Touch/sniff	Frequency	18.4±1.8	15.0±2.1	14.3±1.8$_{h}$	15.2±1.8	17.1±1.8	23.6±2.0	13.9±1.9$_{h}$	15.9±1.6	23.5±1.7$_{l}$
		Duration (s)	85.5±11.7	78.3±14.0	71.6±11.7$_{h}$	76.4±11.7	79.5±11.7	127.9±13.1	52.2±12.3$_{H}$	73.9±10.7$_{h}$	99.8±11.2
		Latency (s)	17.0±7.7	19.0±9.2	4.8±7.7	21.7±7.7	16.2±7.7	14.1±8.6	29.2±8.1	22.6±7.0	9.7±7.3
	Lick pups	Frequency	3.4±0.9$_{h}$	0.0±1.1	0.9±0.9	0.9±0.9	0.5±0.9	4.9±1.0	0.3±1.0$_{h}$	0.2±0.9$_{h}$	1.8±0.9
		Duration (s)	27.3±6.7	0.0±8.0	9.5±6.7	1.0±6.7	0.7±6.7	13.0±7.5	0.5±7.1	0.9±6.4	11.2±6.4
		Latency (s)	619.9±239.1	1800.0±285.8	1541.3±239.1	1281.6±239.1	985.3±239.1	693.7±267.4	1231.0±252.1	1330.7±228.0	873.6±228.0
Non-pup directed	Self-groom	Frequency	6.4±1.8	1.3±2.1	3.7±1.8	5.1±1.8	5.9±1.8	3.8±2.0	0.7±1.9	3.6±1.7	4.2±1.7
		Duration (s)	87.8±25.9	8.5±31.0	42.8±25.9	58.3±25.9	63.0±25.9	24.0±29.0	5.3±27.3	64.8±24.7	30.7±24.7
		Latency (s)	414.0±153.2	1129.9±183.2$_{h}$	218.5±153.2	495.0±153.2	538.2±153.2	306.7±171.3	1486.6±161.5$_{L,M,H}$	441.0±146.1	252.8±146.1
Activity	Other	Frequency	46.6±5.8	27.9±6.9	27.4±5.8	28.9±5.8	28.0±5.8	40.9±6.4	22.1±6.1	25.8±5.5	37.2±5.5
		Duration (s)	580.1±81.9$_{L,M}$	168.8±97.9$_{h}$	345.9±81.9	147.2±81.9	121.2±81.9	614.0±91.6	110.8±86.4$_{h}$	135.2±78.1$_{h}$	386.4±78.1
		Latency (s)	4.0±61.9	7.6±74.0	1.2±61.9	0.6±61.9	8.9±61.9	0.4±69.2	15.7±65.3	165.8±59.0	4.0±59.0

Mean±SEM of selected maternal behaviors on postpartum day 1. Means with capitalized subscripts differ at $p \leq 0.01$ while lower case subscripts differ at $p \leq 0.05$. Different letters indicate differences as follows: L (l) indicates groups significantly different from AFA/FLU low dose, M (m) indicates groups significantly different from AFA/FLU medium dose, and H (h) indicates groups significantly different from AFA/FLU high dose.

3.5.2. Non-pup directed and activity behavior

Significant treatment effects were found on the latency to begin self-grooming [$F(8,77)=6.99$, $p \leq 0.01$] and the duration of other behaviors [$F(8,77)=5.508$, $p \leq 0.01$].

3.5.2.1. AFA/FLU vs. AFA. Dams treated with the high dose of AFA/FLU groomed themselves earlier in the session and performed "other" behaviors longer (duration) than dams treated with the medium dose of AFA ($p \leq 0.05$). AFA/FLU low and medium dose treatment dams performed "other" behaviors for a shorter duration than AFA low dose treated dams ($p \leq 0.01$).

3.5.2.2. AFA/FLU vs. FLU. Dams treated with the all doses of AFA/FLU groomed themselves earlier in the session than those treated with the low dose of FLU ($p \leq 0.01$). AFA/FLU high dose treated dams also performed "other" behaviors for a longer duration FLU low and medium dose treated dams ($p \leq 0.05$).

3.6. Maternal aggression: AFA vs. SAL

3.6.1. Aggressive behaviors

There were no significant differences between groups on any measure of aggressive behavior (See Table 5).

3.6.2. Defensive behaviors

All AFA treated dams threatened for a shorter duration [$F(3,32)=7.20$, $p \leq 0.01$] and high dose AFA treated dams only threatened intruders less often [$F(3,32)=4.04$, $p \leq 0.02$] than SAL treated dams ($p \leq 0.01$).

3.6.3. Activity levels

There were no significant differences between groups on any measure of activity level.

3.7. Maternal aggression: FLU vs. SALY

3.7.1. Aggressive behaviors

Generally, high dose FLU treated dams tended to attack intruders more frequently, for a longer duration, and earlier than low and medium dose FLU groups and controls, though only duration of attack was significantly increased [$F(6,64)=2.36$, $p \leq 0.05$], as shown in Table 6. Dams treated with the high dose of FLU, attacked intruders over a longer duration of time than did SALY controls ($p \leq 0.05$).

3.7.2. Defensive behaviors

There was a significant treatment effect on threat duration [$F(6,64)=4.60$, $p \leq 0.04$]. Post hoc analysis indicated that dams treated with the high dose of FLU threatened intruders for a shorter duration than control dams ($p \leq 0.01$).

3.7.3. Activity levels

Treatment effects were evident for the duration of rear/sniff [$F(6,64)=2.65$, $p \leq 0.02$] and the latency to perform other behaviors [$F(3,40)=3.248$, $p \leq 0.03$]. FLU low and medium treated dams rear/sniffed more than SALY dams ($p \leq 0.05$). All FLU treated dams also began performing "other" behaviors later in the session than SALY dams ($p \leq 0.05$).

3.8. Maternal aggression: AFA/FLU vs. SALY

3.8.1. Aggressive behaviors

There were no significant differences on the frequency, duration, or latency of any aggressive behavior (see Table 6). Interestingly, the AFA/FLU dams all tended to nip or bite the intruder more than controls (ns).

Table 5
AFA vs. SAL: maternal aggression

Postpartum day 6 maternal aggression			Amfonelic acid			Saline
			0.625 mg/kg	1.25 mg/kg	2.5 mg/kg	
Aggressive	Fight/attack	Frequency	3.1±1.2	3.1±1.1	4.4±1.2	5.8±1.2
		Duration (s)	5.7±2.2	5.4±2.1	8.4±2.3	8.1±2.2
		Latency (s)	207.0±69.1	239.1±65.5	154.7±73.3	152.7±69.1
	Nip/bite	Frequency	6.2±2.7	3.3±2.5	5.6±2.8	10.2±2.7
		Duration (s)	8.0±3.0	4.6±2.8	10.0±3.1	6.7±3.0
		Latency (s)	119.2±56.0	183.6±53.1	213.9±56.0	86.1±56.0
Defensive	Threat	Frequency	23.2±4.8	23.1±4.6	$13.4 \pm 5.1_s$	37.4±4.8
		Duration (s)	$54.4 \pm 18.3_s$	$52.6 \pm 17.3_s$	$29.9 \pm 19.4_s$	142.2±18.3
		Latency (s)	61.3±18.8	67.9±17.9	95.0±20.0	105.3±18.8
Activity	Rear/sniff	Frequency	21.6±3.0	16.1±2.8	16.5±3.1	13.0±3.0
		Duration (s)	53.5±10.3	41.1±9.7	38.6±10.9	28.8±10.3
		Latency (s)	19.6±13.7	45.5±13.0	34.5±14.6	63.6±13.7
	Other	Frequency	52.8±5.7	49.6±5.4	49.1±6.1	55.2±5.7
		Duration (s)	326.7±23.7	353.2±22.5	359.2±25.2	300.5±23.7
		Latency (s)	04.±0.2	06.±0.1	0.6±0.2	0.3±0.2

Mean±SEM of selected maternal aggressive behaviors on postpartum day 6. Means with subscripts differ at $p \leq 0.01$ and different letters indicate differences as follows: S (s) indicates groups significantly different from saline control.

Table 6
FLU and AFA/FLU vs. SALY maternal aggression

Postpartum day 6 maternal aggression			Fluoxetine			Yoke-fed saline	Amfonelic acid/fluoxetine		
			2.0 mg/kg	4.0 mg/kg	8.0 mg/kg		0.625/2.0 mg/kg	125/4.0 mg/kg	2.5/8.0 mg/kg
Aggressive	Fight/attack	Frequency	4.3±1.4	4.2±1.2	6.5±1.3	4.3±1.3	4.4±1.4	4.5±1.5	5.7±1.4
		Duration (s)	8.0±2.7	8.7±2.2	$16.3\pm2.4_{y}$	6.3±2.4	4.6±2.6	6.4±2.9	7.5±2.7
		Latency (s)	193.5±67.4	196.6±56.1	92.2±61.0	146.1±61.0	301.2±64.0	102.3±71.5	185.9±67.4
	Nip/bite	Frequency	2.4±1.7	2.6±1.4	1.8±1.5	5.0±1.5	11.2±1.6	10.0±1.8	6.1±1.7
		Duration (s)	4.4±1.8	3.2±1.5	4.1±1.7	3.3±1.7	8.0±1.7	5.9±1.9	4.9±1.8
		Latency (s)	247.9±70.8	294.3±58.9	349.2±64.0	199.3±64.0	138.6±67.2	38.0±75.1	75.0±70.8
Defensive	Threat	Frequency	17.6±4.7	19.4±3.9	20.5±4.2	34.9±4.2	33.7±4.4	38.1±5.0	29.4±4.7
		Duration (s)	105.9±26.8	105.3±22.3	$78.0\pm24.3_{y}$	203.5±24.3	99.3±25.4	143.4±28.4	97.7±26.8
		Latency (s)	67.0±18.6	104.6±15.4	96.1±16.8	53.8±16.8	81.7±17.6	47.9±19.7	56.1±18.6
Activity	Rear/sniff	Frequency	16.2±2.5	15.1±2.1	11.4±2.3	7.5±2.3	$20.2\pm2.4_{m,Y}$	8.1±2.4	9.9±2.5
		Duration (s)	$43.3\pm8.6_{y}$	$38.6\pm7.2_{y}$	22.7±7.8	14.8±7.8	$45.6\pm8.2_{m,Y}$	14.6±9.1	21.7±8.6
		Latency (s)	27.4±39.2	92.7±32.6	72.0±35.5	126.9±35.5	22.7±37.2	96.1±41.6	31.2±39.2
	Other	Frequency	39.7±4.5	40.8±3.7	45.5±4.1	45.8±4.1	60.7±4.3	51.25±4.8	50.0±4.5
		Duration (s)	347.4±22.3	329.7±18.6	358.6±20.2	314.5±20.2	317.2±21.2	340.3±23.7	330.3±22.3
		Latency (s)	$1.8\pm0.4_{y}$	$1.5\pm0.4_{y}$	$2.3\pm0.4_{y}$	0.1±0.4	0.1±0.4	0.2±0.5	0.2±0.4

Mean±SEM of selected maternal aggressive behaviors on postpartum day 6. Means with capitalized subscripts differ at $p \leq 0.01$ while lower case subscripts differ at $p \leq 0.05$. Different letters indicate differences as follows: L (l) indicates groups significantly different from low dose of that drug treatment, M (m) indicates groups significantly different from medium dose of that respective drug treatment, H (h) indicates groups significantly different from high dose of that respective drug treatment and Y (y) indicates groups significantly different from saline control.

3.8.2. Defensive behaviors

There were no significant differences in the frequency, duration, or latency of any defensive behavior.

3.8.3. Activity levels

The frequency [$F(6,64)=3.73$, $p \leq 0.01$] and duration [$F(6,64)=2.65$, $p \leq 0.02$] of rear/sniff were significantly different between groups, although general activity (other) was not. The low dose AFA/FLU treated dams had a higher frequency of rear/sniff compared to AFA/FLU medium treated dams ($p \leq 0.05$), and SALY dams ($p \leq 0.01$), and did it longer (duration) than AFA/FLU medium dose ($p \leq 0.05$) and SALY treated dams ($p \leq 0.01$).

3.9. Maternal aggression: AFA/FLU vs. AFA and FLU

3.9.1. Aggressive behaviors

Table 7 illustrates significant treatment effects on attack duration [$F(8,78)=2.10$, $p \leq 0.05$], and frequency [$F(8,78)=4.76$, $p \leq 0.01$] and latency [$F(8,78)=2.49$, $p \leq 0.02$] of nip/bite.

3.9.1.1. AFA/FLU vs. AFA. AFA/FLU low dose treated dams had higher frequencies of nip/bite than AFA medium treated dams ($p \leq 0.01$).

3.9.1.2. AFA/FLU vs. FLU. AFA/FLU low treated dams attacked for shorter periods (duration) than FLU high treatment dams ($p \leq 0.05$). AFA/FLU medium and low dose dams had higher frequencies of nip/bite than all FLU treated dams ($p \leq 0.05$, 0.01 respectively), and AFA/FLU medium dose dams also had a shorter latency to nip/bite than FLU high treated dams ($p \leq 0.05$).

3.9.2. Defensive behaviors

Threat frequency was significantly different between treatment groups [$F(8,78)=3.06$, $p \leq 0.01$].

3.9.2.1. AFA/FLU vs. AFA. Post hoc analysis revealed that AFA/FLU low and medium dose treated dams threatened more than AFA high dose dams ($p \leq 0.05$).

3.9.2.2. AFA/FLU vs. FLU. There were no significant differences between AFA/FLU treated dams and FLU treated dams on measures of defensive behaviors.

3.9.3. Activity levels

There was a significant treatment effect on frequency of rear/sniff behavior [$F(8,78)=2.59$, $p \leq 0.02$] and latency to begin "other" behaviors [$F(8,78)=4.38$, $p \leq 0.01$].

3.9.3.1. AFA/FLU vs. AFA. There were no significant differences between AFA/FLU treated dams and AFA treated dams on activity levels.

3.9.3.2. AFA/FLU vs. FLU. Medium dose AFA/FLU treatment dams had a lower frequency of rear/sniff behavior than FLU low dose treated dams ($p \leq 0.05$). All AFA/FLU treated dams began performing "other" behaviors earlier than the high dose FLU treated dams ($p \leq 0.01$).

3.10. Oxytocin radioimmunoassay

Mean oxytocin levels (picograms/milligram) and SEM are shown in Table 8. There were no significant between-group differences in oxytocin levels in the amygdala or MPOA, although interestingly, the high dose FLU and AFA/FLU treated dams, which were the most aggressive,

Table 7
AFA and FLU vs. AFA/FLU maternal aggression

Postpartum day 6 maternal aggression			Amfonelic acid			Amfonelic acid/fluoxetine			Fluoxetine		
			0.625 mg/kg	1.25 mg/kg	2.5 mg/kg	0.625/2.0 mg/kg	1.25/4.0 mg/kg	2.5/8.0 mg/kg	2.0 mg/kg	4.0 mg/kg	8.0 mg/kg
Aggressive	Fight/attack	Frequency	3.1±1.4	3.1±1.3	4.4±1.4	4.4±1.3	4.5±1.4	5.7±1.4	4.3±1.4	4.2±1.1	6.5±1.2
		Duration (s)	5.7±2.6	5.4±2.5	8.4±2.8	4.6±2.5	6.4±2.8	7.5±2.6	8.0±2.6	8.7±2.2	16.3±2.4
		Latency (s)	207.0±70.3	239.1±66.7	154.7±74.5	301.2±66.7	102.3±74.5	185.9±70.3	193.5±70.3	196.6±58.5	92.1±96.6
	Nip/bite	Frequency	6.2±1.6	$3.3\pm1.5_{L}$	5.6±1.7	112±1.5	10.0±1.7	6.1±1.6	$2.4\pm1.6_{L,m}$	$2.6\pm1.3_{L,m}$	$1.8\pm1.5_{L,m}$
		Duration (s)	8.0±2.2	4.6±2.1	10.0±2.4	8.0±2.1	5.9±2.4	4.9±2.2	4.4±2.2	3.2±1.9	4.1±2.0
		Latency (s)	119.2±68.0	183.6±64.5	213.9±72.1	138.6±64.5	38.0±72.1	75.0±68.0	247.9±68.0	294.3±56.6	$349.2\pm61.5_{m}$
Defensive	Threat	Frequency	23.2±4.5	23.1±4.3	$13.4\pm4.8_{l,m}$	33.7±4.3	38.1±4.8	29.4±4.5	17.6±4.5	19.4±3.8	20.5±4.1
		Duration (s)	54.4±21.4	52.6±20.3	29.9±22.7	99.3±20.3	143.4±22.7	97.7±21.4	105.9±21.4	105.3±17.8	80.0±19.4
		Latency (s)	61.3±18.7	67.9±17.8	95.0±19.8	81.7±17.8	47.9±19.8	56.1±18.7	67.0±18.7	104.6±15.6	96.1±16.9
Activity	Rear/sniff	Frequency	21.6±2.8	16.1±2.7	16.5±3.0	20.2±2.7	8.1±3.0	9.9±2.8	$16.2\pm2.8_{m}$	15.1±2.3	11.4±2.5
		Duration (s)	53.5±9.5	41.1±9.1	38.6±10.1	45.6±9.1	14.6±10.1	21.7±9.5	43.3±9.5	38.6±7.9	22.7±8.6
		Latency (s)	19.6±28.1	45.5±26.7	34.5±29.9	22.6±26.7	96.1±29.9	31.2±28.2	27.4±28.2	92.7±23.4	72.0±25.5
	Other	Frequency	52.8±5.2	49.6±4.9	49.1±5.5	60.7±4.9	51.3±5.5	50.0±5.2	39.7±5.2	40.8±4.3	45.5±4.7
		Duration (s)	326.7±22.5	353.2±21.3	359.2±23.8	317.2±21.3	340.3±23.8	330.3±22.5	347.4±22.5	329.7±18.7	358.6±20.3
		Latency (s)	0.4±0.4	0.6±0.4	0.6±0.4	0.1±0.4	0.2±0.4	0.2±0.4	1.8±0.4	1.5±0.3	$2.3\pm0.4_{L,M,H}$

Mean±SEM of selected maternal aggressive behaviors on postpartum day 6. Means with capitalized subscripts differ at $p \leq 0.01$ while lower case subscripts differ at $p \leq 0.05$. Different letters indicate differences as follows: L (l) indicates groups significantly different from AFA/FLU low dose, M (m) indicates groups significantly different from AFA/FLU medium dose, and H (h) indicates groups significantly different from AFA/FLU high dose.

Table 8
Oxytocin levels

		Medial preoptic area	Amygdala	Ventral tegmental Area	Hippocampus
Amfonelic acid	0.625 mg/kg	2.344±0.415	0.923±0.035	1.793±0.600	$0.642\pm0.024_{s}$
	1.25 mg/kg	2.122±0.415	0.974±0.033	2.323±0.600	$0.639\pm0.024_{s}$
	2.5 mg/kg	2.313±0.415	0.969±0.033	1.267±0.600	$0.702\pm0.024_{s}$
Fluoxetine	2.0 mg/kg	1.503±0.348	0.969±0.026	1.650±0.275	0.797±0.034
	4.0 mg/kg	2.380±0.305	0.923±0.022	0.959±0.262	0.915±0.031
	8.0 mg/kg	1.464±0.311	0.891±0.024	1.671±0.262	0.894±0.031
Amfonelic acid/fluoxetine	0.625/2.0 mg/kg	1.906±0.348	0.959±0.026	1.169±0.275	$0.705\pm0.032_{S,L,M}$
	1.25/4.0 mg/kg	2.315±0.366	0.931±0.027	1.474±0.290	$0.715\pm0.034_{S,L,M}$
	2.5/8.0 mg/kg	1.675±0.366	0.884±0.027	$2.459\pm0.389_{S,M}$	$0.644\pm0.039_{S,L,M,H}$
Controls	Saline	1.512±0.437	0.967±0.037	1.086±0.632	0.924±0.025
	Yoke-fed saline	1.206±0.331	0.925±0.026	0.857±0.262	0.932±0.031

Mean±SEM of oxytocin levels on postpartum day 7. Means with capitalized subscripts differ at $p \leq 0.01$ while lower case subscripts differ at $p \leq 0.05$. Different letters indicate differences as follows: L (l) indicates groups significantly different from low dose of fluoxetine, M (m) indicates groups significantly different from medium dose of fluoxetine, H (h) indicates groups significantly different from high dose of fluoxetine, and S (s) indicates groups significantly different from their respective saline control.

had the lowest oxytocin levels in the amygdala. There was a significant treatment effect on oxytocin levels in the hippocampus [$F(3,35)=29.164$, $p \leq 0.01$] and VTA [$F(3,32)=5.3$, $p \leq 0.04$]. Only significant group differences are presented in text.

3.10.1. AFA vs. SAL

All AFA treated dams exhibited lower levels of oxytocin in the hippocampus than SAL ($p \leq 0.01$).

3.10.2. AFA/FLU vs. SALY

All AFA/FLU treated animals had lower levels of oxytocin in the hippocampus than SALY dams ($p \leq 0.01$). AFA/FLU high dose treated dams had higher levels of oxytocin in the VTA than SALY dams ($p \leq 0.05$).

3.10.3. AFA/FLU vs. FLU

High dose AFA/FLU treated dams had lower levels of oxytocin in the hippocampus than all FLU treated dams ($p \leq 0.01$) and higher levels of oxytocin in the VTA than medium dose FLU treated dams ($p \leq 0.05$). AFA/FLU treated low and medium dose dams had lower hippocampal oxytocin levels than FLU treated low and medium dose dams only ($p \leq 0.01$).

4. Discussion

Results of the present study, in part support previous work by our lab and others, with respect to maternal behavior and some forms of aggression, in that increased dopamine release or chronic reuptake inhibition, have been shown to increase certain aspects of maternal behavior and decrease aggression (Byrnes et al., 2002; Hansen et al., 1991a,b; Hansen, 1994; Johns et al., 1995, 1996; Keer and Stern, 1999; Numan, 1994a,b; Silva et al., 2001; Stern and Taylor, 1991). Dopamine has been strongly associated with both the onset and maintenance of maternal behavior as well as pup-induced maternal behavior (Byrnes et al., 2002; Hansen et al., 1991a,b; Hansen, 1994; Keer and Stern, 1999; Numan, 1994a,b; Olazabal et al., 2004; Silva et al., 2001, Stern and Taylor, 1991). In the present study, while licking, touching, and activity, all dopamine related behaviors, were increased relative to controls in all the AFA and AFA/FLU treated dams, crouching seems to be altered through other systems, which is in agreement with previous findings comparing serotonin and dopamine antagonists (Keer and Stern, 1999). The duration of crouching was particularly lower in the low dose AFA and high dose AFA/FLU treatment dams, while licking, nest building, touching, and activity were increased the most in these two groups, implicating a dopaminergic influence on at least the appetitive and active aspects of maternal behavior. Crouching behavior has been shown to be increased following treatment with a single dose (1.5 mg/kg) of AFA given gestationally (Johns et al., 1996). Though the low dose AFA dams crouched less in the present study, the two highest doses of AFA did not significantly alter crouch duration, perhaps suggesting that increased activity levels induced by the AFA low dose treatment may have interfered with this behavior.

Serotonin, though more strongly associated with aggression, also affects maternal behavior. Barofsky et al. (1983) found impairments in lactation and pup retrieval, and higher incidences of pup cannibalism following specific serotonergic neurotoxin lesions of the median raphe, a major production site for serotonin. Keer and Stern (1999) reported that following an intracerebral ventricular (ICV) infusion of a serotonergic antagonist, crouching in rat dams on PPD 6 was not disrupted. However, when the antagonist was infused directly into the nucleus accumbens, it increased crouching duration. Furthermore, juvenile rats, which are more likely to be induced to behave maternally, have been shown to have higher levels of serotonin than adults in the MPOA, a region relevant to maternal behavior (Olazabal et al., 2004). In the present study, serotonin reuptake inhibition by FLU slightly decreased crouching except in the low dose FLU treated dams (2.0 mg/kg) who displayed the longest duration of crouching

by any dams, while all doses of FLU increased licking and touching compared to controls, especially the high dose group. It appears that uptake inhibition of dopamine or serotonin systems affects crouching differentially as compared to the more appetitive aspects of maternal behavior such as licking and touching pups. It is interesting that the low and medium dose FLU treatment dams had slightly higher rates of licking and touching pups than control dams, but did not show a corresponding increase in activity and repetitive self-directed behavior (self-groom). Specifically, only the high doses of FLU and AFA/FLU and low dose of AFA treatment most significantly affected both appetitive and activity related pup-directed maternal behaviors. Though we chose a dose of FLU specifically to avoid effects on the dopamine system, it is possible that some of the increased activity by the high dose treatment dams was a result of FLU induced dopamine activity. We did not examine levels of serotonin or dopamine following treatment, but other studies have found that fluoxetine (10 mg/kg) can alter dopamine levels and that AFA treatment can alter serotonin levels in specific brain regions depending on the dose and regiment used (Bymaster et al., 2002; McMillen et al., 1991).

When both neurotransmitter systems were stimulated with the AFA/FLU combination, the results were very interesting. Dams that received the combined treatment, displayed a latency to begin maternal behaviors similarly to AFA treated dams generally, but rates and duration of pup directed maternal behaviors such as crouching and licking were more similar to FLU, as were their activity levels (other, self-groom). The highest dose of AFA/FLU treatment produced effects very similar to the low dose AFA treatment on several maternal behaviors (nest build, lick pups, crouching). Though the high dose AFA/FLU dams had generally higher activity rates than other AFA/FLU treatment dams, their active maternal behavior (with the exception of crouch duration) was in fact very good. This would suggest that with respect to simultaneous serotonin and dopamine reuptake inhibition, at the highest dose the behavioral effects of AFA/FLU are likely having a greater effect on general activity than that seen at lower doses, and perhaps the lower duration of crouching reflects the greater time spent in more appetitive aspects of maternal behavior. These studies in conjunction with others previously mentioned establish another correlation between serotonin and maternal behavior, but more research is needed to make specific conclusions.

There have been conflicting findings concerning dopamine and aggressive behavior. Dopamine transporter knockout mice, which were characterized as being easily aroused by novelty, did not exhibit higher levels of aggressiveness (Spielewoy et al., 2000). Rat dams that were given VTA microinfusions of 6-hydroxydopamine (6-OHDA) during lactation showed a persistent deficit in pup retrieval, but were not impaired with respect to nursing, nest-building, or maternal aggression (Hansen et al., 1991b), whereas ICV infused 6-OHDA resulted in hyperemotional and hyper-aggressive behavior in female rats (Sorenson and Gordon, 1975). Preliminary findings from our lab (Johns et al., 1995) reported that maternal aggressive behavior following 1.5 mg/kg of AFA given to rats during gestation reduced maternal aggressive behavior significantly on PPD 6. Similarly, in the present study all three doses of AFA decreased the frequency of all aggressive and defensive behaviors compared to controls, while only slightly increasing the duration of "other" and stereotypical (rear/sniff) behaviors. This would suggest that hyperactivity alone does not account for the lower aggressive behavior. Changes in dopamine receptors following AFA treatment were not examined in this study and dopamine receptor changes in specific brain regions cannot be ruled out as a factor in behavioral changes.

Gestational treatment with the high dose of FLU was correlated with elevated offensive (attack) and lowered defensive (threat) behavior compared to control dams as reported previously (Lomas et al., 2002). All FLU treated dams were less likely to nip/bite or threaten the intruder and had higher rates of stereotypical behavior (rear/sniff), but performance of other non-aggressive behaviors was similar to controls. Aggression has been most strongly linked to serotonin levels, and serotonin agonist administration is correlated with significantly lower levels of maternal aggression (Olivier and Mos, 1992; Olivier et al., 1995). Serotonin transporter knockout mice and SSRI-exposed mice display lower levels of aggression as well (Holmes et al., 2002; Olivier et al., 1995). These findings indicate that higher synaptic levels of serotonin are correlated with lower levels of aggression. Although FLU treatment and the resulting serotonergic reuptake inhibition would be expected to initially increase serotonin availability, over time a decreased release might result in lower levels. Given that FLU is reported to have relatively long efficacy, it is possible that by PPD 6, levels and receptors are differentially altered. Additionally, lower oxytocin levels in the amygdala have been associated with increased maternal aggression in lactating dams on PPD 6 (Johns et al., 1995, 1998), and though oxytocin levels in the amygdala of the high FLU dose group were not significantly lower than controls, they were lower than most other groups. It was recently reported that gestational treatment with the same dose and regimen of FLU treatment used in the present study resulted in a lower affinity for oxytocin receptor binding in the amygdala of lactating rat dams on PPD 6 (Johns et al., 2004). It may be that the combined effects of slightly lower oxytocin levels and a lower affinity of the receptor for oxytocin in the amygdala could affect maternal aggressive behavior in these dams. Much of the data demonstrating decreases in aggression as a result of increased serotonin levels also come from studies looking at resident-intruder (RI) aggression, which has motivational and hormonal effects very different from maternal aggression. Olivier et al. (1995) reported differing effects on RI and maternal

aggression depending on which serotonin receptor subtype was stimulated by an agonist or antagonist.

The combination of AFA and FLU did not significantly increase maternal aggression, but the high dose AFA/FLU treatment resulted in slightly higher levels of fight attack, second only to the FLU high dose treatment, and they also had lower amygdaloidal oxytocin levels as did the FLU high dose treated dams. Unlike the FLU high dose treatment, gestational treatment with a combined AFA/FLU high dose treatment did not significantly alter oxytocin receptor affinity or binding in the amygdala on PPD 6 (Johns et al., 2004). Overall the AFA/FLU groups look more like FLU treated dams with regard to fighting and more like AFA treated dams on defensive (threat) and activity related (other, rear/sniff) behaviors. AFA/FLU treated dams were more likely to nip/bite intruders and did it sooner than all other dam groups. This was the only behavior that totally differentiated them from all other dams.

Generally, adequate levels of oxytocin in the VTA have been associated with normal maternal behavior during the very early postpartum period (Numan, 1994a,b). We have not previously reported oxytocin level changes in the VTA as far out as PPD 6 following gestational treatment with cocaine or 1.5 mg/kg of AFA (Johns et al., 1997). Since the VTA is the source of the dopamine neurons of the dopaminergic mesolimbic system, the high dose AFA/FLU treatment may initiate a dopaminergic–oxytocinergic interaction, which could possibly result in greater oxytocin release (Sarnyai and Kovacs, 1994; Pedersen et al., 1994), although neither AFA nor high FLU doses produced a similar result to the combined drug which might have been expected.

The biggest treatment effects on oxytocin were seen in the hippocampus, another limbic region of interest. Oxytocin levels were reduced relative to controls in all groups treated with AFA alone or in combination with FLU, indicating dopaminergic involvement in these effects as well. Previously, we have reported that cocaine treatment, which produces simultaneous reuptake inhibition of dopamine, serotonin, and norepinephrine, results in reductions in oxytocin levels in the hippocampus (Johns et al., 1993, 1997), which vary depending on treatment and the time of sacrifice. Although the role of uptake inhibition on oxytocin levels in the hippocampus are not clear, oxytocin levels in this region have been reported to affect both drug dependence, tolerance (Sarnyai and Kovacs, 1994) and spatial learning (Tomizawa et al., 2003). Since so few consistent behavioral effects were found in all the treatment groups associated with reduced hippocampal oxytocin levels in the present study, no particular correlations between behavioral and oxytocin data could be made. Further studies concerning the role of oxytocin in this brain region would prove useful.

It is interesting to speculate whether a behavioral stimulus such as pups or an intruder alters oxytocin levels as a result of the stimulus or oxytocin changes occur directly from drug treatment prior to behavior stimulus presentation. If there is a behavioral and biochemical effect resulting from different drug treatments, then it becomes important to understand if that treatment resulted in either a static (oxytocin response before stimulus) or dynamic (oxytocin response after pup or intruder challenge) biological response, but in either case, effects still are the final result of a specific treatment when all else is held constant. We have previously noted that oxytocin level changes in the amygdala have the same relationship to control levels following cocaine treatment, whether measured before or after an aggressive encounter in the early postpartum period (PPD 6–8). Of course drug treatments in the present study are different from cocaine on several levels and so are not directly comparable. The oxytocin response may also be different when testing for the initial onset of maternal behavior since the pup stimuli are so salient, and thus a time course study would be necessary to answer this question. What is clear, is that both oxytocin system and overt behavioral changes, whether static or dynamic, occur in response to different drug treatments and that the relationship between oxytocin, drugs and behavior is complex and involves multiple variables.

It would have been preferable to be able to assess direct changes in levels of serotonin and dopamine in brain regions of interest following drug treatment, but our focus on oxytocin precluded our doing this. Other studies, while not directly comparable to the present drug regimens, do suggest that treatment with fluoxetine and amfonelic acid can alter levels of dopamine, serotonin or their metabolites in various brain regions (McMillen et al., 1991).

The number of dose groups and independent measures of behavior involved in this experiment necessarily meant there would be a large number of multiple comparisons which always raises the question of a greater chance of Type I errors. We combined groups with the same controls where possible to reduce statistical comparisons and have used stringent post hoc tests (Tukey HSD) to reduce the likelihood of finding significance where there really is none. Given the typical variability within groups, which reduces the chance of finding significance and our use of stringent tests, we feel that the differences we report are fairly robust and present in patterns which are logical given our drug groups.

Effects of noradrenergic uptake inhibition, not reported in the present study, were examined in pilot studies, but preliminary data indicated few effects on maternal behavior/aggression or oxytocin (Hofler et al., 2003). This does not negate the possibility that future studies could find other effects on different aspects of maternal behavior or oxytocin related to noradrenergic or combined noradrenergic and serotonergic uptake inhibition.

The differential association of specific maternal behaviors with either reuptake inhibition of dopamine, serotonin, or both is important and will perhaps lead to future studies

examining how the two systems possibly interact to impact specific aspects of maternal behavior and aggression, such as motivational vs. activity related behaviors. The present study may prove to be very clinically relevant, since new treatments for depression may incorporate the use of combined dopamine and serotonin neurotransmitter uptake inhibition for depression (Skolnick et al., 2003; Beer et al., 2004). Additionally, the effects of combined reuptake inhibition during pregnancy will certainly be intensely studied in the future, given recent reports (Moses-Kolko et al., 2005) of behavioral and physiological effects of SSRIs and other drugs on newborns which are somewhat similar to effects seen in babies exposed prenatally to cocaine (Mayes, 2002). It will also be important to further examine exactly how these systems impact the oxytocin system across different brain regions and whether those changes are time and stimulus dependent. Recently, human studies are emerging suggesting a more relevant role for oxytocin in maternal care than might have been previously suspected in humans (Light et al., 2001, 2004).

We have now demonstrated that dopamine reuptake inhibition generally alters the more appetitive aspects of maternal behavior and general activity but has relatively few effects on crouching and maternal aggression, other than suppression of aggression by dopaminergic inhibition. Behavioral data suggests a stronger dopaminergic influence on activity related maternal behaviors, but crouching behavior seems to be affected differentially with much less of a dopaminergic influence. The relevance this work might have to the human drug use during pregnancy (legal and illegal) remains to be seen, but for previously mentioned populations may prove very timely.

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References

Amico JA, Pomerantz SM, Layden LM, Cameron JL. The oxytocin secretory response to dopamine receptor agonists in male and female monkeys. Ann NY Acad Sci 1992;652:478–80.

Amico JA, Layden LM, Pomerantz SM, Cameron JL. Oxytocin and vasopressin secretion in monkeys administered apomorphine and a D2 receptor agonist. Life Sci 1993;52(15):1301–9.

Bagdy G. Role of the hypothalamic paraventricular nucleus in 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptor-mediated oxytocin, prolactin, and ACTH/-corticosterone responses. Behav Brain Res 1996;73:277–80.

Bagdy G, Kalogeras K. Stimulation of 5-HT1A and 5-HT2/H-HT1C receptors induce oxytocin release in the male rat. Brain Res 1993;611: 330–2.

Bagdy G, Kalogeras K, Szemeredi K. Effect of 5-HT1C and 5-HT2 receptor stimulation on excessive grooming, penile erection and plasma oxytocin concentrations. Eur J Pharmacol 1992;229:9–14.

Barofsky A, Taylor J, Tizabi Y, Kumar R, Jones-Quartey K. Specific neurotoxin lesions of median raphe serotonergic neurons disrupt maternal behavior in the lactating rat. Endocrinology 1983;113(5): 1884–93.

Beer B, Stark J, Krieter P, Czobor P, Beer G, Lippa A, et al. DOV 216,303, a "triple" reuptake inhibitor: safety, tolerability, and pharmacokinetic profile. J Clin Pharmacol 2004;44:1360–7.

Bymaster FP, Zhang W, Carter PA, Shaw J, Chernet E, Phebus L, et al. Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. Psychopharmacology (Berl) 2002;160(4):353–61.

Byrnes EM, Rigero BA, Bridges RS. Dopamine antagonists during parturition disrupt maternal care and retention of maternal behavior in rats. Pharmacol Biochem Behav 2002;73(4):869–75.

Coccaro EF. Central serotonin and impulsive aggression. Br J Psychiatry Suppl 1989;8:52–62.

Coccaro EF. Impulsive aggression and central serotonergic system function in humans: an example of a dimensional brain–behavior relationship. Int Clin Psychopharmacol 1992;7(1):3–12.

Cooper JR, Bloom FE, Roth RH. The biological basis of neuropharmacology. 7th ed. New York: Oxford University Press; 1996.

Crowley WR, Parker SL, Armstrong WE, Spinolo LH, Grosvenor CE. Neurotransmitter and neurohormonal regulation of oxytocin secretion in lactation. Ann NY Acad Sci 1992;652:286–302.

Cunningham KA, Paris JM, Goeders NE. Chronic cocaine enhances serotonin autoregulation and serotonin uptake binding. Synapse 1992; 11:112–23.

De Almeida RM, Lucion AB. Effects of intracerebroventricular administration of 5-HT receptor agonists on the maternal aggression of rats. Eur J Pharmacol 1994;264(3):445–8.

Fahrbach SE, Morrell JI, Pfaff DW. Role of oxytocin in onset of estrogen-facilitated maternal behavior. In: Amico J, Robinson AG, editors. Oxytocin: clinical and laboratory studies. Amsterdam: Elsevier; 1985. p. 372–88.

Ferris C, Foote K, Meltser H, Plenby M, Smith K, Insel T. Oxytocin in the amygdala facilitates maternal aggression. In: Pedersen CA, Caldwell JD, Jirikowski JD, Insel TR, editors. Oxytocin and maternal sexual and social behaviors, vol. 474. New York: New York Academy of Sciences; 1992. p. 226–33.

Fuller RW, Perry KW. Amfonelic acid antagonism of dopamine and norepinephrine depletion by a-methyl-*m*-tyrosine in rat brain. Biochem Pharmacol 1981;30:2025–6.

Gaffori O, Le Moal M. Disruption of maternal behavior and appearance of cannibalism after ventral mesencephalic tegmentum lesions. Physiol Behav 1979;23:317–23.

Giordano AL, Johnson AE, Rosenblatt JS. Haloperidol-induced disruption of the retrieval behavior and reversal with apomorphine in lactating rats. Physiol Behav 1990;48:211–4.

Goldberg H, Nissim R. Psychotropic drugs in pregnancy and lactation. Int J Psychiatry Med 1994;24(2):129–49.

Hansen S. Maternal behavior of the female rats with 6-OHDA lesions in the ventral striatum: characterization of the pup retrieval deficit. Physiol Behav 1994;55:615–20.

Hansen S, Harthon C, Wallin E, Lofbergi L, Svensson K. The effects of 6-OHDA-induced dopamine depletions in the ventral or dorsal striatum on maternal and sexual behavior in the female rat. Pharmacol Biochem Behav 1991a;39(1):71–7.

Hansen S, Harthon C, Wallin E, Lofberg L, Svensson K. Mesotelencephalic dopamine system and reproductive behavior in the female rat: effects of ventral tegmental 6-hydroxydopamine lesions on maternal and sexual responsiveness. Behav Neurosci 1991b;105(4):588–98.

Hofler VE, Greenhill KW, Middleton CL, Knupp K, Elliott DA, Joyner PW, et al. Effects of neurotransmitter uptake inhibitors on maternal behavior in rats. Abstr - Soc Neurosci 2003;728:6.

Holmes A, Murphy D, Crawley J. Reduced aggression in mice lacking the serotonin transporter. Psychopharmacology 2002;161:160–7.

Honda K, Negoro H, Fukuaka T, Higuchi T, Uchide K. Effects of microelectrophoretically applied acetylcholine, noradrenalin, dopamine and serotonin on the discharge of the paraventricular oxytocinergic neurons in the rat. Endocrinology 1985;32:127–33.

Izenwasser S, Kornetsky C. The effect of amfonelic acid or nisoxetine in combination with morphine on brain-stimulation reward. Pharmacol Biochem Behav 1989;32(4):983–6.

Johns JM, Caldwell JD, Pedersen CA. Acute cocaine treatment decreases oxytocin levels in the rat hippocampus. Neuropeptides 1993;24(3): 165–9.

Johns JM, Faggin BM, Noonan LR, Li L, Zimmerman LI, Pedersen CA. Chronic cocaine treatment decreases oxytocin levels in the amygdala and increases maternal aggression in Sprague–Dawley rats. Abstr - Soc Neurosci 1995;21:1954.

Johns JM, Noonan LR, Miles S, Zimmerman LI, Pedersen CA, Faggin BM. A comparison of the effects of cocaine and amfonelic acid treatment on the onset of maternal behavior. Proceedings of international society of behavioral neuroscience, May 2–5; 1996.

Johns J, Lubin D, Walker C, Meter K, Mason G. Chronic gestational cocaine treatment decreases oxytocin levels in the medial preoptic area, ventral tegmental area and hippocampus in Sprague–Dawley rats. Neuropeptides 1997;31(5):439–43.

Johns JM, Lubin DA, Walker CH, Joyner PW, Middleton CL, Hofler VE, et al. Gestational cocaine and fluoxetine treatment upregulates oxytocin receptor number and lowers binding affinity in the amygdala of rats on postpartum day six. Int J Dev Neurosci 2004;22(5–6):321–8 [Special issue: Developmental aspects of addiction—edited by RM Booze and CF Mactutus].

Johns JM, Noonan LR, Zimmerman LI, McMillen BA, Means LW, Walker CH, et al. Chronic cocaine treatment alters social/aggressive behavior in Sprague–Dawley rat dams and in their prenatally exposed offspring. Ann NY Acad Sci 1998;846:399–404.

Keer SE, Stern JM. Dopamine receptor blockade in the nucleus accumbens inhibits maternal retrieval and licking, but enhances nursing behavior in lactating rats. Physiol Behav 1999;67(5):659–69.

Kendrick KM, Keverne EB, Hinton MR, Goode JA. Oxytocin, amino acid and monoamine release in the region of the medial preoptic area and bed nucleus of the stria terminalis of the sheep during parturition and suckling. Brain Res 1992;569:199–209.

Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. Arch Gen Psychiatry 1994;51(1):8–19.

Kimble D, Rogers L, Hendrickson C. Hippocampal lesions disrupt maternal, not sexual behavior in the albino rat. J Comp Physiol Psychol 1967;63:401–7.

Konig JFR, Klippel RA. The rat brain: a stereotaxic atlas of the forebrain and lower parts of the brain stem. New York: Krieger; 1963.

Lemke MR, Fuchs G, Gemende I, Herting B, Oehlwein C, Reichmann H, et al. Depression and Parkinson's disease. J Neurol 2004;251(6): 24–7.

Light KC, Smith TE, Johns JM, Hofheimer JA, Amico JA. Oxytocin responsivity in mothers of infants: an initial study of relationships to laboratory and home blood pressure, affect and perceived support. Health Psychol 2001;19:560–7.

Light KC, Grewen KM, Amico JA, Boccia M, Brownley K, Johns JM. Deficits in plasma oxytocin responses and increased negative affect, stress and blood pressure in mothers with cocaine exposure during pregnancy. Addict Behav 2004;29(8):1541–64.

Lomas L, Joyner P, Ardalan C, Lubin D, Middleton C, Holfler V, et al. A comparison of the effects of a serotonergic and dopaminergic–serotonergic combination reuptake inhibitor on maternal aggressive behavior. Abstr - Soc Neurosci 2002;90:5.

Lubin D, Cannon J, Black M, Brown L, Johns J. Effects of chronic cocaine on monoamine levels in discrete brain structures of lactating rat dams. Pharmacol Biochem Behav 2003a;74:449–54.

Lubin DA, Elliot JC, Black MC, John JM. An oxytocin antagonist infused into the central nucleus of the amygdala increases maternal aggressive behavior. Behav Neurosci 2003b;117:195–201.

Mayer AD, Rosenblatt JS. A method for regulating the duration of pregnancy and the time of parturition in Sprague–Dawley rats. Dev Psychobiol 1998;32:131–6.

Mayes LC. A behavioral teratogenic model of the impact of prenatal cocaine exposure on arousal regulatory systems. Neurotoxicol Teratol 2002;24:385–95.

McMillen BA, Scott SM, Williams HL. Effects of subchronic amphetamine or amfonelic acid on rat brain dopaminergic and serotonergic function. J Neural Transm Gen Sect 1991;83(1–2):55–66.

Montero D, de Ceballos ML, Del Rio J. Down-regulation of 3H-imipramine binding sites in cerebral cortex after prenatal exposure to antidepressants. Life Sci 1990;46:1619–26.

Moses-Kolko EL, Bogen D, Perel J, Bregar A, Uhl K, Levin B, et al. Neonatal signs after late in utero exposure to serotonin reuptake inhibitors: literature review and implications for clinical applications. JAMA 2005;293(19):2372–83.

Numan M. A neural circuitry analysis of maternal behavior in the rat. Acta Paediatr Suppl 1994;39:19–28.

Numan M. Maternal behavior. In: Knobil E, Neill JD, editors. The physiology of reproduction. 2nd ed. New York: Raven Press; 1994. p. 221–301.

Numan M, Smith HG. Maternal behavior in rats: evidence for the involvement of preoptic projections to the ventral tegmental area. Behav Neurosci 1984;98:712–27.

Olazabal D, Abercrombie E, Rosenblatt J, Morrell J. The content of DA, serotonin, and their metabolites in the neural circuit that mediates maternal behavior in juvenile rats. Brain Res Bull 2004; 63:259–68.

Olivier B, Mos J. Rodent models of aggressive behavior and serotonergic drugs. Prog Neuropsychopharmacol Biol Psychiatry 1992;16:847–70.

Olivier B, Mos J, van Oorshot R, Hen R. Serotonin receptors and animal models of aggressive behaviors. Pharmacopsychiatry 1995;28:80–90.

Parker SL, Crowley WR. Activation of central D-1 dopamine receptors stimulates oxytocin release in the lactating rat: evidence for involvement of the hypothalamic paraventricular and supraoptic nuclei. Neuroendocrinology 1992;56(3):385–92.

Pedersen CA, Ascher JA, Monroe YL, Prange Jr AJ. Oxytocin induces maternal behavior in virgin female rats. Science 1982;216(4546): 648–50.

Pedersen CA, Caldwell JD, Johnson MF, Fort SA, Prange AJ. Oxytocin antiserum delays onset of ovarian-steroid induced maternal behavior. Neuropeptides 1985;6:175–82.

Pedersen CA, Caldwell JD, Walker CH, Ayers G, Mason GA. Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. Behav Neurosci 1994;108(6): 1163–71.

Porrino LJ, Goodman NL, Sharpe LG. Intravenous self-administration of the indirect dopaminergic agonist amfonelic acid by rats. Pharmacol Biochem Behav 1988;31(3):623–6.

Quintin P, Thomas P. Efficacy of atypical antipsychotics in depressive syndromes. Encephale 2004;30(6):583–9.

Raap DK, Van de Kar LD. Selective serotonergic uptake inhibitors and neuroendocrine function. Life Sci 1999;65:1217–35.

Ritz M, Cone E, Kuhar M. Cocaine inhibition of ligand binding at dopamine, norepinephrine, and serotonin transporters; a structure-activity study. Life Sci 1990;46:635–45.

Sarnyai Z, Kovacs GL. Role of oxytocin in the neuroadaptation to drugs of abuse. Psychoneuroendocrinology 1994;19:85–117.

Sawabini KA, Watts RL. Treatment of depression in Parkinson's disease. Parkinsonism Relat Disord 2004;10(1):37–41.

Saydoff JA, Rittenhouse PA, van de Kar LD, Brownfield MS. Enhanced serotonergic transmission stimulates oxytocin secretion in conscious male rats. J Pharmacol Exp Ther 1991;257(1):95–9.

Silva MR, Bernardi MM, Felicio LF. Effects of dopamine receptor antagonists on ongoing maternal behavior in rats. Pharmacol Biochem Behav 2001;68(3):461–8.

Silva MR, Bernardi MM, Cruz-Casallas PE, Felicio LF. Pimozide injections into the nucleus accumbens disrupt maternal behaviour in lactating rats. Pharmacol Toxicol 2003;93(1):42–7.

Skolnick P, Popik P, Janowsky A, Beer B, Lippa A. "Broad spectrum" antidepressants: is more better for the treatment of depression? Life Sci 2003;73:3175–9.

Sorenson CA, Gordon M. Effects of 6-hydroxydopamine on shock-elicited aggression, emotionality and maternal behavior in female rats. Pharmacol Biochem Behav 1975;3(3):331–5.

Spielewoy C, Roubert C, Hamon M, Nosten-Bertrand M, Betancur C, Giros B. Behavioral disturbances associated with hyperdopaminergia in dopamine-transporter knockout mice. Behav Pharmacol 2000; 11(3–4):279–90.

Stern JM, Keer SE. Maternal motivation of lactating rats is disrupted by low dosages of haloperidol. Behav Brain Res 1999;99(2):231–9.

Stern JM, Taylor LA. Haloperidol inhibits maternal retrieval and licking, but facilitates nursing behavior and milk ejection in lactating rats. J Neuroendocrinol 1991;3:591–6.

Thomas S, Palmiter RD. Impaired maternal behavior in mice lacking norepinephrine and epinephrine. Cell 1997;91:583–92.

Tomizawa K, Iga N, Lu YF, Moriwaki A, Matsushita M, Li ST, et al. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. Nat Neurosci 2003;6(4):384–90.

Uvnas-Moberg K, Hillegaart V, Alster P, Ahlenius S. Effects of 5-HT agonists, selective for different receptor subtypes, on oxytocin, CCK, gastrin and somatostatin plasma levels in the rat. Neuropharmacology 1996;35(11):1635–40.

Van de Kar LD, Rittenhouse PA, Li Q, Levy AD, Brownfield MS. Hypothalamic paraventricular, but not supraoptic neurons, mediate the serotonergic stimulation of the oxytocin secretion. Brain Res Bull 1995;36(1):45–50.

Van Leengoed E, Kerker E, Swanson HH. Inhibition of postpartum maternal behavior in the rat by injecting an oxytocin antagonist into the cerebral ventricles. J Endocrinol 1987;112:275–82.